Repellency of Two Essential Oils to Monomorium pharaonis (Hymenoptera: Formicidae)

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Repellency of two essential oils to *Monomorium pharaonis* (Hymenoptera: Formicidae)

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Abstract

The pharaoh ant (*Monomorium pharaonis* [L.]; Hymenoptera: Formicidae) is one of the most important pests in populated areas around the world where food and water are abundant. In this study, the repellency of the essential oils from *Curcuma longa* L. (Zingiberaceae) (turmeric) and *Litsea cubeba* (Lour.) Pers. (Lauraceae) (litsea) to the pharaoh ant was evaluated. For the tests, the area choice method was used, and 0.1 mL of essential oil at 1 of 3 concentrations (10,000 ppm, 1,000 ppm, and 100 ppm) was applied on a half disc of filter paper to obtain a final concentration of 31.4 µg/cm^2, 3.1 µg/cm^2, and 0.3 µg/cm^2. The same volume of mixture but without essential oil (distilled water + dimethyl sulfoxide + Tween-20®) was used as a control. The repellency of the oils was tested with and without availability of food to the ants, with the food consisting of small maize kernels. Both oils repelled ants with and without the availability of food at a concentration of 10,000 ppm. Turmeric oil showed a higher repellency than litsea oil in both the absence and presence of food. Furthermore, with food present, the repellency of turmeric oil increased whereas that of litsea oil decreased. The minimum percentages of repellency recorded for turmeric and litsea oils were >80% and >70%, respectively. This study demonstrated that the oils from these plants have a strong repellent effect on pharaoh ants in laboratory tests. Further studies are needed to determine the concentrations of these oils that can be used effectively for ant control in buildings and in open fields.

Key Words: pharaoh ant; *Curcuma longa*; *Litsea cubeba*; repellent effect

Resumen

La hormiga faraón (*Monomorium pharaonis* (L.); Hymenoptera: Formicidae) es una de las plagas más importantes en las zonas pobladas de todo el mundo donde la comida y el agua son abundantes. En este estudio, se evaluó la repelencia de los aceites esenciales de *Curcuma longa* L. (Zingiberaceae) (cúrcuma) y *Litsea cubeba* (Lour.) Pers. (Lauraceae) a la hormiga faraón. Para las pruebas, se utilizó el método de elección de zona, y se aplicó 0,1 ml de aceite esencial a 1 de 3 concentraciones (10.000 ppm, 1.000 ppm y 100 ppm) sobre la mitad de un disco de papel de filtro para obtener una concentración final de 31,4 mg/cm^2, 3,1 g/ cm^2 y 0,3 g/ cm^2. Se utilizó el mismo volumen de mezcla pero sin aceite esencial (agua destilada + sulfóxido de dimetilo + Tween-20®) como control. Se probó la repelencia de los aceites con y sin disponibilidad de alimentos para las hormigas, con la comida que consiste de granos pequeños de maíz. Ambos aceites repelaron las hormigas con y sin la disponibilidad de alimentos a una concentración de 10.000 ppm. El aceite de cúrcuma mostró una repelencia al aceite más alto que el *Litsea* tanto en la ausencia y presencia de alimentos. Por otra parte, con el alimento presente, la repelencia del aceite de cúrcuma aumenta mientras que la del aceite de *Litsea* disminuyó. Los porcentajes mínimos de repelencia registrados para los aceites de cúrcuma y *Litsea* fueron >80% y >70%, respectivamente. Este estudio demostró que los aceites de estas plantas tienen un fuerte efecto repelente de hormigas faraón en pruebas de laboratorio. Se necesitan más estudios para determinar las concentraciones de estos aceites que se pueden utilizar con eficacia para el control de hormigas en los edificios y en campos abiertos.

Palabras Clave: hormiga faraón; *Curcuma longa*; *Litsea cubeba*; efecto repelente

Monomorium pharaonis (L.) (Hymenoptera: Formicidae) is commonly known as the pharaoh ant, which is a small (2 mm), yellow or light brown insect (Bosik 1997). Ants are the most common indoor pest in urban areas; overall, ants have adapted to the artificial environments created by humans, and are now thriving in most of the world's big cities. As a result, they are the most abundant of all social insects (Hansen 2011). In the northern hemisphere, they are commonly found in warm, humid conditions and are considered pests, potential pathogen vectors (Edwards & Baker 1981), and a source of indoor aeroallergens (Kim et al. 2006).

When ants find food, they secrete a trail pheromone as they return to the nest. This pheromone leads the followers directly from the nest to the food source. When all the food is consumed, the workers no longer secrete the trail pheromone, and eventually the trail fades away (Bailey 1999). When ants infest structures, they will eat any kind of substrate, ranging from food items to dead insects. In addition, they can cause structural damage in homes when infesting building materials (Dobesh et al 1993). Ants are also regarded a nuisance to humans, given that some species can sting or bite, and they disturb human routines with their food-searching habits. As of 2011, 14,000 ant species

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have been described, as compared with 8,800 species in 1990. Taking into account the undescribed ant species in the world, the actual number of ant species is possibly more than 30,000 (Hansen 2011). Overall, anti-ant sprays can affect only a small percentage of an ant colony. In a mature pharaoh ant colony, only 0.7 to 5.6% of the ants forage (Vail 1996). Pharaoh ants are also classified as agricultural pests, causing damage to crops either directly or by acting as a vector of noxious pests (Pest Notes 2012).

