Antifungal activities of essential oils from some Iranian medicinal plants against various post harvest moulds

P. Mohammadi¹, N. Lotfi², L. Naseri³ and H. R. Etebarian¹

¹Department of Plant Pathology, College of Aboureyhan, Tehran University, Tehran, Iran.
²Department of Medicinal Plant, Shahid Bakeri High Educational Center of Mandoab, Urmia University, Urmia, Iran.
³Department of Horticulture, Urmia University, Urmia, Iran.

The potential antifungal effects of the essential oils and components of Thymus vulgaris L., Thymus migricus Klokov and Desj-Shost., Thymus koschyanus Boiss., Mentha spicata L., Mentha pulegium L., and Satureja mutica Fisch and C.A. (Lamiaceae) were investigated against four food poisoning and plant pathogens. The essential oils were obtained by hydro distillation of dried plant materials and their composition was determined by gas chromatograph and mass spectrometer (GC-MS) in previous studies. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the oils and their components were determined by dilution assays. Thymus species showed very strong antifungal activities. Culture Potato Dextrose Agar (PDA) media with all oil-enrichment resulted in significant (P≤0.01) reduction on subsequent colony development of Rhizopus oryzae (up to 28%) at 100 ppm; Botrytis cinerea (up to 100%); Penicillium expansum (up to 69%) at 100 ppm and Rhizopus stolonifer (up to 56%) at 100 ppm. Among studied fungi, B. cinerea and P. expansum were the most sensitive pathogens treated with MICs of 100 and 150 ppm, respectively. Differences between high MICs and MFCs of M. spicata and T. vulgaris and low MICs and MFCs of T. koschyanus and T. migricus inhibiting the growth and colony development of tested fungi correlated with simultaneous high concentration of thymol and carvacrol in species of T. koschyanus, T. migricus, M. pulegium and S. mutica. It is concluded that essential oils of Thymus, Satureja and Mentha species possess great antifungal potential and could be used as natural preservatives and fungicides.

Key words: Antifungal activity, plant pathogens, essential oils, fungal growth, Lamiaceae family.

INTRODUCTION

The fungal decay of fruits and vegetables in postharvest storage greatly limits their economic value. Although fungicides treatments have been the main method for controlling postharvest diseases, public concern about fungicides residues in food and the development of fungicides resistance by pathogens has increased the search for alternative means of controlling the disease. Biological control of postharvest decays of fruits and vegetables has emerged recently as a promising alternative to the use of synthetic fungicides (Wilson and Wisniewski, 1989; Wisniewski and Wilson, 1992). Essential oils are complex volatile compounds produced in different plant parts, which are known to have various functions in plants including conferring pest and disease resistance (Goubran and Holmes, 1993). Application of essential oil is a very attractive method for controlling postharvest diseases. Essential oils from plant edible parts which are ecfriendly in nature have been used by several workers for controlling fungi, bacteria, viruses and insect pests (Singh and Upadhyaya, 1993; Singh, 1996). The main reasons for using essential oils as antifungal agents are because they have natural origin and
pathogens have low chance of developing resistance.

The complexity of essential oils is attributed to their terpene hydrocarbons and their oxygenated derivatives such as alcohols, aldehydes, ketones, acids and esters (Tzortazakis et al., 2007). Production of essential oils by plants is believed to be predominantly a defense mechanism against pathogens and pests and indeed, essential oils have been shown to possess antimicrobial and antifungal properties (Ahmet et al., 2005). Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multi-purpose functional use (Jobling, 2000). Various Mentha and Thymus species have been credited with a long list of pharmacological properties. They are used for flavours in cooking, in folk medicine as antiseptic and as antimicrobial agents (Bremnes, 2002). The genus Thymus has numerous species and varieties and their essential oil compositions have been studied earlier (Guillen and Manzanos, 1998; Lozeine et al., 1998; Saez, 1998; Tumen et al., 1998). Thymus sitchensis with native name of "Kahlig Oti" is one of the 14 species from genus Thymus and as an endemic species in northwest of Iran that grows on open rocky and gravelly ground (Mozaffarian, 2008).

