JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Repellent Activity of Essential Oils and Some of Their Individual Constituents against *Tribolium castaneum* Herbst

Karina Caballero-Gallardo,[†] Jesús Olivero-Verbel,[*,†,§] and Elena E. Stashenko[§]

[†]Environmental and Computational Chemistry Group, Faculty of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia

[§]Chromatography Laboratory, Research Centre of Excellence, CENIVAM, Industrial University of Santander, Bucaramanga, Colombia

ABSTRACT: A tool for integrated pest management is the use of essential oils (EOs) and plant extracts. In this study, EOs from *Tagetes lucida*, *Lepechinia betonicifolia*, *Lippia alba*, *Cananga odorata*, and *Rosmarinus officinalis*, species grown in Colombia, were analyzed by gas chromatography−mass spectrometry. These oils as well as several of their constituents were tested for repellent activity against *Tribolium castaneum*, using the area preference method. The main components (>10%) found in EOs were methylchavicol, limonene/α-pinene, carvone/limonene, benzyl acetate/linalool/benzyl benzoate, and α-pinene, for *T. lucida*, *L. betonicifolia*, *L. alba*, *C. odorata*, and *R. officinalis*, respectively. All EOs were repellent, followed a dose−response relationship, and had bioactivity similar to or better than that of commercial compound IR3535. EOs from *C. odorata* and *L. alba* were the most active. Compounds from EOs, such benzyl benzoate, β-myrcene, and carvone, showed good repellent properties. In short, EOs from plants cultivated in Colombia are sources of repellents against *T. castaneum*.

KEYWORDS: Pests, insects, Colombia, *Cananga odorata*, *Lippia alba*

INTRODUCTION

Pest control requires the use of chemicals having great specificity, safety, and ecofriendly properties. This need has increased the search for natural products derived from plants as alternatives to conventional or synthetic compounds.[1] Essential oils (EOs), mixtures containing volatile compounds from plants, have been widely used as bioactive agents for this purpose. In fact, these chemicals have proven to be effective as insecticides,[2−8] ovicidals,[9] antifeedants,[10−12] oviposition inhibitors,[13,14] and repellents,[6,15−17] and may also affect some biological parameters such as growth rate, life span, and insect reproduction.[2,18−20] In industrialized nations, such as the United States, EOs are important alternatives to synthetic insecticides during organic food production, whereas in developing countries, their use could help in agriculture and food protection.[21,22]

Although there is a large diversity of food pests that requires control in crop production and storage, species such as *Tribolium castaneum* Herbst (red flour beetle) are of particular economic importance due to its worldwide distribution and preference for grain products in flour mills, warehouses, and grocery stores.[23,24] Currently, the control of this insect is carried out using several synthetic products, for which adverse effects have been reported in animals and humans.[25] Moreover, botanical extracts and EOs have also been successfully used in controlling this insect.[26,27] It has been reported that EOs from *Ocimum basilicum* L (Labiatae) were highly toxic and repellent against *T. castaneum* adults when applied topically or impregnated on filter papers, on grains, or on glass pebbles.[28] Similar activities have been reported for EOs from *Baccharis salicifolia* (Asteraceae),[24] *Artemisia annua* (Asteraceae),[27,29] *Nigella sativa* (Ranunculaceae), *Trachyspermum ammi* (Umbelliferae), and *Anethum graveolens* (Umbelliferae).[8]

Molecules present in EOs have also shown interesting properties for pest control. For instance, compounds such as α-pinene, eugenol, limonene, terpineol, citronellol, citronellal, camphor, and thymol have been recognized to have good repellent properties.[30−33] The aim of this paper was to evaluate the repellent properties of EOs obtained from plants grown in Colombia, as well as those elicited by individual compounds that are commonly found in EOs, against *T. castaneum* Herbst.

MATERIALS AND METHODS

Plant Material. Plant material was obtained from the experimental agroindustrial station of the Industrial University of Santander (Bucaramanga, Colombia), from species cultivated from certified seeds except for *Lepechinia betonicifolia* (Labiatae), which was obtained from the wilderness. Aerial parts of plants were employed to extract EOs from *Lippia alba* (Verbenaceae), *L. betonicifolia*, and *Tagetes lucida* (Asteraceae). Leaves and flowers were used for EOs from *Rosmarinus officinalis* (Labiatae), and *Cananga odorata* (Annonaceae), respectively.

Essential Oil Isolation. The EOs were obtained from the aerial parts or particular plant parts (ca. 150 g in 1 of L water). Microwave-assisted hydrodistillation (MWHD) was carried out as described elsewhere;[34,35] briefly, a Clevenger-type hydrodistillation apparatus was placed inside a domestic microwave oven (LG, 1100 W, 2.45 GHz) with a side orifice through which an external glass condenser joined the 2 L round flask with the plant material (ca. 300 g) and water (ca. 0.5 L), inside the oven. The oven was operated for 40 min (4 × 10 min) at full power, which caused the water to boil vigorously and reflux. Essential oil was decanted from the condensate and dried with anhydrous sodium

Received: October 13, 2010
Accepted: December 29, 2010
Revised: December 28, 2010
Published: February 03, 2011

dx.doi.org/10.1021/jf103937p | J. Agric. Food Chem. 2011, 59, 1690–1696

sulfate. For chromatographic analysis, neat essential oil (50 μL) and *n*-tetradecane (0.5 μL) were dissolved in dichloromethane (1.0 mL final volume, chromatography-grade reagent; Merck, Darmstadt, Germany).

Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. Compound identification of EOs isolated from plants was based on chromatographic (retention times and Kovats indices) and spectroscopic (mass spectra interpretation and database searching) criteria,[36−40] as well as on comparison of retention indices and mass spectra with those of authentic samples. The spectroscopic and chromatographic data were obtained with a gas chromatograph, GC 6890 Plus (Agilent Technologies, Palo Alto, CA) equipped with a mass selective detector MSD 5975 (electron impact ionization, EI, 70 eV; Agilent Technologies), split/splitless injector (1:30 split ratio), a 7863 automatic injector, and a MS-ChemStation G1701-DA data system, which included the spectral libraries Wiley, NIST, and QUADLIB 2007. A fused-silica capillary column DB-5MS (J&W Scientific, Folsom, CA) of 60 m × 0.25 mm i.d., coated with 5% phenyl poly(methylsiloxane) (0.25 μm film thickness), and a DB-WAX (J&W Scientific) 60 m × 0.25 mm i.d. capillary column, coated with poly(ethyleneglycol) (0.25 μm film thickness), were used. With the DB-5MS column, the GC oven temperature was programmed from 45 °C (5 min) to 150 °C (2 min) at 4 °C/min, then to 250 °C (5 min) at 5 °C/min, and finally to 275 °C (15 min) at 10 °C/min. For the DB-WAX column, the oven temperature was programmed from 45 °C (5 min) to 150 °C (3 min) at 3 °C/min and then to 220 °C (5 min) at 4 °C/min. The temperatures of the injection port, ionization chamber, and transfer line were set at 250, 230, and 285 °C, respectively. Helium (99.995%, Aga Fano, Bucaramanga, Colombia) was used as carrier gas, with 155 kPa column head pressure and 27 cm/s linear velocity (1 mL/min, at constant flow). Mass spectra and reconstructed (total) ion chromatograms were obtained by automatic scanning in the mass range *m*/*z* 30−300 at 5.1 scan/s. Chromatographic peaks were checked for homogeneity with the aid of the mass chromatograms for the characteristic fragment ions and with the help of the peak purity function of the MS-Chemstation G1701-DA software. Extracted ion chromatograms were employed as tools to simplify the chromatographic profile during the peak assignment process.

GC-FID Analysis. A gas chromatograph GC 7890 (Agilent Technology, Palo Alto, CA), equipped with a flame ionization detection (FID), a split/splitless injector (1:30 split ratio), and a data system (HP-ChemStation Rev. B.03.02 [341]), was used for GC analysis and essential oil quantification. The detector and the injector temperatures were set at 250 °C. A DB-5 (J&W Scientific) 60 m × 0.25 mm i.d. capillary column, coated with 5% phenyl poly(methylsiloxane) (0.25 μm film thickness), and a DB-WAX column of the same characteristics as that employed in GC-MS were used for linear retention index determination. The oven temperature for both columns was programmed identically as for the GC-MS analysis described above. Helium (99.995%, Aga Fano) was used as carrier gas, with 170 kPa column head pressure and 22.95 cm/s linear velocity (1 mL/min, at constant flow). Hydrogen and air at 30 and 300 mL/min, respectively, were used in the FID, with nitrogen (30 mL/min) as makeup gas. For semiquantification of the essential oil composition, the normalized peak area of each compound was calculated as the mean value of three injections using the DB-5 column.

Individual Compounds Found in Essential Oils. The following compounds that are frequently found in EOs were evaluated in this study: geranyl acetate, 98%; β-citronellol, 95%; benzyl benzoate, 99%; β-myrcene, 95%; carvacrol, 98%; citral, 95%; geraniol, 98%; nerol, 97%; *p*-cymene, 99%; *R*(−)carvone, 98%; and *S*(+)carvone, 96%. These were purchased from Sigma-Aldrich (Steinheim, Germany). Benzyl salicylate, 99%, was obtained from Fluka (Buchs, Switzerland).

Insects and Rearing Conditions. All experiments were conducted in the laboratory using long-established colonies of *T. castaneum* obtained from a supermarket located in Cartagena, Colombia. In the laboratory the strain was maintained in glass containers covered by a plastic mesh. Insects were reared on a diet of whole oat flour and kept at 26 ± 2 °C and 70−85% relative humidity (RH) with a 10:14 h light/dark photoperiod. Red flour beetle adults of both sexes were collected, and those with size between 3.0 and 4.5 mm were used in the experiments.

Repellency Tests. The repellent effects of the EOs and some of their individual components against *T. castaneum* were tested using the area preference method.[15] The solutions of EOs or compounds were prepared in acetone, and in all cases, a volume of 0.5 mL was applied to a half-filter paper disk, as uniformly as possible, to obtain the desired oil volume per unit area of 0.00002, 0.0002, 0.002, 0.02, and 0.2 μL/cm^2, using acetone as solvent. The other half of the filter paper was treated with an equal volume of acetone as a vehicle control. Test areas consisted of 9 cm Whatman no. 1 filter paper cut in half (31.8 cm^2). As a positive control, a 15% formulation of IR3535 [ethyl 3-(*N*-acetyl-*N*-butylamino) propionate], a well-known arthropod repellent, was used under the same conditions as the oils. The treated and control half disks were air-dried for 10 min to evaporate the solvent. Treated and untreated halves were reattached using adhesive tape and placed in 90 mm glass Petri dishes. Twenty adults of *T. castaneum* of both sexes were released at the center of each filter paper disk. Dishes were covered and placed in darkness at 22−24 °C and 75 ± 10% RH. The numbers of *T. castaneum* present on the treated and untreated portions of the experimental paper halves were recorded for each dish after 2 and 4 h of exposure. Percentage repellency (PR) for a given exposure time was computed as follows: $PR = [(N_c - N_t)/(N_c + N_t)] \times 100$, where N_c and N_t were the number of insects on the untreated (control) and treated areas, respectively. Five replicates were used for each tested concentration of both EOs and individual compounds.

