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Chemical composition and insecticidal activities of the essential oil of the flowering aerial parts of *Aster ageratoides*

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Abstract: Water-distilled essential oil from the flowering aerial parts of *Aster ageratoides* Turcz. (Compositae) was analyzed by gas chromatography-mass spectrometry (GC–MS) for the first time. Forty-three compounds, accounting for 96.4 % of the oil, were identified. The main compounds found were α-terpineol (10.8 %), β-caryophyllene (10.3 %), linalool (7.2 %), D-limonene (6.9 %), spathulenol (6.5 %), bornyl acetate (5.8 %) and bicyclosesquiphellandrene (5.6 %). The essential oil of *A. ageratoides* flowering aerial parts possessed contact toxicity against two grain storage insects *Sitophilus zeamais* and *Tribolium castaneum* adults with LD_{50} values of 27.16 and 8.09 μg per adult, respectively. The essential oil also exhibited fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 13.73 and 12.14 mg L^{-1}, respectively. The essential oil showed potential for development as a possible natural fumigant/insecticide for control of insects in stored products.

Keywords: *Aster ageratoides*; *Sitophilus zeamais*; *Tribolium castaneum*; essential oil.

INTRODUCTION

Aster ageratoides Turcz. (Family: Compositae) is a perennial suffrutescent erect herb with long stoloniferous rhizomes that is distributed widely in Siberia, Mongolia, China, South Korea and Japan.[1] Great variations (many types) were observed in this species and 11 varieties (such as var. *ageratoides*; var. *oophyllus*; var. *pilosus*; var. *ovatus*) were suggested. This plant has long been used in traditional Chinese medicine for the treatment of colds, fever, tonsillitis, bronchitis, snake bites and bee stings.[2] Phytochemical analyses of *A. ageratoides* led to the isolation of a number of chlorogenic acids, sesquiterpene lactones, kaurane diter-

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penoids, diterpene glycosides, pentacyclic triterpenoids and triterpenoid saponins.[3–10] However, the constituents of the essential oil derived from the flowering aerial parts of *A. ageratoides* have not been determined to date. Only the essential oil and headspace constituents from the aerial parts of *A. ageratoides* var. *ovatus* were determined by GC and GC–MS.[11] Moreover, the insecticidal activities of the essential oil of *A. ageratoides* against grain storage insects have not been measured. The present investigation consisted of two parts: determination of chemical composition of the essential oil of the flowering aerial parts of *A. ageratoides* and an evaluation of the essential oil as insecticide/fumigant against two grain storage insect pests.

The maize weevil (*Sitophilus zeamais* Motsch.) and red flour beetle (*Tribolium castaneum* Herbst) are two serious pest species of stored grains worldwide.[12] Infestations not only cause significant losses due to the consumption of grains, but also they result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species.[13] Infestations of grain storage insect pests could be controlled by using synthetic fumigants or insecticides. However, the heavy use of synthetic insecticides/fumigants has led to problems, such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to insecticides and lethal effects on non-target organisms, in addition to direct toxicity to users. Moreover, the use of methyl bromide will be prohibited in the near future because of its ozone depletion potential. These problems have highlighted the need to find new safe, economical and effective fumigants. Fortunately, essential oils or their constituents may provide an alternative to currently used fumigants/pesticides in the control of stored-food insects. Investigations in several countries confirm that some plant essential oils not only repel insects, but possess contact and fumigant toxicity against stored product pests as well as exhibiting feeding inhibition or harmful effects on the reproductive system of insects.[14] The toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects.[15–18] The purpose of the present study was to ascertain the chemical composition of *A. ageratoides* and to evaluate the effectiveness of its essential oil as a fumigant and contact toxicant against two species of stored-product beetles.

