

Repellent effect of *Gardenia jasminoides* ethanol extracted oil on *Blattella germanica* and *Monomorium pharaonis*

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Summary The study examined the repellency of *Gardenia jasminoides* ethanol-extracted oil against the German cockroach, *Blattella germanica*, and the pharaoh ant, *Monomorium pharaonis*, which are serious pests in areas of public health hygiene. For the repellency tests, 31.4 µg of the oil was applied per cm² on one half of filter paper discs (9 or 15 cm diameter for the ant and cockroach, respectively), whereas the other half was treated as control (DMSO + Tween). Repellency effects were observed 1, 2, 3 and 4 h after the insect release. The oil showed high repellency against all life stages of cockroaches and worker ants. The maximum repellency was observed for the cockroach adults (81.7 ± 3.1%) followed by the fourth, third and second nymphal stages (76.7 ± 4.2, 75.0 ± 3.4, and 56.7 ± 8.4%, respectively), after 1h exposure. The repellence effect was strong against worker ants (78.3 ± 4.8%) after 1 h exposure. The repellence effect can last at least four hours for both species. Analysis of *Gardenia* oil with Gas chromatography-mass spectrometry identified 14 major chemical components.

Additional keywords: ants, cockroaches, ethanol-extracted oil, *Gardenia jasminoides*, repellent

Introduction

German cockroaches (*Blattella germanica*) and pharaoh ants (*Monomorium pharaonis*) are the most common indoor and outdoor pests in urban areas; both insect species thriving in most of the world's big cities, wherever they have access to water and food (Bosik, 1997; Hansen, 2011; Mahmoud *et al.*, 2013). Numerous findings have demonstrated that both species cause direct damage by infesting food and structural damage in homes, and indirectly as vectors for many disease-producing pathogens such as fungi, bacteria, viruses, proto-

zoa, and helminths, exposing humans to a contamination risk in residential and public health hygiene areas (Dobesh *et al.*, 1993, Fu *et al.*, 2009, Pavlik and Falkinham, 2009; Menasria, 2009). Some cockroach species emit bad odor from their body, which makes the surrounding environment to smell bad for a long time (Cochran, 1982).

For the control of these species infestation, synthetic chemicals have been introduced in households, hospitals, stores, and gardens, because of their rapid action for repelling, killing, or paralyzing the insects (Kostyukovsky, 2002). Although synthetic pesticides timely kill or repel the targeted insect species, the extensive and regular application of these substances increases the risk of resistance development, environmental pollution and damaging effects on biodiversity, bees and pollination, water resources, and food security (Parveen and Dhanda-pani, 2001; Malaj *et al.*, 2014; Queyrel *et al.*, 2016).

Pest control by natural products such as plant essential oils is a promising alternative to chemicals, as their application is considered to be safe and environmentally friendly (Nerioa *et al.*, 2009). Most of the plant essential oils are not pest killers, but they can

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repel insect pests and affect their growth. Moreover, they are cheap and non-toxic for other organisms (Geetha and Anitha, 2014). Due to these reasons, research on the insecticidal and repellent effects of essential oils on insects is increasing every day (Ayvaz *et al.*, 2010; Suthisut *et al.*, 2011; Faraone *et al.*, 2015). The present study aims to investigate the repellency of *Gardenia jasminoides* ethanol-extracted oil against German cockroaches and pharaoh ants.

Material and Methods

Insect collection and rearing

German cockroaches and pharaoh ants were reared at 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) at 25°C, 50% relative humidity (RH), and 14:10 h (L:D). The German cockroaches were originally collected from public kitchens and were reared on waste (fruit, biscuit, newspapers). The workers of pharaoh ants were reared on aphid honeydew provided by aphid colonies on maize crops.

Biopesticide formulations

Dry fruits of *G. jasminoides* were obtained from a franchise of the Beijing Tongrentang Group located in Wuhan, China. Methodology by Su *et al.* (2009) with some modifications was used for the extraction of the oil. Briefly, dried seeds were cleaned and dried for 3 days at 45°C to get constant weight. Once the plant material was dried, it was ground into powder and passed through a 40 mm mesh sieve. The powder was mixed in a brown bottle with a ratio of 1 g of plant powder and 5 mL of 95% ethanol for 7 days. The bottle was shaken twice a day for maximum dissolution and mixing the powder in the ethanol and placed in a dark room at 20–25°C. The ethanol was filtered, and the residual plant material was again diluted with ethanol at 2.5 mL of ethanol for 1 g of residuals and placed under the same conditions.

