Fumigant toxicity of essential oils from *Citrus reticulata* Blanco fruit peels against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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**Abstract:** Certain compounds of plants such as essential oils, with insecticidal properties have been considered as alternatives to chemical pesticides for pest control. This study reports the fumigant toxicity of *Citrus reticulata* Blanco (Rutaceae) peel essential oils against stored-product insect pest, red flour beetle, *Tribolium castaneum* Herbst (Tenebrionidae) adults. Experiment was carried out at 27 ± 1 °C and 60 ± 5 % relative humidity in darkness. Experimental concentrations were 15, 22, 31, 45, and 63 μl/l air tested on adult (1-7 days old) insects after 24 and 48 h of exposure. Results indicated that essential oils from *C. reticulata* had fumigant toxicity effects against this stored pest. LC50 values were 38.2 and 35.6 μl/l air at 24 and 48 h after exposure of *T. castaneum* adults respectively. The essential oils of *Citrus reticulata* fruit peels at the highest dose of 63 μl/l air caused 76.6 % and 79 % mortality of insects after 24 and 48 hours of exposure, respectively. Mortality of *T. castaneum* increased with both increase in concentration of *C. reticulata* oils as well as exposure time of treated insects. These results suggest the potential of *C. reticulata* oil as a control agent against *T. castaneum*.

**Keywords:** *Citrus reticulata*, essential oil, toxicity, *Tribolium castaneum*

**Introduction**

Insect pests often cause extensive damage to stored grains and their products and this may amount to 5-10 % in the temperate zone and 20-30 % in the tropical zone (Haque et al., 2000). In such a situation, protection of stored grain and agricultural products against insect infestation is an urgent need. Synthetic insecticides and fumigation are the main compounds and methods used for stored products insect pests control. However, an uncontrolled use of these synthetic insecticides causes a great hazard to environment and consumers due to residues (Isman, 2006). Naturally occurring substances are an alternative to conventional pesticides and plant essential oils have traditionally been used to kill insects (Isman, 2000). Potential hazards to mammals from synthetic insecticides, the ecological consequences and the increase in pesticide resistance have led to a search for new classes of insecticides with lower mammalian toxicity and a lower persistence in the environment (Renault-Roger et al., 1993). Therefore, development of bio-insecticides has been focused on, as a viable pest control strategy in recent years (Hashim and Devi, 2003). Many plants are known to have various activities against different stored grain insect pests (Mukherjee and Joseph, 2000). Many of these botanicals are aromatic plants producing essential oils and have been widely investigated against stored product insects (Ngamo et al., 2007). The essential oils have the complex mixture of volatile organic compounds which are produced by different plant genera and have been...
reported to be biologically active and are endowed with insecticidal, antimicrobial and bio-regulatory properties (Kumar et al., 2008). Considering the loss of storage pests and adverse effects of chemical pesticides, use of plant extracts is the best method for controlling storage pests (Papachristos and Stamopoulos, 2002). Essential oils have been explored for their insecticidal properties against field crop (Sharma et al., 2001) and stored grain pests (Dunkel and Sears, 1998). Moreover, extracts of many common species have been evaluated for their repellent and insecticidal activities (Isman, 2000). The toxicity of powdered, sun-dried orange and grape fruit peels to Callosobruchus maculatus has been demonstrated (Don-Pedro, 1985). Sweet orange (Citrus sinensis L.) is a medicinal plant prescribed as traditional medicine to treat diverse illnesses (Intekhab and Aslam, 2009). The essential oil of Citrus sinensis L. also has fumigant toxicity against Aedes aegypti L. mosquitoes (Omomouwajo et al., 2005). The present study was conducted to determine the efficiency of the essential oils from Citrus reticulata peel as plant secondary metabolites against the stored product pest, Tribolium castaneum (Herbst).

Materials and Methods

Insect rearing

*T. castaneum* was reared on wheat flour mixed with yeast (10:1 w/w, respectively). Adult insects, 1-7 days old, were used for fumigant toxicity tests. The cultures were maintained in the dark in a growth chamber set at 27 ± 1 °C and 60 ± 5 % RH. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. All experiments were carried out under the same environmental conditions.

Extraction of the essential oils

Essential oils were extracted from fruit peels of Citrus reticulata (Bam variety, 2011) (Rutaceae). Peels were dried in absence of sun light at room temperature at 30±5°C and ground by domestic mixer (Pars Khazar, Iran). Dried material was hydro-distilled in Clevenger apparatus continuously for four hours to yield the essential oil. Conditions of extraction were: 100 g of dried material; 1:12 plant material/water volume ratio. Extracted oils were transferred to glass flasks and kept at 4 °C in a refrigerator.

