UNIVERSITI PUTRA MALAYSIA

AETHIOLOGY OF STEM CANKER PATHOGEN IN *Jatropha curcas* L. IN PENINSULAR MALAYSIA

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By

ROSLINA BINTI SULAIMAN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Salam kasih dan sayang buat:

Mak Ayah dan keluarga yang sangat memahami

dan mendoakan kejayaan.

&

Guru-guru yang tidak jemu menabur ilmu

serta

Kawan-kawan seperjuangan yang sama-sama

mencorakkan masa depan

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

AETHIOLOGY OF STEM CANKER PATHOGEN IN *Jatropha curcas* L. IN PENINSULAR MALAYSIA

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Chair: Ganesan Vadamalai, PhD

Faculty: Agriculture

Jatropha curcas is a multipurpose shrub that can be found throughout tropic and sub-tropic regions. It is a non-edible plant which is mainly cultivated for production of bio-diesel. This plant is not only tolerant to drought and salinity but it is also resistant to pests and diseases. However, new diseases on *J. curcas* have recently been reported recently and among them is stem canker disease. Stem canker disease caused by *Pestalotiopsis* sp. has been reported in India. Similar stem canker symptoms have also been observed in Malaysia but no research effort has been taken to identify the causal agent and the effect of the disease on *J. curcas* production. The purpose of this study was to identify and characterize the causal pathogen of stem canker on *J. curcas* in Malaysia using morphological and molecular characteristics and to confirm the pathogenicity of the causal agent. The study was carried out in four different phases which started with sampling, isolation, identification and pathogenicity test. Through sampling, the disease incidence was recorded and pure culture was isolated. Identification of pathogen was done through morphology and

molecular characterization using Internal Transcribed Spacer (ITS) regions of ribosomal deoxyribonucleic acid (rDNA). The pathogenicity test was then carried out to confirm the pathogenicity of the pathogen on *J. curcas*. This research found that the disease incidence of stem canker on *J. curcas* was high, from 10% to 80%. A total of 48 samples of stem with canker symptoms were collected from 8 locations in three states of Malaysia but only 27 isolates were used due to sporulating ability on artificial media. Morphological characterization of pure culture indicated that the colony was fast growing with fluffy white aerial mycelia which eventually turned to black when mature. The fungus conidia were two-celled, dark brown, thin cell walled, oval in shape and have longitudinal striations when mature, whereas immature conidia were hyaline, thick cell walled, smooth and one-celled. The average size of conidia was 23.63 µm (length) x 12.72 µm (width) while the average length/width ratio was 1.86 (n = 200). The morphological findings described the pathogen as *Lasiodiplodia theobromae.* Additionally, BLAST results indicated that all the 27 sequences were 99% to 100% identical to that of *L. theobromae*. Phylogenetic tree which grouped the 27 isolates in the same cluster with *L. theobromae* and distinct from other anamorph in genus *Botryosphaeria* proved that the isolate that caused stem canker is *L. theobromae*. The pathogenicity test undertaken using mycelial plugs showed symptoms as canker on *J. curcas* stem within a week after inoculation. The symptoms development included black necrotic lesions that appear sunken and as cracks on the bark and gradual plant wilting and eventually plant death. The pathogen was successfully re-isolated from lesions of inoculated stems, thus fulfilling Koch's postulates and confirmed that *L. theobromae* was indeed the causal agent of stem canker in *J. curcas* in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ETIOLOGI PATOGEN KANKER BATANG PADA *Jatropha curcas* L. DI SEMENANJUNG MALAYSIA