Data Analysis. The mean number of insects on the treated portion of the filter paper was compared with the number found on the untreated area using the paired *t* test. If significant differences occurred, repellency or attractancy was established depending on whether the percentage repellency was positive or negative, respectively. In all cases, normal distribution and equality between variances were previously evaluated by Kolmogorov−Smirnov and Bartlett's tests, respectively. Comparisons between mean PR for different EOs or individual compounds were performed using ANOVA, with Dunn's post-test used to compare treated with control-vehicle groups. Spearman correlation was used to establish relationships between repellency and physicochemical parameters of compounds. Statistical analysis was performed with GraphPad InStat,[41] and the significance was set at $P < 0.05$.

RESULTS

The chemical compositions of examined EOs are presented in Table 1. The results revealed quite different compositions, the most abundant compounds being methylchavicol, limonene/α-pinene, carvone/limonene, benzyl acetate/linalool/benzyl benzoate, and α-pinene for the EOs of *T. lucida*, *L. betonicifolia*, *L. alba*, *C. odorata*, and *R. officinalis*, respectively.

The results of repellency assays for tested EOs are presented in Table 2. Data showed that at tested concentrations, most EOs were strongly repellent against *T. castaneum*, and these bioactivities followed clear dose−response relationships. At the lowest assayed concentrations (0.00002 and 0.0002 μL/cm^2), the oil isolated from *T. lucida* showed some insect attractant properties (−60 ± 3% and −6 ± 15%, 2 h after exposure; and −62% ± 4% and −11% ± 17%, 4 h after exposure).

The PRs for most tested oils were similar when compared at both exposure times (Table 2). After 2 h of exposure, oils from *L. alba* and *C. odorata* were significantly more repellent than IR3535 at concentrations of 0.0002 and 0.2 μL/cm^2. At 4 h after exposure, such statistical differences for both oils were observed

Table 1. Compounds (>0.1%) Found in Tested Essential Oils

compound	Kovats indices		relative amount, %				
	DB-5	DB-WAX	*Tagetes lucida*	*Lepechinia betonicifolia*	*Lippia alba*	*Cananga odorata*	*Rosmarinus officinalis*
α-pinene[a]	933	1028		19.4	0.2	1.5	19.6
camphene[a]	950	1073		3.6	0.5		3.8
sabinene	969	1125		3.9			0.1
β-pinene	975	1112		9.5	0.1	0.6	2.6
β-myrcene[a]	988	1152	5.9	2.5	1.5	0.2	2.1
p-methylanisole	1017	1446				8.9	
β-phellandrene[a]	1025	1165		1.7			0.5
limonene[a]	1026	1196		27.5	35.0	0.1	8.2
1,8-cineole[a]	1028	1223				0.2	9.1
trans-*β*-ocimene	1045	1251	1.3		1.1	0.1	
methyl benzoate	1089	1624				10.0	
linalool[a]	1095	1543	0.3		0.7	14.1	4.5
camphor[a]	1142	1496		3.1			3.7
benzyl acetate	1165	1736				18.2	
borneol[a]	1166	1718		0.7	0.3		4.3
α-terpineol[a]	1192	1685					3.3
methylchavicol[a]	1222	1675	92.1				
carvone[a]	1241	1685		0.2	35.3		
piperitone	1249	1692			3.4		
bornyl acetate	1287	1863		1.0			0.8
β-bourbonene	1387	1524		0.4	1.6		
trans-*β*-caryophyllene[a]	1420	1597		6.8	0.2	3.8	1.4
cinnamyl acetate[a]	1446	2148				4.3	
α-humulene[a]	1452	1674		2.0		1.0	0.1
germacrene D	1485	1710		2.9	0.1	7.8	
bicyclosesquiphellandrene	1488	1624			9.6	0.5	
germacrene D-4-ol	1584	1967		1.2			
caryophyllene oxide[a]	1584	2008		1.2			
benzyl benzoate[a]	1784	2735				12.3	

[a] The identification of these compounds was based on a comparison of retention indices and mass spectra with those of authentic samples.

Table 2. Percentage Repellency[a] (PR) after Two Exposure Times for Five Essential Oils against *Tribolium castaneum*

essential oil	2 h					4 h				
	0.00002 μL/cm^2	0.0002 μL/cm^2	0.002 μL/cm^2	0.02 μL/cm^2	0.2 μL/cm^2	0.00002 μL/cm^2	0.0002 μL/cm^2	0.002 μL/cm^2	0.02 μL/cm^2	0.2 μL/cm^2
Tagetes lucida	−60 ± 3*[a]	−6 ± 15	28 ± 18	62 ± 10*	84 ± 6*	−62 ± 4*[a]	−11 ± 17	26 ± 20	61 ± 6*	90 ± 3*
Lepechinia betonicifolia	8 ± 15	10 ± 9	60 ± 8*	68 ± 4*	94 ± 4*[a]	10 ± 14	12 ± 28	56 ± 16*	60 ± 16*	92 ± 4*
Lippia alba	34 ± 8*	58 ± 10*[a]	60 ± 7*	82 ± 2*	96 ± 2*[a]	32 ± 11*	40 ± 19	54 ± 8*	82 ± 13*[a]	96 ± 2*[a]
Cananga odorata	76 ± 3*[a]	84 ± 2*[a]	88 ± 4*	92 ± 2*[a]	98 ± 2*[a]	82 ± 6*[a]	76 ± 6*[a]	72 ± 9*	80 ± 8*	98 ± 2*[a]
Rosmarinus officinalis	32 ± 14	58 ± 9*[a]	64 ± 11*	74 ± 7*	82 ± 4*[b]	32 ± 14	56 ± 12*	70 ± 15*	74 ± 9*	92 ± 4*
IR3535	2 ± 8	16 ± 9	54 ± 12*	60 ± 13*[b]	78 ± 5*	−6 ± 18	4 ± 22	40 ± 11	50 ± 5	75 ± 8

