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Chemical Composition and Antimicrobial Activity of *Origanum vulgare* subsp. *viride* Essential Oils Cultivated in Two Different Regions of Iran

Leila Mehdizadeh ¹, Hossein Mirzaei Najafgholi ², Rostam Yazdani Biouki ³, Mohammad Moghaddam ¹*

¹ Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
² Department of Plant Protection, College of Agriculture, Lorestan University, Lorestan, Iran
³ National Salinity Research Center, Agricultural Research, Education and Extension Organization (AREEO), Yazd, Iran

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**Abstract:** The objective of this research was to examine the chemical compounds and antimicrobial activity of domesticated *Origanum vulgare* subsp. *viride* essential oils from Mashhad and Yazd, Iran. GC and GC-MS analysis revealed that the essential oils of Mashhad and Yazd samples predominantly contain thymol (20.97-21.6 %), 4-terpineol (16.27-15.85 %), γ-terpinene (7.62-5.46 %), trans-sabinene hydrate (6.11-7.91 %) and p-cymene (4.27-5.69 %). The variations observed between the essential oil compositions were mostly quantitative but also qualitative. The inhibitory effects of the essential oils were tested against 10 phytopathogenic microorganisms, including *Xanthomonas citri*, *Ralstonia solanacearum*, *Erwinia amylovora*, *Pectobacterium carotovorum*, *Xanthomonas oryzae* bacteria and *Bipolaris sorokiniana*, *Fusarium oxysporum*, *Drechslera oryzae*, *Alternaria alternata* and *Stemphylium saraciniforme* fungi. The antimicrobial test results indicated that the essential oils had antibacterial and antifungal activities against all of the tested microorganisms. According to the width of the inhibition zone, *X. citri* was the most sensitive bacterium to the essential oils with MIC (3.5 μg/mL) and MBC (5 μg/mL) in both samples. The most sensitive fungus was *D. oryzae* with 1 and 0.8 μg/mL in Mashhad and Yazd essential oil samples. Also the most susceptible fungi with MIC 2.1 μg/mL were *B. sorokiniana* in essential oil obtained from Mashhad and *S. saraciniforme* with MIC 2.2 μg/mL in the essential oil of Yazd’s sample. In conclusion, due to the antimicrobial activities of *O. vulgare* subsp. *viride* essential oil, the effectiveness of essential oils against serious plant pathogens is considerable. In this way, the essential oil has a good capacity as an alternative to synthetic products in various industries.

**Key words:** *Origanum vulgare* subsp. *viride*, cultivation region, essential oil, biological activity.

**Introduction**

The *Origanum* genus (from Lamiaceae family) is characterized by a wide range of volatile secondary metabolites which are widely used in food, pharmaceutical and cosmetic industries as a culinary herb, flavoring matters of beverages and food products, preserve food, as well as in perfumery because of their spicy fragrance. *Origanum* species are traditionally used as sedative, diuretic, sweeter and antiseptic, also in the treatment of gastrointestinal diseases, menstrual problems, urinary tract disorders, respiratory disor-
oders, and rheumatoid arthritis. Due to the variation in genetic forms, the *Origanum* genus chemo-taxonomy is not known completely. Diversity for genetic, chemical and morphological parameters of oregano was found before. *Origanum* species are widely distributed throughout the Mediterranean area as a wild or cultivated plant which is grown on stony slopes at a wide range of altitudes. The previous work reported six subspecies of *O. vulgare* namely; hirtum, vulgare, gracile, virens, glandulosum and viride. Among 42 species known of *Origanum* genus, only *Origanum vulgare* L. (oregano) species and three subspecies (subsp. viride, subsp. vulgare and subsp. gracile) found in Iran, on the basis of the morphological and the intra specific variability. *Origanum vulgare* subsp. viride has wide distribution in North and Northwestern parts of Iran, but not in warm southern area.

The chemical composition and biological activities of *Origanum* species were investigated previously. The influence of genetic, environmental and agronomical factors on the yield and composition of their essential oils has been extensively described. Furthermore, in previous study, antifungal and antibacterial activities of the essential oil of oregano have been shown, which raised great pharmaceutical and industrial interest in oregano.

