

Seasonal Variations in the Chemical Compositions of Essential Oils of Selected Aromatic Plants Growing Wild in Turkey

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The summer leaves of *Thymbra spicata* var. *spicata* and *Satureja thymbra*, two Labiatae aromatic shrubs growing wild in the East Mediterranean region of Turkey, and, in addition, the leaves of *Salvia fruticosa*, *Mentha pulegium*, *Laurus nobilis*, and *Inula viscosa* were collected in 4-week intervals to follow plant development and essential oil composition. The essential oils extracted from leaves (and flowering tops) were estimated during the growing season by means of GC–FID, and 19 terpenic constituents were identified. The changes in the essential oil content (quantity and composition) varied for all six plant species, according to corresponding environmental and growth factors and the major adaptive strategy toward summer drought that each plant species has evolved. The concentration of the fungitoxic components in the essential oils of *Thymbra spicata* and *Satureja thymbra*, the phenolic constituents carvacrol and thymol, were low in the early phenological stage and increased gradually with plant development. The maximum was reached in June/July, shortly after flowering. Taking this into account, the harvest of these two species in order to obtain their essential oils offers acceptable economic possibilities for their use as a natural fungicide. The main compounds of the essential oils found in *Salvia fruticosa*, *Laurus nobilis*, and *Mentha pulegium* were the oxygen-containing monoterpenes 1,8-cineole and pulegone, respectively, and a periodic increase and decrease in their concentrations was observed. The essential oil of *Inula viscosa* contained only small amounts of some of the investigated components. The best time of harvest to gain the essential oils with the highest active ingredients is July for *Thymbra spicata*, *Satureja thymbra*, *Salvia fruticosa*, and *Mentha pulegium* (Labiatae) and September for *Laurus nobilis* (Lauraceae).

Keywords: Essential oils; seasonal variations; thymol; carvacrol; *Thymbra spicata*; *Satureja thymbra*

INTRODUCTION

Thymbra spicata var. *spicata* and *Satureja thymbra* (Labiatae) were sampled in the countries of the Mediterranean basin for centuries for use as local spices ("Za atar"), together with *Majorana syriaca* and *Coriandrum capitatum* (Ravid and Putievsky, 1983, 1985). *Satureja thymbra* is also used as a herbal home remedy, especially for its antiseptic, tonic, stimulating, gastro-sedative, and diuretic properties (Capone *et al.*, 1989). Because of the harsh phenolic character of the oils, they are reminiscent of the taste and fragrance of commercial oregano and thyme oils. In a set of experiments, *Thymbra spicata* and *Satureja thymbra* were used for controlling stored-product insects (Sarac and Tunc, 1995a,b), and their essential oils were effective toward *Sitophilus oryzae* adults and the last instars of *Ephestia kuehniella*.

In previous studies, Yegen *et al.* (1992) and Müller-Riebau *et al.* (1995) have reported that the essential oils of *Thymbra spicata* and *Satureja thymbra*, and their main phenolic constituents, carvacrol and thymol, show a remarkable antifungal activity by inhibition of the mycelial growth of the soil-borne phytopathogenic fungi *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Phytophthora capsici*, one of the most important plant pathogens of pepper growing in the

East Mediterranean region of Turkey. During the period of these studies, we followed the variations of the essential oil concentrations and their main terpenic constituents in the summer leaves of *Thymbra spicata*, *Satureja thymbra*, and further Turkish aromatic plants during the growing season from early spring to late summer. The purpose of this study was—on the basis of the known antifungal activity of a number of constituents of the essential oils—to determine the variations of the essential oil compositions of the species throughout the time period investigated and to estimate the best time of sampling for their use as antifungal agents.

