Components from the Essential Oils from Two *Origanum* Species as Larvicides Against *Euproctis chrysorrhoea* (Lepidoptera: Lymantriidae)

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**ABSTRACT**  The larvicidal potential of the essential oils from *Origanum onites* L. and *O. minutiflorum* (O. Schwarz & P. H. Davis) (Labiatae) and their commercially available components (carvacrol, thymol, γ-terpinene, and terpinen-4-ol) was investigated against the brown-tail moth, *Euproctis chrysorrhoea* (L.) (Lepidoptera: Lymantriidae), an important pest of agricultural and forest crops in southwestern Turkey. This pest is also a public health concern due to the urticating hairs of its larvae. The chemical composition of these essential oils was also determined by gas chromatography-mass spectrometry (GC-MS). Carvacrol was the major component of both *O. onites* and *O. minutiflorum* essential oil, at 29.6% and 56.1%, respectively. The oils and components were bioassayed against 4th instars of *E. chrysorrhoea* at concentrations ranging from 0.0625% to 0.50%. All materials tested showed larvicidal activities in a concentration-dependent manner. Topical applications of the essential oils from *O. onites* and *O. minutiflorum* applied at 80 μl of solution per larva were highly toxic, with LC50 values of 522 and 1076 ppm, respectively. Of the four commercial components tested, thymol and carvacrol were the most active (LC50 values of 367 and 424 ppm, respectively). The other two components, γ-terpinene and terpinen-4-ol, were also toxic with LC50 values of 1172 and 2126 ppm, respectively. Our overall results suggest that the essential oils from *O. onites* and *O. minutiflorum* and their components may be potential alternatives to synthetic insecticides for the control of brown-tail moth larvae.

**KEY WORDS**  Brown-tail moth, chemical composition, essential oil, *Euproctis chrysorrhoea*, larvicidal activity, Lepidoptera, Lymantriidae, *Origanum* species

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**Introduction**

The brown-tail moth, *Euproctis chrysorrhoea* (L.) (Lepidoptera: Lymantriidae), is an important defoliator of forests, hedgerows, orchards, and ornamental plants in Turkey. The species also has public health significance due to the urticating hairs of its larvae, which can cause severe rashes and irritation on skin and eyes (Dejong 1977, Werno & Lamy 1991). Over the last ten years, populations...
The control of *E. chrysorrhoea* in Turkey is reliant heavily upon broad-spectrum synthetic insecticides, including both organophosphates and pyrethroids. Potential problems associated with continued long-term use of toxic insecticides include pest resistance and negative impacts on natural enemies. In addition, increasing documentation of negative environmental and health impacts of synthetic toxic insecticides and increasingly stringent government regulation of pesticides has prompted the examination of alternative methods for insect pest control. Increased public concern of the potential adverse environmental effects associated with the heavy use of chemical insecticides has resulted in renewed interests in the development and use of botanical pesticides. Studies have indicated that essential oils extracted from many plant species and their components possess insecticidal activity and can be one of the promising alternatives to chemical insecticides (Aslan et al. 2004, Sampson et al. 2005, Cetin et al. 2007, Topuz & Erler 2007).

The genus *Origanum* L., belonging to the family Labiatae (=Lamiaceae), contains aromatic and medicinal plants. They are small shrubs or perennial herbs with several stems, ascending or erect, subsessile or petiolate leaves and flowers in verticillasters aggregated in dense or loose spikes which are arranged in a paniculate or corymbiform inflorescence. Most of the *Origanum* species are native to the Mediterranean Basin, and they have a very local distribution around it (Kokkini 1996, Baser 2002). Twenty-four *Origanum* species are endemic to Turkey (Guner et al. 2001). Oregano species are of great economic importance as a spice. They are also used in traditional medicine to treat health disorders. However, recent studies have pointed out that they can be used in many other ways as their essential oils have antimicrobial, cytotoxic, antioxidant, and insecticidal activities (Lagouri et al. 1993, Sivropoulou et al. 1996, Tun et al. 2000). More recently, the essential oils isolated from *O. onites* L. and *O. minutiflorum* (O. Schwarz & P. H. Davis) and their commercially available components were found to have toxic, repellent, development-slowing, and reproduction-inhibiting effects to various pest species (Tun & Erler 2003, Erler 2005a,b, Erler & Tun 2005, Cetin & Yanikoglu 2006, Cetin et al. 2006, 2007). These insecticidal properties suggest that essential oils from oregano species and their components may be promising candidates as novel pesticides against certain insect pest species.

