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In vitro Antifungal Activities of Essential Oils from selected Species of Family Myrtaceae

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Abstract

Present study was designed to examine *in vitro* antifungal activity of essential oils extracted from different species of family Myrtaceae against five mycotoxigenic fungal species viz. *Aspergillus niger*, *Aspergillus solani*, *Fusarium oxysporum*, *Fusarium solani* and *Penecillium digitatum*. The essential oils were extracted by hydro-distillation and characterized chemically by GC and GC-MS. *In vitro* antifungal studies were done by agar well diffusion and microdilution method. The essential oils exhibited varied toxic effects on mycelium growth of selected fungal species. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of essential oils ranged from 2–8 µg/ml against tested fungal strains. Time kill assay showed significant fungicidal effect of oils for four weeks.

Keywords: *Antifungal activity; Aspergillus niger; Fusarium oxysporum; agar well diffusion; time kill assay*

Introduction

Food and food products are susceptible to spoilage during storage. Mycotoxigenic fungi are generally associated with the spoilage of the food products (Adebayo *et al*., 2014). Food spoilage may result in discolouration, mycotoxin contamination, off-flavours and rotting. Synthetic preservatives are preferred in food industry due to their effectiveness and low price to impede spoilage and microbial infectivity (Bajpai *et al*., 2008). However, a renewed interest in natural preservation has appeared stimulated by present food safety concerns, development of microbial resistance, and demands for production of minimally processed food coupled with green image policies of food industries. Among the emerging natural preservatives, the interest in essential oils has been boosted up owing to their diverse biological (antimicrobial, antioxidants, anticarcinogenic) properties (Gutierrez *et al.,* 2009).

Myrtaceae is one of the most diverse and widespread plant families, rich in essential oils. The main genera of Myrtaceae include *Eucalyptus, Eugenia, Leptospermum, Melaleuca, Myrtus, Pimenta, Plinia, Psidium, Pseuocaryophyllus* and *Syzygium.*
Eucalyptus is one of the important genera of Myrtaceae and comprises about 800 species and subspecies (Gil *et al.*, 2010). Most of the species are native to Australia. However, they have been cultivated throughout the tropics and subtropics including Africa, America, China, Europe, India, Mediterranean Basin and Middle East (Guenther, 1952). *Eucalyptus* species are a rich resource of essential oil of medicinal and commercial importance. *Eucalyptus* essential oils have also exhibited antibacterial (Elaissi *et al.*, 2012), antioxidant, anti-inflammatory (Silva *et al*., 2003), antifungal (Somda *et al.*, 2007), antiviral (Schnitzler *et al.*, 2001) and insecticidal activities (Jemâa *et al*., 2014).
The genus *Melaleuca* L. of Myrtaceae consists of around 260 species and occurs predominantly in Australia but is also domesticated in South-East Asia, the Southern United States and the Caribbean (Tran *et al.*, 2013). They are shrubs or trees, generally found in open forests, woodlands or shrublands, particularly along the watercourses and the edges of swamps. The essential oils from *Melaleuca* have demonstrated antibacterial (Hussein *et al.*, 2007), anti-inflammatory (Caldefie-Chezet *et al.*, 2006), fungicidal (Bagg *et al.*, 2006), acaricidal (Iori *et al.*, 2005), antioxidant (Pino *et al.*, 2010) and antiviral properties (Minami *et al.*, 2003).
Keeping in view the antimicrobial properties of family Myrtaceae and recent trend of use of natural preservatives, ten indigenous species from *Eucalyptus* and *Melaleuca* genera namely *E. crebra*, *E. kitsoniana*, *E. melanophloia*, *E. microtheca*, *E. pruinosa*, *E. rudis, E. tereticornis, M. bracteata* and *M. fulgens* and *M. leucadendron* have been selected to evaluate their antifungal activity against foodborne pathogens.

Methods

2.1. Plant Material

Mature leaves of ten selected species of Myrtaceae were collected from the different localities of Pakistan [Table 1]. The taxonomic authentication was performed by Prof. Dr. A. N. Khalid, at the Herbarium, Department of Botany, University of Punjab, Lahore, Pakistan. Plant specimens were deposited in the same herbarium.

