Efficacy of Orange Essential Oil and Citral after Exposure to UV-C Irradiation to Inhibit Penicillium digitatum in Navel Oranges

M. M. Rahman 1, R.B.H. Wills 1, Michael C. Bowyer 1, John. B. Golding 1,2, Timothy Kirkman 1 and Penta Pristijono 1,*

1 School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258, Australia; mohammad.m.rahman@uon.edu.au (M.M.R); ron.wills@newcastle.edu.au (R.B.H.W); michael.bowyer@newcastle.edu.au (M.C.B); john.golding@dpi.nsw.gov.au (J.B.G); timothy.kirkman@newcastle.edu.au (T.K.)

2 NSW Department of Primary Industries, Ourimbah, NSW 2258, Australia

* Correspondence: penta.pristijono@newcastle.edu.au

Received: 2 December 2020; Accepted: 11 December 2020; Published: 14 December 2020

Abstract: The effect of UV-C irradiation on antifungal properties of orange essential oil (EO) against Penicillium digitatum in inoculated Navel oranges was examined. The UV-C irradiation of orange EO resulted in a 20% loss of the major constituent, limonene, and the generation of three hydroperoxide oxidation products, (2S,4R)-p-mentha-6,8-diene-2-hydroperoxide, (1S,4R)-p-mentha-2,8-diene-1-hydroperoxide, and (1R,4R)-p-mentha-2,8-diene-1-hydroperoxide. The P. digitatum growth in oranges dipped in non-irradiated orange EO at 1000–4000 µL L⁻¹ was not significantly different to control the fruit. Dipping in UV-C treated orange EO inhibited the growth of P. digitatum with 4000 µL L⁻¹ having the greatest effect. No phytotoxic injury to the rind was observed at any concentration. Citral, as a known antifungal chemical, was included for comparison. The non-irradiated citral (1000 µL L⁻¹) was more effective than irradiated orange EO, but elicited rind phytotoxicity. The irradiated citral was less effective in inhibiting P. digitatum growth with the loss of citral, but not hydroperoxide formation. These results suggest UV-C irradiated orange EO as a potential alternative to synthetic fungicides to inhibit P. digitatum decay. The source of orange EO could be waste flavedo generated by the orange juice processing industry.

Keywords: orange essential oil; citral; UV-C irradiation; hydroperoxides; Penicillium digitatum

1. Introduction

Penicillium digitatum Pers. (Sacc.) is the causative fungi for the development of green mould, which is a major postharvest disease of citrus fruits causing considerable losses [1]. Synthetic fungicides are routinely used to control postharvest decay such as P. digitatum wastage [2], but there are some perceived health and environmental concerns over their use in many countries [3,4]. The constant use of synthetic fungicides can also lead to the development of resistance to the fungicides, which results in the loss of fungicide efficacy [5].

An alternative approach to control postharvest decay has been to investigate the efficacy of antimicrobial compounds, which are naturally present in plant essential oils (EO) [6]. Plant EOs are mostly terpenoids derived from units of isoprene (2-methyl-1,3-butadiene) with further structural diversification achieved through the inclusion of heteroatom functional groups such as alcohols, aldehydes, ketones, esters, and ethers [7]. EOs are typically low boiling point liquids, which makes their large-scale extraction and purification by low cost, low technology methods, such as steam
distillation, relatively easy and cost effective. An investigation in the use of citrus EOs particularly from oranges is further driven with waste valorisation by value adding to commercial citrus processing through the recovery of EOs from peel waste. The world citrus production for juicing and processing is estimated at 19 million tonnes [1] with the resultant large quantities of peel waste presenting a significant and costly disposal issue to processors [8].

Limonene, a cyclic monoterpen, is the major constituent of orange EO, averaging greater than 90% by volume across the major commercially cultivated orange cultivars [6,9], but it has, at best, only a weak antifungal activity. Indeed, limonene has been reported to both stimulate P. digitatum development [10,11] and to act as an antifungal agent [12]. Du Plooy et al. [13] reported that limonene gave some in vitro growth inhibition of P. digitatum at 3000 µL L⁻¹, but in vivo trials with “Tomango” oranges showed it to be ineffective. There have been few studies with orange EO, however, Wang et al. [14] found, in an in vitro study, that exposure to “Shatangju” orange EO at 2500 µL L⁻¹ mildly stimulated the growth of P. digitatum, but was inhibitory at higher concentrations.

