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Research article

Insecticidal Properties of Essential Oils and Some of Their Constituents on the Turkestan Cockroach (Blattodea: Blattidae)

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Abstract

The Turkestan cockroach, *Blatta lateralis* (Walker), has become the most important peridomestic species in urban areas of the Southwestern United States. The aim of this study was to evaluate the use of botanical compounds to control this urban pest. We tested the acute toxicity and repellency of six botanical constituents and three essential oils on Turkestan cockroach nymphs. Chemical composition of the essential oils was also determined. Topical and fumigant assays with nymphs showed that thymol was the most toxic essential oil constituent, with a LD_{50} of 0.34 mg/nymph and a LC_{50} of 27.6 mg/liter air, respectively. Contact toxicity was also observed in assays with trans-Cinnamaldehyde, eugenol, geraniol, methyl eugenol, and *p*-Cymene. Methyl eugenol and geraniol had limited fumigant toxicity. The essential oils from red thyme, clove bud, and Java citronella exhibited toxicity against nymphs. Cockroaches avoided fresh dry residues of thymol and essential oils. Chemical analysis of the essential oils confirmed high contents of effective essential oil constituents. Our results demonstrated that essential oils and some of their constituents have potential as eco-friendly insecticides for the management of Turkestan cockroaches.

Key words: Peridomestic cockroach, eco-friendly management, essential oil, toxicity, avoidance behavior

The Turkestan cockroach, *Blatta lateralis* (Walker) (Blattodea: Blattidae), has become an important invasive urban pest throughout the Southwestern United States (Kim and Rust 2013). Turkestan cockroaches were initially introduced to the United States in 1978 in shipments of military equipment from the Middle East and Asia that arrived at Sharpe Army Depot in Lathrop, California (Spencer et al. 1979). Several infestations were then reported in urban areas of Texas (USDA 1980), Arizona (Olson 1985), and Georgia (Petersen and Cobb 2009). In the Southwestern United States, Turkestan cockroaches are regularly found inhabiting areas that offer moisture (e.g., water meter boxes, irrigation boxes), shelter (e.g., cable boxes, sewer systems, crawlspaces, manholes), and food (e.g., leaf litter, garbage). Hundreds of Turkestan cockroaches are also seen in animal feed mills, around sheep feeders, and places where animal manure accumulates (Olson 1985, Kim and Rust 2013, Rios and Honda 2013). These cockroaches occasionally invade human environments through holes, cracks, and gaps between doors and floors (Rios and Honda 2013). Infestations of Turkestan cockroaches in indoor areas can lead to airborne exposure to allergens derived from saliva, feces, and shed exuvia of cockroaches that trigger both asthma and allergies in sensitized people (Robinson 1996). This species is also a potential vector of microorganisms that are pathogenic to animals and humans, including *Salmonella* spp. (Fathpour et al. 2003), a bacterium that is a threat to public health in the Southwestern United States (Edrington et al. 2004).

There are concerns about the escalation of Turkestan cockroach infestations in urban areas and their geographical expansion to other areas of the United States. A higher ootheca production and a faster developmental time may explain the displacement of oriental cockroaches by Turkestan cockroaches that is observed by pest control professionals in outdoor habitats throughout the Southwestern United States (Kim and Rust 2013). The geographical expansion of the Turkestan cockroach may also be aided by the exotic pet industry, where the cockroaches are commonly bought and sold over the Internet as live food for reptiles and other animals (http://www.nyworms.com/turkistan_roach.htm).

Current methods for the control and elimination of peridomestic cockroaches rely on the use of synthetic insecticides (Appel and

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Smith 2002). Application of these insecticides in peridomestic and domestic areas is a public concern due to risk of exposure to these insecticides that can result in toxicity (Kass et al. 2009). Furthermore, continued use of insecticides may lead to insecticide resistance in cockroach populations (Wei et al. 2001). Baits, in the form of gels or granules, have become one of the most common method for controlling cockroaches (Mallis 2011). While baits can be very effective against infestations of cockroaches, its efficacy in outdoor areas against peridomestic cockroaches could be limited by environmental conditions, presence of alternative food sources (e.g., organic matter, manure, spilled animal feed) that outcompete baits, and moisture of harborages where Turkestan cockroaches tend to aggregate (Olson 1985). An alternative to the use of traditional insecticides for management of peridomestic cockroaches is the use of plant essential oils. Essential oils have been used for pest control for hundreds of years in Asia (e.g., China, India) and are considered safe because of their relatively short residual period and their low toxicity to humans, animals, and wildlife (Isman 2006). Some essential oil constituents are considered to be minimum risk pesticides according to section 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act of United States (Isman and Paluch 2011), and mixtures of essential oils (e.g., Essentria IC3 Insecticide Concentrate, Central Garden & Pet Company, Schaumburg, IL) have already established a minor presence in the market for indoor and outdoor applications.

