UNIVERSITI PUTRA MALAYSIA

ULTRAVIOLET DISINFECTION FOR CONTROLLING CROWN ROT DISEASE AND RETAINING QUALITY OF MUSA AAA BERANGAN FRUIT

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By

NURATIKA TAMIMI BINTI SHEIKH MOHAMED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

ULTRAVIOLET DISINFECTION FOR CONTROLLING CROWN ROT DISEASE AND RETAINING QUALITY OF *MUSA* AAA *BERANGAN* FRUIT

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April 2017

Chairman : Associate Professor Phebe Ding, PhD
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Crown rot, caused by pathogen complexes, is one of the main diseases affecting banana fruit. A study was conducted to evaluate the potential of ultraviolet C (UVC), an environmentally friendly technology, to control crown rot disease and the impacts on postharvest quality of banana fruit during ripening. UVC irradiation is known for its efficient germicidal effect and potentially to be used as an alternative strategy to replace fungicide. Three pathogenic fungi identified as *Lasiodiplodia theobromae*, *Colletotrichum musae* and *Fusarium equiseti* were isolated from decaying crown surfaces of *Musa* AAA 'Berangan' banana, a Malaysian local cultivar. These three isolates were confirmed their species level based on cultural, morphological and nucleotide sequences of internal transcribed spacer region (ITS). Pathogenicity tests showed that inoculation with *L. theobromae* and combination of *C. musae* + *F. equiseti* + *L. theobromae* gave the maximum disease expression as compared to the individual isolates of *C. musae* and *F. equiseti*. The results of an increase eight steps UVC doses from 0.01 to 0.30 kJ m^{-2} show random increases and decreases in fungal growth of all fungi species tested. In percentage inhibition radial growth, 0.3 kJ m^{-2} was the best for *F. equiseti*, both 0.2 and 0.3 kJ m^{-2} doses were the best for *L. theobromae*, while 0.01 and 0.60 kJ m^{-2} effectively inhibited the *C. musae* radial growth. Meanwhile, 0.015 kJ m^{-2} UVC was effective in restraining the conidial germination and sporulation of these fungi. The effect of UVC on area under disease progress curve (AUDPC) indicated that the highest AUDPC of 77.5 $unit^2$ was recorded in negative control fruit, whereas, fruit treated with 0.30 kJ m^{-2} showed the lowest AUDPC value of 38.1 $unit^2$. However, the application of UVC irradiation dose on banana fruit was found limited due to the adverse effect of higher doses that causing browning discoloration on fruit peel. All the applied doses including 0.02 to 0.05 kJ m^{-2} showed browning scores that corresponding to 40 to 60% peel browning, whereas, fruit treated with 0.01 kJ m^{-2} UVC showed a much lower of browning score by the end of the ripening period. UVC irradiation at 0.01 kJ m^{-2} could be considered as the optimum dose for Berangan banana, as it synergistically reduced the disease severity by 46.25% without causing browning on the fruit peel. In contrast, UVC irradiation at 0.05 kJ m^{-2} was found

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caused peel browning and induced rapid changes in colour, soluble solids concentration and pH of the fruit. The severity of crown rot disease was lower in banana hands irradiated with UVC at 24 h after artificial inoculation compared to those irradiated 24 h before inoculation. Application of low dose UVC irradiation was able to elicit some desirable responses in banana such as induction of peroxidases, accumulation of lignins, formation of cell wall apposition and phenol storing cells to improve their defense against fungi. This study suggested that UVC irradiation at low dose is promising and environmentally friendly to be used as the alternative method for fungicides treatment of banana at postharvest.

