

## Repellent Constituents of Essential Oil of *Cymbopogon distans* Aerial Parts against Two Stored-Product Insects

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**ABSTRACT:** The screening for bioactive principles from several Chinese medicinal herbs showed that the essential oil of *Cymbopogon distans* aerial parts possessed strong repellency against the booklouse, *Liposcelis bostrychophila*, and the red flour beetle, *Tribolium castaneum*. A total of 36 components of the essential oil were identified by GC and GC-MS. *trans*-Geraniol (16.54%), (*R*)-citronellal (15.44%), (+)-citronellol (11.51%), and  $\alpha$ -elemol (9.06%) were the main components of the essential oil followed by  $\beta$ -eudesmol (5.71%) and (+)-limonene (5.05%). From the essential oil, four monoterpenes were isolated by bioassay-guided fractionation. The compounds were identified as limonene, citronellol, citronellal, and *trans*-geraniol. Geraniol and citronellol were strongly repellent against the booklouse, *L. bostrychophila*, whereas citronellal and limonene exhibited weak repellency against the booklouse. Geraniol and citronellol exhibited comparable repellency against the booklouse relative to the positive control, DEET. Moreover, geraniol and citronellol exhibited stronger repellency against the red flour beetle than DEET, whereas the two other compounds showed the same level of repellency against the red flour beetle compared with DEET.

**KEYWORDS:** *Cymbopogon distans*, *Liposcelis bostrychophila*, *Tribolium castaneum*, repellency, citronellol, citronellal, geraniol, limonene

### INTRODUCTION

The widespread extensive use of synthetic insecticides has led to many negative consequences (i.e., insecticide resistance, toxicity to mammals and other nontarget animals, residue problems, environmental pollution), resulting in increasing attention being given to natural products.<sup>1,2</sup> Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecules. During our screening program for new agrochemicals from the local wild plants and Chinese medicinal herbs, the essential oil of *Cymbopogon distans* (Nees ex Steud.) Will Watson (Gramineae) aerial parts was found to possess strong repellent activity against the booklouse, *Liposcelis bostrychophila* Badonnel, and the red flour beetle, *Tribolium castaneum* Herbst. The red flour beetle is one of the most widespread and destructive insect pests of stored cereals.<sup>3</sup> Infestations not only cause significant losses due to the consumption of grains but also result in elevated temperature and moisture conditions, which lead to accelerated growth of molds, including toxigenic species.<sup>4</sup> *L. bostrychophila* is frequently found in stored-product grains, often in extremely high numbers, in amylaceous products.<sup>5</sup> Currently, psocids are perhaps the most important category of emerging pests in stored grains and related commodities.<sup>6,7</sup> Infestations of stored-product insects could be controlled by fumigation or insecticidal treatment of commodities and surfaces.<sup>8</sup> However, increasing attention is being given to the development of more ecologically and economically sustainable control methods for stored-product pests, mainly due to the problems already mentioned, for example, the management

of the habitat and the use of semiochemical-baited traps, repellents, or biological control.<sup>9</sup> *C. distans* is mainly found at 2000–3500 m high mountain slopes, valleys, and open grassy places of southwestern China (southern Shanxi, southern Gansu, Sichuan, and Yunnan provinces as well as Tibet) and also in northwestern India, Nepal, and Pakistan.<sup>10</sup> The essential oil of *C. distans* has been traditionally used as an effective medicine for the treatment of inflammation and coughs, and the oil exhibits antibacterial activity.<sup>11</sup> The chemical composition of the essential oil of *C. distans* has been analyzed previously.<sup>12–19</sup> However, the constituents repellent against *L. bostrychophila* and *T. castaneum* have not been isolated and identified from this Chinese medicinal herb. In this paper, we report the isolation and identification of five repellents contained in *C. distans* essential oil against the booklouse and red flour beetle.