The antifungal properties of limonene oxidation products have also been investigated with reactive oxygen species, such as hydroperoxides, attracting particular attention. Limonene hydroperoxides are naturally produced as a defensive response to the wounding of the peel in lemons, where they induce the production of the antifungal phytoalexin, scoparone [15]. Ben-Yehoshua [16] investigated the photochemical oxidation of limonene using Rose Bengal with the production of limonene hydroperoxide and showed that it possessed a strong antifungal activity in inoculated lemon fruit. Khayyat and Sameeh [17] and Khayyat [18] also investigated, respectively, the oxidation of geranyl acetate and linalyl acetate both thermally and photochemically resulting in the generation of a number of hydroperoxides possessing strong in vitro antifungal and antibacterial activities. We have been unable to find any study on the effect of photoxidised orange EO on mould growth.

The antimicrobial properties of citral (3,7-dimethyl-2,6-octadienal), a monoterpen aldehyde present in varying amounts in different citrus EOs, have also been investigated. Citral is a mixture of two geometric isomers-geranial (E-isomer) and neral (Z-isomer), with the ratio varying between plant sources [19]. Multiple studies show that citral possesses a wide-ranging antifungal activity, which has been attributed to its ability to disrupt the membrane structure and damage cell organelles [20]. Rodov [21] reported that citral levels in lemon flavedo were much greater in green lemons than mature fruit and the decline in the citral level was correlated with an increasing susceptibility to green mould infection. Rodov et al. [21] and Ben-Yehoshua and Rodov [22] reported that dipping non-inoculated Washington Navel oranges in a solution of citral markedly reduced P. digitatum infection. However, various investigations have reported phytotoxic damage to the rind arising from dipping in citral solutions [23–25]. Rodov et al. [26] found that when citral was dissolved in 25% v/v ethanol, it suppressed P. digitatum decay in “Eureka” lemons, but without visible phytotoxic damage to the rind. We are not aware of any investigation on the antimicrobial properties of citral photooxidation products, despite the potential for the formation of hydroperoxides similar to those generated from limonene, as the C6=C7 double bound in the molecule is susceptible to a singlet oxygen attack [27].

In this study, the potential for an enhanced antifungal efficacy of orange EO through the UV-C irradiation on P. digitatum growth in inoculated Navel oranges was examined. In addition, pure citral was included for comparative purposes as it is not a major constituent of orange EO [6], but has antifungal properties [22]. Both orange EO and citral were irradiated with UV-C (λ = 254 nm) in the presence of atmospheric oxygen and water vapour to accelerate oxidative degradation as described by Li et al. [28]. The change in composition of the orange EO and citral following irradiation was also determined, with particular interest in the generation of hydroperoxides. The overall aim of the study was to examine whether a simple, in situ protocol to modify the EO composition could be utilised with the existing citrus industry juicing practices, which would enhance the antifungal properties of orange EO.
2. Materials and Methods

2.1. Plant Materials

Organic Navel oranges (*Citrus sinensis* L. Osbeck) were harvested at commercial maturity from a New South Wales Department of Primary Industries (NSW DPI) orchard (Somersby, NSW, Australia). Non-blemished fruits were sanitised with a sodium hypochlorite solution (10 mL L\(^{-1}\)) and air dried for about 90 min. The surface sterilised fruits were then randomly distributed into 33 experiment units, each consisting of 45 fruits, with three units assigned to each of 11 treatments. The experiment was replicated on three different batches of Navel oranges, which were obtained over a 2-month period.

2.2. Penicillium Cultures and Inoculum Preparation

The *P. digitatum* culture was obtained from the Citrus Pathology Laboratory of the NSW DPI, Ourimbah, Australia. The pathogen was cultured on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI, USA) and incubated at 25 \(^\circ\)C for 7 days. The conidia of *P. digitatum* were gathered and suspended in sterile distilled water to obtain a stock solution. The clear suspension was then diluted with sterile distilled water to a concentration of 10\(^5\) spores mL\(^{-1}\). An haemocytometer and microscope were used to determine the spore concentration.

