UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGY AND ETIOLOGY OF ganoderma UPPER AND BASAL STEM ROT IN OIL PALM (elaeis guineensis JACQ.) ON PEAT IN SARAWAK, MALAYSIA

MOHD RAKIB MOHD RASHID

FSPM 2015 2

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By

MOHD RAKIB BIN MOHD RASHID

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

March 2015

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

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Chairman : Joseph Bong Choon Fah, PhD
Faculty : Faculty of Agriculture and Food Sciences (Bintulu)

Oil palm (*Elaeis guineensis*) is the world's most important oil producing crop which is mostly cultivated in Southeast Asia. The sustainability of oil palm is threatened by *Ganoderma* species that cause upper stem rot (USR) and basal stem rot (BSR). Basal stem rot is a prominent disease of oil palm, while little is known about USR. Lack of knowledge in terms of epidemiology and etiology of the diseases may lead to inaccurate disease control strategies. A study was conducted in oil palm plantations on peat in Sarawak, Malaysia, namely, Sessang Estate in Betong and Sungai Balim Estate in Miri. A total of 46 isolates of *Ganoderma* species from the USR- and BSR-infected palms were identified using multiplex PCR, and their *in-vitro* cultural and basidiospore morphological characteristics were investigated. It was found that both USR and BSR were associated with similar pathogens, where *G. zonatum* (71.7%) was dominant, followed by *G. boninense* (26.1%) and *G. miniatocinctum* (2.2%). This suggests that *G. zonatum* may have played a more vital role in the epidemiology of the disease than previously believed. All samples were found incompatible based on somatic compatibility test which indicated genetic heterogeneity of *Ganoderma* species in oil palm plantation and was suggestive of disease spread via spore dispersal that generated new genetically distinct individuals. There were also significant variations within and between *Ganoderma* species and no distinct relationship were found between species, disease types or geographical origins in terms of their cultural morphology and basidiospore characteristics. This suggests that any of the *Ganoderma* isolates found in this study have similar possibility of either causing USR or BSR. The pathogenic capability and aggressiveness of *Ganoderma* species were assessed based on external and internal infection symptoms using artificial inoculation on oil palm seedlings throughout 24 weeks period. All 46 *Ganoderma* samples tested were confirmed to be pathogenic to oil palm. Among them, *G. zonatum* isolated from USR-infected palms showed superior aggressiveness as compared with others with average foliar symptoms severity (FSS), disease severity index (DSI), stem bole lesion and primary root decay up to 33.18%, 43.78%, 42.20% and 48.55%, respectively. Hence, besides the prominent *G. boninense* and BSR, more studies should emphasize on *G. zonatum* and USR for more effective disease management since it was found as an increasingly important pathogen and disease in oil palm

plantations, especially on peat areas in Sarawak. The infection of USR and BSR were mainly confirmed based on appearance of *Ganoderma* basidiomata on the stem, while the point of infection on the stem was the distinctive characteristic between them, where infection of *Ganoderma* species about one meter above ground indicated USR, while that at the base indicating BSR. USR and BSR disease coexisted in both sites, with lower USR (1.14-1.32%) as compared with BSR (3.48-4.01%) in Betong, while higher USR (3.66-6.02%) as compared with BSR (0.87-3.78%) in Miri throughout the study period. This also indicated that *Ganoderma* species infection in Miri was relatively higher than that in Betong. Geostatistical analysis of the *Ganoderma* species distribution revealed generally weak spatial dependence (>75%), which indicated that the distribution was random, and related to *Ganoderma* species spread via basidiospore dispersal at greater distance, instead of root-to-root infection. It was revealed that spatial distribution of *Ganoderma* species was at relatively greater density in Miri (15.1-100%) as compared with Betong (15.1-30%). Although the distribution of *Ganoderma* species were random, the hotspot patterns generated allow site specific disease management as in precision agriculture and aid in sampling programmes for further investigations on the factors attributed to the patterns. Studies on macro- and micronutrients composition in relation to spatial distribution of *Ganoderma* species revealed that the foliar macro- and micronutrient elements affected the infection of *Ganoderma* species. Based on the four trials conducted at the two study sites, it was found that higher level of phosphorus (P), and lower and deficient level of copper (Cu) and zinc (Zn) in oil palms were associated with higher *Ganoderma* infection. In addition, lower and deficient level of Cu and Zn also could be related to higher *Ganoderma* infection in Miri as compared with Betong. Furthermore, there was no distinct factor in terms of soil and foliar chemical properties between USR and BSR. Therefore, this finding could be used as general guide to control *Ganoderma* species by giving more concern on manipulation of P, Cu and Zn level in oil palm plantations generally, and specifically in the two sites in this study for site-specific disease management as in precision agriculture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