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DISINFEKSI ULTRAUNGU UNTUK MENGAWAL PENYAKIT REPUT UMBUT DAN MENGEKALKAN KUALITI *MUSA* AAA *BERANGAN*

Oleh

NURATIKA TAMIMI BINTI SHEIKH MOHAMED

April 2017

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Reput umbut, berpunca daripada patogen kompleks merupakan salah satu penyakit utama buah pisang. Satu kajian telah dijalankan untuk menilai potensi sinaran ultraungu C (UVC), satu teknologi mesra alam dalam mengawal penyakit reput umbut dan kesannya terhadap kualiti lepas tuai pisang semasa fasa peranuman. Umumnya, UVC telah diketahui mempunyai kesan germisid yang efisien dan berpotensi untuk digunakan sebagai strategi alternatif kepada racun kulat. Tiga kulat patogenik dikenalpasti sebagai *Lasiodiplodia theobromae*, *Colletotrichum musae* dan *Fusarium equiseti* telah dipencilkan daripada umbut yang mereput pada *Musa* AAA 'Berangan', iaitu kultivar pisang tempatan di Malaysia. Tiga pencilan ini telah disahkan spesisnya melalui pencirian kultural, morfologi dan jujukan nukleotida pada kawasan 'internal transcribed spacer' (ITS). Ujian kepatogenan menunjukkan bahawa *L. theobromae* dan gabungan kulat *C. musae* + *F. equiseti* + *L. theobromae* memberikan ekspresi penyakit yang maksimum pada pisang berbanding pencilan *C. musae* and *F. equiseti*. Keputusan menunjukkan bahawa peningkatan dos UVC dari 0.01 kepada 0.30 kJ m^{-2} telah menyebabkan peningkatan dan pengurangan secara rawak pada pertumbuhan miselia kulat-kulat yang diuji. Dos 0.3 kJ m^{-2} didapati paling berkesan dalam mengurangkan pertumbuhan miselia kulat *F. equiseti*, manakala dos-dos 0.2 dan 0.3 kJ m^{-2} adalah yang terbaik terhadap perencatan pertumbuhan kulat *L. theobromae*, dan 0.01 dan 0.6 kJ m^{-2} pula secara efektifnya dapat mengurangkan pertumbuhan radial kulat *C. musae*. Ujikaji menunjukkan bahawa dos 0.015 kJ m^{-2} secara ketaranya telah merencatkan percambahan konidia dan sporulasi setiap kulat. Kesan UVC pada kawasan keluk di bawah graf (AUDPC) mencatatkan nilai tertinggi (77.5 $unit^2$) pada buah kawalan negatif, manakala telah mencatatkan nilai terendah pada buah yang dirawat dengan dos 0.3 kJ m^{-2} (38.1 $unit^2$). Walaubagaimanapun, penggunaan dos UVC pada buah pisang didapati terhad hanya disebabkan oleh kesan dos-dos lebih tinggi yang menyebabkan pemerangan pada kulit buah. Semua dos dari 0.02 ke 0.05 kJ m^{-2} menunjukkan skor pemerangan sebanyak 40 hingga 60%, manakala buah yang dirawat dengan 0.01 kJ m^{-2} menunjukkan skor pemerangan yang lebih rendah pada akhir tempoh peranuman. Dos 0.01 kJ m^{-2} merupakan dos yang optimum pada pisang Berangan kerana ia berkesan

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untuk mengurangkan sebanyak 46.25% penyakit reput umbut tanpa menyebabkan pemerangan pada kulit pisang. Sebaliknya, radiasi UVC pada 0.05 kJ m^{-2} telah menyebabkan pemerangan yang ketara pada kulit pisang dan mempercepat perubahan warna buah, meningkatkan kepekatan pepejal terlarut dan menurunkan pH buah. Kadar keterukan penyakit reput umbut adalah rendah pada buah yang dirawat pada 24 jam selepas inokulasi *L. theobromae*, *C. musae* dan *F. equiseti* berbanding rawatan UVC dalam tempoh 24 jam sebelum inokulasi kulat. Tambahan lagi, rawatan UVC pada dos yang rendah mampu merangsang tindak balas yang baik di dalam buah seperti meningkatkan enzim perksidase, pengumpulan lignin, pembentukan aposisi pada dinding sel dan sel penyimpanan fenol bagi memperkuat sistem pertahanan buah pisang daripada serangan kulat. Secara keseluruhannya, dapatan kajian ini mencadangkan bahawa penggunaan sinar radiasi UVC pada dos yang rendah adalah berpotensi tinggi dan mesra alam untuk digunakan sebagai pengganti racun kulat pada pisang semasa fasa lepas tuai.

