Photo-oxidation of Orange Essential Oil - Combating Green Mould Disease in Citrus.

(An activated natural bio-fungicide)

Mohammad M. Rahman*, University of Newcastle

Ron B.H. Wills*, Michael C. Bowyer*, John B. Golding**, Timothy Kirkman* and Penta Pristijono*

*University of Newcastle & **NSW Department of Primary Industries

Introduction

Green mould caused by *Penicillium digitatum*, is a major postharvest disease of citrus fruits causing serious losses to the industry [1]. It is currently successfully controlled with commercial chemical fungicides, but there are health and environmental concerns over the use of such chemicals [2-7]. There is a growing interest in sourcing natural alternatives to synthetic fungicides [7]. This project investigates the efficacy of oxygenated essential oil products (EO) extracted from citrus waste (e.g. citrus juice pomace) as bio-fungicidal agents.

Methods

Orange EO was mildly oxidised using ultraviolet-C (UV-C) irradiation.

The antifungal potential of non-oxidised and oxidised orange EOs was assessed *in vitro* by agar diffusion assay and in organic Navel oranges inoculated with *P. digitatum* spores by dipping in aqueous emulsions.

Results

UV-C oxidation of orange EO resulted in 20 % loss of the major constituent – limonene. Three hydroperoxide oxidation products - (2S,4R)-p-mentha-6,8-diene-2-hydroperoxide (**1**), (1S,4R)-p-mentha-2,8-diene-1-hydroperoxide (**2**) and (1R,4R)-p-mentha-2,8-diene-1-hydroperoxide (**3**), were identified by GCMS (Fig.1). These hydroperoxides have capability to alkylate DNA and proteins that relates the key concept of survival of microbial species [8].

In vitro agar diffusion assay studies on *P. digitatum* showed a strong antifungal response from the oxidised orange EO solution relative to the control and non-oxidised EO (Fig. 2).

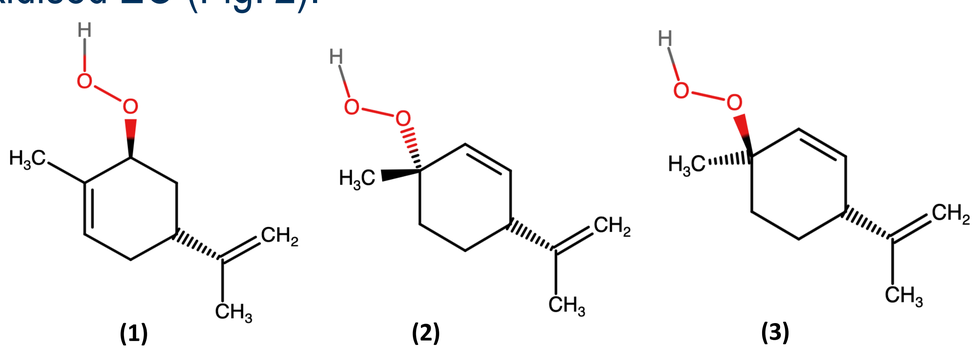

Fig.1 Structure of the developed hydroperoxides following UV-C oxidation of orange essential oil.

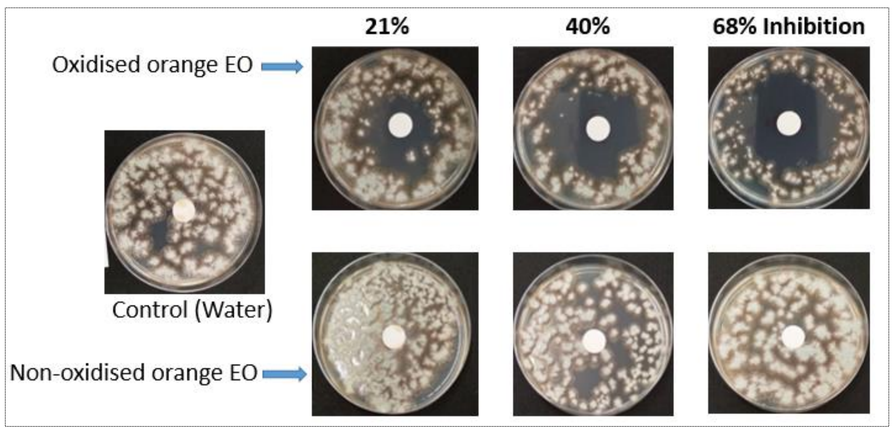

Fig. 2 Radial growth study of the relative antifungal effects of oxidised and non-oxidised orange EO on *P. digitatum. Results shown are* after 3 days at 24 °C.

