UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND CHARACTERIZATION OF FUNGAL SPECIES CAUSING ANTHRACNOSE DISEASE ON MANGO (*Mangifera indica* L.)

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IDENTIFICATION AND CHARACTERIZATION OF FUNGAL SPECIES CAUSING ANTHRACNOSE DISEASE ON MANGO (*Mangifera indica* L.)

BY

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CERTIFICATION

This project report entitled **"Identification and characterization of fungal species causing anthracnose disease on mango (*Mangifera indica* L.) "** prepared by Siti Norliza Binti Mohd Din submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture Science.

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LIST OF ABBREVIATIONS

BLAST = Basic Local Alignment Search Tool

BIOEDIT = Biological Sequence Alignment Editor

DNA = Deoxyribonucleic acid

ITS = Internal Transcribed Spacer

MAFFT = Multiple Alignment Program for Amino Acid or Nucleotide sequences

MEGA = Molecular Evolutionary Genetics Analysis

NCBI = National Center for Biotechnology Information

PDA = Potato Dextrose Agar

PCR = Polymerase Chain Reaction

RNA = Ribonucleic acid

rNA = Ribosomal ribonucleic acid

UPM = Universiti Putra Malaysia

LIST OF UNITS

%	Percentage
°C	Degree Celcius
Cm	Centimeter
g	Gram
h	Hour
mM	Milimol
ml	Mililitre
µl	Microlitre

ABSTRACT

Mango (*Mangifera indica* L.) belongs to the family Anacardiaceae is grown primarily in Malaysia valued for local mango production and has high nutritional value. One of the major problem pre and post-harvest diseases on mango is anthracnose disease caused by many fungal species in the genus *Colletotrichum*. Symptoms of the disease included irregular, circular dark brown spot appear on the young leaves, flowers and fruits. In Malaysia, there is limited research on the composition of fungal species responsible for mango anthracnose. The objectives of this study are; 1) to isolate pure culture of fungal isolates causing anthracnose on mango fruits and leaves; 2) to identify fungal pathogens to species level based on morphological characteristics and polymerase chain reaction (PCR) protocol using ITS4 and ITS5 primers; and 3) to construct internal transcribed spacer (ITS) phylogeny of the fungal species using maximum likelihood analysis. To accomplish these objectives, symptomatic fruits were collected from five different mango trees at Taman Pertanian Universiti (TPU), Universiti Putra Malaysia. Infected tissues (5 x 5mm) from the lesions margin was being surface disinfected for 2 min with 10% Clorox and cultured on potato dextrose agar (PDA). The pure fungal isolates isolated from fruit lesions were identified by conidial and *in vitro* morphological characteristics according to Mordue *et al.*,(1971). The fungal isolates were sub-cultured by single spore isolation and the representative was characterized further. DNA genomic was extracted from fresh fungal mycelium by using protocol of DNeasy Plant Mini Kit from QIAGEN. The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using primers ITS4 and ITS5. The PCR product of the ITS was sequenced and analyzed using BLAST nucleotide query in GenBank. In this study, all fungal isolates

match to the sequence of *Colletotrichum asianum* within *C.gloeosporiodes* species complex. This study is a significant step forward management recommendation in controlling anthracnose in mango production areas.

ABSTRAK

Mangga (Mangifera indica L.) yang tergolong dalam keluarga Anacardiaceace banyak di tanam di Malaysia khususnya untuk pengeluaran mangga tempatan dan mempunyai nilai nutrisi yang tinggi. Salah satu masalah utama berkenaan penyakit sebelum dan selepas tuai adalah penyakit antraknos yang disebabkan oleh pelbagai spesis kulat dalam genus Colletotrichum. Simptom penyakit ini termasuklah hitam coklat dalam bentuk bulatan yang tak sekata yang muncul pada daun muda, bunga, dan buah. Di Malaysia, penyelidikan tentang komposisi spesies kulat yang menjadi penyebab kepada penyakit ini adalah terhad. Objektif bagi kajian ini termasuklah 1) Untuk mengasingkan kultur asli bagi kulat yang menyebabkan antraknos pada buah dan daun mangga ; 2) Untuk mengenalpasti pathogen kulat hingga ke jenis spesies berdasarkan kepada ciri-ciri morfologi dan Tindakan Rantaian Polimerase (PCR) menggunakan protokol primer ITS4 dan ITS5 ; dan 3) Untuk membina filogeni Ruang Tertranskripsi Dalaman (ITS) bagi spesies kulat menggunakan analisis kesamaan maximum. Bagi memenuhi objektif ini, buah yang mempunyai simptom penyakit antraknos dikumpul daripada 5 pokok mangga yang berbeza di Taman Pertanian Universiti (TPU), Universiti Putra Malaysia. Tisu sampel yang dijangkiti (5x5mm) dibersihkan di bahagian permukaan untuk 2 min dengan 10% Clorox dan dikulturkan pada Agar Dextrose Potato (PDA). Kulat asli daripada kultur dikenalpasti melalui conidia dan ciri-ciri morfologi in vitro berdasarkan Mordue et al., (1971). Asingan kulat yang dikultur disub-kulturkan menggunakan asingan spora tunggal. Genomik DNA di ekstrak daripada mycelium kulat yang baru menggunakan kit protocol DNeasy Plant Mini Kit daripada QIAGEN. Ruang Tertranskripsi Dalaman (ITS) bagi DNA ribosome diperolehi

