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

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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 ecofriendly 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

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pathogens have low chance of developing resistance.

The complexicity 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 migricus* 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 this 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 (γ-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 *Saturejae parvifolia* (Muschietti et al., 1996) are the isomers thymol (5-methyl-2-iso- propylphenol) and carvacrol (5-isopropyl-2-methylphe- nol) (Nedyalka et al., 1999).

It is emphasized (Tsimidou and Boskou, 1994) that, in Lamiaceae plants, thymol is always accompanied by its isomer carvacrol. Both compounds are biologically active, thymol has antiseptic and carvacrol possesses antifungal properties (Menphini et al., 1993). Due to these various uses of *Satureja* species or their oils, we were interested in investigating the antifungal activities of the oil of *S. mutica* in Iran. In the literature in most cases, only the main constituents of certain essential oils like terpineol, eugenol, thymol, carvacrol, carvone, geraniol, linalool, citronellol, nerol, safrole, eucalyptol, limonene and cinnamaldehyde are analyzed. Generally, the major components are found to reflect quite well the biophysical and biological features of the essential oils from which they are isolated (Ipek et al., 2005). The amplitude of their effects is just dependent on their concentrations when they are 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. However, it is possible that the activity of the main components is modulated by other minor molecules (Franzios et al., 1997; 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 featured is very important because the distribution of the oil in the cell determines the different types of radical reactions produced, depending on their compartmentation in the cell. In that sense, for biological purposes, it is more informative to study the entire oil rather than some of its components because the concept of synergism appears to be more meaningful. We chose these species for investigation of the antifungal activities of their oil. The essential oil of *T. vulgaris* is a known antiseptic, antiviral and antimicrobial agent (Duke et al., 2000; Lawless, 2002). The essential oil of *T. koschyanus, M. spicata* and *S. mutica* contains a high amount of thymol and carvacrol and is also used in ethnobotany (Kulevanova, 1996). No systematic studies

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 hypo-thesized 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 Azarbaijan 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 (Corticchiato et al., 1998). The obtained essential oils were dried over anhydrous sodium soulfate, filtered and stored in amber bottles at 4°C until required.

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 diameterically in three different directions and the growth inhibition (I) was calculated using the formula:

$$I(\%) = \{(C-d)-(T-d)\}/(C-d) \times 100$$

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 (1×10^7 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) ($P \leq 0.01$) 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 ($P \leq 0.01$) 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.