Using plant essential oils as biopesticides can be very efficient in controlling pests. They can function as fumigants utilizing their topical toxicity, or as antifeedants utilizing their repellent properties. Furthermore, botanical oils can be toxic and repellent to adult insects and can lead to an inhibition of reproduction. This wide range of applications means that botanical oils are considered for both household and industrial uses, and essential oils are presently regarded as eco-friendly tools for pest control (Regnault-Roger 1997). For example, an experiment in which red imported fire ant mounds were drenched with citrus oil formulations containing d-limonene showed that this treatment was as effective as a conventional insecticide (Vogt et al. 2002). *Litsea* species (Lauraceae) have traditionally been used for medicinal purposes and as insect repellents (De Boer et al. 2010; Budin et al. 2012). Crushed leaves, flowers, fruits, and bark smell and show a potential repellency to several insect species (Santiwitchaya 2004; Trongtokit et al. 2005; Tawatsin et al. 2006).

Because many plant extracts are not harmful to non-target organisms (including humans), plant-based repellents could be more environmentally friendly than synthetic products (Bhat & Kempraj 2009). To our knowledge, there are no studies to date on the use of biopesticides against pharaoh ants. Therefore, we conducted this study to determine the repellency of two plant essential oils to adult pharaoh ants and to identify the essential oil components. The results of this study should aid in the development of eco-friendly pest management.

Materials and Methods

INSECT REARING

The pharaoh ant colony was maintained in the laboratory of Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory at the Huazhong Agricultural University, China (30.5931°N, 114.3054°E). Pharaoh ants were first reared on maize seeds and seedlings; adult ants were fed aphid honeydew provided by aphid colonies from maize crops. The maize was grown in the laboratory in 30 × 25 × 12 cm pots at 25 ± 2 °C, 50 ± 5% RH, and a 14:10 h L:D photoperiod.

BIOPESTICIDE FORMULATIONS

Samples were extracted according to methods of Su et al. (2009) and Yao et al. (2011). The plant materials, i.e., rhizomes of *Curcuma longa* L. (Zingiberaceae) (turmeric) and fully ripened fruit of *Litsea cubeba* (Lour.) Pers. (Lauraceae) (litsea), for producing the botanical oils were purchased from a franchised outlet of Beijing Tongrentang Group, China. They were dried in the sun and stored at 25 °C for a maximum of 6 mo after harvest. Before extraction, plant materials were dried in an oven at 45 °C for up to 3 d. The fully dried materials were crushed and sieved though a size 40 mesh. Samples were then added to 5 times their weight 95% ethanol (1 g powder added to 5 mL ethanol), kept in the dark at 20 to 25 °C for 7 d, and shaken twice a day. The solvent was then filtered through a Buchner funnel, the filtrate was collected and preserved at room temperature, and the residue was re-extracted in 95% ethanol (2.5 mL) and kept in the dark and processed as described for the first step. Filtrates from the first and second extraction were combined and concentrated in a rotary evaporator until no more water droplets were observed. The extracted crude oils were then weighed and stored in brown collection bottles at 4 °C in a refrigerator.

REPELLENCY TEST

Crude oil dissolutions and repellency tests were performed according to Su et al. (2009), Yao et al. (2011), and Caballero-Gallardo et al. (2014) with some modifications. The repellency of the extracts was determined with area choice tests in Petri dishes. To prepare the biopesticide, 0.05 g of the crude oil was dissolved in 0.3 mL dimethyl sulfoxide (DMSO), 1% solution of Tween-20® was added, and the volume was subsequently brought to 5 mL with distilled water for a biopesticide concentration of 10,000 ppm; of this stock solution, 0.1 mL was mixed with 0.9 and 9.9 mL distilled water to obtain 1,000 ppm and 100 ppm, respectively. Using a micropipette, 0.1 mL of the prepared biopesticide was uniformly applied on half a filter paper disc (9 cm diameter), in order to get a final concentration of 31.5 μg/cm^2, 3.1 μg/cm^2, and 0.3 μg/cm^2. The same volume of mixture without essential oil (distilled water + DMSO + Tween-20®) was applied to another half disc to serve as a control. To test for repellent effects of DMSO, the same amount of DMSO was used on a half disc as treatment and an untreated half disc served as negative control. When the liquids were fully dried, 2 half discs (1 treatment, 1 control) were placed into 9 cm diameter Petri dishes. Six 1 mm holes were made in the Petri dish cover to allow extra fumes to exit.

Twenty pharaoh ants were placed in the center of each Petri dish. This was replicated 3 times, and each assay was repeated 2 times. Petri dishes were kept in the same environment as described for the rearing conditions, and covered with black plastic to provide darkness. Total numbers of ants were counted on both areas of filter paper (treatment and control) per Petri dish after 1, 2, 3, and 4 h of ant release. The test was repeated using fresh insects and placing food (small maize kernels) in the centers of both filter paper half discs. The percentage of repellency (PR) was calculated using the equation: $PR(\%) = [(C-T) / (C+T)] \times 100$ where C was the number of ants present on the control half disc, and T was the number of ants present on the treated half disc (Liu et al. 2013). To classify the botanical oils in repellency classes from 0 to V (Jilani & Su 1983; Liu et al. 2011), with 0 = no repellent effect and V = very strong repellent effect, the mean PR values were used (Fig. 1).

