



UNIVERSITI PUTRA MALAYSIA

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

MD. ATIQR RAHMAN

FP 2008 12

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

By

MD. ATIQR RAHMAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy
May 2008**



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

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

By

MD. ATIQUR RAHMAN

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 ($\text{Log}_{10} 15.7 \text{ CFU mL}^{-1}$) 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-CaCl₂ throughout the storage period. The combination of *B. cepacia* B23 with chitosan-CaCl₂ 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-CaCl₂ gave the complete control of anthracnose in artificially inoculated fruits stored at 14 °C 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 14° C and 95% RH



plus six days post ripening at 28 ± 2 °C, which was superior to that found with benocide[®] or other treatments tested. Furthermore, fruits treated with the combination of *B. cepacia* B23-chitosan-CaCl₂ showed delayed climacteric respiration and ethylene evolution by at least 7 days compared to control with reduced rate of CO₂ and C₂H₄ 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% CaCl₂ 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.



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

Fakulti : Pertanian

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 *Burkholderia 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. gloeosporioides* 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. gloeosporioides* 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-CaCl₂ sepanjang masa penyimpanan. Kombinasi *B. cepacia* B23 dengan Chitosan-CaCl₂ 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 dengan kombinasi *B. cepacia* B23-chitosan-CaCl₂ menunjukkan kadar respirasi dan evolusi etilena yang rendah sehingga 7 hari berbanding kawalan dengan pengurangan kadar penghasilan CO₂ dan C₂H₄. Kombinasi rawatan ini mengurangkan kehilangan berat lebih daripada 25% berbanding kawalan. Ia juga melambatkan peranakan buah selaras dengan pengejalan 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% CaCl₂ 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.



ACKNOWLEDGEMENTS

All praises and thanks are to Allah, the most beneficent and merciful. The author invokes Allah's blessings of peace for the Holy Prophet Mohammad (peace be onto him), the messenger of Allah, who advised us that education is to be imbibed from cradle to grave.

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.

Grateful and honest thanks are also extended to Associate Professor Dr. Jugah B Kadir and Professor Dr. Russly Abdul Rahman, the members of my supervisory committee for their constructive comments, support and help throughout my studies and in the preparation of this final manuscript.

My admiration and thanks are due to all staffs of postharvest, plant protection and microbiology laboratories for their willing assistance and help during my entire period of research.

I am exceedingly grateful to the Malaysian Government for giving me financial support through IRPA project, without which this study would have been well nigh impossible.



Finally, I also take this opportunity to express my honest gratitude to my wife Mahbuba Begum, sons Mohammad Jubaer Rahman and Mohammad Jarif Rahman. I thank them for their love, sacrifices and spiritual support which made life easy throughout my study at UPM and whole life.



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:

Yahya Awang, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Kamaruzaman Sijam, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Phebe Ding, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Liza Korsten, PhD

Professor
Department of Microbiology and Plant Pathology
Faculty of Natural and Agricultural Sciences
University of Pretoria
South Africa
(External Examiner)

HASANAH MOHD. GHAZALI, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



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:

Mahmud Tengku Muda Mohamed, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Jugah Bin Kadir, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Russly Abdul Rahman, PhD

Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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.

MD. ATIQR RAHMAN

Date:



TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xviii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	6
2.1 Papaya	6
2.1.1 Postharvest losses of papaya	7
2.1.2 Postharvest diseases of papaya	9
2.1.3 Postharvest disease control of papaya	12
2.2 Biological control of postharvest diseases of fruits	14
2.3 Mechanisms of biocontrol of postharvest diseases	17
2.3.1 Antibiosis	17
2.3.2 Competition for nutrients and space	19
2.4 Screening of antagonists	21
2.5 Biocontrol activity of <i>Burkholderia cepacia</i>	22
2.5.1 Biocontrol of postharvest diseases	23
2.5.2 <i>Burkholderia cepacia</i> as a bioremediation agent	24
2.5.3 Mechanisms of biocontrol of <i>B. cepacia</i>	25
2.5.4 Distinguishing between biocontrol and human pathogenic strains of <i>B. cepacia</i> complex	26
2.6 Enhancement of the efficacy of biocontrol agents	27
2.6.1 Addition of additives to enhance antagonist's efficacy	28
2.7 Potential of chitosan to control postharvest diseases and fruit quality	31
2.7.1 Effect of chitosan on <i>in vitro</i> fungal growth	31
2.7.2 Effect of chitosan on hyphal morphology	33
2.7.3 Effect of chitosan on postharvest fruit diseases	34
2.7.4 Effect of chitosan on the quality and storage life of fruit	34