EPIDEMIOLOGI DAN ETIOLOGI REPUT ATAS DAN PANGKAL BATANG *Ganoderma* DALAM KELAPA SAWIT (*Elaeis guineensis* JACQ.) PADA GAMBUT DI SARAWAK, MALAYSIA

Oleh

MOHD RAKIB BIN MOHD RASHID

Mac 2015

Pengerusi : Joseph Bong Choon Fah, PhD
Fakulti : Fakulti Sains Pertanian dan Makanan (Bintulu)

Kelapa sawit (*Elaeis guineensis*) adalah tanaman penghasilan minyak terpenting di dunia yang kebanyakannya ditanam di Asia Tenggara. Kelestarian kelapa sawit diancam oleh species *Ganoderma* yang menyebabkan reput atas batang (USR) dan reput pangkal batang (BSR). Reput pangkal batang adalah penyakit terkenal pada kelapa sawit, manakala sedikit yang diketahui tentang USR. Kekurangan pengetahuan dari segi epidemiologi dan etiologi penyakit-penyakit tersebut boleh menyebabkan strategi kawalan penyakit yang kurang tepat. Satu kajian telah dijalankan di ladang-ladang kelapa sawit di tanah gambut di Sarawak, Malaysia, iaitu, Estet Sessang di Betong dan Estet Sungai Balim di Miri. Sejumlah 46 pencilan spesies *Ganoderma* daripada pokok-pokok sawit dijangkiti USR dan BSR telah dikenal pasti menggunakan multipleks PCR, dan ciri-ciri morfologinya *in-vitro* kultur dan basidiospora telah dikaji. Didapati bahawa kedua-dua USR dan BSR dikaitkan dengan pathogen yang sama, di mana *G. zonatum* (71.7 %) adalah dominan, diikuti oleh *G. boninense* (26.1 %) dan *G. miniatocinctum* (2.2%). Ini menunjukkan bahawa *G. zonatum* mungkin memainkan peranan yang lebih penting dalam epidemiologi penyakit tersebut daripada yang dipercayai sebelum ini. Semua sampel didapati tidak serasi berdasarkan ujian keserasian somatic yang menunjukkan kepelbagaian genetik spesies *Ganoderma* di ladang kelapa sawit dan menandakan penyakit disebarkan melalui penyebaran spora yang menghasilkan individu baru yang genetiknya berbeza. Terdapat juga perbezaan yang ketara dalam dan antara spesies *Ganoderma* dan tidak ada hubungan yang berbeza ditemui antara spesies, jenis penyakit atau asal-usul geografi dari segi ciri-ciri morfologi kultur dan basidiospora mereka. Ini menunjukkan bahawa mana-mana pencilan *Ganoderma* yang ditemui dalam kajian ini mempunyai kemungkinan yang sama, iaitu samada menyebabkan USR atau BSR. Keupayaan patogenik dan keagresifan spesies *Ganoderma* dinilai berdasarkan gejala jangkitan luaran dan dalaman menggunakan inokulasi buatan pada anak pokok kelapa sawit sepanjang tempoh 24 minggu. Kesemua 46 sampel *Ganoderma* yang diuji disahkan sebagai patogen kepada kelapa sawit. Antara mereka, pencilan *G. zonatum* dari pokok sawit yang dijangkiti USR menunjukkan keagresifan unggul berbanding yang lain-lain dengan tahap gejala foliar (FSS), indeks tahap penyakit (DSI), reput batang dan akar utama, masing-masing sehingga 33.18 %, 43.78 %, 42,20 % dan

