academicJournals

Vol. 7(29), pp. 3831-3835, 19 July, 2013
DOI: 10.5897/AJMR2013.5605
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antifungal effects of essential oils of three medicinal plants on post-harvest rot of Valencia oranges at normal and storage temperatures

Sareh Mahdavi[1], Abdulrasoul Zakerin[2], Hamid Sadeghi[3], and Ali Reza Niazmand[4]*

[1]Department of Horticulture, Jahrom Branch, Islamic Azad University, Jahrom, Iran.
[2]Department of Horticulture, Jahrom Branch, Islamic Azad University, Jahrom, Iran.
[3]Department of Plant Physiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran.
[4]Department of Plant Pathology, Jahrom Branch, Islamic Azad University, Jahrom, Iran.

Accepted 29 May, 2013

Post-harvest rots are the main limiting factors in storing horticultural products. Currently, control of postharvest citrus diseases relies mainly on synthetic fungicides. Biological control using naturally occurring substances has been recently explored for managing postharvest decay of citrus fruit. Essential oils of three medicinal plants (*Ocimum basilicum*, *Mentha pulegium* and *Cassia angustifolia*) were tested for their antifungal activity against the principal postharvest fungal pathogens of citrus fruit, that is *Penicillium digitatum*, *Aspergillus flavus* and *Alternaria alternata* at two storage temperatures (6 and 25°C). The three pathogenic fungi were collected from diseased *Citrus sinensis Osbeck* (cv. Valencia) from a citrus storage of Jahrom. Fruit epicarp of cv. Valencia was wounded by the tip of a sterilized pin and 15 ml of three concentrations of each essential oil suspension (200, 400 and 600 ppm) plus 0.5 ml Tween 20 were sprayed on the outer skin. The experimental samples were stored in plastic bags at 6 and 25°C for two months, with six replications per treatment. There were significant differences in infection percentages at the two temperatures. Applying different concentrations of essential oils, at both temperatures, reduced the percentage of fruits infected by fungi compared to controls. At 25°C, *C. angustifolia* and *O. basilicum* essential oils at 200 ppm, and 200 and 400 ppm of *M. pulegium*, caused minimum infection percentages by *Al. alternata*. *Cassia angustifolia* essential oil at 200 and 400 ppm had the same effect on infection percentages and gave the lowest infection percentages by *As. flavus* and *P. digitatum*. At 6°C the effects of all concentrations of tested essential oils on infection percentages by *Al. alternata* and *As. flavus* were the same and differed from controls. In the case of *P. digitatum* there were no differences for the different concentrations of *C. angustifolia* and *M. pulegium* essential oils. For *O. basilicum* essential oil, the highest and the lowest infection percentages by *P. digitatum* were for the 200 and 600 ppm concentrations, respectively. In summary, at 25°C, *C. angustifolia* essential oils cause the lowest infection percentages to all tested fungi. At 6°C the effects of all tested essential oils on infection percentages to all tested fungi were alike.

Key words: *Ocimum basilicum*, *Mentha pulegium*, *Cassia angustifolia*, *Penicillium digitatum*, *Aspergillus flavus, Alternaria alternata*, Valencia cultivar, storage.

INTRODUCTION

During storage, fruits and vegetables are often subject to varying levels of microbial decay, mainly due to pathogenic fungi that usually infect the host through wounds sustained during harvest, handling and/or processing

(El Ghaouth et al., 2002). For citrus fruit, losses are mainly caused by *Penicillium digitatum*, *P. italicum*, *Aspergillus flavus* and *Alternaria alternata* (Eckert and Ogawa, 1985; Diener et al., 1987; Palumbo et al., 2006; Laville, 1971; Wilson and Payne, 1994). Post-harvest diseases destroy 10-30% of the total yield of all crops; however, for perishable crops, especially in developing countries, they destroy > 30% (Kader, 2002). Post-harvest rots are the main limiting factors in storing horticultural products. Currently, control of postharvest citrus diseases relies mainly on synthetic fungicides, principally thiabendazole and imazalil, sprayed on fruit during waxing operations at packing facilities (Brown and Miller, 1999). The emergence of resistant strains of pathogens to these fungicides (Spotts and Cervantes, 1986; Suhr and Nielsen, 2003; Eckert, 1987), as well as the growing concern for human safety and protection of the environment (Suhr and Nielsen, 2003; Wilson et al., 1997), drive the search for alternatives to synthetic fungicides in control of postharvest diseases. Biological control using naturally occurring substances has been recently explored for managing postharvest decay of fruit. Due to their non-phytotoxicity and systemicity (Fawcett and Spencer, 1970), as well as biodegradability, plant-derived products can be potent and valuable reagents in pest management (Mishra and Dubey, 1990; Shukla and Tripathi, 1987; Tripathi and Dubey, 2004; Xuan et al., 2006; Javaid et al., 2006). For example, essential oils and other medicinal and aromatic plant extracts have been suggested for controlling citrus postharvest rots (Wilson et al., 1997; Mari and Guizzardi, 1998; Chebli et al., 2003). In this study, essential oils of three medicinal plants (*Ocimum basilicum*, *Mentha pulegium* and *Cassia angustifolia*) were tested for their antifungal activity against the principal postharvest fungal pathogens of citrus fruits, that is *P. digitatum*, *As. flavus* and *Al. alternata* at two storage temperatures (6 and 25°C). This study is the first using these essential oils in citrus samples.

