UNIVERSITI PUTRA MALAYSIA

POSTHARVEST MANAGEMENT OF ANTHRACNOSE ON QUALITY OF PAPAYA (*CARICA PAPAYA* L.) USING ANTAGONISTIC BACTERIA

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By

MD. ATIQUR RAHMAN

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

To my affectionate parents, beloved wife Mahbuba and sons Mohammad Jubaer Rahman and Mohammad Jarif Rahman, who have encouraged me to the higher ideals of life.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Associate Professor Mahmud Tengku Muda Mohamed, PhD

Faculty : Agriculture

A study was conducted to evaluate the biocontrol potential of antagonistic bacteria to manage anthracnose disease and postharvest quality of papaya during storage. The fruits of papaya cv. 'Sekaki' were found to be highly susceptible to several postharvest fungal diseases. Among them, anthracnose caused by *Colletotrichum gloeosporioides* was the most prevalent, where disease incidence and severity was recorded as 90-98 and 25-38%, respectively. This fungus was isolated from naturally infected papaya fruits and confirmed as pathogenic to papaya fruits. Epiphytic bacteria, isolated from leaf and fruit surfaces of papaya were tested as biocontrol agent against *C. gloeosporioides*. From 27 antagonistic bacteria screened *in vitro* by dual and concomitant test, four isolates namely B23, B19, B04 and B15 had high antagonistic activities against the test fungus. Using the Biolog system, isolates B23 and B19 were identified as *Burkholderia cepacia*, and B04 and B15 as *Pseudomonas aeruginosa*. Both *B. cepacia* and *P. aeruginosa* strongly inhibited the fungal growth by an average of 74 and 68%, respectively. However, *B. cepacia* strain B23 was found to be the most efficacious biocontrol agent in this study, since both cell suspension and filter sterilized culture filtrate of this bacterium

completely suppressed the spore germination of the test fungus, which *P. aeruginosa* could not. These suggest that an antibiotic substance (s) may be produced by *B. cepacia* B23. Effect of different culture media on the production of antifungal substances by *B. cepacia* B23 was investigated to improve the efficacy of this biocontrol agent. The bacterium grew faster in nutrient broth medium and the cell concentration in this liquid medium reached the highest level (Log_{10} 15.7 CFU mL^{-1}) after 72 h of inoculation. Consequently, this bacterium produced more antifungal substances in nutrient broth than other tested media. Higher dilution (1:8) of the antifungal substances in crude supernatant from *B. cepacia* B23 was found to inhibit the mycelial growth and spore germination of *C. gloeosporioides* by 41 and 100%, respectively. Pyrrolnitrin and three other unidentified antifungal compounds were detected on TLC plates, which were resistant to boiling and autoclaving at 121 °C for at least 20 min. This bacterium was found to be highly compatible with chitosan (0.75%) and calcium chloride (3%) or mixture of both. Both of these chemicals have suppressive activity against *C. gloeosporioides* of papaya and could be used as enhancer of biocontrol efficacy of *B. cepacia* B23 during storage. The survival and proliferation of *B. cepacia* B23 in papaya wounds and on fruit surfaces was not affected by chitosan-$CaCl_2$ throughout the storage period. The combination of *B. cepacia* B23 with chitosan-$CaCl_2$ was more effective in controlling the disease than *B. cepacia* B23 alone or other treatments both in inoculated and naturally infected fruits. Combining *B. cepacia* B23 with chitosan-$CaCl_2$ gave the complete control of anthracnose in artificially inoculated fruits stored at 14 ºC and 95% RH for 18 days, which was equal to that obtained with fungicide benocide® (benomyl 50% WP). However, this combination offered a greater control by reducing 99% disease severity in naturally infected fruits at the end of 14 days storage at 14º C and 95% RH

plus six days post ripening at 28 ± 2 ºC, which was superior to that found with benocide® or other treatments tested. Furthermore, fruits treated with the combination of *B. cepacia* B23-chitosan-$CaCl_2$ showed delayed climacteric respiration and ethylene evolution by at least 7 days compared to control with reduced rate of CO_2 and C_2H_4 production. This combined treatment reduced weight loss by more than 25% compared to the control. It also markedly slowed down the ripening of fruits as shown by their retention of firmness 4.17 Newton (N) after storage. Moreover, a delayed change in external colour, titratable acidity and pH without compromising fruit quality was observed in fruits that were subjected to the combined treatment. The storage life was thus extended up to 15 days when compared with control. In addition, the incorporation of 3% $CaCl_2$ into the combined treatment significantly increased (81%) the calcium content in fruits compared to control, thus resulting in improved the nutritional value of the papaya. This study provided an alternative method for fungicides treatment of papaya at postharvest.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGURUSAN LEPAS TUAI ANTRAKNOS TERHADAP KUALITI BETIK (*CARICA PAPAYA* L.) MENGGUNAKAN BAKTERIA ANTAGONIS