48.55 %. Justeru, selain *G. boninense* dan BSR yang terkenal, lebih banyak perhatian perlu diberikan untuk kajian terhadap *G. zonatum* dan USR untuk pengurusan penyakit yang lebih berkesan kerana ia telah didapati sebagai patogen dan penyakit yang semakin penting di lading-ladang kelapa sawit, terutama di kawasan-kawasan tanah gambut di Sarawak. Jangkitan USR and BSR disahkan terutamanya berdasarkan kemunculan basidiomata *Ganoderma* pada batang, manakala bahagian jangkitan pada batang adalah ciri tersendiri yang membezakan di antara penyakit tersebut, di mana jangkitan species *Ganoderma* kira-kira satu meter dari aras tanah menunjukkan USR, manakala di pangkal menunjukkan BSR. Penyakit USR dan BSR wujud bersama di kedua-dua tapak kajian, dengan USR lebih rendah (1.14-1.32%) berbanding BSR (3.48-4.01%) di Betong, manakala USR lebih tinggi (3.66-6.02%) berbanding BSR (0.87-3.78%) di Miri sepanjang tempoh kajian. Ini juga menunjukkan bahawa serangan species *Ganoderma* di Miri adalah lebih tinggi berbanding di Betong. Analisis geostatistik pada taburan species *Ganoderma* umumnya menunjukkan pergantungan lemah spatial (>75%), yang menunjukkan bahawa taburannya adalah rawak dan dikaitkan dengan perebakan species *Ganoderma* melalui penyebaran basidiospore pada jarak yang lebih jauh, dan bukannya jangkitan akar-ke-akar. Ditunjukkan bahawa taburan species *Ganoderma* di Miri adalah lebih padat (15.1-100%) berbanding di Betong (15.1-30%). Walaupun taburan spesies *Ganoderma* adalah rawak, corak titik panas yang dijana membolehkan pengurusan penyakit tapak spesifik seperti dalam pertanian persis dan membantu dalam program persampelan untuk kajian lanjut pada factor-faktor yang dikaitkan dengan corak-corak taburan tersebut. Kajian ke atas komposisi makro dan mikro-nutrien berhubung dengan taburan species *Ganoderma* menunjukkan unsur-unsur makro- dan mikronutrien foliar mempengaruhi serangan species *Ganoderma*. Berdasarkan empat percubaan yang telah dijalankan di dua tapak kajian, didapati bahawa tahap fosforus (P) yang lebih tinggi, dan tahap kuprum (Cu) dan zink (Zn) yang lebih rendah dan kekurangannya dalam kelapa sawit dikaitkan dengan serangan *Ganoderma* yang lebih tinggi. Sebagai tambahan, tahap yang lebih rendah dan kekurangan Cu dan Zn juga boleh dikaitkan dengan serangan *Ganoderma* yang lebih tinggi di Miri berbanding di Betong. Tambahan pula, tidak terdapat sebarang factor berbeza dari segi sifat-sifat kimia foliar dan tanah antara USR dan BSR. Oleh itu, penemuan ini boleh digunakan sebagai panduan umum untuk mengawal spesies *Ganoderma* dengan memberi perhatian yang lebih pada manipulasi P, Cu dan Zn tahap di ladang-ladang kelapa sawit secara amnya, dan khususnya di dua tapak kajian ini untuk pengurusan penyakit khusus kawasan seperti dalam pertanian persis.

ACKNOWLEDGEMENTS

In the name of God, the most gracious, the most merciful. My deepest gratitude goes to my respected supervisory committee chairperson, Assoc. Prof. Dr. Joseph Bong Choon Fah, and other members, namely, Dr. Khairulmazmi Ahmad and Dr. Idris Abu Seman for their expertise, advice, guidance, contributions, supports and encouragements during my study. I also would like to express my gratitude to my beloved family, especially my father, Mr. Mohd Rashid and mother, Mrs. Hasina for their prayers and endless love. Not to forget, thanks to all the supportive staffs in Universiti Putra Malaysia Bintulu Campus (UPMKB), Malaysian Palm Oil Board (MPOB), and Sarawak Oil Palm Berhad (SOPB). Special thanks to all my colleagues and friends for being my "backbone" throughout my study.