### MATERIALS AND METHODS

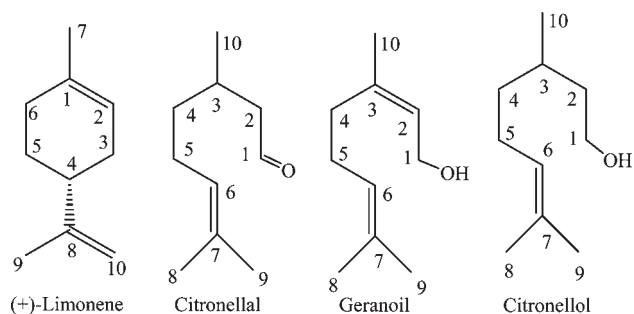
**Extraction of Plant Material.** Dried aerial parts of *C. distans* (5 kg, harvested from Yuxi, Yunnan province, 24.35° N latitude and 102.52° E longitude) were purchased from Anguo Chinese Medicinal Herbs Market (Anguo 071200, Hebei Province, China). The species was identified by Dr. Q. R. Liu, College of Life Sciences, Beijing Normal University, Beijing, China, and a voucher specimen (BNU-Liuzhilong-Lab-09-115) deposited at the Herbarium (BNU) of the College of Life

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**Figure 1.** Structures of repellents isolated from *Cymbopogon distans* essential oil.

Sciences, Beijing Normal University. The plant was ground to powder using a grinding mill (Retsch Muhle, Germany). Each 600 g portion of powder ground was mixed in 1800 mL of distilled water and soaked for 3 h. The mixture was then boiled in a round-bottom flask and steam-distilled for 6–8 h. The essential oil from distillation was collected in a flask. Separation of the essential oil from the aqueous layer was done in a separatory funnel, using the nonpolar solvent *n*-hexane. The solvent was evaporated using a vacuum rotary evaporator (Buchi Rotavapor R-124, Switzerland). The sample was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and kept in a refrigerator (4 °C) for subsequent experiments.

**Analysis of Essential Oil.** Components of the essential oil were separated and identified by gas chromatography–flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC-MS) on an Agilent 6890N gas chromatograph hooked to an Agilent 5973N mass selective detector. The same column and analysis conditions were used for both GC and GC-MS. The column was equipped with a flame ionization detector and a capillary column with HP-5MS (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ). The GC settings were as follows: The initial oven temperature was held at 60 °C for 1 min and ramped at 10 °C  $\text{min}^{-1}$  to 180 °C for 1 min and then ramped at 20 °C  $\text{min}^{-1}$  to 280 °C for 15 min. The injector temperature was maintained at 270 °C. The samples (1  $\mu\text{L}$ ) were injected neat, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0 mL  $\text{min}^{-1}$ . Spectra were scanned from *m/z* 20 to 550 at 2 scans  $\text{s}^{-1}$ . Most constituents were identified by GC by comparison of their retention indices with those in the literature<sup>12–19</sup> or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes ( $\text{C}_8$ – $\text{C}_{24}$ ) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from the literature.<sup>20</sup> Component relative percentages were calculated on the basis of GC peak areas without using correction factors.

**Chromatography.** The crude essential oil (25 mL) was chromatographed on a silica gel (Merck 9385, 1000 g) column (85 mm i.d., 850 mm length) by gradient elution with a mixture of solvents (*n*-hexane, *n*-hexane/ethyl acetate, and acetone). Fractions of 500 mL were collected and concentrated at 40 °C, and similar fractions according to TLC profiles were combined to yield 25 fractions. Fractions (3, 5, 11, and 14) that possessed repellent activity, with similar TLC profiles, were pooled and further purified by preparative silica gel column chromatography (PTLC) until four pure compounds were obtained; structures were determined as limonone (1) (1.4 g), citronellal (2) (2.1 g), citronellol (3) (1.6 g), and *trans*-geraniol (4) (1.5 g) (see Figure 1). The structures of the compounds were elucidated on the basis of high-resolution electron impact mass spectrometry and nuclear magnetic resonance. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker Avance DRX 500 instruments using  $\text{CDCl}_3$  as solvent with TMS as internal standard. EIMS were determined on a ThermoQuest Trace 2000 mass spectrometer at 70 eV (probe).

**Repellent Activity Bioassay.** The red flour beetle, *T. castaneum* and *L. bostrychophila*, were obtained from laboratory cultures in the dark in incubators at 28–30 °C and 70–80% relative humidity. *T. castaneum* was reared on wheat flour mixed with yeast (10:1, w/w). *L. bostrychophila* was reared on a 1:1:1 mixture, by mass, of milk powder, active yeast, and flour. All containers housing insects and the Petri dishes used in experiments were made escape-proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, Edenbridge, U.K.). Adult insects used in all experiments were about 2 weeks old. A commercial repellent, DEET (*N,N*-diethyl-3-methylbenzamide), was purchased from the National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China) and used as a positive control.

