UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION AND PATHOGENICITY OF MARASMIELLUS SPP. ISOLATED FROM OIL PALM AND COCONUT IN WEST MALAYSIA

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By

BUSHRA SUBAIR ABDULSADA

Thesis Submitted to the School Of Graduate Studies, University Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Special dedication

To my beloved parents and my family for their support and patience.

To my husband for his boundless love, understanding, encouragement and patience throughout my study.
Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement of the degree of Doctor of Philosophy

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Chairman: Assoc Prof. Zainal Abidin Mior Ahmad, PhD

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Species of Marasmiellus are of widespread occurrence causing numerous diseases on a wide range of tropical crops. Although Marasmiellus palmivor us has been reported from only a few countries, its records of growth behaviour and habitat suggest the existence of this fungus in countries where oil palm and coconut are cultivated commercially. There is lack of information on the characterization and pathogenicity of species in Malaysia and globally. In addition, the pathogenicity of M. palmivor us has never been proven. The aim of this study was to isolate the fungus from diseased oil palm and coconut, identify and characterize the causal pathogen of bunch rot of oil palm and embryo and shoot rot of germinating coconuts in West Malaysia using morphological and molecular characteristics. The pathogenicity tests and cross inoculations of isolates on oil palm (fruits, seeds, and seedlings) and coconut seeds in the
glasshouse were also conducted. Pure cultures of *Marasmiellus* were isolated from samples of diseased oil palm fruits, coconut seeds, basidiocarps and rhizomorphs. Identification of fungus was done based on characteristics observed from cultural and molecular methods. Pathogenicity tests were conducted to confirm pathogenicity of oil palm and coconut isolates in the glasshouse. Samples were collected from six locations in two states in West Malaysia from which 25 isolates were obtained for study. Pure cultures of isolates produced colonies that were fast growing on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) media, with dense white mycelia and lighter orange colour at the center, binucleate hyphae, were mostly not immersed in agar. Clamp connections were abundantly present in all isolates. The optimum temperature for all isolates growth was 30°C, and all were capable of growing on a wide range of pH. Morphology of basidiocarps and basidiospores was found to be similar with differences in the size and colour of basidiocarps and size of basidiospores. The fungus pileus colour was pale orange in the middle, with a depressed center, and measured 9-30mm in diameter. The stipe was white, pale orange, silky, base was initially filled with tissues then becoming hollow, bulbous and eccentric and measured 7-29mm in diameter. The basidiospores were hyaline, oblong to ellipsoid and smooth. Molecular characterization of isolates based on nLSU region and BLAST results indicated that all 25 isolates sequences were 99% identical to that of *Marasmiellus palmivorus* AY639434 from USA–Hawaii. Phylogenetic analysis of 25 isolates based on neighbor joining method on nLSU region grouped them in the same
cluster with *M. palmivorus* AY639434 from USA–Hawaii and distinct from other genera in *Marasmiaceae* family. Phylogenetic analysis based on neighbor joining method on ITS regions similarly grouped them in the same cluster. There was no previous sequence deposited in GeneBank for *M. palmivorus* and this is the first sequence for this region. Ten different isolates of *Marasmiellus palmivorus* successfully produced high numbers of basidiocarps in the glasshouse when cultured using two artificial methods: whole wheat grain and rubber wood block. Pathogenicity tests on oil palm fruits showed the highest disease severity caused by isolates C3, C4, and C5 with no significant difference between wounded and unwounded fruits. All isolates tested infected shoots and radicles emerging from seed recognizable as two types of damping-off: pre-emergence and post-emergence, decay of hypocotyl near the soil lines and root tissues. The pre and post-emergence damping off ranged from 37.5 to 100 %. Pathogenicity tests on oil palm seedlings showed significant differences in disease incidence between isolates. Five isolates (C3, C4, C5, Bangi1, and MPOB1) were pathogenic to coconut seeds. Isolates C3, C4 and C5 caused highest percentage disease incidence on coconut seeds. Post-emergence disease of roots of coconut seedlings was considered a new finding. Histopathological examinations of the root tissues of oil palm and coconut showed the presence of fungal hyphae within pith, xylem, cortex and epidermis cells with brownish inclusions found in the phloem and pith. The results obtained from pathogenicity tests confirmed *M. palmivorus* to be potentially pathogenic to oil palm and coconut and causing various disorders.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN DAN PATOGENISITI PENCILAN MARASMIELLUS SPP. DARI KELAPA SAWIT DAN KELAPA DI MALAYSIA BARAT

