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Evaluation of fungicidal and fungistatic activity of plant essential oils towards plant pathogenic and saprophytic fungi

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Summary. The contact and vapor effects of essential oils from different plants were studied *in vitro* for fungicidal and fungistatic activity towards different Basidiomycete, Ascomycete, Zygomycete and Oomycete taxa. Of nine essential oils tested, most were fungicidal at very low concentrations to most of the fungi. Hyphae were more sensitive than spores to the formulations. The essential oils citral, β-citronellol, geraniol and oil of lavender, at 1 μL mL^{-1} medium or 12 μL L^{-1} of air, inhibited growth and germination in the fungal species examined. Different species of fungal genera reacted differently to the formulations. Some of the formulations were fungistatic to spore germination.

Key words: plant extracts, contact effects, vapor effects, *Eumycota*, *Oomycota*.

Introduction

In the last few years, there has been increasing interest in essential oils as possible substitutes for conventional synthetic pesticides. This has been due to concern over ecosystem pollution and pesticide resistance in pests and pathogens (Holmes and Eckert, 1999). There are several examples of previous studies which have tested the fungicidal properties of various essential oils (Zambonelli *et al.*, 1996; Edris and Farray, 2003; Plotto *et al.*, 2003; Macias *et al.*, 2007; Neiri *et al.*, 2007). Essential oils have been reported to control *Monilinia laxa* in stone fruits (Neiri *et al.*, 2007), post harvest diseases in tomato (Plotto *et al.*, 2003), citrus (Klieber *et al.*, 2002, Wuryatomo *et al.*, 2003, Hall and Fernandez, 2004), molds (Moleyor and Norasimham, 1987; Nakahara *et al.*, 2003), food-borne pathogens (Kim *et al.*, 1995, Vazquez *et al.*, 2001, Conte *et al.*, 2007,), various bacteria (Stevens *et al.*, 1971, Kim *et al.*, 1995) and weeds (Macias *et al.*, 2007). Greatest attention has been focused on citral and related compounds which have been evaluated for fungicidal properties against various fungi (Wurytamo *et al.*, 2003; Stevens *et al.*, 2007).

Citral, with two isomers geranial and neral, is an acyclic α-β unsaturated monoterpene aldehyde which is a key compound of the lemon-scented essential oils extracted from several herbal plants such as lemongrass (*Cymbopogon citratus*), melissa (*Melissa officinalis*) and verbena (*Verbena officinalis*). The compound is used as a food additive and as a fragrance in cosmetics (Dudai *et al.*, 2005). Citral is present in the oil of several plants at different concentrations. These include lemon myrtle (90–98%), lemongrass (65–85%), lemon tea-tree (70–80%), lemon verbena (30–35%), lime (6–9%) and lemon (2–5%).

The objective of the present study was to evaluate the effects of essential oils, particularly citral, on growth and spore germination by vapor or con-

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tact for fungicidal or fungistatic activity against different classes of fungi and similar organisms. A preliminary report on this research was presented earlier (Banihashemi and Abivardi, 2006).

Materials and methods

Sources of essential oils, fungi and culture media

Essential oils were purchased from different sources and are shown in Table 1 (with reference codes used hereafter).

Several species of pathogenic and saprophytic fungi and Oomycetes were obtained from the fungal culture collection of the Department of Plant Protection, College of Agriculture, Shiraz University, Iran.

Potato dextrose agar (PDA, Merk, Darmstadt, Germany), and water agar supplemented with 1% dextrose (WAD) were used for most of the experiments reported here as culture media for growth and spore germination.

Effect of plant essential oils on growth

Contact effect. The formulations at different concentrations were added to melted agar media and carefully mixed by gentle swirling to avoid bubbles before pouring into 9 cm diam. plastic Petri dishes. After agar was solidified, a 5 mm block from the edge of an actively growing colony of each species was placed in the centre of each Petri dish and incubated at 25°C in the dark. The solvent acetone at different rates was also used in the medium in experimental controls. Daily measurements of radial growth were performed until respective colonies completely covered the medium in Petri dishes. Radial colony growth was converted to mm d^{-1}. For all treatments at least three replications were used and the experiments were repeated three times.

Vapor effect. Petri dishes (8 cm diam.) with medium were inoculated with each species as above and with lids removed, were incubated in air-tight 500 mL capacity clear glass jars at 25ºC in the dark. The candidate extracts were impregnated on 9 cm diam. Whatman No. 1 filter paper at different rates in respect to the volume of the jar and inserted into the inner part of the lid of each jar. Acetone was used for experimental controls. Colony diameter was measured 7 days after incubation or whenever respective colonies covered the medium surface in Petri plates. Plates in which no colony growth occurred were further incubated in the air to check any fungicidal or fungistatic reaction by the formulations. For all treatments at least three replications were used and the experiments were repeated three times.

