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The Fumigating Activity of *Litsea cubeba* oil and Citral on *Solenopsis invicta*

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Abstract

This paper studied the fumigating activity of *Litsea cubeba* oil and citral on *Solenopsis invicta*, identified and analyzed the chemical constituents and volatile components of *L. cubeba* oil via solid-phase microextraction which were then identified via gas chromatography-mass spectrometry. The results showed that citral and (z)-3,7-dimethylocta-2,6-diena were the main components of *L. cubeba* oil, as well as its volatile compounds. According to the experimental results, *L. cubeba* oil and citral had good fumigating activity on workers, and also had significant inhibition on the walking ability and climbing ability of workers. At the same time, the effects of the two agentia on the fumigating activity and behavioral inhibition of microergate were stronger than those of macroergate. After treating with *L. cubeba* oil and citral for 24 hours, the walking rate and grasping rate of microergate were both 0 %. The results showed that *L. cubeba* oil and citral had good control effect on *S. invicta*.

Introduction

Solenopsis invicta (red imported fire ant, RIFA) is an invasive pest native to South America (Dai, 2015). It is widely distributed in China, the USA, New Zealand, Australia, and other parts of the world (Ascunce et al., 2011). RIFA has many characteristics, such as food mixed, diverse dispersal ways, fierce habits, and strong reproductive ability (Lin, 2012). Moreover, it is very aggressive to human beings and seriously affects the biodiversity, agriculture, and forestry production in the invaded area (Huang et al., 2011). Thus, RIFA is considered as one of the 100 most dangerous invading pests in the world. At present, the main methods used to control RIFA include plant quarantine, physical control, chemical control, and biological control (Wang et al., 2005). Among them, chemical control is the most widely used method. Although chemical insecticides can achieve good control effect in a short time, they also bring a series of problems, such as environmental pollution, impact on human and non-target organisms, and emergence of insecticide resistance (Gao, 2010). Therefore, in order to solve these problems, it is necessary to search for natural substances that are nontoxic to non-target organisms and can rapidly degrade in natural environment.

Essential oils are volatile aromatic substances extracted from the roots, stems, leaves, flowers, or fruits of a plant via physical or chemical methods (Adorjan et al., 2010).They are widely used in medicine, chemical industry, food industry, and plant protection (Zhu et al., 2009). They are also used as a fumigant, repellent, and contact insecticide (Palacios et al., 2009; Sfara et al., 2009).Some plant essential oils have a repellent or toxic effect on some insects. The essential oils of *Callistemon citrinus* leaves show repellent and fumigating effects on adults of *Callosobruchus maculatus* (Sohani et al., 2013). The essential oils of *Vitex negundo* exhibit insecticidal activity against *Sitophilus zeamais* (Lu et al., 2009). Volatile compounds of *Michelia alba* leaves can control RIFA populations (Qin et al., 2018).

Litsea cubeba is a typical Chinese perfume plant resource that is mainly found in the southern provinces and regions of the Yangtze River to Tibet. The main component of *L. cubeba* oil is citral, containing 60% ~ 80%, and the oil is mainly derived from *L. cubeba* fruits (Zhao et al., 2010). Several studies emphasized the strong antibacterial effect of *L. cubeba* oil on *Aspergillus flavus* and *Fungi imperfecti* (Huang et al., 1994; Yu et al., 2002; Huang et al., 2005; Zhou et al., 2013; Li et al., 2016) and found that *L. cubeba* oil has strong antioxidant activity (Zhang et al., 2017). Wagan et al. (2016) found that *L. cubeba* oil has a strong repellent effect on *Monomorium pharaonis*. Zhang et al. (2017) found that the extract of *L. cubeba* fruits has strong fumigant and repellent activities on adult *Sitophilus zeamais*, is harmless to human beings, does not pollute the environment, and has a pleasant smell. Therefore, it can be used as a germicide and for pest control and crop disease prevention, and it has broad prospects for development.

