Control of Fusarium spp. causing damping-off of pine seedlings by means of selected essential oils

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a b s t r a c t

Damping-off is regarded as one of the most serious risks to pine plantations and the disease poses a potentially devastating threat to the future sustainability of the South African softwood industry. In spite of numerous prevention methods being tested, no absolute means of controlling the fungus is available to nurseries. In this study, 10 commercially available essential oils were assessed in vitro for their antifungal activity against four strains of Fusarium. Considering the efficacy, as reflected by the MIC values obtained, cost and availability of the essential oils, lemongrass is proposed as the most promising candidate for an in vivo study, first on a small scale and then under commercial conditions.

1. Introduction

The South African forestry industry contributes US $300 million to the economy (about 2% of the gross domestic product) yearly and employs about 100,000 people (New Climate Economy Report, 2014). Associated industries, based on forestry, produce timber products worth US$1.6 billion annually. The most important commercial plantation species is Pinus patula (50%) (Mitchell et al., 2011), followed by Pinus elliotti and Pinus taeda (Ford et al., 2014). Fusarium spp. are known to threaten pine plantations and natural stands throughout the world (Wingfield et al., 2008; Kanzler et al., 2014). Damping-off of young conifer seedlings by Fusarium spp. causes severe crop and economic losses annually. In particular, Fusarium oxysporum has been linked to several forest nursery diseases (Gordon et al., 2001), including pre- and post-emergence damping-off, cotyledon blight of young germinants, as well as stem and root decay of young seedlings (James and Dumroese, 2006). Currently, pine cultivars with innate resistance against F. oxysporum are being developed to offer an alternative approach to disease control (Moraga-Suazo et al., 2014).

Until then, the forestry industry remains heavily reliant on the use of synthetic fungicides to control the disease. The widespread use of these chemicals is of major concern, since many of these fumigants, such as methyl bromide, chloropicrin, metam-sodium and dazomet, are hazardous to the environment. Pine nurseries in South Africa often rely on fungicides that contain Benomyl as the active ingredient, although its use has been banned in many countries (Pearson and Miller, 2014). According to the international policy of the Forest Stewardship Council (FSC), the compound has toxic effects that persist in the environment, particularly in soil and water (FSC, 2012). There is also strong evidence to suggest that pathogens build up resistance to fungicides over time (Mitchell et al., 2011). Restrictive regulations, imposed worldwide on the use of many of these synthetic fungicides (FSC, 2012), drive the search for new or alternative antifungal products. In South Africa, synthetic fungicides are increasingly being confined to fewer products, to reduce the potential threat to the environment and human health. To our knowledge, there are no registered fungicides for the control of Fusarium on either pine seeds or seedlings. The leaflets accompanying fungicides, registered for use on forest seedlings, indicate that they control fungi of the genus Fusarium to some extent. However, according to Starkey and Enebak (2011), the degree of control does not justify the cost of application.

Stakeholders, such as nurseries managers, are under pressure to ensure a regular supply of disease-free trees to growers. Therefore, there is a need for alternative measures to inhibit damping-off of nursery seedlings, in a safe and eco-friendly way. Plant essential oils (EOs) should be evaluated to determine their potential to control diseases in pine nurseries, since they are known to inhibit fungal pathogens (Burt, 2004). Several reports indicate that EOs can effectively kill microorganisms without promoting the acquisition of resistance (Ohno et al., 2003; Ali et al., 2005). Further advantages include low toxicity towards mammals and rapid degradation in soil and water (Karamaouna et al., 2013). The main aim of this study

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Table 1
Percentage mycelial growth inhibition of the four selected Fusarium spp. obtained in a toxic medium assay (N = 10), where the essential oils were incorporated at 1000 J-l/l.

Essential oils	Fusarium oxysporum	Fusarium circinatum	Fusarium circinatum mat 1	Fusarium circinatum mat 2
Orange sweet	18.1 ± 2.2	29.5 ± 15.8	12.9 ± 2.5	25.9 ± 5.6
Citronella	59.3 ± 7.2	69.7 ± 6.0	47.1 ± 8.0	48.2 ± 2.4
Mandarin	14.4 ± 2.0	09.7 ± 2.9	20.2 ± 6.9	13.8 ± 5.7
Grapefruit	21.1 ± 2.5	11.7 ± 1.7	14.5 ± 3.6	15.0 ± 2.7
Camphor	41.6 ± 2.8	36.1 ± 5.5	18.5 ± 4.6	40.8 ± 5.6
Clove	100	100	100	100
Spearmint	24.8 ± 3.7	23.3 ± 5.1	15.5 ± 2.2	34.9 ± 7.8
Lemongrass	100	100	100	100
Rose geranium	100	87.8 ± 1.0	100	100
Thyme	100	100	100	100
Benolyl 500WP	72.2 ± 0.4	78.2 ± 0.9	41.7 ± 1.5	47.2 ± 3.9

was to find an economically viable EO with antifungal properties against selected Fusarium spp., responsible for damping-off.