Other species, namely Thymus kotschyanus grows wild in southern aspect of Alborz Mountains; the native growing site altitude of this species ranges from 1800 to 2800 m. Analysis of the essential oil by GC/MS revealed that the prominent components of these species were Thymol (23 to 29%), Carvacrol (10 to 27%), Linalool (45 to 47.5%), α-Terpinene (39.4%) α-Pinene (7 to 36%) (Habibi et al., 2007). The essential oil of Thymus vulgaris is a known antiseptic, antiviral and antimicrobial agent (Lawless, 2002; Bremnes, 2002). There were 27 identified components accounting for total 97.2% of the oil, in the essential oil of T. vulgaris. The main components were thymol (48.9%) and p-cymene (19.0%) (Marina et al., 2009). However, GC analysis of essential oils of some species of the genus Thymus, Satureja and Mentha showed that the oils obtained from M. spicata and S. mutica contained larger proportions of phenols (= carvacrol plus thymol). Analysis of the essential oil by GC/MS revealed that the prominent components were piperitone (38.0%), piperitenone (33.0%), terpineol (4.7%), 1,8-cineole (4.0%), piperitone oxide (3.4%), menthone (3.1%), borneol (2.9%) and pulegone (2.3%) (Sefidkon and Jamzad, 2005).

As a medicinal plant, Satureja species has been traditionally used as a stimulant, stomachic, carminative, expectorant and aphrodisiac. Remarkable differences between and within the chemical composition of the essential oils of Satureja subspecies has been found (Slavkovska et al., 2001). The essential oil has demonstrated antimicrobial activity because of the phenols in the oil (Sefidkon et al., 2005). The major constituents of essential oils from Satureja species are phenols (y-terpinene (14.9%) and p-cymene (10.3%), carvacrol (30.9%) and thymol (26.5%) (Sefidkon et al., 2005).

Regarding the existence of high amount of thymol and carvacrol in Satureja species, coupled with their good smell and simple cultivation, they are used as a flavoring compound in food, pharmaceutical and cosmetic industries (Zargari, 1990). Main components of the ethanolic extract of Satureja hortensis, as well as of the essential oils of Saturejae montana (Kustrak et al., 1996), Satureja spicigera (Tumen and Baser, 1996), Satureja odora and Satureja parvifolia (Muschietti et al., 1996) are the isomers thymol (5-methyl-2-isopropylphenol) and carvacrol (5-isopropyl-2-methylphenol) (Nedyalka et al., 1999).
have yet been carried out on the evaluation of antifungal activities of some of the Iranian folklore plants for example: *T. koschyanus, S. mutica, T. migricus*. We hypothesized that high level of thymol with carvacrol simultaneously increases antifungal activity of thymol or other essential oil components. The aim of our study was to investigate the activity of essential oils of six medicinal plant species against postharvest pathogens caused by *Rhizopus oryzae, Rhizopus stolonifer, Penicillium expansum* and *Botrytis cinerea* using the *in vitro* culture medium.

**MATERIALS AND METHODS**

**Plant material**

The plants were collected from mountain and pasture areas of Urmia (Band, Nazlu and Gushchi) and Miandoab Agricultural Research Field (MARF) in west Azerbaijan in 2010. Voucher specimen of the plants were deposited in the Medicinal Plants Department, College of Miandoab, University of Urmia and identified by Naser Lotfi from the same department.

**Extraction of essential oil**

Plant materials (leaves, flowers and stem) were dried in the shade at room temperature before grinding to fine powders using a mixer (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex). Essential oils were isolated from air-dried aerial part by hydro-distillation in a Clevenger’s apparatus for two hours (Molinex).

**Isolation of fungi**

Fungi were isolated from decayed fruits collected from trade markets. The isolates were grown and maintained on potato dextrose agar (PDA, Merck) identified with appropriate taxonomic keys (Schipper, 1984; Pitt, 2000). The isolates were maintained on PDA slants at 4°C.

