

Research Article

## Antifungal effects of some medicinal and aromatic plant essential oils against *Alternaria solani*

Sohbat Bahraminejad\*, Behnaz Seifolahpour and Reza Amiri

Department of Agronomy and Plant Breeding, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran.

**Abstract:** In this study, anti *Alternaria solani* effects of essential oils of 11 medicinal and aromatic plant species belonging to four families collected from the west of Iran were investigated based on agar dilution method with five replications at concentration of 1.0 µl/ml. The results showed that the highest inhibitory effect belonged to *Oliveria decumbens*, *Cinnamomum zeylanicum*, *Carum copticum* and *Thymus kotschyanus* which completely inhibited the mycelial growth of the fungus. Therefore, their activity was examined in lower concentrations, too. The essential oil of *O. decumbens* at concentration of 0.50 and 0.25 µl/ml completely suppressed the mycelial growth of the fungus. The major constituents of its essential oil were carvacrol (46.53%), thymol (23.75%), *p*-Cymene (13.70%),  $\gamma$ -terpinene (7.66%) and myristicin (3.65%), respectively. Thus, the essential oil of this species with the highest anti-*Alternaria* activity could be selected for further studies on *in vivo* application as natural fungicide.

**Keywords:** *Alternaria solani*, essential oil, fungicidal activity, GC-MS analysis, *Oliveria decumbens*

### Introduction

Nowadays with the increasing world's global population, reaching food security will be dependent on the development of agricultural sector. Using high yielding cultivars can partly be effective for this purpose, but product-restricting factors such as plant pathogens should not be ignored. Diseases caused by plant pathogens significantly contribute to annual loss in crop yield worldwide (Savary *et al.*, 2006). Among all plant diseases which are caused by various pathogens, fungi are the major pathogens and have the greatest impact with regard to diseases and crop production losses (Bajpai and

Kang, 2010).

Early blight is one of the main diseases of tomatoes, potatoes, and eggplants all over the world. The disease is caused by the species of *Alternaria* and the most destructive and important species is *Alternaria solani* (Ellis & G. Martin) L.R. Jones & Grout (Yazici *et al.*, 2011; Bahraminejad *et al.*, 2015). In Iran, the extent of tomato infection with the disease in "Jiroft" and "Kahnouj" (Kerman province) is reported as 60 to 90% (Shahbazi *et al.*, 2011). Like most other diseases, application of chemical fungicides is the most prevalent and effective control method of the disease which is a serious threat to environment and public health besides causing resistance in pathogen (Goussous *et al.*, 2010). Therefore, in recent years, there has been a clear tendency towards finding safer alternative methods for disease control in agriculture (Moghaddam *et al.*, 2013). Lately there has been a growing interest in biologically natural active

---

Handling Editor: Naser Safaie

\* **Corresponding author**, e-mail: sohbah72@hotmail.com  
Received: 30 May 2016, Accepted: 8 November 2016  
Published online: 1 January 2017

compounds such as essential oils and plant extracts to eliminate different fungi on the plants and food products (Bajpai and Kang, 2010). In this way, numerous studies have investigated the antifungal (Tabassum and Vidyasagar, 2013; Bahraminejad *et al.*, 2015), antibacterial (Ahmady Abchin *et al.*, 2012), nematicidal (Pandey *et al.*, 2000; Bahraminejad *et al.*, 2008), pesticidal and insecticidal (Mann and Kaufman, 2012; Yaghout Nejad *et al.*, 2013) action of essential oils and including plant extracts against phytopathogenic fungi.

Medicinal and aromatic plants have always been known as one of the most important sources of natural active compounds such as secondary metabolites (Chobba *et al.*, 2012) so that, utilization of their essential oils has been known as a new means of crop protection due to being effective, biodegradable, and less toxic to the environment (Djordjevic *et al.*, 2013). In general, essential oils are compounds obtained from basic plant metabolism processes especially under different biotic and abiotic stresses (Bajpai and Kang, 2010; Tajkarim *et al.*, 2010). Usually, mono and sesquiterpenes such as phenols, alcohols, ethers, carbohydrates, aldehydes and ketones are the major constituents of essential oils which are responsible for the biological activity as well as for their fragrance (Tabassum and Vidyasagar, 2013).

Essential oils are known to have antifungal, antiviral, antiparasite, antitermite, and insecticidal activities. Their chance to create new resistant strains will be low since their constituents act as synergists (Jobling, 2000). Therefore, essential oils are one of the most important groups of natural compounds producing safe antifungal materials whose antifungal activity has been well documented against a large number of phytopathogenic fungi under *in vitro* conditions (Sridhar *et al.*, 2003; Viuda-Martos *et al.*, 2007). Kohanmoo and Jamali (2013) showed that the essential oil of *E. globules* caused reduction in the growth of *Fusarium oxysporum* by 30.7%. They stated that this property may be due to the existence of secondary components such as eucalyptol and cineole which constitute more than 80% of the

plant essential oil. Also, the antifungal activity of various plant species products against *A. solani* has been reported by several researchers. Examples of such products are the leaf extract of eucalyptus (Mate *et al.*, 2005), essential oil of *Salvia hydrangea* (Kotan *et al.*, 2008) and the extracts of *Hibiscus sabdariffa* and *Majorana syriaca* (Goussous *et al.*, 2010). The fungitoxic effect of *Mentha spicata* essential oil and the antifungal effects of *Mentha piperita* against *Dreschlera spicifera*, *Fusarium oxysporum* and *Macrophomina phaseolina* have been reported by Ramesh *et al.* (2006) and Moghaddam *et al.* (2013), respectively. In a study, the essential oil of *Carum copticum* caused growth inhibition of *A. solani* by 99.5 and 100% at 0.20 and 0.40  $\mu\text{l/ml}$  concentrations, respectively (Babagoli and Behdad, 2012). Also, in a study by Behdad *et al.* (2013), the essential oil of *C. copticum* completely inhibited the growth of *Rhizopus stolonifer* at 10.0, 2.0, 1.0, 0.50 and 0.30  $\mu\text{l/ml}$  concentrations. Generally, the antifungal activity could be attributed to the presence of some components such as cymene, thymol, carvacrol, pinene and linalool (Cimanga *et al.*, 2002).

Iran -as a vast country with wide ranges of climatic and ecological conditions and presence of rich botanical genetic diversity especially in the west of the country- has a great potential for the development of medicinal plant science, production of medicinal drugs and biosynthesis of active natural components in order to control plant pathogens. The *Oliveria decumbens* Vent. is a native medicinal, aromatic and edible plant from Apiaceae family which grows mainly in the Western foothills of Zagros Mountains and is used for treatment of indigestion, diarrhea, fever and abdominal pains in traditional medicine (Noroozi *et al.*, 2008). Most species of Apiaceae family produce terpenes and other types of volatile compounds, also there are various reports on antibacterial effects of this family (Dehghan *et al.*, 2007). Thymol, p-Cymene,  $\gamma$ -terpinene and carvacrol have been reported as the major components of its essential oil (Amin *et al.*, 2005). The antimicrobial activity of *O. decumbens* essential oil has been previously reported (Sajadi and

Hosseini, 2002; Najafpour Navaei and Mirza, 2003; Mahboubi *et al.*, 2008; Hajimehdipoor *et al.*, 2010).

