Fumigant Activity of Plant Essential Oils and Components from Schizonepeta tenuifolia Against Lycoriella ingenua (Diptera: Sciaridae)

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ABSTRACT

Plant essential oils from 21 plant species were tested for their insecticidal activities against larvae of Lycoriella ingenua Dufour (Diptera: Sciaridae) by using a fumigation bioassay. Good insecticidal activity against larvae of L. ingenua was achieved with essential oils of Acorus gramineus Solander, Schizonepeta tenuifolia Briquet, and Zanthoxylum piperitum De Candolle at 25 µg/ml air. S. tenuifolia oil showed the most potent insecticidal activity among the plant essential oils. At 12.5 µg/ml air concentration, S. tenuifolia oil caused 96.6% mortality, but mortality decreased to 60% at 3.125 µg/ml air. Analysis by gas chromatography-mass spectrometry led to identification of three major compounds from S. tenuifolia oil. These three compounds were tested individually for their insecticidal activities against larvae of L. ingenua and compared with the toxicity of dichlorvos. Pulegone was the most toxic, followed by menthone and limonene with LC50 values of 1.21, 6.03, and 15.42 µg/ml, respectively. LC50 of dichlorvos was 8.13 µg/ml. Effects of S. tenuifolia and its components on growth of Pleurotus ostreatus (Jacq. ex Fr.) Kummer also were investigated.

KEY WORDS plant essential oils, Lycoriella ingenua, fumigant, Schizonepeta tenuifolia, pulegone
Dichlorvos (purity 99.5%) was purchased from Chem Service (West Chester, PA).

**Sample Preparation.** Plant species used are listed in Table 1. Twenty-one plant samples were purchased at Kyungdong medicinal market (Seoul, Korea), and essential oils were extracted by steam distillation as described by Park et al. (2003). In brief, plant samples (100 g) were placed in a 2-liter flask, diluted with distilled water (800 ml) in a 2-liter flask, and steam distilled (100°C). Yields of essential oils extracted with steam distillation in laboratory are given in Table 1.

**Gas Chromatography-Mass Spectrometry (GC-MS).** A 1-μl sample of the essential oil dissolved in hexane [essential oil:hexane, 1:100 (vol:vol)] was analyzed on a gas chromatograph (HP6890, Hewlett Packard, Palo Alto, CA) mass spectrometer (JMS). The GC column was a Packard, Palo Alto, CA)-mass spectrometer (JMS)-analyzed on a gas chromatograph (HP6890, Hewlett Packard, Palo Alto, CA). The GC conditions were as follows: oven temperature, isothermal at 40°C for 5 min then programmed to 220°C at 2°C/min and held at this temperature for 5 min; and ion source temperature, 200°C. Helium was used as the carried gas at the rate of 1.5 ml/min. Effluent of the GC column was introduced directly into the source of the MS. Spectra were obtained in the EI mode with 70-eV ionization energy. The sector mass analyzer was set to scan from 50 to 800 atomic mass units for 2 s. Compounds were identified by comparison of mass spectra of each peak with those of authentic samples in a mass spectra library (John Wiley & Sons, Inc.). Comparison of retention times with authentic samples were analyzed on ZB-WAX column (60 m by 0.25 mm i.d., 0.25-μm film thickness, Phenomenex). The GC conditions were the same as described above.

**Bioassay.** Insecticidal activity was evaluated using a fumigation bioassay. An appropriate concentration of each plant essential oil dissolved in acetone was applied to a filter paper (Whatman No. 2, 4.5 cm in diameter), and the treated filter paper was placed in the bottom lid of a glass cylinder (5 cm in diameter by 10 cm) with a wire sieve fitted at 3.5 cm above the bottom after which lids were tightly sealed with sealing film (Whatman). Test insects were placed on the sieve. This prevented direct contact of insects with the test plant oils and compounds. Twenty 7–10-d-old larvae were used in each replicate. A disk (1 cm in diameter) of P. ostreatus grown on PDA medium was supplied as food for larvae. Treated insects were held at 22 ± 1°C, 80% RH, and a photoperiod of 16:8 (L:D) h. Mortality was determined 1 d after treatment. All treatments were replicated six times. Nominal concentrations in the air in the glass cylinder were calculated using an assumption that all of the oils or compounds volatilized off the filter paper.