Spore germination

Contact effect. Melted WAD was mixed with different rates of the formulations and dispensed in 9 cm diam. Petri dishes as described above. After agar solidified, fresh viable spore suspensions

Table 1. Plant essential oils (and reference codes) used in the present study.

Code	Formulation	Supplier	Article No.	Purity
C5	Oil of peppermint	Dixa (St. Gallen, CH)	1954	PhEur
C6	Oil of geranium	Fluka (Buchs, CH)	48799	Pure
C7	Oil of orange peel	Dixa (St. Gallen)	4323	PhEur
C13	Oil of lavender	Haenseler (Herisau, CH)	1-4850	PhEur
C15	Citral (cis & trans)	Fluka (Buchs)	29450	Pure
C16	(+/-) citronellal	Fluka (Buchs)	27470	Techn
C17	(+/-) β-citronellol	Fluka (Buchs)	27480	Pract
C18	Geraniol	Fluka (Buchs)	48799	Pure
C20	(+/-) linalool	Fluka (Buchs)	62140	Pure

in appropriate spore concentrations were flooded over the surface of the agar and the plates were then incubated at 25ºC in the dark. At different intervals the spore germination was monitored using a light microscope and compared with the experimental control. The inhibition of spore germination was calculated. At least 100 spores in 10 microscope fields were counted for each experimental replication.

Vapor effect. Petri dishes (8 cm diam.) containing WAD were flooded with spore suspension as above and with lids removed, incubated at 25ºC in the dark in air tight 500 mL glass jar containing formulations on filter paper at different rates as above. After 1, 2 or 3 days the Petri dishes were removed from the jars and spore germination was assessed. If no germination was observed the plates were further incubated in the air for a few days and germination was assessed to determine fungicidal or fungistatic effects.

Effect of formulations as soil fumigants

Chlamydospores of *Fusarium oxysporum* f. sp. *sesame* (the causal agent of Fusarium wilt of sesame) were produced in sand (Banihashemi and deZeeuw, 1973). Ten grams of sand inoculum of the pathogen was spread in 6 cm Petri dishes and incubated with the lid off at 25ºC in the dark in 500 mL air tight glass jar with formulations at different rates on filter paper as described above. Three replications were used. Experimental controls consisted of acetone as solvent. After 7 days, jars were opened and sand inoculum was mixed with 0.1% water agar and flooded on *Fusarium oxysporum* semiselective medium (Banihashemi and deZeeuw, 1969). After 7 days, the number of colony forming units was recorded and the percentage of survival was calculated.

Results

Contact effects of formulations

Of nine candidates including C5, C6, C13, C15, C16, C17, C18 and C20, most of them except C7 were very effective at 1 μL mL^{-1} for inhibiting growth of five *Phytophthora* spp. Some of the species were inhibited at 0.1 μL mL^{-1} by some candidates. For example *P. melonis* and to some extent *P. citrophthora* were not inhibited by C5 at 0.1 μL mL^{-1}, whereas *P. cinnamomi* was very sensitive to this extract (Table 2). At concentrations of 1 μL mL^{-1}, almost all of the candidates except oil of orange (C7) was not effective. Among the essential oils, C15, C17, and C18 were the most effective growth inhibitors on *Phytophthora* spp. tested (Table 2).

The same essential oils were tested at 1 μL mL^{-1} for growth inhibition of several different fungi. The sensitivity of the species to different formulations varied. C5 inhibited *Botrytis cinerea* and *Aspergillus niger* but not *Rhizopus stoloni–fera, Penicillium italicum* or *Geotrichum candidum* (Table 3). C7 was the least effective inhibitor of spore germination. The maximum inhibition of spore germination was found by citral (C15), β-citronellol (C17), and geraniol (C18). Spore germination of *Fusarium oxsysporum* f. sp. *sesame* was inhibited by C15, C17 and C18. Several species of *Pythium, Phytophthora, Fusarium, Aspergillus* and other genera were very sensitive to the formulations and no growth was detected after 6 days incubation (data not shown).

Assessment of contact effects of C15, C17 and C18 on two common citrus molds; *Penicillium italicum* and *P. digitatum* showed that only growth of *P. italicum* (the cause of citrus blue mold) was effectively reduced at 0.5 to 1 μL mL^{-1} but was not effective against *P. digitatum* (the cause of citrus green mold) at these concentrations. Lower concentrations of the extracts were not inhibitory. Some formulations, like C15 and C18 at the higher rates, to some extent enhanced growth of *P. digitatum* (Figure 1).