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

The essential oil components were separated and identified by gas chromatography–mass spectrometry (GC-MS) on a Varian 450-GC/320-MS (Varian, Inc., Walnut Creek, California) according to Caballero-Gallardo et al. (2014). The same column and analysis conditions were used

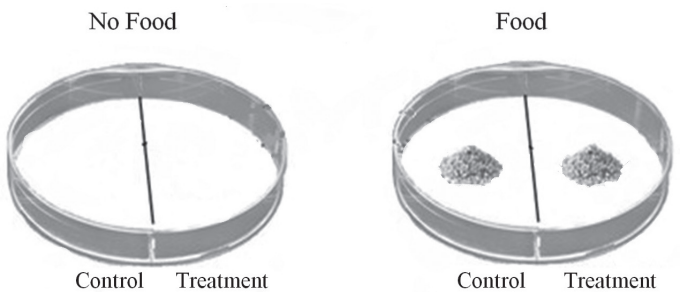

Fig. 1. Experimental setup of the area choice test, with two filter paper half discs fitted in the bottom of a Petri dish. In the "with food" test, food was placed centrally on each half disc.

for both the GC and MS. The spectrophotometer was equipped with a flame ionization detector and a HP-5ms capillary column (30 m × 0.25 mm × 0.25 µm). For the GC, initial oven temperature was held at 60 °C for 3 min, increased at 10 °C/min to 180 °C for 1 min, and then increased at 20 °C/min to 280 °C for 15 min. The injector temperature was maintained at 270 °C. The samples (1 µL, diluted to 1% with hexane) were injected with a split ratio of 1:10, and column pressure was 100 kPa. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The electron ionization source of the detector had an electron energy of 70 eV. Spectra were scanned from 35 to 1,200 m/z at 2 scans per second. MS quad temperature was 150 °C, ion source temperature was 230 °C, the transmission line temperature was 250 °C, and column pressure was 100 kPa. Most constituents were identified from the gas chromatography using MANLIB, REPLIB, PMWTox3N, and Wiley (NIST 2011). The retention indices were determined in relation to a homologous series of n-alkanes (C8–C24) under the same operating conditions.

DATA ANALYSES

Paired *t*-tests were used to compare the mean numbers of ants on the "treated" and "control" areas (filter paper half discs). Mean PR comparisons between "without food" and "with food" for the 4 observation times and between the botanical oils were done with 1-way ANOVA and Tukey's HSD tests. Statistical analyses were performed with SPSS version 20, at a significance level of $P \leq 0.05$.

Results

The two botanical oils tested here showed high repellency at a concentration of 10,000 ppm (Fig. 2). During all hours of observations, without and with food provision, the minimum PR was >80% for turmeric and >70% for litsea (Fig. 3). However, at concentrations of 1,000 and 100 ppm, both essential oils showed no repellent activity to pharaoh ants (Figs. 4 and 5, respectively). DMSO had no repellent effect on pharaoh ants. Paired *t*-tests showed no significant differences in the numbers of ants between the DMSO treatment and the negative control at any of the observation times (1, 2, 3, and 4 h) in both the test without (t = 0.41, 2.74, 2.74, and 1.58, respectively; df = 5; $P > 0.05$) and the test with food (t = 2.24, 1.58, 1.00, and 0.54, respectively; df = 5; $P > 0.05$) (Fig. 6).