2.3. Essential Oils and Chemicals

A commercial orange (*Citrus sinensis*) EO (100% purity), extracted by cold press, was purchased from earthYard (Blaxland East, NSW, Australia). The GC-MS derivatisation reagent N,O-bis(trimethylsilyl) trifluoroacetamide/triethylchlorosilane (BSTFA/TMCS) (99:1), toluene (HPLC Plus grade), citral, and Triton-X100 were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia).

2.4. Irradiation of Essential Oil and Citral

The UV-C irradiation of orange EO and citral was conducted using a custom-made light proof box fitted with two UV emitting germicidal lamps (G20T10 20 Watt, low pressure mercury, Sahkyo Denki, Kanagawa, Japan) at ambient conditions (20 \(\pm\) 4 \(^\circ\)C and 65-0% RH). A SED008/W detector (International Light Technologies, Peabody, MA, USA) with a Precision Infrared Radiometer Irradiance Calibration at \(\lambda = 254\) nm was used to monitor the UV-C intensity. The UV-C intensity (kJ m\(^{-2}\)) was determined prior to the treatment using a research radiometer (International Light Technologies 1700 series, Peabody, MA). The treatment dose (kJ m\(^{-2}\)) was calculated by multiplying the emitting UV light intensity and treatment time (in seconds). The light intensity was evaluated several times during exposure to ensure a consistent output. Orange EO and citral samples were placed into open glass petri dishes approximately 5 cm from the UV-C light source. The UV-C irradiation was applied for 24 h. This exposure time was based on a preliminary study that examined a range of times and found that 24 h was optimal to generate hydroperoxides, as determined by GC-MS analysis.

2.5. Gas Chromatograph/Mass Spectrometry (GC-MS) Analysis

Orange EO and citral were analysed by GC-MS (Shimadzu QP 2010SE, Canby, OR, USA). The irradiated samples were derivatized prior to the analysis using BSTFA/TMCS to be able to detect hydroperoxides and epoxides according to the method of Rudback et al. [29], with slight modifications. Briefly, a stock solution of oil (3 mg mL\(^{-1}\)) was prepared in toluene and stored at -18 \(^\circ\)C overnight. A diluted solution (750 \(\mu\)g L\(^{-1}\)) was then prepared from the stock solution for injection into the GC-MS. For derivatization, BSTFA/TMCS (0.1 mL) was added to a sample of the diluted oil (0.9 mL), vortexed, and held for 24 h for the derivatization reaction to complete. An aliquot (1 \(\mu\)L) of solution was injected into the GC-MS under the following conditions: Non-derivatized oils: Injection temperature 250 \(^\circ\)C, column oven temperature 80 \(^\circ\)C, split ratio 1:16; and for derivatized oils: Injection temperature 230 \(^\circ\)C, column oven temperature 90 \(^\circ\)C, split ratio 1:10. Constituents were identified by comparison of their
mass spectra with mass libraries from The National Institute of Standards and Technology (2010) and by SciFinder—CAS (https://scifinder.cas.org).

2.6. Infection and Postharvest Treatment of Oranges

Oranges were inoculated by wounding the flavedo with a sharpened steel rod (1 mm diameter × 2 mm length) that had been immersed in a spore suspension (10⁵ spores mL⁻¹) of *P. digitatum*. The fruits were then incubated at 20 °C for 24 h. The inoculated fruits were then dipped for 2 min in either an irradiated or non-irradiated orange EO solution. The orange EO dip solutions were prepared by mixing the appropriate volume of orange EO concentrate with absolute ethanol and Triton-X100 to form a stable solution. This mixture was added to 5 L of water with vigorous stirring using a blender (Ozito, Model DMM080, Zhejiang, China) to form an emulsified solution. A unit of fruit was added immediately after preparation of the solution with a fresh solution prepared for each fruit unit. The concentrations of constituents in the dipping solutions was 1000, 2000, 3000, and 4000 µL L⁻¹ orange EO, 1 mL L⁻¹ ethanol, and 24 mg L⁻¹ Triton X100. To examine the effect of citral on *P. digitatum*, oranges were similarly treated with irradiated and non-irradiated citral solutions at 1000 µL L⁻¹, a concentration that preliminary trials with non-irradiated citral showed had a strong antifungal activity against *P. digitatum*. Control fruits were dipped in water containing ethanol (1 mL L⁻¹) and Triton-X100 (24 mg L⁻¹) for 2 min. After the treatment, the fruits were placed on trays and allowed to dry at room temperature (20–26 °C, 55–5% RH) for 1 h, then each treatment unit was transferred into an unsealed low-density polyethylene (LDPE) bag and stored at 20 °C (RH 95–99%). The diameter of the infected fruit lesion on each fruit was recorded daily for 5 days. An individual fruit was considered to be decayed when a soft *P. digitatum* lesion diameter exceeded 4 mm. The percentage of fruits exhibiting decay in each treatment unit was calculated and plotted against the storage time. A regression analysis was performed to calculate the time for 40% of the fruit in that unit to exhibit decay.

