

Fumigation Activity of Essential Oil of *Cinnamomum Loureirii* Against Red Imported Fire Ant (*Solenopsis Invicta*) Workers

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Research Article

Keywords: Cinnamomum loureirii Nees, essential oil, red imported fire ants, fumigation activity

Posted Date: June 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-450369/v1>

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Abstract

The high toxicity, long-lasting residues, and resistance to chemical pesticides and baits used in the control of red imported fire ants could possibly harm human health and ecological balance. Natural compounds would be an excellent source of pesticides because of their good repellency and insecticidal properties. In this study, the fumigation method was used to study the insecticidal effects of essential oils from the bark and leaves of *Cinnamomum loureirii* Nees on red imported fire ants at different concentrations and fumigation time. The fumigation time with *C. loureirii* essential oils was positively correlated with the knockdown ability and mortality in red imported fire ants and was negatively correlated with grasping ability. The insecticidal activity of the essential oil extracted from *C. loureirii* leaves was significantly higher than that of the essential oils extracted from bark at effective concentrations. Cinnamyl acetate, an abundant component in leaf essential oil, plays an important role in improving the insecticidal activity of *trans*-cinnamaldehyde. This study provides a theoretical basis for the development and utilization of cinnamon leaf oil as a new environmentally friendly insecticide resource for the control of red imported fire ants.

1. Introduction

Red imported fire ants (RIFAs), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), are one of the most notorious agricultural pests in the Philippines, Australia, the southern United States, and southern China. They are highly invasive and aggressive and will protect the nest through their quantitative advantages and aggressiveness (Zhang et al. 2007). Moreover, RIFAs can also attack and eliminate local fire ants to destroy the ecological balance of the invaded area. Additionally, they can cause serious damage to public and private properties, such as circuits, traffic, telephone junction boxes, transformers, and light control boxes (Huang et al. 2016). Red imported fire ants also bite humans, animals, and plants, threatening human health and ecological balance (Fu et al. 2015).

Red imported fire ants are native to Brazil, Argentina, Paraguay, and the Panama Canal in South America and can migrate with turf, seedlings, and soil. They can travel through modern transportation to various regions and cities around the world in containers carrying agricultural products, nursery stock, and turf (Fu et al. 2015). Red imported fire ants were accidentally brought into the United States in the 1930s and the 1940s in ballast sand of ships carrying agricultural products from South America (Buren et al. 1974). Traces of RIFAs were found in Taiwan in September 2003. By 2005, RIFAs outbreaks occurred in multiple provinces and regions in China, including Hong Kong, Macau, Guangdong, Guangxi, Fujian, and Hunan (Zhang et al. 2007). With global warming, the habitat of RIFAs has widened, leading to increased destructiveness (Lu et al. 2007).

Chemical pesticides and baits are commonly used to control RIFAs. However, because of their high toxicity, long-lasting residues, and resistance to these chemical pesticides, they could possibly harm human health and ecological balance (Vogt et al. 2002). To reduce the negative effects of chemical pesticides, an increasing number of studies have been conducted on alternative pest control strategies to reduce the use of chemical pesticides, such as the application of plant essential oils as fumigants in plant quarantine (Chang et al. 2001). Many plant essential oils are natural insecticides. Their effects on pest control can be roughly divided into several types, including attracting, repelling, antifeeding, poisoning, and growth inhibition (Isman et al. 2006). Tang et al. (2013) found that wintergreen oil (*Ilex chinensis*), eucalyptus oil (*Eucalyptus globulus*), annual mugwort (*Artemisia annua*), and mugwort oil (*A. argyi*) also have good fumigant activity against RIFAs. Sheppard (1983) suggested that orange peel essential oil has good contact activity against RIFAs. Subsequently, they found that essential oils such as cedar root oil, peppermint oil, clove oil particles, citronella oil, clove oil, and linseed oil demonstrate repellent effects against RIFAs at different concentrations (Wiltz et al. 2007; Chen 2009). These studies revealed that natural compositions could have good repellency and insecticidal properties and may be an excellent source of repellency against RIFAs (Thorvilson and Rudd 2001; Appel et al. 2004).

Cinnamon belongs to the family Lauraceae, which includes plants that are widely planted in southern China, including the Guangdong, Yunnan, and Guangxi provinces (Ribeiro-Santos et al. 2017; Kumar et al. 2019). China has abundant cinnamon resources and produces 80% of the world's cinnamon (Li et al. 2013a). Cinnamon is a traditional Chinese medicine. Essential oils are rich in cinnamon bark and leaves (Li et al. 2013a, b; Li et al. 2020) and have a broad spectrum of biological activities, including antibacterial, insecticidal, anti-inflammatory, antioxidant, antihyperglycemic, antidiabetic, and antitumor effects (Cheng et al. 2009; Wu et al. 2013; Ariaee-Nasab et al. 2014; Yap et al. 2014; Camacho et al. 2015; Jiang et al. 2016; Chang et al. 2017; Benelli et al. 2018; Piotr et al. 2018; Wu et al. 2020). Cinnamon essential oils can be used to treat symptoms such as rheumatoid arthritis, muscle strains, abdominal pain, coughs, and bronchitis (Shi et al. 2016). Recently, the repelling, contact, and fumigation activities of *Cinnamomum zeylanicum* oils were found to exert insecticidal effects, possibly by inhibiting various detoxifying metabolic enzymes in spiral planthoppers, mosquitoes, lice, *Psoroptes cuniculi*, and cotton bollworms (Dugassa et al. 2009; Nerio et al. 2010; Kumar et al. 2012; Jumbo et al. 2014). Although cinnamon essential oils are natural repellents for household pests, they have relatively few applications in the management of agricultural pests, including RIFAs (Chaiyasit et al. 2006). To expand the application of essential oils to the control of RIFAs, in the present study, the insecticidal activities of *C. loureirii* bark and leaf essential oils, including the main components, were evaluated and compared for their efficacy against RIFAs. The results showed that the essential oils from *C. loureirii* bark and leaf had a culling effect on RIFA at appropriate concentrations, and the insecticidal effect of leaf oil was significantly stronger than that of bark oil. Presumably, cinnamyl acetate, an abundant component in leaf essential oil, may play a key role in improving the insecticidal activity of *trans*-cinnamaldehyde. This study provides a theoretical basis for the development and utilization of cinnamon leaf oil as a new environmentally friendly insecticide resource for natural green insecticides against red imported fire ants.

2. Materials And Methods

2.1 Plant materials and preparation

Leaves and barks from 14-year-old plants of *C. loureirii* were collected in Maoming County, one of the major cinnamon-producing areas of Guangdong Province in South China, in May 2019. Samples were washed with tap water and left to shade-dry. Then, the dried samples were ground into powders (60 mesh) and stored in a desiccator for further analysis.

2.2 Essential oil extraction

The volatile oils of the leaves and bark were obtained by hydrodistillation in accordance with the method in the Pharmacopoeia of the People's Republic of China (China Pharmacopoeia Committee 2020) with minor modifications. The powder of the sample (15 g) was weighed into a 1000 mL distillation flask and placed in a sealed vessel along with a mixture of distilled water and zeolites (w/v = 1: 20, 1: 15, and 1: 10) and distilled after soaking for 1, 1.5, or 2 h. Afterwards, the distillate was transferred into a 750 mL separating funnel. Methylene dichloride (40 mL) was used to extract the volatile compounds from the aqueous layer three times. The combined organic layer was transferred into a 250 mL conical flask, dehydrated over anhydrous sodium sulfate for 30 min, and filtered through mid-speed filter paper. After most of the distillate was evaporated over a vapor bath in a fume hood, the concentrated solution was transferred into a dried brown sample bottle and placed at room temperature until the methylene chloride was completely evaporated. The obtained yellow volatile oil was weighed and stored at 4°C until further use. The yield of the essential oil was determined using the following equation:

$$\text{Yield of essential oil (\%)} = \frac{\text{weight of essential oil}}{\text{weight of sample}} \times 100$$

Three repeats were conducted for each sample, and the average essential oil content was calculated.

2.3 Origin and rearing of RIFA micrergates and macrergates

Red imported fire ants were obtained from the suburbs of Zengcheng County of Guangzhou city and stored in the laboratory for bioassays in plastic containers at $25 \pm 2^\circ\text{C}$ and 60%–80% relative humidity. A test tube (25 mm × 200 mm) partially filled with 10% sugar water or tap water and plugged with cotton to reduce the evaporation of water was used as the water source. A Petri dish (8.5 cm × 1.5 cm) containing the larvae of *Tenebrio molitor* (Coleoptera Tenebrionidae) and ham was used as a food source. Red imported fire ants were kept in a dry indoor environment at $25 \pm 2^\circ\text{C}$ during the bioassay experiment. *Tenebrio molitor* was purchased from Guangzhou Insect Market.

2.4 GC-MS analysis

GC-MS analysis was performed on a 7890A gas chromatograph (Agilent, American) interfaced with a 5975C Plus mass spectrometer (Agilent, American). A fused silica capillary Agilent Technology HP-5ms (5% phenyl methyl siloxane) column (Rxi-5MS, 30.0 m × 0.25 mm × 0.25 μm, film thickness = 0.1 m) was used for separation. The injector temperature was 260°C. The initial temperature was 100°C, and the temperature was gradually increased to 150°C at a rate of 4°C/min and held for 3 min at 150°C. Then, the temperature was gradually increased to 180°C at a rate of 4°C/min. Finally, the temperature was gradually increased to 220°C. The linear velocity of the helium carrier gas was 1 mL/min at a split ratio of 1:10. The injection volume was 1 μL.

EI was used as the ion source, and the ion source temperature was 200°C. The sector mass analyzer was set to scan from 45 to 450 amu (m/z). The ionization energy was 70 eV. The interface temperature was 250°C.

The identification of the components was performed by linear retention indices which were calculated using the generalized equation of Van Den Dool and Kratz (1963), with literature data, and by matching their recorded mass spectra with the standard mass spectra from the National Institute of Standards and Technology (NIST 14) library data provided by 5977A HP-MSD (Agilent, American) ChemStation (Adams 2007). The GC-MS peak area normalization method was used to calculate the relative percentage content of each component in the leaf and bark oils of *C. loureirii*. Three replicates were performed for each sample.

2.5 Quantitative analysis of trans-cinnamaldehyde and cinnamyl acetate in samples by GC

The standard solutions were prepared by mixing 3 mg/mL cinnamyl acetate with 10 mg/mL *trans*-cinnamaldehyde and diluted to different concentrations (0.13, 0.26, 0.52, 0.75, 0.98, 1.24, and 1.5 mg/mL) with dichloromethane and were kept at 4°C until further use. The samples were subjected to three parallel determinations to analyze their *trans*-cinnamaldehyde and cinnamyl acetate contents.