EXPERIMENTAL

Plant material and essential oil extraction

The flowering aerial parts of *A. ageratoides* were collected in August 2009 from Xiaolongmen National Forest Park (39.48° N latitude and 115.25° E longitude, Mentougou District, Beijing 102300). The samples were air-dried and identified by Dr. Q. R. Liu (College of Life Sciences, Beijing Normal University, Beijing 100875, China) and a voucher specimen (ENTCAU-Compositae-10044) was deposited at the Department of Entomology, China Agricultural University (Beijing 100193). The samples were ground to a powder using a grinding

mill (Retsch Mühle, Germany). Each 600 g portion of powder 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. Volatile essential oil from the distillation was collected in a flask. Separation of the essential oil from the aqueous layer was performed in a separating funnel, using the non-polar solvent, *n*-hexane. The solvent was evaporated using a vacuum rotary evaporator (Büchi Rotavapor R-124, Switzerland). The sample was dried over anhydrous Na_2SO_4 and kept in a refrigerator (4 °C) for the subsequent experiments.

Insects

S. zeamais and *T. castaneum* were obtained from laboratory cultures maintained in the dark in incubators at 29–30 °C and 70–80 % r.h. The *T. castaneum* were reared on wheat flour mixed with yeast (10:1, w/w) while the *S. zeamais* were reared on whole wheat at 12–13 % moisture content in glass jars (diameter 85 mm, height 130 mm). Unsexed adult weevils/ /beetles used in all the experiments were about 2 weeks old. All containers housing the insects and the Petri dishes used in the experiments were made escape-proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, UK).

Gas chromatography–mass spectrometry

The essential oil of *A. ageratoides* was subjected to GC–MS analysis on an Agilent system consisting of a model 6890N gas chromatograph, a model 5973N mass selective detector (EIMS, electron energy 70 eV), and an Agilent ChemStation data system. The GC column was an HP-5MS fused silica capillary with a 5 % phenyl-methylpolysiloxane stationary phase, with a film thickness of 0.25 μm, a length of 30 m and an internal diameter of 0.25 mm. The following GC settings were employed: the initial oven temperature was held at 60 °C for 1 min and increased at 10 °C min^{-1} to 180 °C held for 1 min, and then increased at 20 °C min^{-1} to 280 °C and held for 15 min. The injector temperature was maintained at 270 °C. The sample (1 μL) was injected neat, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0 mL min^{-1}. Spectra were scanned from 20 to 550 *m*/*z* at two scans s^{-1}. Most constituents were identified by gas chromatography by comparison of their retention indices with those in the literature or with those of authentic compounds. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) run under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in the NIST 08 and Wiley 275 libraries or with mass spectra from the literature.[19] Component relative percentages were calculated based on the normalization method without using correction factors.

Fumigant toxicity bioassay

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. The fumigant toxicity of *A. ageratoides* essential oil was determined using the method of Liu and Ho[20] with some modifications. A serial dilution of the essential oil (5.0–20.0 %, 6 concentrations) was prepared in *n*-hexane. A Whatman filter paper (CAT No. 1001020, diameter 2.0 cm) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 24 mL). Ten microliters of an appropriate concentration of the essential oil was added to the filter paper. The solvent was allowed to evaporate for 15 s before the cap was placed tightly on the glass vial (with 10 unsexed insects) to form a sealed chamber. Preliminary experiments demonstrated that 15 s was sufficient for the evaporation of solvents. The vials were upright and the Fluon (ICI America Inc.) coating restricted the insects to the lower portion of the vial to prevent them from reaching the treated filter paper. *n*-Hexane was used as a control. They were incubated at 27–29 °C and 70–80 %

relative humidity for 24 h. Five replicates were performed for all treatments and controls. The insects were considered dead if their appendages did not move when probed with a camel brush. The mortality of the insects was observed and the results from all replicates were subjected to Probit analysis using the PriProbit Program V1.6.3 to determine the LC_{50} values.[21]

Contact toxicity by topical application

Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil (5.0–25.0 %, v/w, 6 concentrations) was prepared in *n*-hexane. Aliquots of 0.5 μL of the dilutions were applied topically to the dorsal thorax of the insects, using a Burkard Arnold microapplicator. Controls were determined using *n*-hexane. Both treated and control insects were then transferred to glass vials (10 insects per vial) with culture media and kept in incubators. The mortality of insects was observed daily until the end-point mortality was reached one week after treatment. The insects were considered dead if their appendages did not move when probed with a camel brush. The LD_{50} values were calculated using Probit analysis.[21]

