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Research Article

GC-MS Analysis of Insecticidal Essential Oil of Aerial Parts of *Echinops latifolius* Tausch

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The roots of *Echinops latifolius* Tausch (Asteraceae) have been used in the traditional medicine. However, no report on chemical composition and insecticidal activities of the essential oil of this plant exists. The aim of this research was to determine chemical composition and insecticidal activities of the essential oil of *E. latifolius* aerial parts against maize weevils (*Sitophilus zeamais* Motschulsky) for the first time. Essential oil of *E. latifolius* aerial parts at flowering stage was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 35 components of the essential oil of *E. latifolius* aerial parts were identified. The major compounds in the essential oil were 1,8-cineole (19.63%), (*Z*)-β-ocimene (18.44%), and β-pinene (15.56%) followed by β-myrcene (4.75%) and carvone (4.39%). The essential oil of *E. latifolius* possessed contact toxicity against *S. zeamais* with an LD_{50} value of 36.40 μg/adult. The essential oil also exhibited fumigant toxicity against *S. zeamais* with an LC_{50} value of 9.98 mg/L. The study indicates that the essential oil of *E. latifolius* aerial parts has a potential for development into a natural insecticide/fumigant for control of insects in stored grains.

1. Introduction

Insects and fungi create serious quality problems in stored grains. The maize weevil (*Sitophilus zeamais* Motschulsky) is one of the major pests of stored grains and grain products in China, causing serious losses in stored products [1]. The only way to eliminate pests completely from a food grain without leaving pesticide residues is fumigation [2]. Currently, there are only two commonly used fumigants for stored food, methyl bromide (MeBr) and phosphine, which have led to the resurgence of pests. In addition to the resistance developed by the insect pests, chemical application has also exerted undesirable effects on nontarget organisms and fostered environmental and human health concerns [3]. This necessitates the development of natural and safe products that are relatively less hazardous to mammalian health and the environment than existing conventional pesticides, as alternatives to nonselective synthetic pesticides to control the pests of medical and economic importance [4]. Essential oils or their constituents have demonstrated to be potential sources of alternative compounds to currently used fumigants. Essential oils and their constituents have low toxicity to warm-blooded animals, high volatility, and toxicity to stored-grain insect pests [5, 6].

During the screening program for new agrochemicals from Chinese medicinal herbs and wild plants, the essential oil of *Echinops latifolius* Tausch (Asteraceae) (syn. *E. davuricus*) aerial parts at flowering stage was found to possess strong insecticidal toxicity against a cosmopolitan pest of stored products, *S. zeamais*. *Echinops* is comprised of ca. 120 species and distributed all over the world, mostly in the northern hemisphere [7]. *E. latifolius* is a perennial growing to 0.5 m and distributed mainly in the north of China (e.g., Gansu, Hebei, Heilongjiang, Henan, Jilin, Liaoning, Ningxia, Shaanxi, Shandong, Shanxi, and Inner Mongolia) and Russia as well as Mongolia [7]. The root of this plant is anti-inflammatory and galactagogue. It is used in the treatment of breast abscesses with inflammation, mastitis,

lack of milk in nursing mothers, and distension of the breast for a long history and it has been recorded in Chinese Pharmacopoeia as one of the sources for Yuzhou Loulu [8]. In previous studies, several triterpenoids, sesquiterpenoids, and thiophenes were isolated from *E. latifolius* and identified [9–12]. However, a literature survey showed that there is no report on the volatile constituents and insecticidal activity of *E. latifolius*; thus we decided to investigate the chemical constituents and insecticidal activities of the essential oil of *E. latifolius* aerial parts against grain storage insects for the first time.

2. Materials and Methods

2.1. Plant Material. The aerial parts of *E. latifolius* at flowering state were collected in August 2010 from Xiaolongmen National Forest Park (39.48° N latitude and 115.25° E longitude, Mentougou District, Beijing 102300). The sample was air-dried and identified by Dr. Q. R. Liu (College of Life Sciences, Beijing Normal University, Beijing, China) and a voucher specimen (ENTCAU-Compositae-10022) was deposited at the Department of Entomology, China Agricultural University (Beijing).

2.2. Essential Oil Extraction. The sample was 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 *n*-hexane. The solvent was evaporated using 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.

2.3. Insects. The maize weevils (*S. zeamais*) were obtained from laboratory cultures in the dark in incubators at 29-30°C 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). Unsexed 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).

2.4. Gas Chromatography-Mass Spectrometry. The essential oil of *E. latifolius* 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 then heated at 180°C at a rate of 10°C/min, held for 1 min, and then heated to 280°C at 20°C/min and held for 15 min. The injector temperature was maintained at 270°C. The sample (1 μL, diluted 100 : 1 in acetone) was injected, 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 the literature [14]. Component relative percentages were calculated based on normalization method without using correction factors.