Regarding the effect of origin of the plant species on genetic diversity chemical composition, screening studies for novel antifungal compounds in plants grown in different parts of the world and in Iran are needed. Therefore, regarding the importance of screening plant essential oils as first step of the project and the importance of bioactive essential oils as eco-friendly agents, the aim of the present study as a part of the larger screening program was to assess the *in vitro* antifungal activity of the essential oils obtained from 11 collected medicinal and aromatic plant species with an emphasis on *O. decumbens* in the west of Iran against *Alternaria solani*.

## Materials and Methods

### Plant materials

Eleven plant species (Table 1) belonging to four families were mostly collected from various parts of the provinces of Kermanshah and Hamadan in the west of Iran during 2013-2014. As a part of a wider screening program, plants were randomly collected to increase the chance of finding plants with bioactive essential oils. The plants were identified by the curator of Herbarium at Razi University, College of Agriculture and the scientific names were checked in the International Plant Names Index site (<http://www.ipni.org/ipni/plannamesearchpage.do>). Each sample was cleaned, air dried in the shade and ground in a blender to small pieces with an approximate size of 0.4 mm.

**Table 1** *In vitro* screening for anti-*Alternaria* activity of plant extracts.

| Plant species                             | Family    | Location      | Part used     |
|---|-----------|---------------|---------------|
| <i>Echinophora platyloba</i> DC.          | Apiaceae  | Tuiserkan     | Shoot         |
| <i>Salvia officinalis</i> L.              | Lamiaceae | Kangavar      | Shoot         |
| <i>Teucrium polium</i> L.                 | Lamiaceae | Tuiserkan     | Shoot         |
| <i>Vitex pseudonegundo</i>                | Lamiaceae | Tuiserkan     | Shoot         |
| <i>Carum copticum</i> (L.) C.B. Clarke    | Apiaceae  | Market        | Seed          |
| <i>Foeniculum vulgare</i> Mill.           | Apiaceae  | Market        | Seed          |
| <i>Oliveria decumbens</i> Vent.           | Apiaceae  | Sarpole zahab | Inflorescence |
| <i>Mentha pulegium</i> L.                 | Lamiaceae | Sarpole zahab | Shoot         |
| <i>Thymus kotschyanus</i> Boiss. & Hohen. | Lamiaceae | Sahneh        | Shoot         |
| <i>Cinnamomum zeylanicum</i> Blume.       | Lauraceae | Market        | Bark          |
| <i>Eucalyptus globulus</i> Labill.        | Myrtaceae | Sarpole zahab | Leaf          |

### Essential oil isolation and GC-MS analysis

The essential oils were isolated by hydrodistillation of dried plant material for 6h (50 g of sample in 500 ml of distilled water) using a Clevenger-type apparatus. The oils were centrifuged and upper phase were discarded in order to desiccate the oils. After that, the oils were stored in sealed glass vials covered with parafilm and aluminium foil at 4 °C for later use (Xing-dong and Hua-li, 2014). GC-MS analysis was carried out using a Hewlett-Packard 6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary fused silica column

(30 m, 0.25 mm I.D.; 0.25 µm film thickness). Essential oil solution in hexane was injected and column was temperature programmed as follows: 1 minute at 50 °C; then at 4 °C / min to 123 °C; then at 1 °C / min to 128 °C and at 10 °C / min to 245 °C, respectively. Helium was used as carrier gas at a flow rate of 0.9 ml/ min, and 0.2 µl samples were injected in the split mode. Moreover, the injector temperature and split ratio were 250 °C and 1:80, respectively. All mass spectra were acquired in electron-impact (EI) mode with an ionization voltage of 70 eV and ion source temperature, 150 °C. Oil constituents were identified by comparing

linear retention indices with those of standard compounds and by comparison with literature data and MS data obtained from Wiley and NIST libraries (Sandra and Bicchi, 1987; Adams, 2001). Relative percentage amount were calculated from TIC by the computer.

### Fungus preparation

Pure culture of *A. solani* was obtained from the Plant Pathology Laboratory, Razi University, Kermanshah, Iran. The isolate was collected from diseased tomato plants and was maintained on Potato Dextrose Agar (PDA) at 25 °C in incubator.

### In vitro antifungal assay

The antifungal activity of the isolated essential oils was investigated through agar dilution method based on completely randomized design. According to Tripathi *et al.* (2008), final concentration of 1.0 µl/ml of essential oils was prepared by dissolving 0.1 ml essential oil in 0.5 ml of 0.1% Tween 80 and then mixing with 100 ml of PDA medium. The control was prepared similarly using equal amounts of sterilized distilled water including Tween 80 in place of the oil. The culture media immediately was poured into five Petri dishes, 9 mm diameter, as five replicates. A plug of 5-day-old fungal colony, 6mm in diameter, was placed at the centre of the Petri dishes. The Petri dishes were then wrapped with parafilm along the rim, to prevent the escape of the volatile components, inverted and incubated at 25 °C. The diameter of colony was measured daily until the control plates or one of the treated plates was covered by the mycelium completely. Percentage of inhibition of growth for the fungus was calculated based on following formula:

$$IP = [(C - T)/C] \times 100$$
 IP = percentage of mycelial growth inhibition; C = mean diameter (mm) of the control; T = mean diameter (mm) of tested concentration

Moreover, in a separate experiment, lower concentrations of those essential oils which completely inhibited the mycelial growth of the

fungus (at 1.0 µl/ml concentration) were studied. In order to verify the fungicidal or fungistatic activity of the essential oils, the inhibited plugs were subcultured in a new medium without essential oil.

### Statistical analysis

Significant differences between treatments were determined by Duncan's multiple range test at  $P \leq 0.01$ , following one-way ANOVA.