MATERIALS AND METHODS

Plant Species and Sample Collection. The two aromatic perennial shrubs we have studied, *Thymbra spicata* L. var. *spicata* (Labiatae) and *Satureja thymbra* L. (Labiatae), as well as *Salvia fruticosa* Miller syn. *S. triloba* L. (Labiatae), *Mentha pulegium* L. (Labiatae), *Laurus nobilis* L. (Lauraceae), and *Inula viscosa* (L.) Aiton (Compositae), were collected in 1994 in 4-week intervals from three wild-growing populations each in Antalya, Termessus, Aksu, Düden, and Kalkan (Turkey) for analytical examination.

Preparation of the Essential Oils. The necessary quantities for the analyses were always collected from the upper part of the plants. Leaves were separated from the branches. Separation of the essential oils from the plant tissue was conducted by steam distillation in a Clavenger apparatus for about 3 h. In the reproductive phase, the leaves and flowering tops were not distilled separately, except in the cases of *Salvia fruticosa* and *Laurus nobilis*. The quantities analyzed each time were 70-g portions of plant material (fresh weight). The results are the mean of the three wild-growing populations

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Table 1. Dry Weight (% w/w), Essential Oil Contents (% v/w; on the Basis of 100 g of Dried Leaves), and Their Terpenic Constituents (in mg/mL) Extracted from the Summer Leaves of *Thymbra spicata* L. Var. *spicata*, Collected in Aksu

<i>T. spicata</i> , Aksu	t_R , ^a min	Feb	March	April	May	June	July	Aug	Sept
dry wt		<i>b</i>	67.0	78.3	70.7	64.3	46.0	<i>b</i>	<i>b</i>
essential oil content		<i>b</i>	3.1	2.9	4.2	4.8	10.8	<i>b</i>	<i>b</i>
α -pinene	6.60	<i>b</i>	2.5	2.5	2.4	3.1	3.7	<i>b</i>	<i>b</i>
camphene	7.01	<i>b</i>	<i>t</i> ^c	<i>t</i>	<i>t</i>	0.5	0.5	<i>b</i>	<i>b</i>
β -pinene	7.86	<i>b</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>b</i>	<i>b</i>
β -myrcene	8.21	<i>b</i>	5.0	8.1	7.3	7.1	8.6	<i>b</i>	<i>b</i>
limonene	8.76	<i>b</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>b</i>	<i>b</i>
α -terpinene	9.05	<i>b</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>b</i>	<i>b</i>
<i>p</i> -cymene	9.35	<i>b</i>	55.6	20.5	16.8	40.2	54.0	<i>b</i>	<i>b</i>
1,8-cineole	9.68	<i>b</i>	13.4	6.8	7.4	10.2	6.7	<i>b</i>	<i>b</i>
γ -terpinene	10.89	<i>b</i>	40.0	82.9	79.8	40.7	65.2	<i>b</i>	<i>b</i>
linalool	12.45	<i>b</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>	<i>b</i>	<i>b</i>
camphor	14.06	<i>b</i>	nd ^d	nd	nd	nd	nd	<i>b</i>	<i>b</i>
menthone	14.81	<i>b</i>	nd	nd	nd	nd	nd	<i>b</i>	<i>b</i>
borneol	15.49	<i>b</i>	4.9	2.9	1.5	6.8	8.3	<i>b</i>	<i>b</i>
menthol	15.99	<i>b</i>	nd	nd	nd	nd	nd	<i>b</i>	<i>b</i>
terpinen-4-ol	16.16	<i>b</i>	2.0	<i>t</i>	<i>t</i>	3.8	2.8	<i>b</i>	<i>b</i>
pulegone	18.88	<i>b</i>	nd	nd	nd	nd	nd	<i>b</i>	<i>b</i>
thymol	22.05	<i>b</i>	7.7	8.9	8.7	9.1	<i>t</i>	<i>b</i>	<i>b</i>
carvacrol	22.58	<i>b</i>	202.2	353.2	286.1	481.9	489.9	<i>b</i>	<i>b</i>
β -caryophyllene	29.58	<i>b</i>	8.3	10.1	9.2	11.5	9.4	<i>b</i>	<i>b</i>

^a t_R = retention time. % w/w = percent (weight/weight); % v/w = percent (volume/weight). ^b Not investigated. ^c *t* = traces (<1.0 mg/mL). ^d nd, not detected.

collected at each time from the same sites. Essential oil concentration is expressed in milliliters/100 g of dry weight of plant material. The moisture content of the material analyzed was determined after oven-drying at 105 °C for 24 h.