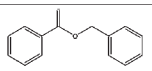
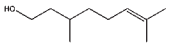
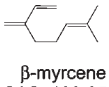
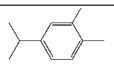
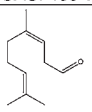
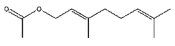
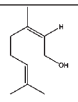
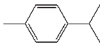
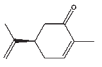
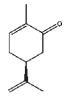
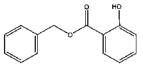
[a] Values are mean ± SE of five replicates. Positive values represent repellency, and negative values show attractant activity. *, significant difference between the number of the organisms on both the treated and untreated halves, using a paired *t* test ($P < 0.05$); [a], significant difference between essential oils and the positive control (IR3535), using ANOVA, with Dunn's post-test; [b], significant difference between the percentage repellency 2 and 4 h after exposure.

only at 0.2 μL/cm^2. Moreover, at concentrations >0.02 μL/cm^2, the repellent properties depicted by the oils were similar to or better than those measured for IR3535.

The essential oil from *C. odorata* reached the highest PR, 98% at 0.2 μL/cm^2. However, together with those from *L. alba* and *L. betonicifolia*, these oils registered PR values of >90% after 2 and 4 h of exposure, indicating that they can be considered excellent candidates as natural repellents.

PRs and some physicochemical properties of individual compounds are presented in Table 3. All tested constituents of the EOs

Table 3. Physicochemical Properties and Percentage Repellency (PR) after Two Exposure Times against *Tribolium castaneum* of 12 Compounds Present in Essential Oils

Main compounds	Molecular weight	Boiling point (°C)	LogP	Concentration (μL/cm^2)	Repellency[a] (%) after exposure time	
					2h	4h
 Benzyl benzoate CAS: 120-51-4	212.25	324	3.97	0.00002 0.0002 0.002 0.02 0.2	3±10 14±9 16±6* 44±10* 93±4*	6±18 18±10 23±10* 44±10* 91±5*
 Citronellol CAS: 106-22-9	156.27	225	3.91	0.00002 0.0002 0.002 0.02 0.2	30±13 45±10* 59±8* 74±8* 96±2*	40±16* 43±13* 60±9* 85±8* 96±3*
 β-myrcene CAS: 123-35-3	136.24	167	4.17	0.00002 0.0002 0.002 0.02 0.2	28±11* 33±14 56±10* 61±9* 85±6*	25±12* 30±14 65±11* 71±13* 93±3*
 Carvacrol CAS: 499-75-2	150.22	238	3.49	0.00002 0.0002 0.002 0.02 0.2	8±10 9±9 -3±42 46±11* 86±4*	5±11 8±11 -6±15 48±14* 90±3*
 Citral CAS: 5392-40-5	152.24	227	3.45	0.00002 0.0002 0.002 0.02 0.2	18±13 64±6* 66±6* 89±4* 98±2*	21±17 51±7* 55±10* 79±5* 98±2*
 Geraniol CAS: 106-24-1	154.25	230	3.56	0.00002 0.0002 0.002 0.02 0.2	6±8 43±4* 64±3* 75±4* 98±2*	4±9 35±5* 58±7* 74±4* 95±3*
 Geranyl acetate CAS: 105-87-3	196.29	240	4.04	0.00002 0.0002 0.002 0.02 0.2	5±10 21±12* 21±6* 60±17* 95±2*	8±13 23±16 24±8* 63±6* 94±2*
 Nerol CAS: 106-25-2	154.25	225	3.47	0.00002 0.0002 0.002 0.02 0.2	8±14 39±6* 64±4* 84±4* 98±2*	8±7 30±7* 60±8* 75±4* 95±4*
 *p*-cymene CAS: 99-87-6	134.22	177	4.10	0.00002 0.0002 0.002 0.02 0.2	-6±12 -10±11 43±8* 61±5* 74±4*	-5±21 -8±13 44±14* 45±7* 69±5*
 R(-) carvone CAS: 6485-40-1	150.22	229	2.71	0.00002 0.0002 0.002 0.02 0.2	21±9 35±9* 55±7* 84±4* 100±0*	19±9* 44±10 68±6* 74±5* 95±2*
 S(+) carvone CAS: 99-49-0	150.22	231	3.07	0.00002 0.0002 0.002 0.02 0.2	-10±12 45±5* 53±5* 91±2* 96±2*	-10±13 21±9* 30±4* 85±5* 94±3*
 Benzyl salicylate CAS: 118-58-1	226.28	320	4.31	0.00002 0.0002 0.002 0.02 0.2	-29±9 29±12* 65±4* 74±4* 94±3*	-34±14 25±8 46±8* 78±7* 99±1*

[a] Values are mean ± SE of five replicates. Positive values represent repellency, and negative values show attractant activity. *, significant difference between number of organisms on both treated and untreated halves, using a paired *t* test ($P < 0.05$).