### Results

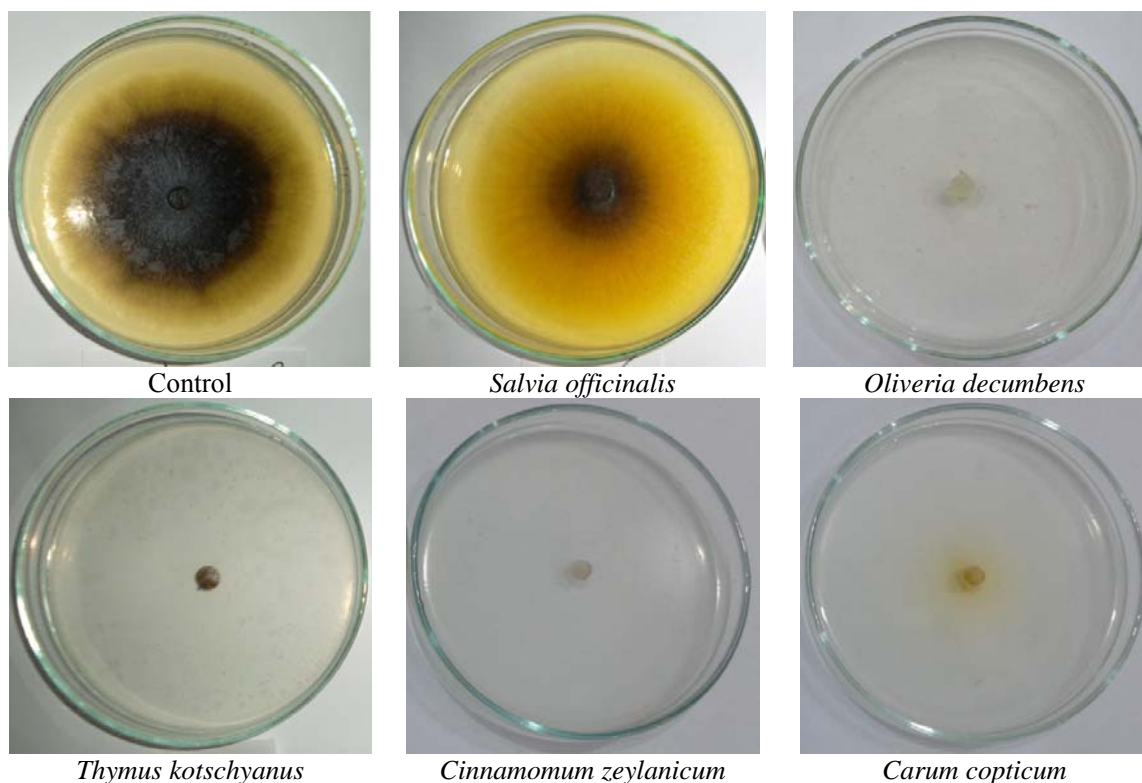
#### Antifungal assay

Seven out of the 11 screened plant species measurably reduced and four of them stimulated the mycelial growth of the fungus, respectively. Therefore, the ANOVA was only performed for these seven species and the results indicated that there was a high significant difference ( $P \leq 0.01$ ) among the activity of the essential oils at 1.0 µl/ml concentration for mycelial growth inhibition (Table 2). Maximum inhibitory effect (100% inhibition) was recorded for the essential oils of *Carum copticum*, *Oliveria decumbens*, *Thymus kotschyanus* and *Cinnamomum zeylanicum* (Fig. 1 and Table 3). The essential oils of *Foeniculum vulgare* and *Mentha pulegium* by 53.81 and 41.94% without significant difference were at the next ranking. *Eucalyptus globules* essential oil showed the lowest inhibitory effect (4%) on the growth of *A. solani*. Whereas, the most stimulating essential oil was *Salvia officinalis* with 9.38% stimulatory effect (Fig. 1).

**Table 2** Analysis of variance for growth inhibition percentage of *Alternaria solani* by seven plant essential oils at 1.0 µl/ml.

| Source of variation               | df | Mean Squares |
|-----------------------------------|----|--------------|
| Essential oil of different plants | 6  | 7493.21**    |
| Error                             | 28 | 132.71       |
| Total                             | 34 |              |
| C. V.%                            | -  | 16.14        |

: ANOVA was done only for the plants that showed inhibitory effect (seven out of the 11 studied species), \*\*: Significantly different at  $p \leq 0.01$ .



**Figure 1** The stimulatory (*Salvia officinalis*) and inhibitory (*Oliveria decumbens*, *Thymus kotschyanus*, *Cinnamomum zeylanicum* and *Carum copticum*) effect of studied essential oils against *Alternaria solani* at 1.0  $\mu\text{l/ml}$  concentrations on potato dextrose agar medium.

**Table 3** Comparison of antifungal effect of the plants essential oils that showed inhibitory effect against *Alternaria solani* at 1.0  $\mu\text{l/ml}$ .

| Plant species                             | Inhibition (%)                 |
|---|--------------------------------|
| <i>Carum copticum</i> (L.) C. B. Clarke   | 100 <sup>a</sup>               |
| <i>Foeniculum vulgare</i> Mill.           | 53.81 $\pm$ 8.27 <sup>b</sup>  |
| <i>Oliveria decumbens</i> Vent.           | 100 <sup>a</sup>               |
| <i>Mentha pulegium</i> L.                 | 41.94 $\pm$ 10.86 <sup>b</sup> |
| <i>Thymus kotschyanus</i> Boiss. & Hohen. | 100 <sup>a</sup>               |
| <i>Cinnamomum zeylanicum</i> Blume.       | 100 <sup>a</sup>               |
| <i>Eucalyptus globulus</i> Labill.        | 4.00 $\pm$ 0.78 <sup>c</sup>   |

<sup>a, b, c</sup>: Percentage of inhibition  $\pm$  standard error; means followed by the same letter are not significantly different (Duncan's multiple range test,  $P < 0.01$ ).

Regarding to 100% inhibition of essential oils of *C. copticum*, *O. decumbens*, *T. kotschyanus* and *C. zeylanicum* against *A. solani*, the fungicidal or fungistatic activity of these plant essential oils was tested. Results of this experiment showed that the observed activity in *C. zeylanicum* and *T. kotschyanus* was fungicidal but the activity in *O. decumbens* and *C. copticum* was fungistatic as mycelial growth was observed when the inhibited plug was cultured in a new medium without essential oil.

In a separate experiment the antifungal activity of lower concentrations of *C. copticum*, *O. decumbens*, *T. kotschyanus* and *C. zeylanicum* was studied. The results of ANOVA for different concentrations of these species showed a high significant difference between tested concentrations of each species

with respect to anti-*Alternaria* activity (Tables 4 and 5). Besides, results of the mean comparisons indicated that the essential oil of *O. decumbens* at 0.50 and 0.25  $\mu\text{l/ml}$  concentrations (Fig. 2 and 3) and the essential oil of *C. zeylanicum* at 0.50  $\mu\text{l/ml}$  concentration (Fig. 4) completely suppressed the growth of the fungus.