The aim of this study was to investigate chemical composition of essential oils from two *Origanum* species (*O. onites* and *O. minutiflorum*) and to evaluate larvicidal potential of these oils and their four commercially available components (carvacrol, thymol, $\gamma$-terpinene and terpinen-4-ol) against 4th instar *E. chrysorrhoea* under laboratory conditions.

**Materials and Methods**

**Extraction and analysis of essential oils.** The plant materials used as the source of essential oils were collected from their natural habitats in Antalya, Turkey. Taxonomic identification was made by botanists from the Biology Department of Akdeniz University, Antalya, Turkey, where voucher specimens of collected species are deposited under the numbers, *O. onites*-21/07 and *O.
minutiflorum-22/07. The essential oils were extracted from air-dried aerial parts of *O. onites* and *O. minutiflorum* by steam distillation using a Clevenger-type apparatus as described by Erler et al. (2006). Conditions of extraction were: 50 g of dried sample; 1:10 plant material/water volume ratio, 3 h distillation. Extracted oils were stored in dark glass tubes in a refrigerator at 4°C until needed.

The essential oils obtained were analyzed by GC-MS (Agilent 6890 GC system 5973 MSD, GMI Inc., Ramsey, MN). Helium (1 ml/min) was used as a carrier gas. The initial temperature was 50°C, which was held for 2 min. The temperature was then increased 5°C/min to 200°C, held for 5 min, increased 10°C/min to 250°C, and held for another 10 min. The GC-MS system was fitted with a HP-1 glass capillary column (50 m × 0.32 mm × 0.52 μm film thickness) that was filled with 100% dimethylpolysiloxane. The oven temperature was programmed from 50°C to 250°C at a 5°C/min dynamic rate and held at 250°C for 15 min. Identification of components in the oils was performed by computer searches in commercial reference libraries. The fragmentation patterns of the mass spectra were compared with those from the WILEY and NIST05 Libraries (Adams 2007).

**Essential oil components.** Four components of the essential oils were purchased from various commercial sources and tested for their larvicidal potential against *E. chrysorrhoea*. These chemicals were carvacrol (Fluka Chemie AG, Buchs, Switzerland, 97% pure), thymol (Merck, Darmstadt, Germany, 99% pure), γ-terpinene (Sigma, Steinheim, Germany, 97% pure), and terpinen-4-ol (Aldrich, Milwaukee, WI, USA, 99% pure). The choice of these components for inclusion in this study was based on previous studies that indicated larvicidal action against several insect pests (Erler 2005b, Cetin et al. 2007).

**Insect material.** Overwintered (4th instar) larvae of *E. chrysorrhoea* were used in the tests. Insects were collected from a pear orchard adjacent to a forest in Korkuteli, Antalya in April 2007. Small branches of pear trees carrying the silken webs in which caterpillars live during the winter were carefully pruned and transported in 5-liter containers to the toxicology laboratory at the Department of Biology, Akdeniz University. A voucher specimen of the collected *E. chrysorrhoea* was deposited at the Plant Protection Department, Akdeniz University, under the catalogue number, 2007/ Lep-18. Emerging larvae were supplied with fresh pear (*Pyrus communis* L. cv. ‘Ankara’) foliage until needed for testing.