2.2. Chemicals: Ethanol and methanol used in this study were purchased from Merck (Darmstadt, Germany). Culture media (Nutrient broth, Nutrient agar, Potato dextrose agar, Plate count agar) were purchased from OXOID Ltd. Hampshire, UK and HiMEDIA, Mumbai. India.

2.3. Isolation of Oils

From each plant, fresh leaves weighing 2 kg were hydrodistilled for 3 hours using Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [28]. Oils were dried over anhydrous sodium sulfate, filtered and stored at -4°C in a freezer until analyzed. The essential oil contents (%) were expressed as volume of essential oil vs. weight of fresh leaves (v/w).

2.4.. Evaluation of Antibacterial Activities of Essential Oils

2.4.1. Tested Microorganisms

Five local fungal strains namely *Aspergillus niger* (AC 1109), *Aspergillus flavus* (AC 1110), *Fusarium oxysporum* (AC 1175), *Fusarium solani* (AC 1199), and

Penicillium digitatum (AC 1160) obtained from fungal bank, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan were selected for *in vitro* antifungal activity of essential oils. All fungal strains were sub-cultured at 25°C for 120 h on potato dextrose agar (PDA) slants to prepare spore suspension before testing.

2.4.2. Agar well Diffusion Method

Antifungal activity of the selected essential oils was checked by agar well diffusion method (Zaika, 1988). Molten agar medium (20 ml) was inoculated with microbial suspension containing the indicator strain having 10^6 cfu/ml concentration. The inoculated medium was poured into Petri plates and allowed to solidify. Wells were made on solidified agar with a sterilized cork borer and 90 µl of the tested oil was added to each. Nystatin was used as a positive control. The plates were incubated at 25°C for 48 h. The diameters of inhibition zones were measured in millimeters and results were recorded in triplicate.

2.4.3. Minimum Inhibitory Concentration (MIC) Assay

Serial dilutions of 4 µg/ml, 8 µg/ml, 15 µg/ml, 65 µg/ml and 250 µg/ml were used in triplicate to determine MIC levels by agar well method (Zaika, 1988). The lowest concentration of oil inhibiting visible growth of each microbe after incubation was taken as the MIC.

2.4.4. Minimal Bactericidal Concentration (MBC) Assay

Minimum fungicidal Concentration (MFC) was determined by broth microdilution method (Rabe, *et al.*, 2002). Fungal spore load (10^6 cfu/ml) was poured in tubes containing respective culture broth and oil with concentration of MIC. Broth tubes with and without fungal load were used as controls. The tubes were incubated for 25°C for 48 h. After incubation, 100 µl from tubes having no visible growth was removed and poured in plates along with agar to enumerate total molds count. The minimum oil concentration that entirely seized the fungal growth and didn't allow slightest growth revival even after 48 hours of incubation was considered as MFC - minimum fungicidal concentration.

2.4.5. Time Kill Assay

A time kill study was carried out with the MIC values found previously by the agar well method to discern whether the tested oils had fungistatic or fungicidal effect over a period of time for use as a food preservative (White *et al.*, 1996). Microorganisms (fungal spores suspension) with 10^6 cfu/ml and oil having concentration equal to MIC were added respectively in the tube of corresponding culture medium. Broth tubes with and without microbial suspension were used as controls. The cultures were incubated for one month at 25°C. An inoculant of 100 µl, removed after 2, 5, 8, 11, 14 and 30 d was poured in agar plates in triplicate to determine the total reduction in viable counts. The mean number of the colonies (cfu/ml) was counted and compared with that in the control culture at the end of the incubation period. The test tubes with turbidity after a certain incubation period depicted fungistatic effect of the tested essential oil at the applied concentration. To determine the fungicidal concentration of essential oils against that particular strain, higher concentrations (8 µg/ml, 15 µg/ml, 65 µg/ml and 250 µg/ml) were applied and lethal effect of essential oils was observed as mentioned above.

Statistical Analysis

The mean values, ± standard deviations were calculated using MS Excel 2007. Data was analysed by using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test by SPSS (version 16.0).

