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Assessment of the growth inhibiting effect of some plant essential oils on different *Fusarium* species isolated from sorghum and maize grains

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Abstract

The antifungal activity of essential oils from clove, cedar wood, *Cymbopogon* species, peppermint, *Eucalyptus* and neem were tested for their efficacy against nine *Fusarium* species, namely *F. verticillioides*, *F. proliferatum*, *F. oxysporum*, *F. anthophilum*, *F. pallidoroseum*, *F. sporotrichioides*, *F. solani*, *F. graminearum* and *F. lateritium*, isolated from maize and sorghum. The results showed that essential oils were antifungal at concentrations of 500–2500 ppm or higher. The oil *Cymbopogon nardus* (referred to as citronella oil) was inhibiting all the *Fusarium* species growth at 500 ppm and higher. The next most potent inhibitors, the oil from *C. citratus* (referred to as lemongrass oil) and peppermint oils, were fully inhibitory from a concentration of 1500 and 2000 ppm and higher, respectively. *Eucalyptus* and neem oils were less effective in inhibiting the growth of *Fusarium* species tested, irrespective of their concentration. Among the seven essential oils tested against all the *Fusarium* species, the citronella oil showed highest inhibitory effect (minimum inhibitory concentration, MIC) at ≥ 1500, 2000, 1000, 500 ppm against *F. verticillioides*, *F. oxysporum*, *F. sporotrichioides*, *F. lateritium* mycelial growth development respectively. However clove, lemongrass oil and citronella showed highest inhibitory effect (≥ 1500 ppm) against *F. proliferatum* and *F. pallidoroseum* respectively. Further lemongrass oil showed highest inhibitory effects at ≥ 1500 and 500 ppm against *F. anthophilum* and *F. lateritum* respectively. The least MIC for *F. graminearum* was observed in peppermint oil at ≥ 500 ppm. The results indicate that the tested toxigenic *Fusarium* spp. are sensitive to the essential oils, and particularly sensitive to the citronella oil. These findings clearly indicate that essential oils should find a practical application to control the growth of *Fusarium* species in stored maize and sorghum grains.

Key words: Antifungal agents, citronella oil, cereals, mycelial growth inhibition.

Introduction

Moulds can colonise diverse food substrates, eventually leading to food spoilage. Species belonging to the families *Fusarium*, *Aspergillus*, *Penicillium* and *Rhizopus* can cause food spoilage (Betts et al. 1999). Species of *Fusarium* are predominantly responsible for deterioration of stored commodities qualitatively and quantitatively (Ramakrishna et al. 1996). Contamination of cereals by *Fusarium* species and their toxins is a global problem. These are often toxigenic, as they produce mycotoxins, such as fumonisins, trichothecenes, or zearalenone (Placinta et al. 1999). Therefore, the presence of *Fusarium* spp. in cereals can lead to a potential health hazard to humans and animals. Thus, elimination of toxigenic *Fusarium* species in cereal grains is of paramount importance to protect the commodity from further bio-deterioration.

Chemical preservatives belonging to the benzimidazoles, aromatic hydrocarbons, sterol biosynthesis inhibitors or other groups have been used to prevent or control the growth of *Fusarium* spp. (Daferera et al. 2003). However, many disadvantages are associated with the use of chemical preservatives as antifungal agents (Basilico & Basilico 1999). Over the past few years, much effort has been directed to searching for new antifungal agents to control the growth of *Fusarium* spp. A number of control and preventive strategies are being used to prevent mould growth and toxin production. Antioxidants (Etcheverry et al. 2002), propionates (Marin et al. 2000), phenolic compounds, parabens, butylated hydroxyanisole (Thompson 1996), phenyllactic acids (Lavermicocca et al. 2003), and plant based products (Soliman & Badeea 2002, Daferera et al. 2003, Leite de Souza et al. 2005) have been used to control the growth of *Fusarium* species. The main reasons for considering the essential oils as antifungal agents include their natural origin and very low risk of pathogens developing resistance.

