Bioactivity and Chemical Constituents of the Essential Oil from *Dendranthema indicum* (L.) Des Moul. against Two Stored Insects

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Abstract: *Dendranthema indicum* (L.) Des Moul. is a perennial herb commonly used as a traditional Chinese medicine for a long time. In this work, we took *Dendranthema indicum* as a target plant and two stored insects which include *Tribolium castaneum* and *Stegobium paniceum* adults as target insects. Essential oil obtained from *Dendranthema indicum* was analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 31 components representing 92.44% of the oil were identified and the main compounds were found to be chamazulene (15.93%), β -caryophyllene (13.78%), germacrene D (9.11%), and *b*-cis-farnesene (6.59%). With a further isolation, three constituents were obtained from the essential oil and identified as chamazulene, β -caryophyllene and eucalyptol. Significantly, in the progress of assay, it showed that the essential oil and chamazulene exhibited stronger insecticidal and repellent activities against *Stegobium paniceum* than *Tribolium castaneum*. The results indicate that additional to its traditional use as Chinese medicinal herb, the essential oil of *Dendranthema indicum* aerial parts and isolated compounds have potential to be developed into natural insecticides or repellents for control of insects in stored grains.

Key words: *D. indicum*, *T. castaneum*, *S. paniceum*, contact toxicity, repellency, chemical composition

1 INTRODUCTION

The red flour beetle, *Tribolium castaneum* Herbst are the most widespread and destructive primary insect pests of stored cereals¹⁾. Drugstore beetle, *Stegobium paniceum*, as an omnivorous stored insects, are widespread in the world. The infestations of stored product insects can not only cause significant losses due to the consumption of grains but also result in the rise of temperature and moisture which lead to an accelerated growth of molds, including toxigenic species²⁾. Synthetic insecticides and fumigants were widespread used in control of insects in stored grains and traditional Chinese medicinal materials, that has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides, and lethal effects on non-target organisms in addition to direct toxicity to users³⁾. These prob-

lems have needed a search for alternative eco-friendly insect pest control methods⁴⁾. Since it has the advantage of providing novel modes of action against insects and reducing the risk of cross-resistance as well as offering new leads for design of target-specific molecules, botanical pesticide was expected to be developed into new natural pesticide^{5, 6)}. According to a report in the literature, the use of essential oils or their constituents with low mammalian toxicity can effectively prevent and/or suppress insect pest especially in storage⁵⁾. Moreover, essential oils from many plants including medicinal herbs, spices and fruits have been evaluated with success for insecticidal/repellency activity

Abbreviations: *D. indicum*: *Dendranthema indicum*; *T. castaneum*: *Tribolium castaneum*; *S. paniceum*: *Stegobium paniceum*; RI: Retention Index; MS: mass spectrum; DEET: N,N-diethyl-3-methyl-benzamide.

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against stored-product insects/mites, in some cases, have been proven more effective than traditionally used organophosphorus pesticides⁷⁻¹⁰. In present study, insecticidal and repellent activities of *D. indicum* essential oil and its three main active ingredients against *T. castaneum* and *S. paniceum* were investigated.

2 EXPERIMENTAL PROCEDURES

2.1 Chemicals

Chamazulene, β -caryophyllene and eucalyptol were isolated from *D. indicum* essential oil on a silica gel column (45 mm $\times$ 500 mm) (Qingdao Marine Chemical Plant, Shandong province, China).

2.2 Material

2.2.1 Plants

Dried aerial parts (10.0 kg of leaves, stems and flowers) of *D. indicum* were harvested in October 2013 from Jining City (35.23° N latitude and 116.33° E longitude), Shandong Province of China. The plant was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing, China) and a voucher specimen (BNU-CMH-Dushushan-2013-10-04-011) was deposited at the Herbarium (BNU) of College of Resource Sciences, Beijing Normal University, China.

2.2.2 Insects

The red flour beetle, *Tribolium castaneum* and drug-store beetle, *Stegobium paniceum* adults (about 7 $\pm$ 2 days old) were collected from laboratory cultures maintained for the last 2 years in dark in incubators at 29 $\pm$ 1°C and 70–80% relative humidity. The insects were kept in glass containers (0.5 L) containing wheat flour at 12–13% moisture content mixed with yeast (10:1, w/w). Adults used in all the experiments were about 7 $\pm$ 2 days old.