**Antifungal activity test**

The antifungal activity test against the pathogens was determined by the poisoned food (PF) (Grover and Moore, 1962) and the volatile activity (VA) tests (Chutia et al., 2009). In PF technique, 20 ml of PDA was poured into sterilized Petri dishes and measured amount of oil was added to get the required concentrations of 0, 50, 100, 150, 250, 350, 450, 650, 1000 and 2000 ppm /100 ml sterile molten PDA (Feng and Zheng, 2007). In media, 0.05 ml/100 ml Tween-80 was added for even distribution of the oil. The test fungi were inoculated with 5 mm mycelial plugs from 7 days old cultures and incubated at 25±2°C. The growth of fungal colonies was recorded after one week and the percentage inhibition was computed after comparison with the control. In VA assay, Petri dishes were filled with 20 ml of PDA and one disc (0.5 cm diameter) of mycelial plug was taken from the edge of a 4 to 6 day old fungal culture and was placed on PDA in the Petri dishes (Sharma and Tripathi, 2006). The Petri dishes were inverted and sterile filter paper discs (4 mm diameter) impregnated with the above concentrations of essential oils was attached to the inverted lid (1 disc per lid). The Petri dishes were then wrapped with parafilm along the rim to check the release of the volatile components, inverted and incubated for 7 days at 25±2°C. The mycelial growth of fungus (mm) in both treated (T) and control (C) Petri dishes was measured diometrically in three different directions and the growth inhibition (I) was calculated using the formula:

\[
I(\%) = \frac{(C-d)-(T-d)}{(C-d)} \times 100
\]

Where d: Diameter of the cut fungus, C: Diameter of the control fungus, T: Diameter of the treated fungus (measurement unit: mm, and two colonies were counted in two dishes). It was repeated three times.

**Spore germination test**

The effects of essential oils on spore germination and germ tube elongation of the pathogens were tested in potato dextrose broth (PDB). Essential oils were added to glass tubes containing 5 mL PDB to obtain final concentrations of 0, 50, 100, 150, 250, 350, 450, 650, 1000 and 2000 ppm. At the same time, aliquots (100 µL) of spore suspensions (1×10<sup>7</sup> spores ml) of pathogens were added to each tube. After 20 h of incubation at 28°C on a rotary shaker (200 rpm), at least 100 spores per replicate were observed microscopically to determine germination rate and germ tube length. Experiments were repeated three times.

**Statistical analysis**

Statistical analysis of the data obtained in the present study was carried out in a completely randomized design layout with three replicates. Each test was run in triplicate. Data were first tested for normality, and then subjected to analysis of variance (ANOVA). Normality of experimental errors was determined using the Univariate and Capability Procedure of SAS Software (SAS Institute Inc., Cary, NC). Analysis of variance, comparison of means, and regression analysis were performed using SAS (SAS Institute Inc.) software. The mean comparisons were done using Duncan's Multiple Range Test (DMRT) (P≤0.01) and the standard error of the means was calculated following one-way ANOVA.

**RESULTS AND DISCUSSION**

Results from PF techniques confirmed the results obtained from VA techniques for all tested traits; so according to data, results showed means of both PF and VA techniques. Culture PDA media with all oil-enrichment resulted in significant (P≤0.01) increases on growth inhibition of four tested fungi (Table 1). In *T. vulgaris*, there were increases in growth inhibition of *R. oryzae* (up to 6.93%) at 150 ppm as well as for *R. stolonifer, P. expansum* and *B. cinerea* (up to 13.36, 24.89 and 58.14%), respectively (Table 1). Moreover, the highest oil concentration employed revealed complete (100%) inhibition on fungal colony development for all the pathogens and all tested plants were different (Tables 1 and 2). In *T. migricus*, there were significant increases in growth inhibition of *R. oryzae* (up to 36.59%) at 150 ppm as well as for *R. stolonifer, P. expansum* and *B. cinerea* (up to 79.57, 100 and 100%), respectively (Table 1). Similar status was observed in essential oil of *T.*
Table 1. Effect of different concentrations of essential oils from medicinal species on the percentage of growth inhibition of *R. oryzae*, *R. stolonifer*, *P. expansum* and *Botrytis cinerea*. Each value represents the mean of three measurements (±SE) from three petri dishes having a similar value of essential oils. Significant differences at the 1% level between values obtained under control and the different concentration of essential oil treatments (*P* ≤ 0.01), according to DMRT.