There is an increasing interest in the use of alternative antimicrobial agents that are not toxic. One such alternative is the use of natural plant products with antifungal activity, as these are more environmentally friendly and accepted more widely by the public. There is a considerable interest in essential oils from aromatic plants with antimicrobial activity to control pathogens and toxin-producing moulds (Soliman & Badeea 2002, Tepe et al. 2005). Although the majority of the essential oils are classified as "Generally Recognised As Safe" (GRAS), their use as preservatives in food is often limited due to flavor considerations (Lambert et al. 2001). Accurate knowledge of the minimum inhibitory concentration (MIC)

of essential oils would enable a balance between the sensory acceptability and antifungal efficacy. Therefore, in the present investigation, essential oils from different plant origin were evaluated at different concentrations for their ability to reduce the growth of *Fusarium* species.

Materials and methods

Sources of essential oils

Pure and natural essential oils (clove, cedar wood, lemon grass, peppermint, citronella and neem oils) were procured by Karnataka State Aromas, Essential Oil Distillery, Bangalore, Karnataka. Eucalyptus oil was purchased from Venus Eucalyptus Oil Distillery, Nilgiris, Tamilnadu (India).

Fusarium species

A total of nine *Fusarium* species viz., *Fusarium verticillioides*, *F. proliferatum*, *F. oxysporum*, *F. anthophilum*, *F. pallidoroseum*, *F. sporotrichioides*, *F. solani*, *F. graminearum* and *F. lateritium* were isolated from maize and sorghum grains, by using both agar plating and standard blotter methods. All *Fusarium* isolates were identified up to species level based on the micro-morphological features, using fungal keys (Leslie & Summerell 2006). The identity was confirmed by PCR, using *Fusarium* genus specific primer sequences (Sreenivasa et al. 2008).

Determining antifungal activity

Antifungal activity of each essential oil was tested by using agar dilution method as described by Viuda-Martos et al. (2006) with minor modifications. The essential oils were incorporated into the PDA medium with 5% Tween 20 as an emulsifying agent, so that the final concentrations of essential oils were 500, 1000, 1500, 2000 and 2500 ppm, respectively. The agar plates were dried at room temperature for 30 min prior to inoculation. Agar discs of 0.5 cm, taken from 4–6 day old *Fusarium* cultures, were placed on the plates, which were incubated at 28 ± 2°C, L:D 12:12 h, for 7 days. Three replicates were maintained for each treatment with the same concentration of essential oils, however controls consisted of PDA medium, mixed with 5% Tween 20 (v/v) without adding any essential oils were also maintained. The average daily radial growth of the fungal colonies was measured with centimeter scale. In the case of full inhibition, the diameter of the initial disk (0.5 cm) was recorded; this was denoted as the MIC.

Statistical analysis

The results were analysed statistically by using The Statistical Presentation System Software (SPSS) for windows version 10.0.1. Three-way ANOVA (oil v/s organism v/s concentrations) was applied to the data to determine if there were differences between concentration and fungal species. In the case of a significant effect, Tukey's multiple comparison tests ($\alpha = 0.05$) was applied to identify which concentrations and which fungal species differed from each other.

Results and discussion

The effect of seven essential oils against nine *Fusarium* species as well as statistical analysis (mean, standard deviation and standard error) were represented in the Tables 1 & 2. The essential oils of clove, cedar wood, lemongrass, peppermint and citronella showed inhibitory effects on all the tested *Fusarium* species, at all concentrations. It could be seen that as the oil concentration increases the inhibitory effect increases. In other words, the inhibitory effect of the oil is proportional to its concentration. Citronella oil has more inhibitory effect than the other oils tested.