**Table 4** Analysis of variance for growth inhibition percentage of *Alternaria solani* by different essential oil concentrations of *Carum copticum*, *Cinnamomum zeylanicum* and *Thymus kotschyanus*.

| Source of variation               | df | Mean Squares       |                      |                       |
|-----------------------------------|----|--------------------|----------------------|-----------------------|
|                                   |    | <i>C. copticum</i> | <i>C. zeylanicum</i> | <i>T. kotschyanus</i> |
| Essential oil of different plants | 3  | 7648.08**          | 3399.54**            | 8687.99**             |
| Error                             | 12 | 103.71             | 54.49                | 15.46                 |
| Total                             | 15 |                    |                      |                       |
| C. V. %                           | -  | 22.26              | 8.88                 | 11.99                 |

\*\* : Significantly different at  $p \leq 0.01$ .

**Table 5** Analysis of variance for growth inhibition percentage of *Alternaria solani* by different essential oil concentrations of *Oliveria decumbens*.

| Source of variation                       | df | Mean Squares |
|---|----|--------------|
| Different concentrations of essential oil | 5  | 7626.09**    |
| Error                                     | 24 | 23.56        |
| Total                                     | 29 |              |
| C. V. %                                   | -  | 6.76         |

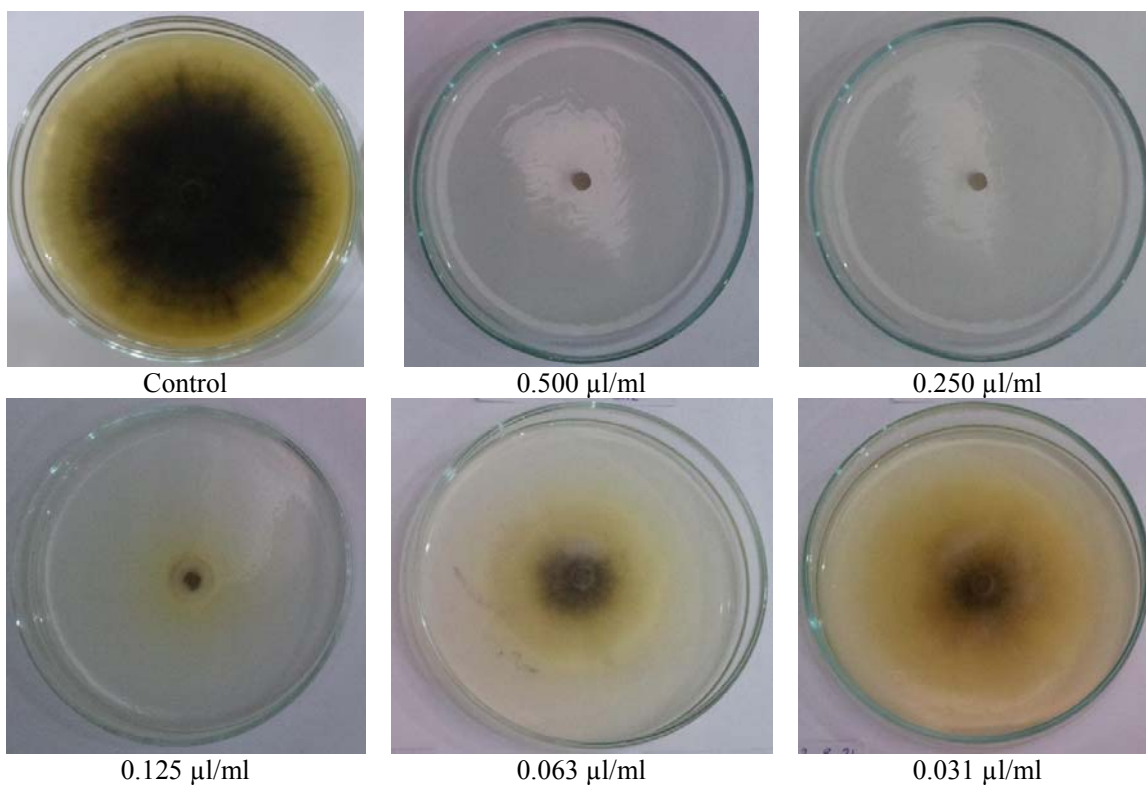
\*\* : Significantly different at the level of  $p \leq 0.01$ .

The essential oils of *C. copticum* completely inhibited the growth of the fungus by fungistatic effect only at 1.0  $\mu\text{l/ml}$  concentration, whereas its inhibition effect was significantly reduced at lower concentrations (Fig. 5). The 1.0 and 0.50  $\mu\text{l/ml}$  concentrations of *C. zeylanicum* essential oil caused complete inhibition of *A.*

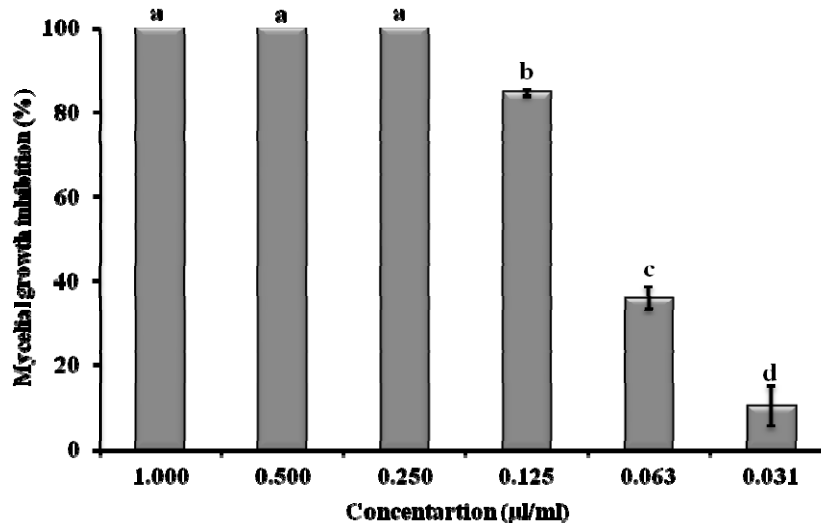
*solani* growth by fungicidal and fungistatic effects, respectively. Moreover, the essential oil of these species indicated 92.91 and 39.72% inhibitions at 0.25 and 0.125  $\mu\text{l/ml}$  concentrations, respectively (Fig. 4). Similar to *C. copticum*, the essential oil of *T. kotschyanus* completely inhibited the growth of the fungus only at 1.0  $\mu\text{l/ml}$  concentration but by fungicidal effect. It inhibited the fungus by 28.38% at 0.50  $\mu\text{l/ml}$  concentration, but it did not have considerable effect on the fungus at 0.25 and 0.125  $\mu\text{l/ml}$  concentrations (Fig. 6). The essential oil of *O. decumbens* had completely inhibitory effect against the fungus at 1.0, 0.50 and 0.25  $\mu\text{l/ml}$  concentrations by fungistatic effects. This oil reduced the growth of the fungus by 84.61, 35.90 and 10.26% at 0.125, 0.0625 and 0.03125  $\mu\text{l/ml}$  concentrations, respectively (Fig. 3). It is concluded that essential oil of *O. decumbens* was the strongest against *A. solani* even at lower concentrations. Therefore, GC-MS analysis was only performed for this essential oil.

