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INTERFERENCE OF PLANT ESSENTIAL OILS ON THE FORAGING BEHAVIOR OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Plant essential oils restrained the course of foraging behavior of individual worker ants and also influenced worker recruitment and food transport. Worker search times increased significantly among the plant oil treatments. The longest search time was observed for ants treated with essential oils of *Capsicum annuum* L. (Solanales: Solanaceae) and *Cedrus deodara* (Roxb.) G.Don (Pinales: Pinaceae). Different essential oils have significantly different effects on the recruitment of the workers, the amount of food transported, and the time spent foraging.

Key Words: fire ants, plant essential oil, foraging behavior, interfering, repellency effects

RESUMEN

Los aceites esenciales de plantas restringieron la dirección de la conducta individual de forrajeo de individuos de las hormigas obreras y también influyeron la selección de los trabajadores y el transporte de alimentos. El tiempo de búsqueda de los trabajadores incrementaron significativamente entre los tratamientos con aceites vegetales. Se observó que el mayor tiempo de búsqueda en las hormigas tratadas con aceites esenciales de *Capsicum annuum* L. (Solanales: Solanaceae) y *Cedrus deodara* (Roxb.) G.Don (Pinales: Pinaceae). Diferentes aceites esenciales tienen efectos significativamente diferentes en la seleción de los trabajadores, la cantidad de alimentos transportados, y el tiempo de forrajeo.

Palabras Clave: hormigas de fuego, aceite esencial de la planta, comportamiento de forrajeo, interferir, efectos de repelencia

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) causes serious damage to humans, animals and the environment. The red imported fire ant was discovered in Southern China at the end of 2004. The ant has invaded schools, rice fields, lawns, and other public areas in both cities and rural locations (Zeng et al. 2005). Chemical treatments have been adopted as the main method for the control of this important medical and agricultural pest (Lofgren et al. 1975; Adams et al. 1983; Lofgren 1986; Adams et al. 1988; Drees & Gold 2003). However, in view of the environmental pollution resulting from such chemicals, the use of many types of pesticides is prohibited in areas within the vicinity of food or water sources (Williams & DeShazo 2004). Therefore, there is a growing interest in treatment methods that are non-toxic or only slightly toxic in the environment. This interest includes fire ant repellents that can potentially be applied in schools, nursing homes, hospitals and other pesticide-sensitive sites. The application of fire ant repellents to quarantined nurseries and equipment used for working the soil could also play an additional role in preventing the spread of fire ants to non-infested areas (Chen 2009).

Previous reports confirmed that a number of materials and compounds were effective repellents of fire ants (Oi & Williams 1996; Vander Meer et al. 1998; Anderson et al. 2002; Vogt et al. 2002; Chen 2005). Certain landscaping materials, such as cedar and cypress mulch, are known to be effective repellents of fire ants (Thorvilson & Rudd 2001). In addition, a recent study in Texas (USA) tested whether a certain type of Old World bluestem grass, 'WW-B Dahl' could effectively repel the red imported fire ant (Sternberg et al. 2006). The results of that study showed that the number of fire ant mounds were much lower in WW-B Dahl pastures than in fields planted with other types of grass. Furthermore, WW-B Dahl grass proved to be an attractive alternative to ranchers and farmers because this grass is favored by cattle and is very productive (Sternberg et al. 2006). City administrators may also find this grass helpful in keeping fire ants out of parks, landscaped areas, and roadsides. Anderson et al. (2002) found that water suspensions of perilla (*Salvia* spp.) leaves, pine (*Pinus* spp.) needles, and cedar shav-

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ings can be effective in repelling fire ants. The repellency and toxicity of peppermint oil particles caused fire ant nests to be abandoned (Appel et al. 2004). Octanoic acid (Vander Meer et al. 1993), bifenthrin and tefluthrin (Oi & Williams 1996) were found to prevent potted plants from becoming contaminated by the red imported fire ant. Chen (2005) found that diethyl phthalate and dimethyl phthalate can be effective in repelling fire ants. Two insect-repellent terpenoids, callicarpenal and intermedeol, isolated from the leaves of American beautyberry (*Callicarpa americana* L.; Verbenaceae) and Japanese beautyberry (*Callicarpa japonica* Thunb.) were evaluated (using digging bioassays) and shown to be effective as repellents against the workers of red imported fire ants (Chen et al. 2008).

