INHIBITORY EFFECT OF SOME MEDICINAL PLANTS ESSENTIAL OILS ON POST-HARVEST FUNGAL DISEASE OF CITRUS FRUITS

M. Azizi[1*], S. Farzad[3], B. Jafarpour[2], M.F. Rastegar[2], and V. Jahanbakhsh[2]
[1]Department of Horticulture Science, Ferdowsi University of Mashhad, Iran.
[2]Department of Plant Disease, Ferdowsi University of Mashhad,Iran.
[3]M.Sc. Student of Plant Disease, Ferdowsi University of Mashhad,Iran.

ABSTRACT

The consequence in misuse of chemical biocides for controlling pest and disease has drawn the attention of policy makers in development of methods potentially available in nature for the purpose. One of the new and safe methods of controlling pest and disease is the usage of essential oils from medicinal plants. In the present investigation, inhibition of radial growth and spore germination of post-harvest important fungi (*Penicillium italicum, P. digitatum* and *Alternaria citri*) exposed to the different concentrations of essential oils of some medicinal plants (*Thymus vulgaris, Mentha piperita, Satureja hortensis, Cuminum cyminum* and *Trachyspermum copticum*) were studied. The concentrations of essential oils examined were 250, 500 and 1000 mg/l. All the data were statistically analyzed. The results showed that the radial growth of *P.italicum* was completely inhibited by *Th. vulgaris* (500 mg/l), *S. hortensis* and *T. copticum* (1000 mg/l). The radial growth of *P.italicum* exposed to *C. cyminum* and *M. piperita* essential oil (1000 mg/l) was decreased (57.17% and 36.8%, respectively). The radial growth of *P. digitatum was* also completely inhibited by *Th. vulgaris*, *T. copticum* (500 mg/l) and *S. hortensis* (1000 mg/l). The radial growth of *P. digitatum* exposed to essential oils of *C.cyminum* and *M. piperita* were decreased 22.8% and 12.15%, respectively. *A. citri*'s radial growth was completely inhibited by *Th. vulgaris* (250 mg/l), *T. copticum* and *S. hortensis* (500 mg/l), *C. cyminum* (1000 mg/l). *M. piperita* essential oils (1000 mg/l) decreased the radial growth of *A. citri* to be 59.44%. Therefore, the inhibitory potency of essential oils on the post-harvest disease of citrus fruits was as *Th. vulgaris*> *T. copticum*> *S. hortensis*> *C. cyminum*> *M. piperita* and the extent of inhibition of fungal growth depended on the concentration of essential oils usage. These results clearly indicated that it is necessary to focus on practical application of the essential oils for inhibition of post-harvest pathogen growth and these compounds could be used as a substitute for chemical fungicides since they are natural and not toxic to humans.

KEYWORDS: Essential oils, Medicinal plants, Post-Harvest disease, Bio-control

1. INTRODUCTION

Worldwide estimates of post-harvest losses of fresh fruits and vegetables amount to 10 to 30% of total yield of crop, with a peak up to 50% and over in several less developed countries (Anonymous, 1981; Hulse, 1982). Post-harvest disease is a major factor in limiting shelf life and storage life of various fruits and vegetables. The main causal organisms are fungi that in addition to causing rot, they also can contaminate foods with highly toxic chemicals (Phillips, 1984; Wilson and Nuovo, 1973). The use of natural products as a supplement or an alternative to synthetic fungicide would reduce the indiscriminate application of phytochemical products which has led to a growing number of resistant strains, besides causing pollution. For these reasons, consumers tend to be suspicious of chemical matter and thus the demand for natural and socially more acceptable materials has been intensified (Skandamis *et al.* 2001).

* Corresponding author. Department of Horticulture Science, Ferdowsi University of Mashhad, Iran.

The activity of several essential oils against plant pathogenic bacteria and fungi has been widely studied, particularly in the last few years (Deans, 1991). The main part of the action of these oils on fungi has been concerned with the genera *Aspergillus* and *Botrytis* (Soliman and Badeaa, 2002; Moretti *et al.* 1998; Bishop and Reagan, 1998; Dwividi and Dubey, 1993). A few studies on the other important pathogens, like *Penicillium* spp., *Alternaria* spp. and *Rhizopus* spp. which can cause fruit and vegetable post-harvest rots have also been conducted (Caccioni and Guizzardi, 1994).