I also would like to thank the MPOB under the Malaysian Ministry of Plantation Industries and Commodities (MPIC) for financial assistance through research grant, and the Malaysian Ministry of Science, Technology and Innovation (MOSTI) for National Science Fellowship (NSF) scholarship award.

I certify that a Thesis Examination Committee has met on 10 March 2015 to conduct the final examination of Mohd Rakib bin Mohd Rashid on his thesis entitled "Epidemiology and Etiology of *Ganoderma* Upper and Basal Stem Rot in Oil Palm (*Elaeis guineensis* Jacq.) on Peat in Sarawak, 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 recommends that the student be awarded the Doctor of Philosophy.

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Associate Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Chairman)

Zainal Abidin bin Mior Ahmad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Julie Flood, PhD
Commonwealth Agricultural Bureau International (CABI)
United Kingdom
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Joseph Bong Choon Fah, PhD
Associate Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Chairman)

Khairulmazmi bin Ahmad, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Idris bin Abu Seman, PhD
Head of Ganoderma and Disease Research for Oil Palm Unit
Biology Research Division
Malaysian Palm Oil Board (MPOB)
(Member)

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Signature: _____
Name of Member of Supervisory Committee: Khairulmazmi bin Ahmad

Signature: _____
Name of Member of Supervisory Committee: Idris bin Abu Seman

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LIST OF ABBREVIATIONS

8-MOP	8-methoxypsoralen
AAS	Atomic absorption spectrometer
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
bp	Base pair
BSR	Basal stem rot
CIRP	Christmas Island Rock Phosphate
CPO	Crude palm oil
D×P	Dura x Pisifera or; Tenera
DI	Disease incidence
DNA	Deoxyribonucleic acid
DNMRT	Duncan's new multiple range test
DPO	Dual priming oligonucleotide
DSI	Disease severity index
FFB	Fresh fruit bunch
FMP	Fused magnesium phosphate
FSS	Foliar symptoms severity
GIS	Geographical Information System
GLM	General linear model
GPS	Global Positioning System
GSM	Ganoderma selective medium
IPM	Integrated pest management
kPa	Kilopascal
ME	Malt extract

MEA	Malt extract agar
MOP	Muriate of potash
MPOB	Malaysian Palm Oil Board
MVSP	Multivariate statistical package
NK Mix	Nitrogen and potassium mixture
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
R^2	Coefficient of determination
RAMS	Random amplified microsatellite
RAPD	Random amplified polymorphic DNA
RCBD	Randomised complete block design
RFLP	Restriction fragment length polymorphisms
RWB	Rubber wood block
SAS	Statistical Analysis System
SOA	Ammonium sulphate
SOPB	Sarawak Oil Palm Berhad
SSI	Spore shape index
UPGMA	Unweighted pair group method of arithmetic averages
UPMKB	Universiti Putra Malaysia Bintulu Campus
USR	Upper stem rot
WAI	Week after inoculation

CHAPTER 1

INTRODUCTION

1.1 Background

Oil palm (*Elaeis guineensis*) is the world's most important oil producing crop as it supplies 65.29 million tonnes, which is approximately 39% of the world vegetable oils and fats, and it is also the most efficient oil producing crop as compared with other major oil producing crops (Kurki *et al.*, 2006; USDA, 2014). Currently, Malaysia is one of the world's largest producers of palm oil contributing 30% towards world palm oil supplies (USDA, 2014). In Malaysia, the oil palm plantations reached 5.23 million hectares with an output of 19.32 million tonnes of crude palm oil (CPO), where the states of Sabah and Sarawak are the largest producers with recent trend showing rapid expansion of plantation on peat in Sarawak (MPOB, 2013). Hence, the utilization of peat lands in Sarawak for oil palm cultivation has become increasingly important (ASOS, 2011; Lim *et al.*, 2012).

