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Full Length Research Paper

Composition and insecticidal activity of the essential oil of *Artemisia igniaria* Maxim. flowering aerial parts against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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The aim of this research was to determine chemical composition and insecticidal activity of the essential oil of *Artemisia igniaria* Maxim. (Asteraceae) aerial parts against the maize weevils (*Sitophilus zeamais* Motschulsky). Essential oil of *A. igniaria* aerial parts was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 40 components of the essential oil were identified. The principal compounds in the essential oil were 1,8-cineole (14.36%), camphor (13.38%), germacrene D (8.77%), caryophyllene (6.91%) and linalool (6.21%) followed by selina-6-en-4-ol (4.69%), 4-terpineol (4.56%), α-terpineol (4.30%) and spatulenol (4.22%). The essential oil exhibited strong contact toxicity against *S. zeamais* with an LD_{50} value of 14.41 μg/adult. The essential oil also possessed fumigant toxicity against *S. zeamais* with an LC_{50} value of 7.22 mg/L. The results indicated that the essential oil of *A. igniaria* showed potential in terms of contact and fumigant toxicity against grain storage insect.

Key words: *Artemisia igniaria*, *Sitophilus zeamais*, contact toxicity, fumigant, essential oil composition.

INTRODUCTION

The maize weevil (*Sitophilus zeamais* Motschulsky) is one of the major pests of stored grains and grain products in the tropics and subtropics (Liu and Ho, 1999). Infestations not only cause significant losses due to the consumption of grains; they also result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species (Magan et al., 2003). Currently, control of stored product insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbance of the environment, increasing application costs, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to the users (Zettler and Arthur, 2000). Thus, there is a considerable interest in developing natural products that are relatively less damaging to mammalian health and the environment than existing conventional pesticides, as alternatives to non-selective synthetic pesticides to control the pests of medical and economic importance (Isman, 2000, 2006).

In recent years, various workers have been concentrating their efforts on the search for natural products as an alternative to conventional insecticides and fumigants, as well as the re-evaluation of traditional botanical pest control agents (Isman, 2006). Essential oils or their constituents may provide an alternative to currently used fumigants/pesticides to control stored-food

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insects. Several essential oils derived from plant species of *Artemisia* have been evaluated for insecticidal activities against stored product insects (Tripathi et al., 2000; Kordali et al., 2006; Liu et al., 2006; Negahban et al., 2006; 2007; Wang et al., 2006; Goel et al., 2007; Mohamed and Abdelgaleil, 2008; Chu et al., 2010a; Yuan et al., 2010). 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 exhibited feeding inhibition or harmful effects on the reproductive system of insects (Isman, 2006; Rajendran and Srianjini, 2008).

During the screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Artemisia igniaria* Maxim. (Family: Asteraceae) flowering aerial parts was found to possess strong insecticidal toxicity against the grain storage insect, *S. zeamais* (Liu et al., 2007). *A. igniaria* is an herbaceous plant distributed mainly in the north of China and, is used as traditional medicinal herb in some areas of China (Folium Artemisiae Argyi) (Ling and Ling, 1991). Several triterpenoids, ρ-coumaric acid, long chain alkyl esters and flavonoids were isolated from the methanol extract of this plant (Hu and Feng, 2000). However, a literature survey has shown that there is no report on the volatile constituents and insecticidal activity of *A. igniaria*; thus we decided to investigate the chemical constituents and insecticidal activities of the essential oil of *A. igniaria* against *S. zeamais,* a grain storage insect for the first time.

MATERIALS AND METHODS

Plant material

The flowering aerial parts of *A. igniaria* were collected in August 2009 from Xiaolongmen National Forest Park (39.48° N latitude and 115.25° E longitude, Mentougou District, Beijing). The samples were air-dried and identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing, China) and a voucher specimen (ENTCAU-Compositae-10005) was deposited at the Department of Entomology, China Agricultural University (Beijing 100193, China). The samples were ground to a powder using a grinding mill (Retsch Mühle, Germany). Each 600 g portion of powder was mixed in 1,800 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 distillation was collected in a flask. Separation of the essential oil from the aqueous layer was done in a separatory funnel, using the non-polar solvent, *n*-hexane. The solvent was evaporated using a vacuum rotary evaporator (BUCHI Rotavapor R-124, Switzerland). The sample was dried over anhydrous Na_2SO_4 and kept in a refrigerator (4°C) for subsequent experiments.