Gas Chromatography. The essential oils were run in random order on a Shimadzu Model GC-14A gas chromatograph, fitted with flame ionization detection (FID), a split-splitless injector, and a linear temperature programmer. The gas chromatograph was connected to a recorder-integrator (Shimadzu Model C-R4A chromatopac). A fused silica column MN 3681-8A (Machery-Nagel, Düren, Germany) was employed, loaded with SE-30 liquid phase (column dimensions: 50 m \times 0.32-mm i.d.). The operating conditions were the following: injector, 230 °C; detector, 250 °C; initial column temperature, 75 °C for 2.5 min, raised at 2.5 °C/min to 175 °C and held for 2.5 min; carrier gas, nitrogen; split ratio, 1:40; volume injected, 1.0 μ L. The terpenic constituents were identified by comparison of their retention times with those of known compounds and cochromatographed with authentic samples of compounds to cross-check the identity of the constituents (Müller-Riebau *et al.*, 1995).

Biological Assays. The antifungal properties of the steam-distilled essential oils of the abovementioned plants and their main constituents (carvacrol, thymol, *p*-cymene, γ -terpinene, 1,8-cineole, camphor, and pulegone) were tested *in vitro*—as described by Müller-Riebau *et al.* (1995)—using the test fungi *Fusarium moniliforme* Sheldon, *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Phytophthora capsici* Leonian. After the addition of 100 μ L of the essential oil to 100 mL of potato-dextrose-agar (Sigma), the mycelial growth was measured on culture plates. Four replicates were inoculated with 7-mm disks of fungus mycelium and incubated in the dark at 23.5 °C for 7 days. Furthermore, appropriate amounts of reference samples were added to the culture medium to give concentrations of 25–250 μ g/mL potato-dextrose-agar.

RESULTS AND DISCUSSION

Thymbra spicata var. *spicata* and *Satureja thymbra*, two perennial Labiatae aromatic shrubs growing wild in the East Mediterranean region of Turkey, are seasonally dimorphic plants, reducing their transpiring surface at the beginning of spring by changing their big winter leaves with small summer leaves. The monthly variations of the amounts and the compositions of essential oils of *Thymbra spicata* and *Satureja thymbra* summer leaves collected prior to, during, and after flowering from different locations in the East Mediter-

ranean region of Turkey were studied. Common trends may be identified, despite the variations in the species and environmental conditions. The amount of oils and the concentrations of the phenolic ingredients are lowest in spring and highest in June/July, the full bloom period. We propose that a close relationship exists between the maturity stages and the production of essential oil and phenolic compounds in the plants. The changes in the essential oil concentrations of *Thymbra spicata* summer leaves from Aksu throughout the growing season and the amounts of their terpenic constituents—according to increasing retention times—are presented in Table 1.

The oxygen-containing phenolic monoterpene carvacrol was found to be the major constituent in the essential oil of *Thymbra spicata*, followed by moderate amounts of the monoterpene hydrocarbons γ -terpinene and *p*-cymene, which are biosynthetically related (Poulose and Croteau, 1978). The qualitative composition of the essential oil from Turkish *Thymbra spicata* shows comparable results to that of plants of Israeli origin (Ravid and Putievsky, 1985). The qualitative compositions of the terpenes identified in the essential oils of *Thymbra spicata* appeared to be constant in the three phenological stages, but there were notable differences in the amounts of several compounds: *p*-cymene, γ -terpinene, and carvacrol always showed the highest concentrations and represented about 30% (in spring) to 60% (in summer) of the essential oil. In particular, the essential oil content and the amount of the main compound, carvacrol, generally increased according to plant development and reached its maximum at plant maturity (10.8 mL/100 g of dried leaves; 489.9 mg/mL), just before leaf fall in August. A marked substantial decrease in the level of carvacrol was found in May, due to intense growth activity. A biosynthetically related increase and decrease in the periodicity of the concentrations can be observed for *p*-cymene and γ -terpinene, while the other values showed no significant change. The dry weight of *Thymbra spicata* plant material notably decreased in July, due to the flowering tops, but the yield of the essential oil attained its highest values.