Vapor effects of formulations

Growth. Several fungi and fungus-like organisms were tested using various formulations at different rates. Growth of *Pythium aphanidermatum* and *Botrytis cinerea* was significantly reduced or inhibited at 12 μL L^{-1} by all formulations except oil of orange (Figures 2 and 3). *Phytophthora* species were inhibited by different formulations at concentration as low as 12.4 μL L^{-1} air (Table 4). Citral was very effective against *P. aphanidermatum* and five species of *Phytophthora* examined. Crude extract of oil of lavender (C13) at 120 μL L^{-1} air and citral at 6.2 μL L^{-1} air inhibited growth of *P. aphanidermatum*. *Phy-*

Table 2. Mean colony growth rate (mm day^{-1})(contact effects) for different *Phytophthora* spp. grown on agar plates amended with different essential oils [a].

Essential oil	Concentration μL mL^{-1}	*P. capcisi*	*P. cinnamomi*	*P. citrophthora*	*P. melonis*	*P. nicotianae*
Oil of lavender	0	5.5 c-e	7.9 a	5.8 b-e	8.2 a	5.8 b-e
	0.5	4.5 gh	0.0 u	5.0 e-g	4.1 hi	4.5 gh
	5	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u
Citral	0	5.5 c-e	7.9 a	5.8b -e	8.2 a	5.8 b-e
	0.1	2.3 m-p	0.0 u	0.6 s-u	0.0 u	0.0 u
	1	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u
Citronellal	0	5.5 c-e	7.9 a	5.8 b-e	8.2 a	5.8 b-e
	0.1	3.0 j-m	6.5 b	3.8 h-j	3.1 j-l	1.2 r-t
	1	0.0 u	0.0 u	0.5 tu	0.0 u	0.0 u
β-Citronellol	0	5.5 c-e	7.9 a	5.8 b-e	8.2 a	5.1 b-e
	0.1	1.6 p-r	0.0 u	3.3 i-k	0.0 u	0.0 u
	1	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u
Geraniol	0	5.5 c-e	7.9 a	5.8 b-e	8.2 a	5.8 b-e
	0.1	1.0 r-t	0.0 u	2.6 k-n	0.6 s-u	1.0r -t
	1	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u
Linalool	0	5.5 c-e	7.9 a	5.8 b-e	8.2 a	5.8 b-e
	0.1	4.9 fg	5.6 c-e	4.9 fg	3.8 h-j	2.4 m-o
	1	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u
Oil of peppermint	0	5.5 c-e	7.9 a	5.8 b-e	8.2 a	5.8 b-e
	0.1	2.4 l-n	0.0 u	5.0 e-g	5.4 d-g	3.5 ij
	1	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u
Oil of geranium	0	5.5 c-e	7.9 a	5.8 b-e	8.2 a	5.8 b-e
	0.1	3.3 i-k	2.0 n-q	1.4 q-s	0.7 s-u	0.7 s-u
	1	0.0 u	0.0 u	0.0 u	0.0 u	0.0 u
Oil of orange	0	5.5c-e	7.9 a	5.8 b-e	8.2 a	5.8 b-e
	0.1	3.8 h-j	6.0 b-d	5.4 d-g	3.8 h-j	3.5 ij
	1	1.4 q-s	6.3 bc	5.8 b-e	6.6 b	1.6 o-r

[a] Means accompanied by the same letter are not significantly different ($P \leq 0.01\%$) (Duncan's multiple range test).

tophthora spp. were more sensitive to most of the formulations than *P. aphanidermatum* at all concentrations tested (Table 4). *P. aphanidermatum* was more sensitive to oil of orange (C7) (Figure 2) than most of the *Phytophthora* species examined (Table 4).

Spore germination. Among the formulations tested on different molds such as *Aspergillus niger, Botrytis cinerea, Geotrichum candidum, Penicillium italicum* and *Rhizopus stolonifer*, different reactions were observed (Table 5). *Aspergillus niger* and *R. stolonifer* were more

Table 3. Mean percentage spore germination of different fungi exposed to different essential oils [a].

