SCREENING OF ANTIFUNGAL PROPERTIES OF ESSENTIAL OILS EXTRACTED FROM SWEET BASIL, FENNEL, SUMMER SAVORY AND THYME AGAINST POSTHARVEST PHYTOPATHOGENIC FUNGI

ALI ABDOLLAHI[1,5], ABBAS HASSANI[2], YOUBERT GHOSTA[3], MOHAMAD HADI MESHKATALSADAT[4] and RAZIEH SHABANI[3]

[1]Department of Medicinal Plants, Faculty of Agriculture and Natural Resource, Sistan and Baluchestan University, Saravan 9951634145, PO Box 99515-143, Iran
Departments of [2]Horticulture and [3]Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran
[4]Faculty of Basic Engineering Sciences, Qom University of Technology, Qom, Iran

[5]Corresponding author. TEL: +98-5485230097; FAX: +98-5485230090; EMAIL: aliabdolahi@ymail.com

Accepted for Publication January 10, 2011

doi:10.1111/j.1745-4565.2011.00306.x

ABSTRACT

The antifungal activity of essential oils of sweet basil (*Ocimum basilicum* L.), fennel (*Foeniculum vulgare* Mill.), summer savory (*Satureja hortensis* L.) and thyme (*Thymus vulgaris* L.) against two well-known postharvest fungi, *Penicillium digitatum* and *Rhizopus stolonifer*, by poison food medium (0, 200, 400, 600, 800 and 1,000 μL/L) and vapor phase (0, 5, 10, 15, 20, 25 and 40 μL) methods were assayed. The results showed that in poison food medium thyme oil had greatest antifungal activity against *P. digitatum* and *R. stolonifer* at 1,000 and ≥600 μL/L, respectively. In vapor phase, thyme oil at ≥5 μL completely inhibited the mycelial growth of pathogens. Summer savory oil at ≥600 μL/L had a significant inhibitory effect on mycelial growth of *R. stolonifer*. Fennel oil showed the lowest antifungal activity against the pathogens. All the essential oil treatments completely inhibited the growth of *R. stolonifer* in vapor phase method. Also *R. stolonifer* were more sensitive against the essential oils. Gas chromatography-mass spectrometry analysis showed that main compounds identified in sweet basil, fennel, summer savory and thyme oils were linalool (65.25%), *trans*-anethole (64.42%), carvacrol (54.14%) and β-ocimene (12.62%), respectively. Therefore, thyme and summer savory oils have a promising potential to use antifungal agent against fruit and vegetable fungi.

PRACTICAL APPLICATIONS

The results of this work show that thyme, summer savory and fennel oils have a high potential to be used as an antifungal agents for control of postharvest phytopatogenic fungi especially in vapor phase method. Therefore, essential oils as safe aromatic compounds that do have not negative effects on human health and environment may be used as a novel and practical tool for preservation of postharvest quality of horticultural crops and are suitable alternative for synthetic fungicides.

INTRODUCTION

Fruits and vegetables are an important part of the human diet. Since the moisture and nutrient content of fresh fruit and vegetables is high, makes them very vulnerable to fungal contamination, which might cause postharvest losses during handling, transport and storage.

Penicillium digitatum (Pers.Fr.) Sacc (green mold) and *Rhizopus stolonifer* (Ehrenb. Fr.) Vuill (rhizopus rot) are well-known postharvest pathogens of citrus and pome fruits (Eckert and Eaks 1989; Qing and Shiping 2000). To prevent development of these pathogens and to limit losses in commercial fruit shipments, treatment with chemical fungicides is a widely used procedure. However, the use of synthetic

fungicides because of some problems related to their use such as environmental contamination and human health risks associated with fungicide residues, decrease effectiveness of fungicides because of proliferation of fungicide-tolerant strains of fungus limited. In response to the problems mentioned earlier, consumer demand for less/eliminate use of synthetic fungicides and increase demand for organic crops in some markets there is considerable interest in finding alternative control approaches for use in integrated fungal decay management strategies for crop protection (Spotts and Cervantes 1986; El Ghaouth 1997; Lima *et al*. 2008).