3.	FUNGI ASSOCIATED WITH MAJOR POSTHARVEST DISEASES OF PAPAYA CV. SEKAKI	
3.1	Introduction	37
3.2	Materials and methods	38
3.2.1	Fruit materials	38
3.2.2	Isolation and identification of pathogenic fungi associated with postharvest diseases of papaya	39
3.2.3	Incidence and severity of postharvest diseases of papaya	39
3.2.4	Pathogenicity of <i>Colletotrichum gloeosporioides</i> on papaya fruits	40
3.3	Statistical analysis	41
3.4	Results	41
3.4.1	Isolation and identification of fungi	41
3.4.2	Disease incidence and severity	45
3.4.3	Pathogenicity test	46
3.5	Discussion	47
4.	SCREENING OF ANTAGONISTIC BACTERIA FOR BIOCONTROL ACTIVITY AGAINST <i>COLLETOTRICHUM GLOEOSPORIOIDES</i> IN PAPAYA	
4.1	Introduction	51
4.2	Materials and methods	52
4.2.1	Isolation of epiphytic bacteria from fruit surface of papaya	52
4.2.2	Preparation of conidial suspension of <i>C. gloeosporioides</i> .	53
4.2.3	Screening, selection and identification of antagonistic bacteria	53
4.2.4	Preparation of aqueous antagonist suspension	54
4.2.5	Preparation of filter sterilized culture filtrate	55
4.2.6	Antagonism	55
4.3	Experimental design and statistical analysis	60
4.4	Results	60
4.4.1	Isolation of epiphytic bacteria from fruit surface of papaya	60
4.4.2	Screening, selection and identification of antagonistic bacteria	61
4.4.3	Production of diffusible and volatile antifungal substances	63
4.4.4	Production of antibiotic substances	64
4.4.5	Antagonistic effect on mycelial growth and spore germination of <i>C. gloeosporioides</i>	65
4.4.6	Study on hyphal morphology	67
4.5	Discussion	68
5.	EXTRACTION OF ANTIFUNGAL SUBSTANCES FROM <i>BURKHOLDERIA CEPACIA</i> WITH ANTIBIOTIC ACTIVITY AGAINST <i>COLLETOTRICHUM GLOEOSPORIOIDES</i> ON PAPAYA	