The major threats to the sustainability of oil palm are pests and diseases. Currently, it is known that the most prominent disease in oil palm plantations across the Southeast Asia region is basal stem rot (BSR) which is caused by a pathogenic basidiomycete fungus, *Ganoderma boninense* (Flood *et al.*, 2000; Corley and Tinker, 2003; Rees *et al.*, 2012; Wong *et al.*, 2012). The disease reduced the yield and shortening the economic life of an oil palm. Moreover, *Ganoderma* species are also found to be closely associated with upper stem rot (USR) in oil palm (Hasan *et al.*, 2005; Pilotti, 2005; Rees *et al.*, 2012). The infection of *Ganoderma* in USR is on the upper portion of the stem or trunk of an oil palm, instead of at the base as in BSR.

Upper stem rot of oil palm in Malaysia has been noticed since 1937 and it was reported to usually occur on deep peat and inland valley soils. Upper stem rot is not considered as a major disease of oil palm at the earlier time (Thompson, 1937) but recently, USR has begun to gain more attention when a few cases of the disease were observed to severely infect the oil palm plantations with the presence of *Ganoderma* species in Sabah, Malaysia (Abdullah *et al.*, 1999), Papua New Guinea (Pilotti, 2005) and Indonesia (Rees *et al.*, 2012).

Over the years, researchers and oil palm planters have put much effort into the management strategies of the diseases caused by *Ganoderma* species through various cultural, chemical and biological aspects of the pest and host. Some of the disease control strategies are through the development of bio-control agents, disease resistant planting materials, methods of pesticides application, cover crops, fertilizers input, early detection of pathogens, and various aspects of estate sanitation (Chung, 2011; Cooper *et al.*, 2011; Hushiarian *et al.*, 2013; Naher *et al.*, 2013). However, until today days, *Ganoderma* species remains the major threats to the sustainability of oil palm.

1.2 Justification

Implementation of effective control strategies on the stem rot diseases and its causative agents in oil palm require for better understandings on the basic information of the diseases and pathogens. Such information include the etiological aspects in terms of symptomology and occurrence of the diseases, and species distribution and aggressiveness of the pathogens, and epidemiological aspects in terms of mode of disease spread and the environmental factors associated with the diseases.

Although it is commonly known that BSR is caused by *Ganoderma boninense*, little is known of the threats by other species of *Ganoderma* in oil palm plantations such as *G. zonatum* and *G. miniatocinctum* that are also implicated in BSR (Idris *et al.*, 2000a; Wong *et al.*, 2012). Moreover, both USR and BSR are associated with similar pathogens, but little is known about the distinctive characteristics between them. In addition, there is little information known about USR due to the lack of comprehensive studies on the disease in terms of epidemiological and etiological aspects. In some cases, USR is misinterpreted as BSR due to lack of information on the disease symptoms. The role of USR and other species of *Ganoderma* (i.e. *G. zonatum* and *G. miniatocinctum*) that may devastate the oil palm industry should not be overlooked by the researchers as well as the planters because in the long run this disease and pathogen may destroy the estates and affect oil palm industries seriously.

The infection mode of *Ganoderma* species in BSR were reported to initiate at the root of an oil palm, and the infection could spread to other healthy neighbouring palms through root-to-root contact (Flood *et al.*, 2005; Khairudin and Chong, 2008). However, the root-to-root infection mode is considered uncommon since *Ganoderma* species in oil palm plantations are genetically heterogeneous which suggests the spread of *Ganoderma* species through basidiospores (Miller *et al.*, 1999; Pilotti *et al.*, 2003; Sanderson, 2005). In addition, USR spread from ground level such as root-to-root contact and infection from inoculum source in the ground was obviously not possible. Hence, the infection modes of *Ganoderma* species are discussed in this study because it is crucial information for effective disease control strategies.

There were several reports on the aggressiveness of *Ganoderma boninense* of basal stem rot (BSR) in oil palms (Rees *et al.*, 2007; Sariah *et al.*, 2007; Chan *et al.*, 2011; Kok *et al.*, 2013). However, until recently, there was no serious attempt on comparative study of *G. boninense*, *G. zonatum* and *G. miniatocinctum*. Moreover, aggressiveness of *Ganoderma* isolates from USR-infected oil palms remains unknown. Difference in aggressiveness among *Ganoderma* species from different sources (USR and BSR) may play a vital role in the intensity of the diseases.