Because of health and economic consideration, the research was made to find out some essential oils of Iranian medicinal plants that could be safely used as substitutes for fungicides to control post-harvest diseases of citrus fruits.

2. MATERIALS AND METHODS

2.1 Essential oils extraction

The essential oils used in this study were prepared using a Clevenger apparatus in the Horticultural Department of Ferdowsi University of Mashhad (FUM). The extracted oils were got rid of water on anhydrous sodium sulphate and stored in a sterilized vial at 4° C until use. Commercially available sup plies of the plant materials used in this study are presented in Table1.

Table 1. Family, scientific name and common name of the plants studied in this experiments.

Family	Scientific name	Common name
Lamiaceae	*Thymus vulgaris*	Thyme
	Mentha piperita	Peppermint
	Satureja hortensis	Summer savory
Apiaceae	*Cuminum cyminum*	Green cumin
	Trachyspermum copticum	Ammi

Penicillium italicum, P. digitatum and *Alternaria citri* were isolated from decaying fruits of several citrus storages in Khorasan province. The isolated fungi were grown on potato dextrose agar (PDA), on Petri dishes, for 4-6 days at 25-28 °C. Each of the tested essential oil was used at different concentratio ns: 250, 500 and 1000 mg/l. Each concentration was mixed with the sterilized semi-solidified PDA (at 45-50 °C) and was then poured into a sterilized plate. A 8-mm diameter plugs of the fungal mycelium fr om the store plates was placed on the surface of the fresh PDA containing essential oils and incubated at 25-28 °C for 8 days (four replicates were used for each treatment). Two perpendicular diameters of g rowth zone were measured after 8-days cultivation.

2.2. Studies on spore germination inhibition of the essential oil

Plates contained essential oils were inoculated with 5µl of spore suspension at 10^5 spores per ml and in cubated at 25-28 °C for 20 h before the germinated spores were counted. Four replicate plates were u sed per experiment.

2.3. In vivo antifungal activity of the essential oils

We selected the treatment in 2.2 with best results for the following initial experiment. Fruits of *Citrus sinensis* sterilized by 1% sodium hypochlorite before being sprayed with 10^8 spore per ml. Spore suspensions were placed in a dessicator for 3 h. The essential oils were then applied to each piece of the fruit in each dessicator with a suitable volume and kept for 12 h at 18-20°C. Five replications were sele cted from each treatment. Each pieace of citrus fruit was drilled by a sterile cork borer (8mm) and then placed in PDA agar plate. The incubation was carried out at 25-28 °C in a 12-h day-night for 7-8 days u ntil fungal colonies were formed. The inoculated samples and percentages were calculated. All data was analyzed by MSTAT-C software and the mean of the fungal colonies were compared by Duncan's Multiple Range Test at 0.05 levels.

3. RESULTS AND DISCUSSION

The inhibition effect of the tested essential oil on the mycelial growth of *P. italicum* was shown in Fig 1. All the treatments decreased the mycelial growth of the fungus significantly. Essential oils of *Th. vulgaris* (500 mg/l), *T. copticum* (1000 mg\l) and *S. hortensis* (1000 mg\l) stopped mycelial growth of *P. italicum* completely. *C. cyminum* and *M. piperita* essential oils were failed to stop growth of the fungus even at 1000 mg/l. The radial growth inhibitions (%) of these essential oils were 57.17 and 36.8 respectively (Table 2). The higher the essential oils concentration, the more the radial growth inhibition. Caccioni and Guizzardi (1998) showed that the mono-terpense of the citrus essential oil decreased the mycelial growth of *P. italicum*. It was mentioned that *P. digitatum* was more sensitive to the essential oils than *P. italicum*. Arras and Arru (1998) reported that *Th. vulgaris* essential oil (250 ppm) had a strong inhibitory effect on *P.italicum, P.digitatum* and *A. citri*. They demonstrated that *Th. capitatus* oil as vapor could change the morphology of *P. digitatum* mycelia. Moreover, carvacrol was the main constituent that affects the fungal mycelium growth.