Recently, there has been a growing interest in the discovery of new safe and effective antimicrobials from natural sources especially from plants for controlling pathogens in food and plant products. Essential oils are usually responsible for the characteristic odors and flavors of the essential oil bearing plants. These compounds synthesized for combating infectious or parasitic agents or generate in response to stress conditions. (Rauha *et al*. 2000; Isman and Machial 2006).

The antimicrobial properties of essential oils have been known for a long time, and numerous studies have documented the antifungal activity of the plant essential oils against postharvest fungal diseases (Reddy *et al*. 1998). According to our knowledge, antifungal activity of the several plant essential oils by several methods against *P. digitatum* and *R. stolonifer* has been assayed before. Authors reported that some essential oils could be effectively inhibiting the growth of this pathogenic fungus (Yahyazadeh *et al*. 2008).

Therefore, among the various alternatives, essential oils may offer a new and effective antifungal agent to control of postharvest pathogens of agricultural crops. Moreover, most of the terpenoids and phenols found in plant essential oils have relatively less toxic effects than synthetic chemicals on plant and mammals, and can be used in foods since are considered generally recognized as safe (GRAS).

This study was undertaken to investigate the antifungal activity essential oils from some aromatic plants of rich Iran flora by poison food medium and vapor phase methods against *P. digitatum* and *R. stolonifer*. In addition, we assayed evaluated the nature of antifungal activity of essential oils. Antifungal means inhibition of fungal growth. The nature of inhibition may be temporary or permanent. Temporary inhibition is fungistatic and the growth of the inhibited fungus revives as the antifungal compounds are removed from the contact. In permanent inhibition (fungicidal), the growth of the fungus does not revive (Sharma and Tripathi 2006).

MATERIALS AND METHODS

Plant Material

The aerial parts of sweet basil, *Ocimum basilicum L*. (Voucher specimen no. 8359), summer savory, *Satureja hortensis L*. (Voucher specimen no. 8406) and thyme, *Thymus vulgaris L*. (Voucher specimen no. 3893) in flowering stage, and ripped seeds of fennel, *Foeniculum vulgare* Mill. (Voucher specimen no. 6227) were collected from research field of Horticulture Department of Agriculture Faculty, Urmia University, Urmia, Iran. All samples were air dried and stored at room temperature in darkness until distillation. The air-dried plant material was water distillated using a Clevenger-type apparatus for 3 h, then essential oils was decanted, dried over anhydrous sodium sulfate and stored at 4C until use and analysis. Voucher specimens were deposited in the Herbarium of Research Institute of Forest and Rengelands Urmia, Iran.

Essential Oil Analysis

Gas chromatography (GC) analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with DB-5 fused silica column (30 m × 0.25 mm; film thicknesses 0.25 μm). Oven temperature was held at 60C for 5 min then programed to 210C at a rate of 3C/min. Injector and detector (flame ionization detector [FID]) temperature were 300 and 280C, respectively. Helium was used as carrier gas with a linear velocity of 32 cm/s. Percentages were calculated by electronic integration of FID peak areas without the use of response factor correction. GC/mass spectrometry (GC/MC) analyses were carried out on a Varian 3400 GC/MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm; film thickness 0.25 μm); oven temperature program was 60–210C at a rate of 3C/min, transfer line temperature 240C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 eV; scan time 1 s; mass range 40–340 amu.

The constituents of the oil were identified by calculation of their retention indices under temperature-programed conditions for identification of individual n-alkanes (C_6-C_{24}) and the oil on DB-5 capillary column and by comparison of their mass spectra with those of the internal reference mass spectra library (NIST 98 and Wiley 5.0) or with authentic compounds or with those of reported in the literature (Davies 1990; Adams 2001). Quantitative data was obtained from FID area percentages without the use of correction factors.