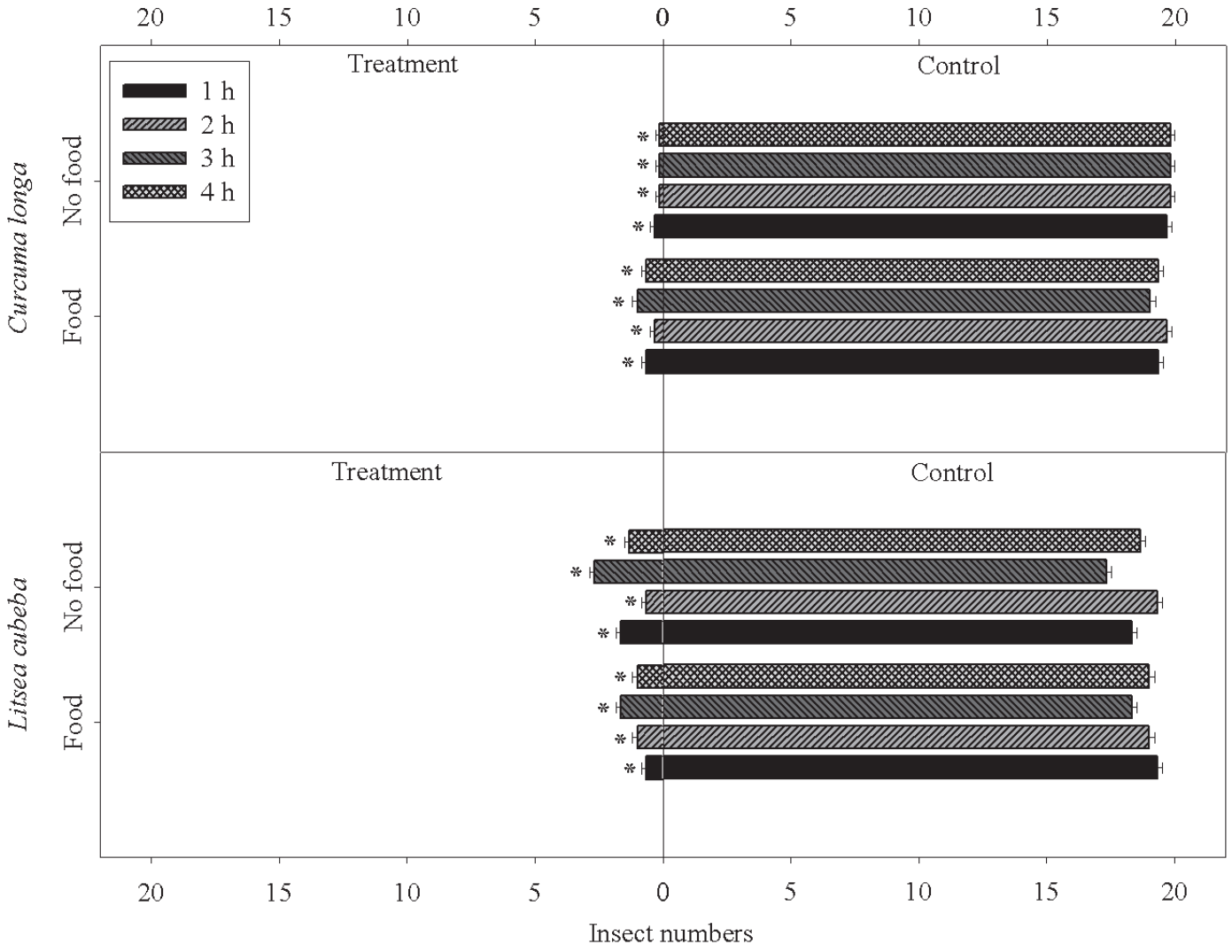

Fig. 2. Mean numbers of ants present on treatment and control filter papers in tests with essential oils at a concentration of 10,000 ppm in the absence or presence of food at different hours of observation. Values are means of 6 replications, and bars indicate the standard error. The mean numbers of ants were compared by paired *t*-tests at a significance level of $P \leq 0.05$. An asterisk indicates a significant difference between treatment and control.

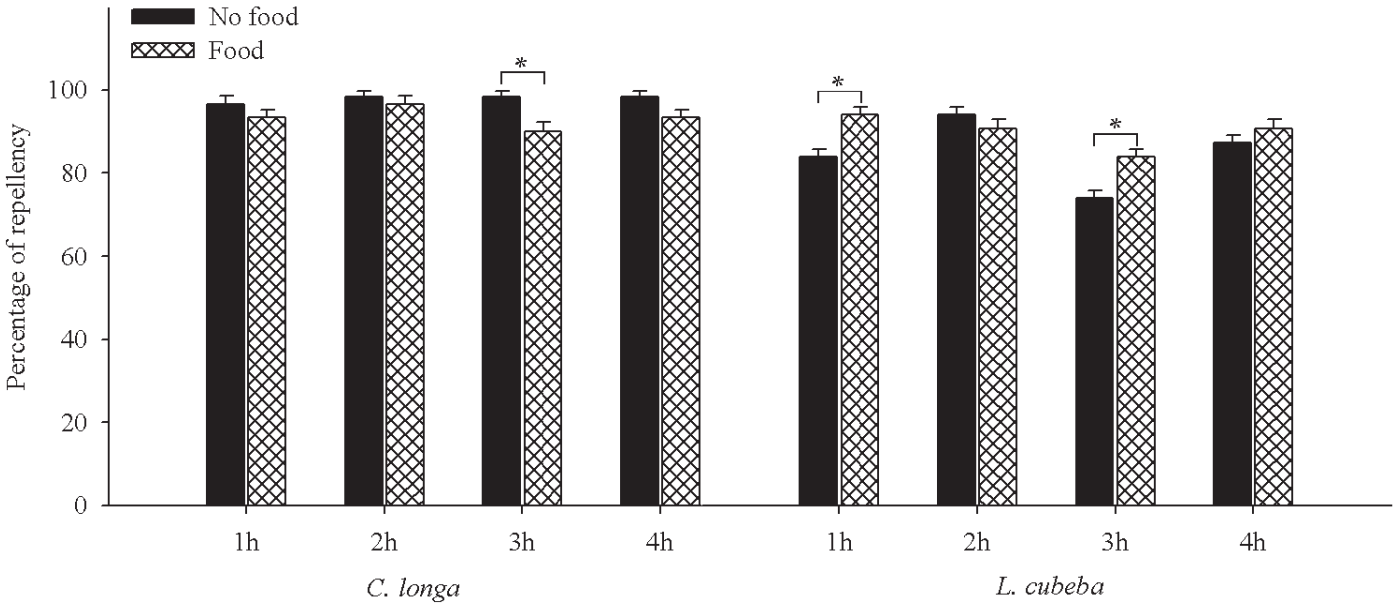

Fig. 3. Percentage of repellency (PR) of *Curcuma longa* and *Litsea cubeba* against *Monomorium pharaonis* in the absence or presence of food at different hours of observation. Values are means of 6 replications, and bars indicate the standard error. The PR values were analyzed by 1-way ANOVA and Tukey's HSD test at a significance level of $P \leq 0.05$. An asterisk indicates a significant difference between absence and presence of food.

CURCUMA LONGA (TURMERIC)

The repellency of turmeric oil at 10,000 ppm to pharaoh ants was highly significant ($P < 0.01$) for all the observations in both tests (without and with food). Numbers of ants on the control filter papers were greater than numbers of ants on the treatment filter papers after 1, 2, 3, and 4 h of exposure when no food was provided (t = 45.85, 59.00, 59.00, and 59.00, respectively; df = 5; $P < 0.01$). These differences were also found when food was present on each filter paper (t = 44.27, 45.85, 34.85, and 44.27, respectively; df = 5; $P < 0.01$) (Fig. 2).