Essential oils are formed in special internal and glandular cells on the leaves and stems of members of aromatic plant families (Guenther 1948). These compounds exhibit a broad spectrum of activity against pathogenic microorganisms (Sameza et al. 2016). Essential oils also possess insecticidal and repellent activity, and disrupt growth, feeding, reproduction, and oviposition of pest insects (Hummelbrunner and Isman 2001, Isman 2006, Zhang et al. 2014, Wu et al. 2014). Essential oils and their constituents have insecticidal and repellent activity against cockroaches and other urban pests (Ngoh et al. 1998, Yeom et al. 2012, Yeom et al. 2013). Essential oil constituents such as geraniol, eugenol, thymol, trans-Cinnamaldehyde, and *p*-Cymene have insecticidal effect on German cockroaches (Phillips et al. 2010, Yeom et al. 2012, Alzogaray et al. 2013, Yeom et al. 2013), while methyl eugenol is toxic to cockroaches (Ngoh et al. 1998). Despite the various reports on the insecticidal properties of these compounds against several species of cockroaches, their toxic effect against the Turkestan cockroach, *B. lateralis*, has not been determined.

The present study was therefore initiated to investigate the contact and fumigant toxicity as well as the behavioral effect of essential oil constituents on *B. lateralis*, a prominent outdoor and indoor pest in the Southwestern United States. Further, we identify essential oils containing effective constituents and their chemical composition was verified. The insecticidal activity of essential oils was also evaluated. Identification of effective essential oils and constituents against Turkestan cockroaches will form the basis for the potential use of these compounds for the management of this cockroach.

Materials and Methods

Insect Colony

The Turkestan cockroach colony was established from samples collected in 2014 from a feed mill (32° 16′45.9″ N, 106° 45′25.3″ W) and a pig rearing facility (32° 16′44.3″ N 106° 45′32.2″ W) at New Mexico State University in Las Cruces, New Mexico. Colonies were reared at 23-26 °C, 30-50% RH, and a photoperiod of 9:15 (L:D) h. Cockroaches were housed in plastic containers with egg crates used as shelters. Cockroaches received a diet containing rabbit, dog, and cat feed and sweetened corn puff cereals (1:1:1:1). Water was given in a plastic bottle through a wet paper towel. In all the experiments, randomly selected, late-instar nymphs (fourth and fifth instar; mean weight: 340 mg/nymph) were used for toxicity evaluation, whereas male last-instar nymphs (fifth) were used for behavioral assays.

Chemicals

The essential oil components were diluted in acetone (99.7% purity; Fisher Scientific, Fair Lawn, NJ). The essential oil components thymol (≥99% purity), geraniol (98% purity), eugenol (99% purity), methyl eugenol (98% purity), trans-Cinnamaldehyde (99% purity), and *p*-Cymene (99% purity) were obtained from Sigma-Aldrich (St. Louis, MO). The pure essential oils evaluated, red thyme oil (*Thymus vulgaris* L.), clove bud oil (*Syzygium aromaticum* L.), and Java citronella oil (*Cymbopogon winterianus* Jowitt), were obtained from the Frontier Natural Products Co-op's brand Aura Cacia (Urbana, IA).

Chemical Analysis of Essential Oils

The essential oils red thyme, clove bud, and Java citronella were analyzed by using gas chromatography–mass spectrometry (GC–MS) to determine their chemical composition. The identification of the essential oil constituents was carried out using an Agilent 7890 GC coupled to a LECO TOFMS (Saint Joseph, Michigan). Data were output to a computer, and ChromaTOF (Saint Joseph, Michigan) was used to identify the compounds. The 30 m by 0.25 mm and 0.25 mm film thickness capillary column was used with helium as a carrier gas. The initial temperature was held at 40 °C for 30 s and increased at 15 °C/min to 280 °C for 5 min. The GC method total time was 21:30 (min:s). The transfer line temperatures were set to 300 °C and equilibrium time was 10 s. The sample volume was 0.2 µl (1:10 in carbon disulfide). The injection rate was 10 µl/s. The acquisition delay was 2:24 min with an acquisition rate of 10 spectra per second. The mass range that was scanned was between 35 and 400 m/z. The ion detector had a voltage of 2,000 V, the electron energy was –70 V, and the ion source temperature was 200 °C. The standard essential oil constituents thymol (≥99% purity), eugenol (99% purity), and geraniol (98% purity) were used to make serial dilutions with carbon disulfide. Dilutions were used to obtain a calibration curve for each compound and the percent concentration of effective components in their respective essential oils was calculated. The essential oil components and their actual retention indices were identified following methodology used by Adams (2007). The retention indices were calculated based on a regression equation obtained from Adams' (2007) actual retention indices values and retention time of known standards that were obtained from GC–MS. Octane was used as an internal standard for the normalization peak abundances between chromatographs. The peak area was quantified from the total ion chromatograph. Each essential oil was analyzed in triplicate.

Contact Toxicity

To facilitate handling during topical application, nymphs were held in a small plastic cup and knocked down with carbon dioxide (CO_2) at 5 liters/min using a blowgun CO_2 releaser regulated by a Flystuff Flowbuddy flow regulator (Genesee Scientific, San Diego, CA). Then, the insect was transferred to a platform, the Flystuff Ultimate Flypad (Genesee Scientific, San Diego, CA), and anesthesia was maintained with a slow release of CO_2. A hand micro-applicator

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(Hamilton, Reno, NV) was used to topically apply essential oil components or essential oil solutions (2-4 µl) in between the metathoracic legs of each nymph. The applied volume varied because some test materials had low toxicity and it was necessary to use more than 2 µl to deliver high concentrations of the compound. Control cockroaches were treated with the same volume of acetone used to dilute each constituent/essential oil. At least five concentrations (0.1–10 mg/nymph) were used for each compound. Seven replicates containing six nymphs each (total $n = 42$) were used for each concentration. After treatment, nymphs were placed in 16-ounce plastic cups with fine-mesh plastic lids (Fabri-Kal, Kalamazoo, MI), provided with water, and maintained in an incubator at 24 °C (RH: 30–50%). The mortality data were recorded 24 h post-application. Those nymphs that were in permanent supine position or did not respond upon prodding were considered dead. Moribund (without the ability to move forward but with occasional movements of the antennae or legs) nymphs were also considered dead.