Insects

The maize weevils (*S. zeamais*) were obtained from laboratory cultures in the dark in incubators at 29-30℃ and 70-80% relative humidity and were reared on whole wheat at 12-13% moisture content in glass jars (diameter 85 mm, height 130 mm). Mixed sex adult weevils used in all the experiments were about one week old. All containers housing insects and the Petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, UK).

Gas Chromatography-Mass Spectrometry

The essential oil of *A. igniaria* was subjected to GC-MS analysis on an Agilent system consisting of a model 6890N gas chromatograph, a model 5973 N 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, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The GC settings were as follows: the initial oven temperature was held at 60℃ for 1 min and ramped at 10℃ min $^{-1}$ to 180℃ held for 1 min, and then ramped at 20℃ min $^{-1}$ to 280℃ and held for 15 min. The injector temperature was maintained at 270℃. The sample (1 µl) was injected neat, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0 ml min^{-1}. Spectra were scanned from 20 to 550 m/z at 2 scans s^{-1}.

Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 08 and Wiley 275 libraries or with mass spectra from literature (Adams, 2007). Component relative percentages were calculated based on normalization method without using correction factors.

Contact toxicity by topical application

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil of *A. igniaria*. A serial dilution of the essential oil (9.94-20.00%, 6 concentrations) was prepared in *n*-hexane. Aliquots of 0.5 µl per insect were topically applied dorsally to the thorax of the weevils, using a Burkard Arnold microapplicator. The control was set up using 0.5 µl *n*-hexane per insect. Ten insects (one week old) were used for each concentration and control, and the experiment was replicated six times. Both the treated and control weevils were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators at 29-30°C and 70-80% relative humidity. Mortality was observed after 24 h. Results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LD_{50} values (Sakuma, 1998).

Fumigant toxicity bioassay

Range-finding studies were run to determine the appropriate testing concentrations of *A. igniaria* essential oil. The fumigant toxicity of *A. igniaria* essential oil was determined by used the method of Liu and Ho (1999) with some modifications. A Whatman filter paper (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 the essential oil (5.39-20.00%, 6 concentrations) 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 mixed sex insects, about one week old) to form a sealed chamber. They were incubated at 27-29°C and 70-80% relative humidity for 24 h.

Mortality of insects was observed and results from all replicates were subjected to probit analysis using the PriProbit Program

Table 1. Chemical constituents of essential oil derived from *Artemisa igniaria* aerial parts.

Compounds	RI*	Peak Area (%)
α-Pinene	931	0.05
Camphene	953	0.05
Benzaldehyde	964	0.32
1,8-Cineole	1032	14.36
Acetophenone	1066	1.38
Linalool	1097	6.21
Camphor	1146	13.38
4-Terpineol	1179	4.56
α-Terpineol	1191	4.30
Myrtenol	1196	1.03
cis-Piperitol	1195	0.46
(S)-Verbenone	1204	0.63
cis-Carveol	1226	0.38
Cuminaldehyde	1236	0.21
cis-Geraniol	1249	0.42
trans-Geraniol	1253	0.91
Geranial	1267	0.13
cis-Isogeraniol	1273	0.38
Perillyl aldehyde	1279	0.29

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

V1.6.3 to determine LC_{50} values (Sakuma, 1998).

RESULTS AND DISCUSSION

The yellow essential oil yield of *A. igniaria* flowering aerial parts was 0.31% (V/W) and the density of the concentrated essential oil was determined as 0.89 g/ml. A total of 40 components of the essential oil were identified, accounting for 86.95% of the total oil. The principal compounds in the essential oil of *A. igniaria* flowering aerial parts were 1,8-cineole (14.36%), camphor (13.38%), germacrene D (8.77%), caryophyllene (6.91%) and linalool (6.21%) followed by selina-6-en-4-ol (4.69%), 4-terpineol (4.56%), α-terpineol (4.30%) and spatulenol (4.22%) (Table 1). Monoterpenoids represented 21 of the 40 compounds, corresponding to 50.13% of the whole oil while 14 of the 40 constituents were sesquiterpenoids (41.93% of the crude essential oil). The essential oil of *A. igniaria* flowering aerial parts exhibited contact toxicity against *S. zeamais* adults with an LD_{50} value of 14.41 µg/adult (Table 2). When compared with the positive control pyrethrum extract, the essential oil demonstrated 3 times less toxic against *S. zeamais*. However, compared with the other essential oils in the literature, the essential oil of *A. igniaria* flowering aerial parts possessed stronger contact toxicity against *S. zeamais* adults, for example, essential oils of *Artemisia lavandulaefolia*, *A. sieversiana*, *A. capillaries*, *A. mongolica*, and *A. vestita* (LD_{50} = 55.2 µg/adult, 113.0 µg/adult, 106.0 µg/adult, 87.9 µg/adult, and 50.6 µg/adult, respectively) (Liu et al. 2010a, 2010b; Chu et al., 2010a), essential oil of *Schizonpeta multifida* (30.2 µg/adult) (Liu et al. 2011), essential oil of *Illicium simonsii* fruits (LD_{50} = 112.7 µg/adult, Chu et al., 2010b).

