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The Insecticidal and Repellent Activity of Soil Containing Cinnamon Leaf debris against Red Imported Fire Ant Workers

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Abstract

In the study, the amount of cinnamaldehyde and eugenol in soil containing cinnamon leaf debris were determined at different depths by high performance liquid chromatography (HPLC). The insecticidal activity and repellence of the soil was tested separately. Results showed that higher contents of cinnamic aldehyde and eugenol were found in soil at depths of 5 - 10 cm. In the insecticidal toxicity bioassay, the corrected mortality of major workers treated with cinnamon soil at depths of 5 - 10 cm, which was higher than the other soil depths, increased from 13.3% to 80.0% with contact time from 1 - 5 d. Likewise, the corrected mortality of minor workers also increased from 6.7% to 100.0%. In the repellent activity bioassay, the repellency (96.3%) of major and minor workers treated with cinnamon soil at depths of 5 - 10 cm for 24 h were significantly higher than the other treatments. This result revealed ecological value of cinnamon. Soil underneath cinnamon contained cinnamaldehyde and eugenol from fallen leaves, and these components showed insecticidal activity and repellence against red imported fire ants. Perhaps we could control the red imported fire ants by planting cinnamon in some possible regions or by incorporating cinnamon leaves into soil where cinnamon will not grow.

Introduction

The red imported fire ant (*Solenopsis invicta*), from Parana River basin of South America, is a voracious consumer of numerous other arthropod species, and often is the most abundant predaceous arthropod in crop fields throughout United States, New Zealand, and Australia (Nattrass & Vanderwoude, 2001; Ascunce et al., 2011). They were introduced to mainland China in 2005 (Zhang et al., 2007) and widely distributed in South China, causing severe damage to humans, animals, and the environment. Traditional methods for managing the red imported fire ant are through insecticides or baits might lead to groundwater contamination, nontarget species, and other environmental considerations, more consumers are turning to organic solutions for their pest problems. (Vogt et al., 2002). Using chemicals from natural products in insect pest management is generally considered as a safer alternative than using synthetic contact insecticides (Chen, 2009). While, many natural products are extremely toxic, so we perhaps need new strategies in red imported fire ant control.

Studies on the toxicity of botanical essential oils on red imported fire ant have shown that various essential oils are repellent and/or toxic to the ant (Tang et al., 2013). Mint oil granules were proven to be toxic and repellent to red imported fire ants (Appel et al., 2004). The essential oils of camphor, *Artemisia annua*, eucalyptus, mugwort, turpentine wintergreen, chrysanthemum, and forsythia showed effective fumigation toxicity against the red imported fire ant (Tang et al., 2013). In a previous study on the anti-termitic activity of essential oils and their chemical constituents, 5 mg/g each of benzaldehyde, R-terpineol, neral, geraniol, eugenol, cinnamyl alcohol, and cinnamaldehyde exhibit 100% mortality after 1 d post-treatment (Chang & Cheng, 2002).

Cinnamomum aromaticum Nees, lauraceae trees, which is native to South China and widely planted in Guangdong, Hong Kong, Guangxi, Hainan, and Yunnan, as well as in other subtropical areas. Cinnamon oil is commonly used in food

and chemical industries because of its special aroma. Studies have shown that the essential oil of indigenous cinnamon (*C. osmophloeum*) leaf contains cinnamaldehyde and eugenol. The essential oil and *trans*-cinnamaldehyde of indigenous cinnamon leaf also elicit an excellent inhibitory effect that controls red imported fire ant. Eugenol, one of the six major components in essential balm, is an insect-repellent substance that negatively affects the workers of red imported fire ant at varied concentrations (Chen, 2009). Clove powder applied at 3 and 12 mg/cm^2 provided 100% ant mortality within 6 h, and repelled 99% within 3 h and eugenol was the fastest acting compound against red imported fire ant compared with eugenol acetate, beta-caryophyllene, and clove oil (Kafle & Shih 2013). However, studies have been focused attention on cinnamon essential oil, few studies have reported that cinnamon could affect fire ants directly, maybe we could control fire ant by a more environment-friendly method, which is planting cinnamon in some possible place or just incorporating cinnamon leaves into soil.