2.7. Statistical Analysis

A randomised experiment design incorporating 11 treatments with three replications from three different batches of oranges was utilised. The linear regression analysis was performed using IBM SPSS (version 25) (Armonk, NY, USA). The analysis using one-way ANOVA was performed using the SAS statistical software (version 9.4) (Cary, NC, USA) and where there was a significant difference between means, the least significance difference (LSD) at *p* = 0.05 was calculated.

3. Results and discussion

3.1. Composition of Orange EO and Citral

Limonene was present at about 90% of the total constituents with a range of minor aliphatic and aromatic compounds (all individually less than 2%) also detected (Table 1). The findings are consistent with previous reports relating to the composition of orange EO [6,9,30]. Post-irradiation with UV-C, the limonene content of orange EO was significantly lower by about 20% and a range of new compounds were identified as various limonene oxidation products including three hydroperoxides, (2S,4R)-p-mentha-6,8-diene-2-hydroperoxide, (1S,4R)-p-mentha-2,8-diene-1-hydroperoxide, and (1R,4R)-p-mentha-2,8-diene-1-hydroperoxide. Previous reports have identified up to six hydroperoxides [31,32]. The variation between studies would seem to be due to different reaction conditions used as detailed by Li et al. [28] who stated that the irradiation-mediated oxidation of limonene was affected by factors such as energy input, water content, and atmospheric oxygen level.
Table 1. Composition of non-irradiated and irradiated orange essential oil (EO).

| Compound            | Non-irradiated Orange EO Peak Area (%) a | Irradiated Orange EO Compound               | Peak Area (%) a |
|---------------------|------------------------------------------|------------------------------------------|-----------------|
| D-Limonene          | 88.8                                     | D-Limonene                               | 60.1            |
| β-Mycene            | 1.5                                      | Benzaldehyde                             | 3.8             |
| Benzaldehyde        | 1.2                                      | Ethylbenzene                             | 1.5             |
| Ethylbenzene        | 0.8                                      | Linalool                                 | 1.3             |
| α-Pinene            | 0.5                                      | α-Pinene                                 | 0.4             |
| Octanal             | 0.5                                      | β-Mycene                                 | 0.3             |
| Linalool            | 0.5                                      | l-Carvone                                | 3.3             |
| 3-Carene            | 0.4                                      | trans-Limonene oxide                     | 2.5             |
| Decanal             | 0.2                                      | trans-α-pinene                           | 2.1             |
|                     |                                          | cis-Limonene oxide                       | 1.8             |
|                     |                                          | (2S,4R)-p-Mentha-6,8-diene 2-hydroperoxide | 1.8             |
|                     |                                          | (1S,4R)-p-Mentha-2,8-diene, 1-hydroperoxide | 1.4             |
|                     |                                          | cis-carveol                              | 1.4             |
|                     |                                          | (1R,4R)-p-Mentha-2,8-diene, 1-hydroperoxide | 1.4             |
|                     |                                          | trans-p-Mentha-2,8-dienol                | 1.3             |
|                     |                                          | p-Mentha-1,8-dien-7-ol                   | 0.8             |
|                     |                                          | p-Mentha-1(7),8(10)-dien-9-ol            | 0.5             |
|                     |                                          | p-Mentha-1(7),8-dien-2-ol                | 0.4             |

a Values are % of total peak area of the mean value of samples obtained from three replicate units. b Group 1 compounds are present in non-irradiated EO, Group 2 are new oxidation products.