No considerable variation in the essential oil composition was encountered when plant material collected in Aksu was compared with the samples from Düden,

Table 2. Dry Weight (% w/w), Essential Oil Contents (% v/w), and Their Terpenic Constituents (in mg/mL) Extracted from the Summer Leaves of *Satureja thymbra* L., Collected in Antalya^a

<i>S. thymbra</i> , Antalya	<i>t_R</i> , min	Feb	March	April	May	June	July	Aug	Sept
dry wt		65.0	69.0	76.0	72.5	66.3	48.0		
essential oil content		3.3	2.6	3.7	4.5	6.8	8.1		
α -pinene	6.60	4.2	4.3	3.2	2.4	4.3	4.1		
camphene	7.01	t	0.5	t	t	t	t		
β -pinene	7.86	2.2	2.5	1.0	t	1.2	t		
β -myrcene	8.21	5.1	5.1	7.2	5.6	8.7	8.9		
limonene	8.76	t	t	t	t	t	t		
α -terpinene	9.05	t	t	t	t	t	t		
<i>p</i> -cymene	9.35	95.4	77.4	31.8	30.4	58.5	74.1		
1,8-cineole	9.68	14.7	20.4	9.9	11.3	9.3	5.0		
γ -terpinene	10.89	21.5	53.8	168.4	103.7	128.5	139.2		
linalool	12.45	6.2	4.1	2.9	3.2	10.1	11.0		
camphor	14.06	nd	nd	nd	nd	nd	nd		
menthone	14.81	nd	nd	nd	nd	nd	nd		
borneol	15.49	3.6	2.6	0.5	t	1.9	3.4		
menthol	15.99	nd	nd	nd	nd	nd	nd		
terpinen-4-ol	16.16	4.8	3.7	1.9	2.4	3.7	4.3		
pulegone	18.88	nd	nd	nd	nd	nd	nd		
thymol	22.05	96.6	92.2	169.1	172.3	366.3	418.2		
carvacrol	22.58	47.2	21.6	23.7	19.8	63.9	55.0		
β -caryophyllene	29.58	14.7	14.3	20.9	15.7	41.4	21.4		

^a Abbreviations are as in Table 1. Blanks mean not investigated.

whereas in samples from Antalya, the thymol contents were much higher prior to flowering onset. In conclusion, only small variations in chemical oil composition were apparent between different location (coastal/inland) types of this species. The dried leaves and flowering tops from Aksu had a greater oil content in July (10.8%) than those from Antalya (9.7%) and particularly from Düden (7.0%).

The concentration changes of *Satureja thymbra* leaf essential oils corresponded to those of *Thymbra spicata*, with low concentrations of phenols in February/March and the highest amounts in late summer. The results for the samples of a thymol-containing chemotype of *Satureja thymbra* from Antalya are contained in Table 2. It should be noted, with regard to the chemical compositions of the essential oils, that the occurrence of a carvacrol-containing chemovariety was only detected in plants from Aksu.

The main constituent in the essential oil of *Satureja thymbra* was the carvacrol isomer thymol, followed by *p*-cymene, γ -terpinene, and carvacrol. Other terpenes were present only in very low amounts or traces. In particular, the highest values of thymol were measured in July (418.2 mg/mL), in the presence of carvacrol in considerable amounts (55.0 mg/mL). Compared to *Thymbra spicata*, the biogenetic precursors of thymol and carvacrol, γ -terpinene and *p*-cymene, were found in higher concentrations. The concentrations of the minor components varied also periodically, but the differences between the highest and lowest values were less than 10 mg/mL. The qualitative composition of the essential oil from Turkish *Satureja thymbra* is similar to that of plants of Israeli (Ravid and Putievsky, 1983, 1985) and Greek origin (Philianos *et al.*, 1984). The same proportionality exists in the primary components of the essential oils from Sardinian *Satureja thymbra* (Capone *et al.*, 1988, 1989), collected before, during, and after the blossoming season.