**Effect on the Mycelial Growth of P. ostreatus.** Effect of S. tenuifolia oil and its components on the mycelial growth of P. ostreatus were examined by the method of Alvarez-Castellanos et al. (2001). PDA plates were prepared using plastic petri dishes (87 mm). Agar-mycelial plugs (7 mm in diameter) of P. ostreatus were inoculated at the center of the dish. The petri dishes were placed with the lid upside down. Essential oil of S. tenuifolia and its components were introduced onto a paper disc (8 mm, Advantec, Tampa, FL), and the disc was placed on the lid without agar. The treatments were incubated at 28°C in the dark. Colony growth diameter was measured after the fungal growth in the control treatment had completely covered the petri dishes. All treatments were replicated four times.

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**Table 1. List of plant essential oils tested**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Family name</th>
<th>Part</th>
<th>Yield (vol wt, %)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acorus gramineus Solander</td>
<td>Araceae</td>
<td>Root</td>
<td>0.07</td>
</tr>
<tr>
<td>Artemisia capillaris Thunberg</td>
<td>Compositae</td>
<td>Whole plant</td>
<td>0.21</td>
</tr>
<tr>
<td>Carpesium abrotanoides L.</td>
<td>Compositae</td>
<td>Fruit</td>
<td>0.25</td>
</tr>
<tr>
<td>Atractylodes japonicus Koizumi</td>
<td>Compositae</td>
<td>Root</td>
<td>0.11</td>
</tr>
<tr>
<td>Saussurea lappa Clarke</td>
<td>Compositae</td>
<td>Root</td>
<td>0.15</td>
</tr>
<tr>
<td>Juniperus chinensis L.</td>
<td>Cupressaceae</td>
<td>Wood</td>
<td>0.51</td>
</tr>
<tr>
<td>Cyperus rotundus L.</td>
<td>Cyperaceae</td>
<td>Root</td>
<td>0.27</td>
</tr>
<tr>
<td>Perilla frutescens Britton</td>
<td>Labiatae</td>
<td>Leaf</td>
<td>0.12</td>
</tr>
<tr>
<td>Schizandraepteta tenuifolia Briquet</td>
<td>Labiatae</td>
<td>Whole plant</td>
<td>0.39</td>
</tr>
<tr>
<td>Forsythia koreana Nakai</td>
<td>Oleaceae</td>
<td>Fruit</td>
<td>2.25</td>
</tr>
<tr>
<td>Pomerac trifoliate Bañesque</td>
<td>Rutaceae</td>
<td>Fruit</td>
<td>0.37</td>
</tr>
<tr>
<td>Zanthoxylum piperitum De Candolle</td>
<td>Rutaceae</td>
<td>Fruit</td>
<td>0.07</td>
</tr>
<tr>
<td>Santalum album L.</td>
<td>Santalaceae</td>
<td>Wood</td>
<td>0.20</td>
</tr>
<tr>
<td>Schizandrae chinensis Baillon</td>
<td>Schizandraeae</td>
<td>Fruit</td>
<td>0.70</td>
</tr>
<tr>
<td>Styrox benzoin Dryander</td>
<td>Styraceae</td>
<td>Resin</td>
<td>0.36</td>
</tr>
<tr>
<td>Angelica dahurica Bentham et Hooker</td>
<td>Umbelliferae</td>
<td>Root</td>
<td>0.05</td>
</tr>
<tr>
<td>Cnidium officinale Makino</td>
<td>Umbelliferae</td>
<td>Root</td>
<td>0.13</td>
</tr>
<tr>
<td>Anomum globosum Loureiro</td>
<td>Zingiberaceae</td>
<td>Fruit</td>
<td>0.09</td>
</tr>
<tr>
<td>Curcuma longa L.</td>
<td>Zingiberaceae</td>
<td>Root</td>
<td>0.44</td>
</tr>
<tr>
<td>Curcuma zedoaria Roscoe</td>
<td>Zingiberaceae</td>
<td>Root</td>
<td>0.27</td>
</tr>
<tr>
<td>Kaempferiia galanga L.</td>
<td>Zingiberaceae</td>
<td>Root</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* (volume of essential oils/dry weight of plant samples) × 100.
Growth inhibition of treatment against control was calculated by percentage, by using the following formula: % inhibition = C – T/C × 100, where C is an average of four replicates of hyphal extension (millimeters) in control, and T is an average of four replicates of hyphal extension (millimeters) of plates treated with either the essential oil or an individual compound.

Statistical Analysis. Larval mortality and mycelial growth inhibition were transformed to arcsine square root values for analysis of variance (ANOVA). Treatment means were compared and separated by Schef- fè’s test, and LC50 values were calculated by probit analysis (SAS Institute 1999).

