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Fumigant Toxicity and Repellence Activity of Camphor Essential Oil from *Cinnamomum camphora* Siebold Against *Solenopsis invicta* Workers (Hymenoptera:Formicidae)

J. T. Fu,¹ L. Tang,¹ W. S. Li,¹ K. Wang,¹ D. M. Cheng,^{1,3} and Z. X. Zhang^{1,2,4}

¹Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agricultural University, Guangzhou 510642, China

²State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangzhou 510642, China

³Department of Plant Protection, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China

⁴Corresponding author, e-mail: zdsys@scau.edu.cn

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ABSTRACT. The red imported fire ant (RIFA) *Solenopsis invicta* Buren causes severe damage to humans and animals as well as the environment. Chemical treatment is the main strategy of RIFA management, which also is potentially toxic to the environment. Plant essential oils (EOs) are considered as potential substance that can be used to control insects. This study aimed to identify the chemical composition of camphor EO and investigate the insecticidal activity on RIFAs. The chemical composition of the EO was analyzed by gas chromatography/mass spectrometry and gas chromatography with flame ionization detection. Results revealed that 36.61% camphor and 30.05% cineole were the major components. The insecticidal activity of camphor EO was assessed against RIFA workers by conducting two different bioassays: fumigant toxicity and repellence. Fumigant toxicity assay results showed that the lethal dose (LC₅₀) of the EO at 24 h was 1.67 and 4.28 µg/ml for minor and major workers, respectively; knockdown time (KT₅₀) was 10.82 and 14.73 h. At 2.55 µg/ml, the highest average mortality of the ants was 84.89% after 72 h. Camphor EO exhibited fumigant toxicity against minor and major workers as indicated by the effects on attacking, feeding, and climbing behaviors. This EO was also strongly repellent to the two size workers of the colony as observed in their behavior against *Tenebrio molitor* treated with 5 µl EO. The fumigant toxicity and repellence of camphor EO against RIFA indicated that this substance could be a potential alternative for the development of eco-friendly products used to control pests.

Key Words: camphor essential oil, fumigant, repellent, *Solenopsis invicta*

Introduction

The red imported fire ant (RIFA) *Solenopsis invicta* is one of the most notoriously known agricultural insect pests in southern United States, Australia, Philippines, Taiwan, and China (Zhang et al. 2007). Numerous ant species exploit and colonize environments altered by human activity, and RIFAs are more aggressive than other ant species. In infested areas, RIFA colonies are commonly found in gardens, lawns, parks, school yards, golf courses, and roadsides (Brenda and Carl 2010). These ants reproduce and infest areas rapidly; for instance, they can be moved with grass sod or nursery stock; they can also be carried across long distances by soil-moving equipment.

RIFA colonies are destructive. These ants dominate their home ranges because of their large numbers and aggressiveness. They can even alter the composition of ecological communities in invaded areas, outcompeting and frequently eliminating the native fire ants (Brenda and Carl 2010). In cities, RIFA occasionally build colonies in electrical circuitry, causing shorted air conditioners; these ants may also inhabit telephone junction boxes, traffic and light control boxes, and transformers (Collins and Scheffrahn 2001). They even cause potholes in sidewalks or highways when a colony underneath these paths eventually dies out. In addition to structural damage, human health problems also arise, particularly from the stings of RIFAs. The presence of RIFA in crops or gardens threatens individuals because of stings, thereby impeding the manual harvesting of fruits and vegetables (Collins and Scheffrahn 2001). Fire ants sting repeatedly; with each sting, venom is injected into organism. RIFA venom contains high toxin concentrations that cause intense burning and itching; then a blister is formed and becomes a white pustule (Brenda and Carl 2010). Broken or scratched pustules can harbor secondary bacterial infections and may result in

permanent scarring. In worse cases, severe allergic reactions can occur, resulting in anaphylactic shock and even death (Dowell et al. 1997).

Current methods used to control RIFAs are extremely costly and inefficient (Drees and Gold 2003). The conventional methods, such as chemical treatment of nests and surrounding areas, may result in environmental contamination. Moreover, the use of non-RIFA-specific agricultural insecticides in some places, such as nurseries, truck stops, and parks, have been considered as high-risk sites of RIFA invasion because RIFAs can easily travel in soil and potted plants, as well as vehicles may pose safety risks.