5.1	Introduction	72
5.2	Materials and methods	73
5.2.1	Culture of <i>Colletotrichum gloeosporioides</i> and conidial suspension preparation	73
5.2.2	Antagonistic bacterial isolate	74
5.2.3	Selection of liquid culture media for the growth of <i>B. cepacia</i> and accumulation of antifungal substances	74
5.2.4	Determination of Pyrrolnitrin in the crude supernatant on Thin Layer Chromatography (TLC)	75
5.2.5	Determination of inhibitory activity units (IAU) of the antifungal substances in the crude supernatant	76
5.2.6	Thermostability of the antifungal substances in the crude supernatant from <i>B. cepacia</i>	77
5.3	Experimental design	77
5.4	Statistical analysis	77
5.5	Results	78
5.5.1	Effect of liquid culture media on the growth of <i>B. cepacia</i> and accumulation of antifungal substances	78
5.5.2	Determination of pyrrolnitrin in the crude supernatant	80
5.5.3	Determination of inhibitory activity units (IAU) of the antifungal substances in the crude supernatant	81
5.5.4	Thermostability of the antifungal substances in the crude supernatant from <i>B. cepacia</i>	82
5.6	Discussion	83
6.	ENHANCING THE EFFICACY OF <i>BURKHOLDERIA CEPACIA</i> WITH CHITOSAN AND CALCIUM CHLORIDE TO CONTROL ANTHRACNOSE OF PAPAYA DURING STORAGE	
6.1	Introduction	89
6.2	Materials and methods	92
6.2.1	Fungal culture and preparation of conidial suspension of <i>C. gloeosporioides</i>	92
6.2.2	Preparation of aqueous suspension of <i>B. cepacia</i>	92
6.2.3	Preparation of chitosan solutions	93
6.2.4	Compatibility test of <i>B. cepacia</i> with sodium carbonate, sodium bicarbonate, calcium chloride and chitosan	93
6.2.5	Effect of chitosan and calcium chloride on <i>in vitro</i> growth and spore germination of <i>C. gloeosporioides</i>	94
6.2.6	Effect of chitosan and calcium chloride on fruit colonization by <i>B. cepacia</i> B23	95
6.2.7	Biocontrol activity of <i>B. cepacia</i> B23 enhanced with chitosan and calcium chloride on papaya fruits pre-inoculated with <i>C. gloeosporioides</i>	96
6.2.8	Biocontrol activity on naturally infected fruits	98

6.3	Statistical analysis	99
6.4	Results	100
6.4.1	Compatibility of <i>B. cepacia</i> B23 with different additives	100
6.4.2	Effect of chitosan on <i>in vitro</i> growth, spore germination and hyphal morphology of <i>C. gloeosporioides</i>	101
6.4.3	Effect of calcium chloride on <i>in vitro</i> growth and spore germination of <i>C. gloeosporioides</i>	105
6.4.4	Effect of chitosan and CaCl ₂ on fruit colonization by <i>B. cepacia</i> B23	108
6.4.5	Biocontrol activity of <i>B. cepacia</i> B23 enhanced with chitosan and calcium chloride on papaya fruits pre-inoculated with <i>C. gloeosporioides</i>	109
6.4.6	Biocontrol activity on naturally infected fruits	114
6.5	Discussion	120
6.5.1	Effect of chitosan and calcium chloride on <i>in vitro</i> growth and hyphal morphology of <i>C. gloeosporioides</i>	120
6.5.2	Biocontrol activity of <i>B. cepacia</i> B23 enhanced with chitosan and calcium chloride on inoculated and naturally infected fruits	125
7.	EVALUATION OF POSTHARVEST APPLICATION OF <i>BURKHOLDERIA CEPACIA</i> IN COMBINATION WITH CALCIUM-INCORPORATED CHITOSAN COATING ON THE STORABILITY AND QUALITY OF PAPAYA	
7.1	Introduction	135
7.2	Material and methods	137
7.2.1	Fruits and treatments	137
7.2.2	Determination of respiration rate and ethylene production in papaya during storage	138
7.2.3	Determination of physical characteristics	139
7.2.4	Determination of chemical characteristics	140
7.3	SEM observation of papaya fruit pericarp	144
7.4	Experimental Design and Statistical analysis	145
7.5	Results	145
7.5.1	Respiration rate and ethylene production	145
7.5.2	Changes in physical characteristics	148
7.5.3	Changes in chemical characteristics	153
7.5.4	SEM observation of papaya fruit pericarp	159
7.6	Discussion	161
8.	SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	178
	REFERENCES	181
	BIODATA OF THE STUDENT	215
	LIST OF PUBLICATIONS	216



