Research reports

In vitro evaluation of the antifungal activity of some essential oils on post-harvest fungal pathogens of tropical fruits

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Summary
Essential oils derived from *Citrus aurantifolia* Swingle, *Citrus limon* (L.) Burm.f., *Cymbopogon citratus* (DC.) Stapf, *Cestrum nocturnum* L., and *Michelia champaca* Linn. were incorporated into potato dextrose agar and evaluated *in vitro* for fungistatic and fungicidal activity against *Fusarium oxysporum* (Fo), *Fusarium oxysporum* (Fo2) and *Glomerella cingulata* (Gc) isolated from the snake fruit, papaya and wax apple, respectively. *Cymbopogon citratus* oil exhibited the most activity against the fungi tested with minimum inhibitory concentrations (MIC) of 1.2, 0.8 and 1.0 μL mL^{-1} against Fo, Fo2 and Gc respectively. Essential oils from *M. champaca* and *C. nocturnum* were moderately effective with fungistatic and fungicidal concentrations ranging from 0.8 to 6.4 μL mL^{-1}. Both citrus oils were found to be the least effective with the MIC ranging from 6.4 to 38.4 μL mL^{-1}. This preliminary study has revealed the potential use of the essential oil from *Cymbopogon citratus* against post-harvest fungal pathogens *F. oxysporum* and *G. cingulata*.

Introduction
Post-harvest fruit losses worldwide are as high as 30 to 40% and often much higher in some developing countries (Farzana 2006). In tropical countries, the warm and humid climate provides a suitable environment for the development and spread of numerous fungal pathogens. Harvested fruit, with their high moisture content and rich nutrients, are easily attacked by fungi which can cause considerable economic losses. Post-harvest diseases are primarily controlled by the extensive use of fungicides such as benomyl, thiabendazole, and imazali as they are effective and economic. However, in recent times the development of fungicide-resistant strains and the increasing demand for fresh products not treated with agrochemicals have warranted the need for alternative control measures. Alternatives to synthetic chemicals include antagonistic microorganisms such as yeasts and bacteria, natural plant- and animal-derived products with fungicidal properties, and induced resistance of plants.

The use of biologically based compounds, such as essential oils obtained from edible plants is suggested as a feasible approach to reduce post-harvest diseases in fruits and vegetables (Isman 2000). Essential oils are complex volatile compounds and have various functions in the plants' metabolism (Goubran and Holmes 1993) as well as having antimicrobial, antifungal and/or insecticidal properties. Previous studies have demonstrated the potential use of essential oils from citrus (Sokovic and Griensven 2006), lemongrass (Pandey *et al.* 1996) and champaka (Baby 2007) against fungal and bacterial pathogens through in vitro evaluations. The use of essential oils may prove to be one of the best ways to manage food or reduce losses to pests in the future (Baby 2007). Thus, this study was conducted in order to determine the concentration necessary for *in vitro* inhibition of selected post-harvest fungal pathogens of tropical fruits by five essential oils.

Materials and methods
Essential oils
A total of five essential oils, from *Citrus aurantifolia* Swingle (lime), *Citrus limon* (L.) Burm.f. (lemon), *Cymbopogon citratus* (DC.) Stapf. (lemongrass), *Cestrum nocturnum* L. (nightqueen) and *Michelia champaca* Linn. (champaka) were tested. The essential oils were 100% pure, and purchased from Buana Amertha Sari, Indonesia and Louis Dreyfus Citrus, Brazil. The essential oils were kept at 4°C in air-tight sealed glass vials covered with aluminium foil, prior to use.

Fungal pathogens
The test fungi *Fusarium oxysporum* (Fo), *Fusarium oxysporum* (Fo2), and *Glomerella cingulata* (Gc) were isolated from decaying snake fruit, papaya and wax apple, respectively. Each isolated strain was maintained on potato dextrose agar (PDA) and stored at 4°C before use. To maintain the pathogenicity of each fungus, periodic inoculations and reisolations from the infected fruits were carried out.