During all observations, the PR was slightly higher in the absence of food than in the presence of food. This difference was statistically significant after 3 h of exposure (F = 7.353, df = 2, $P < 0.03$). For all the recording times, the PR was maintained at class V (Fig. 3).

LITSEA CUBEBA (LITSEA)

Essential oil from *L. cubeba* at 10,000 ppm showed good potential for repelling *M. pharaonis*. The numbers of pharaoh ants were significantly greater on the control filter papers than on the treatment filter papers after 1, 2, 3, and 4 h of exposure in the absence of food (t = 39.53, 44.72, 34.78, and 41.11, respectively; df = 5; $P < 0.01$). These differences occurred also in the presence of food (t = 44.27, 34.85, 39.53, and 34.86, respectively; df = 5; $P < 0.01$) (Fig. 2).

The PR varied considerably among the recording hours when no food was added to the Petri dishes (F = 11.25, 1.00, 11.25, and 1.00; df = 1). When food was present, the PR was both higher and more stable than when no food was offered to the ants (Fig. 3). The PR after 3 h of exposure was the lowest, both when food was absent and when it was present. Significant differences between the PRs in the presence and absence of food were observed after 1 and 3 h of exposure (F = 11.250, df = 1, $P < 0.01$). For all the recording times, with and without food provision, the PR was maintained at class IV or V (Fig. 3).

COMPARISON BETWEEN *CURCUMA LONGA* AND *LITSEA CUBEBA*

In general, the RP of *L. cubeba* oil during the 4 h of observation fluctuated more in the absence than in the presence of food, whereas for *C. longa* oil, the RP practically remained stable in both tests. In a comparison of both oils, we found that the repellent activity of *L. cubeba* was significantly lower than that of *C. longa* at all observation times in the test without food (F = 20.00, 3.46, 86.53, and 18.85 at 1, 2, 3, and 4 h, respectively; df = 1; P £ 0.04). In the presence of food, no statistical difference was observed between the activity of both essential oils (F = 0.00, 4.00, 4.00, and 1.00; df = 1; $P > 0.05$) (Fig. 7).

CHEMICAL COMPONENTS OF *CURCUMA LONGA* AND *LITSEA CUBEBA*

The GC-MS analysis of the essential oils showed complex mixtures of constituents. Twelve major components were identified in the essential oils of the two aromatic plant species (6 per species). The primary chemicals identified from *C. longa* and *L. cubeba* oils are presented in Table 1.

Discussion

This is the first study to show repellency of *C. longa* and *L. cubeba* oils to pharaoh ants both with and without the provision of food. Essential oil from *C. longa* showed a strong repellency to pharaoh ants for up to 4 h of observation in the absence and presence of food. Chahal et al. (2005) showed repellent activities of major constituents of turmeric root powder oil (turmerone and ar-turmerone) to the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Obtaining oils from turmeric leaves and unutilized parts of the turmeric plant is very useful for the production of biopesticides to control pests (Walia 2005). Their fumigants can induce topical toxicity, or have antifeedant or repellent effects on the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Govindaraddi 2005). Jilani et al. (1988) conducted a repellency test with *T. castaneum* in a food preference chamber, where 100, 500, or 1,000 ppm of turmeric oil were applied on rice grains. Their results showed an increase in repellency with increasing concentrations of the oil. In the present study, we observed a lower repellency of turmeric oil when food was provided than when

