Chemical Composition and Fungitoxic Properties to Phytopathogenic Fungi of Essential Oils of Selected Aromatic Plants Growing Wild in Turkey

Frank Müller-Riebau,*,† Bernhard Berger,† and Oktay Yegen‡

Institut für Pflanzenpathologie und Pflanzenschutz der Universität Göttingen, Grisebachstr. 6, 37077 Göttingen, Germany and Akdeniz Üniversitesi, Dumlupinar Bulvari, Kampüs - 07003 Antalya, Turkey

Essential oils of *Thymbra spicata*, *Satureja thymbra*, *Salvia fruticosa*, *Laurus nobilis*, *Mentha pulegium*, *Inula viscosa*, *Pimpinella anisum*, *Eucalyptus camaldulensis*, and *Origanum minitiflorum* plants growing wild in southern Turkey were investigated by means of GC-FID, and 20 components were identified. The main ones were γ-terpinene, *p*-cymene, thymol, and carvacrol as well as 1,8-cineole, pulegone, and anethole. Biological assays showed that fungitoxicity against the soil-borne plant disease-causing fungi *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Phytophthora capsici* was due to different concentrations of the phenolic fraction (especially thymol and/or carvacrol) in the essential oils.

Keywords: *Essential oils; antifungal activity; thymol; carvacrol; 1,8-cineole*

Yegen *et al.* (1992) reported that the aqueous extracts and essential oils of several aromatic plants growing as wild populations in southern Turkey exhibited fungitoxicity against soil-borne phytopathogenic fungi *in vitro*. The essential oils of *Thymbra spicata* and *Satureja thymbra* were most effective in inhibiting mycelial growth of the test fungi *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Phytophthora capsici* with minimum inhibitory concentrations between 400 and 800 μg/mL medium. Investigations with thin-layer chromatography implicated thymol and carvacrol as major antifungal active compounds, but the identification of further substances responsible for this inhibition has not yet been completed.

Thymbra spicata and *Satureja thymbra* (Labiatae) grow wild in some eastern Mediterranean countries. Plants of the two species are traditionally used in local spice ("Za'atar"), together with *Majorana syriaca* (L.) Rafin. and *Coridothymus capitatus* (L.) Rchb. fil. (Ravid and Putievsky, 1983, 1985; Fleisher *et al.*, 1984). *Satureja thymbra* is also being used in folk medicine for its antiseptic, tonic, stimulating, gastrosedative, 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.

The purpose of this study was the chemical analysis of the essential oil of *Thymbra spicata*, *Satureja thymbra*, *Salvia fruticosa*, *Laurus nobilis*, *Mentha pulegium*, *Inula viscosa*, and further Turkish wild aromatic plants and the determination of their fungitoxic components by biological tests—in view of a use, if possible, as natural fungicides.

MATERIALS AND METHODS

Plant Species. Plant material of *Thymbra spicata* L., *Satureja thymbra* L., *Salvia fruticosa* Mill., *Laurus nobilis* L., *Mentha pulegium* L., *Inula viscosa* (L.) Ait., *Pimpinella anisum* L., and *Eucalyptus camaldulensis* Dehn used in this investigation was collected (during the summers of 1991, 1992, and 1993) from different wild populations in southern Turkey in the province of Antalya. Furthermore, wild growing plants, conforming to the botanical description of *Origanum minitiflorum* Shwars et Davis, were collected at the hills near Korkuteli in July 1993.

Isolation of the Essential Oils. Leaves were separated from branches, and the dried leaves (and flowering tops) were ground to a coarse powder: 50 g of powdered plant materials were steam-distilled with 500 mL of double distilled water for about 3 h until the complete extraction of the oil was achieved. The separation from the water was conducted by petroleum ether (boiling range 60–80 °C). The extract was dried over anhydrous Na_2SO_4, the solvent evaporated, and the essential oil stored in the dark at 4 °C until further analysis. Highest yields of essential oil were obtained from flowering *Origanum minitiflorum*: 4.34 mL/100 g of dry leaf weight.

