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Chemical composition and insecticidal activity of *Heracleum moellendorffii* Hance essential oil

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Water-distilled essential oil from *Heracleum moellendorffii* Hance (Apiaceae) flowering aerial parts was analysed by GC-MS for the first time. Fourty-one components comprising 98.7% of the total oil were identified, of which apiol (11.0%), β-pinene (9.2%), α-terpineol (7.5%), myristicin (7.1%), osthole (6.1%) and (*E*)-anethole (5.2%) were found to be the major components. The essential oil of *H. moellendorfii* showed moderate contact toxicity against *Sitophilus zeamais* and *Tribolium castaneum* adults with LD_{50} values of 27.19 μg/adult and 23.01 μg/adult, respectively. The essential oil of *H. moellendorffii* also possessed fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 23.14 mg/L air and 12.09 mg/L air, respectively.

Key words: *Heracleum moellendorffii, Sitophilus zeamais, Tribolium castaneum,* fumigant, contact toxicity, essential oil composition

INTRODUCTION

Heracleum moellendorffii Hance (Family: Apiaceae) is a species of a perennial herbaceous rhizome plant of the Apiaceae family, used in some southeastern area of China as a substitute of the traditional medicinal herb *Radix Angelicae Pubescentis* [1]. Its roots were used in traditional Chinese medicines as a remedy for arthritic disease: anti-inflammatory and analgesic constituents were found in its roots [1]. Several studies on chemical constituents of *H. moellendorffii* have been reported and a number of flavonoides, monoterpenoids, sesquiterpenoids, coumarins and polyacetylenic compounds have been isolated [2–6]. Chemical composition of the essential oil derived from *H. moellendorffii* roots has been also studied [7]. However, the constituents of the essential oil derived from *H. moellendorffii* flowering aerial parts have not been determined so far. Moreover, insecticidal activity of the *H. moellendorffii* essential oil against stored product insects has not been measured. The present investigation consisted of two parts: determination of the chemical composition of the

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essential oil of *H. moellendorffii* flowering aerial parts; and evaluation of the essential oil as an insecticide / fumigant for the control of two stored-product insect pests.

The maize weevil (*Sitophilus zeamais* Motsch.) and red flour beetle (*Tribolium castaneum* Herbst) are two serious pest species of stored grains worldwide [8]. 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 [9]. The control of grain storage insects relies heavily on the use of synthetic insecticides and fumigants. However, repeated use of those fumigants and insecticides for decades 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 users [10]. An alternative to synthetic pesticides is the use of natural compounds, such as essential oils that result from secondary metabolism in plants [11]. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects [12].

EXPERIMENTAL

Plant material and essential oil extraction

Fresh flowering aerial parts (10 kg of leaves, stems and flowers) of *H. moellendorffii* were harvested in August 2009 from Xiaolongmeng 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. Liu, Q. R. (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimen (BNU-zhilongliu-2009-08-29-032) was deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University. The samples were ground to 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.

GC/MS analysis

Gas chromatographic analysis was performed on an Agilent 6890N while the essential oils were identified on a mass spectrometer Agilent Technologies 5973N. They were equipped with a flame ionization detector and capillary column with HP-5MS (30 m × 0.25 mm × 0.25 μm). The GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and increased at 10 °C min^{-1} to 180 °C for 1 min, and then ramped at 20 °C min^{-1} to 280 °C for 15 min. The injector temperature was maintained at 270 °C. The samples (1 μL) were 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 [7] 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 on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from the literature [13]. Component relative percentages were calculated based on GC peak areas without using correction factors.

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. *T. castaneum* were reared on wheat flour mixed with yeast (10 : 1, w/w) while *S. zeamais* were reared on whole wheat at 12–13% moisture content. Unsexed adult weevils / beetles used in all the experiments were about 2 weeks old.