A fused silica capillary Agilent Technology HP-5ms (5% phenyl methyl siloxane) column (Rxi-5MS, 30.0 m × 0.25 mm × 0.25 μm, film thickness = 0.1 m) was used for separation. The injector temperature was set to 200°C. The detector temperature was set to 300°C. The initial column temperature was maintained at 50°C for 2 min, and the temperature was gradually increased to 300°C at a rate of 15°C/min and was then held for 10 min. The linear velocity of the nitrogen carrier gas was 2.57 mL/min at a split ratio of 1:40.

2.6 Physiological indices of RIFAs

2.6.1 Mortality

The biological fumigation activities of plant essential oils and main compounds were evaluated against RIFAs according to the method of Seo et al. (2009), with slight modifications. Cinnamon essential oils were placed into 200 µL capped centrifuge tubes with 10 small holes in the cap. Then, the centrifuge tubes were placed in a cup precoated with polytetrafluoroethylene (PTFE) emulsion and dried for 24 h. Twenty medium-sized RIFA workers were transferred into each cup (body length was 3.0-4.3 mm, head width was 0.7-1.0 mm), which was then sealed with an airtight lid. The test was carried out at a temperature of 25 ± 1°C and a humidity of 80%.

Experiment 1: Essential oils at concentrations of 320, 160, and 80 µg/cm³ were tested. The cumulative mortality caused by essential oil at a concentration of 320 µg/cm³ was determined every 10 min for 1 h. At other concentrations, cumulative mortality was measured every 2 h for a period of 12 h.

Experiment 2: *Trans*-cinnamaldehyde at concentrations of 5, 50, and 100 µg/cm³ and cinnamyl acetate at 2.5, 25, and 50 µg/cm³ were tested, and cumulative mortality by fumigation was measured every 2 h. Mortality was measured continuously for 20 h. The mixtures of *trans*-cinnamaldehyde and cinnamyl acetate (v/v = 2:1) at concentrations of 7.5, 75, and 150 µg/cm³ were tested for cumulative mortality every 2 h for a period of 20 h.

Experiment 3: Mixtures of *trans*-cinnamaldehyde and cinnamyl acetate in different ratios (v/v = 1:2, 2:1, and 4:1) at a concentration of 75 µg/cm³ were tested for cumulative mortality by fumigation every 2 h for a period of 20 h.

Experiment 4: Quantitative cinnamyl acetate was added to the essential oil of *Cinnamomum cassia* bark to obtain a 2:1 (v/v) mixture of *trans*-cinnamaldehyde and cinnamyl acetate, and cumulative mortality by fumigation was measured every 2 h. The rates were measured continuously for 12 h.

Taking an empty centrifuge tube as a control. According to the provisions of the pesticide control institute of the Ministry of Agriculture on the efficacy test methods and evaluation standards of health insecticides, only the mortality and knockdown ability observed in the control group must be lower than 20%, all experiments were considered valid. All treatments were repeated three times. The following formula was used to calculate mortality:

$$\text{Mortality (\%)} = \frac{\text{Number of dead RIFAs}}{\text{Total number of RIFAs}} \times 100$$

2.6.2 Grasping ability

The experimental procedure was the same as that described in section 2.6.1. The RIFA workers were transferred into a disposable plastic cup, covered with white paper, and the disposable cup was gently turned upside-down and allowed to stand for 3 s. Then, the cup was gently turned back, and the number of RIFAs that fell down into the bottom of the cup was recorded. Taking an empty centrifuge tube as a control and assuming that the mortality and knockdown ability observed in the control group must be lower than 20%, all experiments were considered valid. All treatments were repeated three times. The following formula was used to calculate the grasping ability:

$$\text{Grasping ability (\%)} = \frac{\text{Total number of RIFAs} - \text{Falling number of RIFAs}}{\text{Total number of RIFAs}} \times 100$$

2.6.3 Knockdown ability

The experimental operation was the same as that described in Section 2.6.1. The knockdown ability of RIFA workers was observed at the corresponding time point. The total number of ants being knocked down was the sum of the dead

and ant workers who had lost the ability to walk. Taking an empty centrifuge tube as a control and assuming that the mortality and knockdown ability observed in the control group must be lower than 20%, all experiments were considered valid. All treatments were repeated three times. The following formula was used to calculate the knockdown ability:

$$\text{Knockdown ability (\%)} = \frac{\text{Total number of RIFAs} - \text{Number of normal RIFAs}}{\text{Total number of RIFAs}} \times 100$$

2.6.4 Scanning electron microscopy (SEM) observation of the antennae of RIFA treated with cinnamon essential oils

Fumigation was performed with essential oil, *trans*-cinnamaldehyde, cinnamyl acetate, and a mixture of *trans*-cinnamaldehyde and cinnamyl acetate (v/v = 2:1) at concentrations of 320, 100, 50, and 150 µg/cm³, respectively. The worker ants were treated for 24 h and then stored. The test used ant workers without fumigation as a control group.

Sample preparation method for SEM: Under an anatomical lens, the antennae and heads of the test ants were cut off with a blade. The surface attachments were washed with 70% ethanol, and the samples were fixed with glutaraldehyde for 24 h. After dehydration with 70%, 80%, 90%, and 100% ethanol gradients, they were glued onto the SEM sample stage with conductive glue, and after spraying with gold, the antennae of RIFAs were observed and photographed under SEM (Hitachi S-3400N-®, Japan).

2.6.5 GC-EAG analysis of the main components in essential oils

Medium-sized RIFA workers were chosen, and the antennae were cut off quickly with a scalpel. The cut end was connected to a reference electrode filled with Ringer's saline and placed on the electrode. The tip of the antenna was connected to the recording electrode. The electrode was connected to the signal amplifier through a silver chloride-plated silver wire, and the signal was monitored by the probe, and the data were recorded and analyzed by GC-EAG (Agilent 7890B-5977B, SYNTech IDAC-2).

GC-EAG analysis was conducted with an HP-SMS capillary column with an injection volume of 1 µL and no split injection. The inlet temperature was 230°C, the initial temperature of the program was 50°C, and the temperature was gradually increased to 100°C at a rate of 5°C/min. Then, the temperature was gradually increased to 240°C at a rate of 10°C/min and held for 4 min. The total time was 20 min. The detector was a flame ionization detector (FID) with a temperature of 250°C.

2.6.6 EAG analysis of single and mixed components of *trans*-cinnamaldehyde and cinnamyl acetate on the antenna electroantennogram (EAG) responses of RIFA

The antenna EAG responses of RIFA to *trans*-cinnamaldehyde and cinnamyl acetate were measured by the method described by Yan et al. (2012), with slight modifications. First, the heads of medium-sized RIFA workers were completely cut off. Second, a small portion at the end of the antenna was cut off. Finally, the antenna was cut off from the head. The antenna was connected to a PRG-2 electrode painted with conductive glue, and the EAG baseline in the EAG IDAC2 record was measured. The continuous humidified airflow was 500 mL/min, the stimulation airflow was 450 mL/min, the stimulation airflow duration was 0.3 s, the signal drift was automatically controlled, and the maximum recording time was 5 s. The measurement was repeated 10 times, and a new worker antenna was applied in each group. The sample volume was 1 µL each time (at concentrations of 1, 10, 100, and 1000 µg/mL), which was dripped onto a 5 mm × 30 mm filter paper strip. Each repetition interval was 1 min, and *n*-hexane was used as the control.

2.6.7 Statistics

The fumigation effects of different concentrations of *C. loureirii* leaf and bark essential oils, *trans*-cinnamaldehyde, cinnamyl acetate, and the mixture on the mortalities, grasping abilities, and knockdown abilities of ant workers were compared. The variance analysis (ANOVA) of the data was performed by the Tukey method ($P < 0.05$).

3. Results

3.1 The fumigation activity of the essential oils of *C. loureirii* leaf and bark

3.1.1 Mortality

The insecticidal effects of fumigation of essential oils from different organs of *C. loureirii* on RIFA workers were compared. When fumigated with essential oils, the mortality of worker ants was different depending on the concentration, source of the essential oils, and fumigation time ($P < 0.05$) (Fig. 1a, b). After treatment with different concentrations of essential oil for 0–12 h, the mortalities of RIFA showed an increasing trend with the extension of treatment time. However, at the same treatment time, the mortality rate was not positively correlated with the concentration of essential oil. In the experiment with essential oil at concentrations of 80–320 $\mu\text{g}/\text{cm}^3$, a concentration of 320 $\mu\text{g}/\text{cm}^3$ showed the best insecticidal effect. Essential oils from cinnamon leaves and bark could kill all RIFAs within 1 h; in particular, leaf essential oil was more effective than bark essential oil. Moreover, at a concentration of 160 $\mu\text{g}/\text{cm}^3$, cinnamon leaf essential oil killed RIFA within 10 h (Fig. 1b). In contrast, the fumigation effect of cinnamon bark essential oil was weaker. At concentrations of 80 $\mu\text{g}/\text{cm}^3$, treatment with cinnamon leaf and bark essential oils for 12 h did not completely kill RIFAs. Overall, the essential oils of *C. loureirii* leaves showed good fumigation activity.

3.1.2 Grasping ability

The insecticidal effects of fumigation of essential oils from different organs of *C. loureirii* on RIFA workers were compared. When fumigated with essential oils, the grasping abilities of worker ants were different depending on the concentration, source of the essential oils, and fumigation time ($P < 0.05$) (Fig. 1c, d). Treatments with different concentrations of leaf and bark essential oil showed obvious effects on the worker ants compared with the control (Fig. 1c, d). The grasping ability was negatively correlated with fumigation time but was not completely positively correlated with oil concentration. As the concentration of essential oil increased to 320 $\mu\text{g}/\text{cm}^3$, the grasping ability of the RIFA decreased significantly. The leaf essential oil could cause the RIFAs to completely lose their grasping ability within 10 min, while the essential oil from bark had a slightly weaker effect on grasping ability. The latter required 50 min before ants completely lost their grasping ability. In addition, at a concentration of 160 $\mu\text{g}/\text{cm}^3$, leaf essential oil reduced the grasping ability of RIFA to 0% within 8 h. However, bark essential oil required 12 h to reduce the grasping ability of RIFA to 0%. In general, the effect of leaf essential oil on the grasping ability of RIFA was significantly stronger than that of bark essential oil.

3.1.3 Knockdown ability

The insecticidal effects of fumigation using essential oils from different organs of *C. loureirii* on RIFA workers were compared. When fumigated with essential oils, the knockdown abilities of worker ants were different depending on the concentration, source of the essential oils, and fumigation time ($P < 0.05$) (Fig. 1e, f). Different concentrations of *C. loureirii* leaf and bark essential oils had a very obvious effect on worker ants compared to the control (Fig. 1e, f). Knockdown ability was positively correlated with processing time but was not completely correlated with concentration. When the concentration of essential oil increased to 320 $\mu\text{g}/\text{cm}^3$, the essential oil from the leaf could knock down all the RIFA within 10 min. However, the essential oil from the bark took 50 min to achieve the same effect. At an essential oil concentration of 160 $\mu\text{g}/\text{cm}^3$, the knockdown ability of *C. loureirii* leaf and bark essential oils reached 100%.