Gas Chromatography. Essential oil composition was analyzed using 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 (Macherey-Nagel, Düren, Germany) was employed, loaded with SE-30 liquid phase (column dimensions: 50 m × 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 was nitrogen; split ratio 1:40, volume injected 1.0 μL.

Stock solutions for the two-point calibration graph were prepared containing 50 mg/mL of the internal standard (eugenol) with 5 and 50 mg/mL of the following terpenic components (supplied by Fluka Chemie AG, Buchs, Switzerland) in *n*-hexane as solvent: (1*S*)-(−)-α-pinene, (−)-camphene, (1*S*)-(−)-β-pinene, β-myrcene [Sigma M 0382], (±)-limonene, α-terpinene, *p*-cymene, 1,8-cineole, γ-terpinene, (±)-linalool, (±)-camphor, menthone, (−)-borneol, menthol, (+)-terpinen-4-ol, (*R*)-(+)-pulegone, anethole, thymol, carvacrol, and (−)-*trans*-caryophyllene; calibration curves were linear between 0.5 and 100 mg/mL. The essential oils were diluted in *n*-hexane to adequate volumes prior to injection. The identities of the peaks were confirmed by comparison of their retention times (min) with those of reference samples and, in case of doubt, by their mass spectra, obtained by gas chromatography–mass spectrometry (GC-MS).

Test Microorganisms. The antifungal properties of the

† Universität Göttingen.
‡ Akdeniz Üniversitesi.

0021-8561/95/1443-2262$09.00/0

Table 1. Main Components (mg/mL) of Essential Oils Obtained from *T. spicata*, *S. thymbra*, *S. fruticosa*, *L. nobilis*, *M. pulegium*, *I. viscosa*, and *O. minitiflorum*, Collected in 1993

no.	component	t_R, min	*T. spicata* 1993.an[a]	*S. thymbra* 1993.an	*S. fruticosa* 1993.ka	*L. nobilis* 1993.te	*M. pulegium* 1993.ak	*I. viscosa* 1993.an	*O. minitiflorum* 1993.ko
1	α-pinene	6.60	6.0	6.6	27.6	1.3	1.4	t	t
2	camphene	7.01	2.5	1.5	11.7	–	t	–	t
3	β-pinene	7.86	1.6	2.4	20.4	1.6	t	–	t
4	β-myrcene	8.21	14.5	13.7	9.6	t	1.1	t	t
5	limonene	8.76	1.7	2.3	t	t	–	–	–
6	α-terpinene	9.05	t[b]	t	–	–	–	–	–
7	*p*-cymene	9.35	90.1	93.5	12.3	t	t	1.5	6.8
8	1,8-cineole	9.68	4.6	5.7	456.1	121.4	34.7	–	1.2
9	γ-terpinene	10.89	86.4	216.3	9.8	1.7	t	t	1.5
10	linalool	12.45	2.0	8.0	17.0	15.2	2.5	t	t
11	camphor	14.06	–[c]	–	36.4	t	–	–	–
12	menthone	14.81	–	–	–	–	5.4	–	–
13	borneol	15.49	10.4	3.0	13.5	4.7	13.8	t	3.7
14	menthol	15.99	–	–	–	–	–	t	–
15	terpinen-4-ol	16.16	3.9	4.7	4.0	7.8	1.1	–	4.2
16	pulegone	18.88	–	–	–	–	205.5	–	–
17	thymol	22.05	87.2	273.8	39.0	t	t	t	1.5
18	carvacrol	22.58	439.3	27.7	9.2	t	t	5.7	559.4
19	β-caryophyllene	29.58	13.0	32.7	6.9	2.0	t	t	5.6

[a] Collected in: an (Antalya), ka (Kalkan), te (Termessus), ak (Aksu), ko (Korkuteli). [b] t = traces (<1 mg/mL). [c] – means not detectable.

steam-distilled essential oils were tested *in vitro* with *Fusarium moniliforme* Sheldon, *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Phytophthora capsici* Leonian.