The MIC (full inhibition) for *F. verticillioides* was 1500 ppm in the case of citronella oil, and 2500 ppm in the case of clove and peppermint oil. The MIC for *F. proliferatum was* 1500 ppm in the case of clove, lemongrass and citronella oil, and 2500 ppm in the case of cedar wood, and peppermint oil. The MIC for *F. oxysporum* was 2000 ppm in the case of citronella oil and 2500 ppm in the case of peppermint oil. The MIC for *F. anthophilum* was 1000 ppm in the case of lemongrass oil, 1500 ppm in the case of citronella, 2000 ppm in the case of peppermint, and 2500 ppm in the case of clove oil. The MIC for *F. pallidoroseum* was 1500 ppm in the case of lemongrass and citronella oil. The MIC for *F. sporotrichioides* was 1000 ppm in the case of citronella oil, and 1500 ppm in the case of clove and lemon grass oil. The MIC for *F. solani* was 500 ppm in the case of lemongrass oil, 1500 ppm in the case of peppermint and citronella oil. The MIC for *F. lateritium* was 500 ppm in the case of citronella oil, 1500 ppm in the case of lemon grass oil, 2000 ppm in the case of peppermint oil, and 2500 ppm in the case of clove oil. The MIC for *F. graminearum* was 500 ppm in the case of peppermint oil, 1000 ppm in the case of lemon grass oil, and 1500 ppm in the case of clove and citronella oil. For all other combinations of oils and *Fusarium* species, no MIC could be determined.

Tukey's multiple comparison test ($\alpha = 0.05$) showed that all essential oils tested showed anti-fusarial activity against *Fusarium* species isolated from maize and sorghum grains, except Eucalyptus and neem oil. Essential oils were most effective against *F. solani*, followed by *F. anthophilum*, *F. graminearum*, *F. proliferatum*, *F. sporotrichioides*, *F. pallidoroseum*, *F. lateritium and* finally *F. verticillioides*. Essential oils were least effective aginst *F. oxysporum*.

The inhibitory effect of essential oils from 10 Indian plants against growth of the genus *Fusarium* has been reported by Paster et al. (1995) and Rai et al. (1999). Basilico & Basilico (1999) have tested the antifungal activity of essential oils against fungi, such as *Fusarium* and *Aspergillus* species. Thirty seven essential oils screened under *in vitro* conditions against different *Fusarium* species showed that some essential oils were suitable as novel food protectants (Velluti et al.

Table 1: Effect of clove, cederwood, lemongrass and peppermint oils on the growth of different *Fusarium* species.