Test Pathogens

The test fungi *P. digitatum* and *R. stolonifer* were isolated from an infected table grape fruits and identified in the Agriculture Faculty, Plant Pathology Department, Urmia University. The isolated fungi maintained on potato dextrose agar (PDA) medium at 25C. A 7-day-old culture of each fungus was used for bioactivity tests.

Antifungal Activity Measurements

The antifungal activity of essential oil against *P. digitatum* and *R. stolonifer* were tested by poison food medium and vapor

phase assay methods. In poison food medium method, calculated concentrations (0, 200, 400, 600, 800 and 1,000 μL/L) of essential oils were add aseptically to sterile molten PDA medium (≈45C) containing Tween 80 (0.5%, v/v). The resulting media were immediately dispensed (≈20 mL) into sterilized Petri plates (9 cm). A mycelial disk 5 mm in diameter of the test pathogens taken from the 7-day-old culture, with help of a sterilized cork borer, was placed at the center of the medium. The control test without any test oil was performed in the same way.

In vapor phase assay, a mycelial disk of 5 mm diameter of the test pathogens from the 7-day-old culture was placed at the center of Petri plates in a PDA culture media, then calculated concentrations (0, 5, 10, 15, 20, 25 and 40 μL) of essential oils by using micropipettor applied to filter paper (70 mm in diameter, Whatman No.1), and it kept at the lid of the inverted Petri plate. Plates sealed with sealing film.

In both of methods inoculated Petri plates were incubated at 25C in darkness and observations were recorded on 7 days, time by which the growth of the control would have reached the edge of the plate. Each test was replicated four times and fungitoxicity was measured in terms of percent mycelial growth inhibition (MGI %) calculated by the formula $MGI\ (\%) = ([dc - dt]/\mathrm{dc}) \times 100$, where *dc* and *dt* represent mycelial growth diameter in control and treatment Petri plates, respectively.

At the end of the incubation period, fungistatic and/or fungicidal nature of the essential oils was determined by Sharma and Tripathi, 2006. The agar discs of the tested fungi which did not show any visible growth were transferred on to essential oils-free PDA plates and incubated for further 7 days to observe the revival growth. Where there was no growth, the effects were classified as fungicidal, whereas, in the cases that fungi were grown, the effects were classified as fungistatic.

Statistical Analyses

The data obtained as MGI (%) of essential oils were subjected to analysis of variance with MSTATC statistical software (Freed *et al*. 1991). The significance was determined by means of Duncan's multiple-range test ($P < 0.05$).

RESULTS AND DISCUSSION

The results of GC/MS analysis of essential oils is summarized in Table 1. The main compounds identified in sweet basil, fennel, summer savory and thyme oils were linalool (65.25%), *trans*-anethole (64.42%), carvacrol (54.14%) and β-ocimene (12.62%), respectively. The GC/MS analysis of essential oils used in this study showed that phenolic monoterpenes are the most dominant component available in thyme (thymol) and summer savory (carvacrol), whereas linalool as alcoholic compound and *trans*-anethole as ether compound were the main compounds on sweet basil and fennel oils, respectively.

The result of the assessment the antifungal activity of sweet basil, fennel, summer savory and thyme oils on percentage of mycelial growth inhibition (MGI%) of *R. stolonifer* and *P. digitatum* in poison food medium and vapor phase methods showed that the antifungal activity of essential oils were dependent on type of fungi, and type and concentration of the essential oil assayed. The results showed that thyme oil at 600 and 1,000 μL/L had a significant inhibitory effect on mycelia growth of *P. digitatum* (Fig. 1) and completely inhibited the mycelia growth of the *R. stolonifer* (Fig. 2) in poison food medium method. Summer savory oil at ≥600 μL/L had a significant inhibitory effect on mycelial growth of *R. stolonifer* (Fig. 2). Also, sweet basil and fennel oils at ≥800 μL/L concentration had considerable inhibitory effect against *R. stolonifer* (Fig. 2). Fennel oil at 200 μL/L concentration showed lowest inhibitory effect on mycelial growth of *R. stolonifer* and *P. digitatum* by 13.89 and 0%, respectively (Figs. 1 and 2).