Test plant	Test fungus	Concentrations of the essential oils (ppm /100 ml)									
		0	50	100	150	250	350	450	650	1000	2000
T. vulgaris	*R. oryzae*	0.23±0.08	1.89±0.05	3.76±0.07	6.93±0.33	12.45±0.06	44.65±0.02	67.89±0.49	100±0.01	100±0.04	100±0.06
	R. stolonifer	0.37±0.72	2.46±0.12	6.68±0.06	13.36±0.29	22.92±0.15	56.17±0.51	100±0.23	100±0.57	100±0.17	100±0.36
	P. expansum	0.29±0.69	3.57±0.09	8.79±0.73	24.89±0.48	49.27±0.27	100±0.06	100±0.09	100±0.17	100±0.34	100±0.27
	B. cinerea	0.19±0.79	5.83±0.53	18.74±0.89	58.14±0.08	100±0.37	100±0.49	100±0.05	100±0.26	100±0.09	100±0.46
T. migricus Klokov and Desj-Shost.	*R. oryzae*	0.29±0.49	7.10±0.66	23.66±0.05	36.59±0.07	78.44±0.13	100±0.72	100±0.46	100±0.06	100±0.05	100±0.72
	R. stolonifer	0.46±0.05	15.05±0.01	53.28±0.23	79.57±0.06	100±0.05	100±1.03	100±0.06	100±0.09	100±0.07	100±0.48
	P. expansum	0.36±0.06	26.43±0.09	62.90±0.54	100±0.72	100±0.09	100±0.99	100±0.21	100±0.08	100±0.29	100±0.69
	B. cinerea	0.24±0.69	54.23±0.29	100±0.06	100±0.05	100±1.13	100±1.09	100±0.72	100±0.15	100±0.48	100±0.72
T. koschyanus Boiss.	*R. oryzae*	0.45±0.07	12.15±0.48	32.66±0.75	69.51±0.08	100±0.23	100±0.09	100±0.05	100±0.06	100±0.95	100±0.01
	R. stolonifer	0.32±0.12	19.05±0.05	64.28±0.01	100±0.72	100±0.05	100±0.87	100±0.07	100±0.09	100±0.29	100±0.05
	P. expansum	0.25±0.21	54.43±0.15	76.53±1.42	100±0.09	100±1.36	100±0.08	100±0.48	100±0.23	100±0.72	100±0.12
	B. cinerea	0.16±1.08	72.14±0.72	100±0.29	100±0.07	100±0.05	100±0.76	100±0.97	100±0.01	100±0.41	100±0.69
M. spicata	*R. oryzae*	0.56±0.23	0.89±0.01	2.67±0.09	14.69±0.08	23.98±0.06	49.86±0.12	69.45±0.15	91.56±0.05	100±0.21	100±0.05
	R. stolonifer	0.36±0.48	3.45±0.05	7.81±0.07	25.85±0.06	37.39±0.12	61.52±0.84	86.04±0.31	100±0.05	100±0.09	100±0.06
	P. expansum	0.17±0.05	6.92±0.09	15.78±0.06	40.73±0.48	68.73±0.05	89.67±0.21	100±0.08	100±0.07	100±0.29	100±0.01
	B. cinerea	0.28±0.69	18.93±0.15	37.82±0.08	59.82±0.01	93.58±0.23	100±0.05	100±0.72	100±0.09	100±0.25	100±0.67
M. pulegium	*R. oryzae*	0.11±0.29	3.98±0.07	11.57±0.04	38.27±0.05	79.35±0.75	100±0.12	100±0.06	100±0.49	100±0.05	100±0.69
	R. stolonifer	0.16±0.09	7.93±0.05	28.47±0.08	57.83±0.01	100±0.06	100±0.88	100±0.15	100±0.05	100±0.09	100±0.23
	P. expansum	0.31±0.08	19.94±0.72	42.09±0.05	100±0.28	100±0.23	100±0.29	100±0.06	100±0.08	100±0.07	100±0.12
	B. cinerea	0.52±0.12	45.67±0.15	100±0.43	100±0.56	100±0.05	100±0.69	100±0.22	100±0.76	100±0.69	100±0.48
S. mutica Fisch and C.A.	*R. oryzae*	0.48±0.61	0.89±0.23	3.87±0.35	17.82±0.14	31.56±0.08	63.74±0.92	100±0.05	100±0.62	100±0.09	100±0.01
	R. stolonifer	0.63±0.01	2.68±0.06	6.92±0.63	28.46±0.40	59.03±0.05	100±0.61	100±0.06	100±0.72	100±0.08	100±0.41
	P. expansum	0.37±0.15	17.48±0.69	37.33±0.05	71.92±0.35	100±0.26	100±0.55	100±0.09	100±0.77	100±0.15	100±0.05
	B. cinerea	0.42±0.29	37.96±0.08	60.83±0.21	100±0.59	100±0.81	100±0.58	100±0.05	100±0.01	100±0.47	100±0.09

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.*

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.

