EFFECT OF OZONE ON ANTHRACNOSE, PHYSICOCHEMICAL RESPONSES AND GENE EXPRESSION IN PAPAYA (*CARICA PAPAYA* L.)

ONG MEI KYING

DOCTOR OF PHILOSOPHY
THE UNIVERSITY OF NOTTINGHAM
MALAYSIA CAMPUS

2014

EFFECT OF OZONE ON ANTHRACNOSE, PHYSICOCHEMICAL RESPONSES AND GENE EXPRESSION IN PAPAYA (*CARICA PAPAYA* L.)

By

ONG MEI KYING

Thesis Submitted to The University of Nottingham Malaysia Campus, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

August 2014

Dedication of love and gratitude to:

My caring parents and loving husband
whose endless support,
understanding and timely encouragement
inspired me to
strive and fulfil this goal.

ABSTRACT

EFFECT OF OZONE ON ANTHRACNOSE, PHYSICOCHEMICAL RESPONSES AND GENE EXPRESSION IN PAPAYA (*CARICA PAPAYA* L.)

By

ONG MEI KYING

August 2014

Chairman : Associate Professor Asgar Ali, PhD

Faculty : Science

A study was conducted to investigate the effects of varying levels of ozone (0, 1.5, 2.5, 3.5 or 5.0 ppm) for 96 h on 1. the *in vitro* and *in vivo* growth of *Colletotrichum gloeosporioides*, the causal organism of anthracnose; 2. the reactive oxygen species generation and spore mitochondria of *C. gloeosporioides* using transmission electron microscope, fluorescence microscope and laser scanning confocal microscope; 3. the production of defence-related enzymes in papaya; 4. microbiological analysis on ozone-treated and non-treated papaya; 5. the biochemical, physiological, gas exchange and sensory characteristics of papaya fruit during storage (25 ± 3 °C, 70 ± 5 %RH) for 14 days; 6. the changes in total phenols, total carotenoids and antioxidant activity; and 7. gene expression of ozone-fumigated papaya fruit. Data were analyzed using analysis of variance and differences among treatment means were separated by Duncan Multiple Range Test (DMRT). The results of antifungal studies showed that mycelial

growth of *C. gloeosporioides* was reduced significantly ($p < 0.05$) at all concentrations compared to the control. The maximum inhibition in mycelium growth (41.2 %) was obtained at 5.0 ppm ozone. Similarly, conidial germination inhibition was 100 % for 5 ppm ozone. *In vivo* analysis revealed that 2.5 ppm ozone was the optimal concentration for controlling anthracnose disease incidence (72.5 %) and disease severity after 10 days of storage, showing that a moderate concentration of ozone is effective in the reduction of *C. gloeosporioides* in artificially inoculated papaya fruit without affecting the quality aspect of the fruit.

The results of scanning electron microscopy (SEM) also confirmed that ozone fumigated fungus at levels above 3.5 ppm deformed and disintegrated spore and mycelia structure. Further to that, transmission electron microscopy (TEM) illustrated that the mitochondria of ozonized fungus was disintegrated and had ruptured membrane. In spores treated with 3.5 ppm ozone, mitochondrial cristae were distorted, whereas the mitochondria were almost completely degraded in spores treated with 5.0 ppm. Meanwhile, the results from microscopy studies using laser scanning confocal microscope and fluorescence microscope showed that ozone treatment caused production of reactive oxygen species (ROS) in mitochondria of *C. gloeosporioides*. With increased concentration of ozone, higher levels of ROS were induced in the spores.

Besides its direct antifungal activity, the study strongly suggested that ozone induces a series of defense reactions through production of compounds such

as total phenols, polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia-lyase (PAL) in ozone-fumigated papaya. Likewise, content of ascorbic acid, β-carotene, lycopene and antioxidant activity of papaya increased as fruit ripened and was further enhanced by exposure to ozone for 96 hours from day 4 until day 8. Twenty-four hours of ozone treatment at the level of 0.5, 2, 3.5 and 5.8 ppm reduced the total mesophilic microorganism counts of fruit with initial values of 4.48 to 2.18 log $cfug^{-1}$. In addition, no coliform bacteria were initiated after 24 hours at all levels of ozone exposure.