Biological Assays. The antifungal properties of the reference samples were tested *in vitro* according to the poisoned-food technique of Grover and Moore (1962) by determining the minimum concentration inhibiting in a solid medium with six concentrations between 25 and 250 μg/mL in potato–dextrose–agar by dissolving the requisite amount of the component in 0.1 mL of acetone and then mixing with 9.9 mL of the medium separately. Four replicates were inoculated with 7 mm discs of fungus mycelium and incubated in the dark at 23.5 °C, and mycelial growth was measured after 7 days. In addition, the antifungal activities of the synthetic fungicides Folicur EC 250, Previcur N, Sportak, Rovral, Maneb 80, and the active ingredient of Baytan, Triadimenol, were investigated using the test fungi mentioned above. Essential oils were tested in culture plates similar to the bioassay procedures at a concentration of 1000 μL/L. The fungistatic–fungicidal nature of essential oils and reference components were tested by observing revival of growth of the inhibited mycelial disc following its transfer to (nontreated) potato–dextrose–agar: Compounds were classified as fungistatic if any mycelial growth was observed after a further 10 days.

Thin Layer Chromatography. Analytical thin-layer chromatographic studies of the essential oils, with emphasis on their phenolic components, were done on TLC aluminum sheets precoated with a layer thickness of 0.2 mm silica gel 60 (Merck AG, Darmstadt, Germany). Plates of 10 × 10 cm were developed in chloroform–dichloromethane–*n*-hexane (45:45:10 v/v/v). The essential oils and the compounds thymol and carvacrol (for cochromatography) were applied in dichloromethane solutions. After chromatography, the air-dried plates were sprayed with a *p*-anisaldehyde–sulfuric acid solution and heated in an oven at 120 °C to develop the essential oil spots of thymol and carvacrol.

Biological activities of the separated volatile oil constituents were detected after preparative thin-layer chromatography. After development with the respective solvent system, one half of a 20 × 10 cm-plate was sprayed with *Cladosporium herbarum* Link ex Fr. spore suspensions to locate the antifungal spots and incubated at 23.5 °C for 7 days in a moisture chamber. The other (nonsprayed) part was stored at 4 °C. The nonsprayed developed areas, situated at the level of the reference spots, were separately scraped out, and the silica gel was extracted with 1000 μL of methanol and centrifuged (12.000 rpm). The solvent of an aliquot of 900 μL was evaporated, the residue was dissolved in 50 μL *n*-hexane, and 1 μL was injected into the gas chromatograph.

RESULTS

Gas Chromatography. The constituents of the essential oils of the leaves of *T. spicata*, *S. thymbra*, *S. fruticosa*, *L. nobilis*, *M. pulegium*, *I. viscosa*, and *O. minitiflorum* are listed in Table 1 according to increasing retention times.

The thymol isomer carvacrol was the major constituent in the essential oils of *T. spicata* (Labiatae), followed by moderate concentrations of the monoterpene hydrocarbons γ-terpinene and *p*-cymene. The qualitative composition of the oils were similar in all samples, and the content of the phenolic fraction in the oils appeared to be constant. The highest carvacrol content (515.8 mg/mL), in the presence of thymol in small quantities (8.4 mg/mL), was detected in an essential oil of *T. spicata* plants collected near Aksu in July, 1993, while the lowest carvacrol content (439.3 mg/mL), in the presence of thymol in moderate quantities (87.2 mg/mL), was detected in an essential oil collected in Antalya in July, 1993. In the three years of the investigation, the thymol-containing chemovariety of *S. thymbra* (Labiatae) was abundant: Thymol, γ-terpinene, and (with a lower content) *p*-cymene were prevalent in the essential oils with respect to the other components. Carvacrol and β-caryophyllene were still found in considerable amounts. However, GC analysis of essential oils of *T. spicata* and *S. thymbra* showed that the oils obtained from *T. spicata* contained larger proportions of phenols (= carvacrol plus thymol) with a smaller amount of γ-terpinene and β-caryophyllene than in the oils obtained from *S. thymbra*. The relative content of *p*-cymene, a metabolic product of γ-terpinene, and the other essential oil components were comparable in the oils of *T. spicata* and *S. thymbra*.