In the test of essential oils on *P. digitatum* in vapor phase, thyme (at ≥5 μL), summer savory (at ≥10 μL), sweet basil (at ≥25 μL) and fennel (at 40 μL) completely inhibited mycelial growth of this fungal species. But only thyme and summer savory oils showed fungicidal property at ≥5- and ≥20-μL concentrations, respectively (Fig. 3). Test of natural antifungal properties of essential oils against *R. stolonifer* showed that summer savory (at ≥5 μL), thyme (at ≥10 μL), sweet basil (at ≥15 μL) and fennel (at 40 μL) oils showed higher fungicidal properties, respectively (Fig. 4).

Antifungal activity of essential oils from thyme and summer savory oils were higher than sweet basil and fennel oils and the level of fungicidal nature of thyme and summer savory oils against pathogens were higher than sweet basil and fennel oils. These results were agreement with reports of other authors that described that thyme and summer savory oils showed high antifungal activity against *Botrytis cinerea*, *R. stolonifer*, *Penicillium* spp., *Aspergillus parasiticus* and *A. flavus* (Reddy *et al*. 1998; Sokmen *et al*. 2004; Rasooli and Owlia 2005; Viuda-Martos *et al*. 2007; Yahyazadeh *et al*. 2008). On the other hand, the results of this study did not agree with results of Yahyazadeh *et al*. (2008), who reported that fennel oil induced the mycelial growth of *P. digitatum* in direction contact method. In this study, a low concentration of essential oils is fungistatic, and high concentrations of essential oils had fungicidal property. Rasooli and Owlia (2005) described that thyme oil exhibited fungistatic (250 μL/L) and fungicidal (500 μL/L) activities against *A. parasiticus*. Also, the antifungal activities of essential oils in vapor phase were higher than the poison food medium method. This results were in accordance with results of Edris and Farrag (2003), Šegvić Klarić *et al*. (2007) and Yahyazadeh *et al*. (2008) who reported that

TABLE 1. THE MAIN COMPONENTS OF ESSENTIAL OILS IDENTIFIED IN GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

No	Component	RI*	Sweet basil	Fennel	Summer savory	Thyme
1	α-pinene	936	–	–	–	5.24
2	α-terpinene	943	–	0.06	–	–
3	Limonene	964	–	3.37	–	–
4	β-pinene	971	0.38	1.46	0.85	3.84
5	Ocimene	972	–	0.14	–	–
6	Myrcene	977	2.17	–	–	–
7	β-myrcene	981	–	–	0.46	–
8	Champhene	988	–	–	–	2.56
9	γ-terpinene	996	–	1.43	–	–
10	α-phellandrene	997	–	0.62	5.31	8.5
11	β-ocimene	1030	–	–	–	12.62
12	(E)-β-ocimene	1032	1.98	–	–	–
13	ρ-cymene	1040	–	–	3.56	–
14	1,8-cineol	1049	3.62	–	0.04	–
15	Fenchone	1051	–	14.59	–	–
16	Endo fenchole	1054	–	0.14	–	–
17	Pinalol	1059	–	0.48	–	–
18	Terpinolene	1075	–	–	20.59	–
19	α-terpinolene	1080	–	–	–	1.24
20	Camphor	1083	0.66	1.48	–	–
21	Linalool	1090	65.25	0.08	0.02	4.18
22	Nerol oxide	1103	–	–	–	3.24
23	Borneol	1107	–	–	0.01	2.32
24	4-terpineol	1119	0.25	0.98	0.02	0.82
25	α-terpineol	1138	–	0.09	–	–
26	Methyl cavicol	1153	2.47	6.62	–	–
27	2-thujenol	1176	–	–	–	1.19
28	Geraniol	1234	0.82	–	–	–
29	Thymol methyl ether	1235	–	–	0.12	–
30	Trans-anethole	1240	–	64.42	0.02	–
31	Linalyl acetate	1253	–	–	–	1.32
32	β-citronellol	1264	–	–	–	0.53
33	Bornyl acetate	1265	0.48	–	–	–
34	Carvacrol	1285	–	–	54.14	6.85
35	Thymol	1289	–	–	–	10.56
36	Anethole	1305	–	–	–	1.54
37	Thojyl alchol	1305	–	–	–	0.2
38	Geranilacetate	1352	0.37	–	–	2.04
39	α-copaene	1358	–	0.03	–	1.38
40	Methyl eugenol	1368	1.36	–	–	–
41	Neryl acetate	1368	–	–	–	0.23
42	Geranyl propionate	1374	–	–	–	0.45
43	β-elemene	1381	1.8	–	–	–
44	Eugenol	1389	2.89	–	0.04	–
45	Cis-α-bergamotene	1408	0.3	–	–	–
46	z-jasmone	1413	–	–	0.35	–
47	β-caryophyllene	1420	–	–	–	6.31
48	Trans-α-bergamotene	1427	3.86	–	–	–
49	(Z)-β-farnesene	1441	0.57	–	–	–
50	α-ionone	1443	–	–	0.01	–
51	α-humulene	1446	0.26	–	0.05	0.6
52	Cis-farnesol	1450	–	–	–	0.77
53	Neryl acetone	1460	–	–	0.12	–
54	Germacrene-D	1462	–	0.15	–	1.94
55	γ-murolene	1464	1.88	–	–	–
56	α-murolene	1470	–	–	–	0.34
57	δ-elemene	1470	–	–	–	0.6