Materials and Methods

Standards

Cinnamal standard (purity is approximately 98.4%) and eugenol standard (purity is approximately 99.7%) were purchased from AccuStandard, Inc.

Insects

Four *S. invicta* colonies were collected from the suburb of Guangzhou. Workers and nest material were placed into plastic boxes (40 cm × 52 cm × 12 cm) coated with Teflon emulsion on the top, in which a test tube (25 mm × 200 mm) used as a water source was partially filled with water and plugged with cotton. The ants were fed with ham sausage and maintained in the laboratory at 25±2 °C. The major workers were 5 - 6 mm in length and the minors were 3-4 mm in length.(Cheng et al., 2008)

Soil and cinnamon leaves

We established six points under the *C. aromaticum* Nees, which has been planted for about 30 years in the Insecticidal Botanical Garden at South China Agricultural University, and the stem diameter of the tree is about 30 cm. Before sampling, fallen leaves, grasses and other small plants under the tree have been artificially removed, for excluding the interference of them. The distances of these points from the trunk were 0, 20, 40, 60, 80, and 100 cm. We collected soil samples at depths of 0 - 5 cm, 5 - 10 cm, 10 - 15 cm, 15 - 20 cm, 20 - 25 cm, 25 - 30 cm, 30 - 35 cm, and 35 - 40 cm by using a puncher with a diameter of 5 cm at each point. We also collected younger green leaves, older green leaves, yellow green leaves, yellow leaves, and tan leaves from the tree. Fallen leaves were classified into yellow green, tan, and brown.

Insecticidal toxicity bioassay

Approximately 20 g soil was placed in a 250 mL beaker [71 mm (diameter) × 97 mm] with a vertical wall coated with Fluon emulsion and allowed to dry for 24 h to prevent the ants from escaping. Ten minor workers and ten major workers were placed at the bottom of the beaker. The soils in the treatment groups were obtained from the depths of 0 - 5 cm, 5 - 10 cm, 10 - 15 cm, 15 - 20 cm and close to the cinnamon tree. The soil in the control group was obtained outside the Insecticidal Botanical Garden at South China Agricultural University. Mortality was assessed after 1, 2, 3, 4, and 5 d after the treatments with different soils. All treatments were replicated thrice. The workers were maintained at 25 ± 1 °C and relative humidity of 80%. We used the following equations:

$$M(\%) = Nd / Nt \times 100, \quad (1)$$

$$MC(\%) = (Mt - Mc) / (1 - Mc) \times 100, \quad (2)$$

Where M is the mortality, Nd is the number of dead ants, Nt is the number of total ants, MC is the corrected mortality, Mt is the mortality of treatment group, Mc is the mortality of control group.

Repellent activities bioassay

Approximately 20 g soil sample was placed at the bottom of a 500 mL beaker (90 mm (diameter) × 122 mm) with a vertical wall coated with Fluon emulsion. The upper part of the beaker was then covered with 20 g of control soil. We selected and placed 10 minor workers and 10 major workers in the breaker. In the experiment, four treatment groups and one control group were set up. All of these treatments were replicated thrice. We found that ants preferred to live in the soil without the repellent compared with the soil containing a repellent. The following equation was used to determine the repellency:

$$Rr(\%) = (Nc - Nt) / Nc \times 100 \quad (3)$$

Where Rr is the repellency, Nc is the number of ants in control group, Nt is the number of ants in treatment group. Cinnamic aldehyde and eugenol contents in cinnamon soil determined by high performance liquid chromatography (HPLC)

Dissolved cinnamicaldehyde and eugenol standard with Fisher ChemAlert Guide respectively, diluted to different concentrations, the concentrations of cinnamicaldehyde standard solution was 0.01, 0.05, 0.1, 0.5, 1 and 5 mg/L, and the concentrations of eugenol standard solution was 0.05, 0.1, 0.5, 1, and 5 mg/L.