**Larvicidal bioassay.** Bioassays were performed using methods of Cetin et al. (2007) and Erler & Cetin (2007) with minor modifications. Fourth instar *E. chrysorrhoea* (determined according to head capsule width) were exposed to various concentrations (0.0625, 0.125, 0.25, and 0.50%) of both the essential oils and the commercial components that were prepared using distilled water in an emulsion with 0.01% Tween-80 (Merck, Darmstadt, Germany). Each concentration was topically applied to the 8th abdominal sternite, an area less covered with hairs, at 80 μl per larva using a microapplicator (Burkard, Rickmansworth, UK). Twenty-five larvae were treated with each concentration of each test material. Treated larvae (25 per jar) were transferred into glass jars containing fresh pear foliage, which was replenished as needed. Each jar was counted as one replicate. Four replicates were used for each concentration of each test material, and each replicate set contained one control jar containing insects that had been treated with a 0.01% emulsion of distilled water and Tween-80. Twenty holes were drilled into the screw cap of each jar for air circulation. All bioassays were conducted at 26 ± 2°C, 60 ± 10% RH, and a 12:12 h (L:D) photoperiod. Larval mortality was
recorded after 96 h. Mortality was determined by observing those individuals that did not move when prodded with a needle. Mortality was expressed as a percentage of the initial number of larvae in each jar.

Phytotoxicity test. Phytotoxic effects of the test materials were evaluated by using fresh pear shoots placed into plastic bottles containing 0.5 liter tap water to maintain the turgor of the foliage. The shoots were sprayed until runoff with the highest concentration (0.5%) of each test material, and they were observed over a 7-day period for any phytotoxicity. There were five replicates for each test material and the control mixture (0.01% Tween-80).

Statistical analysis. Percent mortality data were converted to arcsine values and then subjected to analysis of variance (ANOVA). Significant differences among the treatment means were separated using the Duncan’s multiple range test (DMRT), and a probability (P) of ≤0.05 was accepted as statistically significant. The LC values (LC\textsubscript{50} and LC\textsubscript{90}) and the 95% confidence limits were calculated by using a probit analysis program (US EPA 1999).

Results

Chemical composition of essential oils. Table 1 lists the main components of the essential oils from the two Origanum species. Carvacrol was the major component of both O. onites (29.6%) and O. minutiflorum (56.1%) essential oil. Thymol (17.1%) and linalool (27.6%) were also major components of O. onites essential oil, while trichloromethane (19.3%) was a major component of O. minutiflorum essential oil (Table 1).

Larvicidal activity. Both O. onites and O. minutiflorum essential oils showed larvicidal activity against 4th instar E. chrysorrhoea (Table 2). Although larval mortality generally increased with increasing concentrations of the test essential oils, the mortalities by O. onites essential oil at two lower concentrations (0.0625% and 0.125%) did not differ significantly from each other. The same were
seen between the mortalities by *O. minutiflorum* essential oil at two higher concentrations (0.25% and 0.50%). Mortalities caused by essential oils from both species did not differ significantly at higher concentrations (0.25% and 0.50%), however, significant differences between the mortalities by *O. onites* and *O. minutiflorum* essential oils were seen at lower concentrations (0.0625% and 0.125%).

All of the components tested were larvicidal against *E. Chrysorrhoea*, with carvacrol showing the strongest toxicity (100%) at its highest concentration (0.50%). This was followed by thymol with 86% mortality at the 0.50% concentration. With the exception of the highest concentration, mortalities caused by these two components did not differ significantly. Also, at every concentration, there were no significant differences between the mortalities caused by γ-terpinene and terpinen-4-ol.

When LC$_{50}$ (ppm) values of the test materials were compared, the order of toxicity was: thymol > carvacrol > *O. onites* essential oil > *O. minutiflorum* essential oil > γ-terpinene > terpinen-4-ol (Table 4). However, based on the estimated LC$_{90}$ (ppm) values, the order of toxicity was: carvacrol > *O. onites* essential oil > *O. minutiflorum* essential oil > thymol > γ-terpinene > terpinen-4-ol.

**Phytotoxicity.** Only thymol and terpinen-4-ol at the highest concentration (0.50%) were phytotoxic to fresh pear shoots. The most obvious phytotoxicity symptoms that appeared on the shoots were burning of young leaves and necrotic spots on older leaves. This level of phytotoxicity could be a major concern for pear growers because more than 50% of the pear foliage was damaged by these two components.

**Discussion**

The results obtained from the GC-MS analysis are in accordance with previously published data on *O. onites* and *O. minutiflorum* essential oil compositions (Baser et al. 1993, Dadalioglu & Evrendilek 2004, Demirci et al. 2004, Kokkini et al. 2004).