Specifically, the knockdown ability of leaf oil reached 100% at 8 h, while the knockdown ability of bark oil reached 100% at 10 h. In summary, regardless of whether the concentration of the essential oil was 160 or 320 $\mu\text{g}/\text{cm}^3$, the effect of leaf oil on RIFA was significantly stronger than that of bark oil.

3.2 Analysis of essential oil compositions

There was a significant difference in the content of essential oils from *C. loureirii* bark and leaves. The essential oil content of *C. loureirii* bark reached 3.75%, which was significantly higher than the essential oil content of leaves by 2.06% (Fig. 2a). Gas chromatography-mass spectrometry (GC-MS) was used to analyze the differences in the composition and relative contents of the *C. loureirii* bark and leaf essential oils (Table 1). According to the peak area normalization method, *trans*-cinnamaldehyde was the main component of cinnamon essential oils (63.2%). In addition, the relative content of cinnamyl acetate in *C. loureirii* leaf essential oil was as high as 27.52% (Table 1, Fig. 3), while the cinnamyl acetate content of bark essential oil was very low.

Gas chromatography (GC) was used to quantitatively analyze *trans*-cinnamaldehyde and cinnamyl acetate in cinnamon essential oils (Fig. 2b-d). The absolute content of *trans*-cinnamaldehyde in *C. loureirii* bark essential oil (803.28 mg/g) was significantly higher than in leaves (532.85 mg/g) (Fig. 2b). The content of cinnamyl acetate (231.62 mg/g) in the essential oil of *C. loureirii* leaf was significantly higher than in the bark (5.94 mg/g) (Fig. 2c). The content of *trans*-cinnamaldehyde and cinnamyl acetate (532.85 and 231.62 mg/g) in the essential oil of *C. loureirii* leaf was approximately 2.3:1 (Fig. 2d). In summary, the leaf essential oil was rich in both *trans*-cinnamaldehyde and cinnamyl acetate.

3.3 Insecticidal effect of *trans*-cinnamaldehyde and cinnamyl acetate

To explore the possible reasons that *C. loureirii* leaf essential oil had a good fumigation effect on ants, the fumigation activities of *trans*-cinnamaldehyde, cinnamyl acetate, and their mixtures were studied. The effect of the mixture of *trans*-cinnamaldehyde and cinnamyl acetate in the essential oil of *C. loureirii* leaf (v/v = 2:1), and the effect of the mixture of *trans*-cinnamaldehyde and cinnamyl acetate at a ratio of 2:1 on the RIFA was studied. The fumigation effect of the mixture (7.5, 75, and 150 $\mu\text{g}/\text{cm}^3$) was compared with that of different concentrations of *trans*-cinnamaldehyde (5, 50, and 100 $\mu\text{g}/\text{cm}^3$) and cinnamyl acetate (2.5, 25, and 50 $\mu\text{g}/\text{cm}^3$). The effects of the treatments on the mortality, knockdown ability, and grasping ability of RIFA at 20 h were observed. The mortality, grasping ability, and knockdown ability against RIFA were different depending on the composition, concentration, and fumigation time ($P < 0.05$) (Fig. 4). After treatment with *trans*-cinnamaldehyde at different concentrations, the mortality and knockdown ability of RIFAs were positively correlated with the treatment time and concentration, and the grasping ability was inversely related. After treatment with 100 $\mu\text{g}/\text{cm}^3$ *trans*-cinnamaldehyde for 20 h, the mortality and knockdown ability of RIFA was 100%, and the grasping ability was zero (Fig. 4a, b, c). Treatments at other concentrations only fumigated RIFA. However, different concentrations of cinnamyl acetate had almost no fumigation effect on RIFA (Fig. 4g, h, i). Compared with the two single-component treatments, after 20 h of treatment, the effect of the mixture on RIFA at a concentration of 150 $\mu\text{g}/\text{cm}^3$ (100 $\mu\text{g}/\text{cm}^3$ *trans*-cinnamaldehyde and 50 $\mu\text{g}/\text{cm}^3$ cinnamyl acetate) was obviously enhanced, which affected the RIFA 4 h earlier than the effect of 100 $\mu\text{g}/\text{cm}^3$ *trans*-cinnamaldehyde, but there was only a slight difference in the effect of completely killing the RIFA (Fig. 4d, e, f). Interestingly, after treatment with 50 $\mu\text{g}/\text{cm}^3$ *trans*-cinnamaldehyde and 25 $\mu\text{g}/\text{cm}^3$ cinnamyl acetate for 20 h, the mortalities of RIFA were only approximately 60% and 5%, respectively (Fig. 4a, g). However, after treatment with a 2:1 mixture of 75 $\mu\text{g}/\text{cm}^3$ (50 $\mu\text{g}/\text{cm}^3$ *trans*-cinnamaldehyde + 25 $\mu\text{g}/\text{cm}^3$ cinnamyl acetate), the mortality of RIFA increased to approximately 90% (Fig. 4d). The mixture of *trans*-cinnamaldehyde and cinnamyl acetate in a 2:1 ratio can significantly improve the insecticidal effect of *trans*-cinnamaldehyde.

To evaluate the fumigation activities of a mixture of *trans*-cinnamaldehyde and cinnamyl acetate, we studied the fumigation effect of mixtures with a concentration of 75 µg/cm³ at different ratios of *trans*-cinnamaldehyde and cinnamyl acetate on the RIFA. The mortality and knockdown ability of all ratio treatments increased with treatment time after 20 h (Fig. 5a, c), and the grasping ability decreased (Fig. 5b) compared to the control. After 10 h, the insecticidal effect of the mixture was not obvious; after 10 h, the effect began to appear. After 14 h, the fumigation effect of the mixtures with different ratios was clearly discernible, and as the content of cinnamyl acetate increased, the fumigation effect of the mixture increased first and then decreased. The insecticidal effect was best when the ratio of *trans*-cinnamaldehyde to cinnamyl acetate mixture was 2:1, and the mortality reached 95% at 20 h. The 2:1 mixture of *trans*-cinnamaldehyde and cinnamyl acetate had the strongest insecticidal effect of all ratios.

To investigate the effect of cinnamyl acetate, we selected *Cinnamomum cassia* bark essential oil with a low content of cinnamyl acetate and a weak fumigation effect on ants as the experimental sample (Fig. 2c). Cinnamyl acetate was added to the *C. cassia* oil sample to prepare a mixture of *trans*-cinnamaldehyde and cinnamyl acetate at a ratio of 2:1. Adding cinnamyl acetate to *C. cassia* bark oil at a concentration of 160 µg/cm³ significantly increased the insecticidal activity of the mixture (Fig. 6a, b, c). A mixture of cinnamyl acetate added to *C. cassia* bark oil at a concentration of 320 µg/cm³ killed the RIFA 20 min earlier than pure *C. cassia* bark oil (Fig. 6d, e, f). Adding cinnamyl acetate to *C. cassia* bark oil in the amount of *trans*-cinnamaldehyde 1:2 significantly improved the insecticidal activity of *C. cassia* bark oil.

3.4 Scanning electron microscopy (SEM) characterization of the antennal morphology of RIFA

Antennae are important sensory organs for RIFA (Backen et al. 2000). In the current study, the effects of 320 µg/cm³ *C. loureirii* leaf and bark essential oils, 100 µg/cm³ *trans*-cinnamaldehyde, 50 µg/cm³ cinnamyl acetate component, and 150 µg/cm³ *trans*-cinnamaldehyde/cinnamyl acetate mixture (v/v = 2:1) on the antennal morphology of middle-sized worker ants were observed by SEM after 3 h of fumigation.

Under normal circumstances, the sensilli trichodea of RIFA antennae have a smooth surface and stand upright, gradually taper, and point toward the top. The sensilli chaetica are thicker and slightly shorter than the sensilli trichodea, with an erect base. The top is relatively blunt, and most of them point to the top (Fig. 7f). However, the morphology of the sensilla trichodea and sensilla chaetica of the antennae rod segments of RIFA in different treatments were different. The 320 µg/cm³ *C. loureirii* leaf and bark essential oils had a significant effect on antennal morphology compared to treatments of 100 µg/cm³ *trans*-cinnamaldehyde, 50 µg/cm³ cinnamyl acetate component, and 150 µg/cm³ *trans*-cinnamaldehyde/cinnamyl acetate mixture (Fig. 7c, d, e). Some of the sensilla trichodea and sensilla chaetica were broken, and most of them were arranged in reverse (Fig. 7a). The effect of 320 µg/cm³ *C. loureirii* leaf essential oil was the second most obvious. Most of the sensilli chaetica in the treatment group were disordered (Fig. 7b). The fumigation effect of 100 µg/cm³ *trans*-cinnamaldehyde and 150 µg/cm³ mixture was stronger than that of the 50 µg/cm³ cinnamyl acetate component (Fig. 7c, e, and d). Some of the sensilli chaetica in the treatment group were disordered (Fig. 7c, e). The 50 µg/cm³ cinnamyl acetate had almost no effect on the antennal morphology, similar to the control group (Fig. 7d). Overall, the effects of the treatments (from strong to weak) were 320 µg/cm³ *C. loureirii* bark essential oils, 320 µg/cm³ *C. loureirii* leaf, 100 µg/cm³ *trans*-cinnamaldehyde, 150 µg/cm³ *trans*-cinnamaldehyde/cinnamyl acetate mixture, and 50 µg/cm³ cinnamyl acetate. It could be observed with the naked eye that the RIFA behaved abnormally during the treatment process, with the test ants exhibiting abnormal friction of the antennae. We speculated that some volatile components from the essential oils had special effects on the receptors of RIFA and caused damage to or even shedding of the antennae.

3.5 Electroantennogram (EAG) responses of RIFA

An EAG is generally assumed to reflect the regular voltage fluctuations caused by the electrochemical demagnetization of olfactory neurons in the antennae of insects (Guo et al. 2003). They have been used mainly to collect very weak original electrical signals from insect test subjects and then amplify them under the automatic control of software, to generate relatively strong electrical signals to investigate insects that perceive the environment through their antennae (Guo et al. 2003). In this experiment, GC-EAG was used to screen the main components of *C. loureirii* essential oils related to antennal reactions, and EAG was used to analyze the effect of gradients containing different concentrations of *trans*-cinnamaldehyde and cinnamyl acetate on the antennae of worker ants.

Through GC-EAG analysis, when *trans*-cinnamaldehyde reached peak levels, the antenna electroantennogram responses of RIFA workers changed significantly, indicating that *trans*-cinnamaldehyde is the main component of cinnamon essential oils that acts on RIFA (Fig. 8a). Using EAG verification, it was found that the effect of *trans*-cinnamaldehyde on the antennae of RIFA workers formed a concentration gradient with significant differences. The EAG transformation values reached 43.16 ± 0.67 at the highest concentration of 1 mg/mL (Fig. 8b), After treatment with the same concentration of cinnamyl acetate at 1 mg/mL, the transformation value of antennae from test ants was only 7.00 ± 0.75 , which was slightly different from the blank control, *n*-hexane (Fig. 8c). Through EAG analysis, it was found that the EAG change of the mixed solution was more obvious at the same concentration (Fig. 8e).