In recent years, a series of studies have shown that essential oils (EOs) derived from plants have proven to be toxic to different pests (Dugassa et al. 2009, Nerio et al. 2010, Kumar et al. 2012). EOs of plants are formed by complex mixes of compounds structurally different which can act synergistically increasing its action potential and efficacy (Berenbaum 1985). The fragrant camphor tree, *Cinnamomum camphora* (L.) J. Presl (Lauraceae), for extraction of its camphor EO rich in camphor, occurs naturally in Asian countries (Chen et al. 2013). Camphor EO has great commercial value to perfumery and cosmetic industry. Camphor exhibits a number of biological properties such as insecticidal, antimicrobial, antiviral, anticoccidial, antinociceptive, anticancer, and antitussive activities, in addition to its use as a skin penetration enhancer (Chen et al. 2013). However, to date, very few studies evaluated the activity of the EO of camphor against RIFA. Our previous study found that the EO of camphor has strong fumigant toxicity against *S. invicta* (Tang et al. 2013). Chen (2009) also investigated the repellency of camphor oil product in China against workers of RIFAs.

In this study, we identified the chemical composition of camphor EO and assessed the fumigant toxicity and repellence activity of camphor EO against the RIFA.

Materials and Methods

Essential Oil. Camphor EO was purchased from Kang Shen Natural Medicinal Oil Refinery, Jiangxi, China, and was stored in plastic bottles at 4°C until further use.

Chemicals. Compounds camphor and cineole were purchased from Jing Chun Chemical Reagent Co., Ltd., Shanghai, China. HPLC grade acetone was purchased from Guangzhou Chemical Reagent Factory.

Worker Ant. *S. invicta* colonies were directly obtained from nests on the suburb of Zengcheng, Guangdong Province. Workers were reared in plastic containers (50 cm in diameter by 20 cm in height) under the natural environment conditions (temperature $25 \pm 2^\circ\text{C}$ and a relative humidity of 60–80%). Workers were allowed free access to a test tube (25 mm by 200 mm), which was used as a water source, partially filled with 10% honey water and plugged with cotton. A Petri dish (8.5 cm by 1.5 cm) containing *Tenebrio molitor* was placed in containers as the food source. *T. molitor* were purchased from the insect–fish market in Guangzhou, fed with wheat bran, and kept in a dry indoor environment at $25 \pm 2^\circ\text{C}$ until use.

Gas Chromatography-Mass Spectrometry Analysis. Chemical composition of camphor EO was determined by a gas chromatography/mass spectroscopic detector (GC–MS) Agilent 6890N GC interfaced with a Agilent 5957C mass spectrometer fitted with a capillary column (HP-5MS, 30 m by 0.25 mm in internal diameter, 0.25 μm in film thickness). Helium was used as the carrier gas (flow rate = 1.0 ml/min). The injector temperature was 25°C , and the detector (or interface) temperature was 300°C . The injection volume of samples was 1.0 μl ; the partition rate of the injected volume was 1:50. The temperature program was as follows: 50°C for 5.0 min, an increase of $10^\circ\text{C}/\text{min}$ to 220°C , and then held at 220°C for 5.0 min, with a total run time of 40.0 min. The mass spectrum was obtained at an ionization voltage of (EI) 70 eV, mass range 50–500 m/z and detector voltage 1.5 V. The chemical constituents were quantified by gas chromatography with flame ionization detection (GC-FID). The samples were then analyzed on same Agilent instrument fitted with the same column and following the same temperature program as above. The amount of each constituent was determined by area normalization (%) and the concentrations were calculated from the GC peak areas. Analytes profile was characterized from their mass spectral data using National Institute of Standards and Technology (NIST05) and Wiley8 mass spectrometry libraries.