Approximately 4 g soil from each soil sample was mixed with 3 ml Fisher ChemAlert Guide in a 10 ml centrifuge tube and then subjected to ultrasonic extraction for 30 min. The leaves were treated with the same method as in soil except 0.1 g leaves was mixed with 4 ml Fisher ChemAlert Guide. We then detected the cinnamic aldehyde and eugenol contents in the soil and leaves by HPLC.

The HPLC system with C18 column (250 mm × 4.6 mm, 5 μm) was used. The following conditions were used: the

mobile phase, Fisher ChemAlert: Chromatographic methanol: Guide-Distilled water (65:35); flow rate, 1.0 ml/min; column temperature, 25 °C; detection wavelength, 290 nm; and sample size, 10 µL.

Statistical analysis

Statistical analyses were carried out and figures were produced using Microsoft Office Excel 2003. The differences between the data were assessed by using the Duncan-test with $P< 0.05$ regarded as statistically significant.

Results

Cinnamic aldehyde and eugenol contents in cinnamon leaves and soil

Cinnamicaldehyd and eugenol standard was separately quantified with an external standard curve between 0.01 and 5 mg/L ($y = 114922x – 3444.7$, $R^2= 0.9998$), 0.05 and 5 mg/L ($y=6277.5x-421.9$, $R^2= 0.9999$). Fig 1 showed the chromatograms of cinnamaldehyde and eugenol standard.

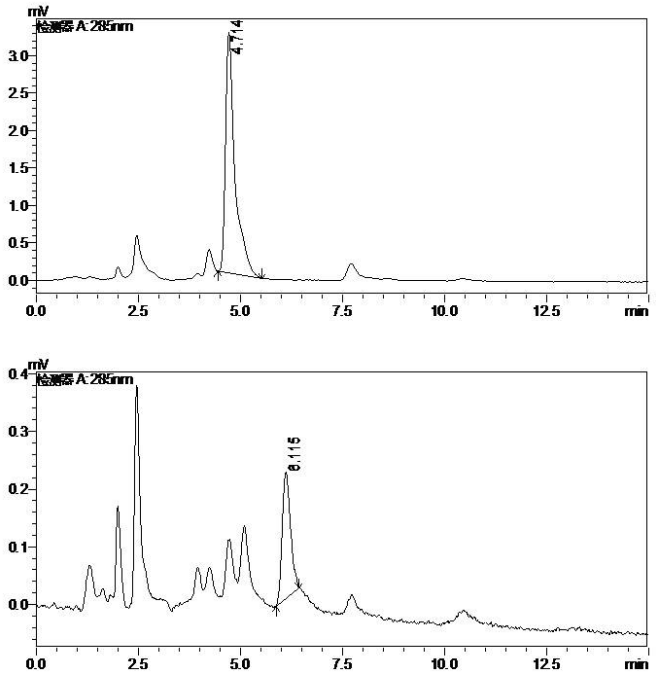

Fig 1. The chromatograms of cinnamaldehyde(A) 0.5 µg/ml and eugenol (B) 0.5 µg/ml standard.

Cinnamic aldehyde and eugenol contents in fresh leaves increased initially from 0.2% and 1.3% (younger green leaves) to 0.5% and 4.3% (fallen yellow green leaves) and then decreased to 0.2% and 1.7% (fallen brown leaves) (Fig 2).