Table 4. Spearman Correlation Values between Percentage Repellency (PR) Observed at Different Exposure Times and Physicochemical Parameters

parameter	PR[a] 0.00002 (2 h)	PR 0.0002 (2 h)	PR 0.002 (2 h)	PR 0.02 (2 h)	PR 0.2 (2 h)	boiling point (°C)	mol wt	LogP
PR 0.00002 (4 h)	1	0.372 (0.234)	0.181 (0.571)	0.083 (0.798)	0.293 (0.355)	**−0.577 (0.050)**	−0.246 (0.442)	−0.270 (0.397)
PR 0.0002 (4 h)	0.626 (0.029)	1	0.688 (0.013)	0.822 (0.001)	0.739 (0.006)	−0.254 (0.425)	0.078 (0.810)	−0.543 (0.068)
PR 0.002 (4 h)	0.546 (0.066)	0.770 (0.003)	1	0.820 (0.001)	0.368 (0.240)	−0.214 (0.505)	−0.199 (0.536)	−0.111 (0.732)
PR 0.02 (4 h)	0.137 (0.671)	0.608 (0.036)	0.424 (0.170)	1	0.743 (0.006)	−0.296 (0.351)	−0.175 (0.585)	**−0.643 (0.024)**
PR 0.2 (4 h)	0.186 (0.564)	0.781 (0.003)	0.484 (0.111)	0.781 (0.003)	1	0.002 (0.996)	0.210 (0.512)	**−0.737 (0.006)**
boiling point	−0.496 (0.101)	−0.317 (0.314)	**−0.670 (0.017)**	−0.178 (0.581)	0.042 (0.896)	1	0.659 (0.020)	0.032 (0.922)
mol wt	−0.076 (0.814)	0.125 (0.698)	−0.207 (0.519)	0.143 (0.657)	0.512 (0.088)	0.659 (0.020)	1	0.247 (0.439)
LogP	−0.158 (0.625)	−0.382 (0.221)	−0.158 (0.625)	−0.379 (0.224)	−0.166 (0.607)	0.031 (0.922)	0.425 (0.169)	1

[a] Data to the right and left of the diagonal correspond to correlations obtained using repellency data taken after 2 and 4 h exposure, respectively.

exhibited bioactivity against *T. castaneum*, with some values better than those observed for the commercial repellent. With the exception of *p*-cymene, most PRs were >90% at a concentration of 0.2 μL/cm^2 at 4 h after exposure. The least active was *p*-cymene, which showed attractant activity at the lower concentrations and both exposure times (−6% ± 12% and −10% ± 11%, 2 h after exposure; and −5% ± 21% and −8% ± 13%, 4 h after exposure).

DISCUSSION

T. castaneum is a major pest of wheat grain flour.[23] The control of this organism is mostly achieved using synthetic chemicals as fumigants, but frequently those might cause adverse effects on nontarget animals and humans.[25] Results presented here have shown that several plants cultivated in Colombia possess a variety of different chemicals with good repellent activities toward this insect. Importantly, these oils have similar or better repellent properties than those elicited by IR3535, a commercial repellent used in public health applications for human skin and clothing.[42] Among examined EOs, *C. odorata* was significantly more effective than IR3535 at most tested concentrations.

The oil from *L. alba* had not been previously reported as repellent against *T. castaneum*, although *Lippia* species have been used as insect repellent against *Anopheles gambiae*.[43] Interestingly, although sharing some minor components, the repellent activity of the essential oil of *L. alba* was similar to that observed for *R. officinalis*. This last essential oil has shown repellent properties against insects such as *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*.[44]

The PRs for most tested oils were similar when compared at both exposure times (Table 2). After 2 h of exposure, oils from *L. alba* and *C. odorata* were significantly more repellent than IR3535 at concentrations of 0.0002 and 0.2 μL/cm^2. At 4 h after exposure, such statistical differences for both oils were observed only at 0.2 μL/cm^2. Moreover, at concentrations >0.02 μL/cm^2, the repellent properties shown by the oils were similar to or better than those measured for IR3535.

Several species belonging to the *Lepechinia* genus have been found to have insecticidal activity against *A. aegypti* larvae.[45] However, to the best of our knowledge, this is the first report presenting the repellent activity of the EO isolated from *L. betonicifolia*, found in Colombia, against *T. castaneum*. Among tested oils, it was the one with the greatest PR and moderately better than the commercial repellent IR3535.

The insecticidal constituents of EOs studied here were mainly monoterpenoids, as also found for most of these mixtures.[46−48] Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects and interfere with their physiological functions.[49] Due to their high volatility, they have fumigant properties and might be of importance for the control of stored-product insects.[48] The insecticidal activity of EOs depends on the action of their components. For instance, limonene, found in *L. alba* (35%), has been referenced to have fumigant toxicity against *T. castaneum*.[50] Linalool, found in *C. odorata* (14.1%) and *R. officinalis* (4.5%), and β-pinene, present in *R. officinalis* (2.6%), have shown toxic effects on *Sitophilus oryzae*.[46,51] Limonene and camphor have also been reported to possess repellent effects,[52] and both are constituents of the essential oil extracted from *R. officinalis* (8.2 and 3.7%, respectively). Similarly, α-terpineol has been shown to work as a repellent against *T. castaneum* Herbst adults,[24] and it is found in *R. officinalis* (3.3%).