The essential oil of *A. igniaria* flowering aerial parts possessed fumigant toxicity against the maize weevils with an LC_{50} value of 7.22 mg/L (Table 2). The commercial grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* adults with an LC_{50} value of 0.67 mg/L (Liu and Ho, 1999), thus the essential oil was 11 times less toxic to *S. zeamais* adults compared with MeBr.

However, compared with the other essential oils in the previous studies, the essential oil of *A. igniaria* exhibited stronger fumigant toxicity against *S. zeamais* adults, for example, essential oils of *S. multifida* (Liu et al. 2011), *Murraya exotica* (Li et al., 2010), and several essential oils from Genus *Artemisa* (Chu et al., 2010a; Liu et al., 2010a, b). Moreover, in the previous reports, two main constituents of the essential oil, 1,8-cineole and camphor have been found to possess fumigant toxicity against several stored product insects, such as *S. granarius*, *S. oryzae*, *Tribolium castaneum, T. confusum*, and *Rhyzopertha dominica* (Aggarwal et al., 2001; Lee et al., 2004; Kordali et al., 2006; Rozman et al., 2007; Abdelgaleil et al., 2009; Suthisut et al., 2011). The above findings suggest that fumigant activity of the essential oil of *A. igniaria* flowering aerial parts is quite promising by considering some synthetic fumigants and it shows potential to be developed as possible natural fumigant/insecticide for control of stored product insects.

Table 1. Contd.

trans-Anethole	1283	1.13
4-Vinylguaiacol	1323	0.55
ρ-Mentha-1,4-dien-7-ol	1330	0.23
γ-Pyronene	1343	0.86
Longipinene	1348	0.97
Eugenol	1356	1.86
α-Cubebene	1350	1.27
β-Bourbonene	1382	2.52
β-Elemene	1389	1.95
cis-Jasmone	1394	0.27
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1-methyl-6-methylene-4-(1-methylethyl)-	1401	1.25
Methyleugenol	1408	0.56
Caryophyllene	1430	6.91
β-Gurjunene	1434	1.94
1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	1456	2.26
allo-Aromadendrene	1459	0.66
α-Amorphene	1479	0.59
Germacrene D	1482	8.77
δ-Cadinene	1523	3.27
Spatulenol	1578	4.22
Selina-6-en-4-ol	1615	4.69
Total		96.16
Monoterpenoids		50.13
Sesquiterpenoids		41.93
Others		4.10

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

Table 2. Contact (CT) and Fumigant toxicity (FT) of *Artemisa igniaria* essential oil against *Sitophilus zeamais* adults.

	Treatment	LD_{50} (µg/adult) LC_{50} (mg/L air)	95% FL	Slope ± SE	Chi square (χ^2)
CT	*A. igniaria*	14.41	13.21-15.92	3.85 ± 0.42	16.43
	Pyrethrum extract*	4.29	3.86-4.72	-	-
FT	*A. igniaria*	7.22	6.61-7.88	2.76 ± 0.30	18.07
	MeBr**	0.67	-	-	-

*from Wang et al. (2011). **from Liu and Ho (1999).

Conclusion

The composition of the essential oil derived from *A. igniaria* flowering aerial parts was determined by GC-MS for the first time. The essential oil was demonstrated to exhibit strong contact and fumigant toxicity against *S. zeamais* adults. These findings suggest that the essential oil of *A. igniaria* flowering aerial parts possessed potential for development as novel natural insecticide/fumigant for stored products. However, for the practical application of the essential oil as 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 cost.

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