<table>
<thead>
<tr>
<th>Test plant</th>
<th>Test fungus</th>
<th>Concentrations of the essential oils (ppm /100 ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><em>T. vulgaris</em></td>
<td><em>R. oryzae</em></td>
<td>0.23±0.08</td>
</tr>
<tr>
<td></td>
<td><em>R. stolonifer</em></td>
<td>0.37±0.72</td>
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<tr>
<td></td>
<td><em>P. expansum</em></td>
<td>0.29±0.69</td>
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<tr>
<td></td>
<td><em>B. cinerea</em></td>
<td>0.19±0.79</td>
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<tr>
<td><em>T. migricus</em> Klokov and Desj-Shost.</td>
<td><em>R. oryzae</em></td>
<td>0.29±0.49</td>
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<tr>
<td></td>
<td><em>R. stolonifer</em></td>
<td>0.46±0.05</td>
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<tr>
<td></td>
<td><em>P. expansum</em></td>
<td>0.36±0.06</td>
</tr>
<tr>
<td></td>
<td><em>B. cinerea</em></td>
<td>0.24±0.69</td>
</tr>
<tr>
<td><em>T. koschyanus</em> Boiss.</td>
<td><em>R. oryzae</em></td>
<td>0.45±0.07</td>
</tr>
<tr>
<td></td>
<td><em>R. stolonifer</em></td>
<td>0.32±0.12</td>
</tr>
<tr>
<td></td>
<td><em>P. expansum</em></td>
<td>0.25±0.21</td>
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<tr>
<td></td>
<td><em>B. cinerea</em></td>
<td>0.16±0.08</td>
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<tr>
<td><em>M. spicata</em> Fisch and C.A.</td>
<td><em>R. oryzae</em></td>
<td>0.56±0.23</td>
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<tr>
<td></td>
<td><em>R. stolonifer</em></td>
<td>0.36±0.48</td>
</tr>
<tr>
<td></td>
<td><em>P. expansum</em></td>
<td>0.17±0.05</td>
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<tr>
<td></td>
<td><em>B. cinerea</em></td>
<td>0.28±0.69</td>
</tr>
<tr>
<td><em>M. pulegium</em></td>
<td><em>R. oryzae</em></td>
<td>0.11±0.29</td>
</tr>
<tr>
<td></td>
<td><em>R. stolonifer</em></td>
<td>0.16±0.09</td>
</tr>
<tr>
<td></td>
<td><em>P. expansum</em></td>
<td>0.31±0.08</td>
</tr>
<tr>
<td></td>
<td><em>B. cinerea</em></td>
<td>0.52±0.12</td>
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</tbody>
</table>

*koschyanus* against all tested fungi. *T. migricus* and *T. koschyanus* were shown to be most potent among *Thymus* species against all tested pathogens (Table 1 and Figure 2). Antifungal activities of *T. koschyanus* essential oils concentration on *R. oryzae*, *R. stolonifer*, *P. expansum* and *Botrytis cinerea*. Each value represents the mean of three measurements (±SE) from three petri dishes having a similar value of essential oils. Significant differences at the 1% level between values obtained under control and the different concentration of essential oil treatments (*P* ≤ 0.01), according to DMRT.
expansum and B. cinerea were 200, 150, 150 and 100 ppm, respectively.

Our results confirmed that Rhizopus species were respectively (Table 1). Also in M. pulegium, significant antifungal activity against tested pathogens increases at 150 ppm (Table 1). Among Mentha species, M. pulegium was shown to be the most potent in comparison with M. spicata, T. vulgaris and T. migricus (Tables 1 and 2). In different concentrations of essential oil of S. mutica, significant inhibition of growth and reduction of colony diameter of R. oryzae, R. stolonifer, P. expansum and B. cinerea was observed at 150, 150, 50 and 50 ppm (Table 1). Our results showed that 100% inhibition of growth of R. oryzae with essential oils of T. vulgaris, T. migricus and T. koschyanus species occurred at concentrations of 650, 350 and 250 ppm, respectively, whereas 100% inhibition of growth of B. cinerea was observed at concentrations of 250, 150 and 100 ppm (Table 1 and Figure 2). Results showed that B. cinerea were the most sensitive ones to Thymus, Mentha and Satureja essential oils (Tables 1 and 2).