After all the mixing and dissolving processes, both filtered solutions were mixed and dried in a rotary evaporator until all the liquid evaporated and glue-like consistency with an oil-type liquid on the surface appeared. The final product was collected in a brown bottle, weighed (159.28 g of crude oil was obtained from 589.54 g of seeds) and stored at 4°C.

For the preparation of the working solution, 0.05 g of *G. jasminoides* crude extract was dissolved in 0.3 mL of dimethyl sulfoxide (DMSO); 1% Tween 20 was added to the solution, and the final volume was adjusted to 5 mL at a final concentration of 10,000 ppm (10 mg/ml) by adding double-distilled water. For the control solution, 0.3 mL of DMSO and 1% Tween 20 were added to the water to make 5 mL.

Gas chromatography and mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) Varian 450-GC/320-MS (Varian, Inc., Walnut Creek, California) was used to separate and identify the chemical components of ethanol-extracted oil. The methodology followed was previously described by Caballero-Gallardo *et al.* (2014). The same column and analysis conditions were used for both the GC and MS. The spectrophotometer was equipped with a flame ionization detector and an HP-5ms capillary column (30 m × 0.25 mm × 0.25 µm). For the GC, the 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 the 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, the ion source temperature was 230°C, the transmission line temperature was 250°C, and the 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.

Ant and cockroach repellency test

An area preference test was performed on 9 cm diameter filter paper for ant bioassay and 15 cm filter paper for cockroaches in Petri dishes. Filter paper discs of either diameter were cut into two halves. With the help of a micropipette, 0.1 mL of the working solution was applied uniformly on one half of the filter paper disc, and the same volume of control solution was applied uniformly on the other half of the filter paper disc, to get a final concentration of 31.4 µg of the ethanol-extracted oil per cm². Both halves were placed at room temperature to dry for one hour. When dry, the two halves were placed into the Petri dishes. Six holes of 1 mm were made on the Petri dish cover for ventilation. Twenty pharaoh ant workers were released in the center of the Petri dishes using an aspirator. Twenty adult cockroaches were released in the center of the Petri dishes using a fine brush. All dishes were covered with black plastic tape (leaving space of 5 cm for ventilation) to provide darkness and kept in the same room as described for the rearing conditions. Individuals were counted on both treated and control halves of the filter paper 1, 2, 3, and 4 h after the insect release. The same experiment was repeated with the fourth, third, second and first nymphal stages of the cockroach. The repellency test was repeated using fresh insects. A total of 8 replications were conducted for each release time of the ant and 6 replications of each treatment combination (life stage x release time) of the cockroach.

For the calculation of the percentage of repellency (PR) the formula "PR = [(Nc-Nt)/20]*100%" was used, where Nc was the number of ants/cockroaches present in the control area, and Nt was the number of ants/cockroaches present on the treatment area

(Liu *et al.*, 2013).

Statistical analysis

A Chi-square test was conducted to compare the repellent effect in the treatment and control parts within the same developmental stage and exposure time. One-way ANOVA and Turkey test was used to achieve the comparisons of the means between the developmental stages within the same exposure time and between exposure times within the same developmental stage. Data were analyzed using SPSS (Ver. 20) at $\alpha = 0.05$ significance level. All the graphs were plotted in Sigma Plot 10.

Results

GC-MS analysis of the *G. jasminoides* oil

Ethanol-extracted oil of *G. jasminoides* was analyzed by GC-MS and 14 major chemical components were identified. The chemicals, their percentage, retention time, and formula are presented in Table 1 (also presented in Wagan *et al.*, 2018).

Repellency of *G. jasminoides* oil to the German cockroach

The ethanol-extracted oil from *G. jasminoides* showed high repellency against all growth stages of German cockroach and pharaoh ant workers in the area preference test in Petri dishes.

