UNIVERSITI PUTRA MALAYSIA

ISOLATION, CHARACTERIZATION AND PATHOGENICITY OF FUNGAL PATHOGENS CAUSING POST-HARVEST SPOILAGE IN *SYZYGIUM MALACCENSE* (MALAY APPLE)

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BY

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A project report submitted to Faculty of Agriculture, University Putra Malaysia, 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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This project report entitled “ISOLATION, CHARACTERIZATION AND PATHOGENICITY OF FUNGAL PATHOGENS CAUSING POST-HARVEST SPOILAGE IN *SYZYGIUM MALACCENSE* (MALAY APPLE)” is prepared by Alawiah binti Daud 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 Agriculture Science.

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LIST OF ABBREVIATION

AP	Extraction buffer
AW	Column wash buffer
BLAST	Basic Local Alignment Search Tool
Bp	base pair
CRD	Complete Randomized Design
DNA	Deoxyribonucleic acid
ERS	Economic Research Services
EtBr	Ethidium bromide
ITS	Internal Transcribed Spacer
LCB	Lactophenol Cotton Blue
NCBI	National Centre for Biotechnology Information
OD	Optical Density
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
rDNA	Ribosomal Deoxyribonucleic acid
TBE	Tris Borate EDTA

ABSTRACT

Syzygium malaccense (Malay apple) is one of Malaysia's local fruit that has demands from the local and international consumers. However, post-harvest spoilage of *S. malaccense* is causing economic loss to exporters in general and local sellers in particular. In addition, spoilage caused by pathogenic microbes may cause health hazards to the consumers. Therefore, in order to curb post-harvest spoilage, the identity of the causal pathogens are crucial for spoilage management. Thus, the objectives for this study were i. To isolate and identify the fungal pathogens at species level from *S. malaccense* obtained from the local markets in Peninsular Malaysia, ii. To conduct phylogenetic relationship among species, and iii. To perform pathogenicity test. In order to achieve these objectives, the samples were obtained from local fruit markets in four different states namely Negeri Sembilan, Kelantan, Terengganu and Selangor. These samples were subjected to isolation step on Potato Dextrose Agar (PDA) prior to cultural and morphological identification based on visibility and microscopic characteristics of each pure culture isolated. This was then followed by molecular identification, where genomic DNA was extracted and amplified using ITS 1 and ITS 4 universal primer sets and the polymerase chain reaction (PCR) products of each isolate subjected to sequencing and later BLAST analysis based on GenBank sequence database. Finally, pathogenicity test was conducted to confirm the isolated fungal pathogens as the causal agent of the post-harvest spoilage of *S. malaccense.* Consequently, in the present study the isolated pathogens were identified as *Lasiodiplodia theobromae, Pestalotiopsis microspora,* and

Penicillium verruculosum. In conclusion, all identified fungal pathogens exhibited host-specificity rather than location specific as the causal of post-harvest spoilage of *S. malaccense*.

ABSTRAK

Syzygium malaccense (jambu merah) adalah salah satu buah tempatan Malaysia yang mempunyai permintaan daripada pengguna tempatan dan antarabangsa. Walau bagaimanapun, fenomena buah ini selepas dituai pembusukan selepas tuai menyebabkan kerugian ekonomi kepada penjual tempatan dan pengeksport. Selain itu, kerosakan yang disebabkan oleh patogen kulat ini boleh menyebabkan masalah kesihatan kepada pengguna. Oleh itu, dalam usaha untuk mengatasi masalah pembusukan selepas dituai, identiti isolat patogen kulat yang menjadi penyebab pembusukan adalah penting untuk pengurusan kerosakan. Oleh itu, objektif kajian ini adalah i. Untuk menjalankan pemencilan dan mengenalpasti patogen kulat pada tahap spesis dari *S. malaccense* diperolehi dari pasaran tempatan di Semenanjung Malaysia, ii. Menjalankan hubungan filogenetik antara spesies, dan iii. Untuk melaksanakan ujian kepatogenan. Untuk mencapai objektif ini, sampel diperoleh dari pasar buah-buahan tempatan di empat negeri yang berbeza iaitu Negeri Sembilan, Kelantan, Terengganu dan Selangor. Pemencilan patogen kulat daripada *S. malaccense* dilakukan di atas Agar Kentang Dekstrose (PDA) sebelum ciri- ciri morfologi dikaji bagi tujuan identifikasi. Ini diikuti dengan pencirian secara molekul, di mana DNA genomic setiap isolat patogen kulat yang berjaya dipencilkan, diekstrak dan diamplifikasi menggunakan set pencetus universal ITS1 dan ITS4. Ini diikuti dengan proses penjujukan nukleotida dan hasil jujukan yang diperolehi digunakan dalam analisis BLAST berdasarkan pangkalan data jujukan di GenBank. Akhirnya, ujian kepatogenan dijalankan untuk mengesahkan patogen kulat yang dipencil

