Application of citral to control postharvest diseases of oranges

Erminawati Wuryatmo (Wati)

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Alhamdulillahhirrabbilalamin…

Kupersembahkan thesis ini kepada ibunda tercinta Hj.St.Aisyah, yang dengan kasih sayangnya tidak pernah lelah mendoakanku,
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Abstract

Green mould, blue mould and sour rot, caused by the fungi *Penicillium digitatum, P. italicum* and *Geotrichum citri-aurantii*, are postharvest diseases which cause significant losses to the citrus industry worldwide. Current control of the diseases raises some problems, such as development of fungicide resistance, concerns about residues harmful to humans, and also restrictions on the use of certain fungicides. Those problems have led to a need to develop alternative fungicides, including exploitation of some natural products such as essential oils.

Application of the essential oil, citral (3,7-dimethyl-2,6-octadienal) to control the fungi and the diseases was assessed in this study. *In vitro*, citral incorporated into agar at 2%, 6% and 15% prevented germination of spores of the fungi, and no mycelial growth was observed by microscopic observation after 17 days of incubation. When citral was applied as a solution on agar, spore germination of *P. digitatum* and *G. citri-aurantii* was inhibited at concentrations of 6% and 15%. However, germination of *P. italicum* spores was not affected. Vapour of citral and its individual isomers, geranial and neral, generated from 6 and 15% aqueous solutions, inhibited spore germination and growth of the three pathogens. Vapour generated from 15% aqueous solutions of citral and geranial were fungicidal to *P. digitatum* and *G. citri-aurantii*, and fungistatic to *P. italicum*, while neral was fungicidal to *G. citri-aurantii* and fungistatic to the other two fungi. The result suggested that method of application and citral concentration affected the efficacy of citral in controlling the fungi. In the three methods of applications examined, citral was effective in controlling *G. citri-aurantii*, especially at high concentration.

As an α, β-unsaturated aldehyde, citral may be degraded over time due to oxidative reactions, resulting in change in its composition, and this may affect its antifungal activity. Storage of citral may result in the oxidation of neral and geranial to produce neric acid and geranic acid. GC/MS results showed that neral, geranial, neric acid and geranic acid were detected, while the related compounds, nerol, geraniol, citronellal, citronellol and citronellic acid were not detected either for citral stored at $5^{o}C$ or at room temperature. At room temperature, geranial and neral content declined more quickly than at $5^{o}C$.

The effect of citral on the incidence of disease on fruit was studied by applying citral as a fumigant. Wounded oranges inoculated with spore suspension (10^6 spores mL^{-1}) of the fungi were placed in 5-litre plastic boxes, fumigated with 2, 6, or 15% citral, and incubated at 5°C or room temperature. Fumigation of oranges with citral in this closed system delayed the onset of sour rot at room temperature by 7 – 10 days and at 5°C, by 13 – 30 days, suggesting that volatile citral controlled *G. citri-aurantii* on fruit as well as *in vitro*. The effects of fumigation with citral on green and blue mould were more variable. Fumigation delayed the onset of green mould and blue mould at 5°C by 2 days at the higher concentrations (6 and 15%) tested, while at room temperature, spoilage was not delayed even at the highest concentration tested. Measurement of citral in the headspace of boxes containing fruit and citral-soaked pads showed that the concentration above the fruit was higher than that measured below the fruit both at 5°C and at room temperature. Phytotoxicity symptoms were observed on the upper surface of some fruit that was close to or in direct contact with the citral-soaked pad at concentrations of 6% and 15%, suggesting that phytotoxicity may have been associated with high volatile citral concentration. However, citral residue was not detected in oranges irrespective of treatment with citral, which suggested that little citral had penetrated into the peel. During storage the citral content decreased due to oxidation of geranial and neral to produce geranic acid and neric acid both at 5°C and room temperature. This may have had an impact on the efficacy of citral against the pathogens.

