Bioactivities and Chemical Constituents of Essential Oil Extracted from *Artemisia anethoides* Against Two Stored Product Insects

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Abstract: The chemical constituents of the essential oil extracted from *Artemisia anethoides* and the bioactivities of essential oil against *Tribolium castaneum* and *Lasioderma serricorne* were investigated. The main components of the essential oil were 1,8-cineole (36.54%), 2-isopropyl-5-methyl-3-cyclohexen-1-one (10.40%), terpinen-4-ol (8.58%), 2-isopropyltoluene (6.20) and pinocarveol (5.08%). The essential oil of *A. anethoides* possessed contact and fumigant toxicities against *T. castaneum* adults ($LD_{50} = 28.80 \mu\text{g/adult}$ and $LC_{50} = 13.05 \text{ mg/L air}$, respectively) and against *L. serricorne* ($LD_{50} = 24.03 \mu\text{g/adult}$ and $LD_{50} = 8.04 \text{ mg/L air}$, respectively). The crude oil showed repellent activity against *T. castaneum* and *L. serricorne*. Especially, the percentage repellency of essential oil was same level with DEET (positive control) against *T. castaneum*. The results indicated that the essential oil of *A. anethoides* had the potential to be developed as insecticide and repellent for control of *T. castaneum* and *L. serricorne*.

Key words: *Tribolium castaneum*, *Lasioderma serricorne*, insecticidal toxicity, repellent activity

1 INTRODUCTION

The red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the cigarette beetle *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) are the two most common species of the insects in stored product. The secreta of *T. castaneum* can cause the flour to agglomerate, change color and go bad, and the secreta contain the benzoquinone which is a kind of carcinogenic substance. They have the characteristics of rapid reproduction, strong adaptation, and worldwide distribution, so the two species of insects cause significant damage during storage of many crops and products¹.

Synthetic chemical pesticides are widely used and have high efficiency against insects on farms and stored products more than 100 years, but more and more negative ecology and health impacts have attracted people's attention². For example, the overzealous used of synthetic chemical pesticides have caused some species of animal to be in the state of extinction or on the edge of extinction³. Therefore, the available alternatives of synthetic chemical insecticides are required and urgent. Many government en-

couraged researchers to study new pesticides which were high-efficiency and low toxicity to mammal and environment⁴.

It was more than 150 years that botanical insecticide was used to protect food crops and stored products from insect pests⁵. Now the botanical insecticides have attracted more and more researchers' interest for the characteristic of fair insecticides, low toxicity and easy biodegradation^{6, 7}. Because their highly volatile nature, the plant essential oils are less likely to leave residues on stored products, and many essential oils have been evaluated for insecticidal activity⁸⁻¹³. These favorable properties and studies suggest that we may find new and viable pesticides based on the essential oil.

Artemisia anethoides Mattf. (Compositae) widely distribute in northern China and the resource of the plant is

Abbreviations: *A. anethoides*: *Artemisia anethoides*; *T. castaneum*: *Tribolium castaneum*; *L. serricorne*: *Lasioderma serricorne*; **RI**: Retention Index; **GC-MS**: Gas Chromatography-Mass Spectrometer; **FID**: flame ionization detector; **DEET**: N,N-diethyl-3-methyl-benzami; **PR**: percentage repellency

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very abundant. *A. anethoides* have been used as a substitute of *Artemisia capillaris* which is famous traditional Chinese medicine¹⁴. The bioactivity about essential oil of *A. anethoides* has been not investigated.

In this work, the insecticidal and repellent activities of the essential oil from *A. anethoides* against *L. serricorne* and *T. castaneum* adults will be researched. It was expected to find new botanical pesticides.

2 MATERIALS and METHODS

2.1 Plant material and extraction of essential oil

The fresh leaves of *A. anethoides* were collected from Lintan county, Gansu Province, China (34° 58' 18" N latitude, 103° 39' 12" E longitude, altitude 2233 m) in August 2014. The species was identified by Dr. Liu, Q.R. and the voucher specimen (BNU-dushushan-2014080205) was deposited at the Herbarium (BNU) of College of Resources Science and Technology, Beijing Normal University. The leaves were air-dried and chopped, then weighed to 500 g and were transferred into a clevenger apparatus equipped with a watercooled oil receiver to reduce overheating artifacts. The hydrodistillation was carried out for 6 h. The distillate was extracted with n-hexane and the essential oil was collected. The extra water was removed by adding anhydrous sodium sulphate. The volumes of pure essential oil were 5.05 mL. The essential oil was stored in airtight containers in refrigerator at 4°C.