Results & Discussion

Genus Eucalyptus

Pure essential oils of all evaluated *Eucalyptus* species established high antifungal activity [**Table 2**]. The degree of inhibition of fungal growth varied depending on the sensitivity of essential oils towards the particular strain. *Eucalyptus crebra*, *E. kitsoniana*, *E. microtheca* and *E. rudis* induced large zones of inhibition (46.0 mm) against all tested fungal strains. *Eucalyptus melanophloia* and *E. tereticornis* showed varied inhibitory effect towards tested strains. Higher inhibition zones were exhibited by *E. melanophloia* and *E. tereticornis* against *A. flavus*, *F. oxysporum* and *F. solani* whereas a low activity was observed for them against *A. niger* (IZ =19.3 mm) and *P. digitatum* (IZ = 27.3 mm) respectively. Tested fungal strains showed different sensitivities towards *E. pruinosa* essential oil. *Eucalyptus* pruinosa induced IZ 19.3–19.5 mm

against *F. oxysporum* and *F. solani* followed by *A. niger* (IZ = 17.8 mm), *P. digitatum* (IZ = 16.0 mm) and *A. flavus* (IZ = 11.3 mm). Our results on antifungal activity showed contradiction to previous report by Ghaffar *et al*., 2015 where larger inhibition zones were reported for *E. melanophloia* (27.0 mm) while smaller for *E. crebra* (25.0 mm), *E. microtheca* (21.0 mm) and *E. tereticornis* (26.0 mm) against *A. niger*. This could be related to different method used for antimicrobial screening, concentration of pure essential oil used and their chemical composition. Antifungal activity of *E. pruinosa* and *E. rudis* essential oils against selected strains has not been reported previously. The degree of antifungal activity was assessed by determination of their minimum inhibitory effect at concentrations 4–100 µg/ml. MIC and MFC values for different *Eucalyptus* species ranged from 2–8 µg/ml **(Table 2)**.

The time kill assay was carried out to check the potency of *Eucalyptus* essential oils to be used as a food preservative. The study was carried out to evaluate the fungistatic or fungicidal effects of tested oils for four weeks. *Eucalyptus* essential oils showed fungicidal effect at 8 µg/ml in time kill assay. Kaur et al., 2011 reported fungicidal effect of *E. tereticornis* essential oil at 500 ppm.

Studies suggested that fungal activity of essential oils depend on their major components absorbed directly on the fungal mycelium. These lipophilic agents execute their action either by interacting with cellular membranes altering their fatty acid composition and thus permeability (Taweechaisupapong *et al*., 2012) or empty the cytoplasmic content of fungal hyphae (Soylu *et al*., 2006) or interfere with cell wall enzymes like chitin synthase/chitinase (Camele *et al*., 2012).

Our previous studies on the chemical composition of selected species revealed 1,8-cineole (31.6–49.7 %) as a main component in *E. crebra, E. microtheca* and *E. rudis* essential oils; *p*-cymene (41.8–58.1 %) in *E. melanophloia* and *E. tereticornis* oils while *E. kitsoniana* and *E. pruinosa* essential oils were dominated by α-pinene (25.8–31.4 %) (Siddique et al., 2017a). The antifungal effect of these major oil components is well established. 1,8-Cineole have exhibited promising antifungal activity against *P. digitatum* and *A. niger* (Gehan *et al*., 2012). Matsuzaki *et al.* 2013 reported antifungal activity of α-pinene and 1,8-cineole against *Candida albicans*. There are few studies on the antifungal activity of *p*-cymene. Klaric *et al.* 2007, reported strong fungicidal and/or fungistatic activities of *T. vulgaris* essential oil with *p*-cymene (36.5%) and thymol (33.0%) as main components against *Aspergillus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Mucor* and *Rhizopus*. *p*-Cymene has been found to be much less effective against *Aspergillus spp*. and *Penicillium spp*., (MIC ≥ 4 or 8%, v/v), when compared with thymol (Hammer et al., 2003). However, thymol and *p*-cymene, alone or in combination, exhibit strong antifungal activity against *Candida spp*. (Pina-Vaz et al., 2003). Mota *et al*., reported low antifungal activity of p-cymene against *R. oryzae*. *E. melanophloia* and *E. tereticornis* oils presented large inhibition zones against tested *Aspergillus*, *Fusarium* and *Penicillium* strains. This could be due to presence of other active components (α-pinene and 1,8-cineole) present in appreciable amounts.