Essential oil	Concentration µL mL^{-1}	*Aspergillus niger*	*Botrytis cinerea*	*Geotrichum candidum*	*Penicillium italicum*	*Rhizopus stolonifer*
Oil of lavender	0	100 a	100 a	100 a	100 a	100 a
	0.5	100 a	100 a	100 a	100 a	60 c-e
	5	0 j	0 j	0 j	53 d-g	11 h-j
Citral	0	100 a	100 a	100 a	100 a	100 a
	0.1	0 j	13 h-j	76 a-d	0 j	0 j
	1	0 j	0 j	0 j	0 j	0 j
Citronellal	0	100 a	100 a	100 a	100 a	100 a
	0.1	100 a	83 a-c	100 a	100 a	70 a-d
	1	60 c-e	70 a-d	17 h-j	0 j	10 h-j
β-Citronellol	0	100 a	100 a	100 a	100 a	100 a
	0.1	100 a	70 a-d	100 a	93 ab	70 a-d
	1	0 j	0 j	0 j	0 j	0 j
Geraniol	0	100 a	100 a	100 a	100 a	100 a
	0.1	93 ab	66 b-d	100 a	100 a	100 a
	1	0 j	0 j	0 j	0 j	0 j
Linalool	0	100 a	100 a	100 a	100 a	100 a
	0.1	100 a	100 a	100 a	100 a	100 a
	1	33 ei	20 h-j	100 a	100 a	0 j
Oil of peppermint	0	100 a	100 a	100 a	100 a	100 a
	0.1	100 a	100 a	100 a	100 a	86 ei
	1	0 j	6 ij	100 a	100 a	33 a-c
Oil of geranium	0	100 a	100 a	100 a	100 a	100 a
	0.1	100 a	36 eh	100 a	100 a	23 g-j
	1	0 j	20 h-j	26 f-j	66 b-d	0 j
Oil of orange	0	100 a	100 a	100 a	100 a	100 a
	0.1	100 a	100 a	100 a	100 a	100 a
	1	100 a	96 a	90 ab	100 a	100 a

[a] See Table 2.

sensitive to the formulations than other species of fungi examined. Spores of *G. candidum* and *Penicillium italicum* were very resistant to most of the formulations except citral (Table 5). Increasing exposure time increased fungicidal activity of the essential oils. Shorter exposure, however, was fungistatic for some formulations. Citral after 24h exposure was fungicidal (data not shown). C5, C13, C15 and C20 were fungistatic to conidia of *Botrytis cinerea* (the cause of strawberry grey mold). Germination of spores of *Penicillium italicum* and *P. digitatum* was completely inhibited by C15 at 0.1 µL L^{-1} of air. C17 and C18 were not effective even up to 1 µL L^{-1} air and resulted in 100% germination (Figure 4).

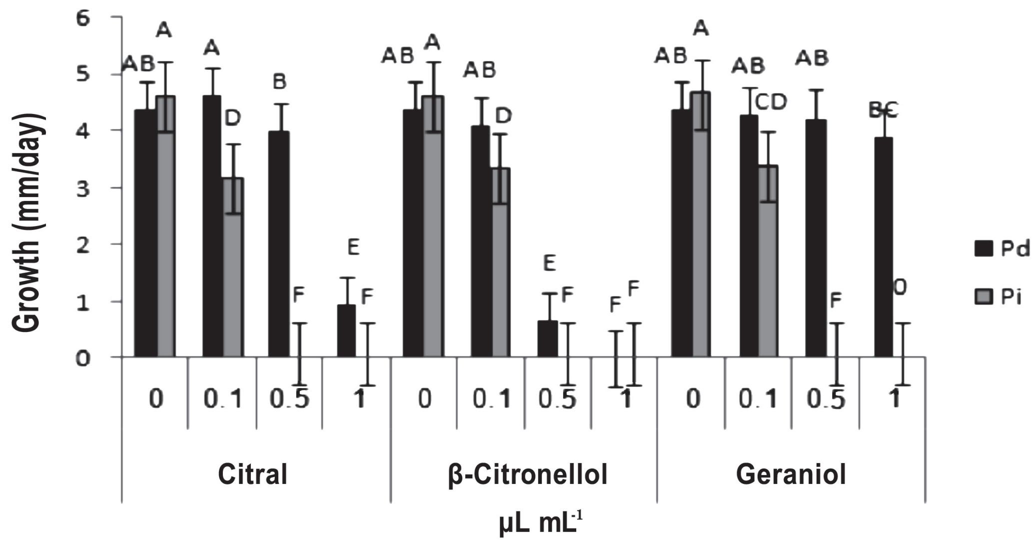

Figure 1. *Penicillium italicum* (Pi) and *P. digitatum* (Pd) grown on agar plates amended with different essential oils (contact effect). Bars not followed by the same letter are not significantly different ($P \leq 0.01\%$), Duncan's Multiple Range Test.

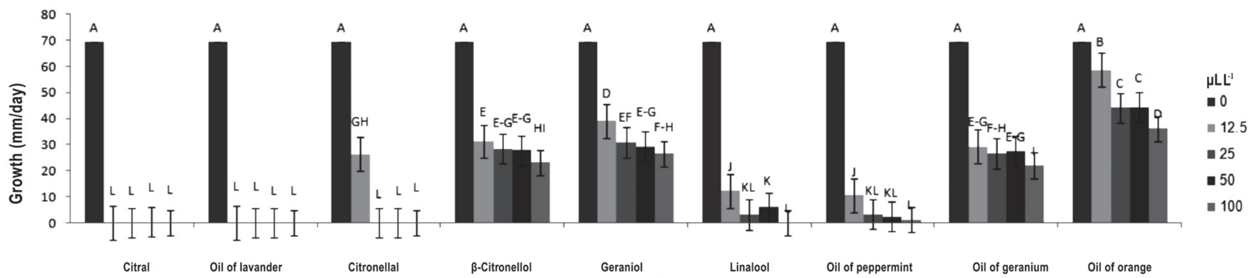

Figure 2. Mean colony growth rate (mm day^{-1}) (fumigant effect) of *Pythium aphanidermatum* grown on agar plates amended with different essential oils. Bars followed by the same letter are not significantly different ($P \leq 0.01\%$), Duncan's Multiple Range Test. (concentration of oil of lavender: 0, 0.25, 0.5, 1, 2 mL L^{-1} jar).

