UNIVERSITI PUTRA MALAYSIA

ANTAGONIST YEAST FOR BIOCONTROL OF POSTHARVEST ANTHRACNOSE DISEASE ON PAPAYA (Carica papaya L.)

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By

HAMIZAH BINTI HASSAN

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

July 2017

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DEDICATION

To my parents, siblings, supervisors, friends and husband.
Thank you.

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

ANTAGONIST YEAST FOR BIOCONTROL OF POSTHARVEST ANTHRACNOSE DISEASE ON PAPAYA (*Carica papaya* L.)

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July 2017

Chairman : Mahmud Tengku Muda Mohamed, PhD
Faculty : Agriculture

Papaya (*Carica papaya* L.) contain high nutritive value and very susceptible to postharvest decay. Major postharvest disease related to papaya fruit is anthracnose, caused by *Colletotrichum gloeosporioides.* Postharvest fungicide treatments are very popular for the disease control due to it fast and broad spectrum effects. However, withdrawal of a few commercial fungicides due to its negative effects on human, environment and produce increased public concerns and demands to other safer alternatives of control measures. Among all, biological control using microorganism to fight against host pathogens provides a positive results. However, limited studies done in Malaysia regarding the use of yeast as biocontrol agent for postharvest disease. Thus, this study was carried out with aim to develop a locally isolated, yeast antagonist agent for controlling anthracnose disease on papaya. In the first experiment, isolation of natural yeast on papaya plant was carried out. A total of 110 yeasts were isolated and tested for their antagonist activity against *C. gloeosporioides.* After a series of initial screening tests for antagonism, two yeasts, namely F001 and FL013 showed higher antagonistic activity against the pathogen were selected. Both F001 and FL013 showed better antagonist activity when concentration of yeast cells were increased from 5 x 10^4 to 5 x 10^8 cells mL^{-1} in fungal spore germination test. Both yeasts also showed their ability to work well in cold storage condition and significantly inhibited the mycelial growth up to 70% using FL013 dipping treatment. However, only F001 has the ability to produce diffusible antifungal compounds which caused total suppression (100%) on the fungal growth. Thus, by referring to previous results, only F001 was identified and used in next experiments. Yeast F001, identified as *Trichosporon asahii*, also exhibited direct attachment on pathogen to control fungal hyphae growth and able to colonize well on papaya peel. In the next experiment, suitable additives for combining with *T. asahii* to improve antagonist activity of the yeast was investigated. Results showed that combination of *T. asahii* with calcium chloride able to totally suppress the pathogen growth in *in vitro* test. For *in vivo* test, combination of *T. asahii* with calcium chloride at 2.5% (w/v) caused reduction in

disease severity until 83% as compare to control fruit. This treatment combination was chosen and used in the subsequent studies. Next, effect of utilizing this treatment combination on disease incidence, disease severity, total phenolic, total flavonoid and antioxidant activity were evaluated. Results showed that *T. asahii* with 2.5% calcium chloride coating on 'Solo' papaya was capable to reduce disease severity but not able to significantly increase the activity of those phytochemicals. The treatment also showed that no interaction effect with storage day on defense-related enzyme, including polyphenol oxidase, phenylalanine ammonia lyase and catalase activity. However, treatment application on the fruit did not influence the quality and storability of papaya after stored at 12 ^{o}C for 28 days.

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

YIS ANTAGONIS SEBAGAI KAWALAN BIOLOGI BAGI PENYAKIT ANTRAKNOS LEPAS TUAI TERHADAP BETIK (*Carica papaya* L.)