Furthermore, little was known about the status of the diseases and pathogens in Sarawak or Borneo since most of the previous reports were based on studies in Peninsular Malaysia and other countries (Turner, 1981; Ariffin *et al.*, 1989; Idris *et al.*, 2000a; Hasan *et al.*, 2005; Pilotti, 2005; Khairudin and Chong, 2008; Rees *et al.*, 2012). The state of Sarawak, which has the major distribution of peat lands in Malaysia has become an important area for oil palm cultivation (ASOS, 2011; Lim *et al.*, 2012). One of the major drawbacks of oil

palm cultivation on peat is nutrient unavailability due to nutrients fixation onto the organic matter (Fageria *et al.*, 2002; Hasnol *et al.*, 2011; Lim *et al.*, 2012) that may lead to unhealthy oil palm which later leads to increased susceptibility of disease infection (Marschner, 1995). Hence, one of the important environmental factors that could be related to the diseases epidemiology is the nutrients availability.

1.3 Objectives

The objectives of this research are to:

i. Investigate the genetic and morphological diversity of *Ganoderma* species associated with USR and BSR in oil palm.
ii. Evaluate the aggressiveness of *Ganoderma* species isolated from USR- and BSR-infected oil palms.
iii. Investigate disease symptoms, occurrence and spatio-temporal distribution of USR and BSR, and hotspot analysis of *Ganoderma* species of the diseases in oil palm.
iv. Investigate the relationship between oil palm nutrients status and infection by *Ganoderma* species in plantation on peat.

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BIODATA OF STUDENT

Mohd Rakib bin Mohd Rashid was born in 4th December 1985 in Beaufort, Sabah, Malaysia. He received his primary education in Sekolah Rendah Kebangsaan Kundasang Pekan Ranau in 1992, Sekolah Rendah Kebangsaan Tanjung Aru 2 in 1995, and Sekolah Kebangsaan Likas in 1996. In 1998, he pursued his secondary education in Sekolah Menengah Kebangsaan Likas. He completed his Sijil Tinggi Pelajaran Malaysia (STPM) in Sekolah Menengah All Saints, Kota Kinabalu in 2004. In the following year, he continued his higher education in Universiti Putra Malaysia Bintulu Campus and graduated with Bachelor of Science Bioindustry (Honours First Class) in 2009. Afterwards, he was enrolled in degree of Doctor of Philosophy in Plant Pathology since year 2009. During the postgraduate studies, he has published two impact factor journal articles, attended several international conferences inside and outside Malaysia, and enrolled in several academician part-time jobs.

LIST OF PUBLICATIONS

Journal Articles Published:

1. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2014. Genetic and morphological diversity of *Ganoderma* species isolated from infected oil palms (*Elaeis guineensis*). *International Journal of Agriculture and Biology* **16**: 691-699.

2. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2014. Occurrence and spatial distribution of *Ganoderma* species causing upper and basal stem rot in oil palm. *Journal of Food, Agriculture and Environment* **12**: 360-364.

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1. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. Aggressiveness of *Ganoderma boninense* and *G. zonatum* isolated from upper- and basal stem rot of oil palm (*Elaeis guineensis*) in Malaysia. *Journal of Oil Palm Research*.

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1. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2013. Genetic and morphological diversity of *Ganoderma* sp. from upper and basal stem rot infected oil palms. In: eds. Nadarajah, K., L.Y. Sze, G. Krishnasamy, F.D.A. Bakar, J. Santanam, M.J. Masarudin, N. Shahab, S.C. Chin, V. Sabaratnam, T.K. Lin and R.A. Rahim, *International Congress of the Malaysian Society for Microbiology*, 12-15 December 2013. Langkawi, Kedah, Malaysia, pp. 80-84.

2. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2014. Association of copper and zinc to *Ganoderma* spp. spatial distribution in oil palm (*Elaeis guineensis*) plantations on peat. In: *International Conference on Plant Physiology*, 26-28 August 2014. Kuta, Bali, Indonesia.

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