Fumigant Toxicity

Fumigant activity was assessed by sealing groups of six nymphs in 473-ml glass jars (Kerr Group Inc., Lancaster, PA) with essential oil components or essential oils diluted in acetone (300 µl) and contained in strips of filter paper (3 by 5 cm; Whatman #1, Maidstone, UK). Treated filter paper was dried for 5 min in a chemical hood to allow for the evaporation of acetone and then attached to the underside of the glass jar lid with double-sided tape. Turkestan cockroaches could not climb glass; therefore, the strips were out of reach of the cockroaches. At least five concentrations were used for each compound (20–1,600 mg/liter air). Acetone was used as a control. Three replicate jars containing six nymphs each (total $n = 18$) were used for each concentration. The jars were maintained in an incubator at 24°C (RH: 30–50%). No water was provided. Mortality was assessed at 24 h.

Behavioral Test

Essential oil constituents were assayed at 1% and this solution was prepared as follows: a 50% solution of essential oil component was initially prepared with acetone. Then, 1 ml of this solution was mixed with 49 ml of deionized water. Five microliters of Tween 80 (Sigma-Aldrich, St. Louis, MO) was used as the surfactant. Control solutions contained the same amount of deionized water, Tween 80, and acetone, excluding essential oil components or essential oils. Essential oils were diluted to 1% based on the absolute amount of the primary constituents thymol, eugenol, and geraniol identified in our GC–MS analysis.

Behavioral responses were tested in plastic arenas (22.5 cm in length, 18.5 cm in width, and 8.5 cm in height; GODMORGON IKEA, Las Vegas, NV). Two pieces of filter paper (11.25 by 18.5 cm; Sigma Aldrich; Whatman #1, St. Louis, MO) were treated either with the 1% experimental solution (essential oil constituent or essential oil) or the control solution. The filter papers were sprayed with a 118-ml fine mist spray bottle (PRO Chemical & Dye, Fall River, MA). The spray bottles were triggered until they dispensed 3 ml for each filter paper at a distance of approximately 10 cm, resulting in a uniform wet surface at rates of 14.40 mg/cm^2. Experimental solutions were weighed before and after each spraying to apply the exact amount of solution. After solvent evaporation in a hood (~30 min), each treated half-paper was then attached lengthwise, edge to edge, to the bottom of the arena with double-sided tape. Individual nymphs were introduced in the control zone of the arena. In total, 15 replications were done for each experimental compound, and each day, one replicate for each compound was made. For each day, a common control group was used. Bioassays were conducted under ambient temperature (25 °C ±2) and relative humidity (40 ± 10%).

A high-resolution monochrome camera (Ikegami Electronics Inc., Maywood, NJ) with a variable focal TV lens (4.5–12.5 MM F1.2; Computar, Cary, NC) and an infrared pass filter (Heliopan, North White Plains, NY) were used to record cockroach activity in the arena. The camera was suspended 36 cm above the arena using a stand. Light for the recordings was provided by one infrared illuminator (AXTON, North Salt Lake, UT) positioned underneath the arena, facing upward, which provided indirect lighting to reduce reflections on the camera lens. EthoVision XT version 11.5 software (Noldus Information Technology Inc. Leesburg, VA; Noldus et al. 2002) was used to capture video images to track the cockroaches during 20-min bioassays. EthoVision XT virtually facilitates the division of the arena into two equal zones known as "treated" and "control." The detection method used for acquisition was subtraction. The detection thresholds were set so that all objects that were different from the background image would be tracked. To achieve this, the image of the arena without the nymph was saved as a reference image. The sampling rate was two samples per second. The sensitivity was set at one unit and background changes were set up at very fast. The minimum and maximum subject size of the object was fixed as 50 and 900 units, respectively. The background noise filter was set up as 10, and contrast was set up on a range of 25 to 255. Variables calculated at both the treated and control zones were time spent, distance traveled, percent of time moving, and velocity.

Statistical Analysis

LD_{50} or LC_{50} was determined for essential oils and essential oil constituents by using probit analysis (Finney 1971) with SPSS 23.0 software (IBM Corp. 2015). Differences among LD_{50} or LC_{50} values were judged as statistically significant when the 95% confidence intervals of these values did not overlap (Payton et al. 2003). The quantitative data from repellency assays with essential oil constituents were analyzed using one-way analysis of variance (ANOVA). The many–to–one comparison was done with Dunnett's test for mean separation. The quantitative data from behavioral assays with essential oils were analyzed with the nonparametric Mann–Whitney *U*-test using SPSS 23.0 software (IBM Corp. 2015).