4. Discussion

Cinnamon essential oils have effective insecticidal activity (Pandey et al. 2012; Jumbo et al. 2014). However, with the exception of *Cinnamomum verum* leaf essential oil, which is rich in eugenol, other varieties of leaf essential oils are considered to have low economic value (Su 1985; Li et al. 2013b; Fu et al. 2015). A recent study indicated that soils containing fallen leaves of cinnamon usually repel RIFA because the soils are rich in *trans*-cinnamaldehyde and eugenol (Zhang 2015). To make full use of cinnamon resources, we conducted a comparative study on the activities of *C. loureirii* leaf and bark essential oils against RIFA. In a fumigation experiment of RIFA with *C. loureirii* leaf and bark essential oils at different concentrations, we found that both leaf and bark essential oils had high insecticidal activity, especially at a concentration of $160 \mu\text{g}/\text{cm}^3$. The insecticidal effect of leaf essential oil was obviously stronger than that of bark essential oil and was able to cause 100% mortality in RIFA within 10 h. Furthermore, at a concentration of $320 \mu\text{g}/\text{cm}^3$, the essential oils of leaf and bark could kill all the RIFA in approximately 1 h, but the essential oil of leaves exhibited stronger insecticidal activity and could kill all the RIFA in 10 min.

The ingredients that confer strong insecticidal activity to leaf essential oil were analyzed. Cheng et al. (2009) studied the insecticidal activity of cinnamon essential oil against *Aedes albopictus*, *Culex quinquefasciatus*, and *Armigeres subalbatus* and found that α -methylcinnamaldehyde, benzaldehyde, and *trans*-cinnamaldehyde in essential oils are important ingredients for insecticidal activity. Ling et al. (2015) used nuclear magnetic resonance (NMR) and Electrospray ionization mass spectrometry (ESI-MS) to identify cinnamaldehyde and cinnamic acid as the insecticidal components of cinnamon against *Dactylogyrus intermedius*. Recently, Kang and Lee (2018) studied the contact and fumigation effects of *trans*-cinnamaldehyde, cinnamyl acetate, and coumarin in cinnamon essential oils on adult *Tyrophagus putrescentiae* mites and found that the three components had no significant difference in contact activity. However, *trans*-cinnamaldehyde showed the highest insecticidal activity, especially when compared with cinnamyl acetate and coumarin against *Sitophilus oryzae* (Linnaeus), *Sitophilus zeamais*, *Sitotroga cerealella*, and *Plodia interpunctella* (Hübener) by contact or fumigation methods. Among them, in both contact and fumigation bioassays, cinnamyl acetate had almost no insecticidal activity against adult insects (Kim et al. 2004; Wang et al. 2011). Our research also shows that cinnamyl acetate has almost no direct insecticidal activity against RIFA in contact or fumigation bioassays. Similarly, the EAG results also support that *trans*-cinnamaldehyde is the main component of cinnamon essential oils acting on RIFA, and cinnamyl acetate has no activity against RIFA. Interestingly, GC-MS

analysis of *C. loureirii* bark essential oil revealed that the relative content of *trans*-cinnamaldehyde (70.31%) in bark was significantly higher than that in leaves (63.20%). In contrast, cinnamyl acetate was not detected in the essential oil of bark but was present at concentrations as high as 27.52% in the essential oil of leaves.

This phenomenon has piqued our interest in the role of cinnamyl acetate. Coincidentally, Cheng et al. (2009) demonstrated that the leaf essential oils of cinnamaldehyde/cinnamyl acetate types had an excellent inhibitory effect compared with the cinnamaldehyde-type effect against *Aedes albopictus* larvae. Tepe and Ozaslan (2020) recently found that the antioxidant activity (FRAP) of *trans*-cinnamaldehyde in *C. verum* essential oil was weak but could be improved by supplementing with different ratios of cinnamyl acetate. Similarly, our results show that the fumigation effects of the *trans*-cinnamaldehyde/cinnamyl acetate mixture at different ratios were distinctive. When the content of cinnamyl acetate in the mixture increased, the fumigation effect first increased and then decreased. In particular, when the concentration ratio of *trans*-cinnamaldehyde to cinnamyl acetate was 2:1, the insecticidal activity was the strongest among all mixtures and single components. Interestingly, the 2:1 concentration ratio of *trans*-cinnamaldehyde and cinnamyl acetate was in accordance with the composition of the essential oil of *C. loureirii* leaves. Furthermore, cinnamyl acetate was added to the essential oil of *C. cassia* bark (which contains almost no cinnamyl acetate) at a *trans*-cinnamaldehyde and cinnamyl acetate concentration ratio of 2:1. The bioassay results showed that cinnamyl acetate also significantly improved the insecticidal ability of *C. cassia* bark oil. GC-EAG analysis further supports that the 2:1 *trans*-cinnamaldehyde/cinnamyl acetate mixture has a stronger effect on RIFA than *trans*-cinnamaldehyde itself.

In summary, our research shows that *C. loureirii* leaf essential oil has good insecticidal activity against RIFA via fumigation and has the potential to become a low-cost biological resource for the development of environmentally friendly insecticides against RIFA, especially in confined spaces such as warehouses and containers. Among its main ingredients, *trans*-cinnamaldehyde showed the highest insecticidal activity, and cinnamyl acetate significantly improved the overall controlling effect. It is possible that cinnamyl acetate could increase other targets; in particular, cinnamyl acetate attacks the membrane and cytoplasm and, in certain situations, may completely alter the morphology of the cells (Vasconcelos et al. 2018). The mechanism by which cinnamyl acetate improves *trans*-cinnamaldehyde insecticidal ability is worthy of further study.

5. Conclusions

The fumigation activity of *C. loureirii* bark and leaf oils was compared at different treatment times and concentrations, and the main components of the oils (cinnamyl acetate and *trans*-cinnamaldehyde) were analyzed at different treatment concentrations and proportions. *C. loureirii* leaf oil can be developed and used as a novel, environmentally friendly insecticide resource for the control of RIFA. With the advantage of a natural source, low-cost, readily available, volatile, and renewable, *C. loureirii* leaf oil is an ideal candidate ingredient for use in integrated pest management and organic agriculture, especially in confined spaces such as warehouses and containers.

As previously reported, we not only found that cinnamyl acetate, an abundant component in the leaf essential oil of *C. loureirii*, could play an important role in improving the insecticidal activity of *trans*-cinnamaldehyde but also found a significant ratio of concentrations of *trans*-cinnamaldehyde and cinnamyl acetate to kill RIFA. In this way, different volatilities of essential oil components are used to achieve different insecticidal effects.

Furthermore, it is important to study the relationships and ratios of other compositions in a high proportion of *C. loureirii* leaf oil and their effect on fumigation activity. This study provides a new environmentally friendly insecticide resource for the control and management of RIFA and has important practical significance for the economic benefits of *C. loureirii* leaves.

Key message

- *Cinnamomum loureirii* leaf oil is new insecticide resource against *Solenopsis invicta*
- *Cinnamomum loureirii* leaf oil has higher insecticidal activity than that of bark
- Cinnamyl acetate can strikingly improve insecticidal activity of *trans*-cinnamaldehyde
- The 2:1 of *trans*-cinnamaldehyde and cinnamyl acetate is the best ratio concentration

Declarations

Acknowledgments

This research was supported by the Science and Technology Innovation Fund Project on Forestry of Guangdong Province (2017KJCX006), the Natural Science Foundation of Guangdong Province (2019A1515011005) and the 2019 South China Agricultural University high-level university construction special fund "Top-notch" project support. We thank Guangdong Institute of Biological Resources for technique support.

Conflicts of interest/Competing interests

We have no conflicts of interest to disclose.

Availability of data and material (data transparency)

Not applicable.

Code availability (software application or custom code)

Not applicable.

Ethics approval (include appropriate approvals or waivers)

Ethical approval This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

Authors' Contributions

HW, HX, ZZ and HX designed the experiments. HX, YH, MB, YL, JL, HB and LY performed the experiments and the data analysis. HX, HX and HW wrote the manuscript. All authors read and approved the final manuscript.

Informed consent

Informed consent was obtained from all individual participants included in the study.

References

1. Adams RP (2007) Identification of essential oil components by gas chromatography / mass spectrometry, 4th edition, vol 456. Allured Pub Corp, 362 S Schmale Rd, ISBN 978-1-932633-21-4, Carol Stream, IL, 60188-2787, USA
2. Appel AG, Gehret MJ, Tanley MJ (2004) Repellency and toxicity of mint oil granules to red imported fire ants (Hymenoptera: Formicidae). J Econ Entomol 97: 575-580. <https://doi.org/10.1093/jee/97.2.575>
3. Ariaee-Nasab N, Vahedi Z, Vahedi F (2014) Inhibitory effects of cinnamon-water extract on human tumor cell lines. Asian Pac J Trop Dis 4, S975-S978. [https://doi.org/10.1016/S2222-1808\(14\)60768-4](https://doi.org/10.1016/S2222-1808(14)60768-4)