Certain components of the pepper plant *Capsicum annuum* L. (Solanales: Solanaceae) have long been known as insect repellents. For example, the negative effects of extracts from the fruits and seeds of *C. annuum* were observed in experiments with the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), (Silvanidae) and the rust-red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae), both insect pests of stored products (Iorizzi et al. 2000). The Himalayan cedar, *Cedrus deodara* (Roxb.) G.Don (Pinales: Pinaceae), is a graceful ornamental evergreen tree that grows extensively on the slopes of the Himalayas and shows repellent effects on the nesting of *S. invicta*. *Cedrus deodara* could also supply repellants for use against other pests. It has been reported that a highly effective oil obtained from *C. deodara* caused complete mortality in *Anopheles stephensi* (Kumar & Dutta 1987).

In the above-mentioned reports, nest digging behavior of workers was used to evaluate the repellent effects of plant essential oils on fire ants. However, foraging is one of the most important daily activities for social insects, including fire ants and other ants. The repellent effects of plant oils on the foraging behavior of the red imported fire ants have not been tested to date. In this study, the repellent effects of several plant essential oils on the workers of the red imported fire ant were evaluated using an ant foraging bioassay. The results of this study identify essential oils that significantly deter the foraging of red imported fire ants. These findings may have practical significance in quarantine procedures for fire ants and for public protection.

Materials and Methods

Materials

Sausage (Xincheng Jinluo Meat Group Co., Ltd., Linyi, Shandong, China) which is made mainly from chicken, starch and sugar was presented as a lure to observe the foraging behavior and recruitment of *S. invicta*. The plant essential oils of *Salvia sclarea* L.; (Lamiales: Lamiacaeae), *C. annuum*, *Mentha Canadensis* L.; (Lamiales: Lamiacaeae), *Mentha longifolia* (L.) Huds., *C. deodara*, and *Pinus* spp. were provided by DaMo Chemical Co. Ltd (Tianjin, China). The essential oil product, which in Chinese is called Feng You Jing (FYJ) (Liang Jiefu Pharmaceutical Pte Ltd., Singapore), was purchased from a Chinese traditional herb medicine store in the city of Guangzhou, Guangdong Province, China in Oct 2010. The percentage of each compound in FYJ reported on the label was 25% for menthol, 20% for methyl salicylate, 3% for camphor, 3% for eucalyptus oil, 14% for eugenol and 35% for liquid paraffin. The hexane and ethanol used in the bioassay were Analytical reagent grade (Sinopharm Chemical Reagent Co. Ltd. and DaMao Tianjin Reagent Factory).

Field Conditions

The field experiments were performed on the campus of South China Agricultural University in Guangzhou, Guangdong, China from Apr to Jun 2011. The number of inseminated queens in the polygyne colonies ranged between 2 and 27 and was determined by dissection (Porter 1992). The fire ant density at the study site was 8 mounds/100 m^2. On the day of the experiments, the weather was sunny with air temperature of 25-35 °C and 40~55% RH. The bioassays below were conducted between 10:00 a.m. to 3:00 p.m.

Deterrent Effects of Essential Oils on the Food-Searching Behavior of the Red Imported Fire Ant

Based on the report of Chen et al (2008) on the bioassay of repellency against the digging behavior of fire ant workers and on our preliminary experiments, we diluted essential oils with hexane to 10^{-6} mL/mL for the following bioassay. First, 100 µL of oil solution or hexane (control) was dropped onto the center of a 70 mm-diam piece of filter paper using a pipette. Five s later, 8 randomized filter paper pieces were each placed 15 cm from the nest border. A 0.5 g piece of sausage was then placed on each piece of filter paper (Fig. 1). The search time (the time required for one of the sausages to be discovered) and the number of recruited workers of the fire ants for each food source were recorded. A video camera (Nikon D90 system) was positioned above each mound by a tripod in order to record the numbers of workers on the filter papers at different times. Each treatment was repeated with 5 nests.