The oil content of *Satureja thymbra* was also seen to generally increase with time (up to 8.1 mL/100 g of dried leaves). This was also observed by Vokou and Margaritis (1986), who reported the highest values of essential oil in *Satureja thymbra* of Greek origin in June, shortly after flowering. This characteristic periodicity in the essential oil content was also referred for other aromatic dimorphic plants of the Labiatae growing in the Medi-

terranean region, like *Thymus capitatus*, *Thymus herbarona* from Sardinia (Falchi Delitala *et al.*, 1983), *Thymus capitatus*, *Teucrium polium* from Greece (Vokou and Margaritis, 1986), and *Thymus serpylloides* from Spain (Arrebola *et al.*, 1994).

The results on essential oils of *Salvia fruticosa* (Kalkan), *Mentha pulegium* (Antalya), and *Laurus nobilis* (Termessus) are summarized in Table 3. The oxygen-containing monoterpene 1,8-cineole (=eucalyptol), one of the few cyclic terpene ethers found in aromatic plants, was detected to be the main constituent in the essential oil of *Salvia fruticosa*, confirming previous notes (Buil *et al.*, 1977; Bayrak and Akgül, 1987). A seasonal variation was noted in the 1,8-cineole content of this perennial Labiatae aromatic shrub; the maximum value was reached in July (297.3 mg/mL). The only other major volatile component was camphor, and its variation correlated closely to those of 1,8-cineole. In August, the contents of 1,8-cineole and camphor decreased slightly. Variations in the concentrations of other terpene hydrocarbons were insignificant. The flowers themselves, collected in March (when Greek sage was in full flower), contained smaller amounts of 1,8-cineole (116.3 mg/mL) and camphor (3.5 mg/mL) in the essential oil than the leaves but a much higher content of β -pinene (68.0 mg/mL). *Salvia fruticosa* attained its highest oil content in August (14.3 mL/100 g of dried leaves).

Although *Mentha pulegium*, a small perennial labiateous plant, can be easily confused with *Mentha spicata*, the ingredients of these two species are different: *Mentha pulegium* contains pulegone, a keto cyclic monoterpene, and menthone as its major ingredients. Pulegone reached its highest concentration in July (419.6 mg/mL), although it never reached the amounts described by Skrubis (1972), indicating significant differences between cultivars. It is interesting that the highest content of menthone was measured in April (166.0 mg/mL). There was a small decrease in dry weight and an increase in essential oil content during the growing season from April to August (from 1.6 to 5.2 mL/100 g of dried leaves).

Laurus nobilis is an evergreen tree belonging to the Lauraceae, and laurel leaves have been used as a spice since antiquity, primarily because of their essential oil content. In Turkey, laurel leaf is harvested mainly from

Table 3. Dry Weight (% w/w), Essential Oil Contents (% v/w), and the Most Abundant Terpenic Constituents (in mg/mL) Extracted from the Leaves of *Salvia fruticosa* L. (Kalkan), *Laurus nobilis* L. (Termessus), and *Mentha pulegium* L. (Antalya)^a

