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Full Length Research Paper

Composition and toxicity of Chinese *Dracocephalum moldavica* (Labiatae) essential oil against two grain storage insects

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Essential oil of *Dracocephalum moldavica* flowering aerial parts was extracted via hydrodistillation. A total of 38 components of the essential oil were identified. 1, 8-Cineol (31.25%) and 4-terpineol (22.82%) were the two main components of the essential oil followed by cumin alcohol (4.29%) and α-terpineol (4.21%). The essential oil exhibited strong fumigant toxicity against *Sitophilus zeamais* and *Tribolium castaneum* adults with LC_{50} values of 2.65 and 0.88 mg/L, respectively. The essential oil also showed contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 22.10 and 18.28 µg/adult, respectively. The essential oil of *D. moldavica* may have the potential to be developed as a new natural fumigant/insecticide for the control stored product insects.

Key words: *Dracocephalum moldavica*, *Sitophilus zeamais*, *Tribolium castaneum*, contact toxicity, essential oil composition.

INTRODUCTION

Sitophilus and *Tribolium* species are two 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). Control of the stored product insects is based on the application of synthetic insecticides/fumigants. However, repeated use of those fumigants for decades has led to resurgence of stored-product insect pests, sometimes resulted in the development of resistance, and had undesirable effects on non-target organisms (United States Environmental Protection Agency, 1993; Zettler and Arthur, 2000). These problems have highlighted the need to develop new types of selective insect-control alternatives with fumigant action. Plant essential oils and their components have been shown to possess potential to be developed as new fumigants and they may have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (Isman, 2006). Essential oils derived from more than 75 plant species have been evaluated for fumigant toxicity against stored product insects so far (Rajendran and Srianjini, 2008).

During our screening program for new agrochemicals from Chinese medicinal herbs and local wild plants, essential oil of *Dracocephalum moldavica* L. (Family: Labiatae) flowering aerial parts was found to possess insecticidal toxicity to the maize weevils, *Sitophilus zeamais* Motschulsky and the red back flour beetles, *Tribolium castaneum* (Herbst). *D. moldavica* is an annual herbaceous aromatic plant and native to central Asia and is naturalized in eastern and central Europe (Tian et al., 2009). In China, it is predominantly found in the north of the country. This plant has been of interest to Uygur traditional medicine, especially in north Xinjiang

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automonous region. It is used as a food ingredient, as a tea, as an herbal drug for its reputed medicinal properties, for example, the treatment of stomach and liver disorders, headaches and congestions, with strong cardioprotective effect (Chinese Pharmacopoeia Commission, 1999).

Previous phytochemical studies on *D. moldavica* resulted in the identification of phenolics (rosmarinic acid, caffeic acid), iridoids, sesquiterpenoids, flavonoids, triterpenoids, and steroids (Gu et al., 2004; 2005; Dastmalchi et al., 2006, 2007; Feng and Li, 2006; Popova et al., 2008; Sultan et al., 2008). Zeng et al. (2010) reviewed the constituents derived from the plants of genus *Dracocephalum* (including terpenoids, flavonoids, alkaloids, lignans, phenols, coumarins, and cyanogenic glucosides) and the biological activities were also discussed. The composition of the essential oil of the aerial flowering parts of *D. moldavica* has been investigated previously (Holm et al., 1988; Shatar and Altantsetseg, 2000; El-Baky and El-Baroty, 2008; Sonboli et al., 2008). The essential oil of *D. moldavica* was evaluated as a seed protectant against the pulse beetle (*Callosobruchus maculatus*) (Aziz and Abbass, 2010). However, no reports on toxicity of *D. moldavica* essential oil against the two grain storage insects were available so far. This study analyses the chemical composition and toxicity of essential oil of *D. moldavica* against the two grain storage insects.

MATERIALS AND METHODS

Plant material

Fresh aerial parts (10 kg of leaves, stems and flowers) of *D. moldavica* were harvested in August 2009 from Xiaolongmeng National Forest Park (Mentougou District, Beijing 102300). The aerial parts were air-dried for one week and ground to a powder using a grinding mill (Retsch Mühle, Germany). The species was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (BNU-zhilongliu-2009-08-29-031) was deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University. The aerial parts were ground to a powder using a grinding mill (Retsch Mühle, 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 to 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*) and red flour beeltes (*T. castaneum*) were obtained from laboratory cultures maintained for the last 15 years in the dark in incubators at 29 to 30°C and 70 to 80% relative humidity. The red flour beetle was reared on wheat flour mixed with yeast (10:1, w/w) while the maize weevils were reared on whole wheat at 12 to 13% moisture content in glass jars (diameter 85 mm, height 130 mm) at 29 to 30°C and 70 to 80% relative humidity. Unsexed adult weevils and beetles used in all the experiments were about 2 weeks old.

