Grasas y Aceites
Vol. 51. Fasc. 4 (2000), 237-240

Inhibitory effect of some spice essential oils on *Penicillium digitatum* causing postharvest rot in citrus

By **Fahri Yigit** [1], **Musa Özcan** [2*] and **Attila Akgül** [2]

[1] Department of Plant Protection, Faculty of Agriculture, Selçuk University, Konya 42031, Turkey
[2] Department of Food Engineering, Faculty of Agriculture, Selçuk University, Konya 42031, Turkey
* e-mail: fyigit@karatay1.cc.selcuk.edu.tr

RESUMEN

Efecto inhibidor de algunos aceites esenciales sobre el *Penicillium digitatum* causante de la putrefacción post-cosecha de cítricos.

En este estudio para controlar las manchas azules causadas por *Penicillium digitatum,* se aplicó aceite esencial de comino en discos de papel de filtro de 6 mm de diámetro, los cuales fueron empapados en 0,04 ml de aceite y su vapor inhibió completamente el crecimiento micelar y la germinación de esporas del patógeno in vitro. Cuando los discos de papel de filtro empapados en aceites esenciales de tomillo, eneldo, culantro y romero se colocaron sobre el medio de cultivo (PDA), no se observó efecto sobre el crecimiento micelar. Los efectos de sus vapores inhibieron el crecimiento micelar de patógenos en un 85,8%, 82,8%, 80% y 71,4% respectivamente. Los aceites de eneldo y romero también evitaron la aparición del color micelar.

PALABRAS-CLAVE: Aceite esencial - Cítrico - Inhibición - Penicillium digitatum - Putrefacción.

SUMMARY

Inhibitory effect of some spice essential oils on *Penicillium digitatum* causing postharvest rot in citrus.

In this study to control blue mould caused by *Penicillium digitatum,* essential oil of cumin was applied with filter paper discs of 6 mm diameter which were soaked in 0,04 ml oil and vapour effect inhibited completely mycelial growth and spore germination of pathogen in vitro. When filter paper discs soaked in essential oils of black thyme, dill, coriander and rosemary were placed on the culture medium (PDA), they had no effect on the mycelial growth. Their vapour effect inhibited mycelial growth of pathogen 85.8%, 82.8%, 80% and 71.4% respectively. Dill and rosemary oils also prevented mycelial colour.

KEY-WORDS: Citrus - Essential oil - Inhibition - Penicillium digitatum - Rot.

1. INTRODUCTION

Citrus fruits are stored at favourable temperature and relative humidity or marketed directly. The fruits are vulnerable to wounding due to the fact that their peel tissue is soft. Wounds occurred on the peels cause the postharvest infection and are important for pathogenesis. It has been reported that volatile compounds from exocarp-wounded oranges induced germination in 50% of *Penicillium digitatum* conidia on water agar, compared with < 5% on water agar alone (Eckert and Ratnayake, 1994). There are several pathogens, such as *Botrytis cinerea, Sclerotinia sclerotiorum, Trichoderma lignorum, Alternaria citri, Fusarium lateridium, F. solani, Penicillium italicum* and *P. digitatum* which damage citrus fruits (Klotz, 1973). *Penicillium* species are the most important of them. To control such pathogens, many investigations were carried out in recent years and focused on the chemical control, but several *Penicillium* species showed resistance against certain chemicals (Wild, 1989, Mahmoud and Hanafy, 1991). Instead of chemical control, some biocontrol studies were conducted on the postharvest decays of citrus fruit in recent years. Many biocontrol agents isolated from these studies achieved effective control (Wilson and Chalutz, 1989, Chalutz and Wilson, 1990, Arras, 1993, Houng *et al.*, 1992, Huang *et al.*, 1993, Arras and D'hallewin, 1994).

One of the control methods is also UV-light treatment but this method had no success by percent of 100 to control such moulds. In addition, it was also used plant extracts to control *Penicillium* species. A methanol-water extract from *Impatiens balsamina* inhibited mycelial growth of *P. italicum* on agar plates. However the results on wounded lemon fruits were inconsistent (Jiratko, 1994). It is known that essential oils of certain plants also prevented growth of fungi as well as some chemicals (Pruthi, 1980, Yousef and Tawill, 1980, Azzouz and Bullerman, 1982, Akgül and Kivanç, 1988a, Akgül and Kivanç, 1988b, Farag *et al.*, 1989, Knobloch *et al.*, 1989). The aim of this study is to assay some essential oils known fungistatic toward *P. digitatum in vitro* and *in vivo.*

2. MATERIALS AND METHODS

Valencia oranges were obtained from marked. The fungus employed for assays of antifungal activity was isolated from decayed Valencia oranges and

identified by Dr. Fahri YIĞIT (S.Ü. Agriculture Faculty, Department of Plant Protection). After identification, it was cultured routinely on Potato Dextrose Agar (Difco) at 25°C.