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I certify that a Thesis Examination Committee has met on 26 April 2017 to conduct the final examination of Nuratika Tamimi bt Sheikh Mohamed on her thesis entitled "Ultraviolet Disinfection for Controlling Crown Rot Disease and Retaining Quality of *Musa* AAA *Berangan* Fruit" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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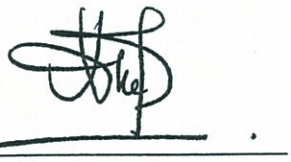

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TABLE OF CONTENTS

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LIST OF TABLES

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LIST OF FIGURES

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LIST OF ABBREVIATIONS

ABTS	2-2'-Azinobis-3-ethylbenzothiozoline-6-sulfonic acid
ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
bp	Base pair
BLAST	Basic local alignment search tool
C*	Chroma
CLA	Carnation leaf agar
cm	Centimeter
cm^2	Centimeter square
CMC	Na-carboxymethyl cellulose
CPD	Critical point drying
CRD	Completely randomized design
CWA	Cell wall apposition
cv.	Cultivar
DAR	Days after ripening initiation
DI	Disease incidence
DS	Disease severity
DMRT	Duncan's multiple range test
DNA	Deoxyribonucleic acid
DPPH	1,1-Diphenyl-2-picryl hydrazyl
EDTA	Ethylene-diaminetetraacetic acid disodium salt
ºC	Degree celsius
FAO	Food and Agriculture Organization

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FRAP	Ferric reducing antioxidant power
FW	Fresh weight
GC	Gas chromatography
GAE	Gallic acid equivalents
g	Gram
H_2O	Water
HCl	Hydrochloric acid
h	hour
h°	Hue angle
ITS	Internal transcribed spacer
kb	Kilobase
kg	Kilogram
kJ m^{-2}	Kilojoule per meter square
L*	Lightness
MEGA	Molecular Evolutionary Genetic Analysis
mg	Milligram
mm	Millimeter
µm	Micrometer
mL	Milliliter
µL	Microliter
mM	Millimolar
M	Molar
N	Newton
NCBI	National Center for Biotechnology Information
NJ	Neighbour-joining

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nm	Nanometer
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PIRG	Percent inhibition of radial growth
%	Percent
rpm	Rotation per minute
rDNA	Ribosomal deoxyribonucleic acid
RH	Relative humidity
S.E.	Standard error
SEM	Scanning electron microscope
SAS	Statistical Analysis System
spp.	Species
SSC	Soluble solids concentration
TA	Titratable acidity
TBE	Tris-borate/EDTA
TE	Trolox equivalent
TEM	Transmission electron microscope
TGA	Thioglycolic acid
Tris	Tris (hydroxymethyl) aminomethane
UV	Ultraviolet
UVC	Ultraviolet C
× *g*	Relative centrifugal force
v/v	Volume per volume
w/v	Weight per volume

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CHAPTER 1

INTRODUCTION

Banana is one of the oldest fruit acknowledged to mankind and popularly known as poor man's fruit and serve as a staple food to millions of people in tropics (Ruane et al., 2013). It is grown in more than 120 countries and ranked the fourth most important global food crop next to rice, wheat and maize (Picq et al., 1999) and ranked second in world fruit production after oranges and before grapes (Lassois et al., 2010). In Malaysia, banana is listed among the six Malaysian premium fruit for development under the Entry Point Project of agriculture, National Key Economic Area for fruit production (ETP Annual Report, 2014). *Musa* AAA 'Berangan' is one of the most famous varieties among local. It has deep yellow flesh colour and balance sweet acidic ratio, which make banana lovers unforgettable at the first bite. Banana fruit also provides significant proportion of antioxidants in human diet such as carotenoid pigments, ascorbic acids, flavonoids and phenolic acids (Sulaiman et al., 2011).