Essential oils, as a plant secondary metabolism, have various applications in pharmaceutical, chemical, cosmetic, hygienic, and food industries. Pathogenic microorganisms are one of the most important economic problems of crops and food productions. Due to the high level toxic residues by applying synthetic fungicides or bactericides, for the control of plant diseases, the exploitation of natural substances such as the essential oil which is safer for consumers and the environment than synthetic materials, is urgently needed.

*O. vulgare* subsp. *viride* is a wild plant that observed in Yazd province as a self-growing plant. Since it is used as a medicinal plants, it is cultivated in recent years and become a domesticate plant. Recently, there has been growing interest in the use of natural substances due to concern about some the safety of synthetic compounds, which have encouraged more detailed studies on originated substances. On this basis, this experiment was performed to investigate and compare the chemical composition and antimicrobial activity of the essential oils of domesticated *O. vulgare* subsp. *viride* plant samples cultivated in two regions of Iran against six bacteria and five fungi strains with the aim of evaluation effect of cultivation sites on yield and constitutes of essential oil and funding new natural bactericides and fungicides.

**Materials and methods**

**Plant material**

*O. vulgare* subsp. *viride* plants were propagated by vegetative propagation. Four-year-old marjoram plants were arranged from a cloning unit in the village of Darberaz in Yazd province. This clone was selected from a wild population growing in Yazd province, Iran.

The experiments were held in two locations: in the Experimental Field of Agricultural Faculty of Ferdowsi University of Mashhad, and in the field of Darberaz village, Khazarabad part, Sadugh town, Yazd, Iran. The meteorological conditions that overcome during the cultivation periods in these research areas are shown in Table 1.

Planting was done in both regions in April 2013, divided marjoram plants were transferred to field spaced 50 cm between the rows and 20 cm on the rows. The soil analysis of each experimental site is reported in Table 1. Hoeing and mechanical weeding were made regularly. Irrigation was regularly applied during the vegetation period. After 3 months of growth, aerial parts of plants were harvested at flowering stage, and then air dried.

**Isolation of the essential oil**

Dried aerial parts of *O. vulgare* subsp. *viride* (50 g) in each cultivation site were subjected to hydrodistillation for 4 h an all-glass Clevenger-type apparatus. Samples were collected over water, dried with anhydrous sodium sulphate and stored in dark at 4°C.

**Gas chromatography (GC)**

A Thermo-UFM Ultra-Fast gas chromatograph equipped with a DB-5 fused silica column (30 m × 0.1 mm i.d., film thickness 0.40 μm) was used
Table 1. Basic characterization of the cultivation sites

<table>
<thead>
<tr>
<th>Localization</th>
<th>Cultivation site</th>
<th>Mashhad</th>
<th>Yazd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude (m)</td>
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<td>1830</td>
<td></td>
</tr>
<tr>
<td>Latitude</td>
<td>36°16’</td>
<td>31°50’</td>
<td></td>
</tr>
<tr>
<td>Longitude</td>
<td>59°36’</td>
<td>53°59’</td>
<td></td>
</tr>
<tr>
<td>Mean of minimum temperature (°C)</td>
<td>7.03</td>
<td>11.79</td>
<td></td>
</tr>
<tr>
<td>Mean of maximum temperature (°C)</td>
<td>21.18</td>
<td>26.58</td>
<td></td>
</tr>
<tr>
<td>Total precipitation (mL)</td>
<td>253.95</td>
<td>55.15</td>
<td></td>
</tr>
<tr>
<td>Soil characteristics</td>
<td>Silty loam</td>
<td>Sandy loam</td>
<td></td>
</tr>
<tr>
<td>Soil texture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC* (ds m⁻¹)</td>
<td>1.4</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>Organic C (%)</td>
<td>0.22</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Available N (mgkg⁻¹)</td>
<td>11</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>Available P (mgkg⁻¹)</td>
<td>12.8</td>
<td>8.48</td>
<td></td>
</tr>
<tr>
<td>Available K (mgkg⁻¹)</td>
<td>125</td>
<td>201</td>
<td></td>
</tr>
</tbody>
</table>

*Electrical conductivity

for Gas chromatography (GC) analysis. The oven temperature was kept at 60°C for 3 min, and then programmed to increase to 280°C at a rate of 80°C min⁻¹. The temperatures of the injector and flame-ionization detector were held at 285°C. Helium was used as carrier gas with a linear velocity of 32 cm s⁻¹. The essential oils were injected manually into the GC instrument without dilution. By using the area normalization method, without consideration of response factors, the percentages of compounds were measured.