4. Backen SJ, Sendova-Franks AB, Franks NR (2000) Testing the limits of social resilience in ant colonies. *Behav Ecol Sociobiol* 48: 125-131. <https://doi.org/10.1007/s002650000219>
5. Benelli G, Pavela R, Giordani C, Casettari L, Curzi G, Cappellacci L, Petrelli R, Maggi F (2018) Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. *Ind Crop Prod* 112: 668-680. <https://doi.org/10.1016/j.indcrop.2017.12.062>
6. Buren WF, Allen GE, Whitcomb WH, Lennartz FE, Williams RN (1974) Zoogeography of the imported fire ants. *J New York Entomol S* 82: 113-124
7. Camacho S, Michlig S, De Senarclens-Bezençon C, Meylan J, Meystre J, Pezzoli M, Markram H, Le Coutre J (2015) Anti-obesity and anti-hyperglycemic effects of cinnamaldehyde via altered ghrelin secretion and functional impact on food intake and gastric emptying. *Sci Rep* 5: 7919. <https://doi.org/10.1038/srep07919>
8. Chaiyasit D, Choochote W, Rattanachanpichai E, Chaithong U, Chaiwong P, Jitpakdi A, Tippawangkosol P, Riyong D, Pitasawat B (2006) Essential oils as potential adulticides against two populations of *Aedes aegypti*, the laboratory and natural field strains, in Chiang Mai province, northern Thailand. *Parasitol Res* 99: 715-721. <https://doi.org/10.1007/s00436-006-0232-x>
9. Chang ST, Cheng SS, Wang SY (2001) Antitermitic activity of essential oils and components from Taiwania (*Taiwania cryptomerioides*). *J Chem Ecol* 27: 717-724. <https://doi.org/10.1023/A:1010397801826>
10. Chang WL, Cheng FC, Wang SP, Chou ST, Shih Y (2017) *Cinnamomum cassia* essential oil and its major constituent cinnamaldehyde induced cell cycle arrest and apoptosis in human oral squamous cell carcinoma HSC-3 cells. *Environ Toxicol* 32: 456-468. <https://doi.org/10.1002/tox.22250>
11. Chen J (2009) Repellency of an over-the-counter essential oil product in China against workers of red imported fire ants. *J Agric Food Chem* 57: 618-622. <https://doi.org/10.1021/jf8028072>
12. Cheng SS, Liu JY, Huang CG, Hsui YR, Chen WJ, Chang ST (2009) Insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against three mosquito species. *Bioresour Technol* 100: 457-464. <https://doi.org/10.1016/j.biortech.2008.02.030>
13. China Pharmacopoeia Committee (2020) Pharmacopoeia of the People's Republic of China 2020, vol 1. Chinese Medical Science and Technology Press, Beijing, China, pp 142-420
14. Dugassa S, Medhin G, Balkew M, Seyoum A, Gebre-Michael T (2009) Field investigation on the repellent activity of some aromatic plants by traditional means against *Anopheles arabiensis* and *An. pharoensis* (Diptera: Culicidae) around Koka, central Ethiopia. *Acta Trop* 112: 38-42. <https://doi.org/10.1016/j.actatropica.2009.06.002>
15. Fu JT, Tang L, Li WS, Wang K, Cheng DM, Zhang ZX (2015) Fumigant toxicity and repellence activity of camphor essential oil from *Cinnamomum camphora* Siebold against *Solenopsis invicta* workers (Hymenoptera: Formicidae). *J Insect Sci* 15: 129. <https://doi.org/10.1093/jisesa/iev112>
16. Guo XR, Yuan GH, Jiang JW, Ma JS (2003) Electroantennogram responses of insects to volatile secondary compounds from plants. *J Henan Agric Univ (Natural science edition)* 37: 18-22. <https://doi.org/10.16445/j.cnki.1000-2340.2003.01.005>
17. Huang RL, Li ZH, Wang SY, Fu JT, Cheng DM, Zhang ZX (2016) Insecticidal effect of volatile compounds from plant materials of *Murraya exotica* against red imported fire ant workers. *Sociobiology* 63: 783-791. <https://doi.org/10.13102/sociobiology.v63i2.972>
18. Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol* 51: 45-66. <https://doi.org/10.1146/annurev.ento.51.110104.151146>
19. Jiang H, Wang J, Song L, Cao XS, Yao X, Tang F, Yue YD (2016) GC \times GC-TOFMS analysis of essential oils composition from leaf, twigs and seeds of *Cinnamomum camphora* (L.) Presl and their insecticidal and repellent activities. *Molecules* 21: 423. <https://doi.org/10.3390/molecules21040423>

20. Jumbo LOV, Faroni LRA, Oliveira EE, Pimentel MA, Silva GN (2014) Potential use of clove and cinnamon essential oils to control the bean weevil, *Acanthoscelides obtectus* Say, in small storage units. Ind Crop Prod 56: 27-34. <https://doi.org/10.1016/j.indcrop.2014.02.038>
21. Kang MS, Lee HS (2018) Acaricidal and insecticidal responses of *Cinnamomum cassia* oils and main constituents. Appl Biol Chem 61: 653-659. <https://doi.org/10.1007/s13765-018-0402-4>
22. Kim HK, Kim JR, Ahn YJ (2004) Acaricidal activity of cinnamaldehyde and its congeners against *Tyrophagus putrescentiae* (Acari: Acaridae). J Stored Prod Res 40: 55-63. [https://doi.org/10.1016/S0022-474X\(02\)00075-9](https://doi.org/10.1016/S0022-474X(02)00075-9)
23. Kumar P, Mishra S, Malik A, Satya S (2012) Compositional analysis and insecticidal activity of *Eucalyptus globulus* (family: Myrtaceae) essential oil against housefly (*Musca domestica*). Acta Trop 122: 212-218. <https://doi.org/10.1016/j.actatropica.2012.01.015>
24. Kumar S, Kumari R, Mishra S (2019) Pharmacological properties and their medicinal uses of Cinnamomum: A review. J Pharm Pharmacol 71: 1735-1761. <https://doi.org/10.1111/jphp.13173>
25. Li YQ, Kong DX, Fu Y, Sussman MR, Wu H (2020) The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol Biochem 148: 80-89. <https://doi.org/10.1016/j.plaphy.2020.01.006>
26. Li YQ, Kong DX, Huang RS, Liang HL, Xu CG, Wu H (2013b) Variations in essential oil yields and compositions of *Cinnamomum cassia* leaves at different developmental stages. Ind Crop Prod 47: 92-101. <https://doi.org/10.1016/j.indcrop.2013.02.031>
27. Li YQ, Kong DX, Wu H (2013a) Analysis and evaluation of essential oil components of cinnamon barks using GC-MS and FT-IR spectroscopy. Ind Crop Prod 41: 269-278. <https://doi.org/10.1016/j.indcrop.2012.04.056>
28. Ling F, Jiang C, Liu G, Li M, Wang G (2015) Anthelmintic efficacy of cinnamaldehyde and cinnamic acid from cortex cinnamon essential oil against *Dactylogyrus intermedius*. Parasitology 142: 1744-1750. <https://doi.org/10.1017/S0031182015001031>
29. Lu YY, Li ND, Liang WG, Zeng L (2007) Spatial correlation of active mounds locative distribution of *Solenopsis invicta* Buren polygyne populations. J Appl Ecol 18: 140-144. <https://doi.org/CNKI:SUN:YYSB.0.2007-01-023>
30. Nerio LS, Olivero-Verbel J, Stanshenko E (2010) Repellent activity of essential oils: a review. Bioresource Technol 101: 372-378. <https://doi.org/10.1016/j.biortech.2009.07.048>
31. Pandey A, Chattopadhyay P, Banerjee S, Pakshirajan K, Singh L (2012) Antitermitic activity of plant essential oils and their major constituents against termite *Odontotermes assamensis* Holmgren (Isoptera: Termitidae) of North East India. Int Biodeter Biodegr 75: 63-67. <https://doi.org/10.1016/j.ibiod.2012.09.004>
32. Piotr S, Magdalena Z, Joanna P, Barbara K, Sławomir M (2018) Essential oils as potential anti-staphylococcal agents. Acta Vet 68: 95-107. <https://doi.org/10.2478/acve-2018-0008>
33. Ribeiro-Santos R, Andrade M, Madella D, Martinazzo AP, Moura LDAG, De Melo NR, Sanches-Silva A (2017) Revisiting an ancient spice with medicinal purposes: Cinnamon. Trends Food Sci Tech 62: 154-169. <https://doi.org/10.1016/j.tifs.2017.02.011>
34. Seo SM, Kim J, Lee SG, Shin CH, Shin SC, Park IK (2009) Fumigant antitermitic activity of plant essential oils and components from Ajowan (*Trachyspermum ammi*), Allspice (*Pimenta dioica*), Caraway (*Carum carvi*), Dill (*Anethum graveolens*), Geranium (*Pelargonium graveolens*), and Litsea (*Litsea cubeba*) oils against Japanese termite (*Reticulitermes speratus* Kolbe). J Agric Food Chem 57: 6596-6602. <https://doi.org/10.1021/jf9015416>
35. Sheppard DC (1983) Toxicity of citrus peel liquids to the house fly and red imported fire ant. J Agric Entomol 1: 95-100
36. Shi XP, Zhang CJ, Liu QH, Zhang Z, Zheng B, Bao MZ (2016) De novo comparative transcriptome analysis provides new insights into sucrose induced somatic embryogenesis in camphor tree (*Cinnamomum camphora* L.). BMC

- Genomics 17: 26. <https://doi.org/10.1186/s12864-015-2357-8>
37. Su HCF (1985) Laboratory evaluation of biological activity of *Cinnamomum cassia* to four species of stored-product insects. J Entomol Sci 20: 247-253. <https://doi.org/10.18474/0749-8004-20.2.247>
38. Tang L, Sun YY, Zhang QP, Zhou Y, Zhang N, Zhang ZX (2013) Fumigant activity of eight plant essential oils against workers of red imported fire ant, *Solenopsis invicta*. Sociobiology 60: 35-40. <https://doi.org/10.13102/sociobiology.v60i1.35-40>
39. Tepe AS, Ozaslan M (2020) Anti-alzheimer, anti-diabetic, skin-whitening, and antioxidant activities of the essential oil of *Cinnamomum zeylanicum*. Ind Crop Prod 145: 1-8. <https://doi.org/10.1016/j.indcrop.2019.112069>
40. Thorvilson H, Rudd B (2001) Are landscaping mulches repellent to red imported fire ants *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Southwest Entomol 26: 195-203
41. Van Den Dool H, Kratz DP (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr 11: 463-471. [https://doi.org/10.1016/s0021-9673\(01\)80947-x](https://doi.org/10.1016/s0021-9673(01)80947-x)
42. Vasconcelos NG, Croda J, Simionatto S (2018) Antibacterial mechanisms of cinnamon and its constituents: A review. Microb Pathogenesis 120: 198-203. <https://doi.org/10.1016/j.micpath.2018.04.036>
43. Vogt JT, Shelton TG, Merchant ME, Russell SA, Tanley MJ, Appel AG (2002) Efficacy of three citrus oil formulations against *Solenopsis invicta* Buren (Hymenoptera: Formicidae), the red imported fire ant. J Agric Urban Entomol 19: 159-171. <https://doi.org/10.1080/09670870210133698>
44. Wang Z, Kim HK, Tao WQ, Wang M, Ahn YJ (2011) Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J Med Entomol 48: 366-371. <https://doi.org/10.1603/me10127>
45. Wiltz BA, Suiter DR, Gardner WA (2007) Deterrency and toxicity of essential oils to Argentine and red imported fire ants (Hymenoptera: Formicidae). J Entomol Sci 42: 239-249. <https://doi.org/10.18474/0749-8004-42.2.239>
46. Wu H, Fu CC, Yu DD, Feng JT, Zhang X, Ma ZQ (2013) Repellent activity screening of 11 kinds of essential oils against *Aedes albopictus* Skuse: microcapsule preparation of *Herba Schizonepetae* oil and repellent bioassay on hand skin. T Roy Soc Trop Med H 107: 471-479. <https://doi.org/10.1093/trstmh/trt045>
47. Wu MT, Ni L, Lu HX, Xu HY, Zou SQ, Zou XX (2020) Terpenoids and their biological activities from cinnamomum: a review. J Chem 2020: 1-14. <https://doi.org/10.1155/2020/5097542>
48. Yan WW, Zhang H, Dong YL, Xu HH, Zhang ZX (2012) Effect of α -terthienyl on the foraging behavior and feeler responses of *Solenopsis invicta* Buren. Chinese J Pestic Sci 14: 277-282. <https://doi.org/10.3969/j.issn.1008-7303.2012.03.06>
49. Yap PSX, Yiap BC, Ping HC, Lim SHE (2014) Essential oils, a new horizon in combating bacterial antibiotic resistance. Open Microbiol J 8: 6-14. <https://doi.org/10.2174/1874285801408010006>
50. Zhang RZ, Li YC, Liu N, Porter SD (2007) An overview of the red imported fire ant (Hymenoptera: Formicidae) in mainland China. Fla Entomol 90: 723-731. [https://doi.org/10.1653/0015-4040\(2007\)90\[723:AOOTRI\]2.0.CO;2](https://doi.org/10.1653/0015-4040(2007)90[723:AOOTRI]2.0.CO;2)
51. Zhang ZX (2015) The insecticidal and repellent activity of soil containing cinnamon leaf debris against red imported fire ant workers. Sociobiology 62: 46-51. <https://doi.org/10.13102/sociobiology.v62i1.46-51>

Tables

Table 1 GC-MS component analysis of *C. loureirii* essential oils.