Chi-square test showed a significant difference in repellency between the treated and the corresponding control area within each developmental stage and time exposure: in the adult stage at 1, 2, 3, and 4 h exposure: $\chi^2 = 48.02, 31.72, 30.00$, and 23.74 , respectively, $df = 1$, $P < 0.01$; in the fourth nymphal stage at 1, 2, 3, and 4 h exposure: $\chi^2 = 41.34, 29.56, 25.21$ and 20.95 , respectively $df = 1$, $P < 0.01$; in the third nymphal stage at 1, 2, 3, and 4 h exposure: $\chi^2 = 39.27, 28.34, 20.95$, and 18.37 , respectively, $df = 1$, $P < 0.01$; in the second nymphal stage at 1, 2, 3, and 4 h exposure ($\chi^2 = 20.95, 17.16, 13.82$, and 10.89 , respectively, $df = 1$, $P < 0.01$; in the first nymphal stage at 1, 2, 3, and 4 h: $\chi^2 =$

Table 1. GC-MS results of the ethanol-extracted oil from *Gardenia jasminoides*.

Components	Retention Time	Percentage of Total	Formula
Isolongifolene	10.10	0.48	C ₁₅ H ₂₄
Thujopsene	10.37	0.24	C ₁₅ H ₂₄
Diethyl phthalate	11.21	0.14	C ₁₂ H ₁₄ O ₄
8.beta.h-cedran-8-ol	11.51	0.16	C ₁₅ H ₂₆ O
Alpha-bisabolol	11.81	0.10	C ₁₅ H ₂₆ O
n-Hexadecanoic acid	13.17	0.60	C ₁₆ H ₃₂ O ₂
Hexadecanoic acid, ethyl ester	13.30	0.76	C ₁₈ H ₃₆ O ₂
Ethylene brassy-late	13.62	0.41	C ₁₅ H ₂₆ O ₄
9-12-Octadecadienoic acid	14.03	1.81	C ₁₈ H ₃₂ O ₂
Ethyl linoleate	14.12	1.30	C ₂₀ H ₃₆ O ₂
9-Octadecenoic acid ethyl ester	14.14	0.99	C ₂₀ H ₃₈ O ₂
Octadecanoic acid, ethyl ester	14.24	0.14	C ₂₀ H ₄₀ O ₂
9-12-Octadecadienoyl chloride	15.28	0.16	C ₁₈ H ₃₁ ClO
Squalene	17.54	0.37	C ₃₀ H ₅₀

Source: Wagan *et al.* (2018)

8.35, 10.00, 5.52, and 6.86, respectively, $df = 1$, $P < 0.01$ (Fig. 1).

The repellency of the oil differed significantly between the cockroach stages (ANOVA) within each exposure time: 1hr ($F = 11.21$, $df = 4, 25$, $P = 0.00$), 2 h ($F = 9.06$, $df = 4, 25$, $P = 0.00$), 3 h ($F = 1.76$, $df = 4, 25$, $P = 0.00$) and 4 h ($F = 7.52$, $df = 4, 25$, $P = 0.00$) of exposure (Fig.

2). Moreover, in each developmental stage the repellency differed significantly between exposure times: in the adult stage: $F = 7.61$, $df = 3, 20$, $P = 0.01$; in the fourth instar: $F = 6.84$, $df = 3, 20$, $P = 0.01$; in the third instar: $F = 9.62$, $df = 3, 20$, $P = 0.00$; in the second instar: $F = 1.31$, $df = 3, 20$, $P = 0.30$; in the first instar: $F = 0.61$, $df = 3, 20$, $P = 0.62$ (Fig. 3).