The antifungal activity of tested essential oils on spore germination is shown in Figure 1. ANOVA revealed spore germination to be significantly (P<0.01) reduced by oil of tested plants in R. oryzae, R. stolonifer, P. expansum and B. cinerea with the impacts of oil dependent species and different oil concentrations. Essential oils of T. migricus Klokov and Desj-Shost., T. koschyanus Boiss. and M. pulegium have the highest potential on spore germination of P. expansum (95 to 97%) and B. cinerea (97 to 98%) and the least on R. oryzae (80%) and R. stolonifer (90 to 92%) (Figure 1). The essential oils of T. migricus, T. koschyanus, M. pulegium and S. mutica tested by PF and the volatile activities (VA) test showed very strong antifungal activity. These oils were active against R. oryzae, R. stolonifer, P. expansum and B. cinerea at the concentration of 150 ppm. At lower concentrations (100 ppm), P. expansum and B. cinerea were inhibited. The essential oils of members of the Lamiaceae family (Tables 1 and 2 and Figure 2) had inhibitory effects on the four toxigenic tested fungi. T. koschyanus, T. migricus, M. pulegium and S. mutica caused complete growth inhibition of all tested fungi. B. cinerea was affected severely by all tested oils, while R. oryzae and R. stolonifer were moderately affected.

Thyme oil was more toxic on tested fungi than the other two members of the Lamiaceae family. Thyme oil drastic concentration was observed at >100 ppm on B. cinerea and P. expansum and at >200 ppm on R. oryzae and R. stolonifer. The essential oils of T. koschyanus and T. migricus have 27 components, accounting for total 97.2% of the oil. The main components are thymol (48.9%) and carvacrol (29.0%) (Marina et al., 2009). Also, the essential oil of T. vulgaris tested by macrolidiation method showed strong antifungal activity (Marina et al., 2009). Marina et al. (2009)'s studies revealed that concentration of 250 ppm of oil inhibited Alternaria alternata, Fusarium tricinctum, all Aspergillus species and dermatomycetes. Pseudomonas helianthi and Cladosporium cladosporioides were inhibited at lower concentrations (150 ppm). T. vulgaris has 27 components, accounting for total 97.2% of the oil, in the essential oil. The main components are thymol (48.9%) and p-cymene (19.0%) (Marina et al., 2009). The MICs of T. vulgaris oil in PF and VA tested against R. oryzae, R. stolonifer, P. expansum and B. cinerea were 350, 250, 150 and 150 ppm, respectively (Table 2). This result showed that Thymol had the same antifungal potential as the oil, while carvacrol exhibited a slightly better effect, with MICs of 50-150 ppm against the tested fungi (Table 2). Differences between high MICs and MFCs of T. vulgaris and low MICs and MFCs of T. koschyanus and T. migricus inhibited growth and colony development of tested fungi related to the simultaneous high concentration of thymol and carvacrol in species of T. koschyanus and T. migricus.

Strong antifungal activities of thymol and carvacrol were also reported in the literature (Lawless, 2002). In this study, essential oils of tested plants were very effective on inhibition of growth and reduction of spore germination of tested fungi, especially P. expansum and B. cinerea. The oil of seven Lamiaceae, which consists mainly of carvacrol, linalyl acetate and thymol as major components, exhibited a complete mycelial inhibition effect on the growth of B. cinerea (Bouchra et al., 2003). Comparing the previous data with the chemical composition of the oils, it becomes evident that there is a

<table>
<thead>
<tr>
<th>Test plant</th>
<th>R. oryzae</th>
<th>R. stolonifer</th>
<th>P. expansum</th>
<th>B. cinerea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
</tr>
<tr>
<td>T. vulgaris</td>
<td>350</td>
<td>650</td>
<td>250</td>
<td>450</td>
</tr>
<tr>
<td>T. migricus Klokov and Desj-Shost.</td>
<td>100</td>
<td>350</td>
<td>100</td>
<td>250</td>
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<tr>
<td>T. koschyanus Boiss.</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>M. spicata</td>
<td>350</td>
<td>1000</td>
<td>150</td>
<td>650</td>
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<td>M. pulegium</td>
<td>150</td>
<td>350</td>
<td>100</td>
<td>250</td>
</tr>
<tr>
<td>S. mutica Fisch and C.A.</td>
<td>250</td>
<td>450</td>
<td>150</td>
<td>350</td>
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</tbody>
</table>