NO.	Component ^a	RI ^b _{exp}	RI ^c _{lit}	Molecularformula	Area Pct(%)		ID ^e
					<i>C.loureirii</i> bark ^d	<i>C.loureirii</i> leaf ^d	
1	<i>cis</i> -cinnamaldehyde	1225	1219	C ₉ H ₈ O	0.21	0.44	a,b,c
2	<i>trans</i> -cinnamaldehyde	1230	1225	C ₉ H ₈ O	70.31	63.20	a,b,c
3	<i>o</i> -Anisaldehyde	1251	1242	C ₈ H ₈ O ₂	Tr	0.40	a,b,c
4	α -copaene	1381	1379	C ₁₅ H ₂₄	20.46	0.86	a,b,c
5	I-caryophyllene	1425	1423	C ₁₅ H ₂₄	Tr	0.17	a,b,c
6	cinnamyl acetate	1449	1445	C ₁₁ H ₁₂ O ₂	Tr	27.52	a,b,c
7	α -muurolene	1504	1505	C ₁₅ H ₂₄	1.81	0.20	a,b,c
8	<i>d</i> -cadinene	1524	1524	C ₁₅ H ₂₈	3.23	0.54	a,b,c
9	2'-methoxycinnamaldehyde	1530	1529	C ₁₀ H ₁₀ O ₂	Tr	5.28	a,b,c
10	naphthalene,1,2,3,4,6,8a-hexahydro-1-isopropyl-4,7-dimethyl- (8Cl)	1532	1532	C ₁₅ H ₂₄	2.75	Tr	a,b,c
11	α -calacorene	1542	1542	C ₁₅ H ₂₀	1.19	Tr	a,b,c
12	<i>cis</i> -nerolidol	1559	1562	C ₁₅ H ₂₆ O	Tr	0.25	a,b,c
13	espatulenol	1577	1577	C ₁₅ H ₂₄ O	Tr	0.35	a,b,c
	total				99.96	99.21	

^aCompounds are listed in order of their elution from a HP-5MS column.

^bLinear retention index on HP-5MS column, experimentally determined using homologous series of C7-C30 alkanes.

^cLinear retention index taken from NIST and literature.

^dValues are given as means of three replicates.

^eIdentification methods: a, based on comparison of RT, RI and MS with those of authentic compounds; b, based on comparison of mass spectrum with those reported in WILEY, and NIST (14) MS Search 2.2 libraries; c, based on comparison of calculated RI with those reported in NIST 14.

Retention indices (R.I.) based on a homologous series of normal alkanes.

Tr, traces (%< 0.1).

Figures

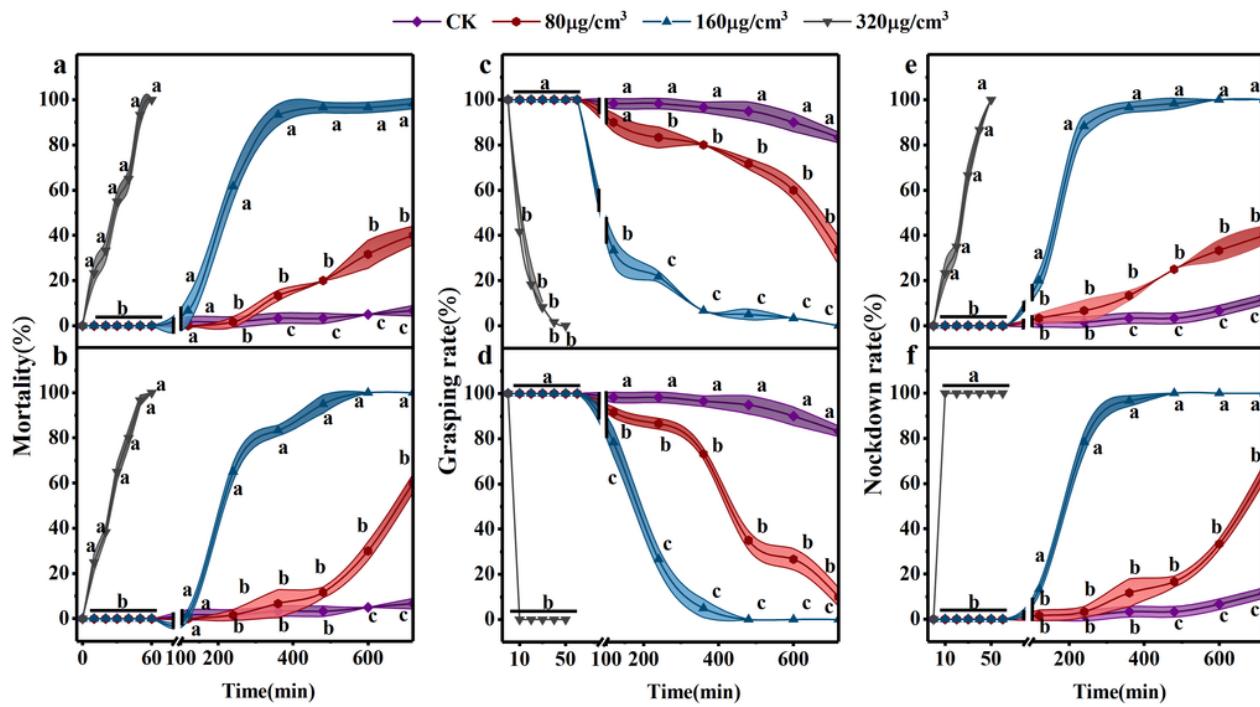


Figure 1

Fumigation mortalities, grasping abilities and knockdown abilities of essential oils from leaf and bark of *C. loureirii* at concentrations of 80-320 µg/cm³. a, c and e: bark essential oil; b, d and f: leaf essential oil; Different letters represent the difference in mortality, grasping ability and knockdown ability of different samples ($P<0.05$); —represent the same letter

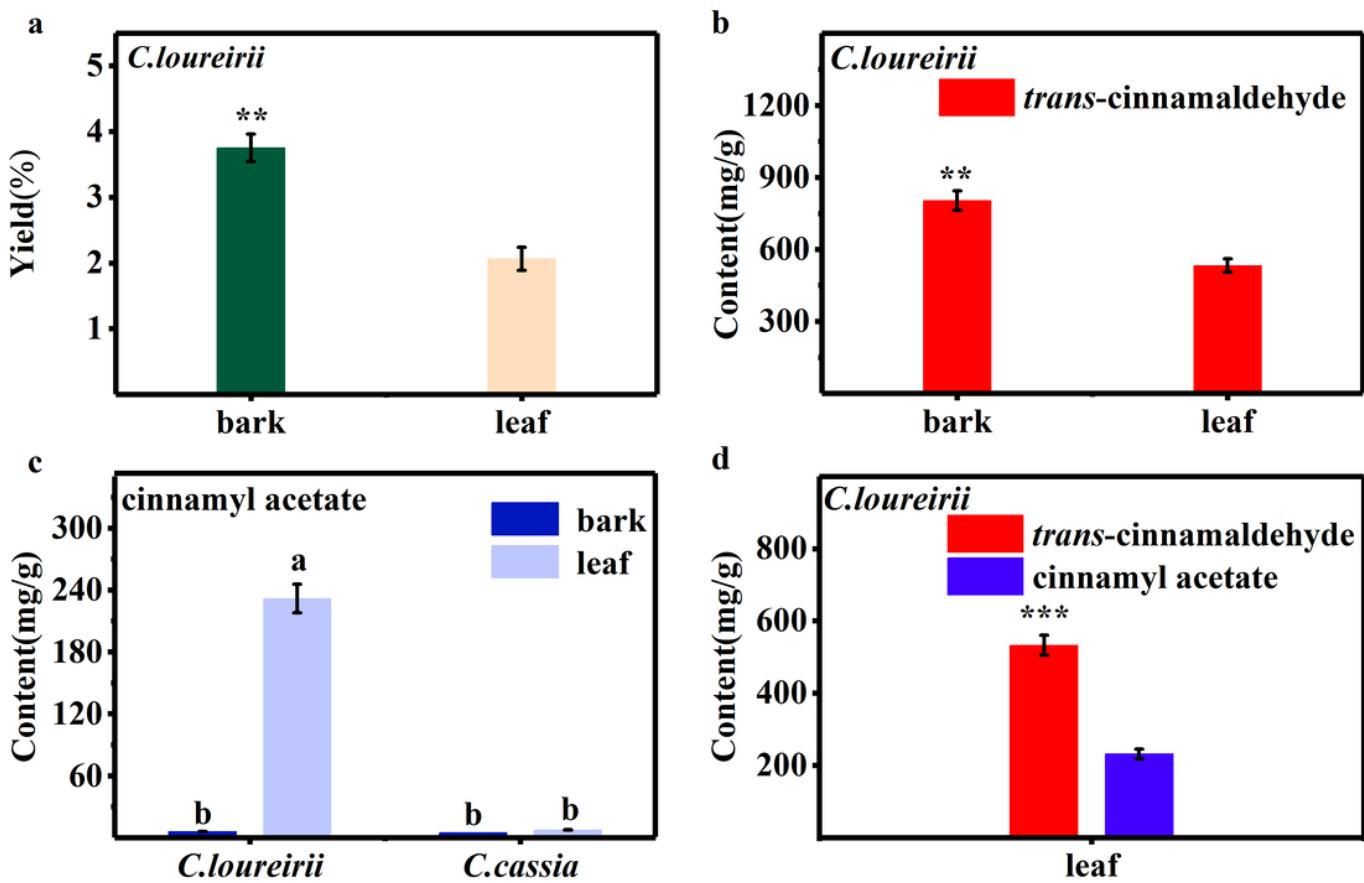


Figure 2

The contents of trans-cinnamaldehyde and cinnamyl acetate in the essential oils from *C. loureirii* bark and leaf. a: essential oil content; b: trans-cinnamaldehyde content in bark and leaf essential oils; c: cinnamyl acetate content in bark and leaf essential oils of *C. loureirii* and *C. cassia*; d: trans-cinnamaldehyde and cinnamyl acetate content in leaf essential oil; **represent the significance at $P<0.01$; *** represent the significance at $P<0.001$; Different letters represent the difference in content of different samples ($P<0.05$)