Gas chromatography-Mass spectrometry (GC-MS)

A Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm) was applied for GC-MS analyses. Following injection, the oven temperature was increased from 50 to 240°C at a rate of 4°C min⁻¹, the temperature of the transfer line was kept at 260°C, and the linear velocity of the helium carrier gas was put at 31.5 cm s⁻¹, with a split ratio of 1:60, an ionization energy of 70 eV, a scan time of 1 s, and a mass range of 40-300 amu.

By comparing the mass spectra of essential oil constituents with those kept in a computer library or achieved using authentic compounds, the essential oil compounds were identified. In comparison the retention indices of authentic compounds with published data in the literature, the identified components were approved. Moreover, mass spectra from the literature were compared. A homologous series of n-alkanes were used for calculating the retention indices of all volatile components.

Antibacterial test

Five phytopathogenic bacteria (Xanthomonas citri, Ralstonia solanacearum, Erwinia amylovora, Pectobacterium carotovorum and Xanthomonas oryzae) obtained from Department of Plant Diseases, Ferdowsi University, Iran. In this study disk diffusion method was used. Whatman No.1 sterile filter paper disks (6 mm diameter) were impregnated with defined concentrations of essential oil (20 μL/disk), prepared in 1% DMSO. The disks were permitted to dry for 15 min and put on the inoculated agar. The same solvents applied for dissolving the samples and prepared controls. The plates were incubated at 28°C for 24 h. The diameter of inhibition zone (in mm) showed the degree of the essential oil activity. Each examination in this study was replicated three times.
MIC and MBC assay

Bacteria were cultured overnight at 28°C for determining minimal inhibitory (MIC) and minimum bactericidal concentrations (MBC) by microtiter plates. The essential oils were dissolved in 1% DMSO. Dilutions were prepared in a 96 well microtiter plates to get final concentrations from 0 to 30 μLmL⁻¹. Finally, 20 μL of inoculums (10⁶-10⁷ CFU mL⁻¹) were inoculated onto the microplates and the tests were performed in a volume of 200 μL. Plates were incubated at 28°C for 24 h. MICs was the lowest concentrations of tested samples (μLmL⁻¹) which did not show any visual growth after macroscopic evaluation. Using the results of the MIC assay, the concentrations exhibiting complete lack of visual growth of bacteria were recognized and 50 μL of each culture broth was moved on to the agar plates and incubated for the indicated time and temperature (28°C, for 48 h). The complete lack of growth on the Nutrient Agar (medium) surface in the lowest concentration of sample was defined as the MBC. For more exact determination of MIC, all the bacterial cultures were co-cultivated with different concentrations of essential oil in 5 mL of nutrient broth medium (in higher volume for less error). After the specified incubation period (28°C, for 24 h) 0.1 mL of cultures from all the test tubes were put on nutrient agar medium to find out the MIC.

Antifungal activity assay

Five plant pathogenic fungi included Bipolaris sorokiniana, Fusarium oxysporum, Drechslera oryzae, Alternaria alternata and Stemphylium sarciniforme were obtained from the collection of Department of Plant Diseases, Lorestan University, Iran. The antifungal activities of the essential oils against above five phytopathogenic fungi were evaluated by disc diffusion method using potato dextrose agar (PDA). The discs (5 mm diameter) of the tested fungi were cut from 5-days-old culture PDA plates and placed mycelia surface down, on opposite edges of the test plates on the centre of dishes and stored under controlled temperature condition of 30°C. The percentage of inhibition of mycelia growth was calculated from the mean values of colony diameter of treated and control. Percentage inhibition was calculated by using the following formula:

\[ % \text{ Inhibition} = \frac{C - T}{C} \times 100 \]

Where C is the mean of diameter of growth in control and T is the mean of diameter of growth with the essential oil.