TABLE 1. CONTINUED

No	Component	RI*	Sweet basil	Fennel	Summer savory	Thyme
58	Allo aromadendrene	1477	0.78	–	–	–
59	Bicyclo germacrene	1483	0.71	–	–	–
60	Germacrene B	1489	–	–	0.11	–
61	α-selinene	1492	1.21	–	–	–
62	Methyl *cis*-Iso-eugenol	1493	–	0.12	–	–
63	γ-cadinene	1500	–	–	–	0.39
64	β-bisabolene	1515	–	–	0.8	–
65	δ-cadinene	1517	–	–	–	2.08
66	1-valancene	1543	–	–	–	0.98
67	Geranyl butyrate	1545	–	–	–	0.78
68	Spathulenol	1550	0.35	–	–	1.71
69	Widrol	1569	–	–	–	2.07
70	Caryophyllene oxide	1586	–	–	0.41	5.7
71	Germacrene-C	1587	–	–	–	0.41
72	Guaiol	1590	–	–	–	0.57
73	Cubenol	1593	0.56	–	–	–
74	Adamantane	1604	–	–	–	0.88
75	γ-eudesmol	1615	–	–	–	0.79
76	Torreyol	1615	–	–	–	0.79
77	β-eudesmol	1625	4.08	–	–	–
78	α-bisabolol	1706	–	–	0.02	–
79	β-farnesene	1838	–	–	–	0.95
80	Eicosane	2000	–	–	0.34	0.45
81	Docosane	2300	–	–	–	2.77
82	Hexatriacontane	2400	–	–	–	0.65

*Retention indices.

the antifungal activity of essential oils in vapor phase were high. Moreover, *R. stolonifer* in both assessment methods were very sensitive in compared with *P. digitatum*. Viuda-Martos *et al*. (2007) reported that the sensitivity of *A. flavus* against essential oil were higher than *A. niger*.