In addition, 2.5 ppm ozone treated fruit showed maximum beneficial effects in reducing weight loss, maintaining firmness, reduced rate of respiration, delaying changes in peel colour and containing the highest soluble solids concentration (SSC) as compared to the control. The titratable acidity declined throughout the storage period with slower rate in ozone-fumigated fruits. Overall sensory assessment of quality after ripening showed fruit were significantly better in quality when fumigated with 2.5 ppm ozone which were assigned highest sensory score in terms of appearance, sweetness, pulp colour, texture, aroma and overall acceptability than the control.

The discovery of the gene expression of papaya in defense response induced by ozone fumigation has further clarified the understanding on how specific gene involved in controlling its expression when the plant changes during stress or in any plant lifecycle event. Among those genes, some involved in ethylene biosynthesis, generation of reactive oxygen species and stress

responses of plant defense were found (mitochondrion, chloroplast, heat shock proteins, polygalacturonase-inhibiting protein, hydroxyproline-rich glycoprotein, ethylene responsive factor and acyl-CoA oxidase).

Thus, the findings from all the experiments carried out during this study showed that 2.5 ppm ozone reduced anthracnose incidence and extended the storage life for up to 12 days while maintaining acceptable quality of papaya fruit. Ozone exposure at 1.5 ppm resulted in poorer quality fruit as compared to 2.5 ppm ozone treated fruit. Higher concentration of ozone exposure at 3.5 ppm and 5 ppm ozone seems non-physiological and caused phytotoxic effect on the quality of papaya fruit. As a non-toxic, biodegradable product, eco-friendly and safe sanitizer, ozone has the potential to become a natural preservative for prolonging the shelf life and retaining quality of papaya by combating fungal disease, particularly fungus *C. gloeosporioides*, thus promoting the marketability of the crop and minimizing postharvest losses in the papaya industry.

ACKNOWLEDGEMENTS

My special and utmost appreciation and gratitude to my supervisor, Associate Professor Dr. Asgar Ali, Director of Centre of Excellence for Postharvest Biotechnology, School of Biosciences, The University of Nottingham Malaysia Campus, for his compassionate and scholastic guidance and mentor. His ceaseless patience, encouragement and constructive criticism have inspired me into a researcher full of high spirit and inner strength.

My heartfelt gratitude to my Co-supervisor, Associate Professor Dr. Feroz Kabir Kazi, Department of Chemical and Environmental Engineering, The University of Nottingham Malaysia Campus for his kindness, patience and continuous motivation to excellence. I am also indebted and wish to express my humble appreciation to my internal assessor, Dr. Susan Azam Ali, Assistant Professor, School of Biosciences, Faculty of Science, The University of Nottingham Malaysia Campus for her pleasing temperament, inspiring guidance and precious suggestions throughout the course of my research.

My appreciation and sincere is also extended to all staff members of biosciences, pharmacy and chemistry laboratories, especially Mr. Wan Ghani, Ms. Siti Norazlin, Ms. Shankari, Ms. Sharmila and Ms. Nurul for their willing assistance and help during my studies. Besides, all the lab comrades and in time of woe and joy companions especially Dr. Mehdi Maqbool, Ms. Noosheen, Ms. Menaka, Ms. Janet, Ms. Nurul Alyaa Alwi, Ms. Maysoun,

Mr. Wei Keat and Ms. Carmen for their ever ready help and support. I would also like to thank all Faculty Office Staff members especially Ms. Sabariah, Ms. Zabidah, Ms. Salma, Ms. Radha, Ms. Ovivi and Ms. Carol for their assistance and help during my PhD programme.

I am certainly grateful to Professor Matthew Dickinson, Department of Plant and Crop Science, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, UK for approving and supporting my research internship on molecular biosciences at main campus in United Kingdom. Moreover, my appreciation and sincere gratitude to Ms. Christina, Assistant Professor, School of Biosciences, Faculty of Science for her valuable guidance and assistance during my research particularly on molecular work. My special appreciation to Dr. Rachael Symonds, Associate Professor, School of Biosciences, Faculty of Science for her helpfulness in qPCR data analysis and timely guidance related to genetic work.