	t _R , min	Feb	March	April	May	June	July	Aug	Sept
<i>S. fruticosa</i> , Kalkan									
dry wt		66.3	67.0	69.0	65.3	47.3	35.0	31.0	
essential oil content		3.5	3.0	4.0	4.1	7.1	12.5	14.3	
β-pinene	7.86	15.4	14.1	9.1	28.3	18.1	25.9	17.2	
1,8-cineole	9.68	219.4	173.0	91.6	130.7	185.3	297.3	193.3	
camphor	14.06	50.2	36.2	9.9	17.2	28.6	75.8	37.3	
β-caryophyllene	29.58	7.2	12.6	7.3	25.8	9.7	12.4	8.7	
<i>L. nobilis</i> , Termessus									
dry weight		52.0	48.0	47.7	48.3	47.7	51.0	46.0	43.0
essential oil content		5.0	6.8	7.8	7.3	5.1	6.2	4.1	10.2
α-pinene	6.60	14.6	12.6	14.7	9.5	17.0	14.6	8.0	7.0
β-pinene	7.86	13.3	10.2	12.1	7.5	13.1	12.3	8.7	7.0
1,8-cineole	9.68	197.8	127.4	178.7	115.9	204.1	201.5	231.1	169.8
terpinen-4-ol	16.16	16.0	5.3	13.8	5.3	8.3	13.3	17.8	11.1
<i>M. pulegium</i> , Antalya									
dry wt				79.0	82.0	72.0	63.0	62.0	
essential oil content				1.6	2.1	3.6	3.6	5.2	
1,8-cineole	9.68			35.2	40.1	19.8	37.9	21.2	
menthone	14.81			166.0	50.8	12.2	73.0	73.4	
borneol		15.49			16.7	47.6	43.4	27.2	10.8
pulegone	18.88			39.6	311.4	378.8	419.6	202.8	

^a Abbreviations are as in Table 1. Blanks mean not investigated.

wild-growing plants. However, the essential oil of *Laurus nobilis* was characterized by the presence of 1,8-cineole as the main constituent. The amount of 1,8-cineole content differed considerably from those of other oils described (Skrubis, 1972; Akgül *et al.*, 1989), but these kinds of variations are known, responding to geographical origin, harvesting time, and growth conditions. Apparently, major changes occurred in the content of 1,8-cineole, with the lowest values in March (during the process of maturation of the flowering tops) and May; minor variations were detected for the other terpenic constituents during the early to late summer months. It should be noted that 1,8-cineole (61.7 mg/mL) and the sesquiterpene β-caryophyllene (27.8 mg/mL) were the most prevalent components in the essential oils of *Laurus nobilis* flowers, collected in March. No periodicity was found concerning the dry weights of the leaves, and the monthly percentages of *Laurus nobilis* leaf volatile oil varied relatively little regardless of the month or stage of harvest. Dry leaves in September had a high essential oil content (10.2 mL/100 g of dried leaves), suggesting that there is an optimal time for harvest. No wide variations were apparent between different types of species: *Laurus nobilis* encountered no significant differences in the foliage essential oil when coastal (Antalya) and inland populations (Termessus) were compared.

Essential oils are known to possess substances that may be fungicidal or fungistatic in nature activity (Yegen *et al.*, 1992; Müller-Riebau *et al.*, 1995). In order to compare the inhibitory effect between the oils and their major constituents, thymol, carvacrol, *p*-cymene, γ-terpinene, 1,8-cineole, camphor, and pulegone were added individually to the culture medium at concentrations similar to those in the neat essential oils. The results show (Table 4) that the inhibitory effect of the essential oils was mainly due to the most abundant components and not to the other associated substances. The phenolic compounds thymol and carvacrol possessed an inhibitory effect with minimum inhibitory concentrations of approximately 100 μg/mL potato-dextrose-agar, while the other components showed up to a concentration of 250 μg/mL medium with only a slight or no mycelial growth inhibition. Furthermore, the results indicate that only the essential oils of *Thymbra spicata* and *Satureja thymbra* containing considerable