Interference Effects of Essential Oils on Food Transport by Red Imported Fire Ants

Prior to the test, the sausages were cut into slices weighing 0.5 g and then divided into 8 smaller fragments each ~ 62.5 mg. A total of 100 µL of oil solution or hexane (control) was dropped onto the center of a piece of filter paper using a pipette. Five

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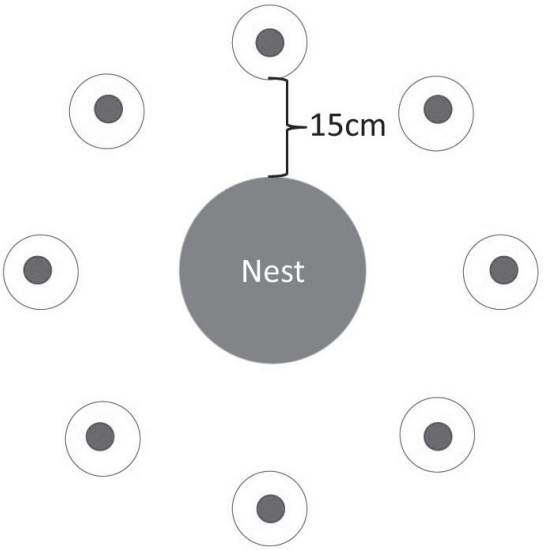


Fig. 1 Schematic map for placing baits on essential oil-treated pieces of filter paper each equidistant from the fire ant nest in the field bioassay of the repellent effects of various essential oils. A dark circle represents the fragment of sausage on a piece of filter paper (white circle). The distance from the edge of each filter paper piece to the nest was 15 cm.

s later each of the filter paper piece was randomly placed on 1 of the 8 locations (Fig. 1) each exactly 15 cm from the nest perimeter. Eight fragments of sausage (each approximately 0.625 mg) were then placed on each piece of filter paper. We recorded the time and observed and counted the number of sausage fragments transported by the workers within consecutive 5 min intervals until all of the sausage pieces had been completely removed. Each treatment was repeated with 5 nests.

Statistical Analysis

The differences in the search times, the number of recruits and number of sausage pieces transported under the different treatments, exposure times, and at different fire ant nests were measured. All data were tested for normal distribution by the Shapiro-Wilk test and for homogeneity of variances by Levene's test. Two-way analysis of variance (ANOVA) was performed to compare the search time of workers and the time required for food transportation exposed to different essential oils. Three-way ANOVA was used to compare the number of fire ant workers recruited and the amounts of food transported from different essential oil treated papers at different exposure times and different nests. When necessary, one way ANOVA was performed for each single factor. All of the statistical analyses were performed using the SPSS 13.0 software package.

Results

Search Times with Different Essential Oils

The experiment showed that the foraging behavior of the fire ants was affected by the 7 types of essential oils (Fig. 2). All of the essential oils

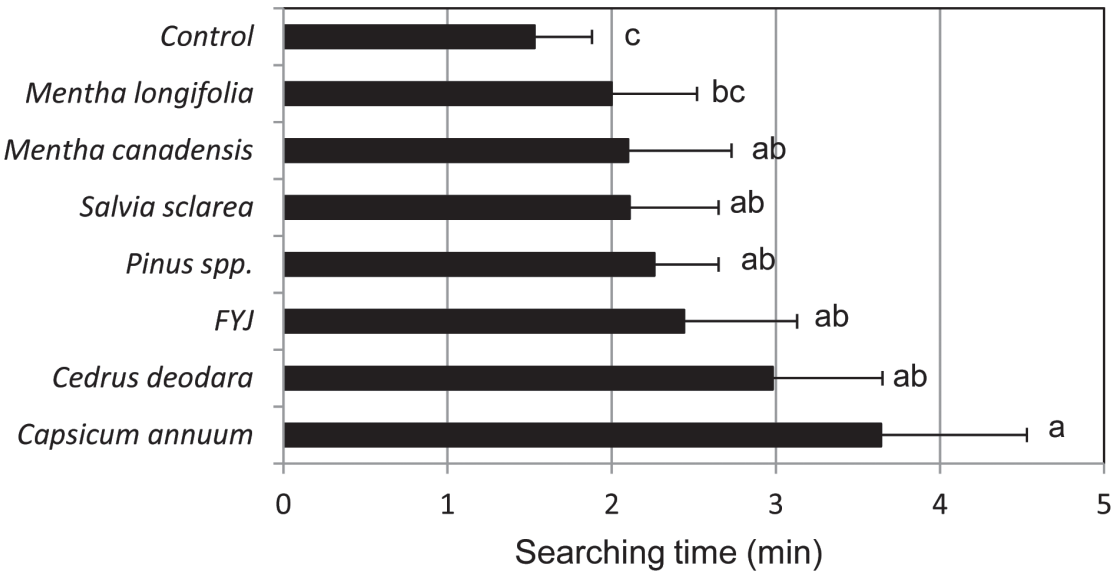


Fig. 2. Time (min) required for *Solenopsis invicta* workers to discover a 0.5 g fragment of sausage placed on a piece of filter paper treated with an essential oil. Each piece of filter paper was placed 15 cm from the perimeter of the fire ant mound. FYJ is a mixture of menthol, methyl salicylate, camphor, eucalyptus oil and eugenol. Means (± SE) followed by the same letter are not significantly different (LSD) at level of 0.05.