Sl. No.	Essential Oils	Concentration (ppm)	Diameter of mycelial growth (cm)								
			*F ver**	*F pro*	*F oxy*	*F ant*	*F pal*	*F spo*	*F sol*	*F lat*	*F gra*
1	Clove	Control	$8.98 \pm 0.02^{a**}$	8.98 ± 0.02^{a}	7.81 ± 0.04^{a}	7.88 ± 0.02^{a}	8.98 ± 0.02^{a}	8.98 ± 0.02^{a}	8.98 ± 0.01^{a}	9.00 ± 0^{a}	8.93 ± 0.02^{a}
		500	6.23 ± 0.03^{b}	3.76 ± 0.09^{b}	7.10 ± 0.06^{b}	6.53 ± 0.03^{b}	8.60 ± 0.06^{b}	8.01 ± 0.06^{b}	8.06 ± 0.03^{b}	8.11 ± 0.04^{b}	5.80 ± 0.06^{b}
		1000	5.33 ± 0.09^{c}	1.13 ± 0.07^{c}	7.10 ± 0.06^{b}	5.53 ± 0.03^{c}	7.56 ± 0.03^{c}	6.25 ± 0.03^{c}	6.85 ± 0.03^{c}	7.33 ± 0.09^{c}	3.85 ± 0.03^{c}
		1500	3.80 ± 0.06^{d}	0.50 ± 0.00^{d}	7.01 ± 0.02^{b}	3.16 ± 0.03^{d}	7.35 ± 0.02^{c}	0.50 ± 0.00^{d}	4.45 ± 0.03^{d}	4.01 ± 0.02^{d}	0.50 ± 0.00^{d}
		2000	2.95 ± 0.03^{e}	0.50 ± 0.00^{d}	7.03 ± 0.03^{b}	1.88 ± 0.04^{e}	6.95 ± 0.03^{d}	0.50 ± 0.00^{d}	2.85 ± 0.03^{e}	2.05 ± 0.03^{e}	0.50 ± 0.00^{d}
		2500	0.50 ± 0.00^{f}	0.50 ± 0.00^{d}	6.40 ± 0.06^{c}	0.50 ± 0.00^{f}	6.67 ± 0.01^{d}	0.50 ± 0.00^{d}	1.16 ± 0.09^{f}	0.50 ± 0.00^{f}	0.50 ± 0.00^{d}
2	Cedar wood	Control	8.98 ± 0.02^{a}	8.95 ± 0.03^{a}	8.98 ± 0.02^{a}	7.20 ± 0.01^{a}	7.78 ± 0.02^{a}	8.95 ± 0.03^{a}	7.53 ± 0.03^{a}	8.93 ± 0.02^{a}	8.95 ± 0.03^{a}
		500	8.45 ± 0.03^{b}	5.70 ± 0.06^{b}	8.88 ± 0.04^{ab}	6.86 ± 0.03^{b}	6.46 ± 0.09^{b}	5.01 ± 0.06^{b}	6.20 ± 0.06^{c}	8.35 ± 0.03^{b}	8.55 ± 0.03^{b}
		1000	7.26 ± 0.03^{c}	4.50 ± 0.06^{c}	8.75 ± 0.03^{b}	6.70 ± 0.06^{b}	5.56 ± 0.07^{c}	3.95 ± 0.03^{c}	6.50 ± 0.06^{b}	7.26 ± 0.03^{c}	8.08 ± 0.04^{c}
		1500	6.55 ± 0.03^{d}	3.10 ± 0.06^{d}	7.48 ± 0.04^{c}	6.43 ± 0.03^{c}	5.33 ± 0.14^{c}	4.15 ± 0.03^{d}	6.06 ± 0.03^{c}	6.36 ± 0.03^{d}	7.50 ± 0.06^{d}
		2000	5.50 ± 0.06^{e}	2.53 ± 0.03^{e}	7.15 ± 0.03^{d}	6.21 ± 0.02^{d}	4.96 ± 0.09^{d}	2.80 ± 0.03^{e}	5.10 ± 0.06^{d}	4.58 ± 0.04^{e}	6.41 ± 0.06^{e}
		2500	4.00 ± 0.06^{f}	0.50 ± 0.00^{f}	6.98 ± 0.04^{e}	6.05 ± 0.03^{d}	4.33 ± 0.07^{e}	2.60 ± 0.03^{f}	5.03 ± 0.07^{d}	3.85 ± 0.03^{f}	6.05 ± 0.03^{f}