Table 2. MIC (ppm/100 ml) and MFC (ppm/100 ml) of six plant species against plant pathogenic fungi R. oryzae, R. stolonifer, P. expansum and B. cinerea using different concentration of their essential oils treatments.
Figure 1. Effect of different concentrations of essential oils of medicinal species on spore germination of *R. oryzae*, *R. stolonifer*, *P. expansum* and *B. cinerea*. Each value represents the mean of three measurements (±SE) from three Petri dishes having a similar value of essential oils. Significant differences at the 1% level between values obtained under control and the different concentration of essential oil treatments (P≤0.01), according to DMRT.
relationship between the high activity of the *Thymus* type oils and the presence of phenol components, such as thymol and carvacrol. The high antifungal activity of these essential oils could be explained by the high percentage of phenol components. It seems possible that phenol components may interfere with cell wall enzymes like chitin synthase/chitinase as well as with the β-glucanases of the fungi (Adams et al., 1996; Kumar et al., 2008).

Consequently, the high content of phenol components may account for the high antifungal activity of oils (Adam et al., 1998). The research showed that essential oils of *Thymus* species, carvacrol and thymol have very high antifungal activities, even higher than the commercial fungicides. On the other hand, our results revealed that in agreement with earlier publications (Reddy et al., 1998; Montes and Carvajal, 1998; Marina et al., 2009), the essential oil of *T. vulgaris* showed very strong antifungal activity at low concentrations of 50 to 1000 ppm. This oil also showed very strong antibacterial activity against food spoilage bacteria (Soković et al., 2007). But the essential oil of *T. vulgaris* possessed slightly lower antifungal potential than *T. koschyanus* and *T. migricus* oil (Figures 1 and 2). MICs and MFCs of *T. koschyanus* and *T. migricus* oils against *R. oryzae*, *R. stolonifer*, *P. expansum* and *B. cinerea* were obtained at low concentration than *T. vulgaris* (Table 2). The lower antifungal effect of *T. vulgaris* oil in comparison with *T. koschyanus* and *T. migricus* oils also can be explained by its lower amount of thymol and its precursors (p-cymene and γ-terpinene) and by the higher percentage of acetates (α-terpinyl acetate and geranyl acetate) which may lead to lower antifungal potential.

The chemical compounds like linalool, Caryophyllene oxide, a-pinene and a-terpineol have antifungal and antibacterial activity (Matasyoh et al., 2007). In this study, *T. koschyanus*, *T. migricus* and *M. pulegium* had shown appreciable amounts of these constituents that act as a fungicidal agent because they formed a charge transfer complex with an electron donor to fungal cells, which resulted in fungal death. Essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite in such plants. Essential oils are very complex natural mixtures which can contain about 20 to 60 components at quite different concentrations (Bakkali et al., 2008). They are characterized by two or three major components at fairly high concentrations (20 to 70%) compared to other components present in trace amounts. For example, carvacrol (20 to 30%) and thymol (15 to 27%) are the major components of the *T. koschyanus*, *T. migricus* and *S. mutica*, piperitone (38%) and piperitenone
Figure 2. Effect of different concentrations of essential oils of medicinal species on spore germination of \( R. \) oryzae, \( R. \) stolonifer, \( P. \) expansum and \( B. \) cinerea. Each value represents the mean of three measurements (±SE) from three Petri dishes having a similar value of essential oils. Significant differences at the 1% level between values obtained under control and the different concentration of essential oil treatments (\( P \leq 0.01 \)), according to DMRT.

(33%) of \( M. \) pulegium essential oils. Generally, these major components determine the biological properties of the essential oils. The components include two groups of distinct biosynthetic origin (Bowles, 2003; Pichersky et al., 2006).

The main group is composed of terpenes and terpenoids and the other, aromatic and aliphatic constituents, all characterized by low molecular weight (Pichersky et al., 2006). Our results showed that \( M. \) pulegium had a high antifungal activity like \( T. \) koschyanus (Table 1 and Figure 1 and 2). Piperitone (38%) and piperitenone mid pulegone (2 to 73%) are the main components of this species. Generally, the major components are found to reflect quite well the biophysical and biological features of the essential oils from which they were isolated (Ipek et al., 2005). The amplitude of their effects is dependent on their concentrations when they were tested alone or with essential oils. Thus, synergistic functions of the various molecules contained in an essential oil, in comparison with the action of one or two
main components of the oil seem questionable. In both VA and PF techniques, the antifungal activities of *Thymus*, *Mentha* and *Satureja* species oils against the pathogens at different concentrations are shown in Figure 2.