Oleh

MD. ATIQUR RAHMAN

Pengerusi : Profesor Madya Tengku Muda Mohamed, PhD

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Kajian dijalankan bertujuan untuk menilai potensi kawalan biologi oleh bakteria antagonis bagi mengawal penyakit antraknos dan kualiti lepas tuai betik semasa penyimpanan. Buah betik kultivar 'Sekaki' didapati mudah dijangkiti oleh penyakit yang disebabkan oleh kulat selepas penuaian. Daripada kulat penyebab penyakit tersebut didapati penyakit antraknos yang disebabkan oleh *Colletotricum gloeosporioides* lazim berlaku, di mana peratusan jangkitan dan keterukan penyakit masing-masing ialah 90-98 dan 25-38%. Kulat diasingkan dari buah betik yang dijangkiti secara semulajadi dan disahkan sebagai patogen kepada buah betik. Bakteria antagonis, diasingkan daripada daun dan bahagian luaran buah betik diuji sebagai agen kawalan biologi terhadap *C. gloeosporioides*. Daripada 27 bakteria antagonis yang disaring secara 'dual' dan ujian 'concomitant', empat yang diasingkan iaitu B23, B19, B04 dan B15 menunjukkan aktiviti antagonis yang tinggi terhadap kulat. Dengan menggunakan sistem biolog, B23 dan B19 yang diasingkan telah dikenalpasti masing-masing sebagai *Burkhoderia cepacia* dan *Pseudomonas aeruginosa*. Kedua-dua *B. cepacia* dan *P. aeruginosa*

merencat pertumbuhan kulat dengan purata 74 dan 68% masing-masing. Walaubagaimanapun, *B. cepacia* B23 didapati paling berkesan sebagai agen kawalan biologi dalam kajian ini. Ini adalah kerana apungan dan kultur tapis nyahkuman ini menghalang secara lengkap percambahan spora kulat yang diuji. Ini tidak berlaku dengan *P. aeruginosa.* Ini menunjukkan bahan antikulat dihasilkan oleh *B. cepacia*. Kesan media kultur yang berbeza semasa pengeluaran bahan antikulat oleh *B. cepacia* B23 didapati dapat memperbaiki keberkesanan agen kawalan biologi ini. Bakteria berkembang dengan pesat dalam media pertumbuhan yang mengandungi nutrien dan kepekatan sel dalam media cecair ini mencapai tahap yang paling tinggi (Log_{10} 15.7 CFU mL^{-1}) selepas 72 jam inokulasi dijalankan. Hal ini menyebabkan bakteria ini menghasilkan lebih banyak bahan antikulat dalam media cecair berbanding media ujian yang lain. Pencairan tinggi (1:8) bahan antikulat dalam supernatant mentah *B. cepacia* B23 didapati dapat menghalang pertumbuhan miselia dan percambahan spora *C. gloeosporiodes* sebanyak 41 dan 100%. Pyrrolnitrin dan tiga antikulat yang tidak dikenalpasti dikesan pada piring TLC dengan rintangan terhadap pendidihan dan pemanasan pada suhu 121 °C selama 20 min. Bakteria ini didapati serasi dengan Chitosan (0.75%) dan Kalsium Klorida (3%) atau campuran kedua-duanya. Kedua-dua bahan kimia ini bertindak secara menindas terhadap *C. gloeosporiodes* pada buah betik dan boleh diguna sebagai peningkat keberkesanan kawalan biologi *B. cepacia* B23 semasa penyimpanan. Kemandirian *B. cepacia* B23 dalam dan pada permukaan buah betik tidak dipengaruhi oleh Chitosan-$CaCl_2$ sepanjang masa penyimpanan. Kombinasi *B. cepacia* B23 dengan Chitosan-$CaCl_2$ memberikan kawalan antraknos yang lengkap dalam buah inokulasi tiruan yang disimpan pada suhu 14 °C dan 95% kelembapan relatif untuk 18 hari, yang mana sama seperti yang didapati oleh racun kulat benocide®

