



UNIVERSITI PUTRA MALAYSIA

**IDENTIFICATION AND PATHOGENICITY TEST OF *Curvularia* SPECIES
CAUSING FRUIT ROT DISEASE ON POMELO (*Citrus maxima*)**

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BY

SITI FAIRUZ SALIM

**A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in
fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of
the degree of Bachelor of Agriculture Science**

Faculty of Agriculture

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CERTIFICATION

This project report entitled of identification and pathogenicity test of *Curvularia* species causing fruit rot disease on pomelo (*Citrus maxima*) is prepared by Siti Fairuz Salim and 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 Agricultural Science.

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LIST OF ABBREVIATIONS

ITS	Internal Transcribed Spacer
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
PDA	Potato Dextrose Agar
°C	Degree celcius
ml	Mililitre
µl	Microliter
ITS5-F	Internal Transcribed Spacer 5- Forwad
ITS4-R	Internal Transcribed Spacer 4- Reverse
dNTPs	Deoxynucleotide triphosphates
dH ₂ O	Distilled water
MgCl ₂	Magnesium chloride
Geldoc	Gel Documentation
UV	UltraViolet
TAE	Tris-acetate-EDTA
Kb	Kilo bases
Bp	Base pairs

ABSTRACT

Pomelo (*Citrus Maxima*) is a citrus tree belongs to the family Rutaceae native to Southeast Asia and Indochina. Pomelo fruit contains high nutritional value including vitamin A, C and B, minerals dietary fiber and many phytochemical which make it as one of the most consumed fruit worldwide. In Malaysia, the production of pomelo is estimated to be 8830 metric tonnes in 2009 and about 1895 ha of pomelo are grown commercially. In Malaysia, there is limited research on the composition of fungal species responsible for fruit rot disease on pomelo. Fruit rots symptoms are not acceptable for sale as fresh fruit and can reduce the market value of the crop. The objectives of this study are; 1) to isolate pure culture of fungal isolates causing fruit rot on pomelo; 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 a phylogenetic tree using ITS analysis. To accomplish these objectives, symptomatic fruits was collected from harvested pomelo fruits at Kampung Gapis, Nyalas Melaka. Infected tissues (5 x 5 mm) from the lesion margins was surface disinfected for 2 min with 10% chlorox and sterile water and transferred on potato dextrose agar (PDA). The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using primers ITS4 and ITS5. The PCR products of the ITS were sequenced and analyzed using BLAST nucleotide search in GenBank. Pathogenicity test was conducted on six asymptomatic pomelo fruits. Both wounded and unwounded pomelo fruit were sprayed with a runoff fungal spore suspension (1×10^6) spores/ml prepared from 27-day-old cultures grown on PDA. Controls were treated with distilled water. The plastic bags were used to cover inoculated pomelo fruits to maintain high humidity. Mycelial growth and development of fruit spot symptoms were observed and

Curvularia lunata was isolated from diseased fruit. This study will add information causing fruit rot on pomelo in Peninsular Malaysia and therefore, effective management of this disease must involve control of this pathogen.



ABSTRAK

Pomelo (*Citrus Maxima*) adalah pokok sitrus kepunyaan keluarga Rutaceae yang berasal dari Asia Tenggara dan Indochina. Buah limau bali mengandungi nilai nutrisi yang tinggi termasuk vitamin A, C dan B, mineral serat diet dan banyak fitokimia yang menjadikan ia sebagai salah satu buah-buahan yang paling banyak digunakan di seluruh dunia. Di Malaysia, pengeluaran limau bali dianggarkan 8830 tan metrik pada tahun 2009 dan kira-kira 1895 hektar limau bali ditanam secara komersial. Di Malaysia, terdapat penyelidikan terhadap komposisi spesis kulat yang menyebabkan penyakit reput buah limau bali. Gejala reput buah tidak laku dijual sebagai buah-buahan segar dan ini telah mengurangkan nilai pasaran tanaman tersebut. Objektif kajian ini adalah; 1) untuk mengasingkan kulat yang menyebabkan reput buah limau bali; 2) untuk mengenal pasti patogen kulat sehingga ke peringkat spesies berdasarkan ciri-ciri morfologi dan tindak balas rantai polimerase (PCR) protokol menggunakan ITS4 dan ITS5 sebagai primers; dan 3) untuk membina pokok filogeni berdasarkan analisis ITS. Bagi mencapai objektif ini, buah yang berpenyakit akan dikutip daripada hasil buah-buahan limau bali yang dituai di Kampung Gapis, Nyalas Melaka. Tisu yang dijangkiti kulat dipotong (5 x 5 mm) dan permukaan hirisan tersebut di bersihkan daripada kuman selama 2 minit dengan menggunakan 10% chlorox dan air steril akan dipindahkan pada kentang dextrose agar (PDA). ITS DNA ribosom akan dikuatkan menggunakan primers ITS4 dan ITS5. Produk PCR daripada ITS akan disusun dan dianalisis menggunakan BLAST carian nukleotida dalam GenBank. Ujian kepatogenan akan dijalankan pada enam buah limau bali yang sihat. Kedua-dua buah limau bali yang dcederakan dan tidak dcedera akan disembur dengan air spora kulat (1×10^6) spora / ml yang disediakan daripada kulat berumur 27 hari di atas PDA. Buah kawalan akan disembur dengan air suling. Beg