Petri dishes 6 cm in diameter were used to confine beetles and booklice, respectively, during the experiment. The essential oil of *C. distans* and the isolated compounds were diluted in acetone to different concentrations (16, 1.6, 0.16, 0.016, and 0.0016 nL  $\text{cm}^{-2}$ ), and absolute acetone was used as the control. Filter paper 6 cm in diameter was cut in half, and 150  $\mu\text{L}$  of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipet. The other half (control) was treated with 150  $\mu\text{L}$  of absolute acetone. Both the treated half and the control half were then air-dried to evaporate the solvent completely (10 s). A full disk was carefully remade by attaching the tested half to the negative control half with tape. Care was taken so that the attachment did not prevent free movement of insects from the one half to the other, but the distance between the filter paper halves remained sufficient to prevent seepage of test samples from one half to the other. Each reassembled filter paper after treatment with solid glue (Glue Stick, Jong Ie Nara Co., Ltd. Hong kong) was placed in a Petri dish with the seam oriented in one of four randomly selected different directions to avoid any insecticidal stimuli affecting the distribution of insects. Twenty insects were released in the center of each filter paper disk, and a cover was placed over the Petri dish. Five replicates were used, and the experiment was repeated three times. Counts of the insects present on each strip were made after 2 and 4 h. The percent repellency (PR) of each volatile oil/compound was then calculated using the formula

$$\text{PR} (\%) = [(N_c - N_t)/(N_c + N_t)] \times 100$$

where  $N_c$  is the number of insects present in the negative control half and  $N_t$  is the number of insects present in the treated half.

As for the red flour beetles, Petri dishes and filter papers were changed to 9 cm in diameter and the concentration of the oils and isolated constituents used in the experiments were 16, 1.6, 0.16, 0.016, and 0.0016 nL  $\text{cm}^{-2}$ . The half filter paper was treated with 500  $\mu\text{L}$  of the solution. Analysis of variance (ANOVA) and Tukey's test were conducted by using SPSS 10 for Windows 98. Percentage was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

## RESULTS AND DISCUSSION

**Chemical Composition of Essential oil.** The yellow essential oil yield of *C. distans* aerial part was 2.26% v/w, and the density of the concentrated essential oil was determined to be 0.85 g/mL. The chemical composition of the essential oil is summarized in Table 1. A total of 36 components of the essential oil were identified. The principal compounds in the essential oil were *trans*-geraniol (16.54%), (*R*)-citronellal (15.44%), (+)-citronellol (11.51%),  $\alpha$ -elemol (9.06%),  $\beta$ -eudesmol (5.71%), and (+)-limonene (5.05%). The chemical composition of the essential oil was different from that reported in other studies. For example, Liu et al.<sup>17</sup> reported piperitone (30%–40%) and geraniol (10%) as the principal constituents of the essential oil, and in another study<sup>15</sup> piperitone (20.02%),  $\delta$ -4-carene (15.65%), and ledol

**Table 1. Chemical Constituents of Essential Oil Derived from *Cymbopogon distans* Aerial Parts**

compound	RI <sup>a</sup>	peak area (%)
$\beta$ -myrcene	986	0.22
6-methyl-5-hepten-2-ol	995	0.42
(+)-limonene	1029	5.05
2,6-dimethyl-5-heptenal	1044	0.49
3,7-dimethyl-2,6-octadiene	1049	2.46
$\alpha$ -terpinolene	1090	0.08
linalool	1094	1.67
rose oxide	1112	0.44
( <i>R</i> )-citronellal	1152	15.44
isopulegol	1156	0.73
4-terpineol	1175	0.14
$\alpha$ -terpineol	1189	0.17
linalyl formate	1219	0.53
(+)-citronellol	1226	11.51
<i>cis</i> -geraniol	1230	0.43
<i>trans</i> -geraniol	1252	16.54
<i>m</i> -eugenol	1362	3.27
$\beta$ -geranyl acetate	1381	2.49
$\beta$ -elemene	1391	0.23
naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1-methyl-6-methylene-4-(1-methylethyl)-	1401	0.45
$\alpha$ -caryophyllene	1454	0.25
germacrene D	1480	1.91
naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	1482	0.44
$\alpha$ -selinene	1494	0.18
$\gamma$ -cadinene	1512	3.41
1 $\xi$ ,6 $\xi$ ,7 $\xi$ -cadina-4,9-diene	1502	0.78
(+)- $\delta$ -cadinene	1520	1.33
(+)- <i>epi</i> -bicyclosesquiphellandrene	1521	0.16
cadine-1,4-diene	1532	2.79
(-)- $\alpha$ -cadinene	1537	0.25
$\alpha$ -calacorene	1540	0.06
$\alpha$ -elemol	1547	9.06
germacrene D-4-ol	1574	1.58
$\gamma$ -eudesmol	1621	2.06
$\beta$ -eudesmol	1648	5.71
<i>trans</i> -farnesol	1722	0.70
total identified		94.15

<sup>a</sup>RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons.