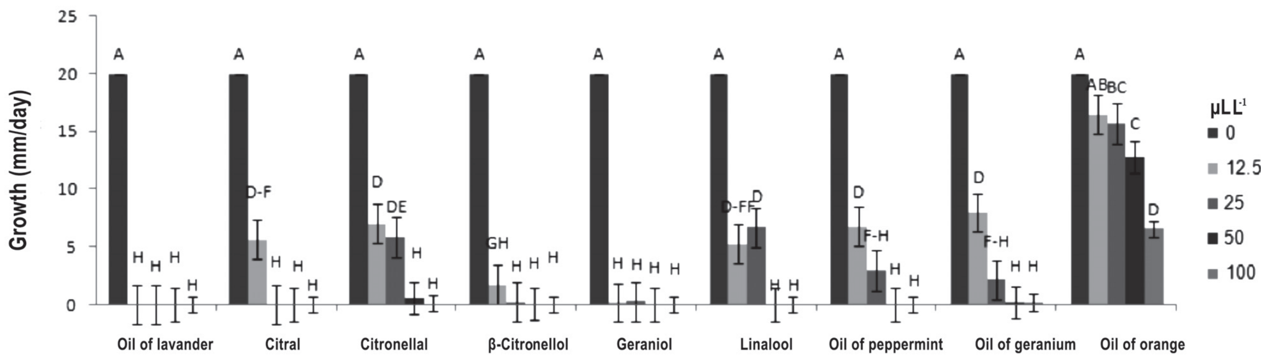

Figure 3. Mean colony growth rate (mm day^{-1}) (fumigant effect) of *Botrytis cinerea* on agar plate amended with different essential oils Bars followed by the same letter are not significantly different ($P \leq 0.01$), Duncan's Multiple Range Test. (Concentration of oil of lavender: 0, 0.25, 0.5, 1, 2 mL L^{-1} jar).

Table 4. Mean colony growth rates (mm day^{-1}) (fumigant effect) for different *Phytophthora* spp. exposed to different essential oils [a].

Essential oil	Concentration μL L^{-1} jar	*P. capsici*	*P. melonis*	*P. nicotianae*	*P. cinnamomi*	*P. citrophthora*
Oil of lavender	0	5.9 ab	5.5 bc	6.4 a	6.1ab	5.9 ab
	250	0.0 r	0.0 r	0.0 r	0.0r	0.0 r
	500	0.0 r	0.0 r	0.0 r	0.0r	0.0 r
	1000	0.0 r	0.0 r	0.0 r	0.0r	0.0r
	2000	0.0 r	0.0 r	0.0r	0.0r	0.0 r
Citral	0	5.9 ab	5.0 b c	6.4 a	6.1 ab	5.9 ab
	12.5	0.0 r	1.7 g-k	2.7 f	1.7 g-l	0.0 r
	25	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	50	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	100	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
Citronellal	0	5.9 ab	5.5 bc	6.4 a	6.1 ab	5.9 ab
	12.5	5.5 ac	2.5f g	2.8 f	2.6 f	2.4 fh
	25	0.0 r	0.8 m-r	0.8 l-r	0.0 r	1.5 in
	50	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	100	0.0 r	0.0 r	0.0 r	0.0 r	0.8 m-r
β-Citronellol	0	5.9 ab	5.5 bc	6.5 a	6.2 ab	5.9 ab
	12.5	2.0 f-j	1.6 h-m	0.0 r	0.0 r	1.4 j-o
	25	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	50	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	100	0.0r	0.0 r	0.0 r	0.0 r	0.0 r
Geraniol	0	5.9 ab	5.5 bc	6.5 a	6.2 ab	5.9 ab
	12.5	2.3 f-i	1.2 j-p	0.6 n-r	0.0 r	0.0 r
	25	0.8 lr	0.0 r	0.0 r	0.0 r	0.0 r
	50	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	100	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
Linalool	0	5.9 ab	5.5 bc	6.5 a	6.2 ab	5.9 ab
	12.5	6.1 ab	4.1 de	3.8 e	2.1 f-j	2.3 f-i
	25	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	50	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	100	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
Oil of peppermint	0	5.9 ab	5.0 bc	6.5 a	6.2 ab	5.9 ab
	12.5	4.9 cd	6.3 ab	4.5 de	0.0 r	1.2 j-p
	25	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	50	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
	100	0.0 r	0.0 r	0.0 r	0.0 r	0.0 r
Oil of geranium	0	5.9 ab	5.5 bc	6.5 a	6.2 ab	5.9 ab
	12.5	2.8 f	4.9 cd	4.4 de	1.1 k-q	1.1 k-q
	25	0.7 m-r	0.3 q-r	2.0 f-k	0.4 p-r	0.5 o-r
	50	0.5 o-r	0.2 q-r	0.5 o-r	0.0 r	0.2 q-r
	100	0.0 r	0.0 r	0.0 r	0.0 r	2.6 f