2.5. Contact Toxicity by Topical Application. Contact toxicity of the essential oil against *S. zeamais* adults was measured as described by Liu et al. [15]. Range-finding studies were run to determine the appropriate testing concentrations of the essential oil of *E. latifolius*. A serial dilution of the essential oil (5.0%, 6.0%, 7.7%, 9.6%, 12.0%, and 15.0%) 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. 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 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. The insects that did not present any movement when touched with a brush were considered as dead.

2.6. Fumigant Toxicity Bioassay. Range-finding studies were run to determine the appropriate testing concentrations of *E. latifolius* essential oil. The fumigant toxicity of *E. latifolius* essential oil was determined based on the method of Liu and Ho [1] 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 unsexed insects) to form a sealed chamber. The vials were placed upright and the Fluon (ICI America Inc.) coating restricted the insects to the lower portion of the vial to prevent them 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.

2.7. Data Analysis. The observed mortality data were corrected for control mortality using Abbott's formula and results from all replicates were subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} or LD_{50} values [16].

3. Results and Discussion

The yellow essential oil yield of *E. latifolius* flowering aerial parts was 0.15% (V/W) and the density of the concentrated essential oil was determined as 0.91 g/mL. A total of 35 components of the essential oil of *E. latifolius* aerial parts were identified, accounting for 98.33% of the total oil. The principal compounds in the essential oil of *E. latifolius* were 1,8-cineole (19.63%), (*Z*)-β-ocimene (18.44%), and β-pinene (15.56%) followed by β-myrcene (4.75%) and carvone (4.39%) (Table 1). Monoterpenoids represented 19 of the 35 compounds, corresponding to 78.45% of the whole oil, while 11 of the 35 constituents were sesquiterpenoids (16.49% of the crude essential oil). This is the first to report chemical composition of the essential oil of *E. latifolius* aerial parts. However, the chemical composition of *E. latifolius* is quite different from that of several essential oils derived from other *Echinops* species [17–23]. Essential oils of some members in genus *Echinops* contained high content of S-containing polyacetylene compounds. For example, the essential oil of Chinese *E. grijsii* roots contained (*Z*)-β-farnesene (25.18%), 5-(but-3-en-1-ynyl)-2,2′-bithiophene (19.67%), β-bisabolene (12.11%), and α-terthienyl (8.36%) [17]. Moreover, essential oils from the roots of *E. bannaticus* and *E. sphaerocephalus* harvested from South Serbia mainly contained 5-(3-buten-1-ynyl)-2,2′-bithienyl (47.3% and 48.9%, resp.) and α-terthienyl (15.5% and 13.7%, resp.) [18]. However, essential oils of other *Echinops* members were characterized by low levels of S-containing polyacetylene compounds or nothing at all. For example, main constituents in the essential oil of *E. giganteus* var. *lelyi* rhizomes purchased from Western Cameroon were silphiperfol-6-ene (26.9%), presilphiperfol-7(8)-ene (9.4%), β-caryophyllene (8.3%), and presilphiperfolan-8-ol (6.7%) [19], while major constituents found in hydrodistilled essential oil of *E. kebericho* collected from Ethiopia were eudesm-7(11)-en-4-ol (14.3%), caryophyllene oxide (9.7%), τ-cadinol (8.3%), and (*E*)-nerolidol (7.2%) [21].

The essential oil of *E. latifolius* aerial parts exhibited contact toxicity against *S. zeamais* adults with an LD_{50} value of 36.40 μg/adult (Table 2). When compared with the positive control pyrethrum extract [13], the essential oil demonstrated 8.5 times less toxicity against *S. zeamais*. However, compared with the other essential oils in the literature using the same bioassay, the essential oil of *E. latifolius* aerial parts possessed stronger or the same level of contact toxicity against *S. zeamais* adults, for example, essential oils of *Artemisia lavandulaefolia*, *A. sieversiana*, *A. capillaris*, *A. mongolica*, *A. vestita*, and *A. eriopoda* (LD_{50} = 55.2 μg/adult, 113.0 μg/adult, 106.0 μg/adult, 87.9 μg/adult, 50.6 μg/adult, and 24.8 μg/adult, resp.) [24–27], essential oil of *Schizonpeta multifida* (30.2 μg/adult) [28], essential oil of *Aster ageratoides* (27.2 μg/adult) [29], essential oil of *Illicium simonsii* fruits (LD_{50} = 112.7 μg/adult) [30], and essential oil of *Cayratia japonica* (LD_{50} = 44.5 μg/adult) [31].