On the other hand, it is important to mention that, taken individually, constituents of EOs have shown better repellent activities against *T. castaneum* than those recorded for EOs and the commercial repellent. Compounds such as β-myrcene, benzyl benzoate, and carvone, which presented good repellent properties, can be part of the composition of one or some of the EOs examined here, including *C. odorata*, *T. lucida*, *R. officinalis*, *L. betonicifolia*, and *L. alba*, suggesting that the activity of these EOs could be linked to the presence of these chemicals.

The physicochemical or structure-based properties that allow molecules to behave as repellent are not well-known. However, some studies have shown that lipophilicity, vapor pressure, boiling point, molecular length, and principal moment of inertia, among others, could be good descriptors for repellency.[53−57] To determine if there are quantitative relationships between measured PRs of tested compounds and some basic physicochemical parameters for these molecules, a Spearman correlation analysis was performed between the biological data at both exposure times and molecular properties such as LogP, boiling point, and molecular weight. The results are presented in Table 4. The data showed that the boiling point presented significant and inverse correlations with the repellent bioactivity observed at the lowest (0.00002 μL/cm^2) tested concentration 2 h after exposure (R = −0.577, P = 0.050) and with an intermediate one (0.002 μL/cm^2) 4 h after exposure (R = −0.670, P = 0.017). On the other hand, LogP was significantly and inversely correlated (R = −0.643, P = 0.024; R = −0.737, P = 0.006) with the bioactivity observed at the highest tested concentrations (0.02 and 0.2 μL/cm^2) 2 h after exposure. These results indicate that both LogP and boiling point are important, but not the only, parameters that determine the repellency properties of these compounds.

It should be kept in mind that although some of the studied EOs presented promising repellent properties against *T. castaneum*,

several issues must be addressed before this use becomes a practical reality. Customer approval, formulation, and nontarget toxicity studies, among others, should be conducted to establish the feasibility of possible applications for these natural products.[58,59] However, being natural ingredients with good organoleptic and antimicrobial characteristics, these essential oils are gaining acceptance not only as insect repellents but also as preservatives or to increase safety in different foodstuffs.[60,61]

In conclusion, the examined EOs had similar or better repellent characteristics than IR3535, and this property could be related to the presence of some particular compounds in these mixtures. These results have shown that EOs from Colombian flora are an important source of natural and potent repellents against *T. castaneum*, being a plausible alternative to the current commercial repellents used to control this organism.

AUTHOR INFORMATION

Corresponding Author

*Postal address: Environmental and Computational Chemistry Group, Faculty of Pharmaceutical Sciences, Campus of Zaragocilla, University of Cartagena, Cartagena, Colombia. Phone: 57-5-6698179. Fax: 57-5-6698323. E-mail: jesusolivero@yahoo.com or joliverov@unicartagena.edu.co.

Funding Sources

This work was supported by COLCIENCIAS (Bogotá, Colombia), CENIVAM, and the University of Cartagena (Cartagena, Colombia), Grant RC 432-2004.

REFERENCES

(1) Pavela, R. Effectiveness of some botanical insecticides against *Spodoptera littoralis* Boisduvala (Lepidoptera: Noctudiae), *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae). *Plant Prot. Sci.* **2009**, *45*, 161–167.

(2) Saxena, C. R.; Dixit, P. D.; Harshan, V. Insecticidal action of *Lantana camara* against *Callosobruchus chinensis* (Coleoptera. Bruchidae). *J. Stored Prod. Res.* **1992**, *28*, 279–281.

(3) Weaver, K. D.; Dunkel, V. F.; Potter, C. R.; Ntezurubanza, L. Contact and fumigant efficacy of powdered and intact *Ocimum canum* Sims (Lamiales: Lamiaceae) against *Zabrotes subfasciatus* (Boheman) adults (Coleoptera: Bruchidae). *J. Stored Prod. Res.* **1994**, *30*, 243–252.

(4) Schmidt, H. G.; Streloke, M. Effects of *Acorus calamus* (L.) (Araceae) oil and its main compound β-asarone on *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *J. Stored Prod. Res.* **1994**, *30*, 227–235.

(5) Verma, N.; Tripathi, A. K.; Prajapati, V.; Bahl, J. R.; Khanuja, S. P. S.; Kumar, S. Toxicity of essential oil from *Lippia alba* towards stored grain insects. *J. Med. Arom. Plant Sci.* **2000**, *22*, 50.

(6) Tripathi, A. K.; Prajapati, V.; Aggrawal, K. K.; Khanuja, S. P. S.; Kumar, S. Toxicity towards *Tribolium castaneum* in the fraction of essential oil of *Anethum sowa* seeds. *J. Med. Arom. Plant Sci.* **2000a**, *22*, 40.

(7) Isman, M. B.; Wan, A. J.; Passreiter, C. M. Insecticidal activity of essential oils to the *tobacco cutworm*, *Spodoptera litura*. *Fitoterapia* **2001**, *72*, 65–68.

(8) Chaubey, M. K. Insecticidal activity of *Trachyspermum ammi* (Umbelliferae), *Anethum graveolens* (Umbelliferae) and *Nigella sativa* (Ranunculaceae) essential oils against stored-product beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *Afr. J. Agric. Res.* **2007**, *2*, 596–600.

(9) Tunc, I.; Berger, B. M.; Erler, F.; Dagh, F. Ovicidal activity of essential oils from five plants against two stored-product insects. *J. Stored Prod. Res.* **2000**, *36*, 161–168.