MATERIALS AND METHODS

Plant material

Seeds of *O. basilicum*, *M. pulegium* and *C. angustifolia* were gotten from the Research Institute of Forests and Rangelands (RIFR), Iran and were sown in research fields of Islamic Azad University branch of Jahrom during 2011 season crops. Plant leaves were harvested before flowering and air dried for one month in darkness at room temperature and then stored in sealed paper bags.

Isolation of essential oils

Dried leaves were subjected to steam distillation for 3 h using a Clevenger-type apparatus. The supernatant was separated by decantation after adding 50% NaCl. The essential oils was collected collected, dried over anhydrous sodium sulfate and stored in sealed glass vials at 4°C in darkness until used. Purity of samples was guaranteed at all times.

Test fungi and growth conditions

In this study three pathogenic fungi were used: *Penicillium digitatum*, *As. flavus* and *Al. alternata* collected from diseased *Citrus sinensis* (cv. Valencia) from citrus storage facility in Jahrom. Samples were cultured on Potato Dextrose Agar media and purified by the single spore method. The plates were incubated at 25°C for 9 days.

Inoculation and storage conditions

Fruit epicarp of cv. Valencia was wounded using the tip of a sterilized pin, and 15 ml of the three concentrations of each essential oil suspension (200, 400 and 600 ppm) plus 0.5 ml Tween 20 were sprayed on the outer skin. The oranges were incubated overnight at room temperature and then inoculated with 15 ml of spore suspensions of each species containing 10^8 spores/ml. The control treatment was sprayed only by spore's suspension. The tests were stored in plastic bags at 6°C and 25°C for two months, with six replications per treatment.

Determination of infection percent

After two months, for each treatment, infection percent was estimated as average of rotten area in proportion to the total area of the fruit.

Statistical analyses

All data analyses were performed by analysis of variance (ANOVA) ($P \leq 0.01$), giving F-values, using SAS program 6.1 (SAS Institute). Significant differences were determined using Duncan's new multiple range test at $P \leq 0.01$.

RESULTS

In vitro experiment

There were significant differences in infection percent-tages at 25 and 6°C (Table 1). At 6°C, the highest and lowest percentages of infections were for *As. flavus* and *P. digitatum,* and *Al. alternata*, respectively (Table 2). At 25°C, the highest and lowest infection percentages were for *As. flavus* and *Al. alternata*, respectively (Table 2).

Cassia angustifolia essential oil caused the more decrease in fungal infection compared with *Ocimum basilicum* essential oil at 25°C. However, at 6°C were no significant differences in infection percentages by each fungal species for the different essential oils (Table 3).

At both temperatures, different concentrations of essential oils reduced the percentage of fruit infected by fungi compared to the controls. Additionally,increasing the concentration of essential oils increased the disease

*Corresponding author. E-mail: Niazmand2003@yahoo.com. Tel: +989177104870. Fax: +987112332517.

Table 1. Mean comparison of infection percentage at two temperatures at P ≤ 0.01.

Sample	Infection percent ± SD	Temperature
A	37.5833 ± 2.05	25
B	20.833 ± 1.84	16

Table 2. Mean comparison of infection percentage to different fungi species at two temperatures.

Fungi species	25°C		6°C	
Without fungi	22.33±3.23	C	66.25±7.75	A
Alternaria alternata	34.33±4.32	B	9.1667±1.29	BC
Penicillium digitatum	48.33±4.02	A	11.4583±1.45	BC
Aspergillus flavus	45.33±3.98	A	13.75±1.75	BC

Means with common letters in the same column are not significantly different at P ≤ 0.01.

infection percentages at 25°C. At 6°C the infection differences between concentrations of essential oils were not significant. At 25°C the maximum infection percentage was for 600 ppm and the minimum for 200 and 400 ppm concentrations of essential oils, respectively (Table 4).