2.3 Isolation of the Essential Oil and Purification of Four Constituent Compounds

The aerial parts were air-dried for one week and ground to a powder and then subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in airtight containers in a refrigerator at 4°C for subsequent experiments. The crude essential oil (8 mL) was chromatographed on a silica gel (Qingdao Marine Chemical Plant, Shandong province, China) column (40 mm i.d., 500 mm length) on three steps. First, chromatographed on by gradient elution with petroleum ether, then with petroleum ether-ethyl acetate, and last with ethyl acetate. Total fractions (200 mL) were collected and maintained at 35°C, and similar fractions according to thin layer chromatography (TLC) profiles were combined to yield 40 fractions. Fractions (1–4, 22–29) were

proned to possess contact toxicity and repellency, with similar TLC profiles, were pooled and further purified by preparative silica gel column chromatography (PTLC) until obtain the pure compounds which were determined as chamazulene (1, 0.82g), β -caryophyllene (2, 0.71g) and eucalyptol (3, 0.06g). The three isolated compounds were identified based on nuclear magnetic resonance. ¹H and ¹³C-NMR spectra were recorded on Bruker Avance DRX 500 instruments using CDCl₃ as solvent with TMS as internal standard.

2.4 GC-FID and GC-MS analysis

GC-MS analysis was run on a Thermo Finnigan Trace DSQ instrument equipped with a flame ionization detector (FID) and a capillary column of HP-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m). The column temperature was programmed at 50°C for 2 min, then increased at a speed of 2°C/min to the temperature of 150°C and held for 2 min, and then increased at 10°C/min until the final temperature of 250°C was reached, where it was held for 5 min. The temperature of injector was maintained at 250°C and the volume injected was 0.1 μ L of 1% solution (diluted in *n*-hexane). The carrier gas was helium at flow rate of 1.0 mL/min. Spectra were scanned from 50 to 550 *m/z*. Most constituents were identified by comparing their retention indices with those reported in the literatures. Under the same operating conditions, the retention indices were determined in relation to a homologous series of *n*-alkanes (C₅–C₃₆). Further identification of the isolated constituents was made by comparing their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature¹¹. Relative percentages of the individual components of the essential oil were obtained by averaging the GC peak area% reports.

2.5 Isolated Constituent Compounds

Chamazulene (1, Fig. 1). Dark blue oil. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 8.19 (1H, s, H-4), 7.65 (1H, d, *J* = 3.6 Hz, H-2), 7.41 (1H, d, *J* = 3.6 Hz, H-1), 7.02 (1H, d, *J* = 10.5 Hz, H-6), 7.00 (1H, d, *J* = 10.5 Hz, H-7), 2.86 (3H, s, H-11), 2.69 (2H, s, H-12), 2.68 (3H, s, H-14), 1.37 (3H, t, *J* = 7.5 Hz, H-13); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 144.32 (C-8),

Fig. 1 Constituent compounds isolated from the essential oil of *D. indicum* aerial parts.

137.32 (C-9), 136.44 (C-10), 136.19 (C-2), 136.13 (C-6), 135.75 (C-5), 134.76 (C-4), 124.96 (C-7), 125.08 (C-3), 112.76 (C-1), 33.85 (C-12), 24.13 (C-14), 17.42 (C-13), 12.90 (Me (C-3)). Its NMR data were identical to the literature data¹².

***β-Caryophyllene* (2, Fig. 1).** Colorless oil. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 5.33 (1H, m, H-5), 4.97 (1H, s, H-12a), 4.85 (1H, s, H-12b), 2.37 (1H, m, H-9), 2.33 (1H, m, H-7b), 2.23 (1H, m, H-7a), 2.11 (1H, m, H-1), 2.02 (1H, m, H-6b), 1.94 (1H, m, H-6a), 1.72 (1H, m, H-2b), 1.69 (1H, m, H-3b), 1.65-1.67 (2H, m, H-10), 1.64 (3H, s, Me-15), 1.60 (1H, m, H-3a), 1.52 (1H, m, H-2a), 1.02 (3H, s, Me-12), 1.00 (3H, s, Me-13); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 154.74 (C-2), 135.58 (C-6), 124.31 (C-5), 111.67 (C-12), 53.54 (C-9), 48.48 (C-1), 40.33 (C-11), 39.96 (C-7), 34.79 (C-3), 33.02 (C-10), 30.09 (C-13), 29.36 (C-8), 28.38 (C-4), 22.66 (C-14), 16.32 (C-15). The ¹H and ¹³C-NMR data were consistent with the literature data¹³.