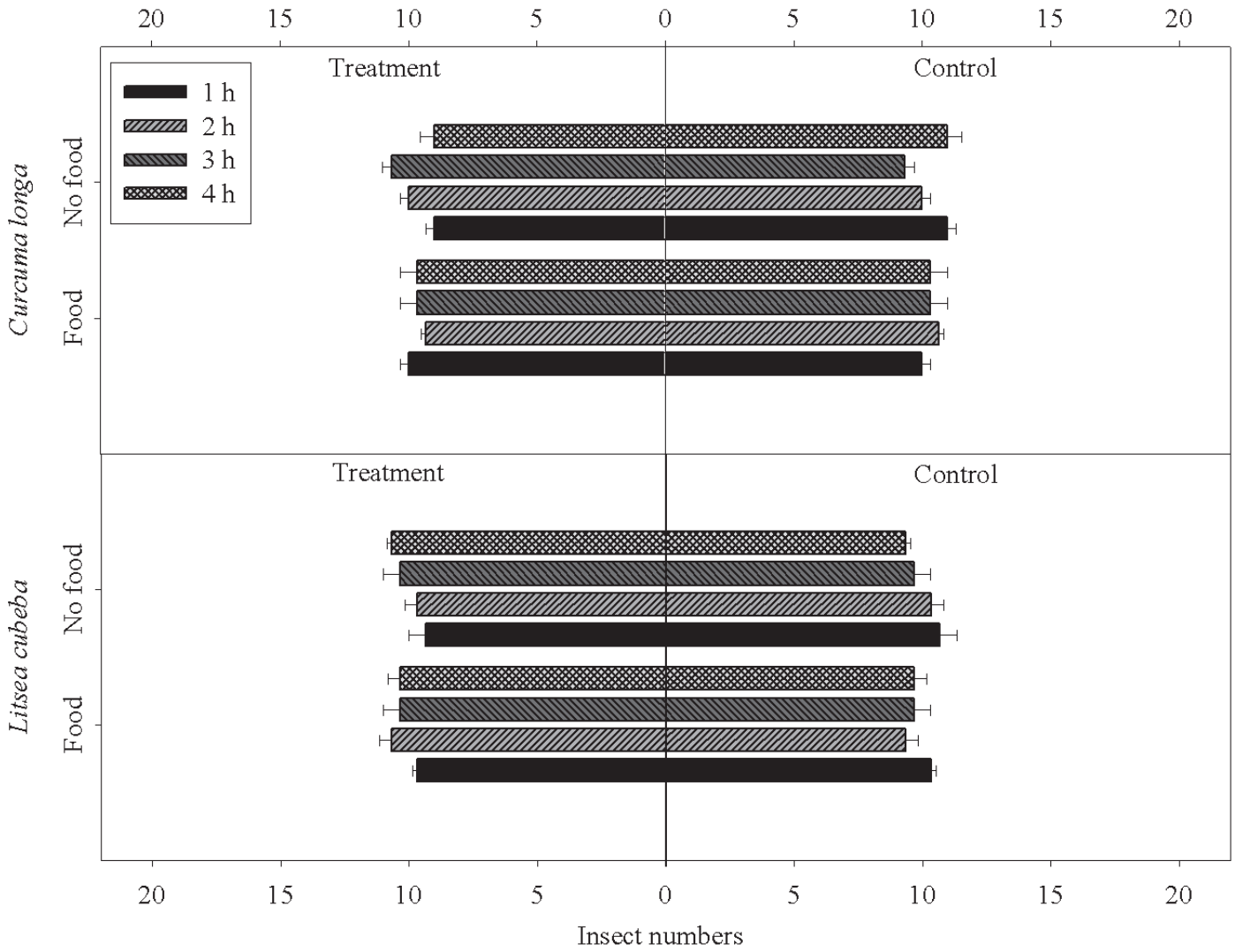

Fig. 4. Mean numbers of ants present on treatment and control filter papers in tests with essential oils at a concentration of 1,000 ppm in the absence or presence of food at different hours of observation. Values are means of 6 replications, and bars indicate the standard error. The mean numbers of ants were compared by paired *t*-tests at a significance level of $P \leq 0.05$. No significant differences between treatment and control were found.

food was absent after 3 h of exposure but not after 1, 2, and 4 h. It is possible that after 3 h of exposure, the turmeric essence was reduced slightly and several ants thus explored the food not only on the control but also on the treatment filter paper.

Essential oil from *L. cubeba* was less repellent than oil from *C. longa* to pharaoh ants in the test without food but not in the test with food. Possibly, although the oil repelled the ants, some of them explored the treated side of the Petri dish for food. Nonetheless, the PR of *L. cubeba* oil was >70% in both tests at all observation times. This finding is consistent with the results of Ko et al. (2009), who demonstrated that *L. cubeba* strongly repelled stored-grain pest, i.e., the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), and *T. castaneum*, even at low concentrations. Furthermore, essential oils of fresh fruits of *L. cubeba* were repellent to mosquitoes of the genus *Armigeres* (Diptera: Culicidae) at concentrations ranging from 1.7 to 6.3 g/cm^2 (Vongsombath et al. 2012).

Unexpectedly, we found that the repellent activity of *L. cubeba* was significantly higher when food was present on both filter papers in the Petri dish (treatment and control) than when food was absent after 1 and 3 h of exposure. It is likely that the ants did not explore the treated side of the Petri dish because they found food on the control side and were repelled by the essential oil on the treatment side; thus, they showed limited movement in the Petri dish. Conversely, in the absence of food, more movement of the ants was noticeable, indicating that this oil did not maintain a constant effect in both tests. Furthermore, the repellency of *L. cubeba* oil was stronger than the food attraction. In contrast, the repellency of *C. longa* oil decreased after food was provided, suggesting that the attraction of the food was greater than the repellency of this oil. This observation needs further investigation in order to understand the interactions between the chemical compounds of the food and the oils.

Our chemical analysis showed that both essential oils were mixtures of 6 major constituents. Previous studies also identified plant essential oils as mixtures of several chemical compounds, of which we found one in the present study. For example, the essential oil of *Pogostemon cablin* (Blanco) Benth. (Lamiaceae) is a mixture of ethymol, *p*-cymene, carvacrol, α-pinene, linalool, myrcene, α-terpineol, and 1,8-cineole. Compounds found in the essential oil of *Corymbia citriodora* (Hook.) K. D. Hill & L. A. S. Johnson (Myrtaceae) include β-patchoulene and α-guaiene. These organic compounds have been found to be poison-