sebagai agen penyebab pembusukkan *S. malaccense* lepas dituai. Dalam kajian ini, identiti semua kulat yang berjaya dipencilkan telah dikenalpasti sebagai *Lasiodiplodia theobromae, Pestalotiopsis microspora* dan *Penicillium verruculosum*. Secara kesimpulannya, kesemua patogen kulat yang dikenalpasti mempamerkan spesifikasi pada perumah dan bukannya berdasarkan lokasi sebagai agen penyebab pembusukkan buah *S. malaccense*.

CHAPTER 1

INTRODUCTION

Syzygium malaccense (L.) Merr. & Perry is from family Myrtaceae and has many different local names based on country where it is planted. It also known as Malay Apple in English and *Jambu air* (Malaysia), *Jambu bol* (Indonesia), Pomarossa malay (Spanish) and Cay roi (Vietnam) (Orwa et al., 2009).

Syzygium malaccense has been cultivated in the tropics for a very long time. *Syzygium malaccense* tree is about 20m tall with straight trunk and branched near the base with broadly ovoid canopy. The leaves of *Syzygium malaccense* is opposite, elliptic oblong in size (15-38cm × 7-20cm) where young leaves appear in red colour (Orwa et al., 2009). The fruits are usually deep red in colour and pear shape with a waxy skin, with crunchy flesh, juicy with a mild sweet flavor (Trade Wind Fruits, 2013)

Post-harvest disease of *S. malaccense* occurs after the fruit was harvested from the plant and during transportation storage and shelf. The spoilage and damage found maybe caused by bacteria and fungi (Coates, and Johnson, 1997). The common post-harvest symptoms that can be seen through naked eyes are rotting and mycelium growth on the

surface of the fruit which indicates infection caused by fungal pathogen (Akinmusire, 2011). Fungi are eukaryotes that can be described as small and microscopic organism that usually branched, filamentous and spore bearing organism that is lack of chlorophyll. According to Agrios (2005), "It is more than 100,000 known fungal species which are saprophytic, and there are more than 10,000 species that can cause disease to plant".

However, post-harvest spoilage of *S. malaccense* is causing economic loss to sellers in particular and exporter countries in general. Retail food loss was particularly difficult to estimate, however, prior to the study done in "Supermarket Loss Estimates for Fresh Fruit, Vegetables, Meat, Poultry, and Seafood and Their Use in the ERS Loss-Adjusted Food Availability Data", the per capita food loss estimates at the retail level are 12 percent on fresh fruit and vegetable commodity itself (Buzby *et al*., 2009). In addition, spoilage caused by these pathogenic fungi may cause health hazards to the consumers. Therefore, in order to curb post-harvest spoilage, the identity of the causal pathogens is crucial for spoilage management.

Thus, the objectives of this study include:

1. To isolate and identify the fungal pathogens from *Syzygium malaccense* fruits obtained from local markets in Malaysia.
2. To conduct phylogenetic relationship among species.
3. To perform pathogenicity test.

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