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**Table 2. Mortality of 4th instar *Euproctis chrysorrhoea* in response to four concentrations of essential oils from *Origanum onites* and *O. minutiflorum* after an exposure period of 96 h.**

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>Control$^b$</th>
<th>0.0625</th>
<th>0.125</th>
<th>0.250</th>
<th>0.500</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. onites</em></td>
<td></td>
<td>2.0 ± 2.0 aA</td>
<td>57.0 ± 4.1 bB</td>
<td>69.0 ± 4.7 bC</td>
<td>80.0 ± 8.0 cA</td>
</tr>
<tr>
<td><em>O. minutiflorum</em></td>
<td>2.0 ± 2.0 aA</td>
<td>35.0 ± 4.7 bA</td>
<td>53.0 ± 5.0 cA</td>
<td>80.0 ± 3.2 dA</td>
<td>85.0 ± 4.4 dA</td>
</tr>
</tbody>
</table>

$^a$80 µl of solution was applied to each larva.
$^b$A mixture of distilled water and 0.01% Tween-80.
$^c$Means within a line followed by the same lower case letter are not significantly different (DMRT, \( P \leq 0.05 \)).
$^d$Means within a column followed by the same capital letter are not significantly different (DMRT, \( P \leq 0.05 \)).
Table 3. Mortality of 4th instar *Euproctis chrysorrhoea* in response to four concentrations of four components of *Origanum onites* and *O. minutiflorum* essential oils after an exposure period of 96 h.

<table>
<thead>
<tr>
<th>Component</th>
<th>Control&lt;sup&gt;b&lt;/sup&gt;</th>
<th>0.0625</th>
<th>0.125</th>
<th>0.250</th>
<th>0.500</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carvacrol</strong></td>
<td>2.0 ± 2.0 a&lt;sup&gt;cA&lt;/sup&gt;&lt;sup&gt;d&lt;/sup&gt;</td>
<td>66.0 ± 4.3 bC</td>
<td>77.0 ± 4.1 bB</td>
<td>87.0 ± 3.7 cB</td>
<td>100.0 ± 0.0 dC</td>
</tr>
<tr>
<td><strong>Thymol</strong></td>
<td>2.0 ± 2.0 aA</td>
<td>57.0 ± 3.7 bBC</td>
<td>75.0 ± 6.8 cB</td>
<td>81.0 ± 2.5 cB</td>
<td>86.0 ± 3.4 cB</td>
</tr>
<tr>
<td><strong>γ-Terpinene</strong></td>
<td>2.0 ± 2.0 aA</td>
<td>39.0 ± 10.1 bAB</td>
<td>52.0 ± 2.8 bcA</td>
<td>64.0 ± 6.7 cA</td>
<td>81.0 ± 4.1 dAB</td>
</tr>
<tr>
<td><strong>Terpinen-4-ol</strong></td>
<td>2.0 ± 2.0 aA</td>
<td>26.0 ± 3.8 bA</td>
<td>37.0 ± 3.0 cA</td>
<td>52.0 ± 4.3 dA</td>
<td>73.0 ± 2.5 eA</td>
</tr>
</tbody>
</table>

<sup>a</sup>80 μl of solution was applied to each larva.

<sup>b</sup>A mixture of distilled water and 0.01% Tween-80.

<sup>c</sup>Means within a line followed by the same lower case letter are not significantly different (DMRT, *P* ≤ 0.05).

<sup>d</sup>Means within a column followed by the same capital letter are not significantly different (DMRT, *P* ≤ 0.05).
The results of larvicidal bioassay showed that both essential oils produced high larval mortalities against *E. chrysorrhoea*. Of the four components tested, the most promising ones were carvacrol and thymol, which were more active than c-terpinene and terpinen-4-ol.

There are many reports on insecticidal activity of essential oils from various *Origanum* species and their components, but many of these studies were based on fumigant activity rather than contact toxicity. The results from this study suggest that essential oils extracted from *Origanum* species and their components have contact toxicity and can be used as contact larvicides against lepidopterans. Similarly, Cetin et al. (2007) reported that the essential oil isolated from *O. onites* and its four components, carvacrol, thymol, c-terpinene and terpinen-4-ol, have great potential as larvicides against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams (Lepidoptera: Thaumetopoeidae).