The essential oils of *S. fruticosa* (Labiatae) and *L. nobilis* (Lauracae) contained mainly 1,8-cineole (= eucalyptol), one of the few cyclic terpene ethers found in aromatic plants, while the main constituent of the essential oil of *Mentha* sp. (Labiatae) was pulegone. The essential oil of *I. viscosa* (Compositeae) contained only small amounts of some of the investigated components. The essential oil of flowering plants of *O. minitiflorum* (Labiatae) contained mainly carvacrol, while *p*-cymene, borneol, terpinen-4-ol, and β-caryophyllene were found in small quantities.

Table 2. Inhibition of Mycelial Growth of the Test Fungi (Colony Diameter Method, Deviation from the Control in %) by 1000 μL of the Essential Oils of Different Plant Species in 1 L Potato–Dextrose–Agar (Average Values)

essential oil	*F. moniliforme*	*R. solani*	*S. sclerotiorum*	*P. capsici*
T. spicata	100.0*	100.0	100.0	100.0
S. thymbra	100.0*	100.0	100.0	100.0
S. fruticosa	47.3	15.0	0.0	48.4
L. nobilis	38.3	4.1	0.0	31.1
M. pulegium	50.5	0.0	0.0	21.7
I. viscosa	35.6	0.0	0.0	39.1
P. anisum	52.4	100.0	100.0	35.9
E. camaldulensis	46.5	59.3	0.0	68.5
O. minitiflorum	100.0	100.0	100.0	100.0

* = inhibition only fungistatic.

In addition, anethole (815.4 mg/mL) was found to be the main component in the essential oil of *P. anisum* (Umbelliferae), and due to the high content of 1,8-cineole (228.6 mg/mL), the essential oil of *E. camaldulensis* (Myrtaceae) can be classified in the same group as those of *S. fruticosa* and *L. nobilis*. It should be noted that eugenol (as the phenolic component) was detected in all of these essential oils as follows: *L. nobilis* (25.7 mg/mL), *S. fruticosa* (3.4 mg/mL), and *E. camaldulensis* (2.2 mg/mL).

Biological Assays. The inhibition of the mycelial growth of *F. moniliforme*, *R. solani*, *S. sclerotiorum*, and *P. capsici* on potato–dextrose–agar containing 1000 μL/L of an essential oil, corresponding to practical fungicide concentrations, is shown in Table 2.

The results indicate that some of the essentail oils tested (*S. fruticosa*, *L. nobilis*, *M. pulegium*, and *E. camaldulensis*) were noninhibitory or slightly inhibitory to the mycelial growth on the test fungi. Only essential oils of thymelike spices (*T. spicata*, *S. thymbra*, and *O. minitiflorum*) containing considerable amounts—more than 234.8 mg/mL—of the phenolic components thymol and/or carvacrol in the essential oil were strongly inhibitory to all the fungi examined. A low phenolic content of 101.5 mg/mL in the essential oil of *Thymus* sp. (not presented in detail here) had no appreciable effect on the mycelium growth to the fungi *in vitro*, while *Thymus* sp. with a phenolic content of 142.9 and 179.4 mg/mL, respectively, were found to be strong inhibitory to *R. solani* (inhibition only fungistatic) and *P. capsici*. The essential oil of *P. anisum* was strongly inhibitory to *R. solani* and *S. sclerotiorum*.

The studies on the antifungal activity of the reference samples showed that only concentrations of more than 100 μg/mL of thymol and carvacrol led to a complete inhibition of fungal growth (Table 3). The phenolic hydrocarbon eugenol showed a moderate inhibition, but it was less efficacious compared to thymol or carvacrol. The components α-pinene, camphene, β-pinene, β-myrcene, limonene, α-terpinene, *p*-cymene, 1,8-cineole, γ-terpinene, linalool, camphor, menthone, borneol, menthol, terpinen-4-ol, pulegone, anethole, and β-caryophyllene showed up to a concentration of 250 μg/mL only a slight or no mycelial growth inhibition. Furthermore, we investigated the efficacies of thymol and carvacrol by comparing them with some synthetic fungicides effective against the test fungi. The results for (for example) *P. capsici*, the agriculturally important soilborne plant pathogen in southern Turkey, showed that these phenolic substances inhibited this fungus better than Previcur N.