Test plant	Test fungi							
	R. oryzae		***R. stolonifer***		***P. expansum***		***B. cinerea***	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
T. vulgaris	350	650	250	450	150	350	150	*250*
T. migricus Klokov and Desj-Shost.	100	350	100	250	50	150	50	100
T. koschyanus Boiss.	100	200	100	150	50	150	50	100
M. spicata	350	1000	150	650	150	450	100	350
M. pulegium	150	350	100	250	50	150	50	100
S. mutica Fisch and C.A.	250	450	150	350	100	250	50	150

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 (Table1 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 ($P \leq 0.01$) 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 macrodilution 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. *Phomopsis 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

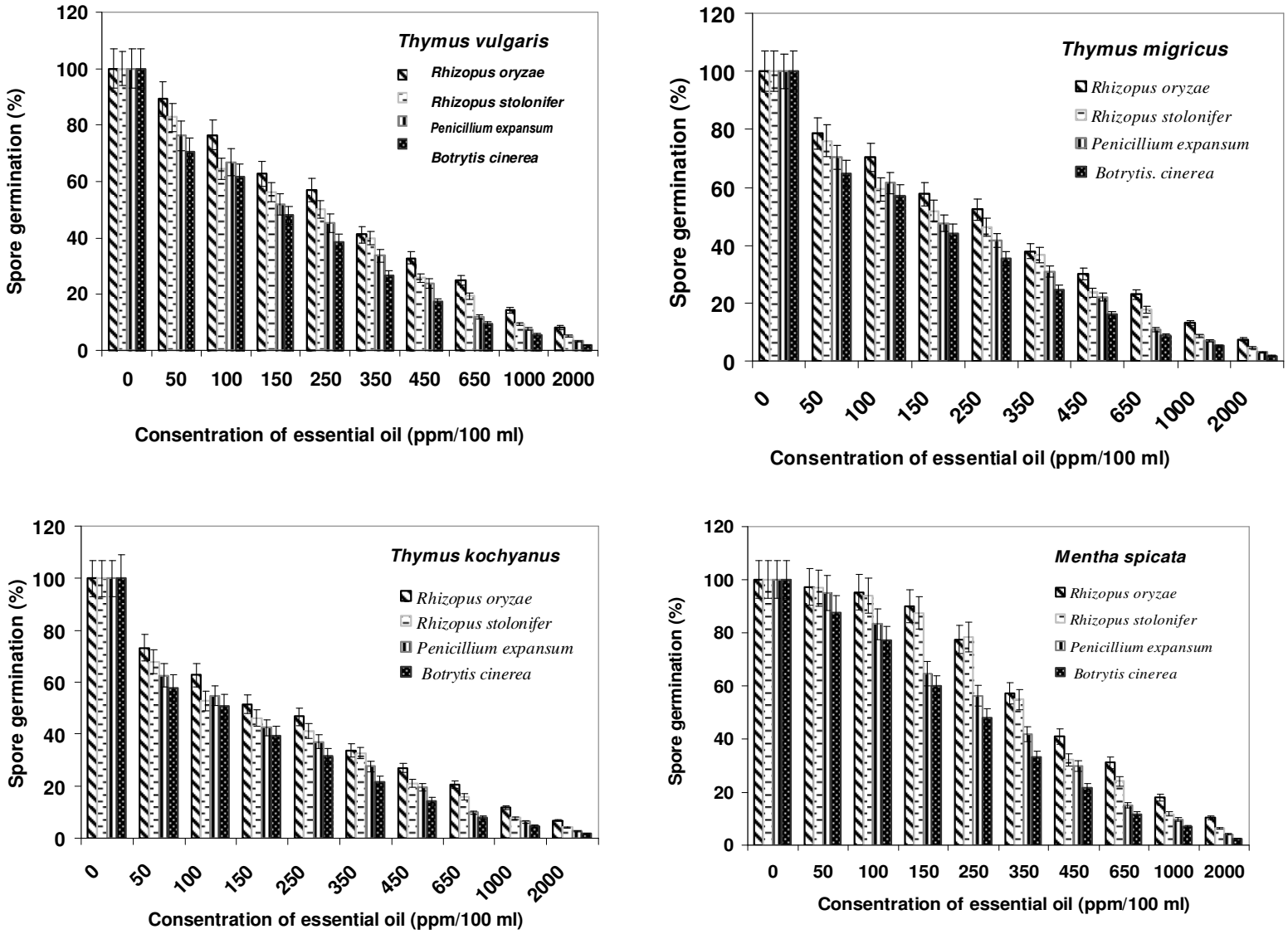

Figure 1. Effect of different concentrations of essential oils of medicinal species on spore germination of *R. oryzae, R. stolonifer, P. expansum* and *B. cinerea*. Each value represents the mean of three measurements (±SE) from three Petri dishes having a similar value of essential oils. Significant differences at the 1% level between values obtained under control and the different concentration of essential oil treatments (P≤0.01), according to DMRT.