At 25°C, *C. angustifolia* and *O. basilicum* essential oils at 200 ppm, and for *M. pulegium* at 200 and 400 ppm, gave the minimum infection percentages by *Al. alternata* and by *As. flavus* at 200 ppm. *Cassia angustifolia* essential oil at 200 and 400 ppm had the same effect on infection percentage and gave the lowest infection percentage by *As. flavus* and *P. digitatum*. *Ocimum basilicum* essential oil of 200 ppm caused the lowest infection percent by *Al. alternata*; and 200 and 400 ppm had similar effects and the minimum infection percentages by *P. digitatum*. Essential oil of *O. basilicum* at 400 ppm showed the minimum infection by *As. flavus*. Different concentrations of *M. pulegium* essential oil had the same effect on infection percentage by *P. digitatum* (Table 5).

At 6°C, the effects of all concentrations of essential oils on infection percentage by *Al. alternata* and *As. flavus* were the same and differed from controls. In the case of *P. digitatum* there were no differences of different concentrations effects of *C. angustifolia* and *M. pulegium* essential oils to infection percent to this fungi species. The *O. basilicum* essential oil gave the highest and lowest infection percentages by *P. digitatum* at 200 and 600 ppm, respectively (Table 6).

DISCUSSION

At both temperatures the percentages of infection varied. Infection percentages were lower at the lower temperature and so oranges remained fresh longer in the cold conditions. These results are in agreement with the results of Grierson and Ben-Yehoshua (1987), who found that the best storage temperature for citrus fruit was 6.7°C. Some researchers concluded that citrus fruit storage at 20°C and higher caused severe weight loss and increased levels of fruit rots (Liu, 2005; Liu et al., 1998).

Table 3. Mean comparison of infection percentage for different plant essential oils at two temperatures.

Plant essential oils	25°C		6°C	
Cassia angustifolia	43.59±3.91	B	24.38±3.76	A
Ocimum basilicum	47.81±3.59	A	25.47±3.78	A
Mentha pulegium	45.16±3.99	AB	25.63±3.85	A

The results show that the highest infection percentages were due to *P. digitatum*, indicating that among tested fungi species, *P. digitatum* is one of the major causes of Valencia orange rot. Research has shown that the *Penicillium* spp. are one the most important and common reasons for postharvest decay of oranges in the world (Brown and Eckert, 1988; Snowdon, 1990; Fatemi and Borji, 2011). *Alternaria alternata* caused the lowest infection percentages, indicating its lower importance in after harvest rots of cv. Valencia than the other species tested.

Among essential oils tested, *C. angustifolia* decreased the infection percentage of tested fungi most efficiently. Other researchers have reported that essential oils of *C. angustifolia* contain antifungal components (example N-butanol) that reduce *As. flavus* and *As. terrus* growth (Gnanavel et al., 2012).

The present study also showed that the effect of various concentrations of essential oils differed (Tables 5 and 6). Infection percentages increased with increasing concentration of essential oils. The concentration that causes the lowest infection percentage was 200 ppm. These results are consistent with the results obtained by Adesegun et al. (2012), who reported that increasing the concentration of tested plant extracts decreased growth inhibition of *Sclerotium rolfsii*. This may be related to changes in osmotic pressure of the solutions, although how osmotic pressure can affect activity and growth of plant pathogenic fungi should be the subject of future research. Many researchers believe that osmotic pressure of environments around a fungus can affect the growth and pathogenicity of imperfect fungi (Motoyama et al., 2001).

The percentages of infection were similar at 6°C for all concentration of essential oils. However, at 25°C, *C. angustifolia* essential oils caused the least infection percentages. This showed that temperature can affect the intensity of effects of essential oils on infection percentages. Temperature also affected the activity of the tested fungi. At 25°C the infection percentages by *As. flavus* and *Al. alternata* were similar but they differed at 6°C. Fungal infections were all lower at 6°C than at 25°C,

Table 4. Mean comparison of effects of different concentrations of plant essential oils (EOs) on infection percentage at two temperatures.

EO concentration	25°C		6°C	
0	88.953**±2.60**	A	66.25±**7.75**	A
200	24.1667**±2.22**	C	9.1667±**1.29**	BC
400	29.1667**±1.97**	C	11.4583±**1.45**	BC
600	39.7919**±2.72**	B	13.75±**1.75**	BC

Means with common letters in the same column are not significantly different at P ≤ 0.01.