***Eucalyptol* (3, Fig. 1).** Colorless oil. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 2.03 (2H, t, H-2), 1.68 (2H, t, H-6), 1.52 (4H, m, H-3, 5), 1.42 (1H, m, H-4), 1.25 (6H, s, H-9, 10), 1.07 (3H, s, H-7); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 73.61 (C-8), 69.77 (C-1), 32.94 (C-4), 31.51 (C-3, 5), 28.89 (C-2, 6), 27.58 (C-7), 22.83 (C-9, 10). The ¹H and ¹³C-NMR data were in agreement with the reported data¹⁴.

2.6 Bioactivity

2.6.1 Contact activity

The contact toxicity of the essential oil and the three isolated compounds against *T. castaneum* and *S. paniceum* adults was measured as described by Liu and Ho¹. The contact toxicity of the essential oil and the three isolated compounds against *S. paniceum* followed the same way. A lot of range-finding studies were conducted to determine the appropriate testing concentrations. Finally, the essential oil and three isolated compounds with five concentrations were prepared in *n*-hexane. Aliquots of 0.5 μL of the dilutions were topically applied to the dorsal thorax of the insects. Controls were determined with *n*-hexane. Ten insects were used for each concentration and control, and the experiment was replicated five times. Then, both treated and control insects were transferred to glass vials with culture media and kept in incubators. Mortality of tested insects was recorded after 24 h and the observed mortality data were corrected for control mortality using Abbott's formula. The Probit analysis (IBM SPSS V20.0)^{15, 10} was used to obtain LD₅₀ values. The positive control, pyrethrins (pyrethrin I, 24%; pyrethrin II, 13%; cinnerin I, 2%; cinnerin II, 2%; jasmolin I, 1%; jasmolin II, 1%) was purchased from Dr. Ehrenstorfer, Germany.

2.6.2 Repellency tests

The repellent activity of the essential oil and three isolated compounds against *T. castaneum* and *S. paniceum* adults was tested using previous method¹⁶. Petri dishes (9

cm in diameter) were used to confine test insects during the experiment. Crude essential oil and its three isolated compounds were diluted to five concentrations (39.32, 7.86, 1.57, 0.31 and 0.06 nL/cm²) with *n*-hexane, and *n*-hexane was used as the control. Filter paper (9 cm in diameter) was cut in half and each concentration with 500 μL was separately applied to half of the filter paper with a micropipette at the same time. 500 μL of *n*-hexane was used in control on the other half. The treated and control half filter paper were air-dried for 30 s to remove the solvent, re-attached with adhesive tape, and kept in 9 cm glass Petri dishes. Twenty insects of both sexes were released at 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 insects present on each strip were made at 2 and 4 h after exposure. The percent repellency (PR) of the volatile oil and its isolated compounds was then calculated using the formula:

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

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. Analysis of variance (One-Way ANOVA and GLM Univariate) and Tukey's test were conducted by using SPSS 20.0 for Windows 2007. Percentage of mortality data were subjected to an arcsine square-root transformation before variance and Tukey's tests. 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.

3 RESULTS and DISCUSSION

3.1 Chemical compounds of the essential oil

The *D. indicum* essential oil was yellow with a yield of 0.08% (v/w) and density of 0.91 g/mL. GC-MS and GC-FID analysis of *D. indicum* essential oil revealed 31 components representing 92.44% of the oil (Table 1) and the main compounds of the essential oil were chamazulene (15.93%), *β*-caryophyllene (13.78%), germacrene D (9.11%) and *b*-cis-farnesene (6.59%).

The chemical composition of the essential oil of *D. indicum* aerial parts in the present study was not the same as that reported in previous studies. For example, compounds in higher amounts present in essential oil of *D. indicum* in Hubei, Guangdong and Guangxi province of China were camphor (31.38%), borneol (41.21%) and isobornyl acetate (46.00%), respectively¹⁷. What's more, the content and composition of the volatile oil of *D. indicum* were various with different extraction methods. In the literature, the essential oil from the dried flowers of *D. indicum* was obtained by supercritical fluid extraction

Table 1 Chemical composition of the essential oil of *D. indicum* aerial parts.