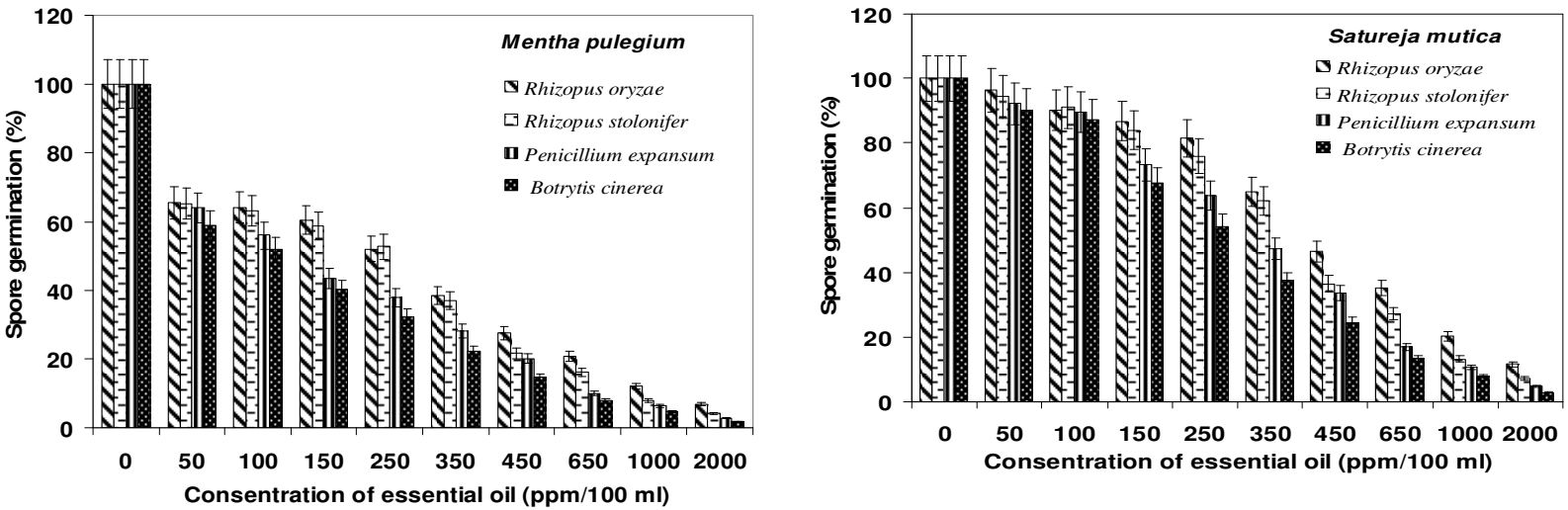

Figure 1. Contd.