Insecticidal activity of essential oils can be attributed to a number of small terpenoid and phenolic compounds, which in pure form have been shown to exhibit various activities against insect pests (Tun & Erler 2003, Erler 2005a,b, Erler & Tun 2005). Chemical analysis of the essential oils tested in this study have shown that the mutual principal active compounds are carvacrol, thymol, p-cymene, c-terpinene, and borneol. However, there are often large differences in the reported insecticidal activity of essential oils from the same species. The reasons for this variability may be due to the geographical source, the harvesting season, the genotype, the climate, the drying regime, and the part of the plant that was extracted. These factors influence the chemical composition and relative proportions of the individual components in the essential oil of the plant (Azevedo et al. 2001, Babu & Kaul 2005, Kosar et al. 2008).

The type of essential oil component is important in insecticidal properties of these chemicals. The components used in this study are classified into three chemical groups: alcohols (terpinen-4-ol), hydrocarbons (c-terpinene), and phenols (carvacrol and thymol). In this study, the phenolics were more toxic than the alcohol and hydrocarbon. In previous studies, these same phenolic components (carvacrol and thymol) were found to be more toxic and repellent to

### Table 4. Larvicidal activity of *Origanum onites* and *O. minutiflorum* essential oils and their four components against 4th instar *Euproctis chrysorrhoea* after an exposure period of 96 h.

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Slope (SE)</th>
<th>Intercept (SE)</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; (ppm) (LCL-UCL)</th>
<th>LC&lt;sub&gt;90&lt;/sub&gt; (ppm) (LCL-UCL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. onites</em> essential oil</td>
<td>1.36 (0.22)</td>
<td>1.30 (0.71)</td>
<td>522 (294–729)</td>
<td>4500 (3230–8379)</td>
</tr>
<tr>
<td><em>O. minutiflorum</em> essential oil</td>
<td>1.72 (0.21)</td>
<td>0.23 (0.69)</td>
<td>1076 (842–1304)</td>
<td>5937 (4366–9551)</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>1.78 (0.47)</td>
<td>0.42 (1.48)</td>
<td>424 (—)</td>
<td>2311 (—)</td>
</tr>
<tr>
<td>Thymol</td>
<td>1.00 (0.21)</td>
<td>2.41 (0.68)</td>
<td>367 (120–609)</td>
<td>6873 (4108–21456)</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>1.26 (0.20)</td>
<td>1.12 (0.65)</td>
<td>1172 (840–1502)</td>
<td>12100 (7233–31172)</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1.41 (0.20)</td>
<td>0.27 (0.68)</td>
<td>2126 (1695–2720)</td>
<td>17031 (10058–42670)</td>
</tr>
</tbody>
</table>

<sup>a</sup>95% confidence limits; LCL, lower confidence limit; UCL, upper confidence limit.  
<sup>b</sup>Could not be determined.
several stored product pests than the other terpenes used in the present study (Tun & Erler 2003, Erler 2005a,b). Carvacrol and thymol have also been shown to be effective against greenhouse insect and mite pests (Erler & Tun 2005). Therefore, we conclude that the larvicidal action of the essential oils in this study may be attributed mostly to these phenol components.

Essential oils are the odorous, volatile products of aromatic plants’ secondary metabolism, normally formed in special cells or groups of cells found in many leaves and stems. For centuries many essential oils have served as flavoring agents in food and beverages without any reported illnesses or side effects resulting from their use (Simon 1990). They are therefore considered less harmful to humans than most conventional insecticides. Furthermore, studies have shown that they are readily biodegradable and less detrimental to non-target organisms (Baysal & Yegen 1997, Yegen et al. 1998).

In conclusion, the oregano essential oils and their main components tested in this study offer great potential as larvicides for the control of E. chrysorrhoea, an important forest, agricultural and urban pest. The use of these botanical derivatives in pest control instead of synthetic pesticides may help reduce adverse environmental effects.

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