MIC assay

Determination of MIC values was performed by a macrodilution method. The 10⁴ cfu/mL suspensions of fungi spores were prepared from 7-day old culture of target fungi, then concentrations of the essential oils (0.1-10 μLmL⁻¹) were prepared in the PDA Broth and inoculation was done with fungi spore suspension 10⁴ cfu/mL. Tubes containing essential oils and fungi spores kept in shaker incubator at 30°C for 72 h and MIC was determined as the lowest concentration of the essential oil causing full growth inhibition.

Statistical analysis

In the disk sensitivity test, zones of inhibition were measured millimeters with a centimeter ruler. Statistical analysis was performed using the SPSS 21.0 software program. The mean comparisons of inhibitory effects of essential oils on bacteria strains were made using Duncan’s multiple range tests at \( p < 0.05 \). Antimicrobial activities data are demonstrated as mean values ± standard deviation (SD).

Results and discussion

Essential oil yield

The essential oil yields (% v/w) of O. vulgare subsp. viride from the two cultivation sites were varied. The highest essential oil percentage (1.58%) was obtained from the plants which were cultivated in Yazd region whereas the samples in Mashhad region produce the lowest essential oil content (1.55%). Although, the findings were close to each other in amount (Table 2).

The essential oil content is generally known to be affected great extent by environmental factors, while the essential oil profile is affected by genetic factors. Moreover, environmental condi-
tions may play an important role in determining essential oil yield.

In comparison with our results the retention indices and percent composition of essential oil separated from aerial parts of *O. vulgare* subsp. *viride* in Behshahr region of Iran was lower (0.4 % v/w) \(^{17}\). Higher elevation and temperature supplied a better growing condition which cause to a higher accumulation of essential oil in the leaves of oregano. The results of other researches indicated that altitude is the most important environmental factor which is influenced essential oil content of *O. vulgare* subsp. *hirtum* \(^{18}\) and *Thymbra spicata* var. *spicata* \(^{19}\). Due to the findings of both mentioned studies, high values of essential oils can be achieved at low altitudes which is compatible with Mediterranean-type ecosystems. But these findings were not in agreement with our results, because the essential oil yield of Yazd from higher altitude was higher. Although a little differentiations between two samples were found.

**Essential oil composition**

The results of chemical composition analysis of *O. vulgare* subsp. *viride* from two regions showed that the percentage of compounds varied greatly in different areas. The GC and GC-MS analysis resulted in identification of 44 and 50 constituents of essential oil composition in the essential oils from Mashhad and Yazd, respectively. Their sum constituted the bulk of the essential oils and ranged from 99.88 up to 99.93% of total essential oil (Table 2).

The analysis of essential oils detected six major compounds viz. thymol (21.6 %), 4-terpineol (15.85 %), *trans*-sabinene hydrate (7.91 %), *p*-cymene (5.69 %), *γ*-terpinene (5.46 %), sabine (3.81 %); and thymol (20.97 %), 4-terpineol (16.27 %), *γ*-terpinene (7.62 %), *trans*-sabinene hydrate (6.11 %), *p*-cymene (4.27 %), sabine (4.49 %) for the essential oil of Yazd and Mashhad, respectively. In addition, the essential oils extracted from both sites contained oxygenated monoterpenes, hydrocarbons monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and other compounds in different percentage according to the cultivation sites. Based on the results thymol was dominant essential oil component for both samples, although some compounds were only found in Yazd sample in trace amounts (Table 2, Fig. 1, 2).

A comparison of our results with essential oil constituents which was reported in other studies showed high chemical variations between the researches. There are several studies regarding composition of *O. vulgare* essential oils from various regions of the world, such as Greece \(^{20}\), India \(^{21}\).