Table 4. Inhibition of Mycelial Growth of the Test Fungi (Colony Diameter Method) by 100 μL of the Essential Oils of Different Plant Species in 100 mL of Potato-Dextrose-Agar (Average Values), and Their Main Constituents (100 μg/mL of Potato-Dextrose-Agar)

essential oil/main constituent	F. moniliforme	R. solani	S. sclerotiorum	P. capsici
<i>Thymbra spicata</i>	100.0 ^a	100.0	100.0	100.0
carvacrol	87.2	100.0 ^a	100.0 ^a	100.0 ^a
γ-terpinene	0.0	0.0	0.0	0.0
<i>p</i> -cymene	0.0	0.0	0.0	0.0
<i>Satureja thymbra</i>	100.0 ^a	100.0	100.0	100.0
thymol	87.9	88.6	100.0	100.0
<i>Salvia fruticosa</i>	47.3	15.0	0.0	48.4
<i>Laurus nobilis</i>	38.3	4.1	0.0	31.1
1,8-cineole	18.2	0.0	0.0	8.8
camphor	0.0	0.0	0.0	0.0
<i>Mentha pulegium</i>	50.5	0.0	0.0	21.7
pulegone	0.0	0.0	0.0	0.0
menthone	0.0	0.0	0.0	0.0
<i>Inula viscosa</i>	35.6	0.0	0.0	39.1

^a Inhibition only fungistatic.

amounts of the phenolic components thymol and/or carvacrol were strongly inhibitory to all the fungi examined.

Thymbra spicata and *Satureja thymbra* grow wild in the East Mediterranean region of Turkey, where their branches are gathered manually by local farmers. The time of flowering onset is an indication for them that the plant is ready to be collected: the product would then consist of leaves with only a few flowering tops. However, concerning the highest yields of antifungal ingredients from the essential oils of *Thymbra spicata* and *Satureja thymbra*, collections in late summer, just before leaf fall in August, were preferable to the onset of flowering. Our results indicate that the phenolic compounds in the essential oils of these two species offer acceptable economic possibilities for their use as a natural fungicide, especially in combination with the common technique of soil solarization (Yügel, 1995). Compared to that, the low yields of phenolic compounds in the volatile oils from *Salvia fruticosa*, *Laurus nobilis*, *Mentha pulegium*, and *Inula viscosa*, a perennial Mediterranean widespread weedy compositae well-known for its strong odor mainly due to various volatile compounds, do not appear to offer alternative economic possibilities. This result is in accordance with Shimoni

et al. (1993), who tested the essential oils of *Satureja thymbra*, *Salvia triloba*, and further aromatic plants for their antifungal activity against the soil-borne plant pathogens *Fusarium oxysporum* f. sp. *vasinfectum* and *Macrophomina phaseolina*. Qasem *et al.* (1995) reported that *Inula viscosa*, incorporated as dried shoot material or crude shoot water extracts in a culture medium, showed slightly antifungal effects on the mycelial growth of tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici*. From gas chromatogram profiles, we could conclude that the essential oils of *Inula viscosa* contained only small amounts of some of the investigated terpenic constituents. The content of the phenolic compounds thymol and carvacrol was rather low, with highest values of approximately 50 mg/mL in May and June.

To use the essential oils of *Thymbra spicata* var. *spicata* and *Satureja thymbra* (and their phenolic compounds) as a natural fungicide, further work is needed to investigate the practical implications and nontoxic dosage of this natural source of antifungal agents. First, investigations under greenhouse and plastic tunnel conditions had indicated that 10 mL of these essential oils in 50 g of perlite/m² of soil seems to be effective against *Phytophthora capsici* in pepper growing. If the essential oils were applied either in their extracted form in low concentrations or as dried aromatic plant material, which implies much reduced costs, the inhibition exerted by the crude herbs was less effective to noneffective to that of the equivalent quantity of the essential oil conditions.

Although it is known that the essential oils extracted from *Satureja thymbra*, *Salvia fruticosa*, *Laurus nobilis*, and *Mentha pulegium* possess potent antibacterial activities (Akgül *et al.*, 1989; Capone *et al.*, 1989; Vokou *et al.*, 1993), it is also known that some soil-borne bacteria are able to catabolize these compounds and use them as carbon and energy sources (Vokou *et al.*, 1984; Vokou and Margaritis, 1988). Further studies are also necessary to learn more about the decomposition of these terpenes by soil microorganisms.

ABBREVIATIONS USED

f. sp., formae specialis; var., variety.

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