The antimicrobial activity of plant extracts, essential oils and their constituents against several pathogens have been reported in different reviews (Bakkali *et al*. 2008). However, the exact mechanism actions of these components against fungi were not well determined. Several authors related the antimicrobial activity of essential oils to main dominant component of essential oils (Nychas 1995). Shelef (1983) described that the antimicrobial property of essential oils can

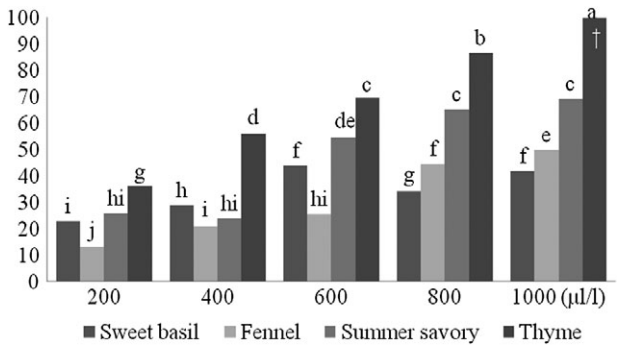

FIG. 1. EFFECT OF ESSENTIAL OILS ON MYCELIAL GROWTH INHIBITION PERCENTAGE (MGI %) OF *P. DIGITATUM* BY POISON FOOD MEDIUM METHOD

Means of treatments with the same letter are not significantly different according to Duncan's multiple-range test at $P < 0.05$.
†Treatment with fungistatic activity.

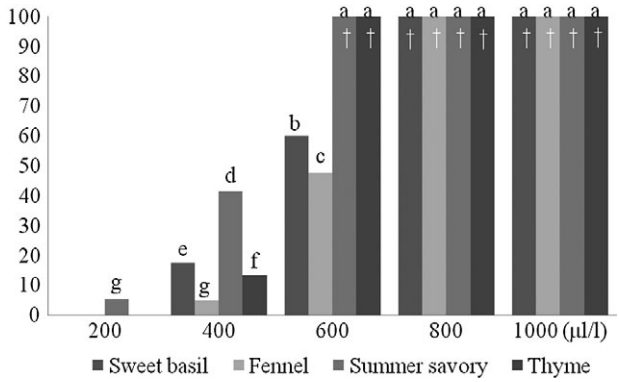

FIG. 2. EFFECT OF ESSENTIAL OILS ON MYCELIAL GROWTH INHIBITION PERCENTAGE (MGI %) OF *R. STOLONIFER* BY POISON FOOD MEDIUM METHOD

Means of treatments with the same letter are not significantly different according to Duncan's multiple-range test at $P < 0.05$.
†Treatment with fungistatic activity.

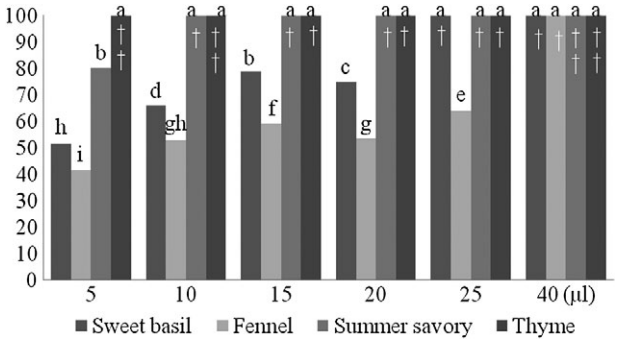

FIG. 3. THE EFFECTS OF ESSENTIAL OILS ON PERCENTAGE OF MYCELIAL GROWTH INHIBITION OF *P. DIGITATUM* IN VAPOR PHASE
Means of treatments with the same letter are not significantly different according to Duncan's multiple-range test at $P < 0.05$.
†Treatment with fungistatic activity. ††Treatments with fungicidal activity.

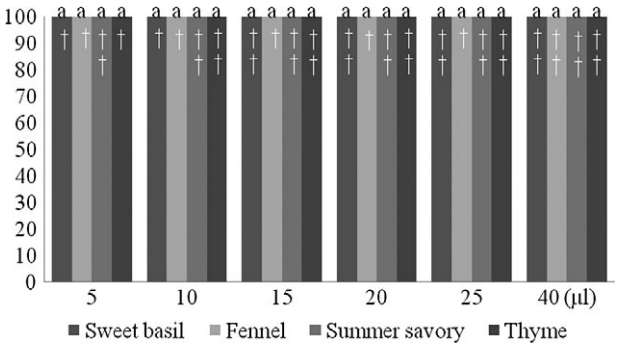

FIG. 4. THE EFFECTS OF ESSENTIAL OILS ON PERCENTAGE OF MYCELIAL GROWTH INHIBITION OF *R. STOLONIFER* IN VAPOR PHASE
Means of treatments with the same letter are not significantly different according to Duncan's multiple-range test at $P < 0.05$.
†Treatment with fungistatic activity. ††Treatments with fungicidal activity.