In the present study, we determined the fumigating activity of *L. cubeba* oil and citral on microergate and macroergate and analyzed the chemical and volatile constituents of *L. cubeba* oil. Our results showed that *L. cubeba* oil and citral can be used to control RIFA.

Materials and Methods

Chemical Reagents

L. cubeba oil and citral were purchased from Guangzhou Suining Biological Instrument Co., Ltd., China. *L. cubeba* oil was stored in a refrigerator at 4 °C. The purity of citral was 95%.

Insects

RIFA colonies were collected from the campus of South China Agricultural University. The ants and nest materials were placed into plastic boxes (40 cm × 52 cm × 12 cm), which were coated with Teflon emulsion on the top. A test tube (25 mm × 200 mm) used as a water source was partially filled with water and plugged with cotton. Mealworms (*Tenebrio molitors*) were used as the food source. *T. molitor* larvae were purchased from the insect–fish market in Guangzhou, fed with wheat bran, and kept in a dry indoor environment at 25 ± 2 °C. This study opted to test microergate and macroergate. The microergates and macroergates were approximately 3 and 5 mm in length, respectively (Lin et al., 2005; Zhang et al., 2007).

Chemical Analysis via Gas Chromatography–Mass Spectrometry

L. cubeba oil was diluted 2000 times with ethyl acetate. Subsequently, 0.5 ìL of the sample was taken to be directly detected using an Agilent 7890 gas chromatograph coupled with an Agilent mass spectrometer detector. A HP-5(MS) capillary column (30 m × 0.25 mm × 0.25 ìm) was used to separate the compounds. Mass spectra were obtained by electron ionization at 70 eV with a spectral range of 35-450 m/z.

The injection temperature was 250 °C. The oven temperature was initially set at 40 °C for 1 min to 100 °C (10 °C/min) for 2 min, then to 200 °C (10 °C/min) for 2 min, and to 280 °C (15 °C/min) for 5 min. The detector was operated at 280 °C. Helium was used as a carrier gas at a flow rate of 1 mL/min and split ratio of 10:1. The compounds were identified via mass spectroscopy. Then, the spectra were compared with those from the computer mass libraries (Chen et al., 2012).

Extraction of Volatiles via Solid-Phase Microextraction (SPME)

Approximately 0.5 mL of essential oil was taken in a 50 mL headspace vial, sealed, and placed in a 35 °C water bath for 20 min. Subsequently, SPME fiber (50/30 ìm, CAR/DVB/PDMS) was inserted for head-space extraction for 20 min. The sampled heat at 250 °C was absorbed for 3 min.

The injection temperature was 250 °C. The oven temperature was initially set at 40 °C for 1 min to 100 °C (10 °C/min) for 2 min and then to 200 °C (50 °C/min) for 2 min. The detector was operated at 280 °C. Helium was used as a carrier gas at a flow rate of 1 mL/min and split ratio of 50:1. The compounds were identified via mass spectroscopy, and the spectra were then compared with those from computer mass libraries.

Toxicity Bioassay of Fumigant

The fumigating activity of *L. cubeba* oil and citral was determined on the basis of preliminary experiments. The *L. cubeba* oil and citral were packed in 1.5 mL centrifuge tube (Axygen Biosciences, USA) with eight pinholes to make them vaporize outside. Then, the centrifuge tube was placed at the bottom of the 250 mL rigid plastic cup. The Fluon emulsion (Guangzhou Xingsheng Street Science and Technology Co., Ltd., China) was evenly coated with the wall of each hard-plastic cup to below 2 cm and dried for 24 h to prevent the ants from escaping. Twenty-five microergates and macroergates were placed into a plastic cup bottom and then sealed with plastic wrap and rubber band. The insects were placed at a temperature of 25 ± 2 °C and a relative humidity of 80%. Two concentrations (4 and 8 ìL/tube) of essential oil and citral were applied to workers, respectively, and the cumulative mortalities were assessed after 4, 8, 12, 16, and 24 h of treatment. Each treatment had three replicates, with 25 workers per replicate.