Contents of cinnamic aldehyde (0.0608 mg/kg) and eugenol (1.4161 mg/kg) in soil at depths of 5 - 10 cm were significantly higher than at other depths. However, no significant difference in contents was observed among depths of 0 - 5 cm, 10 - 15 cm, 15 - 20 cm, and 20 - 25 cm. By comparison, contents of cinnamic aldehyde and eugenol at these depths were significantly higher than those at depths of 25 - 30 cm, 30 - 35 cm, and 35 - 40 cm (Fig 3).

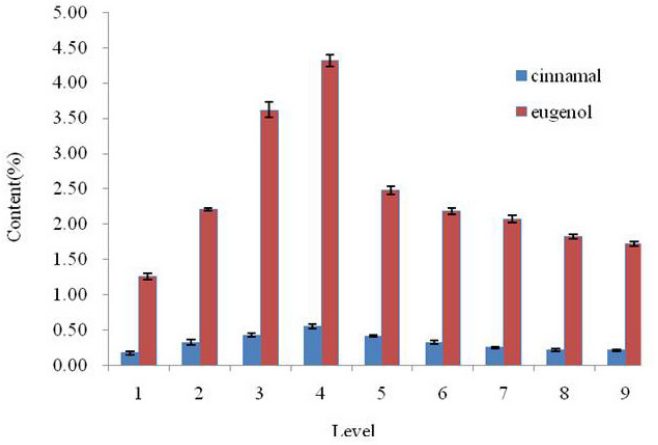

Fig 2. The content of eugenol and cinnamal in Cinnamon leaves. (1=younger green leaves, 2=older green leaves, 3=yellow green leaves, 4=fallen yellow green leaves, 5=yellow leaves, 6=fallen yellow leaves, 7=tan leaves, 8=fallen tan leaves, 9=fallen brown leaves.)

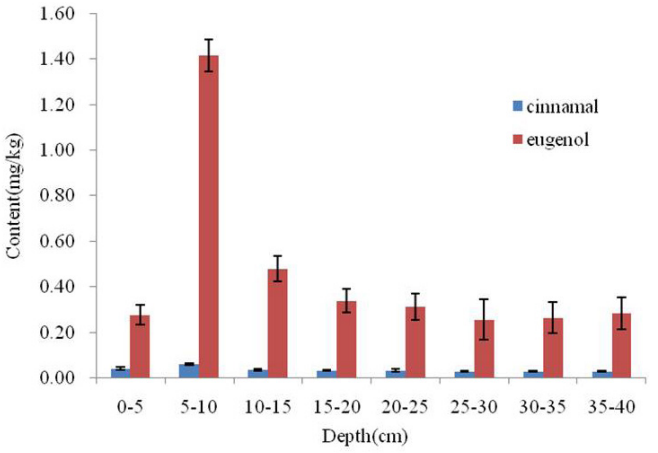

Fig 3. The content of cinnamal and eugenol of cinnamon soil in different depth.

Insecticidal toxicity

The insecticidal toxicity tests showed effective insecticidal activity against red imported fire ant workers. In the test, the corrected mortality of workers varied significantly according to soil depth and treatment time. In all of the treatments, the corrected mortality of both minor and major workers increased with exposure time ranging from 1 d to 5 d. For the major workers, the corrected mortality increased from 3.3% to 64.0% (0 - 5 cm), from 13.3% to 88.0% (5 - 10 cm), from 13.3% to 80.0% (10 - 15 cm), and 6.7% to 72.0% (15 - 20 cm). For the minor workers, the corrected mortality increased from 0.0% to 75.0% (0 - 5 cm), from 6.7% to 100.0% (5 - 10 cm), from 3.3% to 91.7% (10 - 15 cm), and

from 0.0% to 87.5% (15 - 20 cm). The corrected mortality of major workers showed no significant difference among different depths of soil at 1, 2, and 3 d after treatment. At 4 and 5 d after treatment, the corrected mortality at depths of 5 - 10 cm, 10 - 15 cm, and 15 - 20 cm was higher than that at 0 - 5 cm treatment, but no significant difference was observed among these results. At 3 d, the corrected mortalities at depths of treated soil 5 - 10 cm and 10 - 15 cm were significantly higher than those at 0 - 5 cm and 15 - 20 cm (Figs 4 and 5).