2.2 Insects

Tribolium castaneum and *Lasioderma serricorne* were obtained from laboratory-reared maintained in the dark cabinet of an incubator for the last 2 years. The culture condition is 29 ± 1°C and 70–80% relative humidity. Two species of insects were reared in glass containers (0.5 L) containing wheat flour mixed with yeast (10:1, w/w). The unsexed insects used in all of the experiments were 1–2 weeks old adults.

2.3 GC-MS analysis of *A. anethoides* essential oil

GC instrument (Agilent 6890N) which was equipped with a flame ionization detector (FID) and coupled to a mass spectrometer (Agilent 5973N) was carried out to analysis the constituents of *A. anethoides* essential oil. Capillary column was HP-5MS (30 m × 0.25 mm × 0.25 µm). The column temperature was programmed at 60°C for 2 min, then risen at 10°C/min to 180°C for keeping 1 min, and then risen at 20°C/min to 280°C for keeping 15 min. The injector temperature was maintained at 270°C. The samples (1 µL, dilute to 1% with hexane) were injected with a split ratio of 1:10. The helium was used as the carrier gas at a flow rate of 1.0 mL/min. The identification of the constituents were based on comparison of their retention indices

(RI) obtained using n-alkanes (C₈–C₂₄). On the other hand depended on their mass spectra with those stored in NIST 05 (Standard Reference Data, Gaithersburg, MD, USA) and Wiley 275 libraries (Wiley, New York, NY, USA) or with mass spectra from the literature¹⁵. Relative percentages of the individual constituents of the essential oil were obtained by averaging the GC-FID peak area% reports.

2.4 Fumigant toxicity

The fumigant toxicity of the essential oil against two species of insects was tested as described by Liu and Ho¹⁶. A serial dilution of the essential oil was prepared in n-hexane. A Whatman filter paper (diameter 2.0 cm) was impregnated with 10 µL dilution, and then placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 25 mL). The solvent was allowed to evaporate for 20 s before the cap was placed tightly on the glass vial, each of which contained 10 insects inside to form a sealed chamber. n-Hexane was used as a negative control. The MeBr and phosphine were used as positive control against *T. castaneum* and *L. serricorne* respectively. Five replicates were carried out for all treatments and controls, and the dead insects were counted after 24 h.

2.5 Contact toxicity

The contact toxicity was tested based on the methods described by Liu and Ho¹⁶ against *T. castaneum* and *L. serricorne*. The essential oil was dissolved in n-hexane to prepare a serial testing solution. n-Hexane solvent was the negative control and the pyrethrins was the positive control. Aliquots of 0.5 µL of the dilutions were applied topically to the dorsal thorax of two species of insects. Five replicates were implemented for all treatments and controls. The dead insects were counted after 24 h.

2.6 Repellency tests

The repellent activity of the essential oil against *T. castaneum* and *L. serricorne* were investigated based on the method described by Zhang et al.¹⁷. Range-finding studies were run to determine the appropriate testing concentrations. The essential oil of *A. anethoides* was dissolved in n-hexane to prepare serials of testing solutions with five concentrations (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm²), and n-hexane was used as the negative control. Meanwhile a commercial repellent, N, N-diethyl-3-methylbenzamide (DEET) was used as a positive control. All experiments were repeated at least five times. Numbers of the insects present on each strip were counted after 2 h and 4 h. The value of percent repellency (PR) was calculated with following equation:

$$PR(\%) = [(Nc - Nt) / (Nc + Nt)] \times 100$$

here: Nc was the number of insects present on the negative control half, Nt was the number of insects present on

the testing part, and then the averaged PR values and the SE values were derived.

2.7 Data analysis

The data were corrected for control mortality using Abbott's formula. The LC_{50} (Fumigant toxicity) and LD_{50} (Contact toxicity) values were calculated by using Probit analysis (IBM SPSS V20.0)¹⁸⁾. In repellency tests, means and standard errors were conducted by Microsoft Excel 2007 for Windows XP.

3 RESULTS and DISCUSSION

3.1 Chemical composition of the essential oil

The essential oil was extracted from the leaves of *A. anethoides* with the yield of 1.01% (v/w) and the density of 0.98 g/mL. 17 components were identified in *A. anethoides* essential oil and the sum of their contents were accounted for 98.08% of the essential oil. The results were listed in Table 1. The main components were 1,8-cineole (36.54%), 2-isopropyl-5-methyl-3-cyclohexen-1-one (10.40%), terpinen-4-ol (8.58%), 2-isopropyltoluene (6.20), pinocarveol

(5.08%). There was not literature about the constituent of the essential oil of *A. anethoides*. 1,8-Cineole was rich in essential oil of *A. anethoides* and it was similar with many others artemisia species. For example, the percentage of 1,8-cineole was 22.03%, 32.93% and 39.88% in the essential oils of *Artemisia. argyi*, *Artemisia. stolonifera* and *Artemisia. mongolica*¹⁹⁻²¹⁾. 1,8-Cineole may be representative constituent in *Artemisia* Linn.