2. Materials and methods

2.1. Pathogens

Four strains of Fusarium (F. circinatum, F. oxysporum, F. circinatum mat 1, F. circinatum mat 2) were obtained from the culture collection of the Department of Biotechnology and Food Technology, Tshwane University of Technology, South Africa.

Each strain was grown for 7 days on malt extract agar (MEA) plates at 25 °C. A 10 ml volume of a phosphate buffer solution (containing 6.8045 g KH_2PO_4, and 1 ml Tween-80 in 1 l of distilled water) was transferred to a MEA plate containing the mature, sporulating pathogen and spread across the surface of the plate using a Dragalsky needle. The concentration of the resulting spore suspension was standardized at an optical density of 0.583 using a spectrophotometer (Helios Gamma, Thermo Electro Corporation) at 600 nm.

2.2. Essential oils

Five EOs were selected based on their basis of the price and availability in South Africa, namely sweet orange (Citrus sinensis), citronella (Cymbopogon nardus); mandarin (Citrus reticulata); grapefruit (Citrus paradise)and camphor (Cinnamomum camphora) EOs. A further four oils were selected based on reports that they are active against Fusarium spp (Combrinck et al.,2011), namely clove (Syzygium aromaticum); spearmint (Mentha spicata) lemongrass (Cymbopogon citratus) and thyme (Thymus vulgaris). One South African EO, rose geranium (Pelargonium graveolens) was also included. All the oils were purchased from Holistic Emporium (Johannesburg, South Africa). The commercial fungicide, Benomyl 500WP (750 mg/l; Villa Crop Protection (Pty) Ltd, Johannesburg, South Africa) was used as a positive control.

2.3. In vitro antifungal activity of the essential oils

The antifungal properties of the EOs were screened against the four Fusarium spp., by incorporating them into the culture medium. Potato dextrose agar (PDA, Merck, Johannesburg, South Africa) medium was prepared and supplemented with a specific EO at increasing concentrations, ranging from 100 to 1000 J-l/l, respectively. All test substances were pre-mixed with 200 J-l of surfactant (Tween-80, Merck, Johannesburg, South Africa). The negative controls consisted of PDA and the surfactant only. A volume of 10 J-l of the fungal spore suspension was deposited aseptically onto the centre of each PDA plate. After 7 days incubation at 25 °C, the mycelial growth (mm) was measured using a 150 mm digital calliper-KTV150 (Major Tech Pty Ltd, Johannesburg, South Africa). Ten replicates were prepared and the data was expressed as percentage inhibition of mycelial growth relative to the control, according to the method described by Plaza et al. (2004).

3. Results and discussion

The majority of the EOs selected for this study have a single main active component and can be purchased, without difficulty, in large quantities and according to specification. This approach rules out oils that function through synergistic action, since the chemistry of many active ingredients acting in concert is more difficult to control. Of the 10 essential oils tested at 1000 J-l/l, only that of lemongrass, clove and thyme were able to totally control the mycelial growth of all four selected Fusarium spp. (Table 1). Previously, our research group reported on the chemistry of those oils (Combrinck et al., 2011). The commercial oils of lemongrass was found to contain 84.8% citral (42.5% geranial and 31.7% neral), while clove oil contained 88.3% of eugenol and thyme oil was characterized by 63.1% thymol and high levels of linalool (21.3%). In addition, the oil from rose geranium achieved complete growth inhibition of Fusarium oxysporum, Fusarium circinatum mat 1 and Fusarium circinatum mat 2, but only 87.8% inhibition of Fusarium circinatum. Neither the commercial fungicides, nor the citrus EOs, which are the cheapest on the market, were able to achieve significant inhibition of the pathogens. The prices of the three most successful oils vary tremendously, with lemongrass (±23 $/kg) being the most affordable, costing approximately 40% less than both thyme and clove oils.

Oils exhibiting total growth inhibition of all four Fusarium strains (clove, thyme and lemongrass) were tested further to determine their minimum inhibitory concentration (MIC) (Table 2). Although the oil of rose geranium did not completely inhibit the growth of one of the strains (Fusarium circinatum), this oil was also included as it is produced in South Africa, making it affordable and available for blending with other oils. Lemongrass was

Table 2
Minimum inhibition concentration (J-l/l) of the four selected essential oils against the four selected Fusarium spp.