The analysis of mycelial growth of *C. herbarum* inhibitory spots on the thin-layer plates showed that thymol and carvacrol were the major fungitoxic components in *T. spicata*, *S. thymbra*, and *S. fruticosa*. Minor components of *S. fruticosa*, fractionated by thin-layer chromatography, were 1.8-cineole, linalool, and camphor. We were able to associate the antifungal activity of the essential oil of *M. pulegium* with the keto cyclic monoterpene pulegone, although the reference samples showed only a slight toxicity, up to a concentration of 250 μg/mL. None of the investigated components were present in the antifungal spots of *L. nobilis* and *I. viscosa*. Eugenol, a minor component in *L. nobilis* and *S. fruticosa*, was not detectable.

DISCUSSION

Gas Chromatography. The chemical composition of essential oils of *T. spicata* and *S. thymbra* growing wild in Israel were described by Ravid and Putievsky (1983, 1985), and the analysis of the investigated Turkish samples gave similar results: The most abundant components of the oils were (according to increasing retention times): γ-terpinene, *p*-cymene, thymol, or carvacrol, which are biosynthetically related (Poulose and Croteau, 1978), while other terpenes occurred only in low concentrations or in traces.

Capone *et al.* (1988, 1989) reported, similar to our results, that the main components of the oil of *S. thymbra* growing on the Italian island of Sardinia were γ-terpinene, *p*-cymene, thymol, and the sesquiterpene hydrocarbon β-caryophyllene. Ravid and Putievsky (1983) reported that γ-terpinene was found to be the major component of the oil of *S. thymbra* if the plants were collected when they started flowering. The highest values of oxygenated compounds (like thymol) occur in June and July, shortly after flowering (Werker *et al.*, 1985; Vokou and Margaris, 1986).

S. fruticosa (syn. *Salvia triloba* L.) grows wild in Italy (Sicily), Greece, Turkey, and Lebanon. In our investigations the content of 1,8-cineole and minor components, like the keto cyclic monoterpene camphor, exceeded those of other oils of Turkish origin described in the literature by Buil *et al.* (1977) and Bayrak and Akgül (1987). In contrast to these reports, thymol and carvacrol were found as additional minor components. The chemical composition of the Turkish laurel leaf oils, which are obtained from *L. nobilis*, examined in our investigation was comparable to the results of Akgül *et al.* (1989), who reported that a sample of laurel leaf oil collected in the province of Antalya contained mainly 1,8-cineole (followed by high percentages of α-terpinyl acetate and sabinene).

In the essential oils of *M. pulegium*, in accordance to Skrubis (1972), only the keto cyclic monoterpene pulegone was found in considerable amounts. In the current literature only a few references were found on the chemical composition of *I. viscosa* (Bohlmann *et al.*, 1977; Taillade *et al.*, 1980; Grande *et al.*, 1985), especially from plants growing in Turkey (Öksüz, 1976, 1977), but mainly flavonoids have been reported from the investigated varieties. *O. minitiflorum* is less widely distributed, and only small samples of this plant could be collected. Carvacrol was detected as the most prevalent component in the essential oil: This was not unexpected if compared with other representatives of the genus *Origanum* of Turkish origin, like *Origanum smyrnaeum* L. (Buil *et al.*, 1977) and *Origanum majorana* L. (Sarer *et al.*,1982).