3.2 Fumigant toxicity

The fumigant toxicity of *A. anethoides* essential oil against *T. castaneum* and *L. serricornis* were listed in Table 2. The essential oil of *A. anethoides* possessed fumigant toxicity with a LC_{50} value of 13.05 mg/L air and 8.04 mg/L air against *T. castaneum* and *L. serricornis* respectively. When compared with the positive control, the essential oil demonstrated more weak toxicity against two species of insects. Nevertheless, based on similar bioassay tested methods, compared with the fumigant toxicity of other essential oils, the essential oil of *A. anethoides* exhibited stronger toxicity than *Litsea cubeba* (LC_{50} = 22.97 mg/L air)¹²⁾. The essential oil of *A. anethoides* showed weaker fumigant toxicity than the essential oils that their

Table 1 Chemical constituents of the essential oil derived from *A. anethoides*.

Peak No.	RI*	RT	Compounds	Chemical formula	Composition (%)
1	978	3.954	2-Isopropyltoluene	$C_{10}H_{14}$	6.20
2	1017	4.111	1,8-Cineole	$C_{10}H_{18}O$	36.54
3	1174	4.672	β -Terpineol	$C_{10}H_{18}O$	2.68
4	1126	4.899	p-Menth-2-en-1-ol	$C_{10}H_{18}O$	1.39
5	1155	5.091	Pinocarveol	$C_{10}H_{16}O$	5.08
6	1167	5.254	α -Pinocavone	$C_{10}H_{14}O$	3.77
7	1179	5.396	Terpinen-4-ol	$C_{10}H_{18}O$	8.58
8	1184	5.517	α -Terpineol	$C_{10}H_{18}O$	2.52
9	1251	6.021	2-Isopropyl-5-methyl-3-cyclohexen-1-one	$C_{10}H_{16}O$	10.40
10	1272	6.191	Bornyl acetate	$C_{12}H_{20}O_2$	0.37
11	1372	6.930	α -Copaene	$C_{15}H_{24}$	0.92
12	1414	7.278	β -Caryophyllene	$C_{15}H_{24}$	2.44
13	1479	7.740	Germacrene D	$C_{15}H_{24}$	4.49
14	1489	7.853	Bicyclogermacrene	$C_{15}H_{24}$	2.28
15	1527	8.088	4-(methylthio)-Phenol	$C_7H_8O_S$	4.46
16	1608	8.514	Davanone	$C_{15}H_{24}O_2$	4.38
17	1563	8.642	Spathulenol	$C_{15}H_{24}O$	1.58
total					98.08
Monoterpenoids					65.88
Sesquiterpenoids					16.09
Others					16.11

* RI, retention index as determined on a HP-5MS column using the homologous series of *n*-alkanes.

Table 2 Fumigant toxicity of *A. anethoides* essential oil against *T. castaneum* (TC) and *L. serricorne* (LS) adults.

Insect	Treatment	LC ₅₀ (95% FL) (mg/L air)	Slope ± SE	Chi square (χ ²)	p-value
TC	Essential oil	13.05 (11.81-14.39)	4.37 ± 0.42	11.39	0.998
	1,8-Cineole **	5.47 (4.73-6.17)	3.97 ± 0.47	25.30	—
	Terpinen-4-ol **	3.7 (3.3-4.3)	2.49 ± 0.31	27.56	0.233
	MeBr *	1.75	—	—	—
LS	Essential oil	8.04 (7.30-8.85)	0.98 ± 0.10	21.02	0.82
	1,8-Cineole **	5.18 (4.63-5.70)	4.84 ± 0.59	16.79	—
	Terpinen-4-ol **	1.3 (0.8-1.7)	1.50 ± 0.31	12.44	0.963
	Phosphine **	9.23 × 10 ⁻³ (7.13 × 10 ⁻³ -11.37 × 10 ⁻³)	2.12 ± 0.27	11.96	—

* data from Liu and Ho¹⁶⁾, ** data from Wang *et al.*^{22,23)} and be got at the same laboratory with author.

Table 3 Contact toxicity of *A. anethoides* essential oil against *T. castaneum* (TC) and *L. serricorne* (LS) adults.