Essential oils	Fusarium oxysporum	Fusarium circinatum	Fusarium circinatum mat 1	Fusarium circinatum mat 2
Clove	500	500	400	500
Lemongrass	300	700	500	400
Thyme	500	500	500	500
Rose geranium	900	>1000	900	1000

Table 3
Inhibitory effect of mixture of lemongrass and thyme at different concentrations against the four selected Fusarium spp.

	Lemongrass							
	J-l/l		100	200	300	400	500	600
Thyme	100	F 1	59.5 ± 5.9	65.6 ± 3.6	75.4 ± 4.4	100	100	100
		F 2	66.2 ± 1.3	68.7 ± 2.1	76.5 ± 2.6	80.8 ± 2.7	100	100
		F 3	11.2 ± 8.3	19.1 ± 12.3	21.4 ± 6.1	23.2 ± 8.8	43.8 ± 3.2	61.8 ± 5.8
		F 4	59.6 ± 5.5	69.2 ± 1.6	76.0 ± 2.6	84.5 ± 4.5	100	100
	200	F 1	61.1 ± 3.2	79.1 ± 3.4	100	100	100	100
		F 2	71.2 ± 1.3	82.0 ± 2.4	83.0 ± 2.5	100	100	100
		F 3	40.7 ± 2.4	51.0 ± 2.8	51.6 ± 1.7	58.6 ± 9.1	65.8 ± 4.0	80.4 ± 9.4
		F 4	68. ± 4.5	78.0 ± 2.0	83.6 ± 2.4	100	100	100
	300	F 1	78.7 ± 1.3	100	100	100	100	100
		F 2	83.7 ± 1.4	86.7 ± 1.8	94.2 ± 0.8	100	100	100
		F 3	75.7 ± 0.8	79.1 ± 2.3	83.1 ± 0.7	87.6 ± 0.7	89.5 ± 1.2	100
		F 4	80.1 ± 1.0	86.7 ± 0.4	100	100	100	100
	400	F 1	84.9 ± 1.6	100	100	100	100	100
		F 2	76.0 ± 1.2	85.5 ± 0.2	100	100	100	100
		F 3	60.1 ± 4.6	69.2 ± 5.9	80.3 ± 3.8	80.3 ± 3.7	90.4 ± 0.7	100
		F 4	77.4 ± 1.9	80.6 ± 1.3	100	100	100	100
	500	F 1	85.7 ± 1.6	100	100	100	100	100
		F 2	78.3 ± 1.6	100	100	100	100	100
		F 3	58.5 ± 5.3	73.3 ± 4.7	83.9 ± 2.7	88.8 ± 1.1	90.8 ± 0.9	100
		F 4	78.4 ± 1.0	82.3 ± 1.8	100	100	100	100
	600	F 1	100	100	100	100	100	100
		F 2	100	100	100	100	100	100
		F 3	68.4 ± 3.1	74.7 ± 2.4	85.5 ± 1.8	90.9 ± 2.1	100	100
		F 4	80.2 ± 1.8	84.3 ± 0.3	100	100	100	100

the best at inhibiting Fusarium oxysporum and Fusarium circinatum mat 2, at concentrations of 300 J-l/l and 400 J-l/l, respectively. The lowest MIC value for the inhibition of Fusarium circinatum was clove and thyme oil at 500 J-l/l, while Fusarium circinatum mat1 was controlled with clove oil at 400 J-l/l (Table 2). These results are in accordance with those of Linde et al. (2010) and Ćosić et al., 2010, ;1;yme oils were able to totally inhibit the growth of a Fusarium oxysporum isolate. Although clove oil (MIC of 400 J-l/l for F. circinatum mat 1) was slightly more effective than thyme oil (MIC of 500 J-l/l for F. circinatum mat 1), the availability of clove oil in South Africa is limited and therefore not suitable for industrial scale application in nurseries.

A further experiment was conducted to determine whether a mixture of thyme oil and lemongrass oil at different concentrations would result in complete inhibition of all strains tested at lower concentrations. However, none of the mixtures tested achieved synergistic effects (Table 3). Mixing of the oils is therefore not a more economic option.

4. Conclusions

Lemongrass, thyme and clove EOs are able to completely inhibit the mycelial growth of the four Fusarium strains such as Fusarium oxysporum, Fusarium circinatum and Fusarium circinatum mat 1 and mat 2, tested at 1000 J-l/l. The MIC values obtained for the three oils indicate that the performances of the oils were similar against all four pathogens. Therefore, availability and cost of the oils was used as the criteria for the selection of the oils used to evaluate possible synergism of an oil blend. However, no significant synergistic effects were observed when lemongrass and thyme oils were blended. This study has indicated that lemongrass, which is both available and cost effective, is an attractive option for further investigations towards alternatives to synthetic fungicides for the control of damping-off in pine seedlings. However more trials using different treatments should be conducted in nurseries.

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