Genus *Melaleuca*

The tested fungal strains were highly sensitive towards *Melaleuca* essential oils. *Melaleuca leucadendron* essential oil induced larger inhibition zones (46.0 mm) against *A. flavus* followed by *M. bracteata* (40.0 mm) and *M. fulgens* (30.0 mm). The tested *Melaleuca* essential oils showed moderate activity against *A. niger* and *F. solani* with IZ (22.3–27.0 mm) and (21.7–33.3 mm) respectively. *Fusarium oxysporum* was most susceptible to *M. leucadendron* (IZ = 46.0 mm) followed by *M. bracteata* (31.0 mm) and *M. fulgens* (21.7 mm). *Penecillium digitatum* showed highly sensitivity to *M. bracteata* (43.7 mm) essential oil whereas *M. fulgens* and *M. leucadendron* oils demonstrated similar inhibitory effect against it (4.0–25.0 mm). Antifungal activity of *M. bracteata* and *M. fulgens* has not been reported previously. Putarji *el al*., 2012 reported antifungal activity of *M. leucadendron* L. essential oil against wood rot fungi so a comparison could not be made with present study.

The MIC and MFC values of *Melaleuca* essential oils ranged from 2–8 µg/ml for tested fungal strains. The evaluated *Melaleuca* oils showed fungicidal effect at 8 µg/ml for a month.

The antifungal activity of *Melaleuca* species could be related to eugenol methyl ether; their

major component (82.3–95.4 %) (Siddique et al., 2017b) and a compound with good antifungal activity against *F. solani* f. sp. *piperis* (Silva *et al.*, 2014).

The results of the present study showed that the essential oils from selected Myrtaceae species exhibited good antifungal activities. This suggests the use of essential oils as alternative to chemical preservatives in food. However, further studies are needed to authenticate direct application of essential oils in food systems.

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Table 1. The detail of collected plants from different sites of Pakistan.

Plant Name	Harvesting Month	Locality of Collection	GPS coordinates	Voucher Number
Eucalyptus crebra	March, 2013	Pakistan Forest Research Institute, Faisalabad.	Longitude 73.11°E, Latitude 31.28°N	BDSS # 4023
Eucalyptus kitsoniana	March, 2013	Pakistan Forest Research Institute, Faisalabad.	Longitude 73.11°E, Latitude 31.28°N	BDSS # 4024
Eucalyptus melanophloia	March, 2013	Pakistan Forest Research Institute, Faisalabad.	Longitude 73.11°E, Latitude 31.28°N	BDSS # 4025
Eucalyptus microtheca	March, 2013	Pakistan Forest Research Institute, Faisalabad.	Longitude 73.11°E, Latitude 31.28°N	BDSS # 4026
Eucalyptus pruinosa	March, 2013	Pakistan Forest Research Institute, Faisalabad.	Longitude 73.11°E, Latitude 31.28°N	BDSS # 4027
Eucalyptus rudis	March, 2013	Pakistan Forest Research Institute, Faisalabad.	Longitude 73.11°E, Latitude 31.28°N	BDSS # 4028
Eucalyptus tereticornis	March, 2013	Pakistan Forest Research Institute, Faisalabad.	Longitude 73.11°E, Latitude 31.28°N	BDSS # 4029
Melaleuca bracteata F. Muell	April, 2013	Government College University Botanical Garden, Lahore.	Longitude 74.31°E, Latitude 31.57°N	BDSS # 4040
Melaleuca fulgens R. Br. subsp. *Steedmanii*	April, 2013	Qarshi Botanical Garden, Hattar, Abottabad.	Longitude72.85°E, Latitude 33.85°N	BDSS # 4041
Melaleuca leucadendron (L.) L.	April, 2013	Jinnah Garden, Lahore.	Longitude 74.26°E, Latitude 31.50°N	BDSS # 4039

Table 2: Chemical Composition of Essential Oils from different Myrtaceae Species.