(10) Huang, Y.; Tan, J. M. W. L.; Kini, R. M.; Ho, S. H. Toxicity and antifeedant action of nutmeg oil against *T. castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. Stored Prod. Res.* **1997**, *33*, 289–298.

(11) Faini, F.; Labbe, C.; Torres, R.; del le Monache, G.; Coll, J. Eudesmane derivatives from *Flourensia thurifera*: structure and biological activity. *Nat. Prod. Lett.* **1997**, *11*, 1–4.

(12) Huang, Y.; Lam, S. L.; Ho, S. H. Bioactivities of essential oil from *Elletaria cardamomum* (L) Maton to *Sitophilus zeamais* Motsch and *Tribolium castaneum* (Herbst). *J. Stored Prod. Res.* **2000**, *36*, 107–117.

(13) Sarac, A.; Tunc, I. Toxicity of essential oil vapours to stored-product insects. *J. Plant. Dis. Protect.* **1995**, *102*, 69–74.

(14) Ho, S. H.; Koh, L.; Ma, Y.; Huang, Y.; Sim, K. Y. The oil of garlic, *Allium sativum* L (Amaryllidaceae), as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamaiz* (Mostch). *Postharvest Biol. Technol.* **1996**, *9*, 41–48.

(15) Tapondjou, A.; Adler, C.; Fontem, D.; Bouda, H.; Reichmuth, C. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. *J. Stored Prod. Res.* **2005**, *41*, 91–102.

(16) Wang, J.; Zhua, F.; Zhoua, X. M.; Niua, C. Y.; Lei, C. L. Repellent and fumigant activity of essential oil from *Artemisia vulgaris* to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J. Stored Prod. Res.* **2006**, *42*, 339–347.

(17) Ogendo, J. O.; Kostyukovsky, M.; Ravid, U.; Matasyoh, J. C.; Deng, A. L.; Omolo, E. O.; Kariuki, S. T.; Shaaya, E. Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. *J. Stored Prod. Res.* **2008**, *44*, 328–334.

(18) Regnault-Roger, C.; Hamraoui, A. Inhibition of reproduction of *Acanthoscelides obtectus* Say (Coleoptera), a kidney bean (*Phaseolus vulgaris*) bruchid, by aromatic essential oils. *Crop. Prot.* **1994**, *13*, 624–628.

(19) Pascual-Villalobos, J. M. Evaluation of the insecticidal activity of *Chrysanthemum coronarium* L. plant extracts. *Plagas* **1996**, *22*, 411–420.

(20) Gurusubramanian, G.; Krishna, S. S. The effects of exposing eggs of four cotton insects pests to volatiles of *Allium sativum* (Liliaceae). *Bull. Entomol. Res.* **1996**, *86*, 29–31.

(21) Isman, M. B. Plant essential oils as green pesticides for pest and disease management. *Agricultural Applications in Green Chemistry*; Nelson W. M., Ed.; ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

(22) Isman, M. B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **2006**, *51*, 45–66.

(23) Howe, R. W. Losses caused by insects and mites in stored foods and foodstuffs. *Nutr. Abstr. Rev.* **1965**, *35*, 285–302.

(24) García, M.; Donadel, O. J.; Ardanaz, C. E.; Tonn, C. E.; Sosa, M. Toxic and repellent effects of *Baccharis salicifolia* essential oil on *Tribolium castaneum*. *Pest Manag. Sci.* **2005**, *61*, 612–618.

(25) Okonkwo, E. U.; Okoye, W. I. The efficacy of four seed powders and the essential oils as protectants of cow-pea and maize grain against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in Nigeria. *Int. J. Pest Mang.* **1996**, *42*, 143–146.

(26) Emara, T. E.; Ryan, M. F. Effect of two crude botanical extracts on the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *J. Egypt. Ger. Soc. Zool.* **1997**, *23*, 125–140.

(27) Tripathi, A. K.; Prajapati, V.; Aggarwal, K. K.; Khanuja, S. P.; Kumar, S. Repellency and toxicity of oil from *Artemisia annua* to certain stored-product beetles. *J. Econ. Entomol.* **2000**, *93*, 43–47.

(28) Obeng-ofori, D.; Reichmuth, C. H. Bioactivity of eugenol, a major component of essential oil of *Ocimum suave* (Wild) against four species of stored-product Coleoptera. *Int. J. Pest Manag.* **1997**, *43*, 89–94.

(29) Goel, D.; Goel, R.; Singh, V.; Ali, M.; Mallavarapu, G.; Kumar, S. Composition of the essential oil from the root of *Artemisia annua*. *J. Nat. Med.* **2007**, *61*, 458–461.

(30) Ibrahim, J.; Zaki, Z. M. Development of environment-friendly insect repellents from the leaf oils of selected Malaysian plants. *ASEAN Review of Biodiversity and Environmental Conservation (ARBEC)*; 1998; Vol. 6, pp 1−7

(31) Yang, Y. C.; Choi, H. C.; Choi, W. S.; Clark, J. M.; Ahn, Y. J. Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus* capitis (Anoplura: Pediculidae). *J. Agric. Food Chem.* **2004**, *52*, 2507–2511.

(32) Park, B. S.; Choi, W. S.; Kim, J. H.; Lee, S. E. Monoterpenes from thyme (*Thymus vulgaris*) as potential mosquito repellents. *J. Am. Mosq. Control Assoc.* **2005**, *21*, 80–83.

(33) Jaenson, T. G.; Palsson, K.; Borg-Karlson, A. K. Evaluation of extracts and oils of mosquito (Diptera: Culicidae) repellent plants from Sweden and Guinea-Bissau. *J. Med. Entomol.* **2006**, *43*, 113–119.