[a] See Table 2.

Soil fumigation. Two-month-old inocula of *Fusarium oxysporum* f. sp. *sesame* consisted mainly of chlamydospores. Exposure of this inoculum for 7 days to vapors of several formulations at 50 and 100 μL L^{-1} of air effectively reduced populations. C15, C16, C18 and C20 completely killed the inoculum at 50 to 100 μL L^{-1} air (Figure 5). Some formulations such as oil of peppermint (C5) and oil of orange (C7) increased populations at both of the rates tested.

Discussion

Essential oils are complex mixtures of various volatile compounds derived from plants. They are extracted from plant organs and possess a variety of medicinal and antimicrobial properties. The antimicrobial, anti-oxidant and anti-inflammatory properties of these materials have been shown in animal and human food (Kim *et al*., 1995, Vazques *et al*., 2001, Dudai *et al*., 2003, Conte *et al*., 2007, Dalleau *et al*., 2008, Park *et al*., 2009).

An initial investigation carried out to assess the efficacy of certain plant extracts on various fungi and fungus-like organisms indicated the potential antifungal activity of most of the tested candidates. Both contact and vapor effects were confirmed on many plant pathogenic and saprophytic fungi and fungus-like organisms. In most cases hyphae were more sensitive to the formulations than spores. In some instances some of the formulations were very effective as vapors at rates as low as 6 μL L^{-1} air.

There are few reports on the effect of plant essential oils on Oomycete plant pathogens (Singh *et al*., 1992, Zambonelli *et al*., 1996, Bianchi *et al*., 1997). The present study is the most comprehensive on the effect of various plant essential oils on different species of plant pathogenic Oomycetes causing serious diseases on many annual and perennial plants. Among the formulations tested, citral was very inhibitory to these organisms, both by contact and as a vapor. The reaction of Oomycetes including *Pythium* and *Phytophthora* spp. varied to the different formulations. While growth of some species was completely inhibited at the rate of 0.1 μL mL^{-1} of the culture medium, some candidates were only effective if the concentration was increased to the higher levels. Under vapor state, the essential oils were effective on growth inhibition at 12 μL L^{-1} of the air tight containers. Some essential oils, such as oil of orange, were not effective against the fungi examined at the rate of 100 μL L^{-1} of air. Growth of *Pythium aphanidermatum,* a high temperature pathogen, was completely inhibited by contact (at 1 μL mL^{-1} of the medium) and vapor (at 6 μL L^{-1} air) by most of the formulations tested.

The 6 μL L^{-1} air rate inhibited growth of most of the taxa tested in Basidiomycetes, Ascomycetes, Zygomycetes and Oomycetes. The rates of efficacy determined for the most of the formulations used in the present study are much lower than reported elsewhere (Plotto *et al*. 2003, Wuryatmo *et al*. 2003, Hall and Fernandez 2004).

Reactions of spores to the formulations varied. Some essential oils were fungicidal and some fungistatic even at low rates. Killing efficiency of spores was time dependent. Extended exposure time of spores to the formulations resulted in complete mortality. Spores of *R. stolonifer* (common bread mold) were resistant to vapors of nine of the formulations at low rates. Some formulations, such as oil of lavender (crude extract) and citral (pure), respectively at the rates of 0.250 and 12 μL L^{-1} air, resulted in complete death of the spores (Table 5). Spores of *R. stolonifer* that had been exposed at different time intervals for 1 to 3 days and higher rates, gave complete inhibition of spore germination within 24 h. However, few formulations were not fungicidal at higher rates and shorter exposure times. Incubating the treated spores under air resulted in the resumption of germination. Only citral and oil of geranium were fungicidal as vapors at 100 μL L^{-1} air during 24h of exposure. Longer incubation was necessary to achieve inhibition by other formulations (data not shown).