3	Lemon grass	Control	8.96 ± 0.02^{a}	8.96 ± 0.02^{a}	8.95 ± 0.03^{a}	8.96 ± 0.02^{a}	8.96 ± 0.02^{a}	8.96 ± 0.02^{a}	7.06 ± 0.03^{a}	8.96 ± 0.02^{a}	8.98 ± 0.02^{a}
		500	7.38 ± 0.04^{b}	5.85 ± 0.03^{b}	7.15 ± 0.03^{b}	3.26 ± 0.02^{b}	2.11 ± 0.02^{b}	2.16 ± 0.03^{b}	0.50 ± 0.00^{b}	1.88 ± 0.04^{b}	1.06 ± 0.04^{b}
		1000	6.50 ± 0.06^{c}	2.86 ± 0.04^{c}	6.25 ± 0.03^{c}	0.50 ± 0.00^{c}	1.83 ± 0.07^{c}	1.83 ± 0.03^{c}	0.50 ± 0.00^{b}	0.93 ± 0.03^{c}	0.50 ± 0.00^{c}
		1500	6.13 ± 0.03^{d}	0.50 ± 0.00^{d}	5.75 ± 0.03^{d}	0.50 ± 0.00^{c}	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{b}	0.50 ± 0.00^{d}	0.50 ± 0.00^{c}
		2000	5.78 ± 0.02^{e}	0.50 ± 0.00^{d}	5.11 ± 0.06^{e}	0.50 ± 0.00^{c}	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{b}	0.50 ± 0.00^{d}	0.50 ± 0.00^{c}
		2500	5.15 ± 0.03^{f}	0.50 ± 0.00^{d}	4.51 ± 0.06^{f}	0.50 ± 0.00^{c}	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{b}	0.50 ± 0.00^{d}	0.50 ± 0.00^{c}
4	Peppermint	Control	8.95 ± 0.03^{a}	8.98 ± 0.02^{a}	8.63 ± 0.03^{a}	8.98 ± 0.02^{a}	8.98 ± 0.02^{a}	8.98 ± 0.02^{a}	7.16 ± 0.03^{a}	8.96 ± 0.02^{a}	8.96 ± 0.02^{a}
		500	6.78 ± 0.07^{b}	6.61 ± 0.06^{b}	7.00 ± 0.06^{b}	6.78 ± 0.04^{b}	6.05 ± 0.03^{b}	7.96 ± 0.03^{b}	2.20 ± 0.06^{b}	7.00 ± 0.06^{b}	0.50 ± 0.00^{b}
		1000	4.25 ± 0.03^{c}	4.15 ± 0.03^{c}	6.76 ± 0.03^{c}	4.91 ± 0.02^{c}	4.43 ± 0.03^{c}	6.53 ± 0.09^{c}	1.06 ± 0.03^{c}	2.35 ± 0.03^{c}	0.50 ± 0.00^{b}
		1500	3.08 ± 0.04^{d}	2.08 ± 0.04^{d}	5.10 ± 0.06^{d}	0.90 ± 0.06^{d}	1.76 ± 0.03^{d}	3.63 ± 0.03^{d}	0.50 ± 0.00^{d}	1.35 ± 0.03^{d}	0.50 ± 0.00^{b}
		2000	1.65 ± 0.03^{e}	0.91 ± 0.04^{e}	3.43 ± 0.03^{e}	0.50 ± 0.00^{e}	0.96 ± 0.03^{e}	2.40 ± 0.06^{e}	0.50 ± 0.00^{d}	0.50 ± 0.00^{e}	0.50 ± 0.00^{b}
		2500	0.50 ± 0.00^{f}	0.50 ± 0.00^{f}	0.50 ± 0.00^{f}	0.50 ± 0.00^{e}	0.50 ± 0.00^{f}	0.93 ± 0.03^{f}	0.50 ± 0.00^{d}	0.50 ± 0.00^{e}	0.50 ± 0.00^{b}