Gas chromatography-mass spectrometry

The essential oil of *D. moldavica* flowering aerial parts 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, 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°C for 1 min and ramped at 10°C min^{-1} to 180°C held for 1 min, and then ramped 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 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 (El-Baky and El-Baroty, 2008; Sonboli et al., 2008) 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 to 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

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. A serial dilution (4.0 to 10.0% v/v for *S. zeamais*, 0.5 to 5.0% v/v for *T. castaneum*, six concentrations) of the essential oil was prepared in *n*-hexane. Aliquots of 0.5 µl per insect were topically applied dorsally to the thorax of insects, using a Burkard Arnold microapplicator. Controls were determined using 0.5 µl *n*-hexane per insect. Ten insects were used for each concentration and control, and the experiment was replicated six times. Both treated and control insects were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators at 29 to 30°C and 70 to 80% relative humidity. Mortality was observed daily until end-point mortality was reached one week after treatment. 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

A Whatman filter paper (diameter 2.0 cm, CAT No. 1001020) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 24 ml). Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. Ten microliters of 0.2 to 12.0% of essential oil (v:v) was added to the filter paper. The solvent was allowed to evaporate for 30 s before the cap was placed tightly on the glass vial (with 10 unsexed insects) to form a sealed chamber. Fluon (ICI America Inc) was used inside glass vial to prevent insects from the treated filter paper. *n*-Hexane was used as controls. Six replicates were used in all treatments and controls and they were incubated at 29 to 30°C

and 70 to 80% relative humidity for 24 h. The insects were then transferred to clean vials with some culture media and kept in an incubator. Mortality of insects was observed daily until end-point mortality was reached one week after treatment. Results from all replicates were subjected to probit analysis using the PriProbit program V1.6.3 to determine LC_{50} values (Sakuma, 1998).

RESULTS AND DISCUSSION

The steam distillation for 3 h of flowering aerial parts of *D. moldavica* afforded essential oils with a yield of 0.31% and the density of the concentrated essential oil was determined to be 0.78 g/ml. The GC–MS analysis of the essential oil led to the identification and quantification of a total of 38 major components accounting for 98.41% of the total component present (Table 1). 1,8-Cineol (31.25%) and 4-terpineol (22.82%) are the two main components of the essential oil followed by cumin alcohol (4.29%), α-terpineol (4.21%) and sabinene (3.62%). The results are quite different from the previous reports. For example, *D. moldavica* essential oil from Xinjiang automonous region contained citral (31.43%), *n*-hexadecanoic acid (16.48%), and geraniol ester (9.02%) (Tian et al., 2009). The main constituents of *D. moldavica* essential oil from Hungary were geraniol (33.15%) and geranyl acetate (27.48%) (Kakasy et al., 2006). Abd El-Baky and El-Baroty (2008) also demonstrated that the principal constituents of the essential oil of *D. moldavica* collected from Egypt were geranyl acetate (24.93%), geranial (23.67%), geraniol (14.96%), and nerol (11.0%). However, the major constituents of the essential oil of *D. moldavica* collected from North Iran were limonene (19.8%), α-pinene (14.4%), methyl geranate (8.5%), geranyl acetate (7.9%), carvacrol (7.8%) and geranial (5.4%) (Morteza-Semnani et al., 2007). The previous findings suggested that the essential oil content of *D. moldavica* and its composition showed considerable variations due to plant origin, ecological and climatic conditions as well as storage duration of medicinal herbs (Gholizadeh et al., 2010).

Results of topical application indicated that the essential oil of *D. moldavica* flowering aerial parts showed strong contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} value of 22.10 and 18.28 µg/adult, respectively (Table 2). However the essential oil demonstrated weak acute toxicity against the two species of insects (5 to 50 times less active) when compared with the positive control (pyrethrum extract, 25% pyrethrine I and pyrethrine II) because the pyrethrum extract has acute toxicity to maize weevils and red flour beetles with LD_{50} value of 4.29 and 0.36 µg/adult, respectively (Li et al., 2010; Liu et al., 2010).