Findings may contribute to a better understanding of the efficacy of citral when applied to the pathogens *in vitro* and to the development of effective control methods when applied on fruit. The possibility of combining citral treatment with other commonly used practices is also worthy of consideration. For example, citral could be combined with heat treatment to increase the volatility of the citral. In addition, incorporation of citral in a wax formulation may allow a low concentration of citral to be used in direct contact with the pathogens on fruit. Fumigation of fruit with citral may offer potential as a means to control development of sour rot of oranges, and its effects on fruit quality, flavour and nutritional aspects require further investigation.

Declaration

NAME: ERMINAWATI WURYATMO (WATI). PROGRAM: PhD

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Publications and conference proceedings

The following publications and abstracts in conference proceedings were produced during the PhD candidature;

Wuryatmo, E., Klieber, A. and Scott, E. (2001) The effect of citral components on citrus postharvest fungal spoilage in culture. In: Australasian Postharvest Horticulture Conference Proceedings. Oral Presentation *Australasian Postharvest Horticulture Conference*, Adelaide, September 23-27, 2001.

Klieber, A., Scott, E., and Wuryatmo, E. (2002) Effect of method of application on antifungal efficacy of citral against post-harvest spoilage fungi of citrus in culture. *Australasian Plant Pathology,* **31**, 329-332.

Wuryatmo, E., Klieber, A., and Scott, E. (2003) Inhibition of citrus postharvest pathogens by vapor of citral and related compounds in culture. *Journal of Agricultural and Food Chemistry*, **51**, 2637-2640.

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Abbreviations

^oC	degree Celsius
2 ,4 D	2,4 Dichlorophenoxy
ANOVA	analysis of variance
a_w	water activity
$Ca(OH)_2$	calcium hydroxide
$C_2 H_2$	acetylene
$C_2 H_4$	ethylene
cfu	colony forming units
CH_2Cl_2	dichloro methane
CO_2	carbon dioxide
CuCl	cuprous chloride
DBAD	di-tert butyl azo dicarboxilate
FID	flame ionisation detector
kg, g, mg, µg	kilogram, gram, milligram, microgram
Gcd	*Geotrichum candidum* (*Geotrichum citri-aurantii*)
GC	gas chromatography
GC/MS	gas chromatograph/mass spectrometry
h	hours
H_2O	water (dihydrogen oxide)
KPa	kilo Pascal
K_2CO_3	potassium carbonate
L, mL, µL	litre, millilitre, microlitre

LSD	least significant difference
m, mm, μm	metre, millimetre, micrometre
min	minutes
n	number of mole present
NDY	Neutral-Dox Yeast
Pdg	*Penicillium digitatum*
Pit	*Penicillium italicum*
P	pressure
PDA	potato dextrose agar
π	phi
R	gas constant (8.3143 Joules/Moles/K)
r	radius
SARDI	South Australian Research and Development Institute
SPME	solid-phase microextraction
T	temperature
TFE	tetrafluoroethylene
TLC	thin layer chromatography
V	volume
v/v	volume per volume

Glossary of terms used in this study:

2% citral	In Petri dishes (*in vitro* experiment) : 2 μL citral diluted to 100 μL with 400 μL L^{-1} aqueous TritonX solution In box (*in vivo* experiment) : 131 μL citral diluted to 6550 μL with 400 μL L^{-1} aqueous TritonX solution
6% citral	In Petri dishes (*in vitro* experiment) : 6 μL citral diluted to 100 μL with 400 μL L^{-1} aqueous TritonX solution In box (*in vivo* experiment) : 393 μL citral diluted to 6550 μL with 400 μL L^{-1} aqueous TritonX solution
15% citral	In Petri dishes (*in vitro* experiment) : 15 μL citral diluted to 100 μL with 400 μL L^{-1} aqueous TritonX solution In box (*in vivo* experiment) : 983 μL citral diluted to 6550 μL with 400 μL L^{-1} aqueous TritonX solution
100% disease incidence	The time required for all fruit to show disease
Δ value	The time course for disease development, as a measure of the efficacy of citral in delaying the onset of disease