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except that of *Mentha longifolia* significantly prolonged the time required for the workers to arrive at the sausage compared to the untreated control. However only *C. annuum* significantly prolonged the search time more than *M. longifolia,* and the prolongation of the search time of *C. annuum* essential oil was not significantly greater than those of the essential oils of *C. deodara*, FJY, *Pinus* spp., *S. sclarea*, or *M. canadensis*. In addition, we observed that the fire ants made repeated visits to inspect the sausage. This behavior seemed to affect the subsequent recruitment.

The results showed that the search times of workers for the sausage were affected significantly by both the fire ant nest ($F = 21.487$, df = 4, 28; $P < 0.0001$) and essential oil type ($F = 10.382$, df = 7, 28; $P < 0.0001$). Search times for the fire ants with the *C. annuum* oil and *C. deodara* oil treatments were 3.64 min and 2.98 min, respectively; these values were greater than the control (1.53 min).

Recruitment of Fire Ant Workers in Different Essential Oil Treatments

The numbers of worker ants recruited (Table 1) during the observation periods differed significantly with different essential oils ($F = 49.565$, df = 7, 280; $P < 0.0001$), exposure time ($F = 139.701$, df = 6,280; $P < 0.0001$) and the fire ant nests ($F = 30.540$, df = 4, 280; $P < 0.0001$). No significant differences in recruitment occurred during the initial 10 min of exposure time. When the exposure time exceeded 10 min, the number of workers at the sausage fragments differed significantly among the different essential oil treatments. *Capsicum annuum* oil had the greatest effect, followed by the *C. deodara* oil, and *S. sclarea* oil. In the peppermint treatment, the number of worker ants recruited was significantly less than the control when the exposure time is greater than 10 min. In the treatments of *C. deodara* oil and *S. sclarea* oil treatments, the numbers of worker ants recruited were significantly less than in the control for exposure times greater than 15 min and 20 min, respectively. Worker recruitment was significantly lower when exposure times were greater than 25 min and 35 min for the *Pinus* spp. oil and FYJ treatments, respectively. These results show that recruitment after discovery of food depend on both the exposure time and the type of oil used. In our test, the *C. annuum* oil and *C. deodara* oil showed better recruitment deterrence effects on the workers than the other essential oils tested (Table 1).

The Amount of Food Transported and the Time Required for Food Transportation under Different Essential Oil Treatments

The amount of food transported by the worker ants (Table 2) showed an increasing trend with

TABLE 1. THE NUMBERS OF *SOLENOPSIS INVICTA* WORKERS ATTRACTED TO SAUSAGE FRAGMENTS ON 70 MM-DIAM PIECES OF FILTER PAPER EACH TREATED WITH A STANDARD AMOUNT OF AN ESSENTIAL OIL AND PLACED 15-CM FROM THE NEST. THE NUMBER OF RECRUITS WAS RECORDED IN 5 MIN INTERVALS FROM THE TIME OF PLACEMENT.