The analysis of the non-irradiated citral mainly comprised the geometric isomers geranial (60%) and neral (35%). The post-irradiation analysis showed that both isomers were still present in a significant quantity, although the relative proportions had changed, with geranial (the E isomer) falling to about 50% and with neral (the Z isomer) remaining at 36%. Several new oxidation products were identified, including geranic acid (4.2%), neric acid (1.9%), (1S,2R,5R)-2-(2-hydroxypropan-2-yl)-5-methylcyclohexanol (also known as menthoglycol) (2.3%), and 2,7-dimethyl-2,7-octanediol (3.5%). No epoxide or hydroperoxide products were detected. The formation of a cyclohexanol adduct under photochemical reaction conditions has not been previously reported, but menthoglycol is a known cyclisation product from citral [33].

3.2. Effects of Orange EO and Citral on Decay in Inoculated Navel Oranges

The storage life, as the time taken for 40% of the fruit in each treatment to show a decay lesion diameter ≥4 mm, is presented in Figure 1. This shows firstly, that there was no significant difference between the time to 40% decay for the fruit dipped in any concentration of non-irradiated orange EO and the untreated control. However, fruit dipped in irradiated orange EO took a significantly longer time to a 40% decay than the control fruit with the relative effectiveness increasing with the orange EO concentration. While the irradiated EO did not completely inhibit decay, its growth rate was significantly reduced. These findings were confirmed by regression analysis which showed that for the fruit exposed to the irradiated orange EO there was a significant linear relationship between EO concentration and storage time (y = 0.91x + 0.78; R² = 0.92), but no significant relationship for the fruit exposed to non-irradiated orange EO (R² = 0.05). An important observation in this study was that there was no visible phytotoxic injury on the surface of any fruit exposed to orange EO. The enhanced antimicrobial activity of the irradiated orange EO is attributed to the presence of limonene hydroperoxide derivatives, which Ben-Yehoshua [16] has claimed in a patent to reduce P. digitatum induced decay in kumquat and lemon fruits.
with the non-irradiated citral, orange EO had a significantly commercial advantage of not generating any visible skin damage without the need for an ethanolic dip solution. Given the ready availability of orange skin tissue as a processing waste product and the ability to use the EO in an aqueous solution, it would seem worthwhile to conduct further studies to determine if other methods or combinations of methods, such as radiation, heat, oxygen, and water, could enhance the antimicrobial activity of orange EO to a level where it could be a commercially profitable product for the orange processing industry.

**Figure 1.** Time to reach 40% decay of oranges inoculated with *P. digitatum* and dipped with irradiated (Ir) and non-irradiated (NIr) orange EO and citral. The value for each treatment is the mean of nine treatment units (three replicates x three batches of fruit). The bars on each treatment are the mean standard errors. Different letters indicate a significant difference between treatments at *p* = 0.05.

The data presented in Figure 1 also show that dipping oranges in 1000 µL L\(^{-1}\) non-irradiated citral significantly delayed the onset of *P. digitatum* decay and was about twice as effective as dipping in the irradiated 4000 µL L\(^{-1}\) orange EO. This observation is consistent with Ben-Yehoshua et al. [23] who reported that citral extracted from lemon peel displayed a strong inhibitory activity against *P. digitatum* when applied at 2000 µL L\(^{-1}\) to mature green lemons. However, in this study, the irradiated citral was significantly less effective than the non-irradiated citral and gave a similar inhibition as dipping in 4000 µL L\(^{-1}\) irradiated orange EO. The analysis of citral, in this study, showed that irradiation reduced the level of citral, particularly of geranial, without the generation of any hydroperoxide. Therefore, it is reasoned that citral, unlike limonene, has an antimicrobial activity and the reduction in citral during UV-C irradiation, without a corresponding generation of hydroperoxide, resulted in a lower antifungal efficacy. However, for both irradiated and non-irradiated citral, there was a phytotoxic effect on the peel, which was viewed as skin cell collapse, pitting, and discolouration on all fruits. Knight [24] also reported a phytotoxic effect for non-irradiated citral in Navel oranges.