plastik akan digunakan untuk membungkus buah limau bali yang disuntik untuk mengekalkan kelembapan yang tinggi. Pertumbuhan dan perkembangan gejala buah akan diperhatikan dan agen penyebab akan diambil dari buah-buahan yang berpenyakit. Kajian ini akan menambah maklumat yang menyebabkan reput buah-buahan limau bali di Semenanjung Malaysia oleh itu, pengurusan yang berkesan untuk penyakit ini mesti melibatkan kawalan patogen.



CHAPTER 1

INTRODUCTION

Pomelo is the largest fruit from Rutaceae family is native to Malaysia and southeastern Asia. The largest diameter is 30cm and 10 kg in weigh. Pomelo has a very thick rind but yet soft and easily to be peeled off. Some of the product made from the peel are sweet candies and marmelades. Pomelo being one of the fruit consume in a high quantities in the world, but the large amounts of peels being produce leads to difficult for the waste management (Hameed *et al.*, 2007). Pomelo is also known as pummelo, pommelo, shaddock, limau bali and Chinese grapefruit (Morton, J.F. 1987). Malaysian also called it as Limau Besar or Limau Tambun (Abu Kasim Ali, 2010). 53 varieties of pomelo are registered by Agriculture department but only two commercial varieties which are Shating (PO 51) and Tambun (PO 52). In 1998, MARDI produced one variety named as Melomas (KK2). This variety is one of the popular variety in Malaysia (Abu Kasim Ali, 2010). It has been popular among growers especially development of fruit industries in Malaysia under Dasar Pertanian Negara Ketiga (DPN3) (Abu Kasim Ali, 2010).

According to Jones, (1990) pomelo is widely distributed in Thailand and Malaysia. Pomelo was used for edible, medicinal and hybridization. Based on statistic from Department of Agriculture, 2013, Pomelo had been cultivated about 1,690 hectares with 10,519. In 2011, the main state produced pomelo is Johor with 428 hectares and Sarawak with 296 hectares while Sabah with 231 hectares (Department of Agriculture, 2013). The area used for Pomelo cultivation in Malaysia is small compared to other fruits. In 2008, the area used to cultivate this crop is about 1,850 ha and the main states

were at Kedah, Johor and Perak. Pomelo also cultivate at Sabah and Sarawak but the area are small compared to Peninsular Malaysia (Abu Kasim Ali, 2010).

Pomelo has a lot of advantages and one of it is it contain high vitamin (44 mg/100g) and β -carotin (200 mg/100 g). Normally it is eaten as freshly or as a juice. Due to a research done by MARDI, about 56% Malaysian used it as fresh and 24% as juice (Abu Kasim Ali, 2010). However, there is some problem that is happen which is a very high values can be reach for the losses of postharvest on fruit and vegetables. If the handling of postharvest and the conditions of storage are not optimal, more than 25% is from the total production in industrialized countries while more than 50% in developing countries. Because of the low pH, high amount of water content and nutrients, much of the fruit are losses due to attacked by several fungal pathogens. While fruit are attached to their plant, intrinsic resistance protects them but it will be lost after the process of harvesting (Droby *et al.*, 1992).

According to Barkai-Goland, (2001), most of the postharvest pathogen that is attack in the field only penetrate through a wound and cannot directly penetrate the fruit through their cuticle. Normally, the wound appeared on the fruit are caused during harvest, transportation, packinghouse operations and storage processes. Some of fungus species that are caused the most important postharvest diseases are of *Fusarium*, *Aspergillus*, *Botrytis Geotrichum*, *Monilinia*, *Alternaria*, *Penicillium*, *Rhizopus*, *Mucor* and other genera. There are many ways that we can used to reduce postharvest losses by practicing proper sanitation procedures, delay the fruit senescence by maintaining the natural fruit resistance and reduce any mechanical injuries on the fruit (Shweleft, 1986).

Postharvest synthetic fungicides are also used to control all of the disease because the modern storage facilities and all beneficial practices only are not enough (Eckert, 1990). Therefore, it is very important to identify the fungal that contaminate the fruit because some of the molds can produce mycotoxins (Stinson et al. 1980; Tournas and Stack, 2001; Tournas, 2005).

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

- 1) To isolate pure culture of fungal isolate causing fruit rot on pomelo.
- 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 a phylogenetic tree based on ITS analysis.

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