* F ver, *F. verticillioides;* F pro, *F. proliferatum;* F oxy, *F. oxysporum;* F ant, *F. anthophilum;* F pal, *F. pallidoroseum;* F spo, *F. sporotrichioides;* F sol, *F. solani;* F lat, *F. lateritium;* F gra, *F. graminearum*.

** Figures followed by superscript of same letter in a column are not statistically significant ($P = 0.05$) according to Tukey's multiple-range test.

Values given are mean ± standard error (n = 3)

Table 2: Effect of *Eucalyptus*, citronella and neem oils on the growth of different *Fusarium* species.

Sl. No.	Essential Oil	Concentration (ppm)	Diameter of mycelial growth (cm)								
			*F ver**	*F pro*	*F oxy*	*F ant*	*F pal*	*F spo*	*F sol*	*F lat*	*F gra*
1	*Eucalyptus*	Control	$8.95 \pm 0.03^{a**}$	8.98 ± 0.02^{a}	8.81 ± 0.15^{a}	6.56 ± 0.03^{a}	7.83 ± 0.03^{a}	8.95 ± 0.03^{a}	6.66 ± 0.03^{a}	8.98 ± 0.02^{a}	8.95 ± 0.03^{a}
		500	8.18 ± 0.02^{b}	8.30 ± 0.06^{b}	8.55 ± 0.03^{a}	6.05 ± 0.03^{b}	7.35 ± 0.03^{b}	8.88 ± 0.02^{a}	5.66 ± 0.03^{b}	7.80 ± 0.11^{b}	7.85 ± 0.09^{b}
		1000	7.25 ± 0.03^{c}	8.20 ± 0.06^{b}	7.40 ± 0.06^{b}	5.21 ± 0.06^{c}	5.60 ± 0.06^{c}	8.03 ± 0.03^{b}	5.50 ± 0.06^{b}	7.00 ± 0.06^{c}	7.06 ± 0.03^{c}
		1500	6.51 ± 0.04^{d}	7.46 ± 0.03^{c}	6.23 ± 0.03^{c}	4.93 ± 0.03^{d}	5.23 ± 0.03^{d}	7.41 ± 0.06^{c}	4.96 ± 0.07^{c}	6.21 ± 0.06^{d}	6.81 ± 0.04^{c}
		2000	5.65 ± 0.03^{e}	6.98 ± 0.06^{d}	6.01 ± 0.04^{c}	4.05 ± 0.05^{e}	5.16 ± 0.09^{d}	7.00 ± 0.06^{d}	4.33 ± 0.07^{d}	5.05 ± 0.08^{e}	5.00 ± 0.10^{d}
		2500	4.51 ± 0.04^{f}	6.05 ± 0.03^{e}	3.61 ± 0.06^{d}	3.85 ± 0.03^{f}	4.30 ± 0.11^{e}	6.75 ± 0.03^{e}	3.63 ± 0.07^{e}	4.28 ± 0.04^{f}	4.13 ± 0.07^{e}
2	*Citronella*	Control	8.93 ± 0.04^{a}	8.95 ± 0.03^{a}	8.96 ± 0.02^{a}	8.91 ± 0.06^{a}	8.95 ± 0.03^{a}	8.95 ± 0.03^{a}	8.95 ± 0.03^{a}	8.95 ± 0.03^{a}	7.46 ± 0.03^{a}
		500	6.98 ± 0.04^{b}	7.85 ± 0.03^{b}	6.85 ± 0.03^{b}	7.46 ± 0.15^{b}	6.60 ± 0.15^{b}	5.40 ± 0.03^{b}	2.43 ± 0.03^{b}	0.50 ± 0.00^{b}	2.33 ± 0.03^{b}
		1000	3.03 ± 0.03^{c}	6.41 ± 0.06^{c}	5.91 ± 0.04^{c}	3.98 ± 0.08^{c}	1.16 ± 0.09^{c}	0.50 ± 0.00^{c}	1.16 ± 0.09^{c}	0.50 ± 0.00^{b}	1.91 ± 0.04^{c}
		1500	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	4.43 ± 0.03^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{c}	0.50 ± 0.00^{d}	0.50 ± 0.00^{b}	0.50 ± 0.00^{d}
		2000	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{e}	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{c}	0.50 ± 0.00^{d}	0.50 ± 0.00^{b}	0.50 ± 0.00^{d}
		2500	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{e}	0.50 ± 0.00^{d}	0.50 ± 0.00^{d}	0.50 ± 0.00^{c}	0.50 ± 0.00^{d}	0.50 ± 0.00^{b}	0.50 ± 0.00^{d}
3	Neem	Control	7.90 ± 0.06^{a}	8.98 ± 0.02^{a}	8.66 ± 0.07^{a}	7.53 ± 0.30^{a}	7.11 ± 0.06^{a}	8.96 ± 0.02^{a}	7.66 ± 0.07^{a}	8.96 ± 0.03^{a}	8.95 ± 0.03^{a}
		500	7.70 ± 0.06^{b}	8.66 ± 0.09^{b}	8.61 ± 0.06^{a}	6.81 ± 0.04^{b}	6.83 ± 0.09^{a}	8.61 ± 0.06^{b}	7.60 ± 0.06^{ab}	8.93 ± 0.03^{a}	8.16 ± 0.16^{b}
		1000	7.66 ± 0.06^{b}	7.81 ± 0.06^{c}	7.53 ± 0.09^{b}	6.51 ± 0.06^{c}	6.41 ± 0.07^{b}	7.73 ± 0.03^{c}	7.56 ± 0.03^{ab}	8.83 ± 0.03^{a}	8.43 ± 0.07^{b}
		1500	7.23 ± 0.04^{d}	7.75 ± 0.08^{c}	6.81 ± 0.04^{c}	6.48 ± 0.06^{c}	6.13 ± 0.09^{b}	7.26 ± 0.09^{d}	7.56 ± 0.06^{ab}	8.86 ± 0.03^{a}	7.88 ± 0.02^{c}
		2000	7.46 ± 0.03^{c}	7.70 ± 0.06^{c}	6.71 ± 0.04^{c}	5.93 ± 0.07^{d}	5.63 ± 0.09^{c}	6.53 ± 0.14^{e}	7.31 ± 0.06^{ab}	8.20 ± 0.15^{b}	7.88 ± 0.06^{c}
		2500	7.28 ± 0.02^{cd}	7.41 ± 0.06^{d}	4.95 ± 0.08^{d}	3.25 ± 0.08^{e}	5.28 ± 0.06^{d}	6.03 ± 0.09^{f}	7.50 ± 0.11^{b}	8.10 ± 0.06^{b}	7.81 ± 0.04^{c}