Results

Contact Toxicity

All six essential oil constituents had contact toxicity against Turkestan cockroach nymphs. However, based on the method of overlap of 95% confidence intervals, the LD_{50} value of thymol was significantly more toxic to nymphs ($LD_{50} = 0.34$ mg/nymph) than trans-Cinnamaldehyde (LD_{50}: 1.01 mg/nymph), eugenol (LD_{50}: 1.56 mg/nymph), geraniol (LD_{50}: 2.48 mg/nymph), methyl eugenol (LD_{50}: 3.10 mg/nymph), and *p*-Cymene (LD_{50}: 9.85 mg/nymph; $P < 0.05$; Table 1). The essential oils evaluated also exhibited high contact toxicity against Turkestan cockroach nymphs (Table 1). Red thyme oil (LD_{50}: 1.60 mg/nymph) was as toxic as clove bud oil (LD_{50}: 1.65 mg/nymph; $P > 0.05$), but these two essential oils were five times more toxic than the Java citronella oil (LD_{50}: 7.87 mg/nymph; $P < 0.05$; Table 1).

Fumigant Toxicity

Essential oil components in vapor phase had a similar pattern of toxicity of that observed in topical bioassays (Table 2). Thymol

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Table 1. Mortality produced by essential oil constituents and essential oils dissolved in acetone and topically applied on *B. lateralis* nymphs

Essential oil components	n	LD_{50}[a], mg/nymph (95% CI)[b]	Slope ± SE	χ^2	df	*P* value
Thymol	294	0.34 (0.31–0.37)a	2.86 ± 0.34	6.11	4	0.191
trans-Cinnamaldehyde	294	1.01 (0.95–1.07)b	2.91 ± 0.35	5.17	4	0.270
Eugenol	252	1.56 (1.48–1.64)c	4.29 ± 0.52	1.85	3	0.604
Geraniol	294	2.48 (2.38–2.59)d	4.56 ± 0.57	2.27	4	0.686
Methyl eugenol	294	3.10 (2.91–3.35)e	2.60 ± 0.37	1.509	4	0.825
p-Cymene	72	9.85 (9.04–14.18)f	4.24 ± 1.68	0.187	1	0.665
Essential oils						
Red thyme oil	108	1.60 (1.45–1.74)a	4.17 ± 0.75	0.52	3	0.913
Clove bud oil	126	1.65 (1.52–1.76)a	4.68 ± 0.87	4.60	4	0.331
Java citronella oil	126	7.87 (7.22–8.36)b	4.69 ± 0.84	2.48	4	0.647

Mortality in control groups = 0%, except in *p*-Cymene (5.5% mortality).

[a]LD_{50} = dose necessary to kill 50% of individuals.

[b]95% CI = 95% confidence interval.

LD_{50} values with the same letter within essential oil constituents or within essential oils are not significantly different ($P > 0.05$; based on the method of overlap of 95% confidence intervals, Payton et al. 2003).

Table 2. Mortality of late-instar nymphs of Turkestan cockroaches exposed to fumigants of essential oil constituents and essential oils dissolved in acetone

Essential oil components	n	LC_{50}[a], mg/liter air (95% CI)[b]	Slope ± SE	χ^2	df	*P* Value
Thymol	144	27.64 (24.9–29.9)a	3.36 ± 0.53	3.07	5	0.689
trans-Cinnamaldehyde	108	150.76 (124.3–281.3)b	1.54 ± 0.66	2.64	3	0.449
Eugenol	162	251.20 (231.7–281.6)c	2.96 ± 0.52	11.36	6	0.078
p-Cymene	144	441.84 (378.9–483.7)d	2.88 ± 0.66	1.11	4	0.891
Methyl eugenol	108	>1499.30[c]	1.92 ± 1.31	1.00	3	0.801
Geraniol	108	>7510.05[c]	1.05 ± 2.57	3.63	3	0.304
Essential oils						
Red thyme oil	126	160.55 (146.6–179.9)a	3.16 ± 0.61	1.98	4	0.738
Clove bud oil	108	318.97 (268.1–415.9)b	1.61 ± 0.42	0.29	3	0.962
Java citronella oil	144	746.74 (644.5–958.1)c	1.65 ± 0.37	5.22	5	0.389

Mortality in control groups = 0%.

[a]LC_{50} = concentration necessary to kill 50% of individuals.

[b]95% CI = 95% confidence interval.

[c]Low mortality in assays with methyl eugenol and geraniol prevented us from determining accurate confidence intervals.

LC_{50} values with the same letter within essential oil constituents or within essential oils are not significantly different ($P > 0.05$; based on the method of overlap of 95% confidence intervals, Payton et al. 2003).

exhibited significantly higher toxicity (LC_{50}: 27.64 mg/liter air) to nymphs of Turkestan cockroaches than trans-Cinnamaldehyde (LC_{50}: 150.76 mg/liter air), eugenol (LC_{50}: 251.20 mg/liter air), *p*-Cymene (LC_{50}: 441.84 mg/liter air), methyl eugenol (LC_{50}: 1499.30 mg/liter air), and geraniol (LC_{50}: 7510.05 mg/liter air; $P < 0.05$; Table 2). Analysis of mortality from fumigant assays also detected significant differences between essential oils ($P < 0.05$). Red thyme oil (LC_{50}: 160.55 mg/liter air) was two times more toxic than clove bud oil (LC_{50}: 318.97 mg/liter air) and almost five times more toxic than Java citronella oil (LC_{50}: 746.74 mg/liter air; Table 2).