Fumigant Activity Bioassays

Lethal Dose. Fumigation assays for LC_{50} and LC_{90} were conducted in a glass cylinder (1 dm^3) fitted with a screw cap and a cotton yarn (7 cm in length by 1.5 cm in width) was suspended from the center of the inner face of the cap. The vertical wall inside each glass cylinder was coated with fluon emulsion and died for 24 h to prevent the ants from escaping. Five concentrations (2.55, 5.10, 10.19, 15.29, and 25.48 $\mu\text{g}/\text{ml}$) of camphor EO and camphor cineole were applied separately to the yarn for the major workers (body length = 4.3–4.5 mm; head width = 1.0–1.1 mm) and minor workers (body length = 2.8–3.0 mm; head width = 0.6–0.7 mm). In the control glass cylinder, the cotton yarn was not applied with EO. Each group comprised 15 ants placed at the bottom and then put the lid on the glass cylinder. Each assay was replicated three times. The jars were tightly sealed and then kept in a room at $25 \pm 2^\circ\text{C}$ with relative humidity of 80%. The mortality of the workers was evaluated at 12, 24, 48, and 72 h after the bioassays were conducted to verify the increase in toxicity over time. Ants were considered dead when they remained immobile and did not respond to stimulation applied using a brush.

Knockdown Time. To evaluate the knockdown time (KT_{50}) of the camphor EO, we used LC_{90} for each group in accordance with previous fumigant bioassays. In brief, the ants were divided into two groups, the treatment group (with camphor EO) and the control group (no EO). We employed the same experimental design and procedures for the RIFA exposure to oil as the bioassays used to determine LCs; in this experiment, LC_{90} was used. The ants were monitored for 24 h, and those turned over with their abdomen exposed were considered knocked

down. The activity of camphor EO was determined by counting the knocked down ants after the bioassays were performed.

Determination of Attacking and Feeding Behaviors of RIFAs on *T. molitor*. A total of 50 ants for each size workers of the colony (major workers, minor workers, and medium-sized workers, body length = 3.5–3.7 mm; head width = 0.8–0.9 mm) were placed in a glass cylinder and fumigated for 30 min (Seo et al. 2009). The ants were starved for 2 d before the assays were conducted and 10.19 $\mu\text{g}/\text{ml}$ of camphor EO was applied to the cotton yarn. To observe the attack and feeding behaviors on *T. molitor*, we placed one *T. molitor* of the same size in a Petri dish; the treated ants were then placed into the Petri dish. Each treatment was replicated three times. Only the control assay was not fumigated. All of the bioassays were performed at $25 \pm 2^\circ\text{C}$ and relative humidity of $75 \pm 5\%$. The behaviors of RIFAs against *T. molitor* were observed at 1, 5, 10, 15, 20, 25, and 30 min; afterward, the rate of attack activity was calculated using the following equation:

$$A(\%) = A_n/T_n \times 100,$$

Where A (%) is the attack rate, A_n is the number of major and minor RIFAs attacking *T. molitor*, and T_n is the total number of RIFAs. The feeding activities of medium-sized workers were observed and recorded at 24 and 48 h; the rate of feeding activity was then calculated as the percentage reduction in feeding behavior emergence or inhibition rate. Percentage inhibition rate was calculated as follows:

$$F(\%) = (I_w - R_w)/I_w \times 100,$$

Where F is the feeding rate, I_w is the initial weight of *T. molitor*, and R_w is the remaining weight of *T. molitor*.

Determination of Climbing Behavior. To evaluate the climbing behavior of the fumigated ants, we selected and starved 50 medium-sized RIFA workers for 2 d. These ants were subsequently fumigated for 30 min in a glass cylinder. We employed the same experimental design, procedures of ant exposure to EO, and evaluations as previously described bioassays to determine the attack and feeding behaviors of RIFAs except the observation method. In this experiment, the ants were fumigated and then placed into a conical flask. Afterward, the conical flask was gently flipped with the mouth facing downward on a piece of paper for 3 s. The conical flask was then flipped gently back upward. The number of individuals present on the paper was recorded after 1, 30, 60, 90, 120, 150, and 180 min. The control ants were kept under the same conditions without fumigation. The rate of climbing activity was calculated using the following equation:

$$C(\%) = (T_n - F_n)/T_n \times 100,$$

Where C is the climbing rate, T_n is the total number of RIFA, and F_n is the number of RIFA that fell on the paper.

Repellence Bioassay. The repellence of camphor EO on the two size workers of the colony was evaluated. Repellence bioassays were conducted using 50 ants in the presence of one *T. molitor*. The bioassays were performed in a Petri dish (9 mm by 9 cm) and the inner vertical wall of each Petri dish was coated with Fluon emulsion. Approximately 5 μl of camphor EO was applied on the body of *T. molitor* by using a 10 μl microsyringe (Hamilton). These ants were starved for 2 d and placed in the same Petri dish with the treated *T. molitor*. This procedure was performed three times. Camphor EO was not applied on the control *T. molitor*. The repellence of camphor EO was determined using the same observation step of attack and feeding behaviors on *T. molitors* as previous bioassays. The results of the attack behavior of major and minor RIFAs were recorded at 1, 2, 3, 4, 5, 6, 7, and 8 h. The feeding activities of medium-sized workers were recorded after 24 and 48 h.