<table>
<thead>
<tr>
<th>No.</th>
<th>Component</th>
<th>RI</th>
<th>Mashhad</th>
<th>Yazd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-Hexenal</td>
<td>864</td>
<td>-</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>α-Thujene</td>
<td>926</td>
<td>1.23</td>
<td>1.18</td>
</tr>
<tr>
<td>3</td>
<td>α-Pinene</td>
<td>946</td>
<td>0.7</td>
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</tr>
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<td>4</td>
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<td>958</td>
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<td>979</td>
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<td>6</td>
<td>β-Pinene</td>
<td>982</td>
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<td>7</td>
<td>Octane&lt;3-&gt;</td>
<td>985</td>
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<td>No.</td>
<td>Component</td>
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<td>Yazd</td>
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<td>-------</td>
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<td>trans-Sabinene hydrate acetate</td>
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<td>Intermedeol</td>
<td>1665</td>
<td>0.35</td>
<td>0.38</td>
</tr>
<tr>
<td>50</td>
<td>Hexahydrofarnesyl acetone</td>
<td>1843</td>
<td>-</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Grouped components (%)

- Oxygenated monoterpenes: 49.92 \(\rightarrow\) 51.33
- Hydrocarbons monoterpenes: 39.74 \(\rightarrow\) 38.01
- Sesquiterpene hydrocarbons: 5.07 \(\rightarrow\) 3.9
- Oxygenated sesquiterpenes: 4 \(\rightarrow\) 5.31
- Others: 1.2 \(\rightarrow\) 1.33

Total: 99.93 \(\rightarrow\) 99.88

Essential oil content (%): 1.55 \(\rightarrow\) 1.58
and Argentina, Croatia, Turkey, and some regions of Italy. Therefore, the chemical components of *O. vulgare* essential oil depends on geographical and climate conditions of collection sites. The chemotypes of *O. vulgare* L. were carvacrol type, thymol type and chemotype with main constituents: sabinene, (Z)-ocimene, p-cymene, caryophyllene and bisabolene. The subspecies *vulgare* composed mainly of sabinene, germacrene D, β-caryophyllene and spathulenol, respectively.

The major constituents of *O. vulgare* subsp.
viride from wild growing plants in Kodjour region from Iran were identified linalyl acetate, sabinene, \( \gamma \)-terpinene, \((Z)\)- and \((E)\)-ocimene from monoterpenes and \( \beta \)-caryophyllene, caryophyllene oxide, germacrene D and \( \gamma \)-elemene from sesquiterpenes. In addition, higher sesquiterpenes amount in comparison with monoterpenes components was observed. These findings are different from our results which monoterpenes hydrocarbons was the major group. In another study, the essential oil of *O. vulgare* subsp. *viride* in Behshahr region from Iran was described by higher components of thymol, \( \gamma \)-terpinene, and \( \beta \)-pinene, the other important compounds were 3-octanone, carvacrol, sabinene and \( \alpha \)-pinene which was partially same as our results.

This study suggested that the chemical composition of essential oils is related to the geographic distributions among plants which were in conformity with previous study of the chemical variation in essential oils from various areas suggested the same results for *Thymus* species.

Genetic and environmental factors are key reasons for chemical variations between species and subspecies. Habitats located far from each other will have different climate, soil, and biotic factors, which will influence the production of secondary metabolites. There are some reports regarding the effects of plant genetics, as well as the environmental factors like altitude of plant growing habitat, developmental phase of the plant and post-harvest management on essential oil concentration and composition of *Origanum* plants which can result in biochemical and physiological alterations in plant.

**Antimicrobial screening**

**Antibacterial assay**

The *in vitro* antibacterial activity of the essential oils of *O. vulgare* subsp. *viride* from two cultivation sites were assessed against six bacteria strains. The results which are showed in Table 3 represent the inhibition zones of the essential oils including the diameter (6 mm) of the paper disk. The essential oils from two regions were found to show significant antibacterial activity against *X. citri*. However, their activity against *R. solanacearum* and *X. oryzae* were less pronounced. In addition, more exact data on the antibacterial properties were acquired via the determination of bacteriostatic and bactericidal concentrations. The minimum inhibitory concentration (MIC; \( \mu \)g mL\(^{-1} \)) and minimum bactericidal concentration (MBC; \( \mu \)g mL\(^{-1} \)) of essential oil against six bacteria are shown in Fig. 3 and 4. The essential oil had the most bacteriostatic and bactericidal properties against *X. citri*. According to the results of the width of the inhibition zone diameter and MIC and MBC values, *X. citri* was the most sensitive bacterium to the essential oil.