Chemical Analysis of Essential Oils

The chemical composition determined by GC–MS is presented in Table 3. Thirteen components representing 93.2% of the total detected constituents of red thyme oil were identified. The major components identified in red thyme oil were *p*-Cymene (29.5%), thymol (22.7%), and γ-terpinene (18.9%; Table 3). The other components were present in a total amount of less than 23%. Five components representing 93.3% of the total detected constituents of clove bud oil were identified (Table 3). The major components identified in clove bud oil were eugenol (64%) and E-caryophyllene (22.9%; Table 3). Eleven components representing 90.3% of the total detected constituents of Java citronella oil were identified (Table 3). The major components identified in Java citronella oil were citronellal (56.8%) and geraniol (9.3%; Table 3). The absolute values of thymol in red thyme oil, eugenol in clove bud oil, and geraniol in Java citronella oil were 8.0 ±0.32%, 10.6 ±0.75%, and 2.26 ±0.39%, respectively.

Behavioral Test

Among the six essential oils components evaluated, only thymol produced significant avoidance activity in Turkestan cockroach nymphs (Table 4). Nymphs spent significantly less time (7.15 min, 35.8% of total time) in the zone treated with thymol than in the untreated areas of control groups (9.35 min vs. 7.15 min, respectively; $F = 2.56$, df = 6, $P < 0.05$; Table 4). For any of the essential oil constituents, there was no significant difference in distance traveled ($F = 1.29$, df = 6, $P > 0.05$), amount of time moving ($F = 1.64$ df = 6, $P > 0.05$), or velocity ($F = 0.415$, df = 6, $P > 0.05$) for nymphs interacting with treated zones, when compared with the same parameters calculated in essential oil component-free zones of control groups (Table 4).

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Table 3. Major constituents identified from three essential oils by GC–MS and their relative proportions in the pure oil

No.	Red thyme		RI[a]	Clove bud		RI[a]	Java citronella		
	Constituent	Peak area %		Constituent	Peak area %		Constituent	Peak area %	RI[a]
1	α-Pinene	3.5	936	Eugenol	64.0	1350	(–)- Limonene	6.5	1037
2	Camphene	3.0	956	E-caryophyllene	22.9	1411	Citronellal	56.8	1159
3	β-pinene	1.2	992	α-humulene	1.9	1441	Citronellol	6.8	1229
4	α-phellandrene	2.9	1024	β-thujaplicin	4.2	1479	Geraniol	9.3	1252
5	*p*-Cymene	29.5	1032	Caryophyllene oxide	0.3	1543	Citronellyl acetate	1.6	1337
6	1,8-Cineole	0.7	1041				Geranyl acetate	1.2	1363
7	γ-terpinene	18.9	1066				β-Elemene	2.0	1380
8	Linalool	4.4	1107				Germacrene D	1.8	1460
9	Borneol	1.8	1185				trans-cadina-1(6),4-diene	1.4	1487
10	1-terpinen-4-ol	1.3	1192				Germacrene A	1.7	1511
11	Thymol	22.7	1293				Elemol	1.3	1536
12	Carvacrol	1.4	1302						
13	Z-caryophyllene	1.9	1410						
	Total	**93.2**			**93.3**			**90.3**	

[a]RI (retention index) relative to the homologous series of n-hydrocarbons on the HP-5 MS capillary column.

Table 4. Behavior of nymphs of *B.lateralis* in treated zones for the evaluation of avoidance behavior of essential oil constituents and essential oils

Essential oil components		Time spent in zone (min)	Distance traveled (cm)	Percent time moving (min)	Velocity (cm/s)
Control		9.35 ± 0.67a	792.3 ± 124.7a	3.72 ± 0.41a	1.40 ± 0.21a
Thymol		7.15 ± 0.55b	574.8 ± 74.8a	2.92 ± 0.30a	1.32 ± 0.11a
Geraniol		7.46 ± 0.58a	524 ± 55.8a	2.85 ± 0.29a	1.19 ± 0.10a
trans-Cinnamaldehyde		9.53 ± 0.77a	600.5 ± 64.5a	3.24 ± 0.25a	1.11 ± 0.12a
Eugenol		9.29 ± 0.50a	744.4 ± 82a	3.90 ± 0.33a	1.34 ± 0.13a
Methyl eugenol		8.84 ± 0.64a	633.1 ± 94a	3.08 ± 0.28a	1.21 ± 0.18a
p-Cymene		9.72 ± 0.74a	729.6 ± 96.5a	3.55 ± 0.31a	1.33 ± 0.22a
Essential oils					
Red thyme oil	Control	9.31 ± 0.38a	709.4 ± 110.2a	3.76 ± 0.37a	1.24 ± 0.16a
	Treated	2.97 ± 0.61b	206.4 ± 38.6b	0.92 ± 0.19b	1.59 ± 0.28b
Clove bud oil	Control	9.71 ± 0.64a	687.7 ± 106.8a	3.69 ± 0.42a	1.19 ± 0.16a
	Treated	5.50 ± 0.42b	384.4 ± 91.5b	1.88 ± 0.16b	1.18 ± 0.24a
Java citronella oil	Control	9.78 ± 0.59a	625.6 ± 50.4a	3.55 ± 0.24a	1.17 ± 0.08a
	Treated	1.90 ± 0.47b	134 ± 23b	0.76 ± 0.13b	1.09 ± 0.21a

(N = 15 for each component, oils and control group).