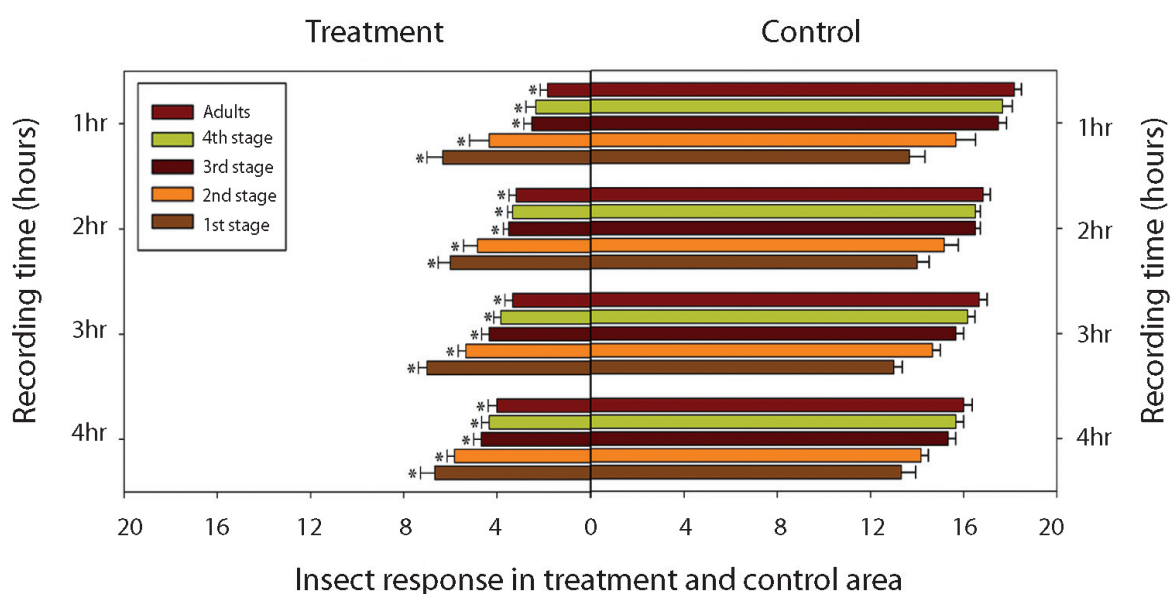


Figure 1. Chi-square test on repellency between the treated area with ethanol-extracted oil from *Gardenia jasminoides* (31.4 $\mu\text{g oil/cm}^2$) and the corresponding control area, to adults and nymphs of the German cockroach, *Blattella germanica*. Bars indicate the standard error.

The maximum repellency was recorded during the first hour of exposure in all stages except for first instar nymphs, and the repellency decreased with the increase of exposure time (Fig. 3). The oil was more effective on the adult cockroaches with a repellency percentage of $81.7 \pm 3.1\%$ at 1 h exposure while this percentage was 76.7 ± 4.2 , 75.00 ± 3.4 , and $56.7 \pm 8.4\%$, on the fourth, third and second instar nymphs, respectively (Fig. 2). In first instar nymphs, the maximum repellency percentage ($40.0 \pm 5.2\%$) was recorded after 2 h exposure to the oil (Fig. 3).

Repellency of *G. jasminoides* oil to worker ants

The chi-square analysis of worker ant repellency showed significant differences between the treated and controlled area during 1, 2, 3, and 4 h of exposure ($\chi^2 = 43.49$, 37.28, 35.36, and 30.00, respectively, $df = 1$, $P < 0.01$) (Fig. 4). The maximum repellency percentage was recorded after 1h exposure ($78.3 \pm 4.8\%$), whereas the repellency decreased with increasing exposure time (Fig. 5). ANOVA analysis showed a significant difference in repellency between exposure times ($F = 15.35$, $df = 3, 20$, $P = 0.00$).

Repellency comparison of *G. jasminoides* oil between worker ants and adult cockroaches

The repellency of *G. jasminoides* oil to adult stages of the insects was higher in the cockroach compared to the ant at 1, 3 and 4h exposure time (ANOVA: 1h: $F = 5.29$, $df = 1, 10$, $P = 0.04$; 3h: $F = 8.00$, $df = 1, 10$, $P = 0.02$; 4h: $F = 14.76$, $df = 1, 10$, $P = 0.01$). No significant difference was observed at 2h exposure time ($F = 0.12$, $df = 1, 10$, $P = 0.76$) (Fig. 6).

Discussion

The ethanol extracted oil from *G. jasminoides* showed a repellent effect to both the German cockroach, *B. germanica*, and the pharaoh ant, *M. pharaonis*, at the concentration of $31.4 \mu\text{g}$ of oil per cm^2 of filter paper in Petri dishes of 15 and 9 cm diameter, respectively.