Table 5. Mean comparison of effects of different concentrations of plant essential oils (EOs) on infection percentage of fungi species at 25°C.

Plant EOs	Concentration	Without fungi		*Alternaria alternata*		*Penicillium digitatum*		*Aspergillus flavus*	
Cassia angustifolia	0	65±9.57	A	92.5±4.78	A	100±0.00	A	97.5±2.50	A
	200	10±4.08	BC	7.5±4.78	C	35±2.88	C	32.5±2.50	C
	400	27.5±4.78	B	25±2.88	B	30±7.07	C	37.5±4.78	C
	600	5±2.88	C	27.5±4.78	B	57.5±4.78	B	47.5±2050	B
Ocimum basilicum	0	57.5±8.53	A	95±5.00	A	100±0.00	A	100±0.00	A
	200	12.5±4.78	B	25±6.45	C	35±2.88	C	42.5±2.50	B
	400	20±7.07	B	30±4.08	BC	42.5±7.50	C	27.5±2.50	C
	600	32.5±6.29	B	42.5±2.50	B	62.5±2.50	B	40±7.07	B
Mentha pulegium	0	67.5±11.08	A	95±2.88	A	100±0.00	A	97.5±11.08	A
	200	12.5±7.50	C	10±4.08	C	37.5±6.29	B	30±7.07	C
	400	10±4.08	C	17.5±7.50	C	37.5±4.78	B	45±2.88	B
	600	15±6.45	B	40±4.08	B	52.5±7.50	B	55±2.88	B

Means with common letters in the same column are not significantly different at P ≤ 0.01.

Table 6. Mean comparison of effects of different concentrations of plant essential oils on infection percentage of fungi species at 6°C.

Plant EOs	Concentrations	without fungi		*Alternaria alternata*		*Penicillium digitatum*		*Aspergillus flavus*	
Cassia angustifolia	0	17.5±8.53	A	67.5±4.78	A	90±7.07	A	87.5±7.50	A
	200	0±0.00	B	5±2.88	B	20±4.08	B	7.5±4.78	B
	400	2.5±2.50	B	7.5±4.78	B	20±4.08	B	12.5±4.78	B
	600	2.5±2050	B	10±5.77	B	27.5±2.50	B	12.5±2.50	B
Ocimum basilicum	0	15±6.45	A	72.5±2.50	A	92.5±4.78	A	85±6.45	A
	200	2.5±2.50	B	5±2.88	B	20±0.00	C	7.5±2.50	B
	400	2.5±2.50	B	7.5±4.78	B	27.5±2.50	BC	12.5±4.78	B
	600	0±0.00	B	12.5±4.78	B	32.5±2.50	B	12.5±4.78	B
Mentha pulegium	0	10±7.07	A	80±4.08	A	90±4.08	A	87.5±4.78	A
	200	2.5±2.50	A	7.5±2.50	B	20±4.08	B	12.5±4.78	B
	400	2.5±2.50	A	12.5±2.50	B	22.5±2.50	B	7.5±4.78	B
	600	2.5±2.50	A	12.5±4.78	B	30±4.08	B	10±0.00	B

Means with common letters in the same column are not significantly different at P ≤ 0.01

and were likely due to differences in fungal growth with temperature. These results are similar to the results obtained by other researchers. The best temperature for growth of *Penicillium* spp. was found to be 23°C, and decreasing the temperature decreased growth (Agostini et al., 2006). Other research has also shown that temperature has a direct effect on the activity of the fungi that cause fruit rots in storage (Bulger et al., 1987).

In the present study, the greatest infection percentages were due to *P. digitatum* at 6°C, indicating that *P. digitatum* can grow even at low temperatures - this species can survive in cold temperatures for 45 d (Rab et al., 2012). Also, the results showed high infection percentage by *As. flavus* at 25°C and this was greatly de-

creased at lower storage temperature. Other researchers reported that the optimum temperature for this fungus was 25°C or above and temperatures below 15°C reduced the growth of *Aspergillus* spp. (Khanna and Chandra, 1975).

Many researches were conducted of using essential oils on growth of fungi under *in vitro* conditions, but this research was conducted under storage environments. Thus the results of this study can be applied in storage condition.

ACKNOWLEDGEMENT

The authors acknowledged Miss. M. Hadadian (Principal Technologist, Niazmand Plant Protection Clinic, Shiraz, Iran) effort and cooperation in the course of this project.

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