Compounds	RI	Content (%)									
		E. cre	*E.rud*	*E.kit*	*E. ter*	*E. pru*	*E. mel*	*E. mic*	*M. bra*	*M. ful*	*M. leu*
α-pinene	930	16.9	32.5	25.8	0.7	29.5	24.1	31.0	0.2	0.1	0.1
Camphene	944	0.2	0.1	0.2	-	0.4	0.3	0.6	-	-	-
β-pinene	974	-	-	-	-	0.3	-	-	-	-	-
4-carene	1001	0.1	tr	0.2	0.2	-	0.3	-	tr	tr	-
α-phellandrene	1002	0.2	tr	2.0	0.3	0.1	1.0	Tr	0.1	0.1	-
D-limonene	1011	4.1	8.4	4.0	-	4.6	6.2	5.7	0.2	0.2	0.1
O-cymene	1026	16.1	1.6	24.8	58.1	8.2	41.8	2.4	1.7	1.2	0.1
1,8-Cineole	1029	49.7	48.5	3.3	6.5	24.2	3.3	31.6	0.3	0.2	0.1
β-Cis-ocimene	1043	tr	0.1	tr	-	-	Tr	-	tr	0.1	-
γ-terpinene	1055	0.2	0.2	1.2	0.5	-	0.8	0.1	tr	0.1	-
Terpinolene	1083	0.1	0.1	0.6	0.2	Tr	0.6	0.1	0.3	0.5	0.1
Terpin-4-ol	1089	4.1	2.4	7.2	2.1	3.9	2.9	4.8	0.9	0.9	0.8
β-Linalool	1095	-	-	Tr	0.5	0.1	0.8	-	1.0	1.0	0.4
α-campholenal	1127	0.1	-	-	-	0.1	0.1	0.1	-	-	-
trans-pinocarveol	1135	2.0	0.9	0.2	0.1	1.3	-	2.2	-	-	-
Camphene Hydrate	1145	0.1	tr	0.1	-	0.2	0.1	0.2	-	-	-
p-cymen-3-ol	1183	0.1	0.4	0.1	0.4	0.1	0.1	tr	-	-	-
p-cymen-8-ol	1196	0.5	0.2	0.3	3.6	0.4	0.8	0.3	0.4	0.2	0.1
2-Isopropenyl-5-methylhex-4-enal	1198	-	-	tr	-	-	-	-	0.1	tr	-
± β-citronellal	1223	-	-	-	-	-	-	-	0.4	0.4	0.1
Nerol acetate	1365	-	0.1	-	0.2	-	-	-	tr	tr	Tr

Table 2: ***Continued***

Compounds	RI	Content (%)									
		E. cre	*E.rud*	*E.kit*	*E. ter*	*E. pru*	*E. mel*	*E. mic*	*M. bra*	*M. ful*	*M. leu*
α-Copaene	1374	-	-	-	0.1	-	-	tr	Tr	0.1	tr
Methyl cinnamte	1379	-	-	-	-	-	-	-	11.4	5.8	0.8
Eugenol methyl ether	1402	0.1	0.8	0.2	0.7	0.1	0.5	-	82.3	87.8	95.4
α-Caryophyllene	1444	tr	0.1	0.1	-	0.2	0.4	0.2	Tr	tr	Tr
Humulen-(IV)	1452	-	-	-	-	-	-	0.1	-	-	-
Germacrene D	1484	tr	0.5	-	0.2	Tr	0.1	tr	0.2	0.6	0.4
Epiglobulol	1532	2.5	1.0	0.5	-	9.7	6.9	2.5	Tr	0.1	Tr
Germacrene B	1559	tr	tr	-	0.2	-	2.8	-	Tr	0.2	0.1
Caryophyllene oxide	1582	-	-	-	-	-	-	-	Tr	0.0	0.1
p-menthan-3-ol	-	-	-	-	-	-	-	-	0.1	-	-
3-allyl,2-methoxy phenol	-	-	-	-	-	-	-	-	0.3	0.3	0.8
Total		98.3	98.4	71.6	75.3	85.2	95.7	85.3	99.9	99.9	99.7
Monoterpenes		21.7	43.0	58.8	59.8	43.0	75.1	40.0	2.8	2.3	0.4
Oxygenated monoterpenes		57.5	53.8	12.5	14.0	32.2	10.4	42.4	96.9	96.7	98.5