	Number of Workers						
Plant oils	5 min	10 min	15 min	20 min	25 min	30 min	35 min
Capsicum annuum	2.00 ± 0.87 aA	2.60 ± 1.47 aA	5.60 ± 2.48 aAB	6.80 ± 2.62 aAB	9.00 ± 4.24 aAB	10.60 ± 4.81 aB	13.00 ± 6.12 aB
FYJ	3.80 ± 2.02 aA	7.40 ± 4.14 abA	14.20 ± 4.75 abAB	20.60 ± 6.03 bcBC	28.20 ± 7.25 cdCD	33.40 ± 7.58 bcD	41.40 ± 7.97 bD
Mentha longifolia	3.40 ± 1.18 aA	9.20 ± 4.10 abA	20.40 ± 6.53 cB	30.80 ± 7.32 cC	42.00 ± 5.07 eD	51.20 ± 4.37 dE	62.00 ± 3.28 cF
Mentha canadensis	3.80 ± 0.92 aA	11.00 ± 4.18 bAB	19.40 ± 7.06 bcBC	27.80 ± 7.41 cCD	36.60 ± 8.51 deDE	45.60 ± 8.22 cdEF	56.00 ±7.23 cF
Salvia sclarea	2.60 ± 1.47 aA	4.40 ± 2.48 abA	9.00 ± 6.24 abAB	12.60 ± 8.54 abAB	19.00 ± 8.63 abBC	29.20 ± 10.25 bCD	37.80 ± 8.10 bD
Cedrus deodara	2.20 ± 0.92 aA	4.40 ± 2.04 abA	7.20 ± 3.06 aAB	11.80 ± 3.98 abBC	13.80 ± 4.56 abBCD	16.60 ± 4.57 aCD	20.20 ± 5.46 aD
Pinus spp.	3.40 ± 1.28 aA	8.60 ± 3.15 abA	12.80 ± 5.32 abA	18.80 ± 4.26 bcB	24.20 ± 3.95 bcB	31.40 ± 2.94 bC	39.60 ± 2.77 bD
Control	3.60 ± 1.18 aA	9.60 ± 3.07 bAB	18.20 ± 4.51 bcB	28.40 ± 5.84 cC	37.80 ± 6.21 deD	45.20 ± 5.95 cdD	56.40 ± 5.82 cE

*Means in the same column followed by the same small letter or in the same row followed by the same capital letter are not significantly different (LSD) at level of 0.05.

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TABLE 2. THE NUMBERS OF SAUSAGE FRAGMENTS TRANSPORTED BY *SOLENOPSIS INVICTA* WORKERS FROM 70 MM-DIAM PIECES OF FILTER PAPER EACH TREATED WITH A STANDARD AMOUNT OF AN ESSENTIAL OIL AND PLACED 15-CM FROM THE NEST. THE NUMBER OF SAUSAGE FRAGMENTS REMOVED FROM EACH PIECE OF FILTER PAPER WAS RECORDED IN 5 MIN INTERVALS FROM TIME OF PLACEMENT.

	Sausage slices transported						
Plant oils	5 min	10 min	15 min	20 min	25 min	30 min	35 min
Capsicum annuum	0.00 ± 0.00 aA	0.00 ± 0.00 aA	0.60 ± 0.39 aA	1.40 ± 0.63 abAB	2.80 ± 1.16 abBC	4.00 ± 1.22 abCD	5.40 ± 1.07 aD
Cedrus deodara	0.00 ± 0.00 aA	0.00 ± 0.00 aA	0.80 ± 0.59 abAB	1.00 ± 0.71 aAB	2.00 ± 1.11 aBC	3.60 ± 1.37 aCD	4.80 ± 1.16 aD
Mentha longifolia	0.60 ± 0.39 abA	2.20 ± 0.92 bB	4.60 ± 1.07 cC	6.20 ± 0.92 deD	7.40 ± 0.38 eD	9.00 ± 0.50 dE	10.00 ± 0.00 cE
Salvia sclarea	0.00 ± 0.00 aA	1.00 ± 0.5 abA	2.20 ± 0.77 abcAB	3.40 ± 1.07 abcBC	4.60 ± 1.28 bcCD	6.20 ± 1.45 bcDE	7.40 ± 1.91 abE
FYJ	0.40 ± 0.63 abA	2.00 ± 1.66 bAB	4.00 ± 2.18 cABC	5.60 ± 2.16 cdeBCD	7.00 ± 1.87 cdCD	8.50 ± 1.35 cdD	7.50 ± 0.71 abCD
Pinus spp.	0.20 ± 0.32 abA	0.80 ± 0.59 abA	2.20 ± 1.05 abcAB	3.80 ± 1.61 bcdBC	5.60 ± 1.70 cdCD	7.00 ± 1.65 cdD	7.50 ± 1.91 abD
Mentha canadensis	1.00 ± 0.71 bA	2.00 ± 1.33 bA	3.0 0± 1.41 abcAB	5.00 ± 1.11 cdeBC	7.00 ± 1.22 cdCD	8.00 ± 1.22 cdD	9.00 ± 1.58 cD
Control	0.40 ± 0.39 abA	1.20 ± 0.92 abAB	3.20 ± 1.16 bcB	6.60 ± 1.63 eC	7.25 ± 0.68 cC	9.50 ± 0.41 dD	10.00 ± 0.00 cD

*Means in the same column followed by the same small letter or in the same row followed by the same capital letter are not significantly different (LSD) at level of 0.05.