Behavior Observation on Walking Ability of Workers

Workers were treated in the same way as above. To observe the walking ability of the workers, the plastic cup was shaken to disperse the workers in the cup. Then, videos of ants walking were recorded, and we watched the worker walking. If the workers could walk continuously for 3 s or more, they were considered to have walking ability. Each treatment had three replicates, with 25 workers per replicate. The following equation was used to calculate the walking rate:

Walking rate (%) = (number of workers that possessed walking ability/number of workers per replicate) × 100

Behavior Observation on Grasping Ability of Workers

Workers were treated in the same way as above. To observe the grasping ability of the workers, the plastic cup was shaken to evenly distribute the workers at the bottom of the cup. After 10 s, the cup was gently rotated 180°. If the ants did not drop from the bottom of the cup, they were considered to have grasping ability. Each treatment had three replicates, with 25 workers per replicate. The following equation was used to calculate the grasping rate:

Grasping rate (%) = (number of workers possessing grasping ability/number of workers per replicate) × 100

Statistical Analysis

The data were represented as means ± SE. Statistical analysis was carried out by using SPSS 24.0 (SPSS Inc., Chicago). Charts were created by using OriginPro 8. Furthermore, the differences between data were compared by using Tukey's test, and values with $P < 0.05$ were regarded statistically significant.

Results

Figure 1 and Tables 1 and 2 show that the chemical constituents of *L. cubeba* oil were mainly citral and (z)-3,7-dimethylocta-2,6-diena (27.76% and 26.13%, respectively). The volatile constituents of *L. cubeba* oil were also mainly citral and (z)-3,7-dimethylocta-2,6-diena (22.43% and 29.34%, respectively). Other compounds, such as D-limonene, (+)-2-bornanone, and eucalyptol, accounted for more than 40% of all the volatiles detected from the essential oil.

Fumigating Toxicity Bioassay

Figure 2 shows that the *L. cubeba* oil and citral had good fumigating activity to microergate and macroergate. With the increase of concentration and time of essential oil and citral, the fumigating activity increased. The mortality of microergate was higher than that of macroergate. After 24 h