Spices used in the experiment are listed in Table I. They were collected from different regions in Turkey. Essential oils to be tested were obtained from these spices by water distillation for tree hours using clevenger apparatus (Miquel *et al.,* 1997). The oils isolated and dried over anhydrous sodium sulfate were maintained in dark bottles colour at cold conditions until used.

Table I
Spices used for distilling essential oils

Common name	Botanical name	Family	Used part
Dill	*Anethum graveolens* L.	Umbelliferae	Fruits
Coriander	*Coriandrum sativum* L.	Umbelliferae	Fruits
Cumin	*Cuminum cyminum* L.	Umbelliferae	Fruits
Rosemary	*Rosmarinus officinalis* L.	Labiatae	Leaves
Thyme (wild)	*Thymbra spicata* L.	Labiatae	Leaves + flowers

Inocula were produced by wound-inoculating orange fruit, incubating the fruit at 25°C for five days and then harvesting the conidia into sterile distilled water for spraying onto wounded fruits and spreading onto the surface of culture medium. Spore suspension was adjusted to 10^6 conidia/ml concentrations.

Fungistatic effect of the essential oils *in vitro* on the pathogen

This effect was tested using two different methods against test organism. a) Addition on the culture medium: 0.2 ml of spore suspension from fresh *P. digitatum* culture was prepared and inoculated onto the surface of Potato-Dextrose-Agar (Difco) in 10 cm diameter petridishes. After inoculation, filter paper discs of 6 mm diameter soaked in 0.04 ml each essential oil were suitably placed on the medium. After incubation for three days at 25°C, spore germination was controlled under light microscopy b) Vapour effect: Filter paper discs were placed on the inside surface of cover of petridishes after they were prepared as the way above. One mycelial disc of 5 mm diameter, cut from the periphery of a 7-day old culture, was aseptically inoculated upside down in the centre of the petri plate. For control, petri plate had no essential oil. After same incubation, the diameters of fungal colonies were measured. Objective of this trial was to determinate effects of essential oils on the mycelial growth and spore germination. All petridishes used in the experiment were covered with parafilm. Each treatment was designated as four replication. Data was analysed by analysis of variance and LSD test.

Effects of the essential oils on the fruit decay

The fruit were dipped into 0.5% sodium hypochloride for surface sterilisation for two minute and washed in sterile water and air dried. Wounds were formed artificially using a sterile needle a diameter of 0.5 cm on fruits at four different directions. Spore suspension (10^6 conidia/ml) was sprayed on the wounds. After two minute, 0.1 ml of each oil concentration adjusted to 300, 600, 900 ppm using propylen glycol, was spread on the wounds using spatula. Propylene glycol used as solvent was spread on the control fruits at the same amount. All fruits were placed in polyethylene bags and incubated at room temperature for four days. After incubation, diameter of each decayed area was measured. Values were average of four different decayed areas on each fruit. All the tests were designed as 10 replicates. Data from fruit treatment with 900 ppm. essential oil concentration of *Thymbra spicata* was analysed by analysis of variance and the LSD test. The other oil concentrations were no found effective sufficiently.

3. RESULTS AND DISCUSSION

When the values were evaluated for fungistatic effect of the essential oils on the pathogen, cumin oil completely inhibited growth of *P. digitatum* in both methods (Table II). There was no effect when the filter paper discs soaked in the essential oil of rosemary, dill, thyme and coriander were placed on the culture medium. However rosemary and dill oils prevented mycelial colour of pathogen. Vapour effect of wild thyme, dill coriander and rosemary oils inhibited mycelial growth and colour as 85.8%, 82.8%, 80% and 71.4%, respectively (Table II). Diameters of moulded areas on the fruits, on which concentrations (300, 600, 900 ppm) of each oil were applied, were measured after incubation (Table III). A concentration of 900 ppm thyme essential oil prevented fruit mould (Fig. 1). There was no fungal growth on the wounded areas although on which

there was slightly soft. Essential oil of rosemary inhibited mycelial growth by 12.5% on the moulded areas. To evaluate the experiment, colony diameters on the affected areas were taken into consideration.

Table II
Vapour effect of the essential oils on *P. digitatum*

Essential oils	Diameter of colony (cm)				Average (cm)	diameter % inhibition	Groups (P: 0.05)*
Anethum graveolens L.	1.2	1.1	1.3	1.2	1.2	82.8	B
Coriandrum sativum L.	1.4	1.5	1.3	1.4	1.4	80	B
Cuminum cyminum L.	0	0	0	0	0	100	A
Rosmarinus officinalis L.	2	2.1	1.9	2	2	71.4	C
Thymbra spicata L.	1	1.1	1.9	1	1	85.8	B
Control	7	7.1	6.9	7.1	7		

* The same letters in the column are not significantly different (P= 0.05).