### Essential oil compositions

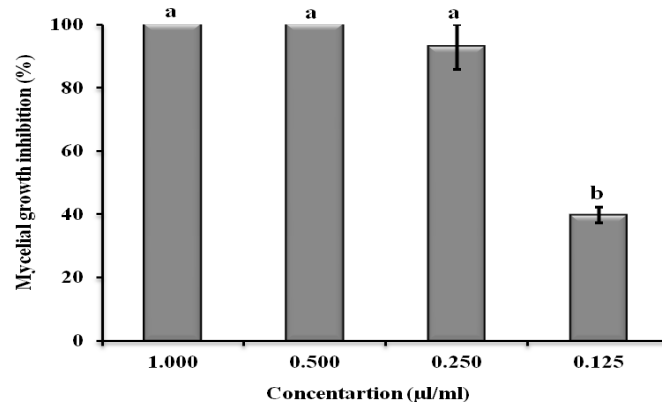
The major constituents of the essential oil in *O. decumbens* are shown in Table 6. A total of 46 components were identified and the major components were carvacrol (46.53%), thymol (23.75%), p-Cymene (13.70%),  $\gamma$ -terpinene (7.66%) and myristicin (3.65%), respectively. Some other compounds were  $\alpha$ -pinene (0.75%),  $\beta$ -pinene (0.71%), caryophyllene oxide (0.63%), trans- $\beta$ -Ocimene (0.33%), di-tert-Butylphenol (0.32%), pyrrolo carbazole (0.21%),  $\alpha$ -Thujene (0.17%) and elemicin (0.16%).



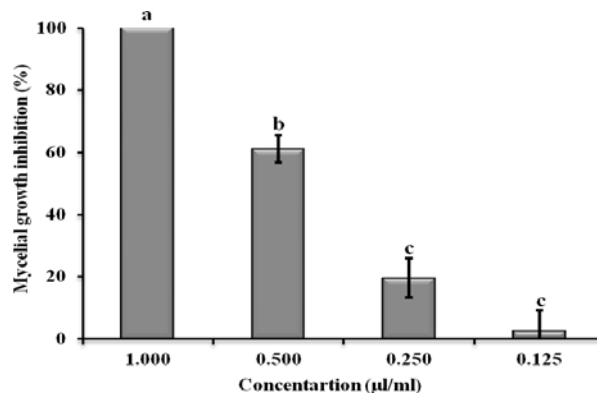
**Figure 2** The inhibitory effect of *Oliveria decumbens* essential oil against *Alternaria solani* in lower concentrations.



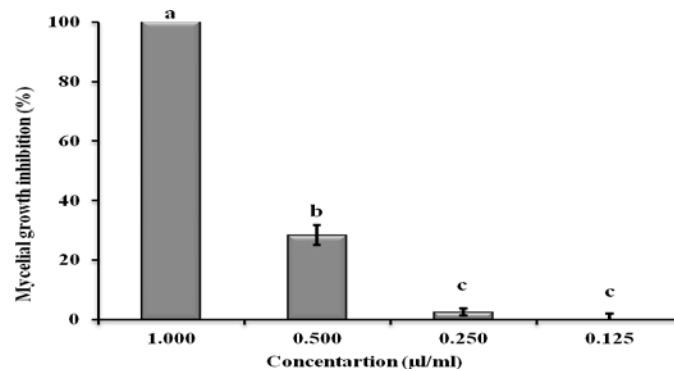
**Figure 3** Mean comparison of different concentrations of *Oliveria decumbens* essential oil on mycelial growth of *Alternaria solani*. Means followed by the same letter are not significantly different (Duncan's multiple range test,  $P < 0.01$ ).



**Figure 4** Mean comparison of different concentrations of *Cinnamomum zeylanicum* essential oil on mycelial growth of *Alternaria solani*. Means followed by the same letter are not significantly different (Duncan's multiple range test,  $P < 0.01$ ).



**Figure 5** Mean comparison of different concentrations of *Carum copticum* essential oil on mycelial growth of *Alternaria solani*. Means followed by the same letter are not significantly different (Duncan's multiple range test,  $P < 0.01$ ).



**Figure 6** Mean comparison of different concentrations of *Thymus kotschyanus* essential oil on mycelial growth of *Alternaria solani*. Means followed by the same letter are not significantly different (Duncan's multiple range test,  $P < 0.01$ ).



**Table 6** Chemical composition identified by the GC-MS analysis of *Oliveria decumbens* essential oil.

| SN | Compound                   | Retention time (min) | Content (%) |
|----|----------------------------|----------------------|-------------|
| 1  | Alpha-Thujene              | 6.37                 | 0.171       |
| 2  | Alpha-Pinene               | 6.56                 | 0.754       |
| 3  | Camphene                   | 6.98                 | 0.012       |
| 4  | Sabinene                   | 7.70                 | 0.079       |
| 5  | Beta-Pinene                | 7.80                 | 0.710       |
| 6  | 1-Octen-3-ol               | 8.02                 | 0.023       |
| 7  | Beta-Myrcene               | 8.22                 | 0.292       |
| 8  | l-Phellandrene             | 8.65                 | 0.026       |
| 9  | Delta 3-Carene             | 8.81                 | 0.023       |
| 10 | Alpha-Terpinene            | 9.04                 | 0.075       |
| 11 | p-Cymene                   | 9.42                 | 13.699      |
| 12 | Trans-Beta-Ocimene         | 9.73                 | 0.332       |
| 13 | Gamma-Terpinene            | 10.50                | 7.659       |
| 14 | Alpha-Terpinolene          | 11.41                | 0.068       |
| 15 | Linalool                   | 11.95                | 0.067       |
| 16 | Gaultheria oil             | 15.21                | 0.021       |
| 17 | Alpha Terpineol            | 15.34                | 0.067       |
| 18 | Thymol methyl ether        | 16.30                | 0.021       |
| 19 | Trans-Anethole             | 18.42                | 0.048       |
| 20 | Thymol                     | 18.99                | 23.752      |
| 21 | Carvacrol                  | 19.34                | 46.531      |
| 22 | Trans Caryophyllene        | 23.29                | 0.087       |
| 23 | Nerolidol                  | 23.78                | 0.006       |
| 24 | Alloaromadendrene          | 23.92                | 0.006       |
| 25 | Trans-Beta-Farnesene       | 24.03                | 0.017       |
| 26 | Jsoascaridole              | 24.98                | 0.008       |
| 27 | Alpha-Curcumen             | 25.14                | 0.011       |
| 28 | Ledene                     | 25.42                | 0.008       |
| 29 | Myristicin                 | 26.17                | 3.645       |
| 30 | Elemicin                   | 26.77                | 0.165       |
| 31 | Cis-Asarone                | 26.88                | 0.047       |
| 32 | Isoseychellene             | 27.03                | 0.032       |
| 33 | Caryophyllene oxide        | 27.28                | 0.629       |
| 34 | Ledol                      | 27.67                | 0.050       |
| 35 | Isospathulenol             | 28.25                | 0.053       |
| 36 | Alpha-Elemene              | 28.51                | 0.034       |
| 37 | Diepi-Alpha-Cedreneopoxide | 28.65                | 0.005       |
| 38 | Beta-Bisabolene            | 28.91                | 0.012       |
| 39 | Isoaromadendrene Epoxide   | 29.54                | 0.022       |
| 40 | Geranyl Linalool Isomer    | 30.14                | 0.013       |
| 41 | Methoxymesitylene          | 31.63                | 0.052       |
| 42 | Cinerson                   | 31.86                | 0.011       |
| 43 | Di-Tert-Butylphenol        | 32.17                | 0.322       |
| 44 | Carveol                    | 32.47                | 0.086       |
| 45 | Pyrrolo Carbazole          | 32.59                | 0.206       |
| 46 | Eicosane                   | 33.26                | 0.045       |
|    | Total                      | -                    | 100         |