(34) Stashenko, E. E.; Jaramillo, B. E.; Martinez, J. R. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N. E. Brown, grown in Colombia and evaluation of its in vitro antioxidant activity. *J. Chromatogr., A* **2004**, *1025*, 93–103.

(35) Stashenko, E.; Martínez, J. R.; Jaramillo, B. E. J. Analysis of volatile secondary metabolites from Colombian *Xylopia aromatica* (Lamarck) by different extraction and headspace methods and gas chromatography. *J. Chromatogr., A* **2004**, *1025*, 105–113.

(36) Kovàts, E. Gas chromatographic characterization of organic substances in the retention index system. *Adv. Chromatogr.* **1965**, *1*, 229–247.

(37) Davies, N. W. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J. Chromatogr.* **1990**, *503*, 1–24.

(38) Jennings, W.; Shibamoto, T. *Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, 1980.

(39) Joulain, D.; König, W. A. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*; E. B. VerlagL Hamburg, Germany, 1998.

(40) Adams, P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*; Allured Publishing: Carol Stream, IL, 2007.

(41) Graphpad Instat version 3.00, GraphPad Sofware, Inc., San Diego, CA, 2003.

(42) Carroll, S. P. Prolonged efficacy of IR3535 repellents against mosquitoes and blacklegged ticks in North America. *J. Med. Entomol.* **2008**, *45*, 706–714.

(43) Omolo, M. O.; Okinyo, D.; Ndiege, I. O.; Lwande, W.; Hassanali, A. Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*. *Phytochemistry* **2004**, *65*, 2797–2802.

(44) Prajapati, V.; Tripathi, A. K.; Aggarwal, K. K.; Khanuja, S. P. S. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresour. Technol.* **2005**, *96*, 1749–1757.

(45) Chantraine, J. M.; Laurent, D.; Ballivian, C.; Saavedra, G.; Ibañez, R.; Vilaseca, L. A. Insecticidal activity of essential oils on *Aedes aegypti* Larvae. *Phytother. Res.* **1998**, *12*, 350–354.

(46) Coats, J. R.; Karr, L. L.; Drewes, C. D. Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. In *Naturally Occurring Pest Bioregulators*, Ed. Hedin, P. A., Ed.; ACS Symposium Series 449; American Chemical Society: Washington, DC, 1991; pp 305−316.

(47) Regnault-Roger, C.; Hamraoui, A. Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Aeanthoseelides obteetus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). *J. Stored Prod. Res.* **1995**, *31*, 291–299.

(48) Ahn, Y. I.; Lee, S. B.; Lee, H. S.; Kim, G. H. Insecticidal and acaricidal activity of caravacrol and β- thujaplicine derived from *Thujopsis dolabrata* var. *hondai* sawdust. *J. Chem. Ecol.* **1998**, *24*, 81–90.

(49) Lee, S.; Peterson, C. J.; Coats, J. R. Fumigation toxicity of monoterpenoids to several stored product insects. *J. Stored Prod. Res.* **2002**, *39*, 77–85.

(50) Lee, S.; Peterson, C. J.; Coats, J. R. Fumigation toxicity of monoterpenoids to several stored product insects. *J. Stored Prod. Res.* **2003**, *39*, 77–85.

(51) Lee, S. E.; Lee, R. H.; Choi, W. S.; Park, B. S.; Kim, J. G.; Campbell, B. C. Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* (L). *Pest Manag. Sci.* **2001**, *57*, 548–553.

(52) Gillij, Y. G.; Gleiser, R. M.; Zygadlo, J. A. Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. *Bioresour. Technol.* **2008**, *99*, 2507–2515.

(53) Davis, E. E. Insect repellents concepts of their mode of action relative to potencial sensory mechanisms in mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* **1985**, *22*, 237–243.

(54) Skinner, W. A.; Johnson, H. L. The design of insect repellents. *Drug Design* **1980**, *10*, 277–302.

(55) Suryanarayana, M. V. S.; Pandey, K. S.; Prakash, S.; Raghuveeran, C. D.; Dangi, R. S.; Swamy, R. V.; Rao, K. M. Structure−activity relationship studies with mosquito repellent amides. *J. Pharm. Sci.* **1991**, *80*, 1055–1057.

(56) Katritzky, A. R.; Dobchev, D. A.; Tulp, I.; Karelson, M.; Carlson, D. A. QSAR study of mosquito repellents using Codessa Pro. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2306–2311.

(57) Wang, Z.; Song, J.; Chen, J.; Song, Z.; Shang, S.; Jiangd, Z.; Han, Z. QSAR study of mosquito repellents from terpenoid with a six-member-ring. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2854–2859.

(58) Katz, T. M.; Miller, J. H.; Hebert, A. A. Insect repellents: historical perspectives and new developments. *J. Am. Acad. Dermatol.* **2008**, *58*, 865–871.

(59) Nerio, L. S.; Olivero-Verbel, J.; Stashenko, E. Repellent activity of essential oils: a review. *Bioresour. Technol.* **2010**, *101*, 372–378.

(60) Ayala-Zavala, J. F.; González-Aguilar, G. A.; del-Toro-Sánchez, L. Enhancing safety and aroma appealing of fresh-cut fruits and vegetables using the antimicrobial and aromatic power of essential oils. *J. Food Sci.* **2009**, *74*, R84–91.

(61) Chulze, S. N. Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Addit. Contam. Part A: Chem. Anal. Control Expos. Risk Assess.* **2010**, *27*, 651–657.