(10.78%) were the main constituents of the essential oil. However, the main components of the essential of *C. distans* harvested from Hubei province (central China) were nerol (36.72%), cadinol (4.28%), and palustrol (3.89%).<sup>18</sup> Moreover, the essential oil of *C. distans* collected from Shanxi province (northwestern China) contained  $\alpha$ -terpinene (27.8%), piperitone (18.2%), intermediol (14.4%), and geranyl acetate (7.31%).<sup>19</sup> The above findings suggest that there are great variations in the chemical composition of essential oil of *C. distans*.<sup>13,14</sup> In fact, *C. distans* has been reported to occur in nature in the form of several geographical races.<sup>13</sup> It was suggested that several chemotypes of

*C. distans* essential oils have marker compounds  $\alpha$ -oxobisabolene-1 (chemotype I); citral, geraniol, and geranyl acetate (chemotype II); piperitone, limonene, and eudesmanediol (chemotype III); and sesquiterpene alcohol (chemotype IV).<sup>13,14</sup> One more chemotype with chemical marker *p*-menthol (66.5%) was reported later.<sup>24</sup> For the practical application of the essential oil as a novel repellent, further studies on plant cultivation and essential oil standardization will be needed because the chemical composition of the essential oil varies greatly with the plant population.

**Data for Isolated Bioactive Compounds.** *Limonene* (1): colorless oil; EI-MS, *m/z* (%) 150 (6.5), 108 (31.9), 107 (16.8), 106 (11.4), 93 (26.5), 82 (100), 79 (10.8), 54 (47.6), 53 (13.4), 41 (18.5), 39 (24.9), 27 (11.3); C<sub>10</sub>H<sub>16</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz),  $\delta$  7.65 (1H, s, H-6), 4.81 (1H, s, H-9), 4.76 (1H, s, H-9), 2.69 (1H, s, H-4), 2.57 (1H, s, H-3), 2.45 (1H, m, H-5), 2.39 (1H, m, H-3), 2.11 (1H, m, H-5), 1.82 (3H, m, 7-CH<sub>3</sub>), 1.78 (3H, m, 10-CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz),  $\delta$  199.34 (C-2), 146.69 (C-8), 144.40 (C-6), 135.45 (C-1), 110.47 (C-9), 43.20 (C-3), 42.55 (C-4), 31.30 (C-5), 20.50 (C-10), 15.63 (C-7). The <sup>1</sup>H and <sup>13</sup>C NMR data were in agreement with the reported data.<sup>21,22</sup>

*Citronellal* (2): colorless oil; EI-MS, *m/z* (%) 154 ([M]<sup>+</sup>, 10), 121 (17), 95 (30), 81 (24), 69 (85), 41 (100); C<sub>10</sub>H<sub>18</sub>O; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>),  $\delta$  9.85 (1H, m, H-1), 5.20 (1H, b, H-6), 2.48 (1H, m, H-2), 2.23 (1H, m, H-2), 1.96 (2H, m, 5-CH<sub>2</sub>), 1.88 (1H, m, H-3), 1.71 (6H, s, 8-CH<sub>3</sub>, 9-CH<sub>3</sub>), 1.29 (2H, m, 4-CH<sub>2</sub>), 1.06 (3H, s, 10-CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz),  $\delta$  202.11 (C-1), 131.25 (C-7), 126.76 (C-6), 51.02 (C-2), 37.51 (C-4), 27.54 (C-3), 25.61 (C-9), 24.12 (C-5), 20.57 (C-10), 19.59 (C-8). The <sup>1</sup>H and <sup>13</sup>C NMR data were in agreement with the reported data.<sup>21–23</sup>