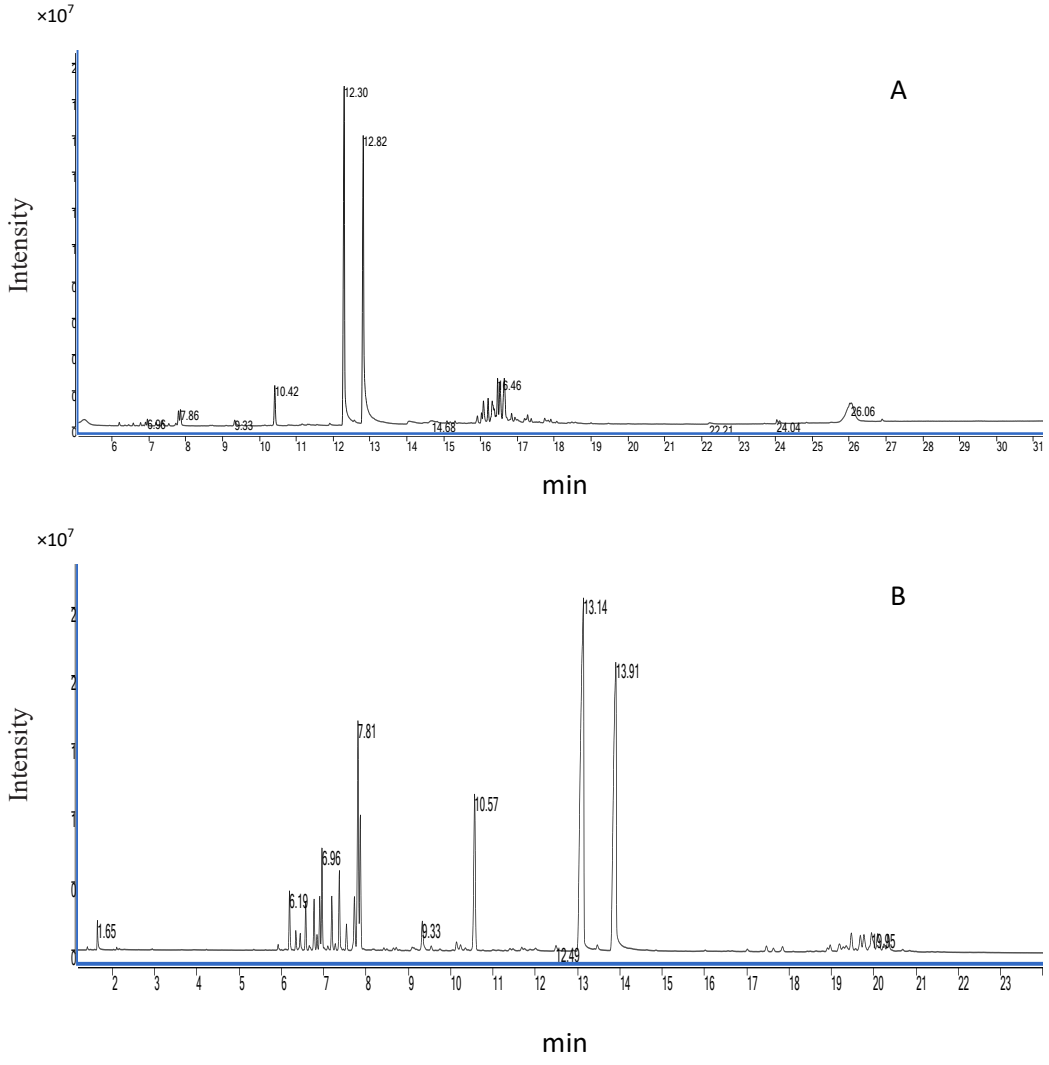

Fig 1. GC-MS total ion chromatograms of chemical compositions (A) and volatile components (B) of *L. cubeba* oil.

Table 1. Chemical compositions of *Litsea cubeba* oil.

Identified peaks	Retention time (min)	Formula	Compound	Relative content (%)
1	6.96	$C_{10}H_{16}$	(1R)-(+)-á-pinene	0.1734
2	7.86	$C_{10}H_{18}O$	Eucalyptol	1.0972
3	9.33	$C_{10}H_{18}O$	Linalool	0.5738
4	10.42	$C_{10}H_{16}O$	(+)-2-Bornanone	2.6873
5	12.30	$C_{10}H_{16}O$	(z)-3,7-dimethylocta-2,6-diena	26.1297
6	12.82	$C_{10}H_{16}O$	Citral	27.7618
7	14.68	$C_6H_{12}O$	3-Hydroxy-2,3-dimethyl-1-butene	0.6092
8	16.21	$C_{15}H_{24}$	(+)-b-Selinene	1.5495
9	16.46	$C_{15}H_{22}$	(+)-Cuparene	2.7297
10	22.21	$C_{16}H_{22}O_4$	Dibutyl phthalate	0.2840

of treatment, the mortalities of *L. cubeba* oil to macroergate were 40% and 60%, and the mortalities of microergate were 40% and 90%. The mortalities of citral to macroergate were 36% and 76%, and the mortalities of microergate were 36% and 96%. This finding indicated that *L. cubeba* oil and citral had strong fumigating activity to microergate ($F = 144.231$, $df = 5$, $p < 0.05$; $F = 243.559$, $df = 5$, $p < 0.05$). During the first 4 h of treatment, the mortality rate of workers was low.

