



UNIVERSITI PUTRA MALAYSIA

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

BUSHRA SUBAIR ABDULSADA

FP 2012 58

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

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**

September 2012

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

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

By

BUSHRA SUBAIR ABDULSADA

September 2012

Chairman: Assoc Prof. Zainal Abidin Mior Ahmad, PhD

Faculty: Agriculture

Species of *Marasmiellus* are of widespread occurrence causing numerous diseases on a wide range of tropical crops. Although *Marasmiellus palmivorus* 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. palmivorus* 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 meluas dan menyebabkan pelbagai penyakit pada tanaman tropika. Walau pun *Marasmiellus palmivorus* telah dilaporkan 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 penciran dari kelapa sawit (buah, biji dan anak benih) dan benih kelapa di rumah kaca. Kultur tulen *Marasmiellus* dipencilkan dari sampel

buah kelapa sawit, biji kelapa, basidiokarpa dan rizomorf. Pengenalpastian kulat dilaku berdasarkan pemerhatian ciri dari kaedah kultur dan molikul. Ujian patogenisiti pencilan kelapa sawit dan kelapa dijalankan di rumah kaca. Sampel telah diperolehi dari enam tempat dalam dua negeri di Malaysia Barat yang memberi 25 pencilan untuk kajian ini. Kultur tulen pencilan menghasilkan koloni yang mempunyai pertumbuhan cepat atas medium PDA dan MEA, dengan miselium putih yang padat dan bewarna jingga cerah di tengah, hifa binuklat, yang kebanyakannya tidak tenggelam dalam agar. Penghubung klam didapati terdapat dengan banyak bagi semua pencilan. Suhu optimum adalah 30°C dan semua dapat tumbuh pada julat pH yang luas. Morfologi basidiokarpa dan basidiospora didapati serupa dengan perbezaan saiz dan warna basidiokarpa dan saiz basidiospora. Warna pileus kulat adalah jingga cerah di tengah, dengan tengah yang mendalam, tepi yang bengkok semasa muda, dan berukuran garispusat 9-30mm. Stip adalah putih, jingga cerah, bersutera, bahagian bawah mulanya berisi dengan tisu kemudian menjadi lompong, bulbusa dan esentrik dan berukuran garispusat 7-29mm. Basidiospora adalah hialin, bujur ke ellipsoid, berselinder pada pandangan depan dan licin. Pencirian molikul pencilan berdasarkan bahagian nLSU dan keputusan BLAST menunjukkan yang kesemua 25 aturan pencilan mempunyai 99% persamaan dengan *M. palmivorus* AY639434USA- Hawaii dan berbeza dari genera lain dalam famili Marasmiaceae. Analisis filogenetik berdasarkan kaedah neighbour bersambung pada bahagian ITS sama mengumpulkan mereka dalam kluster yang sama. Tiada aturan sebelumnya yang telah dimasukkan dalam GenBank

untuk *M. palmivorus* dan ini merupakan aturan yang pertama bagi kawasan ini. Ujian patogenesis 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 menjangkiti pucuk dan akar yang keluar dari biji dicam sebagai dua jenis lecu: pra cambah dan pos cambah, reput di bahagian hipokotil berhampiran paras tanah dan tisu akar. Lecu pra dan pos cambah berjangkit antara 37.5 ke 100%. Ujian patogenesis ke atas benih kelapa sawit menunjukkan perbezaan yang signifikan dalam kejadian penyakit antara pencilan C3, C4 dan C5; MPOB1, Bangi1, dan C2; C6, OP4, UPM4. Lima pencilan (C3, C4, C5, Bangi1 dan MPOB1) adalah patogenik kepada biji kelapa. Pencilan C3, C4 dan C5 menyebabkan 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 menunjukkan kehadiran hifa kulat dalam pith, xylem, korteks dan sel epidermis dengan pengisian perang dijumpai dalam floem dan pith. Keputusan dari ujian patogenesis mengesahkan spesies *Marasmiellus palmivorus* berpotensi patogenik kepada kelapa sawit dan kelapa dan menyebabkan pelbagai kerosakan.