GC-MS analysis of leaf oil

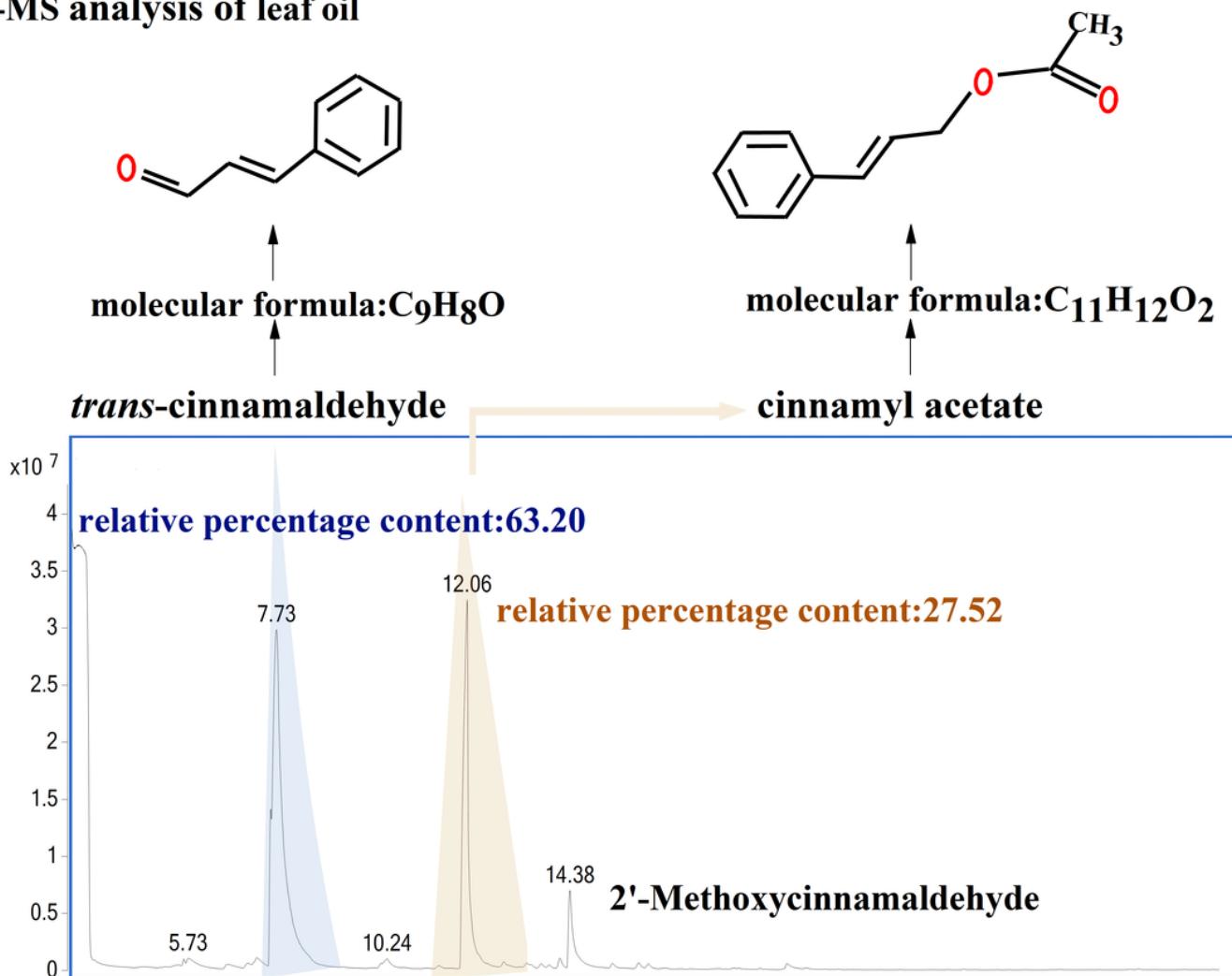


Figure 3

GC-MS analysis of the essential oil of *C. loureirii* leaf

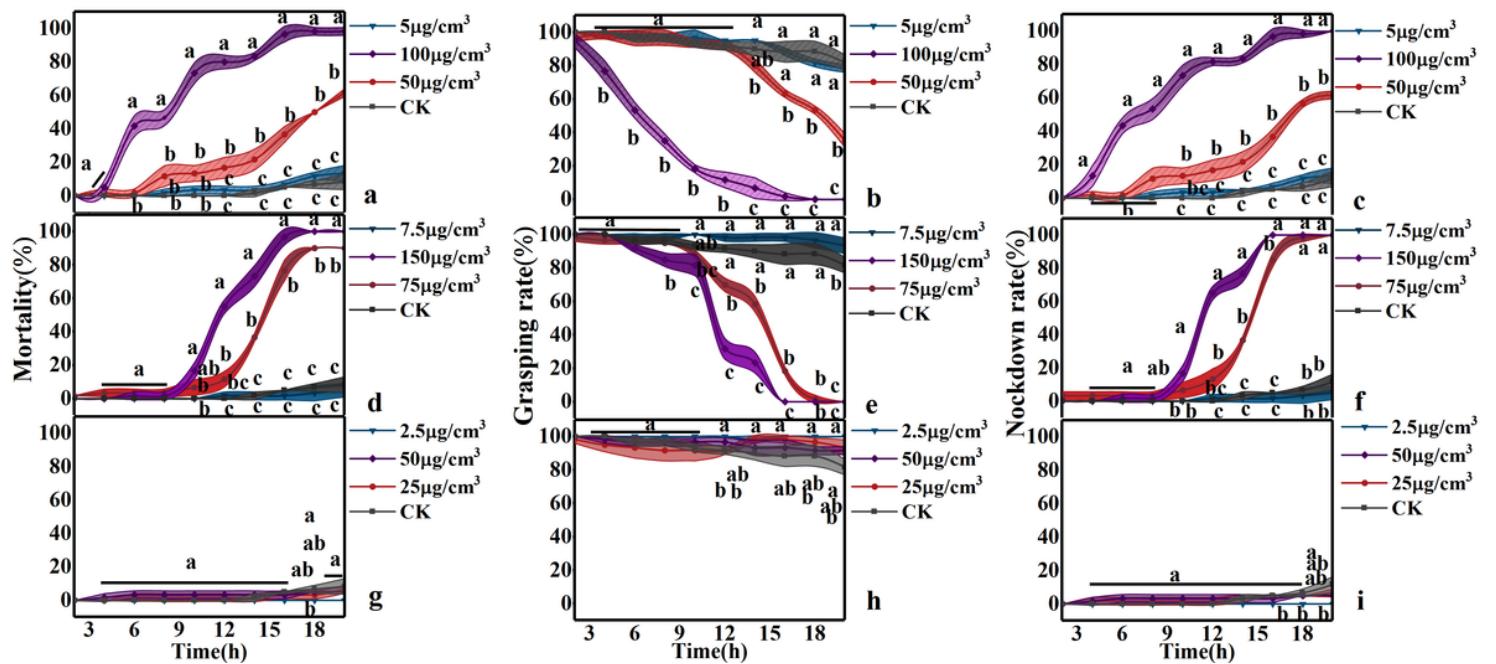


Figure 4

The fumigation effect of single trans-cinnamaldehyde and cinnamyl acetate component and the mixture ratio of 2:1 at different concentrations on the RIFAs after treatment for 20 h. a, b and c: trans-cinnamaldehyde; g, h and i: cinnamyl acetate; d, e and f: mixture; a, d and g: mortality; b, e and h: grasping ability; c, f and i: knockdown ability; Different letters represent the difference in mortality, grasping ability and knockdown ability of different samples ($P < 0.05$); represent the same letter

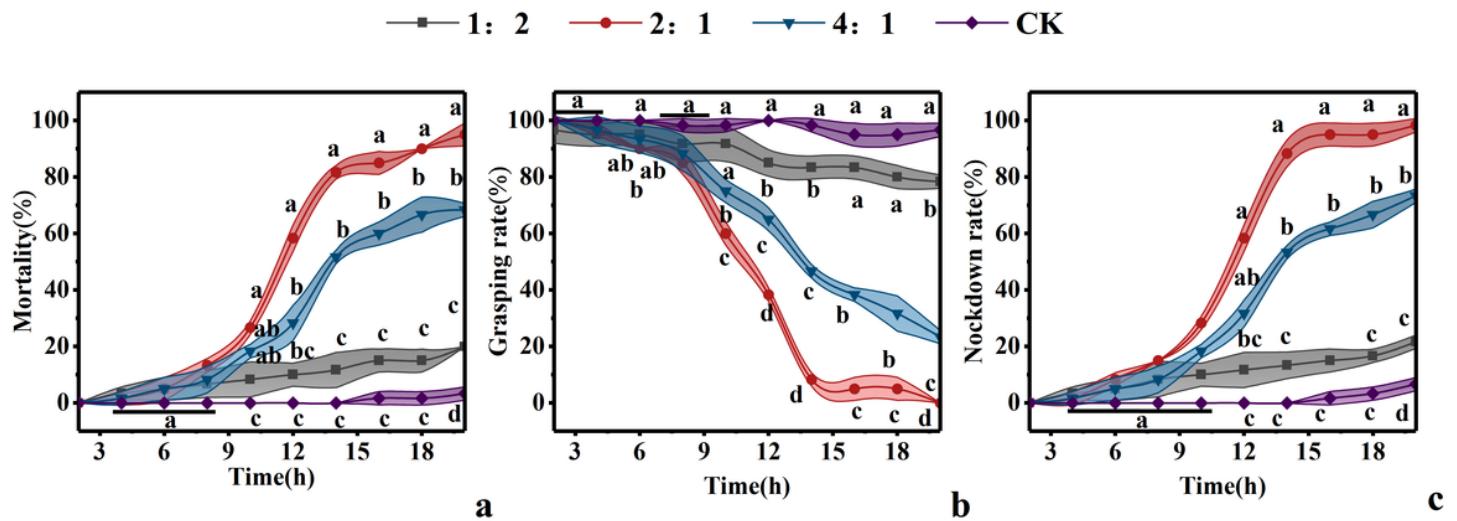


Figure 5

The fumigation effect of mixture of trans-cinnamaldehyde and cinnamyl acetate at a concentration of 75 $\mu\text{g}/\text{cm}^3$ with different ratios on the RIFAs after treatment for 20 h. a: mortality; b: grasping ability; c: knockdown ability; Different letters represent the difference in mortality, grasping ability and knockdown ability of different samples ($P<0.05$); ___represent the same letter