Fumigant toxicity

Range-finding studies were run to determine the appropriate testing concentrations of *H. moellendorffii* essential oil. The fumigant toxicity of the *H. moellendorffii* essential oil was determined by the method of Liu et al. [8] 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 (*n*-hexane as a solvent, 2.0–50.0%, v/w, 6 concentrations) were 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. Fluon (ICI America Inc) was used inside the glass vial to prevent insects from the treated filter paper. 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 the probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} values [14].

Contact toxicity

Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil (6 concentrations, 5.0–20%, v/w) was prepared in *n*-hexane. Aliquots of 0.5 μL of the dilutions were applied topically to the dorsal thorax of the insects. Controls were determined using *n*-hexane. Both treated and control insects were then transferred to glass vials (10 insects / vial) with culture media and kept in incubators. Mortality of insects was observed daily until end-point mortality was reached one week after treatment. The LD_{50} values were calculated by using the Probit analysis [14].

RESULTS AND DISCUSSION

The yellow essential oil yield of *H. moellendorffii* flowering aerial parts was 0.71% (V/W) and the density of the concentrated essential oil was determined as 0.92 g/mL. A total of 41 components were identified in the essential oil of *H. moellendorffii* flowering aerial parts, accounting for 98.66% of the total oil (Table 1). The main components of the essential oil were apiol (11.0%), β-pinene (9.2%), α-terpineol (7.5%), myristicin (7.1%), osthole (6.1%) and (*E*)-anethole (5.2%). Monoterpenoids represented 13 of the 41 compounds, corresponding to 39.6% of the whole oil while 19 of the 41 constituents were sesquiterpenoids (22.1% of the crude essential oil). There were great variations in the chemical composition of the essential oil from *H. moellendorffii* different parts. For example, the main constituents of the *H. moellendorffii* root

Table 1. **Essential oil composition of *Heracleum moellendorffii* flowering aerial parts from Beijing, China**

No.	Compound	RI*	Percentage, %
1	α-Pinene	939	4.2
2	**β-Pinene**	**981**	**9.2**
3	β-Myrcene	991	1.0
4	Limonene	1 027	1.3
5	(*Z*)-β-Ocimene	1 038	3.0
6	γ-Terpinene	1 057	1.4
7	1-Octanol	1 064	1.2
8	Linalool	1 094	3.9
9	ρ-Cymen-8-ol	1 182	3.1
10	**α-Terpineol**	**1 189**	**7.5**
11	Estragole	1 195	3.7
12	γ-Terpineol	1 202	3.4
13	Isothymol methyl ether	1 244	0.1
14	(+)-Carvone	1 247	1.3
15	Phellandral	1 281	0.2
16	**(*E*)-Anethole**	**1 285**	**5.2**
17	4-Vinylguaiacol	1 311	1.1
18	Copaene	1 374	0.3
19	Octyl isobutyrate	1 336	1.6
20	β-Cubebene	1 387	0.5
21	β-Elemene	1 391	0.4
22	Methyleugenol	1 401	0.6
23	α-Cedrene	1 409	0.2
24	(*Z*)-α-Bergamotene	1 413	0.2
25	B-Caryophyllene	1 420	3.4
26	Calarene	1 432	0.2
27	(*E*)-α-Bergamotene	1 434	0.6
28	(*E*)-β-Farnesene	1 452	1.6
29	Allo-aromadendrene	1 458	0.2
30	γ-Muurolene	1 473	2.0
31	Germacrene D	1 485	4.4
32	Eremophilene	1 489	0.7
33	α-Selinene	1 492	0.3
34	1 ξ, 6 ξ,7 ξ-Cadina-4,9-diene	1 502	0.6
35	β-Bisabolene	1 506	0.8
36	**Myristicin**	**1 513**	**7.1**
37	Elemicin	1 554	2.8
38	(+)-Spathulenol	1 578	1.3
39	Caryophyllene oxide	1 583	1.0
40	**Apiol**	**1 682**	**11.0**
41	**Osthole**	**2 139**	**6.1**
	Total		98.7
	Monoterpenoids		39.6
	Sesquiterpenoids		22.1
	Others		36.0