ACKNOWLEDGEMENTS

First and foremost all praises and my endless thanks to Allah, the Most Beneficent, Most Gracious, Most Merciful for his heavenly, luxuriates and blessings over me throughout my life and the period of this study.

I would like to express my deepest thanks to my supervisor, Assoc. Prof. Dr. Zainal Abidin Mior Ahmad for his suggestions for improvement, guidance, invaluable comments, constructive criticisms and full encouragement throughout this study that supported the completion of this thesis. I would also like to extend my thanks and gratitude to my co-supervisors Assoc. Prof. Dr. Jugah Kadir and Dr. Wong Mui Yun for their advice and suggestions. The offer of graduate research fellowship from UPM is appreciated. I am very indebted to University of Baghdad, which provided the funding for my research during the period of study.

My deepest gratitude goes to my family, for their understanding and support throughout the period of study. I give appreciation to my beloved husband, Kamil Aboshkair, for his endless love, care, consistent support and assistance. Last but not least, I offer my regards and blessings to all of those who gave me support especially to my friends Roslina Sulaiman and Mansour Salati.

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 (insert the name of relevant degree).

Members of the Thesis Examination Committee were as follows:

Name of Chairperson, PhD

Title (e.g Professor/ Associate Professor/ Ir)

Name of Faculty

University Putra Malaysia

(Chairman)

Name of Examiner 1, PhD

Title (e.g Professor/ Associate Professor/ Ir)

Name of Faculty

University Putra Malaysia

(Internal Examiner)

Name of Examiner 2, PhD

Title (e.g Professor/ Associate Professor/ Ir)

Name of Faculty

University Putra Malaysia

(Internal Examiner)

Name of External Examiner, PhD

Title (e.g Professor/ Associate Professor/ Ir)

Name of Department and / or Faculty

Name of Organisation (University / Institute)

country

(External Examiner)

BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
University Putra Malaysia

Date:

This thesis was submitted to the senate of University Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were s follows:

Zainal Abidin Mior Ahmad, PhD

Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Chairman)

Jugah Kadir, PhD

Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Member)

Wong Mui Yun, PhD

Associate Professor
Faculty of Agriculture
University Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
University Putra Malaysia

Date:

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:

Declaration Form for Thesis Written in Bahasa Melayu

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

Tarikh:

TABLE OF CONTENT

ABSTRACT	Page
ABSTRAK	i
ACKNOWLEDGMENT	iv
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	x
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xviii
CHAPTER	xxi
1 GENERAL INTRODUCTION	1
2 LITERATURE REVIEW	4
2.1 Oil Palm	4
2.1.1 Oil palm Diseases	8
2.2 Coconut Palm	17
2.2.1 Coconut Palm Diseases	22
2.2.2 Diseases Caused by <i>Marasmiellus spp.</i> on Coconut	28
2.3 The Marasmioid Fungi	31
2.3.1 Taxonomy and Nomenclature	31
2.3.2 Morphology of <i>Marasmiellus spp.</i>	35
2.3.3 Host range of <i>Marasmiellus spp.</i>	36
2.3.4 Distribution of <i>Marasmiellus spp.</i>	38
2.3.5 Biology and Ecology of <i>Marasmiellus spp.</i>	38
2.3.6 Diseases caused by <i>Marasmius spp.</i> and <i>Marasmiellus spp.</i> on Miscellaneous Host Plants	40
2.3.7 Molecular Studies on <i>Marasmioid Fungi</i>	44
3 ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF <i>MARASMIELLUS SPP.</i> FROM OIL PALM AND COCONUT	49
3.1 Introduction	49
3.2 Materials and Methods	51
3.2.1 Collection of Samples	51
3.2.2 Isolation of <i>Marasmiellus spp.</i> From Palms Diseased and Fungi Structures	51
3.2.3 Morphology of Basidiocarps and Basidiospores	53
3.2.3.1 Basidiospore Germination Tests	54