(benomyl 50% WP). Walaubagaimanapun, kombinasi ini menawarkan kawalan yang lebih baik dengan mengurangkan 99% penyakit dalam buah betik yang dijangkiti secara semulajadi pada penghujung 14 hari penyimpanan pada 14 °C dan 95% kelembapan relatif ditambah enam hari pemasakan pada dalam 28 ± 2 °C, yang mana lebih baik daripada benocide® atau rawatan lain yang diuji. Tambahan pula, buah yang dirawat dengon kombinasi *B. cepacia* B23-chitosan-$CaCl_2$ menunjukkan kadar respirasi dan evolusi etilena yang rendah sehingga 7 hari berbanding kawalan dengan pengurangan kadar penghasilan CO_2 dan C_2H_4. Kombinasi rawatan ini mengurangkan kehilangan berat lebih daripada 25% berbanding kawalan. Ia juga melambatkan peranuman buah selaras dengan pengekalan kepejalan 4.17 N selepas disimpan. Selain itu, ia melambatkan perubahan warna luaran, asid tertitrat dan pH tanpa mengkompromi kualiti buah pada buah yang dirawat dengan kombinasi rawatan. Jangkahayat simpanan dapat dipanjangkan sehingga 15 hari apabila dibandingkan dengan kawalan. Tambahan lagi, gabungan 3% $CaCl_2$ ke dalam kombinasi rawatan meningkat secara signifikan (81%) terhadap kandungan kalsium berbanding rawatan, seterusnya meningkatkan nilai nutrisi dalam betik. Kajian ini menyediakan kaedah alternatif untuk rawatan kulat betik semasa lepas tuai.

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I would like to express my sincere gratitude and thanks to Associate Professor Dr. Mahmud Tengku Muda Mohamed, the chairman of my supervisory committee for his dedicated efforts, invaluable advices, ethical support and intellectual guidance in conducting my research and the preparation of this thesis. His immeasurable kindness, encouragement and patience are commendable.

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I certify that an Examination Committee has met on 09 May 2008 of viva voce to conduct the final examination of **Md. Atiqur Rahman** on his **Doctor of Philosophy** thesis entitled **"Postharvest management of anthracnose on quality of papaya (*Carica papaya* L.) using antagonistic bacteria"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 12 June 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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MD. ATIQUR RAHMAN

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
C*	Chroma
CA	Controlled Atmosphere
CFU/cfu	Colony Forming Unit
CF	Cystic Fibrosis
cm	Centimeter
cm^2	Square centimeter
CPD	Critical Point Drying
CRD	Completely Randomized Design
DI	Disease Incidence
DS	Disease Severity
DR	Disease Reduction
DNA	Deoxyribonucleic Acid
º C	Degree Celsius
EPA	United States Environmental Protection Agency
FAMA	Federal Agricultural Marketing Authority, Malaysia
FAO	Food and Agriculture Organization
FID	Flame Ionization Detector
GC	Gas Chromatography
GRAS	Generally Regarded as Safe
g	Gram
HCl	Hydrochloric Acid
HWT	Hot Water Treatment
h°	Hue angle
IAU	Inhibitory Activity Unit
kg	Kilogram
L*	Lightness
MAP	Modified Atmosphere Packaging
MARDI	Malaysian Agricultural Research and Development Institute
mg	Milligram
µg	Microgram
mm	Millimeter
µm	Micrometer
mL	Milliliter
µL	Microliter
mM	Millimolar
M	Molar
N	Newton
NAS	US National Academy of Science
NA	Nutrient Agar
NB	Nutrient Broth
nm	Nanometer

PDA	Potato Dextrose Agar
PIRG	Percent Inhibition of Radial Growth
%	Percent
rpm	Rotation Per minute
RH	Relative Humidity
Rf	Retention Factor
SEM	Scanning Electron Microscope
SAS	Statistical Analysis System
Spp	Species
SSC	Soluble Solids Concentration
TA	Titratable Acidity
TCD	Thermal Conductivity Detector
TLC	Thin Layer Chromatography
TPU	Taman Pertanian Universiti
UV	Ultra Violet
v/v	Volume per volume

CHAPTER 1

INTRODUCTION

Papaya (*Carica papaya* L.) is considered one of the most important fruit crops throughout the tropical and subtropical countries with high consumer demand worldwide. It is rapidly becoming an important fruit internationally, both as a fresh and processed products (Sankat and Maharaj, 1997). In Malaysia, papaya is a smallholders' crop and planting is widespread throughout the country. At present, Sekaki is considered as a leading cultivar of papaya for export as well as domestic consumption. In 2002, Malaysia occupied the first position for export papaya to Hong Kong and ranked second in the world among the papaya exporter countries after Mexico. According to the Federal Agriculture Marketing Authority of Malaysia, the export value of papaya was accounted for 28.5% of the total world exports in 2002 (FAMA, 2006). However, the major constraint that hinder the expansion of export for this fruit are short storage life, susceptibility to postharvest diseases, high shipment cost and residual from fungicides that is harmful to the consumers.

Postharvest fruit decay is a major constraint in food production causing decreases in both quantity and quality of produce. The greatest postharvest losses are certainly due to pathogenic microorganisms, which can infect fruit through wounds or latent infections during the pre-harvest period (Arras and Maltoni, 2004). Among the postharvest pathogens, fungal diseases are, in fact, one of the major causes of fruit decay as they account for 80-90% of all losses in postharvest industry and to the consumer (Gullino, 1995; Sommer, 1985).