Effect of species and different concentration of oils on colony diameter of tested fungi were significant at p ≤ 0.01 level. The essential oils of *T. migricus* and *T. koschyanus* at concentrations of 100 ppm/100 ml significantly reduced (p ≤ 0.01) the colony diameter of *R. oryzae* (25 and 33%), *R. stolonifer* (53 and 65%), *P. expansum* (63 and 77%) and *B. cinerea* (100 and 100%) in both PF and VA techniques. The highest antifungal activity was observed in essential oils of *T. migricus*, *T. koschyanus* and *M. pulegium* (Figure 2). MIC of *T. migricus*, *T. koschyanus* and *M. pulegium* was 100 ppm/100 ml, whereas this concentration completely reduced colony diameter of *B. cinerea*. *T. vulgaris* and *M. spicata* had the least antifungal activity (Table 1 and Figure 1 and 2). Also MIC and MFC concentration of essential oils were different in all of the tested plants and fungi (Figure 1 and 2 and Table 2). Fungal colony diameter and spore germination of *B. cinerea* and *P. expansum* at 50 ppm significantly (p≤0.01) reduced with essential oils of *T. migricus*, *T. koschyanus* and *M. pulegium* (Table 2). This result shows that these species had a lowest MIC level compared to the other species, and *M. spicata* with the highest MIC and MFC level had a low antifungal activity (Table 2). However, it is possible that the activity of the main components is modulated by other minor molecules (Santana-rios et al., 2001; Hoet et al., 2006).

Moreover, it is likely that several components of the essential oils play a role in defining the fragrance, the density, the texture, the colour and above all, cell penetration (Cal, 2006), lipophilic or hydrophilic attraction and fixation on cell walls and membranes, and cellular distribution. This last feature is very important because the distribution of the oil in the cell determines different types of radical reactions produced, depending on their compartmentation in the cell. Results of this study could explain the results reported by Aydin et al. (2005), who noticed that non-cytotoxic low concentrations of thymol, carvacrol and γ-terpinene protected against deoxyribo-nucleic acid (DNA) strand breakage induced by semiquinone and oxygen radicals formed by 2-amino-3-methylimidazo (4,5-f)-quinoline (IQ) and mitomycin C (MMC) in tissues, whereas high concentrations increased DNA damage. So our results (high antifungal activity of *T. koschyanus*, *T. migricus*, *M. pulegium* and *S. mutica* essential oils at low MICs and MFCs concentration), in agreement with Fan and Lou (2004), found that some polyphenols were good antioxidants at low concentrations, but at higher concentrations, they induced cellular DNA damage. Results discussed here could explain that components such as piperitone (38%) and piperitenone mid pulegone of *M. pulegium*, p-cymene and γ-terpinene mid carvacrol and thymol of *S. mutica* and linalool mid carvacrol and thymol of *T. koschyanus* are the syner-gistic functions of the various molecules contained in the essential oils of these species.

**Conclusion**

The results obtained from this study indicate that these plant species, particularly *T. koschyanus*, *T. migricus*, *M. pulegium* and *S. mutica* contain chemical constituents that can be developed as potential antifungal agents for agricultural use. These plants may be ecologically adapted to withstand fungal infection. That is they seem to have developed a huge armament of secondary metabolites (phytoalexins) to resist fungal attack because of their constant exposure to fungi and co-existence with crop plants. Based on both MICs and MFCs and VA and PF techniques results, these species appear to be the best plant materials for isolation of antifungal compound(s). This study demonstrated the potential of plant species as sources of extracts or pure compounds with activity against plant pathogenic fungi.

**ACKNOWLEDGEMENTS**

We acknowledge the Associate Dean for Research Affairs, College of Miandoab. Also we gratefully acknowledge the University of Urmia and Iran National Science Foundation (INSF) for financial support of this research.

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