## Discussion

In the present study, the essential oil of *O. decumbens* completely inhibited the growth of *A. solani* by fungistatic effect at 1.0, 0.50 and 0.25  $\mu\text{l/ml}$  concentrations. Moreover, it reduced the growth of the fungus by 84.61, 35.90 and 10.26% at 0.125, 0.0625 and 0.03125  $\mu\text{l/ml}$  concentrations, respectively. Therefore, the 0.125  $\mu\text{l/ml}$  can be introduced as the minimum inhibitory concentration (more than 50% inhibition) for control of *A. solani*. The high antimicrobial activity of the essential oil of *O. decumbens* grown in the west of Iran has been reported against *Escherichia coli*, *Aspergillus niger* and *Candida albicans* (Mahboubi et al., 2008). In this study, the major constituents of the essential oil in *O. decumbens* were carvacrol, thymol, p-cymene,  $\gamma$ -terpinene and myristicin, respectively. Ten main compounds have been found by Hajimehdipoor et al. (2010) in this essential oil of which  $\gamma$ -terpinene, myristicin, thymol, cymene and carvacrol were the major ones. The composition and or the ratio of each of these compounds in *O. decumbens* essential oil can be affected by the growing region (Hajimehdipoor et al., 2010). Sajadi and Hosseini (2002) introduced most of these compounds as a major secondary compounds in *O. decumbens* flower essential oil as follows: thymol, carvacrol, p-Cymene and  $\gamma$ -terpinene. Mahboubi et al. (2008) reported that the major constituents of the essential oil of *O. decumbens* shoots were thymol (26.9%), p-Cymene (13.3%),  $\gamma$ -terpinene (11%). The yield of essential oil extracted from *O. decumbens* flowers in Kermanshah has been reported to be about 0.1% with 15.11% thymol, 29.7% carvacrol, 15.4% p-Cymene and 20.46%  $\gamma$ -terpinene (Najafpour Navaei and Mirzam, 2003) which our results are in accordance with theirs.

Results of this study indicated that the *C. zeylanicum* essential oil completely inhibited the growth of *A. solani* at 1.0 and 0.50  $\mu\text{l/ml}$  concentrations. Also, it was fungicidal and fungistatic at 1.0 and 0.50  $\mu\text{l/ml}$  concentrations, respectively. The 0.25  $\mu\text{l/ml}$  concentration of

this essential oil was introduced as the minimum inhibitory concentration (more than 50% inhibition) in the control of *A. solani*. *C. zeylanicum* is an evergreen tree 5-7 meters tall with aromatic smell in all parts. The inhibitory effect of its essential oil against phytopathogenic fungi has been formerly reported (Trajano *et al.*, 2012). Chemical composition of *C. zeylanicum* bark essential oil has shown that cinnamaldehyde by 60.41% followed by linalool, ortho-methoxy cinnamic aldehyde,  $\beta$ -caryophyllene, cineol and eugenol are the major compounds (Ojagh *et al.*, 2012).

The essential oil of *C. copticum* in this study completely inhibited the growth of the fungus by fungistatic effect only at 1.0  $\mu$ l/ml concentration, whereas its inhibitory effect was significantly reduced at lower concentrations. *C. copticum* belongs to Apiaceae family. Its seed contains 2-3% yellowish essential oil named "Ajowan". The major compound of this oil constitutes thymol (40 to 50%) followed by tryphenen, cymene, pinene and carvacrol (Ballba *et al.*, 1973). In this study, the inhibitory effect of *C. copticum* essential oil was reduced by reduction of its concentration; nonetheless it had more than 58% inhibitory effect against *A. solani* at 0.50  $\mu$ l/ml concentration. Rezaei Kakhkha *et al.* (2014) reported that *C. copticum* essential oil has high potential in the growth inhibition of *Asergillus parasiticus* and production of aflatoxin. Moreover, the highest inhibitory effect (100%) was obtained at 1000  $\mu$ g/ml concentration. They ascribed this effect to thymol. Thymol is known as very strong antifungal compound (Mahmoud, 1994). The GC-MS analysis of *C. copticum* essential oil indicated that thymol (71.07%), terpinolene (13.08%) and ortho-cymene (10.20%) are the most important compounds in this species (Behravan *et al.*, 2007).

The essential oil of *T. kotschyanus* completely inhibited the growth of the fungus only at 1.0  $\mu$ l/ml concentration. It inhibited the fungus by 28.38% at 0.50  $\mu$ l/ml concentration, but its inhibitory effect was not considerable at 0.25 and 0.125  $\mu$ l/ml concentrations. The species of *Thymus* from Lamiaceae family have

medicinal properties which are mainly used as seasoning in foods, disinfecting agent and antimicrobial agent. *T. kotschyanus* grows wildly in southern parts of "Alborz" mountains and also in northern and northwestern parts of Iran (Habibi *et al.*, 2007). The GC-MS analysis of *T. kotschyanus* essential oil showed that thymol (23-29%), carvacrol (10-27%), linalool (45-47.5%), terpinen (39.4%) and pinene (7-36%) were the major compounds (Habibi *et al.*, 2007).

As Chitwood (2002) stated, the results of such research could help develop new natural fungicides and chemically synthesized derivatives. These results will also help find out the active metabolites in plants and subsequently use them in reverse genetic engineering from metabolites to genes.