Biological Assays. The mycelial growth of test fungi responded differently to the various essential oils, which

Table 3. Inhibition of Mycelial Growth of *F. moniliforme*, *R. solani*, *S. sclerotiorum*, and *P. capsici* (Colony Diameter Method, Deviation from the Control in %) at Different Concentrations (in μg/mL Potato–Dextrose–Agar) of Thymol and Carvacrol, and Inhibition of Mycelial Growth of *P. capsici* at Different Concentrations of a Soil-Applied Systemic Fungicide, Previcur N (Propamocarb Hydrochloride in μg/mL Potato–Dextrose–Agar)

	F. moniliforme		*R. solani*		*S. sclerotiorum*		*P. capsici*		
c	thymol	carvacrol	thymol	carvacrol	thymol	carvacrol	thymol	carvacrol	Previcur N
50	70.6	68.4	34.2	60.6	0.0	0.0	81.9	80.3	15.4
100	87.9	87.2	88.6	100.0*	100.0	100.0*	100.0	100.0*	23.8
150	100.0*	100.0*	100.0*	100.0	100.0	100.0	100.0	100.0	30.8
200	100.0*	100.0*	100.0*	100.0	100.0	100.0	100.0	100.0	34.4
250	100.0	100.0	100.0*	100.0	100.0	100.0	100.0	100.0	33.0

* = inhibition only fungistatic.

indicated that the oils may have different modes of action or that the metabolism of some fungi was able to better overcome the effect of the oil or adapt to it, especially *S. sclerotiorum*.

The essential oils of *T. spicata*, *S. thymbra*, and *O. minitiflorum* were strongly inhibitory to the mycelial growth of all the fungi examined. In addition, Akgül and Kivanc (1988) reported that *T. spicata* and *S. thymbra* showed also inhibitory effects on some common food-borne bacteria. Capone *et al.* (1989) found an antibacterial activity in the essential oil of *S. thymbra* with minimum inhibitory concentrations between 100 and 200 μg/mL for *Staphylococcus aureus* and 400 μg/mL for *Klebsiella pneumoniae*.

Antifungal activities of *S. fruticosa* and *I. viscosa* were reported by Akgül and Kivanc (1989) and Benjilali *et al.* (1984), respectively, but they were found to be fairly weak. Furthermore, Akgül and Kivanc (1989) and Akgül et al. (1989) reported that at a concentration of 0.01%, the leaf essential oil of *L. nobilis* did not affect any of the test microoganism investigated (several strains of bacteria and yeasts as well as some molds). In the current literature no references could be found to research of *M. pulegium* and *E. camaldulensis* having antifungal properties.

The essential oil of *P. anisum* was strongly inhibitory to the mycelial growth of *R. solani* and *S. sclerotiorum*, and this is due to the very high anethole content. Antifungal activities of *P. anisum* were reported by Shukla and Tripathi (1987) and Akgül and Kivanc (1989).

The studies on the antifungal activity of the reference samples showed that concentrations of more than 100 μg/mL of thymol (and carvacrol) led to a complete inhibition of fungal growth. The results confirm those reported by Hitokoto *et al.* (1980), Buchanan and Shepherd (1981), Farag *et al.* (1989), and Karapinar (1990), who demontrated that thymol concentrations ≥150 μg/mL completely inhibited the growth of *Aspergillus parasiticus* and *Aspergillus flavus*, respectively. Eugenol caused a moderate inhibition, but it was less efficacious compared to thymol and carvacrol. This was in accordance to Farag *et al.* (1989) but in contrast to Bullerman *et al.* (1977), who demonstrated that the essential oil of clove inhibited growth and subsequent toxin production by *A. parasiticus* at 250 ppm, and that eugenol, the principal component of clove oil, was inhibitory at a level of 125 ppm. The other components investigated showed up to a concentration of 250 μg/mL only a slight or no mycelial growth inhibition. Karapinar (1990) found an amount of 600 μg/mL as minimal inhibition concentration for menthol, while 1000 ppm was detected for *p*-cymene against *A. flavus* and *A. niger* (Tripathi *et al.*, 1986).