Walking Ability Bioassay

Figure 3 shows that *L. cubeba* oil and citral have good inhibitory effects on the walking ability of microergate and macroergate. With the increase of concentration and time of essential oil and citral, the inhibitory effect increased. After treatment with 2.67 iL/mL and 5.33 iL/mL of *L. cubeba* oil for 24 h, the walking rate of macroergate decreased to 60% and

Table 2. Volatile components of *Litsea cubeba* oil.

Identified peaks	Retention time (min)	Formula	Compound	Relative content (%)
1	1.65	C_3H_6O	Acetone	0.6461
2	6.19	$C_{10}H_{16}$	(1S)-2,6,6-Trimethylbicyclo [3.1.1]hept-2-ene	1.5081
3	6.34	$C_{10}H_{18}$	R(-)3,7-Dimethyl-1,6-octadiene	0.4769
4	6.96	$C_{10}H_{18}$	Dihydropinene	2.6908
5	7.81	$C_{10}H_{16}$	D-Limonene	7.2376
6	9.33	$C_{10}H_{18}O$	Linalool	1.3316
7	10.57	$C_{10}H_{16}O$	(+)-2-Bornanone	6.4628
8	13.14	$C_{10}H_{16}O$	(z)-3,7-dimethylocta-2,6-diena	29.3397
9	13.91	$C_{10}H_{16}O$	Citral	22.4322
10	19.95	$C_{15}H_{22}$	(+)-Cuparene	1.4533

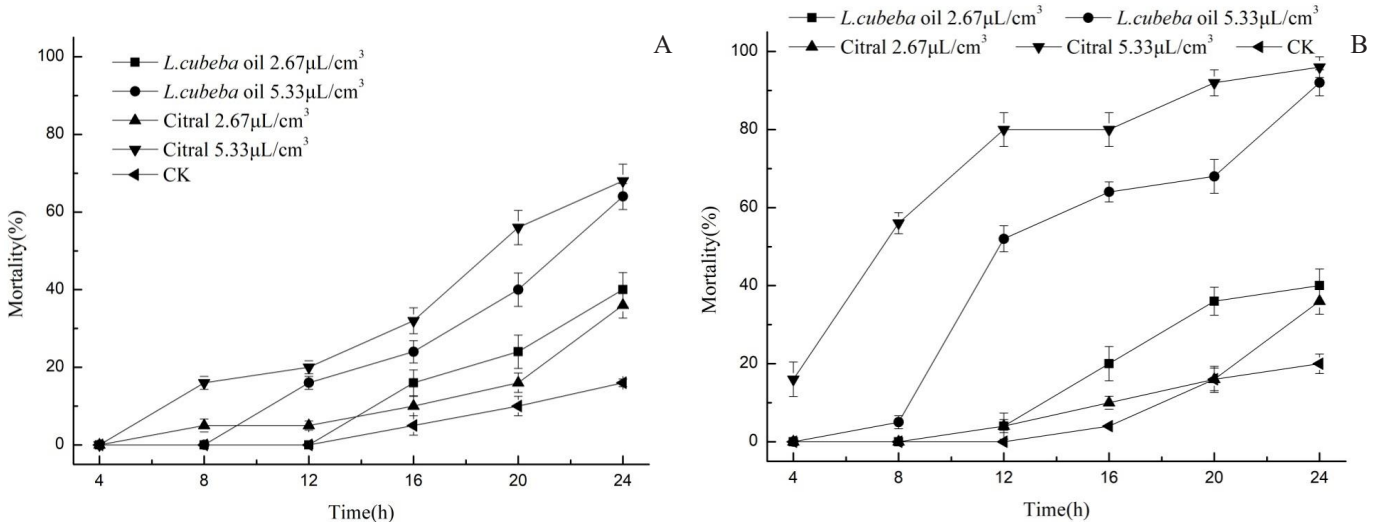

Fig 2. Mortality of workers (A: macroergate, B: microergate) after treating for 24 hours of *Litsea cubeba* oil and citral.