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

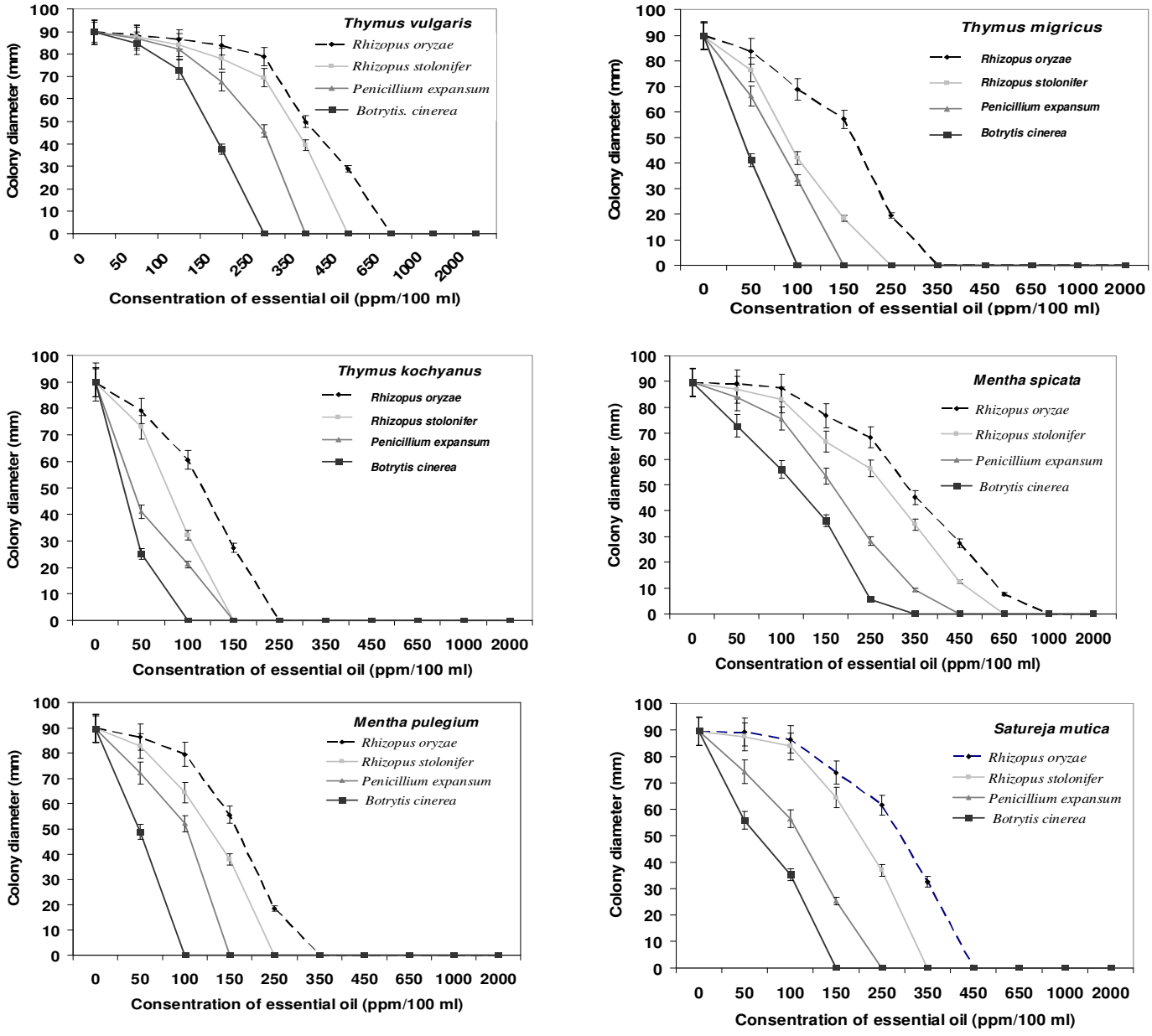

Figure 2. Effect of different concentrations of essential oils of medicinal species on spore germination of *R. oryzae, R. stolonifer, P. expansum and B. cinerea*. Each value represents the mean of three measurements (±SE) from three Petri dishes having a similar value of essential oils. Significant differences at the 1% level between values obtained under control and the different concentration of essential oil treatments (P≤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 biosynthetical 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 $p \leq 0.01$ level. The essential oils of *T. migricus* and *T. koschyanus* at concentrations of 100 ppm/100 ml significantly reduced ($p \leq 0.01$) 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 ($p \leq 0.01$) 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 deoxyribonucleic 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 synergistic 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.