Results

Insecticidal Activity of Plant Essential Oils. Twenty-one plant essential oils tested varied in toxicity to larvae (Table 2). Of these, good insecticidal activity against larvae of L. ingenua was achieved with essential oils of Acorus gramineus, Schizonepeta tenuifolia, and Zanthoxylum piperitum at 25 μg/ml air. S. tenuifolia oil showed the most potent insecticidal activity among the plant essential oils. At 12.5 μg/ml air concentration, S. tenuifolia oil caused 96.6% mortality, but mortality decreased to 60% at 3.125 μg/ml air.

Chemical Components of S. tenuifolia Oils. Fig. 1 shows a chromatogram of S. tenuifolia essential oil by using a ZB-WAX fused silica capillary column. The main components of S. tenuifolia oil were 1) (+)-limonene (4.5%), 2) (-)-menthene (49.7%), and 3) (-)-pulegone (43.3%). The first peak on the chromatogram at ~15 min was solvent (hexane) peak.

Insecticidal Activity of Individual Compounds. Insecticidal activity of S. tenuifolia oil, three main compounds and dichlorvos are shown in Table 3. The LC50 value of S. tenuifolia oil was 2.25 μg/ml. Among the test compounds, pulegone was the most toxic, followed by menthone and limonene with LC50 values of 1.80, 0.92, and 1.3 μg/ml, respectively. The LC50 for dichlorvos was 8.13 μg/ml.

Effect on Growth of P. ostreatus. Effect of S. tenuifolia oil and its components on the mycelial growth of P. ostreatus is shown in Table 4. Inhibition rates of S.

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**Table 2.** Fumigant activity of plant essential oils against larvae of L. ingenua

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Concentration (μg/ml)</th>
<th>Mortality (mean ± SE, %)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. gramineus 25</td>
<td>93.3 ± 3.3ab</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>13.3 ± 3.3defg</td>
<td></td>
</tr>
<tr>
<td>A. capillaris 25</td>
<td>46.6 ± 3.3bcdef</td>
<td></td>
</tr>
<tr>
<td>C. abrotanoides 25</td>
<td>0 g</td>
<td></td>
</tr>
<tr>
<td>A. japonica 25</td>
<td>0 g</td>
<td></td>
</tr>
<tr>
<td>S. lappa 25</td>
<td>3.3 ± 3.3fg</td>
<td></td>
</tr>
<tr>
<td>F. koreana 25</td>
<td>6.6 ± 3.6defg</td>
<td></td>
</tr>
<tr>
<td>P. trifoliate 25</td>
<td>13.3 ± 8.6defg</td>
<td></td>
</tr>
<tr>
<td>Z. piperitum 25</td>
<td>96.6 ± 3.3ab</td>
<td></td>
</tr>
<tr>
<td>S. album 25</td>
<td>13.3 ± 3.3defg</td>
<td></td>
</tr>
<tr>
<td>S. chinensis 25</td>
<td>0 g</td>
<td></td>
</tr>
<tr>
<td>S. benzoin 25</td>
<td>73.3 ± 6.5abed</td>
<td></td>
</tr>
<tr>
<td>A. dahurica 25</td>
<td>10 ± 5.7efg</td>
<td></td>
</tr>
<tr>
<td>C. officinale 25</td>
<td>3.3 ± 3.3fg</td>
<td></td>
</tr>
<tr>
<td>A. globosum 25</td>
<td>46.6 ± 6.6bedef</td>
<td></td>
</tr>
<tr>
<td>C. longa 25</td>
<td>0 g</td>
<td></td>
</tr>
<tr>
<td>C. zedoaria 25</td>
<td>0 g</td>
<td></td>
</tr>
<tr>
<td>K. galanga 25</td>
<td>30 ± 5.7cdefg</td>
<td></td>
</tr>
</tbody>
</table>

*Means within a column followed by same letters are not significantly different (P = 0.05, Scheffe’s test).

**Table 3.** Fumigant activity of oil and constituents from S. tenuifolia and dichlorvos against larvae of L. ingenua

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope ± SE</th>
<th>LC50 (μg/ml)</th>
<th>95% CL</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. tenuifolia oil</td>
<td>2.6 ± 0.36</td>
<td>2.25</td>
<td>1.80–2.80</td>
<td>3.6</td>
</tr>
<tr>
<td>(+)-Limonene</td>
<td>4.01 ± 0.63</td>
<td>15.42</td>
<td>13.01–18.29</td>
<td>0.5</td>
</tr>
<tr>
<td>(-)-Pulegone</td>
<td>2.85 ± 0.43</td>
<td>1.21</td>
<td>0.92–1.50</td>
<td>6.7</td>
</tr>
<tr>
<td>(-)-Menthene</td>
<td>2.36 ± 0.32</td>
<td>6.03</td>
<td>4.73–7.69</td>
<td>1.3</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>2.52 ± 0.42</td>
<td>8.13</td>
<td>6.19–10.29</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*LC50 values based on four concentrations with six replicates of 10 insects per concentration.

bDenotes confidence limit.