One of the most important families of plants that produced essential oils with antimicrobial properties is Lamiaceae. According to the previous researches, essential oils are more active against pathogenic Gram-positive than Gram-negative bacteria. The antibacterial activity of some of *Origanum minutiflorum* and oregano against *Erwinia* spp. and *Xanthomonas* spp., and *Xanthomonas vesicatoria* were studied before. In our previous research the inhibitory effect of the essential oil of *Ocimum ciliatum* was tested against ten phytopathogenic strains. The antibacterial test results showed that the essential oil had antibacterial activity against ten tested bacteria. Similar phytopathogenic bacteria to this study included *X. citri*, *R. solanacearum* and *X. oryzae*. The lowest MIC value was obtained from the essential oil of the plant against *R. solanacearum*. In conformity with our findings, the most bactericidal property of the essential oil from *O. ciliatum* with the lowest MBC was observed against *X. citri* strain.

The strong antibacterial activity of *O. vulgare* is consistent with the chemical composition of essential oil which has been ascribed to the phenolic monoterpenes, carvacrol and thymol, which have similar, synergistic, antagonistic and non-selective antimicrobial activity. Furthermore, a possible synergistic effect with other minor components such as the monoterpenes hydrocarbons \( \gamma \)-terpinene and \( p \)-cymene is existed, which are biosynthetic precursors of thymol and carvacrol. Although \( p \)-cymene is a very weak antibacterial component, it swells bacterial cell membranes to a greater extent than carvacrol does. By this mechanism \( p \)-cymene en-
ables carvacrol to be more easily transported into the bacterial cell, therefore, when both compounds are simultaneously present; a synergistic effect is possible.³⁵

Antifungal assay

Preliminary screening or the putative antifungal activity in vitro of the essential oil was studied against five tested phytopathogenic fungi. Our findings indicated that all of the tested fungi could exhibit antifungal activity. By comparing the results of percentage of inhibition of mycelia growth for each fungus, a difference between the resistances against the tested fungi in relation to the
essential oil region can be observed. The inhibition of mycelia growth percentage is shown in Table 2. As mentioned in our results various cultivation sites had different effects on each fungus. The essential oil exhibited antifungal activity at 20 (μL⁻¹) concentration against the tested fungi with various mycelia growth due to their location from 2.170.21 to 5.10±0.17 mean values. Our data indicated that the most sensitive fungus was D. oryzae in both essential oil samples. But the most resistance fungi were B. sorokiniana in the essential oil obtained from Mashhad and S. sarciniforme in the essential oil from Yazd samples. Moreover, further investigation on the antifungal properties was obtained through the determination of fungistatic concentration.

The minimum inhibitory concentration (MIC; μg mL⁻¹) of essential oil against 5 fungi are shown in Fig. 5. Our findings indicated that the most fungistatic property was observed against D. oryzae in both essential oil samples. So, the most resistance fungus was D. oryzae. Also the most susceptible fungi were B. sorokiniana in the essential oil obtained from Mashhad sample and S. sarciniforme in the essential oil of Yazd sample.

According to the previous investigations the essential oil contains more oxygenated monoterpenes included thymol 4-terpineol, carvacrol in indicated the highest antifungal activity against various phytopathogenic fungi 32, 36. For example, the antifungal activities of O. acutidens essential oil against F. oxysporum, F. solani, F. oxysporum 37, and Botrytis cinerea, Fusarium sp. and Clavibacter michiganensis subsp. michiganensis 38 were studied before.

The lipophilicity of hydrocarbon skeleton and the hydrophilicity of the major functional groups of essential oil are affected the antimicrobial action of it. Expression of different levels of antifungal activity probably due to the variation in the content of known antimicrobial compounds in each essential oil 20. In addition, the respective components of the herbal essential oil are influenced on the antifungal activity of it, the structural configuration of the components and their functional groups and possible synergistic interactions between components 39. The efficacy of the essential oils can be described by the activity of phenol compounds, carvacrol and thymol. Essential oils of Origanum and Thymus species contain mainly aromatic monoterpenes, carvacrol, thymol and p-cymene and their activity are often attributed to these compounds 1, 8. In aromatic ring hydroxyl group is important in antifungal activity of the constituents, include p-cymene skeleton.