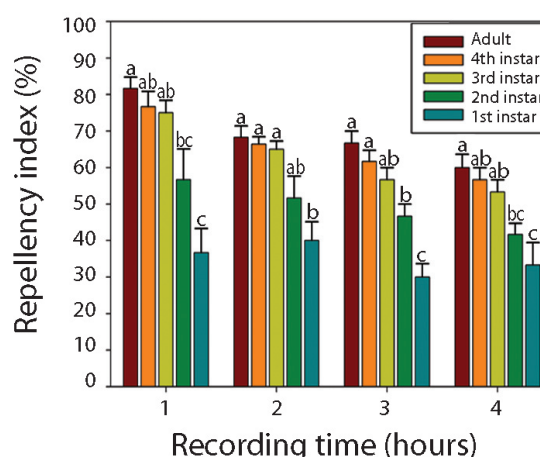


Figure 2. Repellency percentage (Mean + SE) of ethanol-extracted oil from *Gardenia jasminoides* to adults and nymphs of the German cockroach, *Blatella germanica*, at $31.4 \mu\text{g}$ oil/ cm^2 . Letters on the top of the columns show significant differences between developmental stages within the same exposure (recording) time.

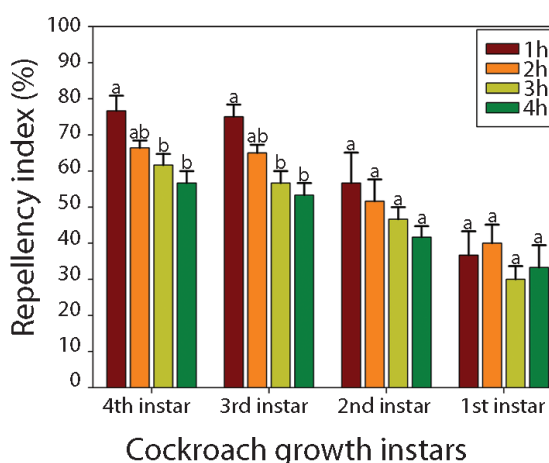


Figure 3. Repellency percentage (Mean + SE) of ethanol-extracted oil from *Gardenia jasminoides* to all nymphal instars of the German cockroach, *Blatella germanica*, at $31.4 \mu\text{g}$ oil/ cm^2 . Letters on top of the columns show significant differences between exposure times within the same instar.

Studies on bioactivity of *G. jasminoides* ethanol-extracted oil on insects are scarce. A repellent effect of *G. jasminoides* oil has been reported for *Tetranychus urticae* (Tetranychidae) 24h after treatment and the repellency was decreased with increasing of exposure time at 72 h (Kim et al., 2005). Potential toxicity, repellency, and anti-oviposition activities of the oil have been reported against *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *T. urticae* in laboratory and greenhouse ex-