*Citronellol* (3): colorless oil; EI-MS, *m/z* (%) 156 ([M]<sup>+</sup>, 9), 138 (6), 123 (18), 109 (11), 95 (23), 82 (30), 81 (29), 69 (100), 67 (27), 55 (33), 41 (72); C<sub>10</sub>H<sub>20</sub>O; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>),  $\delta$  5.20 (1H, t, *J* = 7.2 Hz, H-6), 3.53 (2H, m, H-1), 1.96 (2H, m, 5-CH<sub>2</sub>), 1.71 (6H, s, 8-CH<sub>3</sub>, 9-CH<sub>3</sub>), 1.65 (1H, m, H-3), 1.44 (2H, m, 2-CH<sub>2</sub>), 1.29 (2H, m, 4-CH<sub>2</sub>), 1.06 (3H, s, 10-CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz),  $\delta$  131.21 (C-7), 124.68 (C-6), 61.14 (C-1), 39.87 (C-2), 37.19 (C-4), 29.15 (C-3), 25.67 (C-9), 24.38 (C-5), 19.48 (C-10), 17.59 (C-8). The <sup>1</sup>H and <sup>13</sup>C NMR data were in agreement with the reported data.<sup>21–23</sup>

*Geraniol* (4): colorless oil; EI-MS, *m/z* (%) 154 ([M]<sup>+</sup>, 4), 136 (8), 123 (11), 93 (30), 69 (100), 68 (19), 67 (10), 41 (68), 39 (11); C<sub>10</sub>H<sub>18</sub>O; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>),  $\delta$  5.40 (1H, t, *J* = 6.6 Hz, H-2), 5.01 (1H, t, *J* = 6.6 Hz, H-6), 4.13 (2H, d, H-1), 2.03 (4H, m, H-4, H-5), 1.67 (6H, s, H-8, 9), 1.60 (3H, s, H-10); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz),  $\delta$  139.07 (C-3), 131.61 (C-7), 124.07 (C-2), 123.71 (C-6), 59.16 (C-1), 39.64 (C-4), 26.51 (C-5), 25.66 (C-8), 17.66 (C-9), 16.24 (C-10). The <sup>1</sup>H and <sup>13</sup>C NMR data were in agreement with the reported data.<sup>21–23</sup>

#### Repellent Activity of the Oils and Isolated Constituents.

The results of repellency assays for the essential oil and isolated constituents against the two species of insects are presented in Tables 2 and 3. Data showed that at tested concentrations, *trans*-geraniol and (+)-citronellol were strongly repellent against the booklouse, *L. bostrychophila*. At the lowest assayed concentration (1.6 mL cm<sup>-2</sup>), the two compounds still showed repellency (44 and 32%, respectively) against the booklouse at 4 h after exposure (Table 2). (*R*)-Citronellal and limonene exhibited weak repellency against the booklouse. At the highest concentration (26 nL cm<sup>-2</sup>), the compounds exhibited only 34 and 40%, respectively, repellency against the booklouse at 4 h after exposure

Table 2. Percentage Repellency (PR) after Two Exposure Times for the Essential Oil and Isolated Constituents against *Liposcelis bostrychophila*<sup>a</sup>

treatment	2 h					4 h				
	26 nL/cm <sup>2</sup>	13 nL/cm <sup>2</sup>	6.4 nL/cm <sup>2</sup>	3.2 nL/cm <sup>2</sup>	1.6 nL/cm <sup>2</sup>	26 nL/cm <sup>2</sup>	13 nL/cm <sup>2</sup>	6.4 nL/cm <sup>2</sup>	3.2 nL/cm <sup>2</sup>	1.6 nL/cm <sup>2</sup>
DEET	96 ± 2 a	100 ± 0 a	84 ± 7 a	82 ± 9 ab	46 ± 7 a	94 ± 2 a	94 ± 2 a	92 ± 6 a	64 ± 5 ab	42 ± 12 a
oil	88 ± 2 a	64 ± 5 b	44 ± 13 bc	26 ± 24 c	-32 ± 15 c	90 ± 4 a	90 ± 4 a	48 ± 7 b	30 ± 11 cd	-28 ± 6 d
(R)-citronellal	36 ± 7 b	-10 ± 8 c	20 ± 13 c	-4 ± 16 cd	-10 ± 18 bc	34 ± 12 b	34 ± 12 b	6 ± 19 c	12 ± 16 d	-10 ± 16 cd
(+)-citronellol	96 ± 4 a	66 ± 4 b	66 ± 9 b	52 ± 8 b	28 ± 10 b	82 ± 6 a	82 ± 6 a	64 ± 9 b	44 ± 12 bc	32 ± 13 b
limonene	36 ± 12 b	-4 ± 7 c	-20 ± 12 d	-10 ± 16 d	-6 ± 11 b	40 ± 22 b	40 ± 22 b	-18 ± 11 c	-22 ± 23 e	-4 ± 13 c
<i>trans</i> -geraniol	86 ± 7 a	95 ± 5 a	90 ± 4 a	84 ± 9 a	42 ± 16 a	92 ± 6 a	92 ± 6 a	96 ± 4 a	74 ± 9 a	44 ± 12 a