Oleh

HAMIZAH BINTI HASSAN

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Betik (*Carica papaya* L.) mengandungi nilai pemakanan yang tinggi dan sangat mudah terdedah kepada kerosakan lepas tuai. Penyakit lepas tuai utama yang berkaitan dengan buah betik adalah antraknos yang disebabkan oleh *Colletotrichum gloeosporioides*. Rawatan racun kulat lepas tuai adalah sangat popular untuk kawalan penyakit ini kerana ia memberi kesan yang lebih cepat dan meluas. Walau bagaimanapun, pengharaman beberapa racun kulat komersial disebabkan oleh kesan negatif kepada manusia, alam sekitar dan hasil tanaman telah meningkatkan kebimbangan orang ramai dan permintaan kepada alternatif lain yang lebih selamat sebagai langkah-langkah kawalan. Antara alternatifnya adalah, kawalan biologi menggunakan mikroorganisma lain untuk melawan patogen hasil tanaman turut memberikan hasil yang positif. Walau bagaimanapun, kajian yang dilakukan di Malaysia mengenai penggunaan yis sebagai agen kawalan biologi bagi penyakit lepas tuai masih terhad. Oleh itu, kajian ini dijalankan dengan tujuan untuk mengenalpasti yis yang diperoleh secara lokal, sebagai agen antagonis untuk mengawal penyakit antraknos pada buah betik. Dalam kajian pertama, pemencilan yis yang terdapat secara semula jadi pada tumbuhan betik telah dijalankan. Sebanyak 110 yis telah diperolehi dan diuji untuk aktiviti antagonis terhadap *C. gloeosporioides*. Selepas beberapa siri ujian saringan awal, dua yis, iaitu F001 dan FL013 yang menunjukkan aktiviti antagonis yang lebih berkesan ke atas patogen telah dipilih. Kedua-dua yis, F001 dan FL013 menunjukkan aktiviti antagonis yang lebih baik apabila kepekatan sel yis ditingkatkan dari 5×10^4 kepada 5×10^8 sel mL^{-1} dalam ujian percambahan spora fungi. Kedua-dua yis turut mempamerkan keupayaan untuk bertindak dengan lebih baik dalam suhu dingin dan secara signifikannya menghalang diameter miselium sehingga 70% menggunakan rawatan celupan FL013. Namun, hanya F001 yang berupaya menghasilkan kompoun antikulat yang boleh meresap seterusnya menyebabkan penindasan secara total (100%) ke atas pertumbuhan patogen. Oleh itu, merujuk keputusan sebelumnya, hanya F001 dikenal pasti dan digunakan dalam eksperimen yang seterusnya. Yis F001, dikenal pasti sebagai *Trichosporon asahii*, juga menunjukkan pelekatan secara

langsung kepada patogen untuk mengawal pertumbuhan kulat hifa dan berupaya menghuni kulit buah betik dengan baik. Dalam eksperimen seterusnya, kajian telah dijalankan bagi mencari bahan tambahan yang sesuai untuk digabung bersama *T. asahii* bagi meningkatkan aktiviti antagonis yis. Hasil kajian menunjukkan bahawa kombinasi *T. asahii* dengan kalsium klorida dapat menghalang pertumbuhan patogen sepenuhnya (100%) di dalam ujian *in vitro*. Bagi ujian *in vivo*, gabungan *T. asahii* dengan 2.5% kalsium klorida (w/v) telah menyebabkan pengurangan tahap jangkitan sehingga 83% berbanding buah yang tidak dirawat. Kombinasi rawatan ini telah dipilih dan digunakan dalam kajian seterusnya. Seterusnya, kesan menggunakan gabungan rawatan terhadap jangkitan penyakit, tahap jangkitan, jumlah kandungan fenolik dan flavonoid serta aktiviti antioksidan telah dinilai. Hasil kajian menunjukkan bahawa salutan *T. asahii* dengan 2.5% kalsium klorida pada betik 'Solo' mampu untuk mengurangkan tahap jangkitan tetapi tidak mampu untuk menyebabkan peningkatan aktiviti fitokimia secara ketara. Rawatan ini juga menunjukkan tiada kesan interaksi dengan tempoh penyimpanan ke atas enzim berkaitan pertahanan, termasuk aktiviti polifenol oksidase, fenilalanina amonia liase dan katalase. Walau bagaimanapun, aplikasi rawatan tersebut pada buah tidak menjejaskan kualiti dan tempoh simpanan betik selepas disimpan pada 12 °C selama 28 hari.

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I certify that a Thesis Examination Committee has met o 4 July 2017 n to conduct the final examination of Hamizah binti Hassan on her thesis entitled "Antagonist Yeast for Biocontrol of Postharvest Anthracnose Disease on Papaya (*Carica papaya* L.)" 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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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 Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