Statistical Analysis. Data obtained from each dose and time-response bioassay were subjected to regression analysis by probit to

generate LC_{50} , LC_{90} , and KT_{50} (Finney 1971, SPSS 2012). A one-way analysis of variance was performed to compare the effects of exposure period in the fumigant and repellence bioassay. Data were expressed as the average number \pm standard error and evaluated using Data Processing System statistics. Statistical analyses were performed using SPSS version 20.0 (SPSS 2012).

Results and Discussion

Chemical Composition of the EO. Camphor EO contains 18 compounds that account for 99.9% of the total oil compositions (Table 1). Camphor is the major component, accounting for 36.61% of the

compounds, followed by cineole (30.02%), α -terpineol (5.22%), and safrole (5.15%). Camphor is also a main EO component in many aromatic plant species (Hammer Schmidt et al. 1993, Kamdem and Gage 1995, Tirillini et al. 1996, Juteau et al. 2002, Viljoen et al. 2003). Other studies have found that cineole accounts for 90% of EOs in plant species with the generic product eucalyptus oil; this compound is also commonly used as an insecticide and insect repellent (Boland et al. 1991). Considering that the insecticidal efficacy of EO is possibly dependent on monoterpene, we may characterize this EO to determine the oil components responsible for the insecticidal activity (Kumar et al. 2012). The amount of the major composition of camphor EO was moderate in this study compared with that in previous studies.

Bioassay. Fumigant toxicity was dependent on time and oil concentration used in the experiment. The mortality of ants was significantly different among the tested concentrations ($F = 61.53$, $P < 0.0001$) and sizes ($F = 78.39$, $P < 0.0001$). However, the mortality was not significantly different between camphor EO and the major chemical compounds of EO, namely, camphor and cineole ($F = 1.42$, $P = 0.296$).

The mortality of the ants is presented in Fig. 1. There were no workers found dead in the control groups. In all treatment groups, a noticeable variation of ant mortality was observed as the exposure interval was prolonged. A significant difference ($F = 78.39$, $P < 0.0001$) in the mortalities between minor and major workers was found; in particular, minor workers were more susceptible to camphor EO at 2.55 $\mu\text{g}/\text{ml}$ concentration. An evident increase in mortality of workers was observed at different exposure times, in which the average mortality was 13.3–74.7% for the major workers and 20.0–100.0% for the minor workers. The highest average mortality percentage of the ants at 2.55 $\mu\text{g}/\text{ml}$ was on 84.89% after 72 h.

LC_{50} and LC_{90} of camphor EO and the major components (camphor and cineole) work on the major and minor RIFA workers are presented in Table 2. An increase in toxicity of these components was observed at all concentrations (2.55–25.48 $\mu\text{g}/\text{ml}$) in major and minor workers.

Table 1. Chemical composition of camphor EO

	Composition	RRT (min)	Percentage
1	(1R)-(+)- α -pinene	7.915	2.47
2	Camphene	8.272	1.17
3	Sabinene	8.898	1.92
4	β -Pinene	8.963	1.31
5	2-N-propylpyridine	9.282	1.14
6	Cineole	10.265	30.02
8	γ -terpinene	10.697	0.50
9	Camphor	12.55	36.61
10	DL-Isoborneol	12.755	1.32
11	Terpinen-4-ol	12.934	3.04
12	α -Terpineol	13.182	5.22
13	Safrole	14.646	5.15
14	α -Santalene	16.391	2.73
15	α -Bergamotene	16.558	0.89
16	2-methyl-3-methylene -2-(4-methylpent -3-enyl)norbornane	16.899	1.37
17	Cubebene	17.212	0.44
18	Cis-nerolidol	18.146	4.69

RRT, relative retention time.