The essential oil of *D. moldavica* flowering aerial parts also exhibited strong fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} value of 2.65 and 0.88 mg/L air, respectively (Table 3). The adults of maize weevils were 3 times (based on the LC_{50} value) more tolerant to the fumigant toxicity of the essential oil than *T. castaneum* adults. The currently used grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} value of 0.67 and 1.75 mg/L air, respectively (Liu and Ho, 1999). Compared with the positive control (MeBr), the essential oil of *D. moldavica* flowering aerial parts showed 3 and 2 times less toxicity against *S. zeamais* and *T. castaneum* adults, respectively. However, compared with the other essential oils in the previous studies, the essential oil of *D. moldavica* exhibited stronger fumigant toxicity against *S. zeamais* and *T. castaneum* adults, for example, essential oils of *Mentha microphylla*, *Citrus reticulata*, *Schinus terebenthifolius* (Mohamed and Abdelgaleil, 2008), *Drimys winteri* (Zapata and Smagghe, 2010), *Schizonpeta multifida* (Liu et al., 2011), *Murraya exotica* (Li et al., 2010), and several essential oils from genus *Artemisa* (Chu et al., 2010; Liu et al., 2010a, b). Moreover, in the previous reports, two main constituents of the essential oil, 1,8-cineol and 4-terpineol have been found to possess fumigant toxicity against several stored product insects, such as *S. granarius*, *S. oryzae*, *T. castaneum, T. confusum* and *Rhyzopertha dominica* (Lee et al., 2004; Kordali et al., 2006; Rozman et al., 2007; Abdelgaleil et al., 2009; Sener et al., 2009).

Considering the fumigants currently used are synthetic insecticides, fumigant activity of the essential oil is quite promising and *D. moldavica* essential oil shows potential for development as a novel natural insecticide for grain storage products.

However, for the practical application of the essential oil as novel 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. Moreover, further studies should be done on plant cultivation and essential oil standardization because chemical composition of the essential oil varies greatly with the plant population.

Conclusion

The composition of the essential oil derived from *D. moldavica* flowering aerial parts was determined by GC-FID and GC-MS. The essential oil was demonstrated to exhibit strong fumigant toxicity against the two grain storage insects. Although, its fumigant toxicity against the two grain storage insects were 3 or 2 times less than that of the positive control, the essential oil exhibited stronger fumigant toxicity than most of the reported essential oils in the literature. These findings suggest that the essential oil of *D. moldavica* flowering aerial parts possess potential for development as a novel natural insecticide for stored products. Further studies are required to isolate and identify these active components from the essential oil.

Table 1. Chemical constituents of essential oil derived from *Dracocephalum moldavica* flowering aerial parts.

Compounds	RI*	Peak area (%)
α-Pinene	931	1.01
Sabinene	952	3.62
δ-2-Carene	1002	1.97
1,8-Cineol	1032	31.25
(*Z*)- β-Ocimene	1038	0.47
γ-Terpinene	1057	2.64
Acetophenone	1066	1.64
trans-ρ-Menth-2-en-1-ol	1145	1.41
Sabina ketone	1160	0.83
Pinocarvone	1165	0.16
δ-Terpineol	1174	1.46
4-Terpineol	1179	22.82
Crypton	1186	1.44
α-Terpineol	1191	4.21
Myrtenal	1193	0.79
cis-Carveol	1226	0.49
Carvone	1242	0.60
Phellandral	1281	0.93
Cumin alcohol	1288	4.29
4-Vinylguaiacol	1323	0.48
3-Allylguaiacol	1362	1.73
α-Cubebene	1350	0.20
trans-β-Damascenone	1382	0.43
β-Cubebene	1392	0.37
(-)-β-Elemene	1393	0.40
Caryophyllene	1420	1.57
α-Caryophyllene	1454	0.48
Germacrene D	1477	2.23
α-Muurolene	1498	0.44
α-Farnesene	1511	0.56
γ-Cadinene	1512	0.30
(+)-δ-Cadinene	1520	1.69
Cadina-4,9-diene	1546	0.29
Spatulenol	1578	0.77
Caryophyllene oxide	1584	1.00
τ-Muurolol	1642	1.12
α-Cadinol	1652	1.91
Apiol	1682	0.41
Total		98.41

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

Table 2. Contact toxicity of *Dracocephalum moldavica* essential oil against *Sitophilus zeamais* (SZ) and *Triboilum castaneum* adults (TC).

Insects	Essential oils	LD_{50} (μg/adult)	95% FL	Slope ± SE	Chi square (χ^2)
SZ	*D. moldavica*	22.10	20.87-23.69	3.65 ± 0.32	14.55
	Pyrethrum extract*	4.29	3.86-4.72	-	-
TC	*D. moldavica*	18.28	17.09-19.34	4.67 ± 0.45	10.33
	Pyrethrum extract*	0.36	0.32-0.41	-	-

* Data from Li et al. (2010) and Liu et al. (2010a).

Table 3. Fumigant toxicity of *Dracocephalum moldavica* essential oil against *Sitophilus zeamais* (SZ) and *Triboilum castaneum* adults (TC).

Insects	Essential oils	LC_{50} (mg/L air)	95% FL	Slope ± SE	Chi square (χ^2)
SZ	*D. moldavica*	2.65	2.52 - 2.86	3.65 ± 0.32	9.67
	MeBr*	0.67	-	-	-
TC	*D. moldavica*	0.88	0.75 - 1.02	4.67 ± 0.45	15.34
	MeBr*	1.75	-	-	-

* Data from Liu and Ho (1999).

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