Same letter in the column for essential oil components indicates that parameter values are not significantly different from control group (one-way ANOVA, Dunnett's test, $\alpha = 0.05$). Same letter in the column for each essential oil indicates that parameter values are not significantly different from individual control group (Mann–Whitney *U*-test, $\alpha = 0.05$).

Red thyme, clove bud, and Java citronella essential oils exerted strong avoidance activity in cockroach nymphs (Table 4). Nymphs spent significantly less time in halves treated with red thyme oil (red thyme oil = 2.97 min vs. control = 9.31 min; $U = 4$, $P < 0.05$), halves treated with clove oil (clove oil = 5.50 min vs. control = 9.71 min; $U = 17$, $P < 0.05$), and halves treated with Java citronella oil (Java citronella oil = 1.90 vs. control = 9.78 min; $U = 2$, $P < 0.05$; Table 4). Nymphs traveled significantly less distance in halves treated with red thyme oil (206.4 cm vs. 709.0 cm; $U = 13$, $P < 0.05$), clove oil (384.4 cm vs. 687.7 cm; $U = 32$, $P < 0.05$), or Java citronella oil (134 cm vs. 625.6 cm; $U = 0.00$, $P < 0.05$; Table 4). Nymphs in essential oil-treated zones were significantly less mobile than those in essential oil-free zones (Table 4). In zones treated with red thyme oil, nymphs were four times less active than in red thyme-treated zones (0.92 min vs. 3.76 min, respectively; $U = 6$, $P < 0.05$), while nymphs in clove bud oil were two times less active (1.88 min vs. 3.69 min; $U = 22$, $P < 0.05$), and nymphs in Java citronella oil were four times less active (0.76 min vs. 3.55 min; $U = 0.00$, $P < 0.05$). Nymphs walked significantly faster in areas treated with red thyme oil (velocity = 1.59 cm/s) than nymphs in red thyme oil-free zones (velocity = 1.24 cm/s; $U = 97$, $P < 0.05$; Table 4). However, nymphs in areas treated with clove bud oil or Java citronella oil did not walk significantly faster than their respective essential oil-free areas (Table 4).

Discussion

We used contact, fumigant, and behavioral assays to evaluate the effect of essential oil constituents, and their natural sources on the Turkestan cockroach, a common outdoor and indoor pest in the Southwestern United States. We initially evaluated the bioactivity of six essential oil constituents that were previously reported effective against other cockroach species to determine whether they were also toxic to Turkestan cockroaches. Results from topical and fumigant bioassays showed differences in toxicity among essential oil

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constituents. By direct contact, thymol exhibited the most toxic effect, followed by trans-Cinnamaldehyde, eugenol, and methyl eugenol. *p*-Cymene required larger doses to kill 50% of the nymphs (at least three times the dose), when compared with the killing doses estimated for the other essential oil constituents. Similarly, thymol exhibited the highest toxic activity as a fumigant, followed by trans-Cinnamaldehyde, eugenol, and *p*-Cymene. Very low mortality was observed in groups of nymphs exposed to vapors of methyl eugenol and geraniol, indicating that these constituents only exert their killing effect by penetration through the integument of the insects. The insecticidal activity of thymol against Turkestan cockroach nymphs is consistent with previous studies that reported thymol having high toxicity against German cockroaches (Jang et al. 2005, Phillips 2009, Phillips and Appel 2010). Structural characteristics of essential oil constituents (Kumbhar and Dewang 2001) may explain the differences in toxicity detected in our study. Aromatic compounds (containing benzene rings) are not easy to detoxify by the insect metabolic system; therefore, they are more toxic than aliphatic compounds. In our study, this structure–activity relationship was observed in the contact assays, where the aromatic compounds thymol, trans-cinnamaldehyde, and eugenol were shown to be more toxic than the nonaromatic compound geraniol. Presence of hydrocarbons in the chemical structure of essential oil components is related to the toxicity of fumigants (Kumbhar and Dewang 2001) and would explain the higher fumigant toxicity of *p*-Cymene compared with that of geraniol in our study and in other reports (Jang et al. 2005, Alzogaray et al. 2013). The opposite phenomenon occurs with the addition of the methoxy functional group to aromatic compounds. When compared with eugenol, the presence of the methoxy functional group in methyl eugenol caused a twofold and sixfold decrease in toxicity of topical and fumigant toxicity, respectively. These results are consistent with studies on American cockroaches that found that methyl eugenol was less toxic than eugenol (Ngoh et al. 1998).

