Antifungal Effects of Three Plant Essential Oils Against *Botrytis cinerea* : The Cause of Gray Mold on Strawberry

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**ABSTRACT**

Post-harvest agricultural products are damaged as a result of fungal infections. This damage is estimated on about 20% of strawberries. Chemical fungicides are used usually to control these infections, but these synthetic chemicals are commonly harmful for human health. It is expected that some plant extracts or essential oils can be used against fungal agents instead of the chemicals. The most common fungus disease of strawberries is gray mold (*Botrytis cinerea*). Three essential oils of *Satureja hortensis*, *Zataria multiflora* and *Carum copticom* were tested at first *in vitro* on PDA with three concentrations 100, 200 and 300 ppm for control of the mycelium growth of *Botrytis cinerea*. After that, strawberries were inoculated in spore solution (10⁶ spores per ml) and then treated with three essential oil solutions and a fungicide (Iprodion+Carbendazim 52.5%). The results showed that a 200 ppm concentration of all three essential oils was able to effectively control the growth of the *Botrytis* mycelium on both PDA and the actual fruit.

**Keywords:** Essential oils, *Botrytis cinerea*, *Carum copticom*, *Zataria multiflora*, *Satureja hortensis*, Antifungal effects

**INTRODUCTION**

Generally, consumers prefer to have healthy foods and organic agricultural products. Many countries have special regulations for pesticide residue for import of agricultural crops, especially the fruits and vegetables. Recently, the farmers and agricultural producers are trying to use non-chemical manures and pesticides (Chen et al., 1997). Strawberry plants belong to the Rosaceae family and constitute a large portion of the fresh fruits on the market during the spring. In the regions where they are grown, they are in great demand. Due to its wide adaptation to different climate and soils, the strawberry (hybrid between *Fragaria virginiana* × *F. chiloensis*) is available from the tropics to the sub-arctic region. Iran is the fourteenth largest
producer in the world, and cultivation area of this plant in Iran is around 3800 hectares. Because of the high rate of metabolic activity and respiration as well as the need for high water control, strawberries are very susceptible to microbial rots and mechanical damages during transportation. The most destructive fungal rot of strawberry is gray mold which is caused by *Botrytis cinerea*, especially on post-harvest product (Behnamian and Masiha, 2002). The farmers treated the fruits in chemical solution, such as a benomyl fungicide, which is not safe for consumers. In this study, usage of three essential oils (*Satureja hortensis, Zataria multiflora* and *Carum copticom*) against *Botrytis* *in vitro* and *in vivo* instead of chemical fungicides was studied.

**MATERIALS AND METHODS**

*In vitro, on agar*

Three essential oils were obtained from Barijessance Co. (Kashan, Iran) and analyzed with the GC and GC/MS apparatus. The *Botrytis cinerea* was isolated from infected strawberries and cultivated on PDA (Potato Dextrose Agar) in Petri-dishes. PDA was provided by Haly-Liofilchom Co., (46g/LDW. PH = 5.6 ±0.2).

In the PDA, each of the essential oils was tested on daily mycelium growth of *Botrytis* in three concentrations (100, 200, 300 ppm). Medium PDA was provided in one liter Erlenmeyer and sterilized in an autoclave at 121°C and 1.5 atm pressure for 20 minutes. After cooling in 40-45°C for 20 minutes, the essential oil solution was added, and poured in 4 petri-dishes (as 4 replications of each treatment). The control treatment did not have any essential oil. Instead it had acetone. All 40 petri-dishes were inoculated with young mycelium from the margin of a fungal colony which was cut with a cork-borer (5 mm Ø). The dishes were kept in incubator at 25±1°C. Mycelium growth was measured daily as diameter of fungal colony in millimeters.

*Satureja hortensis*
- Thymol 28.18%
- Py-symen 19.56%
- Gamma-Terpinen 15.97%
- Carvacrol 11.02%

*Zataria multiflora*
- Thymol 38.67%
- PY-cymene 19.56%
- Gamma-terpinen 15.97%
- Carvacrol 15.29%

*Carum copticum*
- Thymol 45.97%
- PY-cymene 20.64%
- Gamma-terpinen 19%

*In vivo, on fruit*

The *Selva* cultivar of strawberry fruits (red in color and about 4-5 cm long and 3.5 cm in diameter) was collected. (Reddy *et al.*, 2000 and Asghari marjanloo *et al.*, 1387).

For the following experiment, the fungus *Botrytis* was cultivated and poured in petri-dishes. For coating all the fruits, 200 ml of the spore suspension was prepared by collecting spores of the above fungus. The solution was prepared in one breaker with 10⁶ spores s/ml (measured with a homocytometer). The strawberry fruits were surface sterilized and placed in the spore suspension solution for 2 min. Then, they were placed in essential oil solutions and put in pots in groups of five. All treatments were kept in room temperature about 24±1°C. After three days, the fruit infect situation was determined.
**RESULTS**

*On PDA*

As shown in Fig.1, maximum mycelia growth of *Botrytis* was in the control group. The minimum growth was in the *Carum copticum* treatments. *Carum copticum* in 100 ppm was able to have a strong inhibitory effect on the mycelia growth. On the other hand, *Satureja hortensis* and *Zataria multiflora* in 100 ppm both had a less significant effect on the growth. However, all three essential oils were complete effective at stopping all growth at a concentration of 200 ppm. Therefore, the approximate minimum effective dose for all three is 200 ppm. Table 1 shows the effect of different essential oils on the mycelia growth of *Botrytis*.