the elapse of time (F = 5.147, df = 6, 280; P < 0.0001) and differed significantly for the same essential oil treatment (F = 28.098, df = 7, 280; P < 0.0001) and the fire ant nest (F = 154.085, df = 4, 280; P < 0.0001). No significant differences were found in the amounts of food transported for exposure times of 5 min and 10 min. For exposure times greater than 10 min, the amounts of food transported differed significantly in the different essential oil treatments (Table 2). *Cedrus deodara* oil had the greatest suppressive effect on the amount of food transported by the workers followed by the *C. annuum* oil. When the exposure time was 10 min or less, no food transport was found in the experiments using *C. deodara* oil and *C. annuum* oil; however, when the time interval was as long as 35 min, the average amount of food transported was 4.8 and 5.4 pieces, respectively. In contrast, the amount of food transported was 7 pieces or more for the other essential oils tested.

The time required to transport all of the food differed (Fig. 3) markedly among the different essential oil treatments (F = 15.257, df = 7, 28; P < 0.0001) and the fire ant nest (F = 2.774, df = 7,28; P = 0.046). It should be noted that all of the treatments were presented in the same habitat. The time required to transport all the food was the greatest under the *C. annuum* oil treatment (53.87 min), followed in declining order by the *C. deodara* oil (50.23 min), the *M. canadensis* oil (41.98 min), FYJ (40.57 min) and the control (27.22 min). Thus, the *C. annuum* oil and the *C. deodara* oil have significant impacts on the amount of food transported and the time required to transport all of the food.

DISCUSSION

Foraging trails of *S. invicta* colonies range in length from 1 cm to 2 m (Markin et al. 1975), which indicate the distance from the food resource to the tunnel opening. Therefore, we set up the sausage only 15 cm away from the nest border to achieve a uniform the trail length. Our field observations confirmed that the foraging trails were almost between 5 cm to 10 cm in our experiments. During foraging, fire ant workers detect the smell of food with their antennae, and the type, location and weight of the food can affect their subsequent recruitment behavior (Xu et al. 2007). The results of the study showed that the presence of essential oils reduced the number of ants arriving at the filter paper, which in turn, influenced the search time, the recruitment and the transport of the food by the workers.

The essential oils were clearly repellent against the foraging of fire ants, and the type of essential oil used made a substantial difference in this effect. Under outdoor conditions, the *C. annuum* oil and *C. deodara* oil were the most effective repellents among the 7 essential oils

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Fig. 3. Effect of plant essential oils on the time (min) required by *Solenopsis invicta* workers to remove all 8 of the ~ 62.5 mg fragments of sausage placed on a piece of filter paper treated with an essential oil. Each piece of filter paper was placed 15 cm from the perimeter of the fire ant mound. FYJ is a mixture of menthol, methyl salicylate, camphor, eucalyptus oil and eugenol. Means (± SE) followed by the same letter are not significantly different (LSD) at level of 0.05.

tested. The concentration of essential oils used in this experiment was 10^{-6} ml/ml, but different concentrations may alter the effectiveness of a repellent (Chen 2009). Future research should consider this possibility. Our study also revealed that the number of worker ants involved in foraging activities tended to increase over time. This may due to the gradual decrease of the repellent effect of essential oils. It is probable that lower repellency occurred because the outdoor air-flow accelerated the evaporation of the essential oils. Perhaps micro-capsules could be developed to ensure a greater stability of the repellent effects.

Plants use secondary metabolites to resist potential damage by herbivores. These secondary metabolites are the result of the long-term coevolution between herbivorous animals and plants (Hartmann 2004). Compared with chemical pesticides, plant essential oils can serve as environmentally friendly pesticides because they are usually safer and less toxic to humans and domestic animals, and they are more readily degraded in the environment (Isman 2000). Many scientists are beginning to focus on essential oils to develop efficient new, minimally toxic pesticides for use against pests that threaten both health and agricultural production. According to Chen (2009), FYJ could be improved to act as a repellent against red imported fire ants workers. Our results indicated that the repellent properties (especially the persistence effect) of *C. annuum* and *C. deodara* were stronger than that of FYJ. Although *C. annuum* and *C. deodara* showed effectiveness in repelling foraging workers, the number of workers recruited was greater after 30 min than 10 min. Modification of *C. annuum* and *C. deodara* oils to produce longer effects over longer periods are needed if these plant oils are to be used as repellents for preventing food contamination by fire ants. These oils, may also prove useful in repelling fire ants from nesting soil (Chen & Allen 2006).

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