## Conclusion

The findings of this study demonstrated existence of anti-*Alternaria* compounds in *C. copticum*, *O. decumbens*, *T. kotschyanus* and *C. zeylanicum* which suggest their potential as a natural fungicides in the control of early blight disease caused by *A. solani*. Moreover, the essential oil of *O. decumbens* with the highest anti-*Alternaria* activity could be selected for further studies on *in vivo* application.

## Acknowledgements

This research was financially supported by Razi University of Kermanshah under Project No. 136370. The authors would like to thank Dr. Saeed Abbasi for providing fungal isolates and valuable comments and Dr. Sohrab Ghousivand for his assistance in GC-MS analysis.

## References

- Adams, R. P. 2001. *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. Allured Publishing Corporation, Carol Stream, IL, USA. pp, 63-344.

- Ahmady Abchin, S., Nasrolahi Omran, A., Jafari, N., Mostafapour, M. J. and Kia, S. M. 2012. Antibacterial effects of *Lavandula Stoechas* essential oil, on gram positive and negative bacteria *in vitro*. Medical Laboratory Journal, 6 (2): 34-41.
- Amin, G., Salehi Sourmaghi, M. H., Zahedi, M., Khanavi, M. and Samadi, N. 2005. Essential oil composition and antimicrobial activity of *Oliveria decumbens*. Fitoterapia, 76 (7-8): 704-707.
- Babagoli, M. A. and Behdad, E. 2012. Effects of three essential oils on the growth of the fungus *Alternaria solani*. Journal of Research in Agricultural Science, 8 (1): 45-57.
- Bahraminejad, S., Amiri, R. and Abbasi, S. 2015. Anti-fungal properties of 43 plant species against *Alternaria solani* and *Botrytis cinerea*. Archives of Phytopathology and Plant Protection, 48: 336-344.
- Bahraminejad, S., Asenstorfer, R. E., Williams, K. J., Hayasaka, Y., Zwer, P. K., Riley, I. T. and Schultz, C. J. 2008. Metabolites correlated with cereal cyst nematode resistance in oats (*Avena sativa*) identified using single seed descent lines. Nematologia Mediterranea, 36: 145-152.
- Bajpai, V. K., Kang, S. C. 2010. Antifungal activity of leaf essential oil and extracts of *Metasequoia glyptostroboides* Miki ex Hu. Journal of the American Oil Chemists' Society, 87: 327-336.
- Ballba, S. I., Hilal, S. H. and Haggag, M. Y. 1973. The volatile oil from the herb and fruits of *Carum copticum* at different stages of growth. Planta Medica, 23: 312-320.
- Behdad, M., Etemadi, N. A., Behdad, E. and Zeinali, H. 2013. Antifungal effects of three plant essential oils against *Rhizopus stolonifer*, the cause of soft rot on strawberry fruit. Iranian Journal of Medicinal and Aromatic Plants, 29: 399-411.
- Behravan, J., Ramezani, M., Hassanzadeh, M.K. and Ebadi, S. 2007. Evaluation of antibacterial activity of the essential oils of *Zataria multiflora*, *Carum copticum* and *Thymus vulgaris* by a thin layer chromatography-bioautography method. Journal of Essential Oil Bearing Plants, 10: 259-264.
- Chitwood, D. J. 2002. Phytochemical based strategies for nematode control. Annual Review of Phytopathology, 40: 221-249.
- Chobba, I. B., Bekir, A., Mansour, R. B., Drira, N., Gharsallah, N. and Kadri, A. 2012. *In vitro* evaluation of antimicrobial and cytotoxic activities of *Rosmarinus officinalis* L. (Lamiaceae) essential oil cultivated from South-West Tunisia. Journal of Applied Pharmaceutical Science, 2 (11): 034-039.
- Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T. and Hermans, N. 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of Ethnopharmacology, 79: 213-220.
- Dehghan, Gh., Solaimanian, R., Shahverdi, A., Amin, Gh., Abdollahi, M. and Shafiee, A. 2007. Chemical composition and antimicrobial activity of essential oil of *Ferula szovitsiana* D.C. Flavour and Fragrance Journal, 22: 224-227.
- Djordjevic, M., Djordjevic, O., Djordjevic, R., Mijatovic, M., Kostic, M., Todorovic, G. and Ivanovic, M. 2013. Alternative approach in control of tomato pathogen by using essential oils *in vitro*. Pakistan Journal of Botany, 45(3): 1069-1072.
- Goussous, S. J., Abu el-Samen, F. M. and Tahhan, R. A. 2010. Antifungal activity of several medicinal plants extracts against the early blight pathogen (*Alternaria solani*). Archives of Phytopathology and Plant Protection, 43: 1745-1757.
- Habibi, H., Mazaheri, D., Majnoon Hosseini, N., Chaechi, M. R., Fakhr-Tabatabaee, M. and Bigdeli, M. 2007. Effect of altitude on essential oil and components in wild thyme (*Thymus kotschyanus* Boiss) Taleghan region. Pajouhesh & Sazandegi, 73: 2-10.
- Hajimehdipoor, H., Samadi, N., Mozaffarian, V., Rahimifard, N., Shoeibi, Sh. and Piralı Hamedani, M. 2010. Chemical composition and antimicrobial activity of *Oliveria*