![Figure 1. Comparison of the different mean essences on botrytis fungus growth on PDA](image)

**Table 1. Mean comparison effect of different essential oils and concentration on *Botrytis* growth on PDA**

<table>
<thead>
<tr>
<th>Essence</th>
<th>Average of fungi colony diameter growth (mm)</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Satureja hortensis</em></td>
<td>33.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td><em>Satureja hortensis</em></td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>200</td>
</tr>
<tr>
<td><em>Satureja hortensis</em></td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>300</td>
</tr>
<tr>
<td><em>Zataria multiflora</em></td>
<td>44.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td><em>Zataria multiflora</em></td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>200</td>
</tr>
<tr>
<td><em>Zataria multiflora</em></td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>300</td>
</tr>
<tr>
<td><em>Carum copticum</em></td>
<td>10.75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td><em>Carum copticum</em></td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>200</td>
</tr>
<tr>
<td><em>Carum copticum</em></td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>300</td>
</tr>
<tr>
<td>Control</td>
<td>90.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

Means in each column without common letters are significantly different (p< 0.05).
Strawberry experiment results

Percentage of infected fruits was determined in 3rd day of the experiment. Measuring method was observational based on percentage of fruit surface which contamination and the incidence with fungi. Amount of infection was scored between 0 for uninfected fruit and 100 for fully covered fruit with fungi. Effect of oils and fungicides with 200 ppm on Botrytis treatments had well situation except in two treatments that was briefly polluted. Based on table 4, fewer infections were belonging to Carbendazim + eprodion (52.5%), Savory, thyme, and Ajowan treatments, respectively. Meanwhile, witness fruits were fully infected. Percentage of fruit infection was shown in Figure2.

Table 2. Mean comparison effect of different essential oils with 200 ppm on percent pollution of Botrytis on strawberry fruit

<table>
<thead>
<tr>
<th>Essence</th>
<th>Control fungicide</th>
<th>Carum copticom</th>
<th>Zataria multiflora</th>
<th>Satureja hortensis</th>
<th>Fruit pollution %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iprodion + Carbendazim</td>
<td>73.33a</td>
<td>0.00d</td>
<td>4.00b</td>
<td>3.33c</td>
</tr>
<tr>
<td>Essence</td>
<td>Fungicide Treatment</td>
<td>4.00b</td>
<td>3.33c</td>
<td>0.33c</td>
<td></td>
</tr>
</tbody>
</table>

Means with the same letters are not significantly different at 1% level

Figure 2. Mean comparison effect of different essential oils with 200 ppm on percent pollution of Botrytis on strawberry fruit
DISCUSSION

The results show the essential oils of Satureja hortensis, Zataria multiflora, and Carum copticum have antifungal activity and are effective on post-harvest strawberry produce. These essential oils are effective entirely at 200 ppm concentration on the fungus on PDA and against the gray mold on strawberry fruits at same concentration.

The result of Bhaskara et al., (1997) on Thymus vulgaris essential oil on Botrytis efficiency confirms our result. In addition, Zataria multiflora essential oil inhibits the mycelium growth of Aspergillus parasiticus on agar at 250 ppm concentration. Omid Beigi (2007) tested three plant extracts, Satureja hortensis, Zataria multiflora, in 350 ppm on medium against Aspergillus flavus.

This concentration inhibited the mycelium growth. The antifungal compounds in essential oils are different as are analyzed in Barijessance Co. Vagelas et al. (2007) believed that the phenol compounds in essential oils are main factor that inhibit the fungal growth. The compounds thymol, carvacrol, Py-cimene and gamma terpinen are effective in Carum copticum 20.64, Satureja hortensis 15.97 and in Zataria multiflora 9.75%.

Nabigol et al., 2008 in a research showed the effect of Salvia officinalis, Artemisia aucheri, Satureja sp. on strawberry plants which confirm current research.

Nery et al. (2006) tested nine plant volatile oils in laboratory and field conditions to control expansum Penicillium (blue mold disease) but the treatment wasn't effective. Resae et al. (2009) showed that amount of chemical components were not same in different plants organs which is probably due to use of the Ajowan fruits in oil extraction. This organ has not high level of thymol and carvacrol, which are important factor in inhibiting the growth of fungi. Karami et al. (2010) showed that bacterial are inhibited by thymol (in oregano) and carvacrol (in fennel).

Based on these studies, we can concluded that plant oils have anti-fungal compounds as an alternative to industrial fungicide to control fungal infections off the products after harvest.

REFERENCES