<sup>a</sup> Means in the same column followed by the same letters do not differ significantly ( $P > 0.05$ ) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

Table 3. Percentage Repellency (PR) after Two Exposure Times for the Essential Oil and Isolated Constituents against *Tribolium castaneum*<sup>a</sup>

treatment	2 h					4 h				
	16 nL/cm <sup>2</sup>	1.6 nL/cm <sup>2</sup>	0.16 nL/cm <sup>2</sup>	0.016 nL/cm <sup>2</sup>	0.0016 nL/cm <sup>2</sup>	16 nL/cm <sup>2</sup>	1.6 nL/cm <sup>2</sup>	0.16 nL/cm <sup>2</sup>	0.016 nL/cm <sup>2</sup>	0.0016 nL/cm <sup>2</sup>
DEET	92 ± 5 a	6 ± 7 a	0 ± 11 a	0 ± 5 a	-34 ± 17 a	90 ± 5 a	-12 ± 6 a	-16 ± 18 a	-4 ± 13 a	-46 ± 7 a
oil	96 ± 2 a	90 ± 6 c	58 ± 12 b	2 ± 6 a	-2 ± 19 a	78 ± 7 a	82 ± 7 b	60 ± 8 c	6 ± 16 ac	-10 ± 8 b
(R)-citronellal	16 ± 22 a	-12 ± 23 a	10 ± 23 a	4 ± 18 a	-20 ± 26 a	-4 ± 32 b	-16 ± 22 a	6 ± 19 a	-16 ± 27 ab	0 ± 18 bc
(+)-citronellol	88 ± 8 a	82.5 ± 8 bc	64 ± 15 b	44 ± 16 c	40 ± 27 b	96 ± 2 a	75 ± 10 b	66 ± 15 c	48 ± 21 c	36 ± 27 d
limonene	20 ± 27 a	-22 ± 22 a	2 ± 20 a	-36 ± 24 b	20 ± 24 b	-4 ± 29 b	-44 ± 22 a	8 ± 18 a	-36 ± 26 b	12 ± 32 cd
<i>trans</i> -geraniol	96 ± 2 a	58 ± 13 b	14 ± 13 a	-12 ± 30 a	-10 ± 15 a	86 ± 7 a	60 ± 19 b	14 ± 27 ab	-10 ± 29 a	-14 ± 15 b

<sup>a</sup> Means in the same column followed by the same letters do not differ significantly ( $P > 0.05$ ) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests.

(Table 2). At the other concentrations, the two compounds showed some insect-attractant properties. Compared with the positive control, DEET, *trans*-geraniol and (+)-citronellol exhibited the same level of repellency against the booklouse (Table 2). However, (*R*)-citronellal and limonene possessed less repellency against the booklouse. Compared with the positive control, *trans*-geraniol and (+)-citronellol as well as the essential oil exhibited stronger repellency against the red flour beetle, *T. castaneum* (Table 3), because at 4 h after exposure, DEET showed repellency against the beetles only at the highest concentration of 16 nL cm<sup>-2</sup>, and the two isolated compounds and the crude oil exhibited repellency at a concentration of 0.16 mL cm<sup>-2</sup>. Moreover, at the lowest concentration (0.0016 nL cm<sup>-2</sup>), (+)-citronellol still exhibited 36% repellency against the red flour beetle at 4 h after exposure (Table 3). However, the other two compounds showed lower repellency against the beetle when compared with the positive control; at 2 h after exposure, the two compounds exhibited weak repellency at the highest concentration, and no repellency was observed at 4 h after exposure (Table 3). Many essential oils and their constituents have been evaluated for repellency against insects.<sup>25</sup> However, there have been only a few studies on the booklouse.<sup>26</sup> In this paper, we report the isolation of four repellent constituents from the essential oil of *C. distans* for the first time. However, in the previous papers, citronellol and *trans*-geraniol were demonstrated to repel mosquitoes, sand flies, human body lice, aphids, and stored-product insects.<sup>27–31</sup> These findings, considered together, suggest that the essential oil and the four compounds show potential for development as a natural repellent for stored products.

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