Sesquiterpenes	0.5	0.6	0.1	1.5	0.2	3.3	0.3	0.2	0.9	0.7
Oxygenated sesquiterpenes	2.5	1.0	0.6	-	9.8	6.9	2.5	0.1	0.1	0.2
Unidentified	1.7	1.6	28.4	24.7	14.8	4.3	14.7	0.1	0.1	0.7
Oil Content (%)	0.5	0.2	0.4	0.4	0.9	1.8	0.1	0.1	2.1	0.4

Plant abbreviations: *Eucalyptus crebra*; *E. cre*; *Eucalyptus kitsoniana*: *E. kit*; *Eucalyptus melanphloia*: *E.mel*; *Eucalyptus microtheca*: *E. mic*;*Eucalyptus pruinosa*: *E.pru*; *Eucalyptus rudis*: *E. rud*; *Eucalyptus tereticornis*: *E.ter*;*M. bracteata*; *M. brac*; *M. fulgens*: *M. fulg*; *M. leuaodendron*:*M. leuco*; RI: Retention Index; - : not detected; tr; traces.

Table 3. Antifungal activity of Myrtaceae Essential oils by Agar well diffusion method.

Essential Oil	Conc. µg/ml					
		A. flavus	*A. niger*	*F. oxysporum*	*F. solani*	*P. digitatum*
E. crebra	250	46.0±0.0a	26.7±0.6b	46.0±0.0a	23.8±0.3b	29.3±0.6b
	100	28.0±1.0a	21.3±1.5c	29.3±1.2b	18.7±0.6c	21.8±0.3d
	65	21.0±1.0b	18.5±0.5d	25.0±0.0c	17.5±0.5c	20.8±1.0d
	15	19.3±1.2e	12.2±0.3e	16.0±1.0d	15.2±0.3cd	15.2±0.3e
	8	19.0±0.9e	11.7±0.6e	14.3±0.6e	14.5±0.5e	13.8±0.8ef
	4	15.8±0.3f	10.3±0.3e	12.2±0.3e	12.0±0.5e	12.0±0.0f
	Pure oil	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a
E. kitsoniana	250	46.0±0.0a	46.0±0.0a	25.8±0.3	27.5±0.5	23.0±0.5
	100	31.0±1.0b	31.3±0.8b	21.0±0.0c	21.0±0.9c	18.6±0.5d
	65	24.3±1.2c	24.0±1.3c	20.0±0.0c	17.2±0.3d	17.0±0.5d
	15	16.2±1.0d	23.0±0.0cd	17.7±0.8d	16.3±0.3d	16.2±0.3de
	8	14.7±0.6e	20.0±0.0d	15.8±0.8e	14.8±0.3e	14.8±0.3e
	4	12.7±0.3e	15.5±0.5e	13.0±0.5f	12.7±0.3e	12.8±0.3f
	Pure oil	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a
E. melanophloia	250	17.8±1.4c	24.7±0.6a	46.0±0.0a	24.7±0.6b	26.7±0.6b
	100	17.2±0.3c	18.3±0.6b	27.7±0.6b	17.3±0.6c	18.7±0.6c
	65	16.8±0.3c	16.5±0.5bc	20.5±0.5c	15.5±0.5c	17.7±0.6c
	15	14.7±0.3cd	12.2±0.3c	14.7±0.6e	14.5±0.5cd	14.5±0.5d
	8	13.7±0.3d	11.7±0.6c	13.5±0.5e	13.3±0.6d	12.7±0.6e
	4	12.0±0.0d	10.7±0.3c	12.0±0.0f	11.8±0.3d	11.5±0.5e
	Pure oil	46.0±0.0a	19.3±1.2b	46.0±0.0a	46.0±0.0a	46.0±0.0a
E. microtheca	250	32.0±1.0b	21.7±0.6c	21.8±0.8b	23.5±0.5b	29.5±0.5b
	100	32.0±1.0b	16.8±0.8d	17.5±1.3c	18.0±1.0c	24.5±0.5c
	65	22.7±1.2c	16.3±0.6d	16.0±0.0c	17.5±0.5c	20.5±0.5d
	15	16.0±1.0d	14.5±0.5de	15.5±0.5cd	16.2±0.3c	16.3±0.6e
	8	14.3±0.6e	12.8±0.8e	14.0±1.0d	14.5±0.5cd	14.3±0.6e
	4	12.8±0.3e	11.5±0.5e	12.8±0.3d	12.7±0.3d	13.0±0.0f
	Pure oil	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a
E. pruinosa	250	21.2±0.3a	18.5±0.5b	24.5±0.5a	18.2±0.3b	26.5±0.5a
	100	19.7±0.3b	16.3±0.6b	21.3±0.6b	15.5±0.5c	22.0±1.0b
	65	18.8±0.3b	14.8±0.3bc	16.8±0.8cd	13.8±0.8d	16.2±0.3c
	15	16.8±0.3bc	13.3±0.3bc	12.5±0.5d	12.2±0.3d	12.7±0.6d
	8	14.8±0.3c	12.5±0.5c	11.2±0.3d	11.5±0.5d	11.8±0.3d
	4	12.5±0.5c	11.2±0.3c	10.3±0.6d	11.0±0.0d	11.2±0.3d
	Pure oil	11.3±0.6b	17.8±0.3b	19.5±0.5c	19.3±0.3b	16.0±0.8c