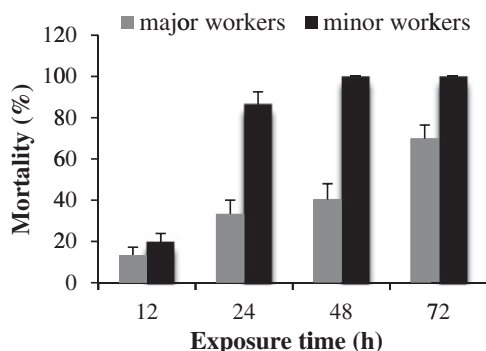


Fig. 1. Mortality (%) of minor and major workers of RIFAs caused by the camphor EO at 2.55 $\mu\text{g}/\text{ml}$ in the fumigation bioassay.

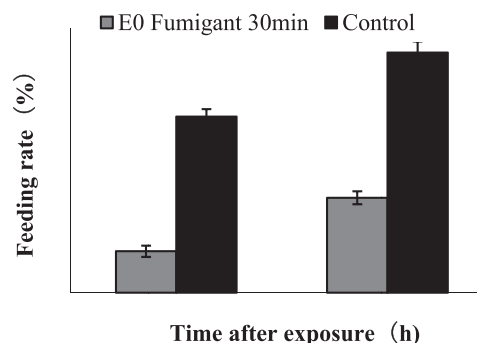


Fig. 2. Feeding rate of medium-sized workers of RIFAs 30 min after fumigation treatment with camphor EO at 10.19 $\mu\text{g}/\text{ml}$ on *T. molitor*.

Table 2. LC_{50} and LC_{90} of camphor EO, cineole, and camphor for minor and major RIF workers after 24 h fumigating exposure^a

Chemical constituents	N^b	Species	LC_{50} (95% CI) ^c	LC_{90} (95% CI) ^c	β^d	χ^2	P
Camphor EO	270	Minor	1.67(1.32–2.90)	2.73(2.36–3.91)	6.023	0.184	0.980
	270	Major	4.28(3.94–4.63)	7.29(6.55–8.39)	5.534	4.516	0.211
Cineole	270	Minor	2.34(2.05–2.54)	3.53(3.18–4.38)	7.194	0.776	0.855
	270	Major	5.99(5.50–6.70)	7.70(6.83–9.70)	11.744	0.466	0.792
Camphor	270	Minor	1.91(0.87–2.22)	2.97(2.69–4.22)	6.708	0.217	0.975
	270	Major	5.59(4.27–7.08)	11.06(8.52–17.83)	4.331	4.930	0.186

^a LC_{50} and LC_{90} were not determined for the species due to the low toxicity (mortality of $<30\%$) observed after 24 h of exposure.

^b Number of insects.

^c LC: lethal concentration in μg of EO/ml.

^d Slope of the curve.

After 24 h, the following values were, respectively, obtained for minor and major workers: LC_{50} of camphor EO, 1.67 and 4.28 $\mu\text{g}/\text{ml}$; KT_{50} , 10.82 and 14.73 h; and LC_{90} of EO, 2.73 and 7.29 $\mu\text{g}/\text{ml}$. After camphor EO was applied, the minor workers displayed signs of intoxication.

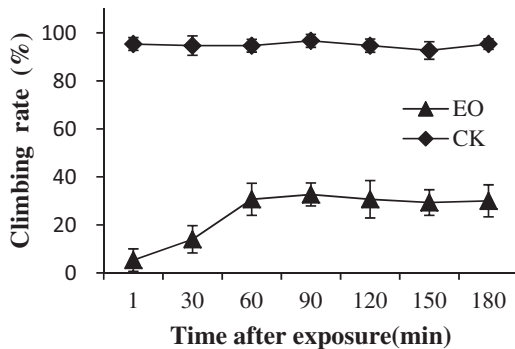


Fig. 3. Climbing rate of medium-sized workers of RIFAs 30 min after fumigation treatment with camphor EO at 10.19 $\mu\text{g}/\text{ml}$ tube (Phelan et al.).

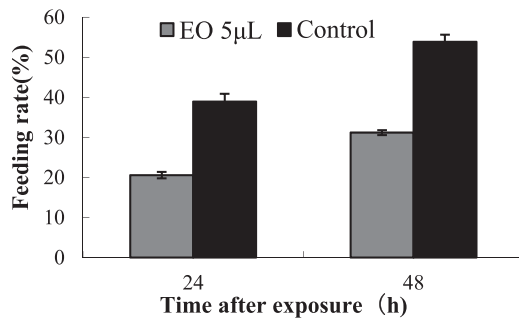


Fig. 4. Feeding rate of medium-sized workers of RIFAs on *T. molitor* treated with camphor EO at 5 $\mu\text{L}/T. molitor$.