We identified from an essential oil database (https://phytochem.nal.usda.gov/phytochem/search), natural sources of essential oil constituents that had exhibited insecticidal activity against Turkestan cockroaches, and then we determined their chemical composition. We pursued this analysis to confirm the presence of the bioactive essential oil constituents. A GC–MS analysis of red thyme oil showed that *p*-Cymene (29.4%), thymol (22.6%), and γ-terpinene (18.9) account for 71% of all oil constituents. The composition of red thyme essential oil is similar to that of indigenous Romanian red thyme crops, in which thymol (30.86%) and *p*-Cymene (30.53%) were the most abundant constituents (Grigore et al. 2010). Our results, however, contrasted with those of Shabnum and Wagay (2011), who, in Italy, found that the most abundant constituents of wild thyme were thymol (46.21%), γ-terpinene (14.08%), and *p*-Cymene (9.91%). In our study, a major constituent of clove bud oil was eugenol (64%), and the percentage did not differ greatly from other analyses of clove bud oil in China (88%; Tian et al. 2015) and Cameroon (83%; Nana et al. 2015). Even though the constituents in clove bud oils can differ, it is very likely that eugenol is the major constituent of clove essential oil in all places (Chaieb et al. 2007). Remarkable differences in content of constituents were observed in the Java citronella oil. While we identified citronellal (56.7%) as the most abundant constituent, followed by geraniol (9.3%), analysis made in Brazil reported geraniol (28.6%) and citronellal (23.6%) as the main constituents of this oil (Pinheiro et al. 2013). Intraspecific variability in the chemical composition of plant essential oils might be due to several factors, including phenological state of the plant (Salehi et al. 2014), plant part extracted (Santos et al. 2016), harvesting time (Salim et al. 2015), climatic and soil variation (Jianu et al. 2015), water level (Alavi-Samani et al. 2015), and presence of distinct chemotypic races of populations (Medina-Holguín et al. 2008). Despite existing variability in the chemical composition of plant essential oils, the bioactivity of plant essential oils, either as insecticides or repellents, is consistent across reported studies (Isman and Machial 2006).

We evaluated the biological activity of red thyme, clove bud, and Java citronella essential oils against Turkestan cockroach nymphs. During topical exposure, red thyme essential oil was as toxic as clove bud essential oil, but both were more toxic than Java citronella essential oil. In fumigant exposures, red thyme essential oil was the most toxic, followed by clove bud and Java citronella essential oils. The higher toxicity of red thyme oil, when compared with other oils, was probably due to the synergistic effect of thymol and *p*-Cymene with other secondary essential oil constituents. The acute toxicity of essential oils and constituents on cockroaches reported in this study may have implications for the control of this urban pest. Detection and direct sprays of harborages of cockroaches in peridomestic areas might reduce populations and prevent individuals from invading indoors areas.

Due to their volatile nature, certain essential oils produced by plants are known for their repellent effects on insects, and the use of a number of these compounds as perimeter treatments against cockroaches, ants, and termites has been proposed (Isman 2000). Effective repellents can be applied around doorframes, windows, and other potential entrance points to prevent the entrance of Turkestan cockroaches. We found in our study that all fresh dry residues of essential oils and one essential oil constituent, thymol, were avoided by Turkestan cockroaches. During analyses of locomotor activity in cockroach nymphs, it became clear that, when half of the arena was treated with thymol or essential oils, nymphs avoided (spent less time in) these areas (Fig. 1). Unlike responses of Chagas's vectors *Rodnius prolixus* and *Triatoma infestans* to some monoterpenes in which some compounds enhanced locomotor activity of the insects (Moretti et al. 2013), thymol and the other essential oil constituents in our study did not affect movement parameters of Turkestan cockroaches. The excitability of insects caused by essential oil constituents seems to vary in a concentration-dependent manner, and in some cases, it is observed only when insects are exposed to high concentrations of the chemicals (Moretti et al. 2013). Although we used relatively high concentrations of the compounds (14 mg/cm^2), it is very likely that the avoidance behavior displayed by the cockroaches could have reduced their exposure to doses that cause locomotor hyperactivity.

Nymphs tended to avoid areas with dry residues of geraniol, but no significant differences were detected. These results were unexpected, as geraniol has high repellent activity against other species of cockroaches (Phillips 2009, Alzogaray et al. 2013). As with evaluations with thymol, cockroaches avoided areas treated with the essential oils red thyme, clove bud, and Java citronella. Unlike evaluations with essential oil constituents, evaluations with the essential oils red thyme, clove bud, and Java citronella showed that locomotor activity of cockroaches is affected upon their contact with dry residues of these oils. Movement parameters such as distance traveled and percent of time moving were reduced more in essential oil-treated areas than in control untreated areas. These results indicate that these essential oils do not cause hyperactivity to Turkestan cockroaches, an undesirable effect that could promote dispersion of cockroaches to uninfested areas. Although repellent activity of essential oils is generally attributed to a specific compound, the observed effect on Turkestan cockroach nymphs might be due to a synergistic effect among essential oil constituents, which results in