E. rudis	**250**	46.0±0.0a	32.0±1.0b	23.8±0.3b	30.3±0.6b	46.0±0.0c
	100	26.5±0.5b	20.7±1.5d	18.3±1.2c	21.3±0.6c	26.7±0.6b
	65	22.3±0.6c	16.3±1.5e	16.0±1.0cd	16.3±0.6d	20.0±0.0c
	15	16.3±0.6d	14.7±0.6ef	15.7±1.5cd	15.3±1.5d	18.7±0.8cd
	8	14.3±0.6de	12.7±0.6f	13.3±0.6d	14.0±1.0e	15.8±0.3d
	4	12.8±0.3e	12.8±0.3f	11.5±0.5d	12.8±0.8e	13.5±0.5d
	Pure oil	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a
E. tereticornis	**250**	46.0±0.0a	21.5±0.5c	18.8±0.8b	20.2±0.3b	21.5±0.5b
	100	24.8±0.8b	18.5±0.5d	15.0±0.0c	16.3±0.3c	16.8±0.3c
	65	20.3±0.6c	16.8±0.3de	13.7±0.3cd	15.3±0.3c	16.3±0.3c
	15	17.3±0.6cd	14.0±0.5e	12.8±0.3d	14.3±0.3cd	15.0±0.9cd
	8	16.0±0.0d	12.5±0.9e	12.2±0.3d	13.5±0.5d	13.5±0.5d
	4	13.5±0.5e	11.5±0.0e	12.0±0.0d	12.2±0.3d	12.3±0.3d
	Pure oil	46.0±0.0a	46.0±0.0a	46.0±0.0a	46.0±0.0a	27.3±0.8a
M. bracteata	**250**	19.7±0.6c	46.0±0.0a	19.7±0.6b	22.3±0.6b	27.8±0.8b
	100	14.8±0.8d	22.3±0.6c	15.5±0.3bc	16.8±0.3c	18.2±0.8c
	65	12.7±0.3d	17.0±0.9d	13.3±0.3c	13.5±0.3d	14.8±0.3d
	15	12.3±0.3de	13.7±0.8e	12.8±0.3c	12.8±0.3d	13.7±0.3d
	8	11.2±0.3e	12.5±0.5e	11.8±0.3c	12.2±0.3d	12.7±0.6d
	4	10.7±0.6e	12.2±0.e	11.3±0.3c	11.8±0.3d	12.3±0.3d
	Pure oil	40.0±0.0a	26.0±0.9b	31.0±1.0a	33.3±1.5a	43.7±0.6a
M. fulgens	**250**	-	-	-	-	-
	100	36.3±0.6a	46.0±0.0a	40.5±0.9a	43.5±0.5a	31.0±1.0a
	65	33.8±0.3b	38.7±1.2b	35.5±1.3b	35.2±0.8b	28.0±1.0b
	15	23.0±1.0d	29.0±0.0c	17.0±1.0d	20.5±0.5c	25.3±1.5c
	8	18.3±0.6e	17.5±0.5f	15.8±0.8e	12.0±1.0d	17.0±1.0d
	4	13.8±0.3f	14.5±0.5g	14.5±0.5e	11.3±0.3d	15.8±0.3d
	Pure oil	30.0±0.0c	22.3±0.6e	21.7±1.5c	21.7±0.6c	24.0±1.0c
M. leucadendron	**250**	-	-	-	-	-
	100	-	38.3±0.6a	-	44.3±1.5a	44.0±1.0a
	65	-	33.8±0.8b	-	38.3±1.2b	34.3±1.5b
	15	35.0±1.0b	29.2±0.8c	35.0±1.0b	22.3±1.5d	26.0±1.0c
	8	26.3±0.6c	19.3±0.6d	29.0±1.0c	19.8±0.8d	21.3±1.5d
	4	12.5±0.5e	14.8±0.3e	14.3±0.6d	14.8±0.8e	16.2±0.3e
	Pure oil	46.0±0.0a	27.0±0.9c	46.0±0.0a	28.0±1.0c	25.0±1.0c
Nystatin	**8**	25.66±0.4	25.33±0.4	17.33±0.4	22.66±0.4	26.33±0.4