Antifungal assay
For each essential oil, at least five concentrations were tested using the direct contact method. The concentrations used ranged from 0–38.4 μL mL^{-1} for both *C. aurantifolia* and *C. limon*; 0–1.2 μL mL^{-1} for *C. citratus*; 0–6.4 μL mL^{-1} for *Cestrum nocturnum* and 0–2.4 μL mL^{-1} for *M. champaca*. The essential oils (at each prepared concentration) were mixed with 0.01% Tween 20 surfactant and dissolved in molten PDA medium. The medium was supplemented with the same amount of distilled water containing 0.01% of Tween 20 instead of the essential oil for the control.

Fungal discs (5 mm) were cut from the periphery of 4-day-old stock cultures using a sterile cork borer. Each disc was inoculated onto the centre of a sterilized Petri dish containing PDA. Each Petri dish was sealed with Para film and incubated at 25 ± 2°C for seven days. The cultures were checked daily and the diameter of the colonies were measured before hyphae in the control treatment reached the edge of the plate.

The lowest concentration without any visible growth under the binocular microscope is defined as minimum inhibition concentration (MIC). Fungal discs, which did not show any visible growth after seven days, were transferred onto fresh PDA plates with no essential oils incorporated and incubated for another seven days to observe the revival of growth. When there was visible growth of the fungus, it meant that the concentration of the essential oil used was fungistatic. However, when there was no visible growth of the fungus, it meant that the concentration of essential oil used was fungicidal.

Statistical analysis
All the experiments were laid out in a completely randomized design. Each treatment was replicated three times and the experiment was repeated twice. The results from two experiments were combined for statistical analysis. The Tukey's Honestly Significant Difference (HSD) Test was used to detect differences in antifungal activity among the oils at the 5% level of significance.

Results and discussion

Table 1 shows the minimum inhibitory concentration (MIC) of the five types of essential oils tested by measurement of the mycelial growth of *Fusarium oxysporum* (Fo), *Fusarium oxysporum* (Fo2), and *Glomerella cingulata* (Gc) isolated from the snake fruit, papaya and wax apple, respectively. The results of this study have clearly demonstrated that the essential oil from *C. citratus* was overall the most active *in vitro* in reducing the rate of mycelial growth of the three fungi tested, although a similar level of performance was observed on Fo2 with oil from *C. nocturnum* and *M. champaca*. *Cymbopogon citratus* oil was fungicidal in this study at very low concentrations of 1.2, 0.8 and 1.0 μL mL^{-1} for Fo, Fo2 and Gc, respectively (Table 1).

Similar results have been reported of the anti-*Fusarium oxysporum* f. sp *cicer* and the anti-*Alternaria porri* effects of 75 different essential oils where the most active essential oils found were those from lemongrass (Pawar and Thaker 2007). The minimum bioactive concentration with fungicidal action for *Cymbopogon flexuosus* oil was found to be 0.4 μL mL^{-1} on *F. oxyporum* Schlecht (Shahi *et al*. 2003). Another study showed that among 49 essential oils tested, palmarosa (*Cymbopogon martini*) oil demonstrated the most antifungal activity against *Botrytis cinerea* (Wilson *et al*. 1997). The finding that lemongrass oil has a broad spectrum of antifungal activity is in line with the findings of previous studies. Shahi *et al*. (2003) reported that *C. flexuosus* oil exhibited a broad antifungal spectrum against 25 types of fungi, and all were inhibited (fungicidal).

Although many plants belonging to different families of angiosperms have been screened for their antifungal properties, this is probably the first time that *M. champaca* oil has shown moderate antifungal activity. The minimum inhibitory concentrations with fungistatic action were recorded as 0.8 μL mL^{-1} for Fo2 and 2.4 μL mL^{-1} for Fo and Gc. The minimum inhibitory concentration with fungicidal action was found to be 1.2 μL mL^{-1} for Fo2 (Table 1). The antifungal property of *M. champaca* oil shows great potential for use in future studies. Leaf extracts of *M. champaca* were toxic to the rice fungus, *Pyricularia oryzae* (Baby 2007). A related study, where *Michelia champaca* oil was one of 75 different essential oils tested revealed that its inhibition of hyphal growth and spore formation in *Aspergillus niger* was low (Pawar and Thaker 2006).

Table 1. Minimum inhibitory concentration (MIC) of five essential oils measured with mycelial growth of fungi tested.