LIST OF TABLES

Table		Page
3.1	List of fungal pathogens associated with major postharvest diseases of papaya.	42
4.1	Screening of antagonistic bacteria against <i>Colletotrichum gloeosporioides</i> in papaya.	62
4.2.	Growth inhibition of <i>C. gloeosporioides</i> by diffusible and volatile antifungal substances produced by antagonistic bacteria at seven days after incubation at 28 ± 2 °C.	64
4.3	Antagonistic effect of <i>B. cepacia</i> and <i>P. aeruginosa</i> on the radial growth and spore germination of <i>C. gloeosporioides</i> on PDA after seven days and seven hours of incubation, respectively at 28 ± 2 °C.	66
5.1	Inhibition of radial growth and spore germination of <i>C. gloeosporioides</i> by different concentration of crude supernatant after seven days and seven hours of incubation, respectively at 28 ± 2 °C.	81
5.2	Effect of the different thermal treatments on the antifungal activity of crude supernatant from <i>B. cepacia</i> against the radial growth of <i>C. gloeosporioides</i> after seven days of incubation at 28 ± 2 °C.	82
6.1.	Compatibility of <i>B. cepacia</i> with different additives.	101
6.2.	Effect of chitosan, calcium chloride and their combination on the populations of <i>B. cepacia</i> B23 in papaya wounds and on surface during storage at 14 °C and 95% RH for 18 days	108
6.3	Effect of <i>B. cepacia</i> , chitosan, calcium chloride and combinations on the reduction of anthracnose disease in pre-inoculated papaya fruits stored at 14 °C for 18 days	112
6.4	Effect of <i>B. cepacia</i> , chitosan, calcium chloride or their combinations on the reduction of anthracnose disease in naturally infected papaya fruits stored at 14 °C for 14 days and six days post ripening under ambient temperature (28 ± 2 °C).	118
7.1	Calcium content in papaya fruit treated with calcium chloride or chitosan solution containing calcium chloride.	159



LIST OF FIGURES

Figure		Page
3.1	Cultural and morphological characteristics of <i>C. gloeosporioides</i> , the causal agent of anthracnose in papaya.	43
3.2	Incidence of major postharvest diseases of papaya.	45
3.3	Severity of major postharvest diseases of papaya.	46
3.4	Pathogenicity of <i>C. gloeosporioides</i> on wounded and unwounded papaya fruits.	50
4.1	Dual culture assay on the inhibition of mycelial growth of <i>C. gloeosporioides</i> by <i>B. cepacia</i> and <i>P. aeruginosa</i> after seven days of incubation at 28 ± 2 °C.	61
4.2	Growth of <i>B. cepacia</i> and <i>P. aeruginosa</i> on NA medium after 24 h of incubation at 28 ± 2 °C.	63
4.3	Mycelial growth inhibition by diffusible antifungal substance (s) produced by <i>B. cepacia</i> and <i>P. aeruginosa</i> on PDA after seven days of incubation at 28 ± 2 °C.	64
4.4	Bioassay test for the production of antibiotic substances by <i>B. cepacia</i> and <i>P. aeruginosa</i> against <i>C. gloeosporioides</i> on PDA after 24h of incubation at 28 ± 2 °C.	65
4.5	Effect of cell suspension and filter sterilized culture filtrate of antagonistic bacteria on the radial growth of <i>C. gloeosporioide</i> on PDA after seven and four days of incubation, respectively at 28 ± 2 °C.	66
4.6	Effect of <i>B. cepacia</i> and <i>P. aeruginosa</i> on spore germination of <i>C. gloeosporioide</i> on PDA after seven hours of incubation at 28 ± 2 °C.	67
4.7	Hyphal morphology of <i>C. gloeosporioides</i> as affected by <i>B. cepacia</i> and <i>P. aeruginosa</i> .	68
5.1	Growth curve of <i>B. cepacia</i> in different liquid culture media.	78
5.2	Inhibition of radial growth of <i>C. gloeosporioides</i> by antifungal substances produced by <i>B. cepacia</i> in different liquid culture media after seven days of incubation.	79