### 4. Conclusions

This study showed that UV-C irradiation of orange EO significantly inhibited the development of *P. digitatum* decay on Navel oranges. While, the level of inhibition was not as high as that achieved with the non-irradiated citral, orange EO had a significantly commercial advantage of not generating any visible skin damage without the need for an ethanolic dip solution. Given the ready availability of orange skin tissue as a processing waste product and the ability to use the EO in an aqueous solution, it would seem worthwhile to conduct further studies to determine if other methods or combinations of methods, such as radiation, heat, oxygen, and water, could enhance the antimicrobial activity of orange EO to a level where it could be a commercially profitable product for the orange processing industry.
Author Contributions: R.B.H.W. conceived the research hypothesis that led to the experiment, contributed to the experimental design, and edited the manuscript draft; M.M.R. carried out the experiment, analysed the data, and wrote the manuscript draft; M.C.B. contributed to the experimental design and edited the manuscript draft; J.B.G. contributed to the experimental design and edited the manuscript draft; T.K. contributed to supervision and validation; P.P. analysed the data and edited the manuscript draft. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Newcastle, Australia, NSW DPI and Horticulture Innovation “Citrus Postharvest Program” (CT19003).

Acknowledgments: M.M.R. also wishes to thank the University of Newcastle for the scholarship that enabled him to conduct his doctoral research program.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. 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; pp. 65–90.

2. 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. [CrossRef]

3. Ismail, M.; Zhang, J. Post-harvest Citrus Diseases and their control. In Outlooks on Pest Management; Research Information: London, UK, 2004; Volume 15, pp. 29–35. [CrossRef]

4. 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. [CrossRef]

5. Eckert, J.W.; Sievert, J.R.; Ratnayake, M. Reduction of imazalil effectiveness against citrus green mold in California packinghouses by resistant biotypes of Penicillium digitatum. Plant Dis. 1994, 78, 971–973. [CrossRef]

6. 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. [CrossRef]

7. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. Food Chem. Toxicol. 2008, 46, 446–475. [CrossRef]

8. Bustamante, J.; Van Stempvoort, S.; García-Gallarreta, M.; Houghton, J.A.; Briers, H.K.; Budarin, V.L.; Matharu, A.S.; Clark, J.H. Microwave assisted hydro-distillation of essential oils from wet citrus peel waste. J. Clean. Prod. 2016, 137, 598–605. [CrossRef]

9. Svoboda, K.P.; Greenaway, R.I. Lemon scented plants. Internat. J. Aromather. 2003, 13, 23–32. [CrossRef]

10. Droby, S.; Eick, A.; Macarins, D.; Cohen, L.; Rafael, G.; Stange, R.; McColum, G.; Dudai, N.; Nasser, A.; Wisniewski, M.; et al. Role of citrus volatiles in host recognition, germination and growth of Penicillium digitatum and Penicillium italicum. Postharvest Biol. Technol. 2008, 49, 386–396. [CrossRef]

11. Simas, D.L.R.; De Amorim, S.H.B.M.; Gouart, F.R.V.; Alviano, C.S.; Alviano, D.S.; Da Silva, A.J.R. Citrus species essential oils and their components can inhibit or stimulate fungal growth in fruit. Indust. Crops Products 2017, 108, 108–115. [CrossRef]

12. Tao, N.; Jia, L.; Zhou, H. Anti-fungal activity of Citrus reticulata Blanco essential oil against Penicillium italicum and Penicillium digitatum. Food Chem. 2014, 153, 265–271. [CrossRef]

13. Du Plooy, W.; Regnier, T.; Combrinck, S. Essential oil amended coatings as alternatives to synthetic fungicides in citrus postharvest management. Postharvest Biol. Technol. 2009, 53, 117–122. [CrossRef]

14. Wang, H.; Tao, N.; Huang, S.; Liu, Y. Effect of Shatangju (Citrus reticulata Blanco) Essential Oil on Spore Germination and Mycelium Growth of Penicillium digitatum and P. italicum. J. Essent. Oil Bear. Plants 2012, 15, 715–723. [CrossRef]

15. Afek, U. Accumulation of Scoparone, a Phytoalexin Associated with Resistance of Citrus to Phytophthora citrophthora. Am. Phytopathol. Soc. 1988, 78, 1678–1682. [CrossRef]

16. Ben-Yehoshua, S. Microbiocidal Formulation Comprising Essential Oils or Their Derivatives. US Patent 7,465,469 B2, 16 December 2008.