RESULTS AND DISCUSSION

The yield of the yellow essential oil of the flowering aerial parts of *A. ageratoides* was 0.79 % (vol/wt) and the density of the concentrated essential oil was 0.87 g mL^{-1}. A total of 43 components were identified in the essential oil of the flowering aerial parts of *A. ageratoides*, accounting for 96.4 % of the total oil (Table I). The main compounds found were *α*-terpineol (10.8 %), *β*-caryophyllene (10.3 %), linalool (7.2 %), D-limonene (6.9 %), spathulenol (6.5 %), bornyl acetate (5.8 %) and bicyclosesquiphellandrene (5.6 %) (Table I). Monoterpenoids represented 21 of the 43 compounds, corresponding to 53.6 % of the whole oil while 20 of the 43 constituents were sesquiterpenoids (41.1 % of the crude essential oil).

TABLE I. Chemical constituents of the studied essential oil

Peak No.	Compound	*RI*[a]	Content, %
1	*α*-Pinene	931	2.3
2	*β*-Pinene	974	1.3
3	*p*-Cymene	1024	0.2
4	D-Limonene	1027	6.9
5	1,8-Cineole	1031	3.6
6	(*Z*)-*β*-Ocimene	1037	1.1
7	(*E*)-*β*-Ocimene	1048	1.2
8	Linalool	1097	7.2
9	*β*-Phenylethanol	1116	0.5
10	Borneol	1167	1.7
11	4-Terpineol	1177	3.7
12	*p*-Cymene-8-ol	1182	1.8
13	*α*-Terpineol	1189	10.8
14	Verbenone	1204	0.7
15	*cis*-Carveol	1222	0.5

TABLE I. Continued

Peak No.	Compound	*RI*[a]	Content, %
16	*cis*-Geraniol	1229	0.2
17	Isothymol methyl ether	1244	0.7
18	*trans*-Geraniol	1252	2.0
19	Perillaldehyde	1274	0.8
20	Bornyl acetate	1285	5.8
21	Cuminic alcohol	1292	0.7
22	Neric acid	1347	0.4
23	Eugenol	1356	1.2
24	Cyclosativene	1364	0.2
25	Ylangene	1372	0.3
26	Copaene	1375	0.6
27	β-Bourbonene	1383	0.3
28	β-Elemene	1390	1.5
29	10*S*,11*S*-Himachala-3(12), 4-diene	1401	0.5
30	β-Caryophyllene	1420	10.3
31	α-Ionone	1427	1.2
32	Geranyl acetone	1452	0.5
33	α-Caryophyllene	1456	1.3
34	*allo*-Aromadendren	1461	0.9
35	γ-Gurjunene	1473	1.5
36	α-Selinene	1495	1.3
37	Bicyclosesquiphellandrene	1499	5.6
38	1ξ,6ξ,7ξ-Cadina-4,9-diene	1502	1.9
39	γ-Cadinene	1512	1.7
40	δ-Cadinene	1523	1.8
41	Ledol	1565	1.7
42	Spathulenol	1578	6.5
43	Caryophyllene oxide	1584	1.5
	Total		96.4

[a]Retention index as determined on a HP-5MS column using a homologous series of *n*-hydrocarbons

No death of insects was observed in the control under the current concentration. The essential oil of the flowering aerial parts of *A. ageratoides* possessed contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 27.16 and 8.09 μg per adult, respectively (Table II). However, the essential oil *A. ageratoides* demonstrated only one-fifth and one-twentieth of the toxicity of a pyrethrum extract against *S. zeamais* and *T. castaneum* (LD_{50} = 4.29 and 0.36 μg per adult, respectively).[22]

The essential oil of the flowering aerial parts of *A. ageratoides* exhibited fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 13.73 and 12.14 mg L^{-1}, respectively (Table II). No death of insects was observed in the control under the current concentration. The commercial grain fumigant, methyl bromide (MeBr) exhibited fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 0.67 and 1.75 mg L^{-1}, respectively.[20]