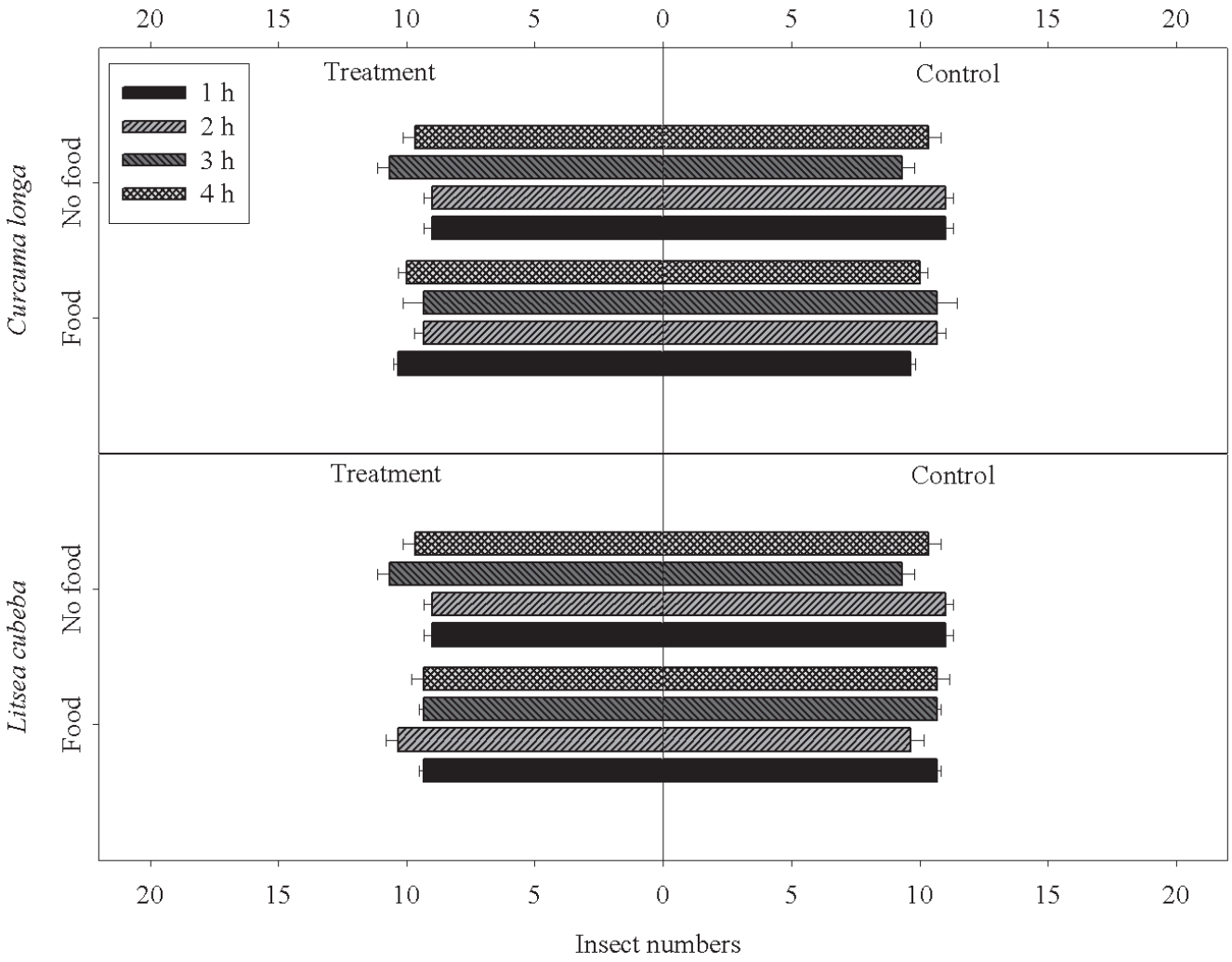

Fig. 5. Mean numbers of ants present on treatment and control filter papers in tests with essential oils at a concentration of 100 ppm in the absence or presence of food at different hours of observation. Values are means of 6 replications, and bars indicate the standard error. The mean numbers of ants were compared by paired *t*-tests at a significance level of $P \leq 0.05$. No significant differences between treatment and control were found.

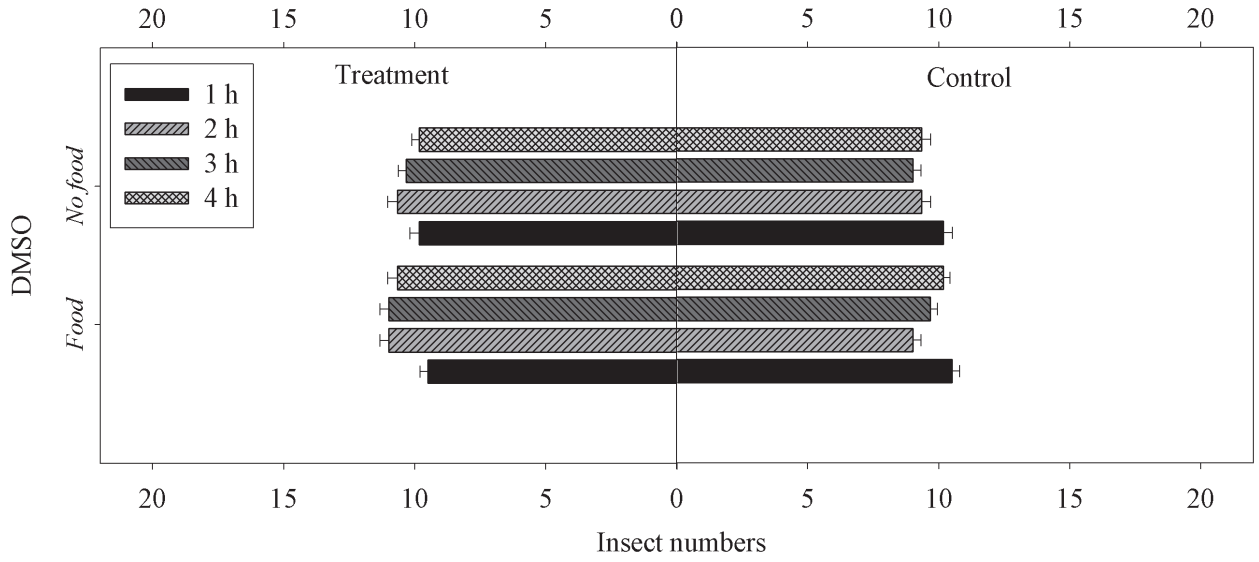

Fig. 6. Mean numbers of ants present on DMSO-treated and untreated control filter papers in the absence or presence of food at different hours of observation. Values are means of 6 replications, and bars indicate the standard error. The mean numbers of ants were compared by paired *t*-tests at a significance level of $P \leq 0.05$. No significant differences between DMSO and untreated control were found.