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LIST OF ABBREVIATIONS

µL	Microliter
BEN	Benomyl 50% WP
BLAST	Basic local alignment search tool
C*	Chroma
CAT	Catalase
cells mL^{-1}	Cells/mililiter
CFU	Colony forming units
CM	Coating material
cm day^{-1}	Centimeter/ day
CRD	Completely randomized design
DMRT	Duncan's multiple range test
DNA	Deoxyribonucleic acid
DOA	Department of Agriculture
DPPH	2,2-diphenyl-1-picrylhydrazyl radical scavenging
DS	Disease severity
FAMA	Federal Agricultural Marketing Authority
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FRAP	Ferric reducing antioxidant power
g L^{-1}	Gram/liter
h^o	Hue
ITS	Internal transcribed spacer
L*	Lightness
LSD	Least significant difference
M	Moles per liter
MAFC	Malaysian AgriFood Corporation
MF	*Metschnikowia fructicola*
mL	Milliliter
mm	Milimeter
mM	Milimoles per liter
MOA	Ministry of Agriculture
NCBI	National Center for Biotechnology Information
NYDA	Nutrient yeast dextrose agar
NYDB	Nutrient yeast dextrose broth
PAL	Phenylalanine ammonia-lyase
PCR	Polymerase chain reaction
pH	Potential of hydrogen
PIRG	Percent inhibition of radial growth
PPO	Polyphenol oxidase
R^2	Coefficient detemination
rDNA	Ribosomal deoxyribonucleic acid
rpm	Revolutions per minute
SBC	Sodium bicarbonate
SD	Storage duration
SDW	Sterile distilled water
SEM	Scanning electron microscope
SE	Standard error

spores mL^{-1}	Spores/mililiter
SSC	Soluble solids concentration
TA	*Trichosporon asahii* combined with 2.5% calcium chloride
TFC	Total flavonoid content
TPC	Total phenolic content
w/v	Weight/volume
WP	Wettable powders

CHAPTER 1

INTRODUCTION

Papaya (*Carica papaya* L.) is an important income generator fruit in Malaysia. According to the Ministry of Agriculture (MOA), in 2001, papaya recorded the highest total export value for fruits with more than RM 90 million followed by watermelon which was RM 60 million (MOA, 2002). However, the export value exhibited a gradual decrease since papaya production decreased from 65,000 tonnes in 2001 to 32,000 tonnes in 2013 (FAOSTAT, 2015; MOA, 2015).

Anthracnose, caused by *Colletotrichum gloeosporioides*, is a common postharvest disease of papaya fruit observed in the market chain which limits exportation (Paull et al., 1997; Ong and Ali, 2015). The control of anthracnose has been accomplished almost exclusively by fungicide application. However, the withdrawal of a number of fungicides that previously used to manage postharvest diseases of fruit had increased public concerns on the potential harmful consequences of fungicide toxicity to the human health and environment. The shifting of global trend towards lessen use of fungicides on fresh produce, safer and eco-friendly alternatives for reducing the decay loss in the harvested commodities, such as using antagonistic microorganisms like yeasts, fungi and bacteria, seem more promising and gaining popularity (Droby, 2006; Korsten, 2006).

Considerable success has been achieved when utilizing antagonistic microorganisms to control postharvest diseases. According to Irtwange (2006) and Qing and Shiping (2000), among all antagonistic organisms, yeasts are efficient biological control agents. Yeasts own many positive natural properties that make them useful for control purposes. In addition, yeasts generally do not produce allergenic spores or mycotoxins as many mycelial fungi do or antibiotic metabolites likely to be produced by bacterial antagonists. Other than requiring only simple nutritional condition, yeasts are also able to colonize on dry surfaces for long periods of time and resistant to many pesticides used in the postharvest environment (El-Tarabily and Sivasithamparam, 2006). Furthermore, past studies suggested that modes of action of biocontrol yeasts indicated low possibility of any hazard to consumers (Chanchaichaovivat et al., 2007).

Generally, antagonistic yeasts alone are often less effective than many of the commercial fungicides currently in use (Janisiewicz and Korsten, 2002). Some substances or methods appear to be useful when in combination with biocontrol yeasts. These include chitosan (Yu et al., 2007), calcium (Janisiewicz and Korsten, 2002; Tian et al., 2002a; Cao et al., 2008), sodium bicarbonate (Droby et al., 2003b; Yao et al., 2004), commercial fruit waxes (Lahlali et al., 2014) and microwave (Zhang et al., 2006).The studies showed that an integrated disease control strategy provides more effective and stable alternatives to synthetic fungicides.

A considerable number of antagonistic microorganisms have been tested in several countries for controlling different pathogens in different fruit-pathogen interaction systems especially on temperate fruits. However, the introduction of new microorganisms from foreign sources or regions to the local host may contribute risk to the produce and ecosystem. Since very few studies have been published from Malaysia on the control of anthracnose diseases on papaya using antagonistic yeast, this study was done with the following objectives:

1. To isolate, screen and identify yeast from papaya plant for their ability to control *C. gloeosporioides*.
2. To investigate the effect of additives (sodium bicarbonate or calcium chloride) in improving the biocontrol efficacy of *T. asahii.*
3. To examine the outcome of *T. asahii* combined with calcium chloride on host defence mechanism of 'Solo' papaya.
4. To evaluate the effect of antagonistic yeast supplemented with calcium chloride on the storability and quality of postharvest papaya.

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