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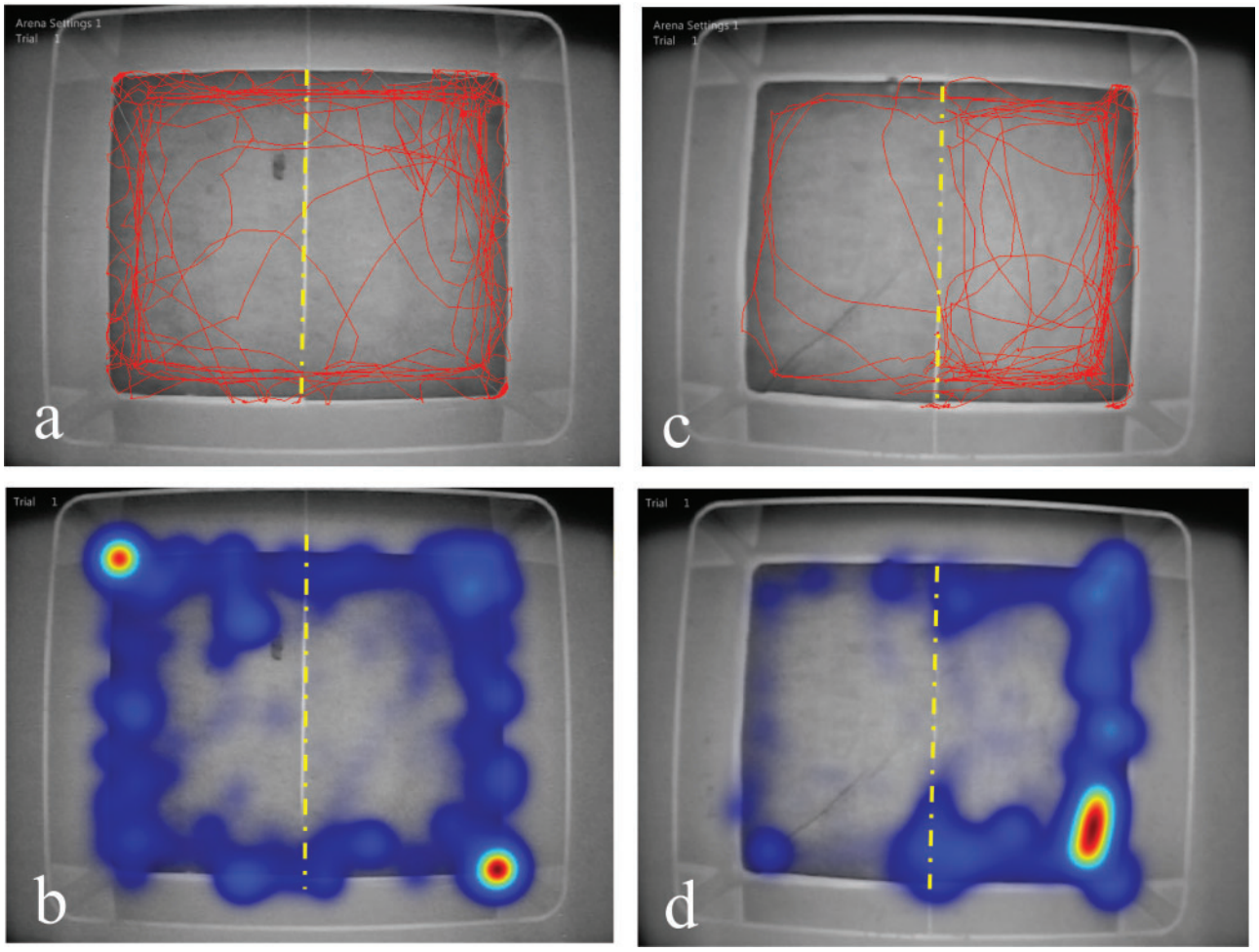

Fig. 1. Representative tracks and "heat maps" showing movement of individual nymphs over a 20-min period in an insecticide-free arena (**a and b**) (left half zone is treated with control solution and right half zone remains untreated); and a thymol-treated arena (**c and d**) left half zone is treated with 1% thymol solution and right half zone is treated with control solution. EthoVision generated "heat maps" that visualize a subject's frequency at specific positions based on a color gradient. Clustered data points appear near the red end of the gradient scale. In (**b**), the two clusters indicate that activity of nymphs was equally distributed in both zones, while in (**d**), a cluster in the insecticide-free zone (right half zone) indicates that the nymph was avoiding the thymol-treated zone (left half zone).

a higher bioactivity compared with that of isolated components (Hummelbrunner and Isman 2001, Nerio et al. 2010, Wu et al. 2015). Identifying the key synergistic constituents within complex essential oil mixtures could allow for the development of highly effective repellent agents (Hummelbrunner and Isman 2001). The results of our experiments potentially have valuable implications for the management of Turkestan cockroaches in areas where this species has become a problem. If laboratory evidence of the insecticidal activity of essential oils and essential oil constituents against *B. lateralis* applies under field conditions, these compounds could become an alternative to synthetic insecticides, mitigating potential issues related to environmental contamination and human insecticide exposure. In addition, essential oils may be useful as part of an integrated pest management program to repel cockroaches from points of entry into a structure or eliminate a harborage area such as a utility meter box. However, the formulations would not be effective as a stand-alone treatment for perimeter application against the Turkestan cockroach and other tactics (e.g., exclusion) need to be incorporated in these management programs.

A limiting factor that will need to be addressed is that plant-derived products are relatively volatile materials, and are likely to evaporate quickly when applied on different substrates. Furthermore, these compounds are meant to be applied mostly in outdoor areas, where exposure to sun and air might decrease their insecticidal properties. Several systems have been proposed to delay the volatilization of active ingredients and to protect them from environmental factors that cause their degradation. Microencapsulated techniques with cotton textile (Specos et al. 2010), silicone (Tarelli et al. 2009), starch (Soottitantawat et al. 2005), or polyester textile (Anitha et al. 2011) are alternatives to prolong the effectivity of essential oil compounds.

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