Generally, the evaluation of several aromatic plants growing as wild populations in southern Turkey for their antifungal activities indicates that they can be divided into groups according to the chemical structure of their volatile oil: High fungicidal activities can be found in the phenol-containing labiaceous species as opposed to weak activities in the 1,8-cineole-containing species. Indifferent activities can be found in species, characterized by the presence of other major components, like anethole or pulegone. The essential oils of the species we examined differed chemically as well as quantitatively, but the inhibitory effects of the oils were mainly due to the most abundant components and not to the other associated substances.

Yegen *et al.* (1992) reported that the essential oils of *T. spicata* and *S. thymbra* had a higher toxicity *in vitro* to *P. capsici* than the fungicides carbendazim and pentachloronitrobenzene. In view of possible antifungal applications, we investigated the efficacies of thymol and carvacrol on test fungi by comparing them with further synthetic fungicides and found that these phenolic substances had a good biological activity compared to six fungicides investigated. Thymol and carvacrol were characterized as highly fungitoxic, and it indicates that there is a relationship between the chemical structure and its antifungal effects (Farag *et al.*, 1989): Thymol and carvacrol (and eugenol) had higher inhibitory action than anethole, which might be due to the presence of a phenolic OH-group. It is well-known that the OH-group is much more reactive and can easily form hydrogen bonds with active sites of enzymes. This suggests that the possibility of using essential oils of aromatic plants growing wild in Turkey as "natural fungicides", approaching realistic fungicide levels, depends on their phenolic content of thymol and carvacrol.

LITERATURE CITED

Akgül, A.; Kivanc, M. Inhibitory effects of six Turkish thyme-like spices on some common food-borne bacteria. *Nahrung* **1988**, *32*, 201–203.

Akgül, A.; Kivanc, M. Sensitivity for four foodborne moulds to essential oils from Turkish spices, herbs and citrus peel. *J. Sci. Food Agric.* **1989**, *47*, 129–132.

Akgül, A.; Kivanc, M.; Bayrak, A. Chemical composition and antimicrobial effect of Turkish laurel leaf oil. *J. Essent. Oil Res.* **1989**, *1*, 277–280.

Bayrak, A.; Akgül, A. Composition of essential oils from Turkish *Salvia* species. *Phytochemistry* **1987**, *26*, 846–847.

Benjilali, B.; Elaraki, A. T.; Ayadi, A.; Ihlal, M. Method to study antimicrobial effects of essential oils. Application to the antifungal activity of six Moroccan essences. *J. Food Prod.* **1984**, *47*, 748–752.

Bohlmann, F.; Czerson, H.; Schöneweiss, S. New Components of *Inula viscosa* Ait. *Chem. Ber.* **1977**, *110*, 1330–1334.

Buchanan, R. L.; Shepherd, A. J. Inhibition of *Aspergillus parasiticus* by thymol. *J. Food Sci.* **1981**, *46*, 976–977.

Buil, P.; Garnero, J.; Guichard, G.; Robertet, P.; Konur, Z. Sur quelques huiles essentielles en provenance de Turquie

(Investigations on essential oils from Turkey). *Riv. Ital. E.P.P.O.S.* **1977**, *59*, 379–384.

Bullerman, L. B.; Lieu, F. Y.; Saxena, S. A. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. *J. Food Sci.* **1977**, *42*, 1107–1109, 1116.

Capone, W.; Mascia, C.; Melis, M.; Spanedda, L. Determination of terpenic compounds in the essential oil from *Satureja thymbra* L. growing in Sardinia. *J. Chromatogr.* **1988**, *457*, 427–430.

Capone, W.; Mascia, C.; Spanedda, L; Chiappini, M. Chemical composition and antibacterial activity of the essential oil from Sardinian *Satureja thymbra*. *Fitoterapia* **1989**, *60*, 90–92.

Farag, R. S.; Daw, Z. Y.; Abo-Raya, S. H. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *J. Food Sci.* **1989**, *54*, 74–76.

Fleisher, A.; Fleisher, Z.; Abu-Rukun, S. Chemovarieties of Coridothymus capitatus L. Rchb. growing in Israel. *J. Sci. Food Agric.* **1984**, *35*, 495–499.