5.3	Growth inhibition of <i>C. gloeosporioides</i> by antifungal substances of <i>B. cepacia</i> produced in different liquid culture media taken after seven days of incubation.	80
5.4	Effect of different thermal treatments on the antifungal activity of crude supernatant from <i>B. cepacia</i> against the radial growth of <i>C. gloeosporioides</i> after seven days of incubation at 28 ± 2 °C.	82
6.1	Effect of different concentrations of chitosan on the radial growth of <i>C. gloeosporioides</i> after seven days of incubation at 28 ± 2 °C.	101
6.2	Effect of different concentrations of chitosan on the radial growth of <i>C. gloeosporioides</i> after seven days of incubation at 28 ± 2 °C.	102
6.3	Effect of different concentrations of chitosan on spore germination of <i>C. gloeosporioides</i> after 7, 9, 11, and 13 hours of inoculation at 28 ± 2 °C.	103
6.4	Effect of different concentrations of chitosan on spore germination of <i>C. gloeosporioides</i> after seven hours of incubation at 28 ± 2 °C.	104
6.5	Effect of chitosan (0.25 to 1%) on hyphal morphology of <i>C. gloeosporioides</i> .	105
6.6	Effect of different concentrations of calcium chloride on the radial growth of <i>C. gloeosporioides</i> after seven days of incubation at 28 ± 2 °C.	106
6.7	Effect of different concentrations of calcium chloride on the conidial germination of <i>C. gloeosporioides</i> after seven hours of incubation at 28 ± 2 °C.	107
6.8	Effect of different concentrations of calcium chloride on the radial growth and spore germination of <i>C. gloeosporioides</i> after six days and seven hours of incubation, respectively at 28 ± 2 °C.	107
6.9	Effect of <i>B. cepacia</i> , chitosan and calcium chloride and combinations on lesion diameter of anthracnose caused by <i>C. gloeosporioides</i> in pre-inoculated papaya fruits stored at 14 °C and 95% RH for 18 days.	110
6.10	Effect of <i>B. cepacia</i> , chitosan and calcium chloride and combinations on the lesion diameter of anthracnose caused by <i>C. gloeosporioides</i> in pre-inoculated papaya fruits stored at 14 °C and 95% RH for 18 days.	113



6.11	Effect of <i>B. cepacia</i> , chitosan and calcium chloride and combinations on the incidence of anthracnose in naturally infected papaya fruits stored at 14 °C for 14 days and six days post ripening under ambient temperature (28 ± 2 °C).	115
6.12	Effect of <i>B. cepacia</i> , chitosan, calcium chloride and combinations on the severity of anthracnose in naturally infected papaya fruits stored at 14 °C for 14 days and six days post ripening under ambient temperature (28 ± 2 °C).	116
6.13	Effect of <i>B. cepacia</i> , chitosan, calcium chloride and combinations on the incidence and severity of anthracnose in naturally infected papaya fruits stored at 14 °C and 95% RH for 14 days and six days under ambient temperature (28 ± 2 °C).	119
7.1	Effect of different treatments on the respiration rate of papaya fruits during storage at 14 °C and 95% RH for 28 days.	146
7.2	Effect of different treatments on the ethylene production from papaya fruits during storage at 14 °C and 95% RH for 28 days.	147
7.3	Effect of different treatments on the weight loss of papaya fruits during storage at 14 °C and 95% RH for 28 days.	149
7.4	Effect of different treatments on the flesh firmness of papaya fruits during storage at 14° C and 95% RH for 28 days.	150
7.5	Effect of different treatments the peel colour of papaya fruits during storage at 14 °C and 95% RH for 28 days.	152
7.6.	Effect of different storage periods on the titratable acidity of papaya fruits during storage at 14° C and 95% RH for 28 days.	153
7.7	Effect of different treatments on the titratable acidity of papaya fruits during storage at 14 °C and 95% RH for 28 days.	154
7.8	Effect of different treatments on the pH of papaya fruits during storage at 14 °C and 95% RH for 28 days.	155
7.9	Effect of different treatments on soluble solids concentration of papaya fruits during storage at 14 °C and 95% RH for 28 days.	156
7.10	Effect of different treatments on ascorbic acid content of papaya fruits during storage at 14 °C and 95% RH for 28 days.	158
7.11	Scanning Electronic Microscope observation of the fruit pericarp of papaya coated with chitosan solution amended with CaCl ₂ .	160



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
° 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	Titrateable 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).