Carvacrol and thymol cause to modifications in the hyphae morphology and hyphae aggregates, resulting decreased hyphae diameters and lyses.

Fig. 5. The antifungal activity of essential oil of Origanum vulgari subsp. viride (MIC =μg L⁻¹)
of hyphae wall interacting with the cell membrane of the pathogen. On the other hand, for antimicrobial activity of essential oil, carvacrol and its hydroxyl group is not only essential, but aliphatic ring substituent of carvacrol also affect its antimicrobial effect. Furthermore, in plant kingdoms the volatile aromatic compounds of essential oils were more fungi toxic than non-aromatic ones.

Previously the antimicrobial nature of *Origanum* species essential oils were investigated which were apparently related to its phenolic components, such as thymol, carvacrol and other monoterpenes such as carvacrol methyl ether, p-cymene, α-terpinene, γ-terpinene, γ-terpineol, sabinene, myrcene, caryophyllene, germacrene, and spathulenol, which are responsible for its prominent antimicrobial activity which are in conformity with our findings. In addition, it has been suggested that phenolic derivatives can cause membrane-disturbing activities. The functional groups of the components play an important role for antimicrobial activity. Phenolic components are capable of disrupting the microbial membrane, thus penetrating inside the cell, where they interact with cellular metabolic mechanisms. In this study, if we consider the data in relation to the compound of the essential oils, it seems that antimicrobial activities of *O. vulgare* subsp. *viride* essential oils from Mashhad and Yazd were mainly linked to the presence of phenolic constituents such as associated with high levels of thymol and 4-terpineol. Thymol present in high concentrations in both samples. Moreover, other constituents individually provided the antibacterial activity, which may play a synergistic interaction with other components of the essential oils.

Our findings in agreement with other researches, allow us to suggest that constituents such as α-terpinene and p-cymene alongside hydrocarbons monoterpenes might contribute in antimicrobial activity in agree with earlier report that explain very similar and strong antifungal activity of savory, oregano and thyme essential oils. Also, the synergistic activity between thymol and carvacrol and their mutual interaction plays an important role in the overall activity of the essential oils.

The essential oils of *O. vulgare* subsp. *viride* from two regions containing the highest percent-ages of thymol were effective against the pathogenic bacteria. As shown in Table 2, the major chemical constituents of these essential oils are essentially oxygenated monoterpenes and hydrocarbons monoterpenes, respectively.

Monoterpenes disrupt the microbial cytoplasmic membranes, so high impermeability of the membrane for protons and larger ions lost. The activity of a complex mixture to a single or particular components attributed, although it is hard. Major or trace constituents in the essential oil might increase the antimicrobial activity. Synergistic influence of components in the essential oil should need to be taken into consideration. Therefore, the susceptibility of the microorganisms to an essential oil depends on the content of active substances and properties of them. Many reports have shown bactericide and fungicide influences of essential oils. Their light toxicity confirm the correctness of the hypotheses stating the necessity to study effects of essential oils on fungal pathogenic species.

**Conclusion**

The results obtained in this study indicated that essential oils composition and biological activity of *O. vulgare* subsp. *viride* affected by environmental conditions. Essential oils of aromatic plants have been recognized to possess biological activity, such as antimicrobial. There is increasing interest in assessing the antimicrobial properties of substances from natural sources that can potentially be used by the food and pharmaceutical industries. Synthetic fungicide and bactericide usage have several disadvantages such as production of dangerous secondary metabolites and the number of resistant fungal pathogenic and toxinogenic species is rising. Moreover, in particular, owing to the antimicrobial activities of *Origanum* species essential oil, our interest is focused on the effectiveness of essential oils against serious plant pathogens. In this way, the essential oil has a good capacity as an alternative to synthetic products in various industries. Although further works are necessary to explore the effective factors on the essential oil compounds and efficacy of the essential oil on other plant pathogenic microorganisms.
References


naturally growing in the wild úora of east Mediterranean and southeastern Anatolia regions of Turkey. Ind. Crops Prod. 32: 593-600.