3.2.3.2	Chemical Reaction Tests	54
3.2.4	Morphological Characteristics of Colony Cultures	55
3.2.4.1	Effect of Media on Colony Growth	55
3.2.4.2	Effect of Temperature on Colony Growth	56
3.2.4.3	Effect of PH on Colony Growth	56
3.2.5	Morphological Characteristic of Hyphae	56
3.2.6	Scanning Electron Microscopy (SEM)	58
3.2.7	Statistical Analysis	59
3.3	Results and Discussion	
3.3.1	Collection of Samples and Isolation of <i>Marasmiellus spp.</i> from diseased palms and fungi structures	60
3.3.2	Morphology of Basidiocarps and Basidiospores	63
3.3.2.1	Basidiospore Germination and Chemical Reaction Tests	70
3.3.3	Morphological and Characteristics of Colony Cultures	72
3.3.3.1	Effect of Media on Colony Growth	74
3.3.3.2	Effect of Temperature on Colony Growth	77
3.3.3.3	Effect of pH on Colony Growth	79
3.3.4	Morphological and Characteristic of Hyphae	79
3.4	Conclusion	81
4	MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF <i>MARASMEILLUS PALMIVORUS</i> USING NUCLEAR LARGE SUBUNIT nLSU AND INTERNAL TRANSCRIBED SPACERS ITS REGIONS	82
4.1	Introduction	82
4.2	Materials and Methods	85
4.2.1	DNA Extraction	85
4.2.2	Amplification of Target DNA	87
4.2.2.1	PCR Amplification of Ribosomal DNA- nuclear Large Subunit (nLSU) (rDNA-nLSU)	87
4.2.2.2	PCR Amplification of Ribosomal DNA - Internal Transcribed Spacers (rDNA - ITS)	88
4.2.3	Agarose Gel Electrophoresis and Staining	89
4.2.4	Purification and Sequence Analysis	89
4.2.5	Phylogenetic Analysis	90
4.3	Results and Discussion	92
4.3.1	Amplification of Target DNA	92
4.3.1.1	PCR Amplification of Ribosomal DNA- nuclear	92

	large subunit (nLSU) (rDNA-nLSU)	
4.3.1.2	PCR Amplification of Ribosomal DNA - Internal Transcribed Spacers (rDNA - ITS)	100
4.4	Conclusion	109
5	PATHOGENICITY OF <i>MARASMIELLUS PALMIVORUS</i> ON OIL PALM AND COCONUT	111
5.1	Introduction	111
5.2	Materials and Methods	113
5.2.1	Fungus Isolates	113
5.2.2	Pathogenicity Tests	113
5.2.2.1	Pathogenicity Tests on Detached Oil Palm Fruits in the Laboratory	113
5.2.2.2	Pathogenicity Tests on Germinated Oil Palm Seeds in the Glasshouse	115
5.2.2.3	Pathogenicity Tests on Oil Palm Seedlings in the Glasshouse	116
5.2.2.4	Pathogenicity Tests of on Coconut Seeds in the Glasshouse	117
5.2.3	Histopathology of Oil Palm and Coconut Root Tissues	118
5.2.4	Statistical Analysis	119
5.3	Result and Discussion	120
5.3.1	Pathogenicity tests	120
5.3.1.1	Pathogenicity Tests on Detached Oil Palm Fruits in the Laboratory	120
5.3.1.2	Pathogenicity Tests on Germinated Oil Palm Seeds in the Glasshouse	124
5.3.1.3	Pathogenicity Tests on Oil Palm Seedlings in the Glasshouse	127
5.3.1.4	Pathogenicity Tests on Coconut Seeds in the Glasshouse	133
5.3.2	Histopathology of Oil Palm and Coconut Root Tissues	138
5.4	Conclusion	144
6	SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	146
6.1	Summary and Conclusions	146
6.2	Recommendations for Future Research	149

REFERENCES	151
APPENDICES	178
BIODATA OF STUDENT	246
LIST OF PUBLICATIONS	247

151
178
246
247