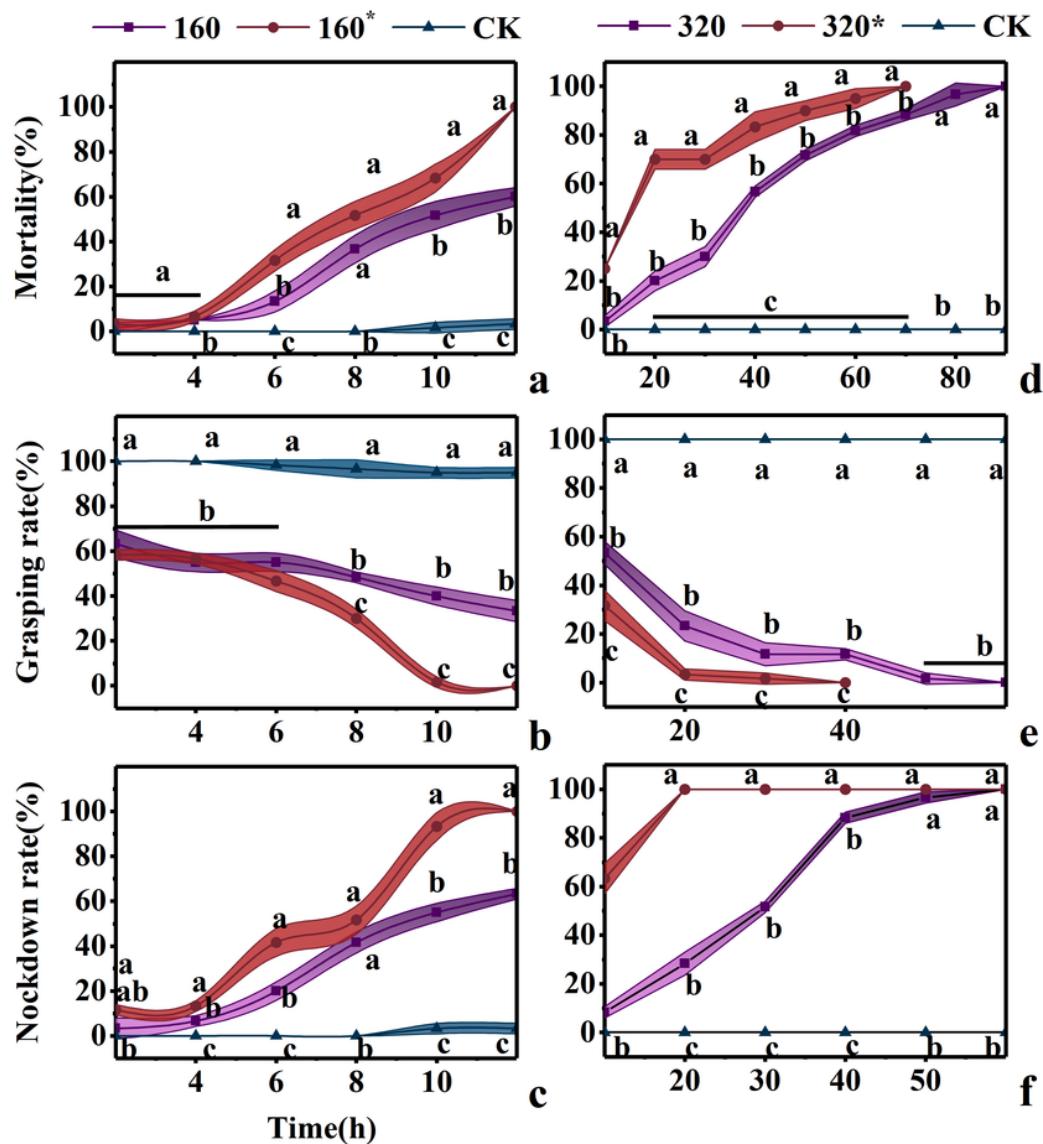


Figure 6

The bark oil of *C. cassia* with two portions of trans-cinnamaldehyde was added a portion of cinnamyl acetate at the concentrations of 160 $\mu\text{g}/\text{cm}^3$ and 320 $\mu\text{g}/\text{cm}^3$, and the fumigation effect on RIFAs after 12 h and 100 min treatments was showed. a, b and c: 160 $\mu\text{g}/\text{cm}^3$ was essential oil, *160 $\mu\text{g}/\text{cm}^3$ was essential oil added with cinnamyl acetate; d, e and f: 320 $\mu\text{g}/\text{cm}^3$ was essential oil, *320 $\mu\text{g}/\text{cm}^3$ was essential oil added with cinnamyl acetate; a and d: mortality; b and e: grasping ability; c and f: knockdown ability; Different letters represent the difference in mortality, grasping ability and knockdown ability of different samples ($P<0.05$); ___represent the same letter

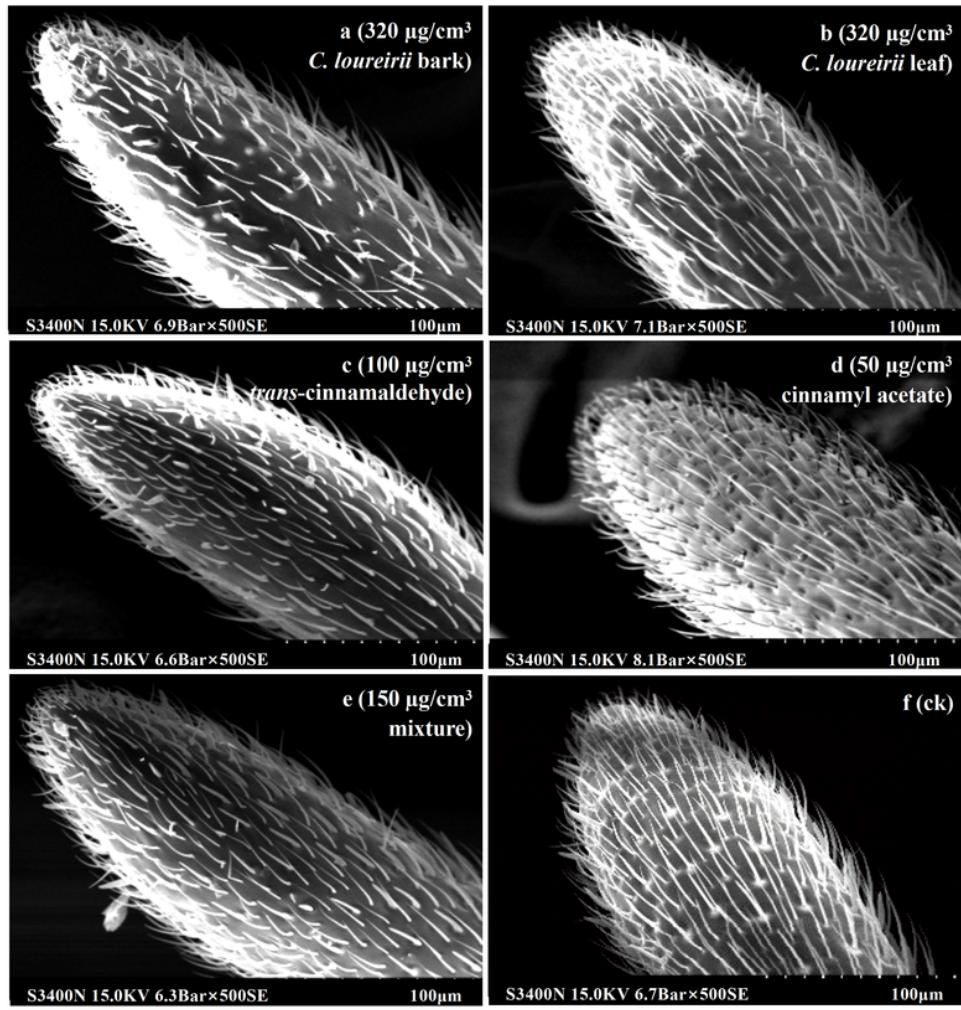


Figure 7

The effect of *C. loureirii* bark and leaf essential oils, trans-cinnamaldehyde and cinnamyl acetate on the antennae of RIFA. *C. loureirii* bark essential oil (a) and leaf essential oil (b) with a concentration of $320 \mu\text{g}/\text{cm}^3$, and $100 \mu\text{g}/\text{cm}^3$ trans-cinnamaldehyde (c), $50 \mu\text{g}/\text{cm}^3$ cinnamyl acetate (d), $150 \mu\text{g}/\text{cm}^3$ trans-cinnamaldehyde and cinnamyl acetate mixture (e); (f) was the blank control

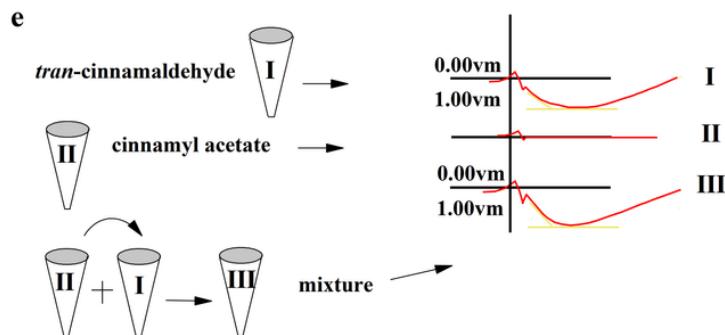
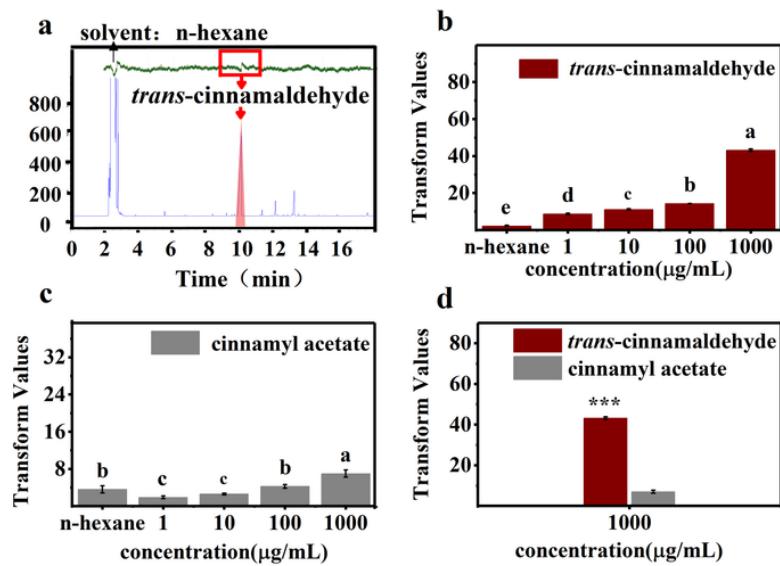


Figure 8

The effect of trans-cinnamaldehyde and cinnamyl acetate along the concentration gradient on the antennae of RIFA. a: GC-EAG analysis diagram; b: trans-cinnamaldehyde EAG analysis diagram; c: cinnamyl acetate EAG analysis diagram; d: significant difference of EAG analysis diagram between cinnamyl acetate and trans-cinnamaldehyde; e: single trans-cinnamaldehyde and cinnamyl acetate component, and mixture EAG; Different letters represent the difference in transform values of different samples ($P<0.05$); *** represent the significance at $P<0.001$

Supplementary Files

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- Graphicalabstract.png