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REFERENCES

Adam K, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1998). Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. J. Agric. Food. Chem., 46: 1738-1745.

Adams S, Kunz B, Weidenbörner M (1996). Mycelial deformations of *Cladosporium herbarum* due to the application of Eugenol and Carvacrol. J. Essent. Oil Res., 8: 535-540.

Ahmet C, Saban K, Hamdullah K, Ercan K (2005). Antifungal properties of essential oil and crude extracts of *Hypericum linarioides* Bosse. Biochem. Syst. Ecol., 33: 245-256.

Aydin S, Basaran AA, Basaran N (2005). The effects of thyme volatiles on the induction of DNA damage by the heterocyclic amine IQ and mitomycin C. Mutat. Res., 581: 43-53.

Bouchra C, Achouri M, Hassani LMI, Hmamouchi M (2003). Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers. J. Ethnopharmacol., 89: 165–169.

Bowles EJ (2003). Chemistry of Aromatherapeutic Oils. Allen & Unwin, ISBN 174114051X.

Bremnes L (2002). Herbs. Eyewitness-Handbooks; DK Publishing: New York, USA.

Cal K (2006). Skin penetration of terpenes from essential oils and topical vehicles. Planta Med., 72: 311-316.

Chutia M, Deka BP, Pathak MG, Sarma TC, Boruah P (2009). Antifungal activity and chemical composition of Citrus reticulata Blanco essential oil against phytopathogens from North East India. Food Sci. Technol., 42: 777-780.

Corticchiato M, Tomi F, Bernardini AF, Casanova J (1998). Composition and infraspecific variability of essential oil from *Thymus herba-barona* lois. Biochem. Syst. Ecol., 26: 915-932.