Relative toxicity = LC50 value of dichlorvos/LC50 value of each chemical.

**Fig. 1.** Gas chromatogram of S. tenuifolia oil. Peak 1, (+)-limonene (4.5%); peak 2, (-)-menthene (49.7%); and peak 3, (-)-pulegone (43.3%).
S. tenuifolia oil against *P. ostreatus* were 39.6 and 38.5% at 49.7 and 24.8 μg/ml, respectively. *S. tenuifolia* oil did not inhibit the mycelial growth of *P. ostreatus* at 12.4 μg/ml. Limonene was the least toxic to *P. ostreatus* among test compounds. Inhibition by pulegone was 75.4% at 49.7 μg/ml, but mortality decreased to 40.4% at 24.8 μg/ml. At 3 μg/ml, no inhibition was observed. Inhibition rates of menthone were 36.8 and 30.7% at 49.7 and 24.8 μg/ml, respectively.

### Discussion

Plant essential oils have potential for *L. ingenua* control because many of them have little or no harmful effects on the environment and humans, and they may be applied in the same way as conventional insecticides. Furthermore, plant essential oils are highly volatile and therefore do not leave toxic residues. Strong insecticidal activity was produced from the plants belonging to the families Labiatae (*S. tenuifolia*), Araceae (*A. gramineus*) and Rutaceae (*Z. piperitum*). *S. tenuifolia* was the most effective against *L. ingenua*. Jacobson (1989) pointed out that the most promising sources of novel plant-based insecticides are species of the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, and Canellaceae.

*S. tenuifolia* has been cultivated in China, Japan, and Korea and is used for the treatment of common colds, fever, sore throat, allergic dermatitis, eczema, and psoriasis (Fung and Lau 2002). Early studies have indicated that *S. tenuifolia* essential oil possesses insecticidal activity against *Reticulitermes speratus* Kolbe (Park and Shin 2005), antioxidant activity (Kirby and Schmidt 1997), antipruritic activity (Tohda et al. 2000), and hemostatic and antimicrobial activity (Zhu 1998). *S. tenuifolia* contains ≈0.5–1.8% volatile oils, consisting mainly of menthone and pulegone and a small amount of *d-*limonene (Fung and Lau 2002). In this study, these same three major components were identified by GC-MS analysis. Menthone was the most abundant compound followed by pulegone and limonene.

Two of the main components of *S. tenuifolia* showed very strong insecticidal activity against *L. ingenua*. Pulegone, menthone, and limonene have been reported to show insecticidal activity against stored product insects (Lee et al. 2003). In our study, pulegone (LC50 = 1.21 μg/ml air) was ≈6.7 times as effective as dichlorvos (LC50 = 8.13 μg/ml air). Menthone, with an LC50 value of 6.03 μg/ml air, was ≈1.3 times as effective as dichlorvos. Only limonene (LC50 = 15.42 μg/ml air) was found to be less toxic than dichlorvos in this test.

Kalberer and Vogel (1977) reported that carboutran decreased the output of *A. bisporus* when it was applied for control of *L. auripila*. Cantelo (1981) investigated the effect of seven commercial pesticides on mushrooms, and they reported that there was no effect on growth of mushrooms except with diazinon. In our study, the essential oil of *S. tenuifolia* inhibited the mycelial growth of *P. ostreatus* at higher concentration (38.5% inhibition at 24.8 μg/ml air). However, no inhibition was observed at 12.4 μg/ml air. At this concentration, *S. tenuifolia* oil caused 96.6% larval mortality. This result indicates that *S. tenuifolia* oil might be useful for control of *L. ingenua* without any deleterious effect on the mycelial growth of *P. ostreatus* at higher concentration. Pulegone produced a similar result, inhibiting mycelial growth of *P. ostreatus* at higher concentration (75.4% inhibition at 49.7 μg/ml air). However, we did not investigate the effect of *S. tenuifolia* oil or its components on production of *P. ostreatus* fruit bodies. Such studies are required before *S. tenuifolia* oil or its component can be considered for the practical use as novel fumigants.

### References Cited


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