17. Khayyat, S.A.; Sameeh, M.Y. Bioactive epoxides and hydroperoxides derived from naturally monoterpane geranyl acetate. Saudi Pharm. J. 2018, 26, 14–19. [CrossRef]
18. Khayyat, S. Thermal, photo-oxidation and antimicrobial studies of linalyl acetate as a major ingredient of lavender essential oil. *Arab. J. Chem.* **2020**, *13*, 1575–1581. [CrossRef]

19. Wilson, N.D.; Ivanova, M.S.; Watt, R.A.; Moffat, A.C. The quantification of citral in lemongrass and lemon oils by near-infrared spectroscopy. *J. Pharm. Pharmacol.* **2002**, *54*, 1257–1263. [CrossRef]

20. Leite, M.C.A.; Bezerra, A.P.D.B.; Sousa, J.P.D.; Guerra, F.Q.S.; Lima, E.D.O. Evaluation of Antifungal Activity and Mechanism of Action of Citral against *Candida albicans*. *Evid. Based Complement Alternat. Med.* **2014**, *2014*, 378280. [CrossRef]

21. Rodov, V.; Ben-Yehoshua, S.; Fang, D.Q.; Kim, J.J.; Ashkenazi, R. Preformed antifungal compounds of lemon fruit: Citral and its relation to disease resistance. *J. Agric. Food Chem.* **1995**, *43*, 1057–1061. [CrossRef]

22. Ben-Yehoshua, S.; Rodov, V. Developing a novel environmentally friendly microbicidal formulation from peel of citrus fruit. *Acta Hortic.* **2006**, 275–284. [CrossRef]

23. Ben-Yehoshua, S.; Rodov, V.; Kim, J.J.; Carmeli, S. Preformed and induced antifungal materials of citrus fruits in relation to the enhancement of decay resistance by heat and ultraviolet treatments. *J. Agric. Food Chem.* **1992**, *40*, 1217–1221. [CrossRef]

24. Knight, T.G. Investigation of the Physiological Basis of the Rind Disorder Oleocellosis in Washington Navel Oranges (Citrus Sinensis [L.] Osbeck). Ph.D. Thesis, University of Adelaide, Adelaide, Australia, 2002.

25. Wuryatmo, E.; Klieber, A.; Scott, E.S. Inhibition of Citrus Postharvest Pathogens by Vapor of Citral and Related Compounds in Culture. *J. Agric. Food Chem.* **2003**, *51*, 2637–2640. [CrossRef]

26. Rodov, V.; Nafussi, B.; Ben-Yehoshua, S. Essential oil components as potential means to control Penicillium digitatum pers. (Sacc.) and other postharvest pathogens of citrus fruit. *Fresh Prod.* **2011**, *5*, 43–50.

27. Elgendy, E.M. Photooxygenation of Natural Limonene. *J. Chin. Pharm. Sci.* **1998**, *50*, 225–231.

28. Li, L.J.; Hong, P.; Jiang, Z.D.; Yang, Y.F.; Du, X.P.; Sun, H.; Wu, L.M.; Ni, H.; Chen, F. Water accelerated transformation of d-limonene induced by ultraviolet irradiation and air exposure. *Food Chem.* **2018**, *239*, 434–441. [CrossRef]

29. Rudbäck, J.; Ramzy, A.; Karlberg, A.-T.; Nilsson, U. Determination of allergenic hydroperoxides in essential oils using gas chromatography with electron ionization mass spectrometry. *J. Sep. Sci.* **2014**, *37*, 982–989. [CrossRef]

30. González-Mas, M.C.; Rambla, J.L.; López-Gresa, M.P.; Blázquez, M.A.; Granell, A. Volatile Compounds in Citrus Essential Oils: A Comprehensive Review. *Front. Plant Sci.* **2019**, *10*. [CrossRef]

31. Calandra, M.J.; Impellizzeri, J.; Wang, Y. An HPLC method for hydroperoxides derived from limonene and linalool in citrus oils, using post-column luminol-mediated chemiluminescence detection. *Flavour Fragr. J.* **2015**, *30*, 121–130. [CrossRef]

32. Schieberle, P.; Maier, W.; Firl, J.; Grosch, W. HRGC separation of hydroperoxides formed during the photosensitized oxidation of (R)—(−)-Limonene. *J. High Resolut. Chromat.* **1987**, *10*, 588–593. [CrossRef]

33. Price, C.C.; Dickman, M.L. Kinetics of the Acid-Catalyzed Cyclization of Citral and Citronellal. *Indust. Eng. Chem.* **1948**, *40*, 257–261. [CrossRef]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).