* F ver, *F. verticillioides;* F pro, *F. proliferatum;* F oxy, *F. oxysporum;* F ant, *F. anthophilum;* F pal, *F. pallidoroseum;* F spo, *F. sporotrichioides;* F sol, *F. solani;* F lat, *F. lateritium;* F gra, *F. graminearum*.

** Figures followed by superscript of the same letter in columns are not found significantly different ($P = 0.05$) according to Tukey's multiple-range test.

Values given are mean ± standard error (n = 3)

2004). Recently, 75 essential oils were tested in India against *F. oxysporum* and a large numbers of essential oils were found to have potent antifungal properties against a broad spectrum of fungi. These oils could be used for the control of fungal infections in plants as well as in stored grains (Pawar & Thaker 2007). Mishra & Dubey (1994) reported that citronella oil inhibited *F. verticillioides* growth by 90% and 100% at 500 and 1000 ppm, respectively. Its potency remained unaltered even after many days of storage. Similarly, Velluti et al. (2004) observed that cinnamon leaf and clove oils reduced the colony growth of *F. verticillioides*, *F. proliferatum* and *F. graminearum* under all conditions tested. Clove and cinnamon oils (1000 mg ml^{-1}) reduced the colony growth by 62% and 80%, respectively.

Aromatic essential oils originating from oregano, thyme, clove, lemongrass, lavender, rosemary, *Dictamnus*, pennyroyal, marjoram, sage, *Eucalyptus*, neem etc. have shown to have antifungal properties (Tzortzakis et al. 2007). In the present work, clove, lemongrass, peppermint and citronella oils fully inhibited growth of *F. proliferatum*, *F. anthophilum*, *F. graminearum* and *F. lateritium*, whereas eucalyptus and neem oils did not fully inhibited growth of any of the *Fusarium* species tested, irrespective of the concentrations used. Cedar wood oil only fully inhibited *F. proliferatum* at a concentration of 2500 ppm.

The antimicrobial activity was correlated with a high percentage of monoterpenes, eugenol, cinnamic aldehyde and thymol (Lis-Balchin & Deans 1997). The major constituents of essential oils are terpenes and phenolic compounds, which could, in turn, be responsible for the antimicrobial properties (Davidson 2001). The possible antimicrobial activity could be due to the ability of essential oils to damage the enzymatic cell systems, including those associated with energy production and synthesis of structural compounds (Conner & Beuchat 1984). The effect of phenolic compounds in essential oils is concentration dependent. At low concentrations, phenolic compounds affect the enzymatic activity but at higher concentrations, they cause protein denaturation (Prindle & Wright 1977).

Conclusion

The present study clearly indicates that citronella oil and essential oils from lemongrass, peppermint and clove have broad-spectrum antifungal properties and are effective against many species of *Fusarium*. The use of essential oils, such as clove, cedar wood, lemongrass, peppermint, and citronella oils, as antifungal agents will be suitable for applications in the food industry. These natural plants based essential oils and their active components may successfully replace synthetic chemicals and provide an alternative method of preservation. However, their use in foods as preservatives is often limited due to flavor. Furthermore, *in-vivo* studies need to be carried out on maize and sorghum grains, to examine the potentials of these essential oils (for example, citronella and lemongrass) to protect grains under different environmental conditions in sub-tropical countries like India.

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