**Fumigant toxicity of Citrus reticulata peel essential oil**

The experimental concentrations of *Citrus reticulata* peel essential oils on *Tribolium castaneum* were determined by preliminary tests. Experimental concentrations were 15, 22, 31, 45, and 63 μl/l air for adult (1-7 days old) insects. For fumigant toxicity assays, filter papers (Whatman No. 1, cut into 4 × 5 cm paper strip) were impregnated with different concentrations using a microsampler. Thirty adult (1-7 days old) insects of *T. castaneum* were placed in small plastic tubes (3.5 cm diameter and 5 cm in height) with open ends covered with cloth mesh. The tubes were hung at the geometrical centre of glass bottles, which were then sealed with air-tight lids. Thirty adult insects of *T. castaneum* were treated in three replicates per treatment. Two experiments were done separately with 24 and 48 h of insect exposures. Treated insects were incubated at 27 ± 1 °C. After this time, the number of dead adults was counted. Those insects that did not move when lightly probed or shaken in light and mild heat were considered dead. Mortality in the controls was not observed in any of the experiments.

Data analysis

Mortality data were analyzed with SPSS software (SPSS Inc, 1993). Probit analysis was used to determine LC₅₀ and LC₉₅ values. The values significance of $\chi^2$ was estimated according to Robertson and Preisler (1992). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test to estimate statistical differences between means at $\alpha = 0.05$. 116
Results

Result of fumigant toxicity of essential oil of *Citrus reticulata* fruit peels is presented in table 1. Analysis of variances showed that the insect mortality was completely correlated with essential oils concentrations at 24 and 48 h exposure. Mean comparisons of percent mortality data with honestly significant differences (HSD) showed that all treatments were statistically different in their lethality to insects. By comparing the mean mortality values, it was seen that with increase in concentrations of *Citrus reticulata* peel essential oils, mortality rate of adults of *Tribolium castaneum* was increased accordingly (Table 1). The insecticidal activity varied with plant derived material, different concentrations of the oils and exposure time. The essential oil of *Citrus reticulata* fruit peels at the highest dose of 63 μl/l air imposed 76.6 % and 79 % mortality after 24 and 48 hours of exposure, respectively (Table 1). No dead insects were observed in the control treatments. The highest dose of essential oil showed significantly higher (P < 0.05) mortality. Table 2 shows the probit analysis results and appropriate LC50 and LC95 values. The LC50 and LC95 values of *C. reticulata* oil were estimated 38.2 and 136.7 μl/l air, and 35.6 and 114.8 μl/l air respectively at 24 and 48 h against adults of *T. castaneum* (Table 2).

Discussion

Essential oils and components from more than 75 plant species belonging to different families have been proved to possess high fumigant activity against stored product insect pests (Rajendran and Sriranjini, 2008). Rutaceae is a large family containing 130 genera in seven subfamilies with many important fruits and essential oils products. In this family, peel has the highest value of all essential oils and is widely used as flavouring agent in bakery, as fragrance in perfumery and also for pharmaceutical applications (Weiss, 1997). In this study, results showed a considerable insecticidal effect of essential oils from peel of citrus fruits on *Tribolium castaneum* after 24 and 48 h of exposure. Several essential oils of plants have been reported for their insecticidal activities against *Tribolium castaneum* (Herbst). The essential oil of *Citrus sinensis* L. showed contact toxicity against adult stage of *Zabrotes subfasciatus* L. after 24, 48, 72 and 96 h exposure (Zewde and Jembere, 2010). Essential oil derived from orange peels is known to have toxic, feeding deterrent and poor development effects on lesser grain borer, *Rhyzopertha dominica* (F.), rice weevils, *Sitophilus oryzae* (L.) and red flour beetle, *Tribolium castaneum* Herbst. (Tripathi et al., 2003).

<table>
<thead>
<tr>
<th>Exposure times (h)</th>
<th>Concentrations (μl/l air)</th>
<th>control</th>
<th>15</th>
<th>22</th>
<th>31</th>
<th>45</th>
<th>63</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0* ± 0.0E</td>
<td>18.8 ± 0.3*</td>
<td>25.5 ± 0.3*</td>
<td>46.6 ± 0.5*</td>
<td>64.4 ± 0.8*</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td>0.0 ± 0.0E</td>
<td>19.9 ± 0.6*</td>
<td>26.6 ± 0.6*</td>
<td>45.5 ± 0.58*</td>
<td>68 ± 0.0*</td>
</tr>
<tr>
<td>48</td>
<td></td>
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</tbody>
</table>

* Means followed by the same letter in each row are not significantly different (HSD, p = 0.05).
Table 2 Probit analysis of T. castaneum mortality treated with different concentrations of Citrus reticulata peel essential oil in 24 and 48 h after exposure.