32% (F = 54.000, df = 5, p < 0.05), and that of microergate decreased to 32% and 16% (F = 48.000, df = 5, p < 0.05). After treatment with 2.67 ìL/mL and 5.33 ìL/mL of citral for 4–24 h, the walking rate of macroergate decreased to 44% and 16% (F = 27.000, df = 5, p < 0.05), and that of microergate decreased to 40% and 0% (F = 300.000, df = 5, p < 0.05). The walking rate of microergate was lower than that of macroergate.

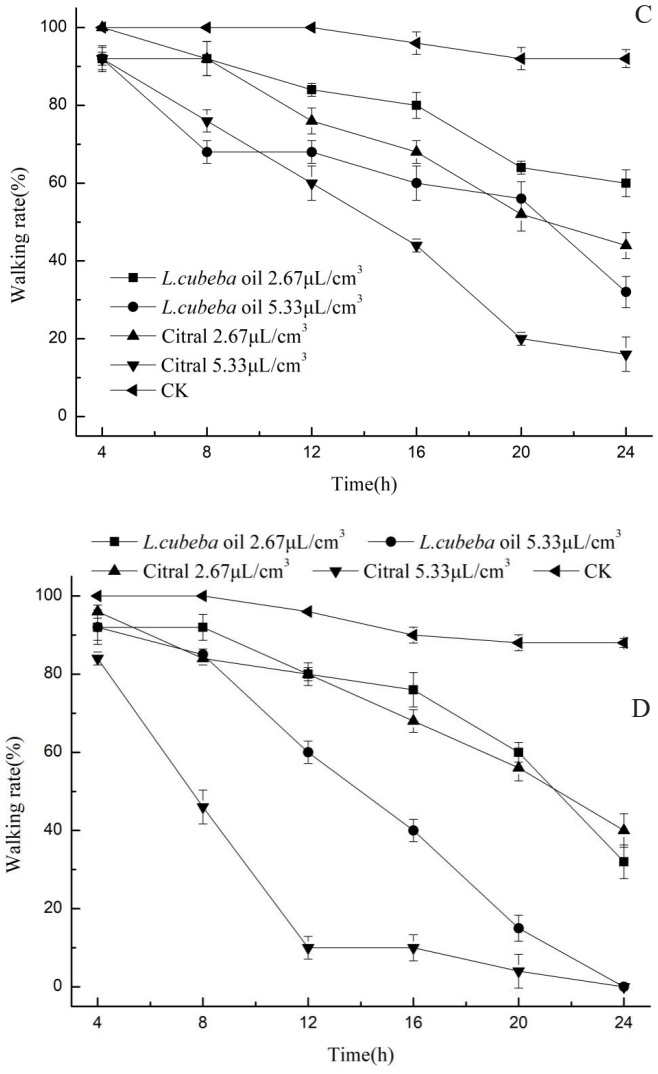

Fig 3. Effects on walking ability of workers (C: macroergate, D: microergate) after treating for 24 hours of *Litsea cubeba* oil and citral.

Grasping Ability Bioassay

Figure 4 shows that *L. cubeba* oil and citral have good inhibitory effect on the climbing ability of microergate and macroergate. After treatment with 5.33 ìL/mL of *L. cubeba* oil and citral for 24 h, the climbing rate of macroergate was 20% and 16%, respectively, and that of microergate was 0%. The climbing rate of microergate was lower than that of macroergate, indicating that the inhibitory effect of microergate was more significant. Moreover, with the increase of essential oil and citral concentration, the inhibitory effect increased (F = 75.000, df = 5, p < 0.05; F = 192.000, df = 5, p < 0.05).

Discussion

Our results showed that *L. cubeba* oil and citral have certain toxicity to RIFA and have significant inhibitory effect on the walking and climbing ability of the workers. High concentration of essential oil and pure products had obvious toxic effects on RIFA.