Oleh

ROSLINA BINTI SULAIMAN

Mei 2011

Pengerusi: Ganesan Vadamalai, PhD

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Jatropha curcas merupakan tumbuhan renek yang boleh didapati di kawasan tropika dan iklim sub-tropika. Tumbuhan ini tidak boleh dimakan dan hanya ditanam untuk penghasilan bio-diesel kerana ia tidak sesuai digunakan sebagai makanan. Tumbuhan ini bukan sahaja bertoleransi terhadap kekeringan dan kemasinan yang tinggi, tetapi ia juga mempunyai ketahanan terhadap serangan serangga perosak dan penyakit. Walaubagaimanapun, pelbagai penyakit baru telah dilaporkan sejak kebelakangan ini, antaranya ialah penyakit kanker pada batang. Penyakit kanker pada batang Jatropha yang disebabkan oleh *Pestalotiopsis* sp. telah dilaporkan di India. Simptom kanker batang yang juga turut didapati di Malaysia tetapi tiada kajian yang dijalankan untuk mengenalpasti agen penyebab dan kesan daripada penyakit tersebut pada pengeluaran *J. curcas*. Maka, kajian ini dijalankan untuk mengenalpasti dan mencirikan patogen yang menyebabkan penyakit kanker pada batang pokok Jatropha yang terdapat di Malaysia dengan menggunakan ciri-ciri kultur dan molekul serta mengesahkan kepatogenan agen penyebab tersebut terhadap pokok *J. curcas*. Penyelidikan dilakukan dalam empat fasa yang berbeza bermula dengan persampelan, pengasingan, pencirian dan ujian kepatogenan. Melalui persampelan,

kejadian penyakit direkodkan dan kultur tulen dihasilkan. Pengenalpastian patogen dilakukan melalui ciri- ciri morfologi dan serta ciri- ciri molekul menggunakan Internal Transcribed Spacer (ITS) dari asid deoksiribonukleik pada ribosom (rDNA). Ujian patogenisiti dilakukan untuk mengesahkan sama ada patogen yang telah diasingkan merupakan penyebab kanker batang yang sebenarnya. Penyelidikan ini mendapati bahawa insiden kejadian penyakit kanker batang pada pokok *J. curcas* adalah tinggi daripada 10% sehingga mencapai 80%. Sebanyak 48 sampel batang dengan simptom kanker dikumpulkan dari 8 lokasi yang berlainan di 3 negeri yang berbeza dan 8 lokasi dari tiga negeri di Malaysia tetapi hanya 27 pencilan yang digunakan sepanjang kajian berdasarkan kemampuan patogen tersebut mengeluarkan spora dalam media buatan. Ciri-ciri morfologi daripada kultur tulen menunjukkan bahawa patogen tersebut mempunyai kadar pertumbuhan yang cepat dengan miselia berwarna putih yang akhirnya berubah menjadi hitam apabila matang. Konidianya pula mempunyai dua sel, berwarna coklat, berdinding sel nipis, berbentuk oval dan mempunyai jalur membujur apabila matang, manakala konidia yang belum matang hanya mempunyai satu sel, berdinding sel tebal, berbentuk oval dan lut cahaya. Purata saiz konidia ialah 23.63µm (panjang) x 12.72µm (lebar) manakala nisbah panjang/lebar konidia ialah 1.86 (n = 200). Penemuan morfologi menunjukkan patogen ini sebagai *Lasiodiplodia theobromae*. Selain itu, amplifikasi tindak balas rantaian polimer (PCR) menggunakan primer ITS1 dan ITS4, menghasilkan jalur DNA bersaiz 500 bp. Keputusan kajian BLAST menunjukkan bahawa semua jujukan DNA adalah 99% hingga 100% menyamai *Lasiodiplodia theobromae*. Hubungan '*phylogenetic'* yang menghimpunkan 27 isolat di dalam kluster *L. theobromae* dan mengasingkannya daripada anarmorph lain dari genus *Botryosphaeria* membuktikan bahawa isolat yang menyebabkan kanker batang *J. curcas* adalah *L. theobromae*.

Ujian kepatogenan yang dilakukan dengan menggunakan plag miselia menghasilkan simptom seperti kanker pada batang *J. curcas* dalam masa seminggu selepas inokulasi. Simptom yang terhasil adalah luka nekrotik hitam yang kemudiannya menjadi cekung dan kelihatan seperti retak pada kulit pokok, seterusnya secara berperingkat menjadi layu dan akhirnya pokok tersebut mati. Kepatogenan berjaya disahkan apabila patogen berjaya dipencil kembali dari pokok yang telah diinokulat dan seterusnya memenuhi syarat-syarat postulat Koch dan menegaskan bahawa *L. theobromae* adalah agen penyebab kanker batang pada pokok *J.curcas* di Malaysia.

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I certify that an Examination Committee has met on 6th of May 2011 to conduct the final examination of Roslina Binti Sulaiman on her degree thesis entitled "Aetiology of Stem Canker on *Jatropha curcas* L. In Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science

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

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DECLARATION

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

ROSLINA BINTI SULAIMAN

Date: 6th of May 2011

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