There were great differences in sensitivity between *P. italicum* and *P. digitatum* to some candidates (Figures 1 and 4)). *Penicillium italicum* was more sensitive to citral, β-citronellol and geraniol than *P. digitatum*, which was inhibited at the rate of 0.5 μL mL^{-1} of the media as contact effect. Under vapor conditions, spore germination by *P. italicum* was completely inhibited at 0.1 μL mL^{-1} by C15 but not affected by C17 and C18 even at 1 μL mL^{-1} (Figure 1). There are several reports of effective post harvest treatments with citral and related compounds for inhibition of citrus molds caused

Table 5. Mean percentage spore germination (fumigant effect) for different fungi exposed to different essential oils [a].

Essential oil	Concentration μL L^{-1} jar	*Aspergillus niger*	*Botrytis cinerea*	*Geotrichum candidum*	*Penicillium italicum*	*Rhizopus stolonifer*
Oil of lavender	0	100 a	100 a	100 a	100 a	100 a
	250	0 f	0 f	100 a	100 a	0 f
	500	0 f	0 f	100 a	100 a	0 f
	1000	0 f	0 f	100 a	100 a	0 f
	2000	0 f	0 f	100 a	100 a	0 f
Citral	0	100 a	100 a	100 a	100 a	100 a
	12.5	0 f	27 e	0 f	0 f	0 f
	25	0 f	0 f	0 f	0 f	0 f
	50	0 f	0 f	0 f	0 f	0 f
	100	0 f	0 f	0 f	0 f	0 f
Citronellal	0	100 a	100 a	100 a	100 a	100 a
	12.5	100 a	100 a	73 b	100 a	100 a
	25	66 bc	100 a	0 f	100 a	100 a
	50	0 f	100 a	0 f	100 a	100 a
	100	0 f	100 a	0 f	63 bc	6 f
β-Citronellol	0	100 a	100 a	100 a	100 a	100 a
	12.5	70 b	100 a	100 a	100 a	100 a
	25	55 c	100 a	100 a	100 a	53 c
	50	0 f	100 a	100 a	100 a	66 bc
	100	0 f	100 a	100 a	100 a	0 f
Geraniol	0	100 a	100 a	100 a	100 a	100 a
	12.5	40 d	100 a	100 a	100 a	100 a
	25	0 f	100 a	100 a	100 a	100 a
	50	0 f	100 a	100 a	100 a	100 a
	100	0 f	100 a	100 a	100 a	6 f
Linalool	0	100 a	100 a	100 a	100 a	100 a
	12.5	100 a	100 a	100 a	100 a	100 a
	25	33 de	100 a	100 a	100 a	0 f
	50	0 f	0 f	0 f	100 a	0 f
	100	0 f	0 f	0 f	100 a	0 f
Oil of peppermint	0	100 a	100 a	100 a	100 a	100 a
	12.5	100 a	100 a	100 a	100 a	100 a
	25	0 f	0 f	100 a	100 a	53 c
	50	0 f	0 f	100 a	100 a	66 bc
	100	0 f	0 f	100 a	100 a	0 f
Oil of geranium	0	100 a	100 a	100 a	100 a	100 a
	12.5	100 a	100 a	100 a	100 a	100 a
	25	100 a	100 a	100 a	100 a	39 d
	50	65 bc	100 a	100 a	100 a	0 f
	100	7 f	100 a	100 a	100 a	0 f
Oil of orange	0	100 a	100 a	100 a	100 a	100 a
	12.5	100 a	100 a	100 a	100 a	100 a
	25	100 a	100 a	100 a	100 a	100 a
	50	100 a	100 a	100 a	100 a	100 a
	100	100 a	100 a	100 a	100 a	76 b

[a] See Table 2.