- decumbens* volatile oil from West of Iran. Journal of Medicinal Plants, 9 (6): 39-44.
- Jobling, J. 2000. Essential oils: A new idea for postharvest disease control. Good Fruit and Vegetables Magazine, 11 (3): 50-54.
- Kohanmoo, M. A. and Jamali, F. 2013. Antifungal activities of some medicinal plant essential oils against *Fusarium oxysporum* f. sp. *Lycopersici*. Biological Control of Pests and Plant Diseases, 2: 27-33.
- Kotan, R., Kordali, S., Cakir, A., Kesdek, M., Kaya, Y. and Kilic, H. 2008. Antimicrobial and insecticidal activities of essential oil isolated from Turkish *Salvia hydrangea* DC. ex. Biochemical Systematics and Ecology, 36: 360-368.
- Mahboubi, M., Feizabadi, M. M., Haghi, G. and Hosseini, H. 2008. Antimicrobial activity and chemical composition of essential oil from *Oliveria decumbens* Vent. Iranian Journal of Medicinal and Aromatic Plants, 24: 56-65.
- Mahmoud, A. L. 1994. Antifungal action and antiaflatoxicogenic properties of some essential oil constituents. Letters in Applied Microbiology, 2: 110-113.
- Mann, S. R. and Kaufman, E. P. 2012. Natural product pesticides: their development, delivery and use against insect vectors. Mini-Review in Organic Chemistry, 9: 185-202.
- Mate, G. D., Deshmukh, V. V., Jiotode, D. J., Chore, N. S. and Dikkar, M. 2005. Efficacy of plant products and fungicides on tomato early blight caused by *Alternaria solani*. Research on Crops, 6: 349-351.
- Moghaddam, M., Pourbaige, M., Kourosh Tabar, H., Farhadi, N. and Ahmadi Hosseini, S. M. 2013. Composition and antifungal activity of peppermint (*Mentha piperita*) essential oil from Iran. Journal of Essential Oil Bearing Plants, 16: 506-512.
- Najafpour Navaei, M. and Mirza, M. 2003. Essential oil of *Oliveria decumbens* Vent. Iranian Journal of Medicinal and Aromatic Plants, 15: 23-31.
- Noroozi, J., Akhiani, H. and Breckle, S-W. 2008. Biodiversity and phytogeography of the alpine flora of Iran. Biodiversity and Conservation, 17: 493-521.
- Ojagh, S. M., Rezaei, M., Razavi, S. H. and Hosseini, S. M. H. 2012. Investigation of antibacterial activity cinnamon bark essential oil (*Cinnamomum zeylanicum*) *in vitro* antibacterial activity against five food spoilage bacteria. Iranian journal of Food Science and Technology, 35 (9): 67-76.
- Pandey, R., Kalra, A., Tandon, S., Mehrotra, N., Singh, H. N. and Kumar, S. 2000. Essential oils as potent sources of nematicidal compounds. Journal of Phytopathology, 148: 501-502.
- Ramesh, S. Y., Sandeep, K. and Anupam, D. 2006. Antifungal properties of essential oil *Mentha spicata* L. var. MSS-5. Indian Journal of Crop Science, 1: 197-200.
- Rezaei Kahkha, M. R., Amanloo, S. and Kaykhaiim, M. 2014. Antiaflatoxicogenic activity of *Carum copticum* essential oil. Environmental Chemistry Letters, 12: 231-234.
- Sajadi, S. E. and Hosseini, S. A. 2002. Essential oil constituents of *Oliveria decumbens* Vent. Journal of Essential Oil Research, 14: 220-221.
- Sandra, P. and Bicchi, C. 1987. Capillary Gas Chromatography in Essential Oil Analysis. Alfred Huethig-Verlag: Heidelberg, pp: 259-274.
- Savary, S., Teng, P. S., Willocquet, L. and Nutter, F. W. 2006. Quantification and modeling of crop losses: a review of purposes. Annual Review of Phytopathology, 44: 89-112.
- Shahbazi, H., Aminian, H., Sahebani, N. and Lak, M. R. 2011. Evaluation of resistance of potato cultivars to isolates of *Alternaria solani* the causal agent of early blight and study of totalphenol contents in resistance and susceptible cultivars. Seed and Plant Improvement Journal, 27-1: 1-14.
- Sridhar, S. R., Rajagopal, R. V., Rajavel, R., Masilamani, S. and Narasimhan, S. 2003. Antifungal activity of some essential oils. Journal of Agricultural and Food Chemistry, 51: 7596-7599.

- Tabassum, N. and Vidyasagar, G. M. 2013. Antifungal investigations on plant essential oils: a review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5 (2): 19-28.
- Tajkarim, M. M., Ibrahim, S. A. and Cliver, D. O. 2010. Antimicrobial herb and spice compounds in food. *Food Control*, 21: 1199-1218.
- Trajano, V. N., Lima, E. O. and De Souza, F. S. 2012. Antifungal activity of the essential oil of *Cinnamomum zeylanicum* Blume and eugenol on *Aspergillus flavus*. *Journal of Essential Oil Bearing Plants*, 15 (5): 785-793.
- Tripathi, P., Dubey, N. K. and Shukla, A. K. 2008. Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*. *World Journal of Microbiology and Biotechnology*, 24: 39-46.
- Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J. and PérezÁlvarez, J. A. 2007. Antifungal activities of thyme, clove and oregano essential oils. *Journal of Food Safety*, 27: 91-101.
- Xing-dong, L. and Hua-li, X. 2014. Antifungal activity of the essential oil of *Zanthoxylum bungeanum* and its major constituent on *Fusarium sulphureum* and dry rot of potato tubers. *Phytoparasitica*, 42: 509-517.
- Yaghout Nejad, F., Radjabi, R. and Palvaneh, N. 2013. A review on evaluation of plant essential oils against pests in Iran. *Persian Gulf Crop Protection*, 2 (4): 74-97.
- Yazici, S., Yanar, Y. and Karaman, I. 2011. Evaluation of bacteria for biological control of early blight disease of tomato. *African Journal of Biotechnology*, 10: 1573-1577.

اثرات ضدقارچی اسانس برخی گونه‌های گیاهی دارویی و معطر علیه قارچ *Alternaria solani*

صحبت بهرامی‌نژاد\*، بهناز سیف‌اله‌پور و رضا امیری

پردیس کشاورزی و منابع طبیعی، دانشگاه رازی، کرمانشاه، ایران.

\* پست الکترونیکی نویسنده مسئول مکاتبه: sohbah72@hotmail.com

دریافت: ۱۰ خرداد ۱۳۹۵؛ پذیرش: ۱۸ آبان ۱۳۹۵

چکیده: در این مطالعه اثرات ضدقارچی اسانس ۱۱ گونه گیاه دارویی و معطر از چهار تیره جمع‌آوری شده از غرب ایران علیه قارچ *Alternaria solani* به روش اختلاط اسانس با محیط کشت با پنج تکرار و در غلظت یک میکرولیتر بر میلی‌لیتر بررسی شد. براساس نتایج، بیش‌ترین اثر بازدارندگی مربوط به اسانس لعل کوهستان *Oliveria decumbens*، دارچین *Cinnamomum zeylanicum*، زنیان *Carum copticum* و آویشن کوهی *Thymus kotschyanus* بود که به‌طور کامل مانع رشد قارچ مورد مطالعه شدند. بنابراین اثرات غلظت‌های پایین‌تر اسانس این گونه‌ها نیز بررسی شد. اسانس لعل کوهستان در غلظت‌های ۰/۵ و ۰/۲۵ میکرولیتر بر میلی‌لیتر نیز به‌طور کامل از رشد میسلیمی قارچ جلوگیری کرد. ترکیبات اصلی اسانس این گونه به‌ترتیب عبارت بودند از carvacrol (۴۶/۵۳٪)، thymol (۲۳/۷۵٪)، p-Cymene (۱۳/۷۰٪)،  $\gamma$ -terpinene (۷/۶۶٪) و myristicin (۳/۶۵٪). بنابراین، اسانس این گونه می‌تواند با دارا بودن فعالیت ضد آلترناریایی بالا، جهت مطالعات بیش‌تر از جمله انجام آزمایشات *in vivo* و هم‌چنین به‌عنوان قارچ‌کش طبیعی انتخاب شود.

واژگان کلیدی: *Alternaria solani*، اسانس، فعالیت قارچ‌کشی، تجزیه GC-MS، *Oliveria decumbens*