Peak No.	Compound	RI ^a	Relative area ^b (%)	Identification Methods ^c
1	<i>p</i> -Xylene	800	2.60	MS, RI
2	<i>o</i> -Xylene	811	2.79	MS, RI
3	3-Carene	1003	1.35	MS, RI
4	<i>D</i> -Limonene	1027	2.56	MS, RI
5	Eucalyptol	1031	3.78	MS, RI, Co
6	2-Isopropyltoluene	1104	0.36	MS, RI
7	Camphor	1143	2.46	MS, RI, Co
8	α -Caryophyllene	1156	2.25	MS, RI
9	Terpinen-4-ol	1176	1.04	MS, RI
10	α -Terpineol	1188	1.02	MS, RI
11	Isobornyl acetate	1287	0.59	MS, RI
12	Terpinyl acetate	1296	0.69	MS, RI
13	Senecioic acid	1310	0.35	MS, RI
14	γ -Elemene	1340	1.02	MS, RI
15	<i>b</i> -Elemene	1389	2.63	MS, RI
16	β -Caryophyllene	1426	13.78	MS, RI
17	(<i>Z,Z</i>)- α -Farnesene	1438	1.34	MS, RI
18	α -Farnesene	1440	2.44	MS, RI
19	Germacrene D	1457	9.11	MS, RI
20	<i>b-cis</i> -Farnesene	1458	6.59	MS, RI
21	α -Calacorene	1543	2.42	MS, RI
22	α -Elemol	1547	1.55	MS, RI
23	β -Eudesmol	1632	0.92	MS, RI
24	Chamazulene	1644	15.93	MS, RI
25	(-)- <i>b</i> -Cadinene	1650	0.70	MS, RI
26	α -Bisabolol	1681	1.77	MS, RI
27	α -Patchoulene	1735	1.70	MS, RI
28	3,3-Dimethylhexane	1742	1.19	MS, RI
29	4,4'-Dimethylbiphenyl	1756	2.77	MS, RI
30	γ -Patchoulene	1879	2.33	MS, RI
31	Diisooctyl phthalate	2135	2.51	MS, RI
Total			92.44	

^a Retention index (RI) relative to the homologous series of *n*-hydrocarbons on the HP-5 MS capillary column.

^b Relative area (peak area relative to the total peak area).

^c MS = mass spectrum, Co = co-injection with standard compound.

(SFE) and steam distillation (SD), and the highest contents were tetracosane (7.50%) and bisabolol oxide (11.87%)¹⁸⁾. Besides, different collection parts also led to different content and composition. The compositions of the essential oil from the leaves of *D. indicum* are more complex and diverse than that isolated from the flowers of *D. indicum*¹⁹⁾. These differences of chemical content and composition of

the essential oils might be attributed to different harvest time and local, climatic and seasonal factors, storage duration of medicinal herbs as well as extraction method, and these differences may result in different biological activities²⁰⁾.

3.2 Bioactivity

3.2.1 Insecticidal activity

3.2.1.1 Contact toxicity

In Table 2, though the essential oil of *D. indicum* aerial parts exhibited no contact toxicity against *T. castaneum* adults, it showed strong contact toxicity against *S. paniceum* adults with LD₅₀ value of 5.82 µg/adult. Compared with the positive control, pyrethrins (LD₅₀ = 1.22 µg/adult), the essential oil presented 5 times less toxicity against *S. paniceum* adults. What's more, chamazulene exhibited stronger contact toxicity against *S. paniceum* adults than *T. castaneum* adults with the LD₅₀ values of 4.30 and 29.52 µg/adult, respectively. Actually, chamazulene only showed 4 times less contact toxicity than the positive control, pyrethrins. Moreover, as one of the main active compounds, chamazulene was proven to possess anti-inflammatory activity in vivo, by inhibiting the leukotriene synthesis, and to inhibit lipid peroxidation^{21, 22}. Chamazulene is a very promising compound and will be paid more attention in the future. While, β-caryophyllene exhibited least contact toxicity against both *T. castaneum* and *S. paniceum* adults with LD₅₀ value of 46.77 and 30.08 µg/adult, respectively. Eucalyptol showed better contact toxicity against *T. castaneum* (LD₅₀ = 18.83 µg/adult) than *S. paniceum* (LD₅₀ = 20.77 µg/adult), but less toxicity compared with the positive control, pyrethrins. What's more, both β-caryophyllene and eucalyptol were proven to possess insecticidal activity against *Lasioderma serricorne*²³ and *Sitophilus zeamais*²⁴. However, considering the currently used insecticides are synthetic insecticides and promising contact activity of *D. indicum* essential oil and its isolated compounds, they have exhibited potential to be developed into possible natural insecticides for the stored product insects.