RIFAs live in complex environments, and a large number of workers may appear in fields, lawns, and roadside habitats (Tang et al., 2016). Their small size and good grasping and walking abilities enable them to adapt to various environments. The ability of workers to avoid natural enemies and resist harsh environment can be reduced by lowering the ability of workers to grasp and walk. Moreover, this process can significantly inhibit the escape and migration of ants, thereby seriously affecting their quality of life (Li et al., 2015). Our results showed that *L. cubeba* oil had strong inhibitory effect on the activity of workers and could effectively control RIFA.

In China, the natural resources of *L. cubeba* are very rich, and this plant has a wide range of applications. *L. cubeba* can be used as a medicine and flavoring agent and

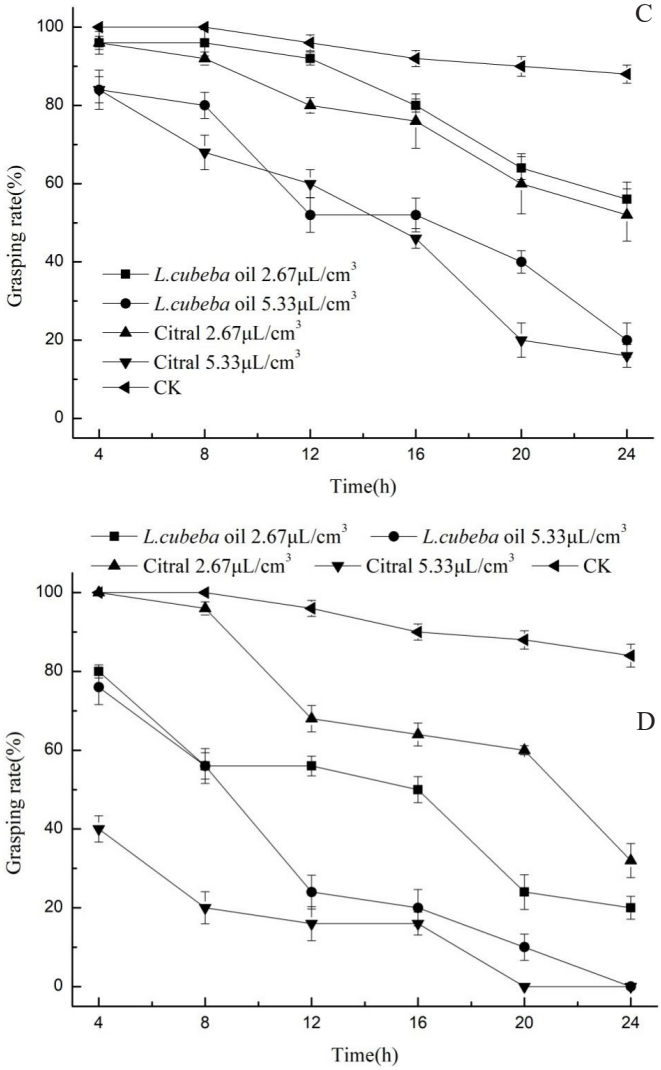

Fig 4. Effects on grasping ability of workers (C: macroergate, D: microergate) after treating for 24 hours of *Litsea cubeba* oil and citral.

preservative in food (Fang, 2007; Chen et al., 2013). Citral is the main component of *L. cubeba* oil. It can be extracted from essential oils, such as *L. cubeba* oil, lemon grass oil, and citrus oil (Liu et al., 2013). Citral can also be synthesized by appropriate methods to improve its volatility and stability (Zhong et al., 2015). Given the nature of *L. cubeba* oil and citral and their fumigating activity against RIFA, people can apply them to their bodies or spray them around the house where RIFAs invade, thereby achieving certain insecticidal and repellent effects.

In conclusion, *L. cubeba* oil and its main component, citral, have good feasibility and potential in the prevention and control of RIFA and are sources of natural insecticides for the control of RIFA.

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