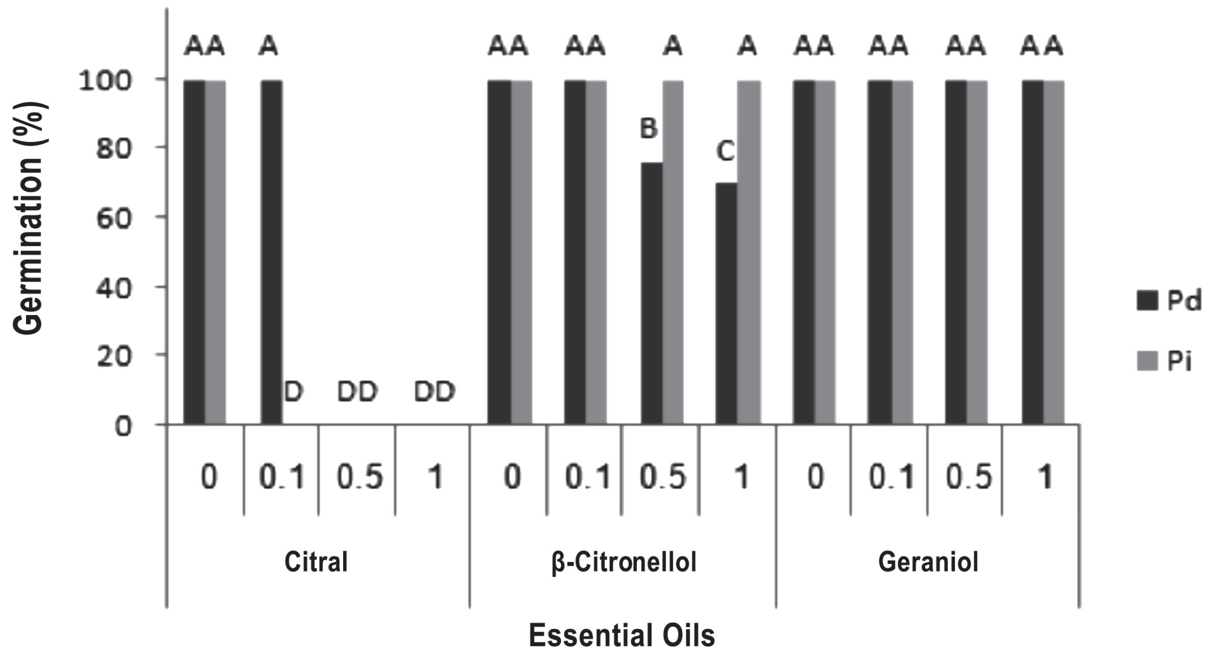

Figure 4. Mean percentage spore germination (fumigant effect) of *Penicillium italicum* (Pi) and *P. digitatum* (Pg) exposed to different essential oils. Bars followed by the same letter are not significantly different ($P \leq 0.1\%$), Duncan's Multiple Range Test.

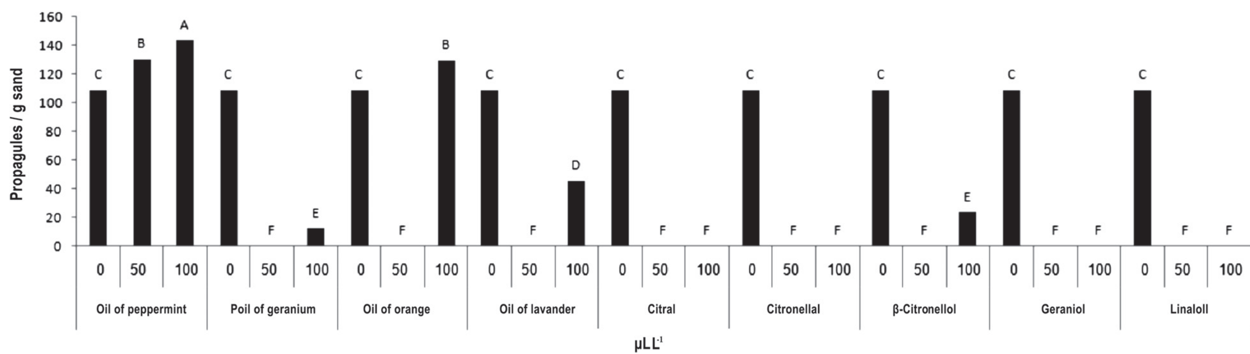

Figure 5. Mean numbers of propagules of *Fusarium oxysporum* f. sp. *sesame* in sand (fumigant effect) after exposure to different essential oils. Bars followed by the same letter are not significantly different ($P \leq 0.01\%$), Duncan's Multiple Range Test.

by *P. italicum*, *P. digitatum* and *Geotrichum candidum* (Wuryatmo *et al*., 2003), of food borne microorganisms (Conte *et al*., 2007) and of brown rot of stone fruits (Neiri *et a*l., 2007).

Citral effectively killed the resistant propagules (chlamydospores) of *F. oxysporum* f. sp. *sesame* in sand at low rates (Figure 5). Some formulations like oil of lavender (crude extract) at the rate of 250 μL L^{-1} air and citral (pure) at 12 μL L^{-1} air resulted in complete destruction of the propagules (Figure 5).

Conclusions

Plant essential oils are one of the promising safe and environmentally-friendly candidates for future use as alternatives to conventional synthetic pesticides for managing fungi and fungus-like organisms as plant pathogens, food contaminants and decays. Most of the formulations used in the present study *in vitro,* showed high efficacy as contact and fumigants on different fungal taxa at very low concentrations. Among the candidates used, citral, its isomers and geraniol effectively inhibited mycelial growth and spore germination through fungicidal and fungistatic actions. The active ingredients of the candidates and their isomers could be used as seed and soil treatments, and also as preventives of post harvest decay and as food preservatives. Their efficacy of these materials *in vivo* must be confirmed, however, before any conclusion can be made on their general application.

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