be related to phenolic compounds in the essential oils of species. Farrag *et al.* (1989) described that the antimicrobial property of essential oil related to phenolic compounds and presence of an aromatic nucleus and OH group of phenolic compounds caused cell wall degeneration. Sinha and Gulati (1990) reported that antifungal activity of sweet basil oil can be related to its constituents, linalool and methylchavicol. Nychas (1995) found that the phenolic components of essential oils denaturized the enzymes that control spore germination. Zambonelli *et al.* (1996) reported that antifungal effects of thyme oil can be related to effect of essential oil on degeneration of fungal hyphae. Ultee *et al.* (2002) reported that carvacrol and cymene affected the cell membrane integrity and they described that between carvacrol and cymene being a synergistic correlation. Also Daferera *et al.* (2003) described that the antifungal activity of essential oils can be attributed to presence of synergistic correlation between total components of essential oil. Rasooli and Mirmostafa (2003) and Rasooli and Owlia (2005) found that the antimicrobial activity of essential oils against fungi and bacteria is caused by the presence of carvacrol and thymol as phenolic compounds of essential oils. They described that essential oils caused severe damage to cell walls, cell membranes and cellular organelles such as mitochondria. Cox and Markham (2007) described that optimum range of hydrophobicity characteristic of essential oil and accumulation of these hydrophobic compounds in the lipid-rich environments of cell membrane structures and damage to cell membrane are some of the factors that affected the antimicrobial property of essential oil.

According to these observations we can conclude that strong antifungal activity of thyme and summer savory oils could be related to their phenolic components such as thymol and carvacrol, and/or could be attributed to synergism correlation between these components with other components essential oils. Also, distinguished that some of essential oils such as thyme and summer savory show promising antifungal activities and may be provide a human and environmentally safer and more acceptable antifungal agents that could be exploited in treatment of fruits and vegetables against postharvest fungal infections, especially in vapor phase. However, further *in vivo* studies are needed to improve our knowledge about the influence of essential oils on postharvest fungi.

REFERENCES

ADAMS, R.P. 2001. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, Allured Publishing Corp, Carol Stream, IL.

BAKKALI, F., AVERBECK, S., AVERBECK, D., ZHIRI, A. and IDAOMAR, M. 2008. Biological effects of essential oils. Food Chem. Toxicol. *46*, 446–475.

COX, S.D. and MARKHAM, J.L. 2007. Susceptibility and intrinsic tolerance of *Pseudomonas aeruginosa* to selected plant volatile compounds. J. Appl. Microbiol. *103*, 930–936.

DAFERERA, D., ZIOGAS, B. and POLISSIOU, M. 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea, Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Prot. *22*, 39–44.

DAVIES, N.W. 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and carbowax 20M phases. J. Chromatogr. *503*, 1–24.

ECKERT, J.W. and EAKS, I.L. 1989. Postharvest disorders and diseases of citrus fruits. In *The Citrus Industry* (W. Reuther, E.C. Calavan and G.E. Carman, eds.) pp. 179–260, University of California Press, Berkeley, CA.

EDRIS, A.E. and FARRAG, E.S. 2003. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapour phase. Nahrung/Food *47*, 117–121.

EL GHAOUTH, A. 1997. Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases. J. Ind. Microbiol. Biotechnol. *19*, 160–162.

FARRAG, R.S., DAW, Z.Y. and ABO-RAYA, S.H. 1989. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J. Food Sci. *54*, 74–76.