menggunakan primer ITS4 dan ITS5. Produk PCR bagi ITS ini dijujuk dan dianalisis menggunakan Nukleotide BLAST dalam GenBank. Dalam kajian ini,kesemua asingan kulat adalah sama dengan jujukan Colletotrichum asianum dalam spesis kompleks C.gloeosporiodes. Kajian ini merupakan satu langkah kehadapan bagi pengesyoran pengurusan dan kawalan bagi penyakit antraknos bagi kawasan pengeluaran mangga.

CHAPTER 1

INTRODUCTION

Mango (*Mangifera indica L.*) is a type of climacteric fruit which is widely consumed by people worldwide and it is native to South and Southeast Asia. Mango is one of the most popular fruit in the tropics due to high nutritional content as well as delicious flavor. This fruit has been grown in many countries in the world including Thailand, Malaysia, Indonesia, Mexico, India, and Philippines. However, mango fruit is prone to infection during post-harvest, transportation and poor storage condition (Zeng *et al.*, 2006).

Anthracnose disease on mango caused by fungus can lead to high economic losses which is reported to be the most important destructive disease worldwide and considered as a major pre and post-harvest disease of mangoes in several tropical countries (Jeffries *et al.*, 1990). Infection can occur in many parts of mango plant including young leaves, stems, young flowers, fruit, panicle and petiole with the appearance of the black sunken lesions on the surface of the mango fruit during ripening. Anthracnose caused by *Colletotrichum gloeosporioides* species complex is reported to be the most serious mango disease worldwide (Sangeetha and Rawal, 2009). Anthracnose can cause up to 30-60% of yield losses and the disease incidence can reach almost 100% in fruit under wet conditions (Akem, 2006).

Anthracnose can be managed with pre-harvest applications of fungicide such as chemical benzimidazole. It could effectively suppressed and control the spread of this

disease during growing season. However, fungal pathogen can become resistance to chemical pesticides as they can increase their populations rapidly (Farungsang *et al*., 1994; Kim *et al*., 2008). However, from previous research and current epitypification reveals that *Colletotrichum gloeosporioides* is not the only one causal agent for anthracnose in the tropical countries. For example in northeastern Brazil, the phylogenetic study and isolation reveals that four previously described species *(C.asianum, C.fructicola,C.tropicale,C.karstii*) and one new species *C.dianesei* caused anthracnose disease in main area of mango production (Lima *et al*., 2013*).*

Anthracnose disease is not only infect mango but also other fruits such as chilli, avocado, rubber, papaya, apples, almond, Arabica coffee and guava (Freeman *et al*., 1998;Amusa *et al*., 2005). However since mango anthracnose is the major constraint to the mango production, it is important to identify the causal agent of the anthracnose disease in Malaysia. This study includes morphological and polymerase chain reaction protocol by using species specific primer to identify sub-species in the *Colletotrichum gleoeosporiodes* complex causing anthracnose disease in Malaysia.

In view of this, the objectives of this study include:

1. To isolate pure culture of fungal isolates causing anthracnose on mango fruits and leaves.
2. To identify fungal pathogens to species level based on morphological characteristics and polymerase chain reaction (PCR) protocol using ITS4 and ITS5 primers.

3. To construct internal transcribed spacer (ITS) phylogeny of the fungal species using maximum likelihood analysis.

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