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 LD50 value of 36.40 μg/adult. The essential oil also exhibited fumigant toxicity against S. zeamais with an LC50 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. davi-ricus) 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 flow-
erating stage were collected in August 2010 from Xiaolong-
men 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 Agricul-
tural University (Beijing).

2.2. Essential Oil Extraction. The sample was ground to
a powder using a grinding mill (Retsch M¨uhle, Germany).
Each 600g 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
Na₂SO₄ 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
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 chromato-
graph, a model 5973N mass selective detector (EI/MS, 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 tem-
perature 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 (C₆–C₁₃) 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 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 cor-
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 LC₅₀ or
LD₅₀ 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-myrceene (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 polycyclicene 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 *S. phaerocephalus* 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 polycyclicene compounds or nothing at all. For example, main constituents in the essential oil of *E. giganteus* var. *leyi* 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%), γ-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 lavandulacofolia*, *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 *Schizonecta 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>
<thead>
<tr>
<th>Number</th>
<th>Compounds</th>
<th>RI</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Pinene</td>
<td>939</td>
<td>1.49</td>
</tr>
<tr>
<td>2</td>
<td>Camphene</td>
<td>954</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>β-Pinene</td>
<td>981</td>
<td>15.56</td>
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<tr>
<td>4</td>
<td>β-Mycene</td>
<td>991</td>
<td>4.75</td>
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<tr>
<td>5</td>
<td>β-Phellandrene</td>
<td>1027</td>
<td>0.15</td>
</tr>
<tr>
<td>6</td>
<td>Limonene</td>
<td>1029</td>
<td>0.98</td>
</tr>
<tr>
<td>7</td>
<td>1,8-Cineole</td>
<td>1031</td>
<td>19.63</td>
</tr>
<tr>
<td>8</td>
<td>(Z)-β-Ocimene</td>
<td>1037</td>
<td>18.44</td>
</tr>
<tr>
<td>9</td>
<td>Artemisia ketone</td>
<td>1062</td>
<td>2.25</td>
</tr>
<tr>
<td>10</td>
<td>γ-Terpine</td>
<td>1059</td>
<td>1.22</td>
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<tr>
<td>11</td>
<td>Linalool</td>
<td>1094</td>
<td>2.74</td>
</tr>
<tr>
<td>12</td>
<td>Camphor</td>
<td>1146</td>
<td>1.50</td>
</tr>
<tr>
<td>13</td>
<td>(Z)-β-Terpineol</td>
<td>1147</td>
<td>0.89</td>
</tr>
<tr>
<td>14</td>
<td>4-Terpineol</td>
<td>1177</td>
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<tr>
<td>15</td>
<td>α-Terpineol</td>
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<td>1.43</td>
</tr>
<tr>
<td>16</td>
<td>Carvone</td>
<td>1254</td>
<td>4.39</td>
</tr>
<tr>
<td>17</td>
<td>Perilla aldehyde</td>
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<td>1.21</td>
</tr>
<tr>
<td>18</td>
<td>Cuminic alcohol</td>
<td>1288</td>
<td>0.60</td>
</tr>
<tr>
<td>19</td>
<td>Dihydrocrolulan I</td>
<td>1290</td>
<td>1.10</td>
</tr>
<tr>
<td>20</td>
<td>Citronelly acetate</td>
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</tr>
<tr>
<td>21</td>
<td>Eugenol</td>
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<tr>
<td>22</td>
<td>β-Cubebene</td>
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<td>Methylcubebene</td>
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<tr>
<td>24</td>
<td>β-Caryophyllene</td>
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<td>26</td>
<td>Bicyclomacraene</td>
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</tr>
<tr>
<td>27</td>
<td>δ-Cadinene</td>
<td>1523</td>
<td>1.52</td>
</tr>
<tr>
<td>28</td>
<td>(–)-Spathulenol</td>
<td>1578</td>
<td>0.56</td>
</tr>
<tr>
<td>29</td>
<td>Caryophyllene oxide</td>
<td>1583</td>
<td>0.82</td>
</tr>
<tr>
<td>30</td>
<td>γ-Eudesmol</td>
<td>1631</td>
<td>0.99</td>
</tr>
<tr>
<td>31</td>
<td>τ-Murolol</td>
<td>1642</td>
<td>1.54</td>
</tr>
<tr>
<td>32</td>
<td>β-Eudesmol</td>
<td>1650</td>
<td>3.10</td>
</tr>
<tr>
<td>33</td>
<td>α-Cadinol</td>
<td>1654</td>
<td>1.19</td>
</tr>
<tr>
<td>34</td>
<td>5-(3-Buten-1-ynyl)-2,2′-bithienyl</td>
<td>1935</td>
<td>0.83</td>
</tr>
<tr>
<td>35</td>
<td>α-Terthienyl</td>
<td>2240</td>
<td>1.21</td>
</tr>
</tbody>
</table>

*RI, retention index as determined on an HP-5ms column using the homologous series of n-hydrocarbons*
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 LC₅₀ values of 1.82 mg/cm² and 3.82 mg/cm², respectively [36], and also possessed contact toxicity against *S. zeamais* adults with 7 d LD₅₀ values of 48 μg/mg and 113 μg/mg, respectively [37, 38]. β-Ocimene exhibited fumigant toxicity against *Tribolium castaneum* adults with LC₅₀ values (72 h) of 14.8 μL/L and against *S. oryzae* adults with an LC₅₀ value of 3.2 μL/L [39]. Moreover, (Z)-β-ocimene also exhibited fumigant toxicity against *S. zeamais* adults with a 24 h LC₅₀ 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.

### Acknowledgments

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### References


some of their major components to granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae),” *Industrial Crops and Products*, vol. 23, no. 2, pp. 162–170, 2006.