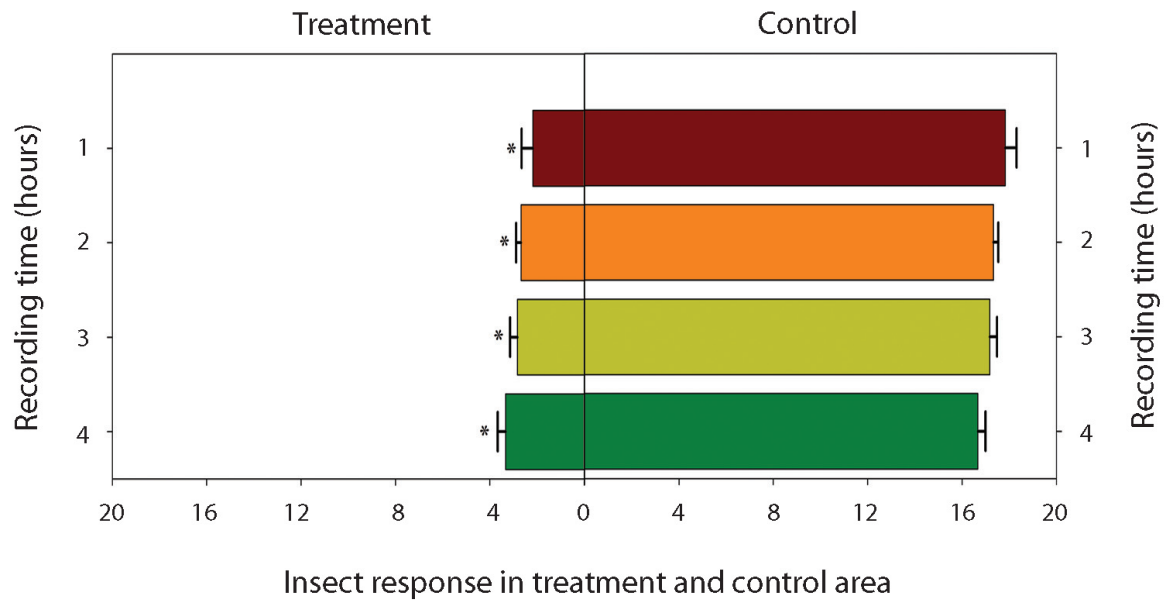


Figure 4. Chi-square test on repellency between the treated area with ethanol-extracted oil from *Gardenia jasminoides* ($31.4 \mu\text{g oil/cm}^2$) and the corresponding control area, to worker ants of *Monomorium pharaonic*. Bars indicate the standard error.

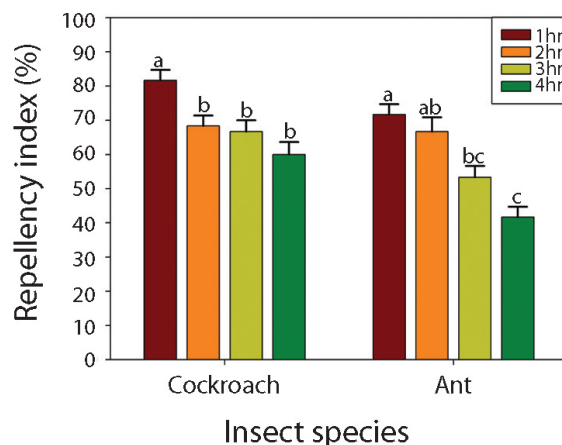


Figure 5. Repellency percentage (Mean + SE) of ethanol-extracted oil from *Gardenia jasminoides* to adults of the German cockroach, *Blattella germanica*, and the worker ants of *Monomorium pharaonic* at $31.4 \mu\text{g oil/cm}^2$. Letters on the top of the columns show significant differences at different exposure times (1, 2, 3, 4 h) for each insect species.

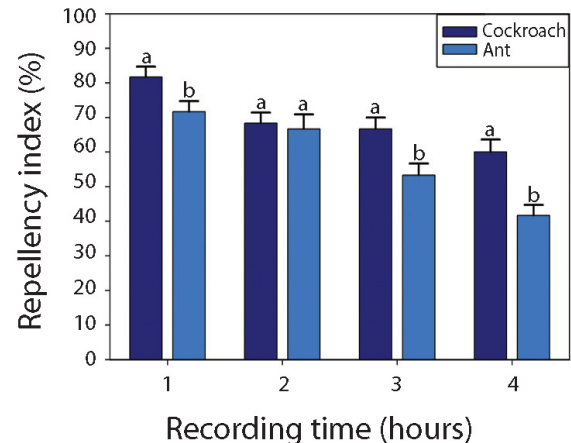


Figure 6. Repellency percentage (Mean + SE) of ethanol-extracted oil from *Gardenia jasminoides* to adults of the cockroach *Blattella germanica* and the worker ants of *Monomorium pharaonic* at $31.4 \mu\text{g oil/cm}^2$. Letters on the top of the columns show significant differences between the insect species at different exposure times (1, 2, 3, 4 h).

periments (Wagan *et al.*, 2018).

In German cockroaches, the *G. jasminoides* oil was more repellent to adults compared to nymphal stages. The weak effect of the oil on first instar nymphs could be related to differences in their chemoreceptor organs which probably are not fully de-

veloped in this stage. Several studies have proved that essential oils of several plant species have repellent activities against German cockroaches. For example, Zibae *et al.* (2016) evaluated the repellency from the mixture of *Eucalyptus globulus*, *Rosmarinus officinalis* essential oils against *B. ger-*

manica, the American cockroach *Periplaneta americana* (Blattodea: Blattellidae), and the brown banded cockroach *Supella longipalpa* (Blattodea: Ectobiidae, formerly Blattellidae), which showed 95%, 100%, and 100% repellency, respectively in laboratory experiments. The same study demonstrated 97% reduction of cockroaches in the field when these oils were applied as a mixed formulation with 10% active ingredient in water. The essential oil of *Citrus hystrix* showed 100% repellency against *B. germanica* and *P. americana* and 87.5% repellency against *Neostylopyga rhombifolia* in a laboratory experiment, whereas it reached 86% repellency when *Citrus hystrix* essential oil was formulated as a 20% active ingredient in ethanol (Thavara et al., 2007).