FREED, R., EISENSMITH, S.P., GOETZ, S., REICOSKY, D., SMALL, V.W. and WOLBERG, P. 1991. *User's Guide to MSTATC*, Michigan State University, East Lansing, MI.

ISMAN, M.B. and MACHIAL, C.M. 2006. Pesticides based on plant essential oils: From traditional practice to commercialization. In *Naturally Occurring Bioactive Compounds* (M. Rai and M.C. Carpinella, eds.) pp. 29–44, Elsevier Pub, Amsterdam, Netherlands.

LIMA, G., DE CARTIS, F. and DE CICCO, V. 2008. Interaction of microbial biocontrol agents and fungicides in the control of postharvest disease. Stewart Postharvest Rev. *4*, 1–7.

NYCHAS, G.J.E. 1995. Natural antimicrobials from plants. In *New Methods of Food Preservation* (G.W. Gould, ed.) pp. 58–69, Blackie Academic Professional, New York, NY.

QING, F. and SHIPING, T. 2000. Postharvest biological control of Rhizopus rot of nectarine fruits by *Pichia membranefaciens*. Plant Dis. *84*, 1212–1216.

RASOOLI, I. and MIRMOSTAFA, S.A. 2003. Bacterial susceptibility to and chemical composition of essential oils from *Thymus kotschyanus* and *Thymus persicus*. J. Agric. Food Chem. *51*, 2200–2205.

RASOOLI, I. and OWLIA, P. 2005. Chemoprevention by thyme oils of *Aspergillus parasiticus* growth and aflatoxin production. Phytochemistry *66*, 2851–2856.

RAUHA, J.P., REMES, S., HEINONEN, M., HOPIA, A., KAHKONEN, M., KUJAJA, T., PIHLAJ, A.K., VUORELA, H. and VUORELA, P. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiol. *56*, 3–12.

REDDY, M.V.B., ANGERS, P., GOSSELIN, A. and ARUL, J. 1998. Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochemistry *47*, 1515–1520.

ŠEGVIĆ KLARIĆ, M., KOSALEC, I., MASTELIĆ, J., PIECKOVÁ, E. and PEPELJNAK, S. 2007. Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. Lett. Appl. Microbiol. *44*, 36–42.

SHARMA, N. and TRIPATHI, A. 2006. Fungitoxicity of the essential oil of *Citrus sinensis* on post-harvest pathogens. World J. Microbiol. Biotechnol. *22*, 587–593.

SHELEF, L.A. 1983. Antimicrobial effects of spices. J. Food Saf. *6*, 29–44.

SINHA, G.K. and GULATI, B.C. 1990. Antibacterial and antifungal study of some essential oils and some of their constituents. Indian Performer *34*, 126–129.

SOKMEN, A., GULLUCE, M., AKPULAT, H.A., DAFERERA, D., TEPE, B., PPOLISSIOU, M., SOKMEN, M. and SAHIN, F. 2004. The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control *15*, 627–634.

SPOTTS, R.A. and CERVANTES, L.A. 1986. Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in packinghouses. Plant Dis. *70*, 106–108.

ULTEE, A., BENNIK, M.H.J. and MOEZELAAR, R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. *68*, 1561–1568.

VIUDA-MARTOS, M., RUIZ-NAVAJAS, Y., FERNÁNDEZ-LÓPEZ, J. and PÉREZ-ÁLVAREZ, J.A. 2007. Antifungal activities of thyme, clove and oregano essential oils. J. Food Saf. *27*, 91–101.

YAHYAZADEH, M., OMIDBAIGI, R., ZARE, R. and TAHERI, H. 2008. Effect of some essential oils on mycelial growth of *Penicillium digitatum* Sacc. World J. Microbiol. Biotechnol. *24*, 1445–1450.

ZAMBONELLI, A., ZECHINI D'AULERIO, A., BIANCHI, A. and ALBASINI, A. 1996. Effects of essential oils on phytopathogenic fungi. J. Phytopathol. *144*, 491–494.