Duke OS, Dayan EF, Romagani JG, Rimando MA (2000). Natural

products as sources of herbicides current status and future trends. Weed Res., 40: 99-111.
Fan P, Lou H (2004). Effects of polyphenols from grape seeds on oxidative damage to cellular DNA. Mol. Cell. Biochem., 267: 67-74.
Feng W, Zheng X (2007). Essential oils to control *Alternaria alternata in vitro* and *in vivo*. Food. Control, 18: 1126-1130.
Franzios G, Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG, Mavragani-Tsipidou P (1997). Insecticidal and genotoxic activities of mint essential oils. J. Agric. Food. Chem., 45: 2690-2694.
Goubran FH, Holmes RJ (1993). The development of alternative fungicides from essential oils Victoria, Australia: Institute for Horticultural Development, Knoxfield, Department of Agriculture.
Grover RK, Moore JD (1962). Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. Phytopathology, 52: 876-880.
Guillen MD, Manzanos MJ (1998). Study of composition of different parts of a Spanish *Thymus vulgaris* L. plant. Food Chem., 3: 373-383.
Habibi H, Mazaheri D, Majnoon Hosseini N, Chaeechi MR, Fakhr-Tabatabaee M, Bigdeli M (2007). Effect of altitude on essential oil and components in wild thyme (*Thymus kotschyanus* Boiss). J. Pajou Sazan, 73: 2-10.
Hoet S, Ste´vigny C, He´rent MF, Quetin-Leclercq J (2006). Antitrypanosomal compounds from leaf essential oil of *Strychnos spinosa*. Planta Med., 72: 480-482.
Ipek E, Zeytinoglu H, Okay S, Tuylu BA, Kurkcuoglu M, Husnu CBK (2005). Genotoxicity and antigenotoxicity of Origanum oil and carvacrol evaluated by Ames Salmonella/microsomal test. Food Chem., 93: 551-556.
Jobling J (2000). Essential Oils: A new idea for postharvest disease control. Good Fruit Vegetables Mag.11:50.
Kulevanova S (1996). Analysis of essential oils and flavonoids from *Thymus* L. genera in flora of Republic of Macedonia. Ph.D, Thesis, Cyril and Methodius University, Skopje, Macedonia.
Kumar A, Shukla R, Singh P, Prasad CS, Kishore R (2008). Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against postharvest fungal infestation of food commodities. Innov. Food Sci. Emerg. Technol. 9:575-580.
Kustrak D, Kuftinec L, Blazevic N, Maffei M (1996). Comparison of the essential oil composition of two species of *Satureja montana*. J. Essent. Oil Res. 8:7-13.
Lawless J (2002). The encyclopedia of essential oils. Thorsons. London. UK. pp. 115-117.
Lozeine K, Vauciunine J, Venskutonis P (1998). Chemical composition of the essential oil of creeping thyme (*Thymus serpyllum* l.) growing wild in Lithuania. Planta Med. 64:772-773.
Menphini A, Pagiotti R, Capuccella M (1993). Antifugal activeity of carvacrol chemotypes of winter savory harvested in Italy. Riv. Ital. E.P.P.O.S. 4:566-571.
Montes-Belmont R, Carvajal M (1998). Control of *Aspergillus flavus* in maize with plant essential oils and their components. J. Food Protect. 61:616-619.
Mozaffarian V (2008). Dictionary of Plants Name of Iran. Current Farhang Publisher. Tehran, Iran, Pp. 740.
Muschietti L, Van BC, Coussio J, Vila R, Clos M, Canigueral S, Adzet T (1996). Chemical composition of the leaf oil of *Saturejae odora* and *Saturejae parvifolia*. J. Essent. Oil Res., 8: 681-684.
Nedyalka V, Yanishlieva EM, Marinovaa MH, Gordon V, Raneva G (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem., 64: 59-66.
Pichersky E, Noel JP, Dudareva N (2006). Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science, 311: 808-811.
Reddy BYM, Angers P, Gosselin A, Arul J (1998). Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochemistry, 47: 535-550.
Santana-Rios G, Orner GA, Amantana A, Provost C, Wu SY, Dashwood RH (2001). Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay. Mutat. Res., 495: 61-74.
Schipper MAA (1984). A revision of the genus *Rhizopus*. I The *Rhizopus stolonifer*- group and *Rhizopus oryzae*. Study Mycol. 25:20-34.
Sefidkon F, Jamzad Z (2005). Chemical composition of the essential oil of three Iranian *Satureja* species (*S. mutica* Fisch & C.A., S. *macrantha* and *S. intermedia*). Food Chem., 91: 1– 4.
Singh G (1996). Studies on fungicidal activity of essential oil. Eur. Cosmet. 4:27-32.
Singh G, Upadhyaya RK (1993). Essential oils. A potent source of natural pesticides. J. Sci. Ind. Res. 52:676-683.
Soković M, Marin PD, Brkć D, Van Griensven LJLD (2007). Chemical composition and antibacterial activity of essential oils of ten aromatic plants against human pathogenic bacteria. Food, 1: 220-226.
Tsimidou M, Boskou D (1994). Antioxidant activity of essential oils from the plants of the Lamiaceae family. In Spices, Herbs and Edible Fungi, ed. G. Charalambous. Elsevier, Amsterdam. Pp. 273-284.
Tumen G, Baser KHC (1996). Essential oil of *Saturejae spicigera* (C. Koch) from Turkey. J. Essent. Oil Res., 8: 57-58.
Tzortazakis NG, Costas D, Economakis G (2007). Antifungal activity of lemongrass (*Cympopogon citrates* L.) essential oil against key postharvest pathogens. Inn. Food Sci. Emerg. Technol., 8: 253-258.
Wilson CL, Wisniewski ME (1989). Biological control of postharvest diseases of fruits and vegetables: an emerging technology. Ann. Rev. Phytopathol., 27: 25-41.
Wisniewski ME, Wilson CL (1992). Biological control of postharvest diseases of fruits and vegetables: recent advances. HortScience, 27: 94-98.
Zargar A (1990). Medicinal Plants. 4th Edn., Tehran University Publications. Tehran pp. 42-45.