Similarly, *G. jasminoides* oil showed repellent activity against worker pharaoh ants in area preference tests up to 4h exposure. Some studies have already proved the repellency of botanical substances introducing new plant-based repellents against different ant species. For example, the aqueous extracts of a plant mixture of cucumber-mint, lemon-garlic, and garlic-mint exhibited 100% repellency against ants (Chaudhari et al., 2013). Chemical component cineole and D-camphor from *Artemisia annua* L oil can repel red imported fire ant workers. These substances showed significant repellent effect at 100, 10, and 1 mg/kg (Zhang et al., 2014).

Fourteen major chemical components were found in *G. jasminoides* ethanol-extracted oil. The bioactivities of these chemicals on insects, mites, rodents and some pathogens are already identified by previous researchers (Wagan et al., 2018; Parthipan et al., 2015; Sato et al., 2007; Friedman et al., 2002). *Gardenia jasminoides* essential oil and its four major compounds, i.e., squalene, ethyl linoleate, n-hexadecanoic acid and 9-12-octadecadienoic, effectively control the adult and nymphal stages of whiteflies and mites and they affect whitefly oviposition (Wagan et al., 2018).

In conclusion, the ethanol-extracted oil of *G. jasminoides* possesses repellent activ-

ities against German cockroaches and pharaoh ants, which could last for four hours. Further research is necessary to elaborate in these results with different concentrations and field tests. As ethanol-extracted oils are non-toxic for mammals, the *G. jasminoides* could be used as an alternative natural product in urban pest management and in the areas of public health hygiene (Kumar et al., 2008; Ling et al., 2009).

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Απωθητική δράση αιθανολικού εκχυλίσματος ελαίου του φυτού *Gardenia jasminoides* στην κατσαρίδα *Blattella germanica* και το μυρμήγκι *Monomorium pharaonis*

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Περίληψη Η μελέτη εξέτασε την απωθητική δράση αιθανολικού εκχυλίσματος ελαίου του φυτού *Gardenia jasminoides* στη Γερμανική κατσαρίδα *Blattella germanica* και στο μυρμήγκι Φαραώ *Monomorium pharaonis*, τα οποία είναι σοβαροί εχθροί υγειονομικής σημασίας. Στις δοκιμές απωθητικής δράσης εφαρμόστηκαν 31,4 μg ελαίου/cm² στο μισό δίσκου διηθητικού χαρτιού (διαμέτρου 9 ή 15 cm για τα μυρμήγκια και τις κατσαρίδες, αντίστοιχα), ενώ το άλλο μισό αποτέλεσε τον μάρτυρα (DMSO + Tween). Παρατηρήθηκε απωθητική δράση 1, 2, 3 και 4 ώρες μετά την απελευθέρωση των εντόμων. Το έλαιο έδειξε υψηλή απωθητική δράση σε όλα τα στάδια ανάπτυξης των κατσαρίδων και στα μυρμήγκια-εργάτες. Στις κατσαρίδες, η μέγιστη απωθητική δράση παρατηρήθηκε στα ενήλικα άτομα ($81,7 \pm 3,1\%$) και ακολούθως στις νύμφες τετάρτου, τρίτου και δεύτερου σταδίου ($76,7 \pm 4,2$, $75,0 \pm 3,4$ και $56,7 \pm 8,4\%$, αντίστοιχα), μετά από μία ώρα έκθεσης. Στα μυρμήγκια-εργάτες, η απωθητική δράση ήταν ισχυρή ($78,3 \pm 4,8\%$) μετά από μία ώρα έκθεσης. Η απωθητική δράση μπορεί να διαρκέσει τουλάχιστον τέσσερις ώρες και για τα δύο είδη εντόμων. Η χημική ανάλυση του ελαίου *Gardenia* με αέρια χρωματογραφία-φασματομετρία μάζας εντόπισε 14 κύρια χημικά συστατικά.

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