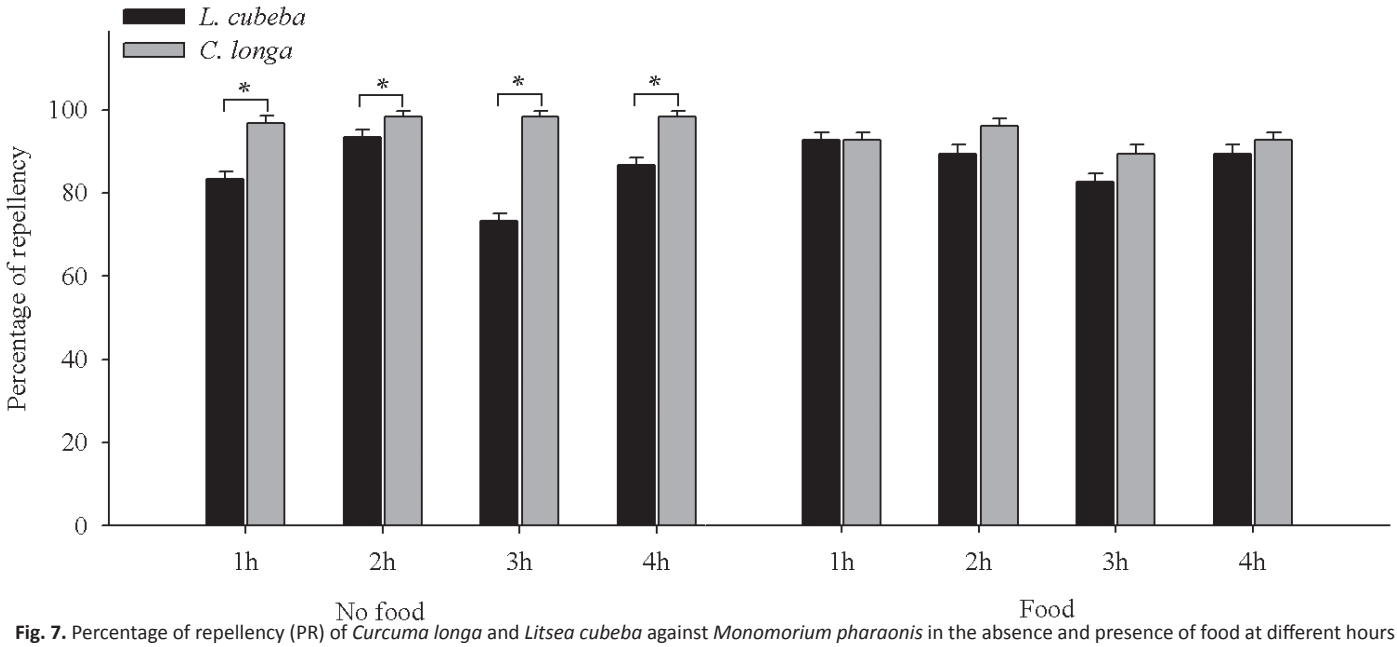

Fig. 7. Percentage of repellency (PR) of *Curcuma longa* and *Litsea cubeba* against *Monomorium pharaonis* in the absence and presence of food at different hours of observation. Values are means of 6 replications, and bars indicate the standard error. The PR values were analyzed by 1-way ANOVA and Tukey's HSD test at a significance level of $P \leq 0.05$. An asterisk indicates a significant difference between the two plant oils.

Table 1. Chemical constituents of *Litsea cubeba* and *Curcuma longa* essential oil based on GC-MS analysis.

Plant	No.	Component	Retention time	% of total
L. cubeba	1	sabinene	5.346	0.028
	2	1,8-cineole	6.093	0.190
	3	*E*-citral	8.614	5.349
	4	dodecanoic acid (CAS)	11.364	12.469
	5	dodecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)	15.017	2.486
	6	(*Z*,*Z*)-9,12-octadecadienoic acid	15.327	18.747
C. longa	1	eucalyptol (1,8-cineole)	6.094	0.312
	2	benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	10.564	3.273
	3	[*S*-(*R**,*S**)]-5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene	10.682	3.383
	4	1-phenyl-2-(*p*-tolyl)-propane	11.733	1.313
	5	curlone	12.506	13.842
	6	tumerone	12.831	1.207

ous to insect and mite pests (Traboulsi et al. 2002, 2005; Miresmailli et al. 2006).

In conclusion, the essential oils of *C. longa* and *L. cubeba* showed high repellency to pharaoh ants in both the absence and presence of food. Both oils may be used effectively in the production and development of ant control strategies. Further studies are needed to clarify what concentrations of these essential oils will be appropriate for use as repellents in buildings, as well as in the open field.

Acknowledgments

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