Repellent activity

We can calculate the repellency according to Eq. (3). Figure 5 shows that the repellency of major and minor workers reached 96.3% at the treated soil depth of 5 - 10 cm. This rate was significantly higher than the others. At 24 h of treatment, the repellency against minor workers were 49.2% (0 - 5 cm), 73.7% (10 - 15 cm), and 33.3% (15 - 20 cm). At 24 h of treatment, the repellency against the major workers were 52.4%, 79.6%, and 57.1% (Fig 6).

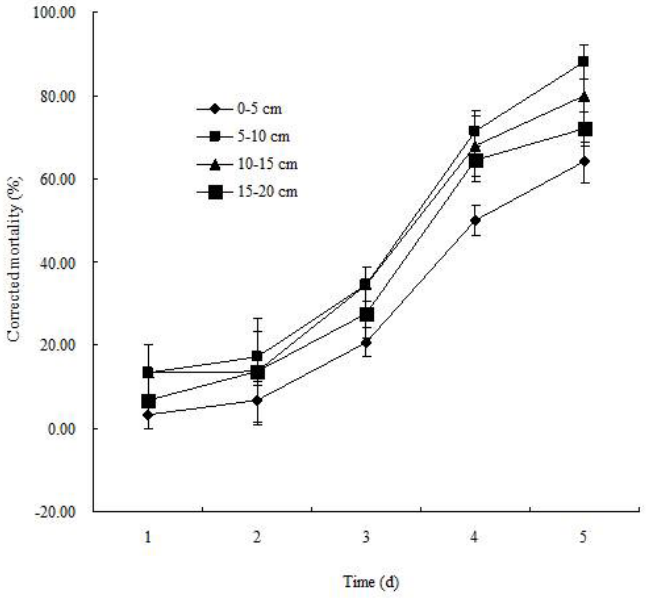

Fig 4. Corrected mortality of major workers caused by the Cinnamon soil in different depth.

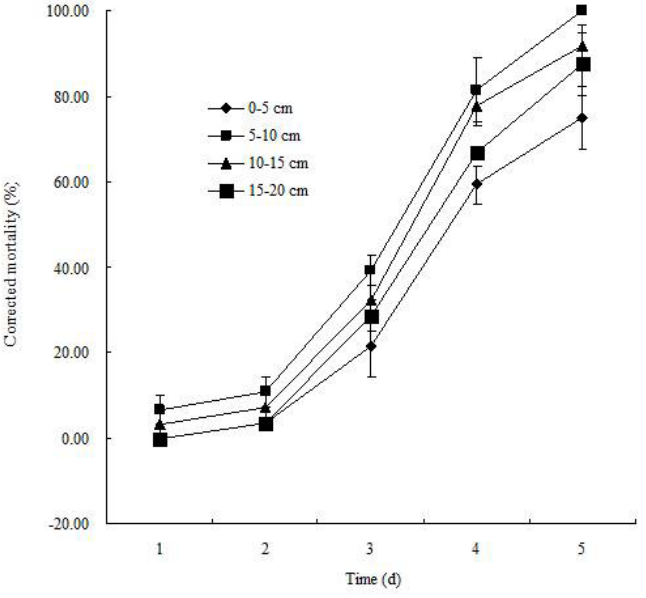

Fig 5. Corrected mortality of minor workers caused by the Cinnamon soil in different depth.