Results

Dipping citrus in oxidised orange EO significantly inhibited growth of *P. digitatum. Optimal* treatment concentration was 4000 µL L^{-1} (Fig.3 & 4). No phytotoxic injury to the rind was observed.

P. digitatum growth in oranges dipped in non-oxidised orange EO at 1000 - 4000 µL L^{-1} was not significantly different to the control (H_2O).

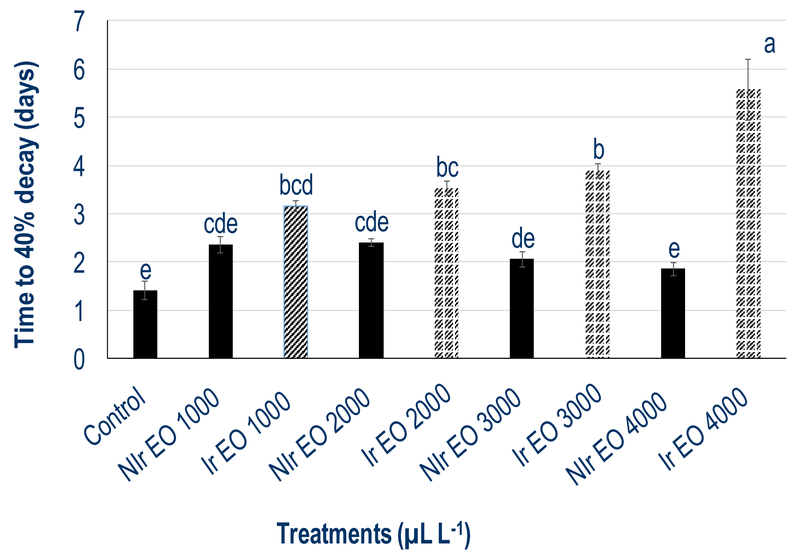

Fig.3 Time for citrus to reach 40% decay following inoculation with *P. digitatum* and dipped with oxidised (Ir) and non-oxidised (Nlr) orange EO. Letters indicate significant difference between treatments (p=0.05).

Non-treated fruits after 5 days

Oxidised orange essential oil (4000 µL L^{-1}) treated fruits after 5 days

Fig.4 Fungal wastage of inoculated oranges during storage at 20 °C following dipping in oxidised orange essential oil (EO) at 4000 µL L^{-1}.

Conclusions

UV-C oxidised orange EO has potential as an alternative treatment to synthetic fungicides for controlling green mould (*P. digitatum)* decay in citrus.

Extraction of orange EO from waste flavedo generated from juice processing has potential to value add to a currently unwanted by-product.

Acknowledgments

M.M.R. wishes to thank the University of Newcastle, Australia, NSW DPI and Horticulture Innovation "Citrus Postharvest Program" (CT19003) for funding and facilities to conduct his doctoral research program.

References

1. Eckert, J. W., & Brown, G. E. Postharvest citrus diseases and their control; W.F. Wardowski, S. Nagy, W. Grierson Ed. Fresh Citrus Fruits, AVI Publishing: Westport, CT, **1986,** 315-360
2. Golding, J.; Archer, J. Advances in postharvest handling of citrus fruit. In Achieving Sustainable Cultivation of Tropical Fruits; Yahia, E.M., Ed.; Burleigh Dodds Science Publishing: Cambridge, UK, **2019**; 65–90.
3. Palou, L.; Smilanick, J.L.; Droby, S. Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Postharvest Rev. **2008**, 4, 1–16.
4. Ismail, M.; Zhang, J. Post-harvest Citrus Diseases and their control. In Outlooks on Pest Management; Research Information: London, UK, **2004**; 15, 29–35.
5. Talibi, I.; Boubaker, H.; Boudyach, E.H.; Ait Ben Aoumar, A. Alternative methods for the control of postharvest citrus diseases. J. Appl. Microbiol. **2014**, 117, 1–17.
6. Eckert, J.W.; Sievert, J.R.; Ratnayake, M. Reduction of imazalil e_ectiveness against citrus green mold in California packinghouses by resistant biotypes of Penicillium digitatum. Plant Dis. **1994**, 78, 971–973.
7. Torres-Alvarez, C.; Núñez González, A.; Rodríguez, J.; Castillo, S.; Leos-Rivas, C.; Báez-González, J.G. Chemical composition, antimicrobial, and antioxidant activities of orange essential oil and its concentrated oils. J. Food **2017**, 15, 129–135.
8. Suzan, A.K.; Selva, R. Recent progress in photochemical reaction on main components of some essential oils. J. Saudi Chem. Soc. **2018**, 22, 855–875.