Cineole (36.61%; LC_{50} of 2.34 and 5.99 $\mu\text{g}/\text{ml}$) and Camphor (30.02%; LC_{50} of 1.91 and 5.59 $\mu\text{g}/\text{ml}$) were also toxic to minor and major RIFA workers. Camphor EO exhibits a stronger toxicity than natural camphor possibly because of the presence of cineole, which contributes to camphor toxicity. Camphor EO displayed fumigant toxicity against the two sizes of RIFA workers. This result was observed in a greater effect on attack, feeding, and climbing behaviors of the fumigated ants than of the control ants (Figs. 2, 3, and 5). This result also indicated that camphor EO could reduce the attack and feeding rate of RIFAs compared with the control ants after fumigation. In addition, camphor EO elicited a higher inhibitory effect on the climbing ability of RIFAs, this ability of RIFAs to climb plants, soil, stones, and nest walls is crucial for survival. From Figs. 2 and 4, it is also evident that the feeding rate of ants in the control group was <60% after 48 h. This could be attributed to the amount of food they consume every day. We determined and compared the toxicity of camphor EO obtained in this study with that in previous studies (Phelan 1976, Rabl et al. 1997, Love et al. 2003). Although the overall knowledge on the toxicity of camphor EO to RIFAs is poor, several published studies show that it exhibits toxic effects against stored-product insects. For instance, contact toxicity, grain treatment, and repellence assays have been conducted to investigate the toxicity of camphor EO and its major component (camphor) to four beetles, namely, *Sitophilus granarius*, *Sitophilus zeamais*, *Tribolium castaneum*, and *Prostephanus truncatus*, after 24 h of exposure (Chen et al. 2013). Another report has indicated that pure camphor compound shows contact and fumigant activity against *Sitophilus oryzae* and *Rhizopertha dominica* but does not affect *Tr. castaneum* after 24 h of exposure at 0.1 $\mu\text{l}/720$ ml (Rozman et al. 2006). It was demonstrated that the insecticidal activity of camphor EO against *R. dominica* and *Si. zeamais* is attributed to camphor and the combined effects of different components, but camphor alone does not affect rice weevil (*Si. oryzae*) at an $LC_{50} > 100$ $\mu\text{l}/\text{liter}$ (Bekele and Hassanali 2001).

The repellence of camphor EO to RIFAs was presented in Figs. 4 and 6. No significant differences were observed in the percentage of individuals in the Petri dish treatment in terms of exposure time ($F = 11.34$; $P = 0.002$) and members of RIFA colonies ($F = 11.12$;