Essential oils from:	Type of fungi[A]	MIC (μL mL^{-1})	
		Fungistatic	Fungicidal
Citrus aurantifolia	Fo	38.4 a[B]	–[C]
	Fo2	6.4 b	38.4 a
	Gc	38.4 a	–
Citrus limon	Fo	38.4 a	–
	Fo2	6.4 b	12.8 b
	Gc	38.4 a	–
Cymbopogon citratus	Fo	0.8 d	1.2 d
	Fo2	0.6 e	0.8 f
	Gc	0.8 d	1.0 e
Cestrum nocturnum	Fo	6.4 b	–
	Fo2	0.8 d	1.6 c
	Gc	6.4 b	–
Michelia champaca	Fo	2.4 c	–
	Fo2	0.8 d	1.2 d
	Gc	2.4 c	–

[A] Fo: *Fusarium oxysporum* (isolated from snake fruit), Fo2: *Fusarium oxysporum* (isolated from papaya), Gc: *Glomerella cingulata*.
[B] Means followed by different letter are significantly different within the same column after determined by Tukey's Honestly Test (HSD) at 5% of significant level.
[C] Cannot be determined.

Prior to this work there was no evidence to show that the essential oil of *C. nocturnum* has antifungal properties against post-harvest fungal pathogens. This study has revealed that *C. nocturnum* oil exhibited a moderate inhibitory effect against the three fungi tested. The oil was able to exert fungistatic and fungicidal activity against Fo2 at 0.8 and 1.6 μL mL^{-1}, respectively. The concentration of 6.4 μL mL^{-1} was fungistatic to Fo and Gc (Table 1).

The least effective of the essential oils observed, were the *C. aurantifolia* and the *C. limon* oils. Both the citrus oils were fungistatic against Fo and Gc at a high concentration of 38.4 μL mL^{-1}. *Citrus aurantifolia* oil at 12.8 μL mL^{-1} and *C. limon* oil at 38.4 μL mL^{-1} were only able to exhibit fungicidal action against Fo2 (Table 1).

This result is in agreement with previous findings on fungitoxicity of citrus oil against pathogens of button mushroom where the lowest activity was observed for the essential oils from citrus and *Matricaria chamommilla* with the active concentrations ranging from 7.0 to 40.0 μL mL^{-1} (Sokovic and Griensven 2006). It has been demonstrated that *Citrus sinensis* which has 84.2% limonene, showed fungicidal activity against post-harvest pathogens such as *Aspergillus niger*, *Botryodiplodia theobromae*, *Botrytis cinerea* and *Penicillium expansum* at concentrations of 700 to 1000 ppm (Sharma and Tripathi 2006). However, the fungi tested in the current study were not sensitive to the *C. aurantifolia* and *C. limon* oils. The low activity of both the citrus oils could be due to some specific reasons as seen in other studies. The occurrence of phytochemicals that do not show antifungal activity, could suggest probable resistance and invasion capability of the fungal strains used as test microorganisms and could be cited as important interfering factors to the antifungal efficiency of the essential oils and phytochemicals included in the antifungal assays (Souza *et al*. 2005).

Conclusion

The five essential oils tested were shown to possess antifungal properties. *Cymbopogon citratus* oil had the most potential as it demonstrated strong efficacy with fungicidal action at 1.2, 0.8 and 1.0 μL mL^{-1} against *Fusarium oxysporum* (Fo), *Fusarium oxysporum* (Fo2) and *Glomerella cingulata* isolated from the snake fruit, papaya and wax apple, respectively. *Cymbopogon citratus* shows great potential as an

antifungal agent, and may be used as a botanic fungicide against post-harvest fungal pathogens in the future. Further studies are required to examine the efficacy of this oil *in vivo*.

References

Baby, P.S. (2007). Aromatic plant. Horticulture Science Series 1, 1st edition, pp. 270. (New India Publishing Agency, Pitam Pura, New Delhi, India).

Farzana, P. (2006). Post harvest technology of fruits and vegetables. ECO Services International. Available at: http://www.eco-web.com/editorial/060529.htm, date 29 May 2007.

Goubran, F.H. and Holmes, R.J. (1993). The development of alternative fungicides from essential oils. Department of Agriculture, Institute for Horticultural Development, Knoxfield, Victoria, Australia. Rural Industries Research and Development Report, pp. 45.