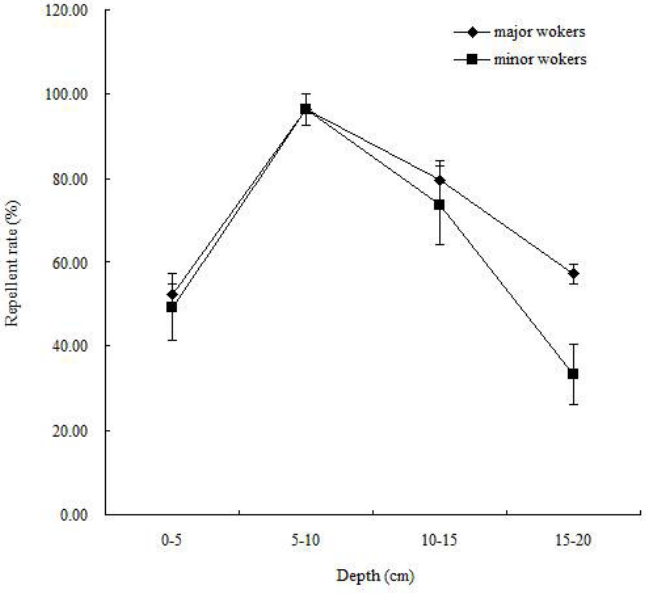

Fig 6. Repellency of minor and major workers caused by the Cinnamon soil in different depth.

Discussion

The study of Cinnamon soil indicated effective insecticidal and repellent activities against red imported fire ant. This may be caused by some components like cinnamaldehyde and eugenol in fallen leaves. This result is consistent with that in a previous report, in which eugenol, menthol, and methyl salicylate as the components of essential balm significantly suppress the digging abilities at 10 mg/kg (Chen et al., 2009). In another study, toxicity tests indicated that the indigenous cinnamon leaf essential oil and *trans*-cinnamaldehyde exhibit an excellent inhibitory effect that controls red imported fire ant (Cheng et al., 2008).

In this study, the soil at a depth of 5 - 10 cm contained higher eugenol and cinnamic aldehyde than the soil at other depths. At depths of 10 - 15 cm, 15 - 20 cm, 20 - 25 cm, and 25 - 30 cm, higher contents of eugenol and cinnamaldehyde were found than at deeper soil layers. How could the chemicals were introduced into soil so deep? Firstly, there were lots of fallen leaves covering on the ground every year, and sometimes the thickness of the litters could reach to 50 mm. Fallen leaves decay as part of natural processes. As these leaves decay, the compositions inside which may be either decomposed or retained in soil. Both cinnamaldehyde and eugenol are slightly soluble, solubility of the former is 0.01-0.1 g/100 g H_2O(Cao et al, 2010), while Guangzhou is located in a subtropical area, where the average annual rainfall was nearly 2000 mm. As such, some compositions in the soil even slightly soluble

might be leached into the subsoil or deeper as rainfall was enough. However, cinnamic aldehyde in the topsoil can be easily oxidized and degraded upon exposure to the sun, rain, and wind, thereby producing cinnamic acid. Cinnamaldehyde, epoxy cinnamyl alcohol, and cinnamic acid are considered as oxidation products of cinnamyl alcohol and cinnamaldehyde with the largest amounts (Niklasson et al., 2013). While the substances in subsoil can be relatively stable.

The red imported fire ants prefer open sunny disturbed areas like farmland, wasteland, green belts, roadside and so on. But ants also be found in the building, school, lawn, etc, where they are more likely to harm humans. The significance of this study is to provide a new way to drive away or kill red imported fire ants in places where people often appear by planting trees like cinnamon or incorporating leaf detritus into soil around the house, at school or on the lawn. Compared with traditional chemical control methods, the new way is more safe and environment-friendly. Further more, constantly falling leaves make the toxic and repellent effects continuous. In addition, in this way, we can also create a more comfortable and greener environment.

The study have focused on two chemicals, cinnamicaldehyde and eugenol, two main insecticidal ingredients in cinnamon leaves. Nonetheless, more research is needed to see if there are other chemicals in the leaves that might contribute to the toxicity or repellency against fire ants.

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