Banana fruit, however, are highly susceptible to crown rot disease especially during ripening, storage and shipping to their final market (Slabaugh and Grove, 1982). This consequently has led to banana quality depreciation and thereby negatively impact on market value of banana (Lassois et al., 2010). The disease is mainly caused by development of several nonspecific pathogens such as *Colletotrichum musae* (Berk. and Curt.), *Lasiodiplodia theobromae*, *Fusarium roseum* and *Ceratocystis paradoxa* (Dade) Moreau (Eckert and Ogawa, 1985). Many researchers agreed on the pathogenicity of *C. musae* which can trigger the crown rot infection at a very small inoculum level (Lassois et al., 2008).

In banana fruit industry, the routine of postharvest fungicide treatments such as benomyl, thiabendazole and imazalil are still the primary means for efficiently control the pathogenic fungi involved in crown rot disease since it has been introduced in the late 1960s (Lassois and de Lapeyre de Bellaire, 2014). The prolonged use of chemical fungicides had reduced their effectiveness against development of pathogen especially strains those are resistant to fungicide and as well as could cause accumulation of chemical residues in fresh produces (Cia et al., 2007). Due to most of people nowadays are highly concerned about their food safety and the demands are increasing for non-chemically treated fruit, therefore, an alternative to the chemical treatments is urgently required and have to be developed.

During the past few decades, postharvest technology of UVC germicidal irradiation has been proposed as surface disinfector on fresh fruit and vegetables (Civello et al., 2006). UVC utilized the shortest wavelength of ultraviolet light at 200 to 280 nm to effectively destroy the genetic information in microorganisms without forming significant toxic residues in the irradiated products. UVC irradiation has potential to be used as an alternative method to reduce the dependency on postharvest agrochemicals

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due to its germicidal properties and could promote resistance against pathogens (Bintsis et al., 2000). UVC light is also approved by US Food and Drug Administration (FDA) as a disinfectant technology for food surface treatment and permissible for its application in major fruit-producing countries (Guerrero-Beltran and Barbosa-Canovas, 2004).

The effect of using UVC treatment to control postharvest diseases have been investigated in several horticultural commodities and the results obtained have positioned this technology as promising way to reduce decay, maintain quality and extending the postharvest shelf life (Baka et al., 1999). Moreover, the non-ionizing and non-thermal irradiation able to induce beneficial responses in fresh produces which lead to natural disease resistance and enhancement of fruit storability (Chang-Hong et al., 2012). Many studies have demonstrated the ability of UVC irradiation in reducing postharvest diseases of fruit and vegetables, such as in orange (Gunduz et al., 2015; Canale et al., 2011), pepper (Rodoni et al., 2015), fresh-cut watermelon (Artes-Hernandez et al., 2010), 'Tatsoi' baby leaves (Tomas-Callejas et al., 2012), 'Haden' mango (Gonzalez-Aguilar et al., 2007), button mushroom (Guan et al., 2012), sweetpotato (Stevens et al., 1999), bell pepper (Mercier et al., 2001) and boysenberry fruit (Vicente et al., 2004).

Given these findings, the effectiveness of UVC irradiation has been proven in numerous horticultural commodities to control postharvest diseases. However, no study has been conducted on the effects of UVC on crown rot disease control, postharvest quality and changes of resistance mechanism in the cultivar of 'Berangan' banana during ripening. Therefore, the general objective of this study is to determine the potential of UVC irradiation on crown rot disease control, changes in postharvest physiology, biochemical and reinforcement of cell wall ultrastructure in Berangan banana fruit during ripening. The specific objectives were:

i. to confirm the fungus complex that are associated with crown rot disease of Berangan banana fruit using cultural, morphological, ribosomal DNA internal transcribed spacer (rDNA-ITS) and pathogenicity tests;
ii. to assess the inactivation properties of UVC irradiation on crown rot fungal growth;
iii. to find out the optimum dose of UVC in reducing crown rot disease on Berangan banana fruit and its effects on postharvest quality and antioxidant properties of fruit during ripening;
iv. to evaluate the effectiveness of differential UVC irradiation timing in suppressing crown rot-infected fungi; and
v. To determine the effect of UVC on resistance mechanisms (biochemical and ultrastructural modifications) in Berangan banana fruit against fungal pathogens.

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