<table>
<thead>
<tr>
<th>Intercept ± SE</th>
<th>Slope ± SE</th>
<th>χ² [df = 5, 12]</th>
<th>LC₉⁵ (µl/l air)</th>
<th>LC₅₀ (µl/l air)</th>
<th>Exposure times (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.43 ± 0.5</td>
<td>5.6 ± 0.22</td>
<td>2.9</td>
<td>136.7 (107.2 – 169)</td>
<td>38.2 (23 – 49.9)</td>
<td>24</td>
</tr>
<tr>
<td>-0.4 ± 0.22</td>
<td>6.7 ± 0.3</td>
<td>2.4</td>
<td>114.8 (87.22 – 130)</td>
<td>35.6 (21.1 – 45.8)</td>
<td>48</td>
</tr>
</tbody>
</table>

* 95% lower and upper confidence intervals are shown in parentheses.

The essential oil from Citrus reticulata fruit peels has also been reported to have fumigant toxicity toward Culex pipiens (L.) (Mwaiko and Savaeli, 1992) and cowpea weevils, Callosobruchus maculatus (F.) (El-Sayed and Abdel-Razik, 1991). Furthermore, the essential oil from Citrus reticulata fruit peels has fumigant action against Xenopsylla cheopis (R.) (Weinzierl and Henn, 1992), household insects, Blatella germanica (L.) and Musca domestica (L.) and stored product Sitophilous oryzae (L.) (Kan and Coats, 1988). Anna senegalensis (P.), Hyptis specigera (J.) and lippie regosa (T.) essential oils were tested against the four major stored product insect pests Sitophilous zeamais, Sitophilous oryzae, Callosobruchus maculatus and Tribolium castaneum. Hyptis specigera (J.) essential oil was the most active towards Sitophilous oryzae (L.) and T. castaneum was the least sensitive insect when exposed to three essential oils (Ngamo et al., 2007). The essential oils from different parts of plants; fruits of Schyzgium aromaticum L., leaves of Aegle marmelos L., seeds of Corriandrum sativum L. and peels of C. reticulata fruits extracted by a water distillation method showed strong repellency against T. castaneum even at low concentrations (Mishra and Tripathi, 2011). The plants volatile essential oils of fruit peels of some citrus species have been reported to have insecticidal properties against stored grain insect pests (Tripathi et al., 2003). The essential oils extracted from Citrus genus have monocyclic monoterpenoids and their major component is d-limonene (j-mentha-l,8-dene) and they have insecticidal activities against insect pests (Kan and Coats, 1988). Similarly, in the present study the essential oil of C. reticulata was found to have remarkable fumigant toxicity at 24 and 48 h exposure against T. castaneum adults. The results of this experiment showed that all ingredients of the essential oil of C. reticulata fruit peels were relatively toxic to Tribolium castaneum.

References


نمی‌شود

چکیده: به‌پیازاری از گیاهان ترکیبات خاصی با خواص حشره‌کشی دارند که در سال‌های اخیر استفاده از این اساس‌های گیاهی به‌عنوان جایگزین سموم شیمیایی در کنترل آفات بیمار مورد توجه قرار گرفته است. در این تحقیق سمیت تدخینی اساس پوست میوه نارنگی عليه مرحله بالغ یک آفت انبازی مهم مورد استفاده اساس نارنگی در خوارند. در طول 24 و 48 ساعت سمیت تدخینی اساس نارنگی شامل 15، 22، 43، 63 میکروآرت بر لیتر هوا بودند. نتایج نشان دهنده سمیت تدخینی اساس نارنگی عليه این آفت انبازی بود. مقادیر LC50 برای بپاده‌های زمانی 24 و 48 ساعت بترتیب 48/2 و 35/6 میکروآرت بر لیتر هوا تعیین شدند. اساس پوست میوه نارنگی در بالاترین میزان مورد استفاده (32 میکروآرت بر لیتر هوا) برای پاپی‌های زمانی 24 و 48 ساعت به ترتیب 79/6 و 79 درصد مرگ و میر را در حشرات کامل ایجاد نمود. میزان مرگ و میر شیب‌های قرمز آزاد با افزایش میزان نارنگی و زمان اساس مشابه احتمال آماری افزایش می‌یابد. این نتایج نشان می‌دهند که اساس نارنگی یکعامل کنترل کننده برای پیشگیری از بیماری افرازی می‌باشد.

واژگان کلیدی: میوه نارنگی، اساس گیاهی، سمیت، شیب‌های قرمز آرد