P.digitatum growth was also affected by application of the essential oils (Fig 2.). *Th. vulgaris*, *T. copticum* (500 mg/l.) and *S. hortensis* (1000mg/l.) stopped mycelium growth completely. The 250 mg/l essential oils of *T. copticum* and *S. hortensis* presented the similar effect by reducing the fungal growth about 65.42% and 68.6%, respectively (Table 2). *C. cyminum* and *M. piperita* oil at 250 mg/l failed to decrease the mycelial growth of *P.digitatum* and there was no significant difference between the treatments and the control in radial growth inhibition (Fig 2). Therefore, *P. digitatum* was more sensitive to the treatments than *P. italicum*.

Alternaria citri was more susceptible to the essential oils than *Penicillium spp*. It was controlled at 100% by *Th. vulgaris* (250 mg/l), *T. copticum* and *S. hortensis* (500 mg/l) and *C. cyminum* (1000 mg/l) (Fig 3). *Th. vulgaris* essential oil had more inhibitory effect than the other two species of the family Lamiaceae, *S. hortensis* and *M. piperita*. *Th. vulgaris* essential oil completely inhibited the fungal growth at the concentration of 250 mg/l while *S. hortensis* had the same effect at 500 mg/l. *M. piperita* oil at high concentration (1000 mg/l) only decreased mycelial growth of the fungus by 59.64 % (Table 2). Platto *et al.* (2003) showed that vapors of *Th. vulgaris* essential oil at 50 mg/l completely inhibited the growth of *A. arborescens* within 8 hours exposure and when *Th. vulgaris* oil incorporated to PDA it was fungistatic at 500 mg/l and fungicidal at 1000 mg/l.

In comparisons of the essential oil, it was shown that *Th. vulgaris* oil completely stopped mycelial growth of *Penicillium spp* at 500 mg/l but lower concentration (250 mg/l) was enough for stopping the mycelial growth of *A. citri*. *M. piperita* essential oils failed to inhibit mycelial growth of the fungus even at 1000 mg/l (Fig 1-3). Other literatures also showed inhibitory effects of *Th. vulgaris* oil on *P. italicum, P. digitatum* and *A. citri* (Arras and Arru, 1998).

As the results from Table 3, essential oils decreased the spore germination of the fungus significantly (except that of peppermint oil at 250 mg/l). Spore germination of *P.italicum* and *P.digitatum* were inhibited completely by *Th. vulgaris* and *T. copticum* essential oils at the high concentration (1000 mg/l) but that of *A. citri* was stopped only by *Th. vulgaris* oil at 500 mg/l or over. It also could be seen that as the oil concentration increases the germination inhibition increases. In other words, the inhibitory effect of the essential oil was proportional to the concentration.

In vivo bioassay of *T. copticum* and *Th. vulgaris* essential oils on infection potential of the fungus was shown in Table 4. Our results showed that *Th. vulgaris* and *T. copticum* oils can also decrease infected fruits percentage significantly when applied after inoculation of the fruits. *P. digitatum* inoculated fruits was controlled by *T. copticum* oil at 250 mg/l. *Th. vulgaris* oil at 150 mg/l completely controlled *P. digitatum* and *A. citri* inoculated fruits and 250 mg/l thyme oil was needed for controlling of *P. italicum* inoculated fruits (Table 4). Moretti *et al.* (1998) also showed that S*alvia officinalis* essential oils inhibited mycelial growth of *Botrytis cinerea* significantly and it was related to that obtained in Dichlorofluanid, one of the most effective synthetic fungicides against pathogens.

Fig1-Effect of different concentrations of essential oil on radial growth of Penicillium italicum