3.2.2 Repellent activity

In Fig. 2, the essential oil of *D. indicum* and its isolated

constituents exhibits potential repellent activity against *T. castaneum* and *S. paniceum* adults, especially towards *T. castaneum* adults. The results presented in Fig. 2 showed that the essential oil and chamazulene possess much stronger repellent activity against *T. castaneum* adults at both 2 h and 4 h after exposure at five tested concentrations. However, β-caryophyllene and eucalyptol exhibited much weaker repellent activity compared with the positive control, DEET. Hence, the strong repellency against *T. castaneum* adults might be attributed to the synergistic effects of its diverse major and minor components.

When it comes to *S. paniceum* adults, the essential oil and its isolated compounds showed weaker repellent activity than that against *T. castaneum* adults. In Fig. 3, β-caryophyllene showed the weakest repellent activity against *S. paniceum* adults at all tested concentrations at both 2 h and 4 h after exposure. At tested concentration of 0.31 nL/cm², eucalyptol exhibited the strongest repellency against *S. paniceum* adults at both 2 h and 4 h after exposure. What's more, at the concentration of 39.32 nL/cm², the essential oil and chamazulene showed the same repellency against *S. paniceum* adults at 2 h after exposure, and stronger repellent activity at 4 h after exposure when compared with DEET.

The above results indicates that the difference insecticidal and repellent activity between *T. castaneum* and *S. paniceum* adults might be attributed to the different mechanism. However, further research should be conducted in the future.

4 CONCLUSION

Based on mass screening, the essential oil of *D. indicum* and its three isolated constituents were examined for in-

Table 2 Contact toxicity of the essential oil of *D. indicum* aerial parts and its constituents against *T. castaneum* and *S. paniceum* adults.

Insects	Treatment	LD ₅₀ * (µg/adult)	95% FL **	Slope ± SE	Chi square (χ ²)
Tribolium castaneum	<i>D. indicum</i>	—	—	—	—
	Chamazulene	29.52	27.91 – 31.04	6.38 ± 0.80	6.129
	β-Caryophyllene	46.77	41.99 – 52.36	3.02 ± 0.37	17.26
	Eucalyptol	18.83	17.13 – 20.69	4.86 ± 0.50	16.56
	Pyrethrins ***	0.26	0.22 – 0.30	3.34 ± 0.32	13.11
Stegobium paniceum	<i>D. indicum</i>	5.82	4.97 – 6.59	2.77 ± 0.39	9.81
	Chamazulene	4.30	3.63 – 4.93	2.23 ± 0.32	13.43
	β-Caryophyllene	30.08	24.01 – 33.72	3.93 ± 0.79	7.83
	Eucalyptol	20.77	18.59 – 23.17	3.03 ± 0.36	10.26
	Pyrethrins ***	1.22	0.67 – 1.80	0.69 ± 0.15	6.21

* 50% of lethal dose, ** fiducial limits, *** data from Yang¹⁰.

Fig. 2 Percentage repellency (PR) of the essential oil from *D. indicum* aerial parts and its isolated constituents against *T. castaneum* at 2 h and 4 h after exposure^a.

^a 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.

secticidal and repellent activity against *T. castaneum* and *S. paniceum* adults. The essential oil and chamazulene possessed strong contact toxicity against *S. paniceum* adults, although they were 5 and 4 times less toxic to the *S. paniceum* adults compared to commercial insecticide Pyrethrins. The essential oil and chamazulene also exhibited strong repellent activity against *T. castaneum* adults compared with the positive control, DEET. These findings, considered together, suggest that the essential oil and its isolated constituents show potential for the development as natural insecticides or repellents for stored products.

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Fig. 3 Percentage repellency (PR) of the essential oil from *D. indicum* aerial parts and its isolated constituents against *S. paniceum* at 2 h and 4 h after exposure^a.

^a 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.

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