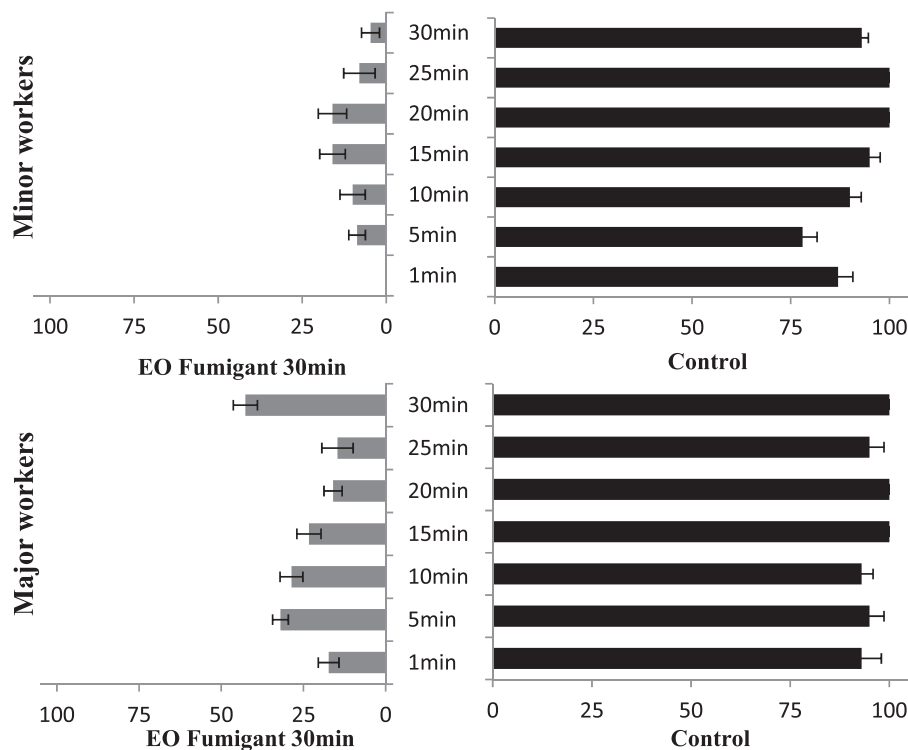


Fig. 5. Attack rate of minor and major workers of RIFAs fumigation treatment 30 min with camphor EO at 10.19 $\mu\text{g}/\text{ml}$ along the time on *T. molitor*.

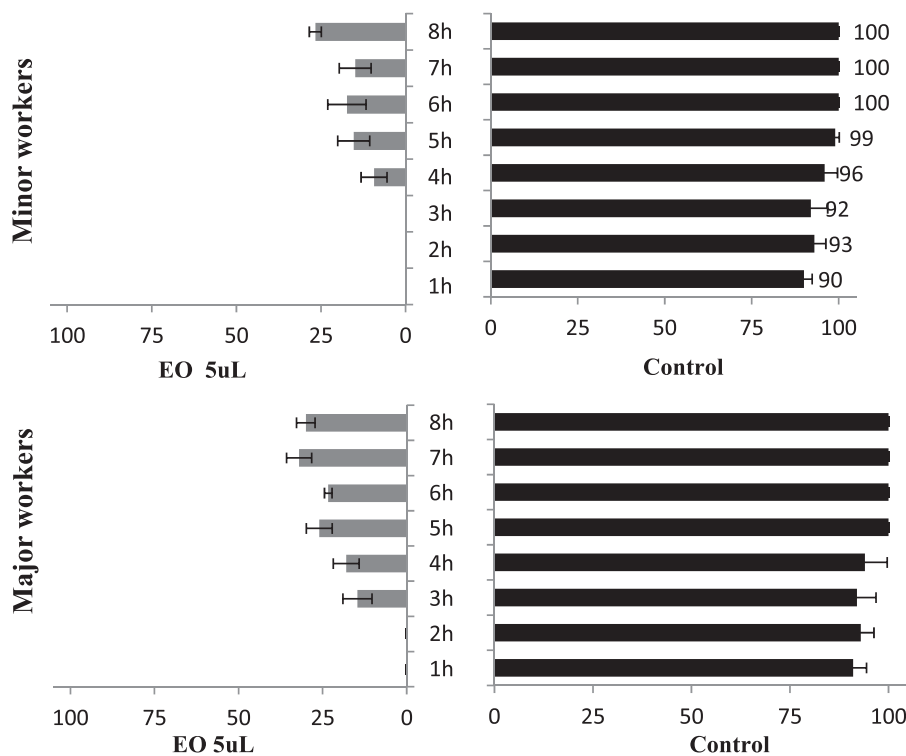


Fig. 6. Repellence of the camphor EO to minor and major workers of RIFAs along the time on *T. molitor* treated with EO at 5 µl/*T. molitor*.

$P = 0.012$). Camphor EO elicited high repellence for minor and major workers. Approximately 70% of the attack rate of major and minor RIFAs was also reduced. This repellence has also been observed in other insects. As a major component of the EO of aromatic plants, camphor exhibits repellence against *Anopheles culicifacies*, *Culex quinquefasciatus*, *Anopheles. gambiae*, and *Anopheles funestus* (Ansari and Razdan 1995, Seyoum et al. 2002, 2003). As RIFAs can easily travel in potted plants and vehicles, as well as other places such as nurseries, parks, and truck stops, insect repellent activities of natural substances could be considered as a practical alternative for the control of RIFAs.

Conclusions

The results obtained in this study demonstrated that camphor EO was biologically effective against RIFAs. Fumigation and repellence assay results also showed significant detrimental effects on the ants. Positive results indicated that camphor EO could be potentially used to develop eco-friendly RIFA control products, in which fumigation and repellence application are employed as delivery methods. As an economically viable product, camphor EO is a good alternative for harmful chemical insecticides used to control RIFAs.

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