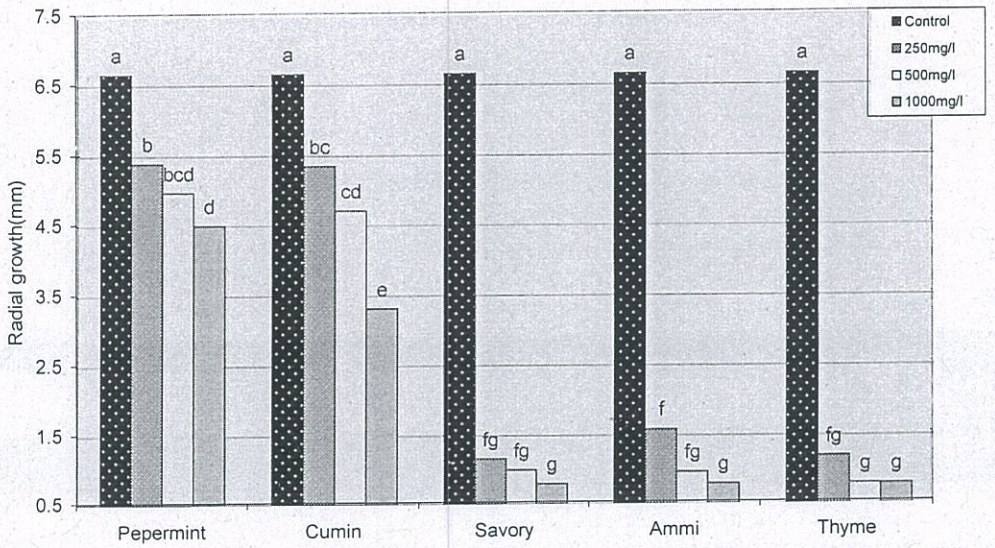

Figure 1 Effect of different concentrations of essential oil on radial growth of Penicillium italicum.

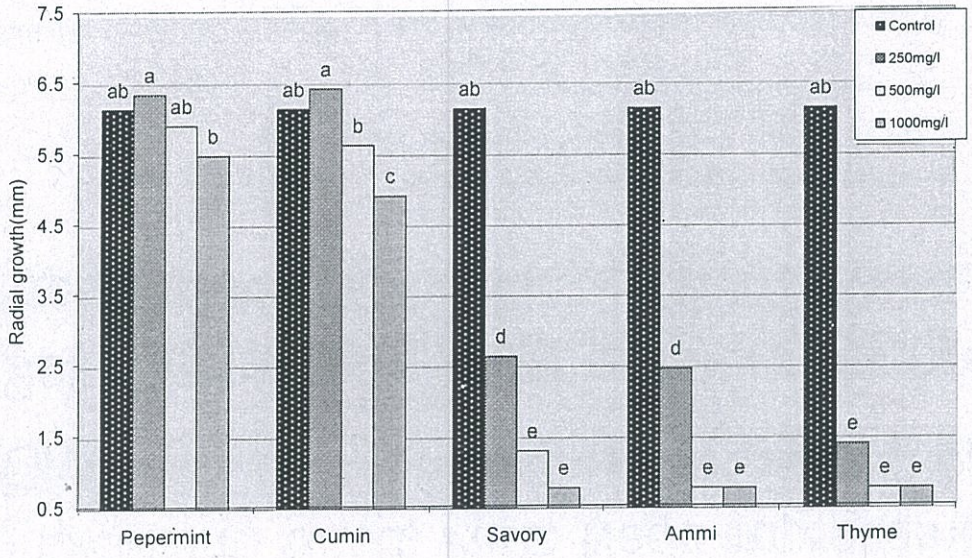

Figure 2 Effect of different concentrations of essential oils on radial growth of penicillium digitatum.

Table 2. Relatives percentage inhibitory effect of plant essential oil on the radial growth inhibition (%) of *Penicillium italicum, P. digitatum* and *Alternaria citri.*

	Penicillium italicum				*Penicilium digitatum*				*Alternaria citri*			
	Control	250 mg/l	500 mg/l	1000 mg/l	Control	250 mg/l	500 mg/l	1000 mg/l	Control	250 mg/l	500 mg/l	1000 mg/l
Mentha piperita	0	21.67	28.8	36.8	0	-3.93	4.3	12.15	0	20	23	59.24
Cuminum cyminum	0	22.35	33.28	57.17	0	-5.42	9.95	22.8	0	25.98	48.22	100
Satureja hortensis	0	93.86	96.50	100	0	65.42	90.28	100	0	74.5	100	100
Trachyspermum copticum	0	86.69	97.1	100	0	68.6	100	100	0	97.5	100	100
Thymus vulgaris	0	93.17	100	100	0	88.6	100	100	0	100	100	100

Inhibition effect was measured as percentage control= [1-(diameter treatment /diameter control)]×100

Table 3. Effect of different concentrations of some plant essential oils on spore germination (%) of caused post-harvest diseased fungi.