Thus, the toxicity of the essential oil was one-twentieth and one-sixth that of MeBr towards *S. zeamais* and *T. castaneum* adults, respectively. However, compared with other essential oils that were tested in the previous studies using a similar bioassay, the essential oil of the flowering aerial parts of *A. ageratoides* exhibited stronger fumigant toxicity against *S. zeamais* and *T. castaneum* adults than the essential oils of *Caryopteris incana*,[23] *Kadsura heteroclite*,[16] *Illicium fragesii*,[24] *I. simonsii*,[17] *Murraya exotica*[22] and several essential oils from the genus *Artemisia*.[15] Moreover, the three main constituent monoterpenoids, *α*-terpineol, linalool and D-limonene have been demonstrated to possess fumigant and contact toxicity activity against several stored-product insects.[25–29] The isolation and identification of the bioactive constituent compounds in the essential oil of the flowering aerial parts of *A. ageratoides* are of utmost importance so that their potential application in controlling stored-product pests can be fully exploited.

TABLE II. Contact and fumigant toxicity of *Aster ageratoides* essential oil against *Sitophilus zeamais* and *Tribolium castaneum* adults

Insect	Treatment	Contact toxicity			Fumigant toxicity		
		LD_{50} / μg adult^{-1} (95 % confidence limit)	Slope ±*SE*	χ^2	LC_{50} / μg mL^{-1} air (95 % confidence limit)	Slope ±*SE*	χ^2
S. zeamais	*A. ageratoides*	27.16 (20.67–26.08)	2.45 ±0.21	12.43	13.73 (11.32–15.12)	3.23 ±0.25	11.26
	Pyrethrum extract[22]	4.29 (3.86–4.72)	–	–	–	–	–
	MeBr[20]	–	–	–	0.67	–	–
T. castaneum	*A. ageratoides*	8.09 (7.89–9.12)	3.23 ±0.32	17.49	12.14 (11.67–13.73)	3.51 ±0.27	16.21
	Pyrethrum extract[22]	0.36 (0.32–0.41)	–	–	–	–	–
	MeBr[20]	–	–	–	1.75	–	–

The present findings suggest that the fumigant activity of the essential oil of the flowering aerial parts of *A. ageratoides* is quite promising considering that the currently used fumigants are synthetic insecticides. The essential oil of the flowering aerial parts of *A. ageratoides* could play an important role in stored grain protection and reduce the need for and the risks associated with synthetic insecticides. However, for the practical application of the essential oil as a novel insecticide/fumigant, further studies on the safety of the essential oil to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce costs.

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И З В О Д

ХЕМИЈСКИ САСТАВ И ИНСЕКТИЦИДНА АКТИВНОСТ ЕТАРСКОГ УЉА НАДЗЕМНИХ ДЕЛОВА У ЦВЕТУ БИЉКЕ *Aster ageratoides*

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Етарско уље надземних делова у цвету биљке *Aster ageratoides* Turcz. (Compositae) је добијено дестилацијом воденом паром и анализирано је методом гасне хроматографије-масене спектрометрије (GC–MS). Идентификована су 43 једињења, што чини 96,4 % уља. Главни састојци су α-терпинеол (10,8 %), β-кариофилен (10,3 %), линалоол (7,2 %), D-лимонен (6,9 %), спатуленол (6,5 %), борнил-ацетат (5,8 %) и бициклосесквифеландрен (5,6 %). Етарско уље надземних делова у цвету биљке *A. ageratoides* испољило је цитотоксичност спрам одраслих инсеката који настањују силосе жита *Sitophilus zeamais* и *Tribolium castaneum*. LD_{50} вредности су биле 27,16 и 8,09 μg по јединки. Етарско уље је, такође, имало фумигантну активност спрам *S. zeamais* и *T. castaneum*. LC_{50} вредности су биле 13,73 и 12,14 mg L^{-1}. Испитивано етарско уље има потенцијал за даљу примену као природни фумигант/инсектицид у контроли инсеката у силосима.

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