Isman, B.M. (2000). Plant essential oils for pest and disease management. *Crop Protection* 19(8), 603-8.

Pandey, M.C., Sharma, J.R. and Dikshit, A. (1996). Antifungal evaluation of the essential oil *Cymbopogon pendulus* (Nees ex. Steud.). *Flavour and Fragrance Journal* 11, 257-60.

Pawar, V.C. and Thaker, V.S. (2006). *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. *Mycoses* 49, 316-23.

Pawar, V. and Thaker, V.S. (2007). Evaluation of the anti-*Fusarium oxysporum* f. sp *cicer* and anti-*Alternaria porri* effects of some essential oils. *World Journal Microbiology Biotechnology* 23, 1099-1106.

Shahi, S.K., Patra, M., Shukla, A.C. and Dikshit, A. (2003). Use of essential oil as botanical-pesticide against post harvest spoilage in *Malus pumilo* fruits. *BioControl* 48, 223-232.

Sharma, N. and Tripathi, A. (2006). Fungitoxicity of the essential oil of *Citrus sinensis* on post-harvest pathogens. *World Journal of Microbiology and Biotechnology* 22 (6), 587-93.

Sokovic, M. and Griensven, L.J.L.D. (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. *European Journal of Plant Pathology* 116, 211-24.

Souza, E.L., Lima, E.O., Freire, K.R.L. and Sousa, C.P. (2005). Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. *Brazilian Archives of Biology and Technology* 48 (2), 245-50.

Wilson, C.L., Solar, J.M., Ghaouth, A. and Wisniewski, M. (1997). Rapid evaluation of plant extract and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Disease* 81, 204-210.

Book review

The Flowering of Australia's Rainforests

By Geoff Williams and Paul Adam

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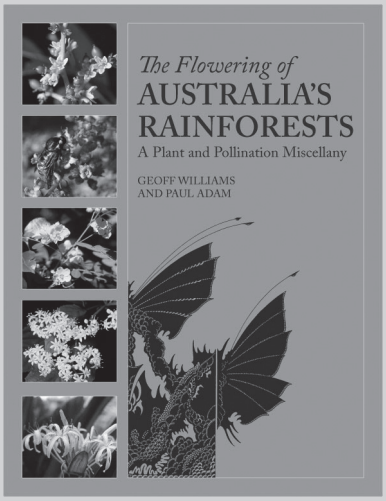

Rainforests are major reserves of biological diversity and their existence is dependent on their regeneration. While many processes including habitat loss and fragmentation, invasion by weeds and pest animals, and climate change threaten regeneration, it is still dependent on pollination processes.

The purpose of this book is to make some of the specialist knowledge available on pollination processes accessible to the general reader. The authors have summarized what is known of the pollination of rainforest plants, both in Australia and overseas, and the evolutionary pathways that have brought them into being. The agents and mechanisms that facilitate pollination have been reviewed and, though there are relatively few studies on the pollination of Australian rainforest species, overseas studies are included.

The Flowering of Australia's Rainforests progresses through introductory sections that cover pollination in lore and legend; plant and flower evolution and development; and the role and function of colour, fragrance and form. Later chapters deal with breeding systems; mimicry; spatial, temporal and structural influences on plant-pollinator interactions; and a discussion and overview of floral syndromes. The book concludes with a section on conservation and fragmentation, and individual plant pollination case studies.

Geoff Williams is a pollination ecologist and conservation biologist, with an additional background in entomology and invertebrate biogeography. He received a PhD from the University of New South Wales, is a Research Associate with the Australian Museum (Sydney), and has particular interests in the pollination of rainforest plants, ecosystem management and forest rehabilitation.

Paul Adam is a botanist, plant geographer and ecologist, and received his PhD from Cambridge University. He holds a senior academic position with the University of New South Wales, and was awarded honorary membership in the general division of the Order of Australia in recognition of his contributions to science, biodiversity conservation and science education.

This reference work is aimed at field naturalists, ecologists, conservation biologists, botanists, ecosystem managers, environmentalists, community groups and individuals involved in habitat restoration, students, and those with a broad interest in natural history. It is an excellent text for anyone wanting to know the whys and wherefores of the relationship between plants and pollinators that has given us such diverse ecosystems.

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