	Penicillium italicum				*Penicilium digitatum*				*Alternaria citri*			
	Control	250 mg/l	500 mg/l	1000 mg/l	Control	250 mg/l	500 mg/l	1000 mg/l	Control	250 mg/l	500 mg/l	1000 mg/l
Mentha piperita	100a	98.4a	84.6b	72.2c	100a	92.5b	75.2d	51.7f	100a	89.7b	84.3c	62.3d
Cuminum cyminum	100a	87.3b	73.2c	68.5d	100a	85.3c	63.4e	44.6g	100a	57.4e	37.4f	26.7g
Satureja hortensis	100a	28.5e	14.8f	2.3h	100a	42.7g	14.7i	3.2j	100a	38.6f	8.4ij	4.4k
Trachyspermum copticum	100a	16.2f	9.8g	0h	100a	22.4h	3.8j	0j	100a	16.5h	10.3i	3.2k
Thymus vulgaris	100a	10.3g	2.3h	0h	100a	19.3hi	5.4j	0j	100a	6.7jk	0k	0k

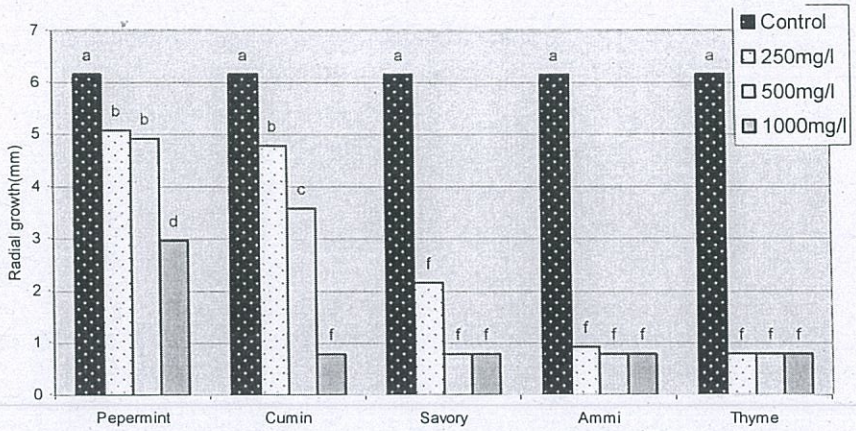

Figure 3 Effect of different concentration of plant essential oil on the radial growth of post-harvest disease control of Alternaria citri.

Table 4. In vivo bioassay of different concentrations of some plant essential oil on fruit infection (total number of fruit tested was 12).

	Penicillium italicum			*Penicilium digitatum*			*Alternaria citri*		
	Means of infected fruits	Percentage of infected fruit	Inhibition (%)	Means of infected fruits	Percentage of infected fruit	Inhibition (%)	Means of infected fruits	Percentage of infected fruits	Inhibition (%)
Control	11a	91.67	0	11a	91.67	0	10.33a	86.11	0
Ammi 75 mg/l	2.67b	22.22	75.76	4.67b	41.67	54.55	3.33b	27.78	67.74
Ammi150mg/l	0.67cd	5.56	93.94	0.67d	8.33	90.91	1.67c	13.89	83.87
Ammi250mg/l	0.33cd	2.78	96.97	0d	0	100	0.33d	2.78	96.77
Thyme 75mg/l	1.33c	11.11	87.88	2.67c	16.67	81.82	0.67d	8.33	90.32
Thyme150mg/l	0.33cd	2.78	96.97	0d	0	100	0d	0	100
Thyme250mg/l	0d	0	100	0d	0	100	0d	0	100

4. CONCLUSION

Our results were agreeable with most of the literatures that described that thyme and other medicinal plant essential oils could inhibit germination and growth of most post-harvest pathogens (Caccioni *et al.*1994). This suggested that the sensitivity of various pathogens may depend on the morphological and physiological characteristics of the fungal hyphae and spores. Volatile essential oils could be used in storage room. The development of low cost application for "appropriate technology" especially in developing countries is another area of further researches. These findings clearly indicated that essential oils should be applied for the inhibition of post-harvest pathogen growth and could be used as a substitute for chemical fungicides since they are natural and non-toxic to humans. Further investigations are being conducted to evaluate the economics of the essential oils in pilot and commercial applications.

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