Oleh

BUSHRA SUBAIR ABDULSADA

September 2012

Pengerusi: Assoc Prof. Zainal Abidin Mior Ahmad, PhD
Fakulti: Pertanian

Spesies Marasmiellus terdapat dengan melnas dan menyebabkan pelbagai penyakit pada tanaman tropika. Walau pun Marasmiellus palmivorus telah dilapor dari hanya beberapa negara, catatan perlakuan pertumbuhan dan kehidupan menunjukkan kehadiran kulat ini dalam semua negara di mana kelapa sawit dan kelapa ditanam secara komersial. Terdapat maklumat yang kurang mengenai pencirian dan patogenisiti spesies di Malaysia dan global. Tambahan pula, patogenisiti M. palmivorus belum pernah terbukti. Tujuan kajian ini adalah untuk memencil kulat dari kelapa sawit dan kelapa yang berpenyakit, mengenal pasti dan mencirikan patogen penyebab reput tandan kelapa sawit dan reput embrio dan pucuk kelapa bercambah di Malaysia Barat menggunakan ciri morfologi dan molikul. Ia juga menjalankan ujian patogenisiti dan inokulasi silang pencilan dari kelapa sawit (buah,biji dan anak benih) dan benih kelapa di rumah kaca. Kultur tulen Marasmiellus dipencilkan dari sampel
untuk *M. palmivorus* dan ini merupakan aturan yang pertama bagi kawasan ini. Ujian patologenisiti ke atas buah kelapa sawit menunjukkan keterukan penyakit yang disebabkan oleh pencilan C3, C4 and C5 dengan perbezaan yang signifikan antara biji diluka dan yang tanpa luka. Kesemua pencilan yang diuji menjangkkiti pucuk dan akar yang keluar dari biji dicam sebagai dua jenis lecuh: pra cambah dan pos cambah, reput di bahagian hipokotil berhampiran paras tanah dan tisu akar. Lecuh pra dan pos cambah berjulat antara 37.5 ke 100%. Ujian patogenisiti ke atas benih kelapa sawit menunjukkan perbezaan yang signifikan dalam kejadlian penyakit antara pencilan C3, C4 dan C5; MPOB1, Bangi1, dan C2; C6,OP4,UPM4. Lima pencilan (C3, C4, C5, Bangi1 dan MPOB1) adalah patogenik kapada biji kelapa. Pencilan C3, C4 dan C5 menyebobkan peratus kejadian penyakit paling tinggi pada biji kelapa. Penyakit pos - cambah pada akar benih kelapa merupakan satu penemuan baru. Pemerhatian histopatologi tisu akar kelapa sawit dan kelapa menury ukkan kehadiran hifa kulat dalam pith, xylem, korteks dan sel epidermis dengan pengisian perang dijumpai dalam floem dan pith. Keputusan dari ujian patogenisiti mengesahkan spesies *Marasmiellus palmivorus* berpotensi patogenik kepada kelapa sawit dan kelapa dan mengyebabkan pelbagai kerosakan.
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I certify that a Thesis Examination Committee has met on (insert the date of viva voce) to conduct the final examination of (Bushra Subair Abdulsada) on his thesis entitled “Characterisation and Pathogenicity of Marasmiellus spp. Isolated from Oil Palm and Coconuts in West Malaysia ” 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 recommended that the student be awarded the (interest the name of relevant degree).

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DECLARATION

I declare that the thesis is my original work except for quotations and citation, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institution.

_____________________________
BUSHRA SUBAIR ABDULSADA

Date:
PERAKUAN

Saya memperakui bahawa tesis ini adalah hasil kerja saya yang asli melainkan petikan dan sedutan yang tiap-tiap satunya telah dijelaskan sumbernya. Saya juga memperakui bahawa tesis ini tidak pernah dimajukan sebelum ini, dan tidak dimajukan serentak dengan ini, untuk ijazah lain sama ada di Universiti Putra Malaysia atau di institusi lain.

______________________________
BUSHRA SUBAIR ABDULSADA

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