The essential oil of *E. latifolius* aerial parts possessed fumigant toxicity against the maize weevils with an LC_{50} value of 9.98 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 [1]; thus the essential oil was 15 times less toxic to *S. zeamais* adults compared with MeBr. However, compared with fumigant activity of the other essential oils in the literature using the same bioassay, the essential oil of *E. latifolius* exhibited stronger or the same level of fumigant toxicity against *S. zeamais* adults, for example, essential oils of *S. multifida* [28], *Kadsura heteroclite* [32], *Murraya exotica* [13], *Ostericum grosseserratum* [33], *Saussurea nivea* [34], and *Illicium pachyphyllum* [35] and several essential oils from genus *Artemisia*

Table 1: Chemical constituents of essential oil derived from *Echinops latifolius* aerial part.

Number	Compounds	RI*	Peak area (%)
1	α-Pinene	939	1.49
2	Camphene	954	0.13
3	β-Pinene	981	15.56
4	β-Myrcene	991	4.75
5	β-Phellandrene	1027	0.15
6	Limonene	1029	0.98
7	1,8-Cineole	1031	19.63
8	(*Z*)-β-Ocimene	1037	18.44
9	Artemisia ketone	1062	2.25
10	γ-Terpinene	1059	1.22
11	Linalool	1094	2.74
12	Camphor	1146	1.50
13	(*Z*)-β-Terpineol	1147	0.89
14	4-Terpineol	1177	0.87
15	α-Terpineol	1188	1.43
16	Carvone	1254	4.39
17	Perilla aldehyde	1279	1.21
18	Cumic alcohol	1288	0.60
19	Dihydroedulan I	1290	1.10
20	Citronellyl acetate	1355	0.22
21	Eugenol	1360	0.33
22	β-Cubebene	1387	0.97
23	Methyleugenol	1403	0.35
24	β-Caryophyllene	1420	3.58
25	α-Caryophyllene	1454	1.29
26	Bicyclogermacrene	1513	0.93
27	δ-Cadinene	1523	1.52
28	(−)-Spathulenol	1578	0.56
29	Caryophyllene oxide	1583	0.82
30	γ-Eudesmol	1631	0.99
31	τ-Muurolol	1642	1.54
32	β-Eudesmol	1650	3.10
33	α-Cadinol	1654	1.19
34	5-(3-Buten-1-ynyl)-2,2′-bithienyl	1935	0.83
35	α-Terthienyl	2240	1.21
	Total identified		98.33
	Monoterpenoids		78.45
	Sesquiterpenoids		16.49
	Others		3.82

*RI, retention index as determined on an HP-5ms column using the homologous series of *n*-hydrocarbons

Table 2: Contact toxicity and fumigant toxicity of *Echinops latifolius* essential oil against *Sitophilus zeamais* adults.

	Treatment	LD_{50} (μg/adult) LC_{50} (mg/L air)	95% FL	Slope ± SE	Chi square (χ^2)
Contact	*E. latifolius*	36.40	33.56–40.47	2.81 ± 0.29	8.52
	Pyrethrum extract*	4.29	3.86–4.72	—	—
Fumigant	*E. latifolius*	9.98	8.98–10.83	2.39 ± 0.24	7.96
	MeBr**	0.67	—	—	—

*Data from Li et al. [13]. **Data from Liu and Ho [1].

[24–27]. Moreover, insecticidal activity of 1,8-cineole, (*Z*)-β-ocimene, and β-pinene (main constituents of the studied essential oil) against grain storage insects had been reported [36–46]. For example, 1,8-cineole and β-pinene exhibited fumigant toxicity against *S. zeamais* adults, with 24 h LC_{50} values of 1.82 mg/cm^2 and 3.82 mg/cm^2, respectively [36], and also possessed contact toxicity against *S. zeamais* adults with 7 d LD_{50} values of 48 μg/mg and 113 μg/mg, respectively [37, 38]. β-Ocimene exhibited fumigant toxicity against *Tribolium castaneum* adults with LC_{50} values (72 h) of 14.8 μL/L and against *S. oryzae* adults with an LC_{50} value of 3.2 μL/L [39]. Moreover, (*Z*)-β-ocimene also exhibited fumigant toxicity against *S. zeamais* adults with a 24 h LC_{50} value of 28.66 mg/L air [40]. The above findings suggest that fumigant activity of the essential oil of *E. latifolius* aerial parts is quite promising. Considering the currently used fumigants and synthetic insecticides, the oil shows potential to develop a possible new natural fumigant/insecticide for control of stored product insects. 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 costs.

4. Conclusion

The study indicates that the essential oil of *E. latifolius* aerial parts has a potential for development into a new natural insecticide/fumigant for control of insects in stored grains. However, further studies on the safety of the oil in humans as well as development studies are required to optimize the efficacy and stability of this extract and to reduce costs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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