Grande, M.; Piera, F.; Cuenca, A.; Torres, P.; Bellido, I. S. Flavonoids from *Inula viscosa*. *Planta Med.* **1985**, *53*, 414–419.

Grover, R. K.; Moore, J. D. Toximetric studies of fungicides against the brown rot organisms, *Sclerotinia fructicola* and *S. laxa*. *Phytopathology* **1962** *52*, 876–880.

Hitokoto, H.; Morozumi, S.; Wauke, T.; Sakai, S.; Kurata, H. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. *Appl. Environ. Microbiol.* **1980**, *39*, 818–821.

Karapinar, M. Influence of menthol and thymol on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *Mitt. Gebiete Lebensm. Hyg.* **1990**, *81*, 287–295.

Öksüz, S. Flavonoidal compounds of *Inula viscosa* Part I. *Planta Med.* **1976**, *29*, 343–347.

Öksüz, S. Flavonoidal compounds of *Inula viscosa* Part II. *Planta Med.* **1977**, *31*, 270–273.

Poulose, A. J.; Croteau, R. Biosynthesis of aromatic monoterpenes. Conversion of γ-terpinene to *p*-cymene and thymol in *Thymus vulgaris* L. *Arch. Biochem. Biophys.* **1978**, *187*, 307–315.

Ravid, U.; Putievsky, E. Constituents of the essentail oils from *Majorana syriaca*, *Coridothymus capitatus* and *Satureja thymbra*. *Planta Med.* **1983**, *49*, 248–249.

Ravid, U.; Putievsky, E. Composition of essentail oils of *Thymbra spicata* and *Satureja thymbra*. *Planta Med.* **1985**, *53*, 337–338.

Sarer, E.; Scheffer, J. J. C.; Svendsen, A. B. Monoterpenes in the essential oil of *Origanum majorana*. *Planta Med.* **1982**, *46*, 236–239.

Shukla, H. S.; Tripathi, S. C. The antifungal substances in oil of anise (*Pimpinella anisum* L.). *Agric. Biol. Chem.* **1987**, *51*, 1991–1993.

Skrubis, B. G. Seven wild aromatic plants growing in Greece and their essential oil. *Flavour Ind.* **1972**, *3*, 566–571.

Taillade, C.; Susplugas, P.; Balansard, G. Sur les flavonoïdes d'*Inula viscosa* Ait (Composées) (Investigations on flavonoids from *Inola viscosa* Ait (Compositae). *Plant. Med. Phytother.* **1980**, *14*, 26–28.

Tripathi, S. C.; Singh, S. P.; Dube, S. Studies on antifungal properties of essential oil of *Trachyspermum ammi* (L.) Sprague. *J. Phytopathol.* **1986**, *116*, 113–120.

Vokou, D.; Margaris, N. S. Variation of volatile oil concentration of mediterranean aromatic shrubs *Thymus capitatus* Hoffmag et Link, *Satureja thymbra* L., *Teucrium polium* L. and *Rosmarinus officinalis*. *Int. J. Biometeor.* **1986**, *30*, 147–155.

Vokou, D; Margaris, N. S.; Lynch, J. M. Effects of volatile oils from aromatic shrubs on soil microorganisms. *Soil Biol. Biochem.* **1984**, *16*, 509–513.

Werker, E.; Ravid, U.; Putievsky, E. Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiatae. *Isr. J. Bot.* **1985**, *34*, 31–45.

Yegen, O.; Berger, B.; Heitefuss, R. Untersuchungen zur fungitoxischen Wirkung der Extrakte sechs ausgewählter Pflanzen aus der Türkei auf phytopathogene Pilze (Investigations on the fungitoxicity of extracts of six selected plants from Turkey against phytopathogenic fungi). *Z. Pflanzenkrankh. Pflanzenschutz* **1992**, *99*, 349–359.

Received for review January 10, 1995. Accepted June 12, 1995.[⊗] This work was financially supported by the Volkswagen Foundation, Wolfsburg, Germany.

JF950022I

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1995.