Table III
Effect of the essential oils on the fruit mould

Essential oils	Doses (ppm)	Diameter of wounded areas on the fruits (cm)										Average diameter (cm)	Groups (p:0.05)*	% Inhibition
Anethum graveolens	300	4	4	4.2	4	4.1	4	3.9	4	4	4	4.02		0.5
	600	4	4	4.1	3.8	3.9	4	4.1	4	4	4	3.9		3.5
	900	4.1	4	3.8	4	4	4.1	4.2	3.9	4	4	4.01	A	0.5
Coriandrum sativum	300	4	3.8	3.8	4	4	4.1	4	4.2	4	4	3.9		3.5
	600	3.8	3.7	4	4	4.1	4.2	4	3.9	4	4	3.9		3.5
	900	4	4.2	4	3.9	3.8	4.1	4.2	4	4	4	4.04	A	0
Cuminum cyminum	300	4	4	4.1	4	4.2	3.9	4.2	4	4	4	4.04		0
	600	3.9	4	4.2	3.8	4.1	4	3.9	4.1	3.8	3.7	3.9		3.5
	900	4	3.8	4.1	3.7	4.1	3.9	4	3.8	4.2	4	3.9	A	3.5
Rosmarinus officinalis	300	4.2	3.7	4.1	4	4	4.1	4.2	4	4	3.8	4.01		0.5
	600	4	4	3.8	4.2	4.1	4	4	4.1	4	3.7	3.9		3.5
	900	4	3	3	3.5	4	4	3.2	3.5	4	3	3.5	B	13.3
Thymbra spicata L.	300	4	4.2	4	4	3.8	4	4.1	4	3.9	4	4		1
	600	4	3.9	4	3.8	4	4.1	4	4	3.8	3.9	3.9		3.5
	900	2	2	1	2.5	3	2	2.5	2	1.5	2	2.05	C	49.2
Control		4	4	4.1	4.2	4	4	4.2	3.9	4	4.1			
		4.1	4	4.2	4	3.8	4	4	4.2	4	4	4.04	A	
		4.2	3.9	4	4	4.1	4.1	4.2	4	4	4			

* The same letters in the column are not significantly different (P= 0.05).

In this study conducted *in vitro* to determinate the effects of some spice essential oils on the mycelial growth and spore germination of *P. digitatum*, none of the oils showed fungicide effect. Although vapour effect of cumin essential oil was found highly effective, when it was moved from petridishes, the pathogen spores began to germinate again. If thyme and cumin oil were absorbed to packing paper which is used to protect citrus fruits from water loses and pathogens caused postharvest rots. They might probably prevent fruits from moulding. This protection effect can be likely due to cumin aldehyde. It has been suggested that this compound among the aromatic aldehydes showed the most antifungal effect (Kurita *et al.*, 1981). When the pathogen mycelium in the petriplate containing dill and rosemary oils was examined on the light microscopy, they were found more effective on both sporulation and spore colour. Density of conidia and conidiophore on the mycelium was found low. Addition, colour of conidia

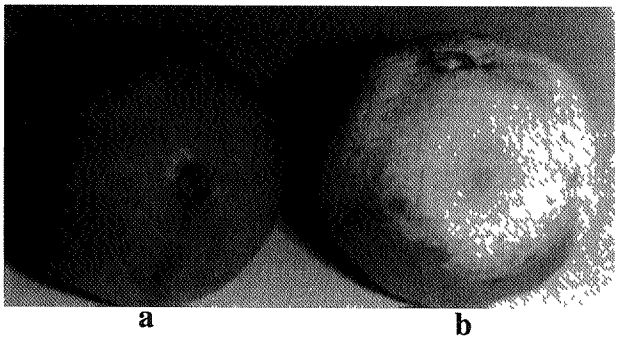

Figure 1
Inhibitory effect of thyme esential oil on the fruit mould a) citrus fruit showing treated with 900 ppm Wild Thyme essential oil b) control fruit treated with only propylene glycol.

was pale. This effect might decrease virulens of pathogen, so that incidence of disease would decrease. Results from investigation showed that these oils would be promise for future studies.

Concentration of 900 ppm thyme oil was found highly effective to control fruit decay. In addition, the peel tissue treated with thyme oil showed shrivelling and hardness. Consequently it is though that cited variations prevented penetration of pathogen through peel tissue. Wounds are very important point of attack of postharvest moulds. For instance volatile compounds from wounded peel stimulates germination (Eckert and Ratnayake, 1994). Therefore it is noticeable that essential oil of thyme improves wound areas and inhibits germination. Such effects result from thymol and carvacrol which are important compounds of this oil (Akgül and Kivanç, 1988b, Akgül *et al.*, 1991). It was concluded that artificial conditions formed for mould occurrence in polyethylene bags might give raise to overlook the effect of other essentials oils on the mould. It may hope that essential oils of cumin and thyme black will be much more effective under conditions for the disease. From view of this it will be continued studies in the future.