The most profound thanks go to MedKlinn International Sdn. Bhd. and Ministry of Higher Education, Government of Malaysia (MOHE), represented by The University of Nottingham Malaysia Campus, for providing me the Research Assistantship (RA), MyBrain scholarship and financial support under the project grant (MOO.51.54.01) and (M0007.54.02), respectively.

The completion of this research work would not be possible without the love, care, support, sacrifices and faith I receive from my dearest parents, my loving husband, my caring mother in law and my daughters, Annrose and

Brendaly. My deepest and heartfelt thanks and gratitude to each and everyone of you for being there whenever I needed your support. Lastly but not the least, to all my dedicated teachers, friends, family members, relatives and to those unnamed, I would like to present this thesis as testimony of each and everyone's unique loving kindness, endless support and contribution directly and indirectly.

SUPERVISORY COMMITTEE

The thesis submitted to the School of Biosciences, Faculty of Science, The University of Nottingham Malaysia Campus has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Asgar Ali, PhD

Associate Professor

School of Biosciences

Faculty of Science

The University of Nottingham Malaysia Campus

(Principal Supervisor)

Feroz Kabir Kazi, PhD

Associate Professor

Department of Chemical and Environmental Engineering

Faculty of Engineering

The University of Nottingham Malaysia Campus

(Co-supervisor)

DECLARATION

I hereby declare that the thesis is based on my original work except for the quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at the University of Nottingham Malaysia Campus or other institutions.

_____

ONG MEI KYING

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND NOTATIONS

ANOVA	Analysis of Variance
°C	degree centigrade
DMRT	Duncan's Multiple Range Test
h	hour (s)
HPO_3	metaphosphoric acid
NaOH	sodium hydroxide
H_2O_2	hydrogen peroxide
DCPIP	2,6-dichlorophenol-indophenol
l	litre
V	volt
M	Molar
mM	miliMolar
µg	microgram
min	minute
sec	second
ml	millilitre
µl	microlitre
cm	centimetre
mm	millimetre
nm	nanometre
µm	micrometre
µM	micromolar (10^{-6} M)
%	percentage

mt	metric ton
ft	feet
N	Newtons
O_3	ozone
C_2H_4	ethylene
CO_2	carbon dioxide
cv.	cultivar
ppm	part per million
ppb	part per billion
SAS	Statistical Analysis System
UPM	Universiti Putra Malaysia
UV	ultraviolet
UV/Vis	ultraviolet / visible
v/v	volume per volume
w/v	weight per volume
RH	relative humidity
FRAP	ferric reducing antioxidant power
DPPH	2,2-diphenyl-1-picrylhydrazyl
TPTZ	2,4,6-tripyridyl-s-triazine
PVPP	polyvinyl polypyrrolidone
GAE	gallic acid equivalent
APHA	American Public Health Association
EPA	Environmental Protection Agency
PCR	polymerase chain reaction

RT-PCR	reverse transcriptase PCR
qPCR	real time quantitative PCR
bp	base pair
L*	Lightness
h°	hue angle
C*	Chroma
SSC	soluble solids concentration
GRAS	generally recognized as safe
CA	controlled atmosphere
EDTA	Ethylene Diamine Tetra Acetic acid
SEM	scanning electron microscopy
TEM	transmission electron microscopy
cfu	colony forming unit
Ct	threshold cycle
ABA	abscisic acid
GC	gas chromatography
TE	Trolox equivalent
TCD	thermal conductivity detector
FID	flame ionization detector
AFLP	amplified fragment length polymorphism
PAL	phenylalanine ammonia lyase
POD	peroxidase
PPO	polyphenol oxidase
PDA	potato dextrose agar

ROI	reactive oxygen intermediates
ROS	reactive oxygen species
RAPD	random amplified polymorphic DNA
SSR	simple sequence repeat
RFLP	restriction fragment length polymorphism