Insect	Treatment	LD ₅₀ (95% FL) (μg/ adult)	Slope ± SE	Chi square (χ ²)	p-value
TC	Essential oil	28.80 (26.16-31.77)	4.47 ± 0.43	11.95	0.997
	1,8-Cineole *	18.83 (17.13-20.69)	4.86 ± 0.50	16.56	—
	Terpinen-4-ol *	19.7 (17.1-22.7)	2.13 ± 0.29	19.28	0.685
	Pyrethrins *	0.26 (0.22-0.30)	3.34 ± 0.32	13.11	0.925
LS	Essential oil	24.03 (22.01-26.30)	5.41 ± 5.06	13.34	0.946
	1,8-Cineole *	15.58 (12.88-18.02)	3.87 ± 0.55	15.18	—
	Terpinen-4-ol *	5.4 (4.0-6.6)	1.70 ± 0.30	11.63	0.976
	Pyrethrins *	0.24 (0.16-0.35)	1.31 ± 0.20	17.36	0.916

* data from Wang *et al.*^{22,23)} and be got at the same laboratory with author.

main constituents were similar against *L. serricorne*, for example, *A. argry* (LC₅₀ = 8.04 mg/L air), *A. stolonifera* (LC₅₀ = 0.99 mg/L air) and *A. mongolica* (LC₅₀ = 6.08 mg/L air)¹⁹⁻²¹⁾. 1,8-Cineole and terpinen-4-ol showed excellent fumigant toxicity (Table 2) against two species of insects. This may be helpful to the fumigant toxicity of the essential oil of *A. anethoides*.

3.3 Contact toxicity

The contact toxicity of *A. anethoides* essential oil against *T. castaneum* and *L. serricorne* were listed in Table 3. The results exhibited the essential oil of *A. anethoides* possessed contact toxicity against *T. castaneum* (LD₅₀ = 28.80 μg/adult) and *L. serricorne* (LD₅₀ = 24.03 μg/adult). When compared with the positive control, pyrethrins, the essential oil demonstrated more weak toxicity against *T. castaneum* and *L. serricorne*. However, compared with other essential oils tested using a similar bioassay in the literature, the essential oil of *A. anethoides* possessed stronger contact toxicity against *T. castaneum* adults, e.g. essential oils of *Evodia lepta* (LD₅₀ = 166.94 μg/adult), *Citrus wilsonii* (LD₅₀ = 48.49 μg/adult)^{24,25)}. On the

other hand, the essential oil of *A. anethoides* showed weaker contact toxicity than the essential oils that their main constituents were similar against *L. serricorne*, for example, *A. argry* (LC₅₀ = 6.42 μg/adult) and *A. mongolica* (LC₅₀ = 22.32 μg/adult)^{19,21)}. 1,8-Cineole and terpinen-4-ol showed obvious contact toxicity (Table 3) against two species of insects.

3.4 Repellent activity

The essential oil of *A. anethoides* exhibited obvious repellent activity against *T. castaneum* and *L. serricorne* (Fig. 1). The PR values of essential oil were 100% at the testing concentrations of 78.63 and 15.73 nL/cm² against *T. castaneum* at 2h and 4 h after exposure, meanwhile the PR values of essential oil were same level with DEET at five testing concentrations against *T. castaneum* at 2h and 4 h after exposure, even the PR values of essential oil were 45% at the lowest testing concentrations of 0.13 nL/cm² against *T. castaneum* at 2h. The results indicated the essential oil of *A. anethoide* possessed approximate repellency activity with positive control DEET against *T. castaneum*. The essential oil of *A. anethoides* showed same

Fig. 1 Percentage repellency of DEET and the essential oil of *A. anethoides* against *T. castaneum* and *L. serricorne* at 2 h and 4 h after exposure.

repellent activity level with DEET at the testing concentrations of 78.63 and 15.73 nL/cm² at 2h after exposure against *L. serricorne*. The PR values of essential oil were more low than DEET at others low testing concentrations against *L. serricorne*. The repellent activity of essential oil was more obvious against *T. castaneum* than *L. serricorne*.

4 CONCLUSION

The repellency method is one of common pest control, the essential oil of *A. anethoides* exhibited excellent repellency activity against *T. castaneum* and *L. serricorne*. Meanwhile it showed certain fumigant and contact toxicity against two species of insects. The essential oil of *A. anethoides* possessed potential insecticidal and repellent affect for control of insects. Now many biopesticide products have entered the market. For example, there are about 400 registered biopesticides active ingredients and over 1250 products in the United States, and the biopesticides has been substantial growth in recent years, but they still comprise less than 4% of the global pesticides market²⁶⁾. Like other crop protection technologies, biopesticides also will become smarter through research and innovation.

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