REFERENCES

Akgül, A. and Kivanç, M. (1988a).—«Sensitivity of four foodborne moulds to essential oils from Turkish spices, herbs and citrus peel».—*J. Sci. Food Agric.* **47**, 129-132.

Akgül, A. and Kivanç, M. (1988b).—«Inhibitory effects of selected Turkish spices and oregano components on some foodborne fungi».—*Int. J. Food Microbiol.* **6**, 263-268.

Akgül, A., Kivanç, M. and Sert. S. (1991).—«Effect of carvacrol on growth and toxin production by *Aspergillus flavus* and *Aspergillus parasiticus.—Sci. Aliments* **11**, 361-370.

Arras, G. (1993).—«Inhibition of postharvest fungal pathogens by *Bacillus subtilis* strains isolated from citrus fruit».— *Adv. Hort. Sc.* **7**, 123-127.

Arras, G. and D'hallewin, G. (1994).—«*In vitro* and *in vivo* control of *Penicillium digitatum* and *Botrytis cinerea* in citrus fruit by *Bacillus subtilis* strains».—*Agric. Mediter.* **124**, 56-61.

Azzouz, M.A. and Bullerman, L.B. (1982).—«Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents».— *J. Food Protect.* **45**,1298-1301.

Chalutz, E. and Wilson, C.L. (1990).—«Postharvest biological control of green and blue mold and sour rot of citrus fruit by *Debaryomyces hansenii*».—*Plant Diseases* **74**, 134-137.

Eckert, J.W. and Ratnayake, M. (1994).—«Role of volatile compounds from wounded oranges in induction of germination of *Penicillilum digitatum* conidia».— *Phytopathology* **84**, 746-750.

Farag, R.S., Daw, Z. and Abo-Raya, S.H. (1989).—«Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxin in a synthetic medium».—*J. Food Sci.* **54**, 74-76.

Houng, Y., Wild, B.L., Morris, S.C. (1992).—«Postharvest biological control of *Penicillium digitatum* decay on citrus fruit by *Bacillus pumilus*».—*Ann. Appl. Biol.* **120**, 367-372.

Huang, Y., Deverall, B.J. and Morris, S.C. (1993).—«Effect of *Pseudomonas cepacia* on postharvest biocontrol of infection by *Penicillium digitatum* and on wound responses of citrus fruit».— *Austr. Plant Pathol.* **22**, 84-93.

Jiratko, J. (1994).—Effect of plant extracts on blue mould of citrus fruit *(Penicillium italicum* Wehler) and grey mould *(Botrytis cinerea* Pers)».—*Ocrana Rostlin* **30**, 273-382.

Klotz, L.I. (1973).—«Color handbook of citrus diseases. Citrus research center and agricultural experiment station».— Riwerside, California.

Knobloch, K., Pauli, A., Iberl, B., Weigand, H. and Weis, N. (1989).—«Antibacterial and antifungal properties of essential oil components».—*J. Essent. Oil Res.* **1**, 119-128.

Kurita, N., Miyagi, M., Kurane, R. and Takahara, Y. (1981).—«Antifungal activity of components of essential oils».— *Agric. Biol. Chem.* **45**, 945-952.

Miquel J., Rhicard, H. and Sandret, F., (1997).—«Volatile constituents of Moraccan thyme oil».— *J. Agric. Food Chem.* **16**, 1054-1057.

Mahmoud, M.B. and Hanafy, S.M. (1991).—«Acquired resistance of *Penicillium digitatum* and *Penicillium italicum* to some postharvest fungicides on navel oranges».— *Ann. Agric. Sci.* Cairo **36**, 681-691.

Pruthi, J.S. (1980).—«Species and condiments: chemistry, microbiology and technology. Academic».—Press, New York.

Wild, B.L. (1989).—«Increased infection potential of benomyl-resistant strains of *Penicillium digitatum* in the presence of sensitive strains».—ISSPP Chem. Cont. Newstletter **12**, 32-33.

Wilson, C.L. and Chalutz, E. (1989).—«Postharvest biological control of *Penicillium* rots of citrus with antagonistic yeasts and bacteria».—*Sci. Hort.* **40**, 105-112.

Yousef, R.T. and Tawil, G.G. (1980).—«Antimicrobial activity of volatile oils».— *Pharmazie* **35**, 698-701.

Recibido: Enero 1999
Aceptado: Julio 1999