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

Table 2. **Toxicity of essential oil of *Heracleum moellendorffii* against *Sitophilus zeamais* (SZ) and *Tribolium castaneum* (TC) adults**

Insect	Treatment	Fumigation		Contact toxicity	
		LC_{50}, mg/L air	95% fiducial limits	LD_{50}, µg/adult	95% fiducial limits
SZ	Essential oil	23.14	20.67–26.08	27.19	25.67–29.43
	Pyrethrum extract*	–	–	4.29	3.86–4.72
	MeBr**	0.67	–	–	–
TC	Essential oil	12.09	10.67–13.34	23.01	21.39–25.45
	Pyrethrum extract			0.36	0.32–0.41
	MeBr**	1.75	–	–	–

* Liu et al. (2010); ** Liu and Ho (1999).

essential oil were β-pinene (24.3%), α-pinene (8.2%) and limonene (8.1%) [7].

The essential oil of *H. moellendorffii* possessed moderate contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 27.19 µg/adult and 23.01 µg/adult, respectively (Table 2). However, the essential oil demonstrated 6 and 63 times less acute toxicity against *S. zeamais* and *T. castaneum* when compared with the positive control (pyrethrum extract, LD_{50} = 4.29 µg/adult and 0.36 µg/adult, respectively) [15].

H. moellendorffii essential oil also showed fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 23.14 mg/L air and 12.09 mg/L air, respectively (Table 2). The commercial grain fumigant, methyl bromide (MeBr), was reported to have fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 0.67 mg/L and 1.75 mg/L air [16], thus the essential oils were 7–34 times less toxic to the two grain storage insects compared with MeBr. However, compared with other essential oils in the literature, the essential oils of *H. moellendorffii* exhibited the same level of fumigant toxicity against the two grain storage insects, e. g. essential oils of *Murraya exotica*, *Artemisia lavandulaefolia*, *A. sieversiana*, *A. vestita*, and *Illicium simonsii* [15, 17–19]. Considering that the currently used fumigants are synthetic insecticides, fumigant activity of the essential oil is quite promising.

In the previous reports, two of the main constituents of the essential oil of *H. moellendorffii* (apiol and myristicin) have been reported to be toxic to several insects / ticks (e. g. house flies, *Musca domestica*, fruit flies, *Drosophila melanogaster*, hairy caterpillar, *Spilarctia obliqua*, and the cattle tick, *Rhipicephalus microplus*) and also to be synergistic for carbamate and organophosphorus insecticides [20–23]. Another three main constituents of the essential oil, β-pinene, α-terpineol and (*E*)-anethole, are very common monoterpenoids and were found in many essential oils. The three monoterpenoids have also been shown to possess strong contact and fumigant toxicities against several insects and mites (e. g. German cockroaches, *Blattella germanica*, cowpea weevil, *Callosobruchus maculatus*, Colorado potato beetle, *Leptinotarsa decemlineata*, the western corn rootworm, *Diabrotica virgifera virgifera*, house flies, *Musca domestica*, the head louse, *Pediculus humanus capitis*, the yellow fever mosquito, *Aedes aegypti*, and the two-spotted spider mite, *Tetranychus urticae*) [24–31]. The isolation and identification of the bioactive compounds in the essential oil of *H. moellendorffii* flowering aerial parts are of utmost importance so that their potential application in controlling stored-product pests can be fully exploited.

The above findings suggest that the essential oil of *H. moellendorffii* flowering aerial parts can play an important role in stored grain protection and reduce the need for the same, and also 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 cost.

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HERACLEUM MOELLENDORFFII HANCE ETERINIO ALIEJAUS CHEMINĖ SUDĖTIS IR INSEKTICIDINIS AKTYVUMAS

S a n t r a u k a

Heracleum moellendorffii Hance žiedynų eterinis aliejus buvo tiriamas dujų chromatografijos ir masių spektrometrijos būdu. Rasta daugiau kaip 40 eterinių aliejų komponentų, kurie kartu sudaro 98,7 % aliejaus.