*The diameter of the inhibition zones (mm), including the well diameter (6mm), are given as mean ± SD of triplicate experiments. ND: not detected.

*The values with the same lower case letters are not statistically significant at P = 0.05% according to Duncan's Multiple Range Test.

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) µg/ml of Myrtaceae essential oils after 48 h and four weeks.

Essential Oil		*A. flavus*	*A. niger*	*F. oxysporum*	*F. solani*	*P. digitatum*
E. crebra	MIC	4	4	4	4	4
	MFC_{48h}	8	8	8	8	8
	MFC_{4w}	8	8	8	8	8
E. kitsoniana	MIC	4	4	4	4	4
	MFC_{48h}	8	8	8	8	8
	MFC_{4w}	8	8	8	8	8
E. melanophloia	MIC	4	4	4	4	4
	MFC_{48h}	8	8	8	8	8
	MFC_{4w}	8	8	8	8	8
E. microtheca	MIC	4	4	4	4	4
	MFC_{48h}	8	8	8	8	8
	MFC_{4w}	8	8	8	8	8
E. pruinosa	MIC	4	4	4	4	4
	MFC_{48h}	8	8	8	8	8
	MFC_{4w}	8	8	8	8	8

Table 4: Continued

Essential Oil		*A. flavus*	*A. niger*	*F. oxysporum*	*F. solani*	*P. digitatum*
E. rudis	MIC	4	4	4	4	4
	MFC_{48h}	8	8	8	8	8
	MFC_{4w}	8	8	8	8	8
E. tereticornis	MIC	4	4	4	4	4
	MFC_{48h}	8	8	8	8	8
	MFC_{4w}	8	8	8	8	8
M. bracteata	MIC	4	4	4	4	4
	MFC_{48h}	4	4	4	4	4
	MFC_{4w}	8	8	8	8	8
M. fulgens	MIC	2	2	2	2	2
	MFC_{48h}	2	2	2	2	2
	MFC_{4w}	8	8	8	8	8
M. leucadendron	MIC	4	2	2	2	2
	MFC_{48h}	4	2	2	2	2
	MFC_{4w}	8	8	8	8	8

*Abbreviation: w = week