UNIVERSITI PUTRA MALAYSIA

EFFECTS OF MECHANICAL WOUNDING AND INFECTION PATTERNS OF *Fusarium solani* ON GAHARU FORMATION IN *Aquilaria malaccensis* Lam.

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By

JONG PHAI LEE

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

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MASTER OF SCIENCE

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2012

SPECIALLY DEDICATED TO

My Beloved
Late Grandfather and Grandmother,
Jong San & Lee Moi Fah
Parents, Jong Siew Thong & Khoo Gek Hiang
Brothers & Sisters
and all my Friends...

'Thanks God for blessing me in all the aspects to be done successfully'...

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

EFFECTS OF MECHANICAL WOUNDING AND INFECTION PATTERNS OF *Fusarium solani* ON GAHARU FORMATION IN *Aquilaria malaccensis* Lam.

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Chair: Rozi Mohamed, PhD
Faculty: Faculty of Forestry

Gaharu is the oleoresin found in karas (*Aquilaria malaccensis*). Recently, many studies have been conducted in order to produce the most suitable and effective inocula in the market. Indeed, there is a need to produce more gaharu in karas tree especially in plantation field. Various fungi have been associated with gaharu formation, however their roles and patterns of colonization are not fully understood. In general, gaharu formation is associated with natural wounding or artificial inoculation by different fungi or chemicals. This study focused on the patterns of *Fusarium solani* colonization at different wounding time-point via real - time quantitative PCR (qPCR) technique and the effects of artificial inoculation on young karas trees as compared to naturally infected wounded karas in the wild. In addition, artificial inoculation technique with different fungal isolates were also aimed to determine their effects on producing gaharu. This was done by comparing their degree of discoloration formed over a period of 1, 3, 6 months respectively and the chemical constituents. Gaharu compounds produced from

both induction methods were identified and compared to commercial samples via GC-MS.

When tracking the amount of *F. solani* inocula in two wounded tree over time in the wild, the fungus was found to colonize wounded karas most frequently at the early stage of wounding, often most abundantly during the first 2 weeks. The highest amount of target DNA copies was quantified at 2 weeks in both investigated trees. This indicates that the infection of fungi might take place at the early stage of the wounding process. The decreasing in gene copy number detected in the latter stages, from 6 to 12 months showed that there was no evidence to state that fungi colonized more abundantly in darker wood. In fact, more resins were produced in order to form gaharu. The high level of terpenes were maintained to control the pathogen attacked. The presence of *F. solani* consistently at every wounding time - point, except for two points in one of the trees, revealed the association of this microbe to gaharu formation. However, its direct role in gaharu formation has to be further investigated, perhaps in a more controlled environment.

The study on fungal inoculation was carried out on young 4-year-old trees in a shade house conditions. Trees were harvested at 1, 3 and 6 months after inoculation. The discoloration on the wound and its intensity were measured. ANOVA analysis concluded that the five species of fungal inocula tested were not giving significant differences in terms of discoloration length and intensity. However, the length of discoloration and its intensity increased progressively from pale yellow to dark brown

over the time period tested. This implies that time plays an important role for producing gaharu-impregnated wood.

When analyzing gaharu compounds using GC-MS, a total of 39 compounds mainly from the group monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, chromone derivatives and fatty acids were identified. Samples analyzed were from commercial gaharu, artificial inoculation and mechanically wounded wood. From GC-MS profiles, all five commercial gaharu, SA, A, B, C and D grades had similar important compounds. Among them were (-)-Aristolene, Palustrol (derivatives of guaiene), γ. Eudesmol, (-)-Aristolene, α-Elemol, Anisylacetone, Benzaldehyde, Benzylacetone, 6-(Benzyloxy)-4,4-dimethyl-2- chromaone and Agarospirol. When compared to artificial inoculated wood, there were some important fragrant compounds found such as Guaiene, Benzenepropanoic acid,2,5-dimethoxy, Palustrol, Benzylacetone, Benzaldehyde, 6-(Benzyloxy)-4,4-dimethyl-2-chromaone. A new compound of 6-(Benzyloxy)-4,4-dimethyl-2- chromaone was detected in the wood sample and it was also known as 4,4-dimethyl-6-phenylmethoxy-3H-chromen-2-one.

In overall, the existence of these compounds proved that gaharu was formed in the fungal inoculated wood. This study demonstrated that fungal inoculation could produce gaharu in young trees in just 6 months time. Fungal invasion could be a method to prolong agression since it could indirectly increase resin production. However, this should be tested on a larger scale under field trial conditions.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

KESAN PENCEDERAAN MEKANIKAL DAN POLA JANGKITAN KULAT *Fusarium solani* KE ARAH PEMBENTUKAN GAHARU DALAM *Aquilaria malaccensis* Lam.

Oleh
JONG PHAI LEE

April 2012

Pengerusi: Rozi Mohamed, PhD
Fakulti: Fakulti Perhutanan

Gaharu merupakan sejenis resin yang terhasil pada pokok karas yang terjangkit. Baru-baru ini, banyak kajian telah dijalankan untuk menghasilkan inokula yang paling sesuai dan berkesan di pasaran. Sememangnya, terdapat keperluan untuk menghasilkan lebih banyak gaharu dalam pokok karas terutamanya di ladang tanaman. Pelbagai kulat dijangka berkait rapat dalam penghasilan gaharu tetapi pemahaman tentang peranan masing-masing dan arah pengkolonian adalah sangat tidak ketara dan jelas. Secara amnya, penghasilan dikaitkan dengan luka semulajadi atau inokulasi buatan atau penggunaan bahan kimia sebagai perangsang pada permukaan pokok karas. Kajian dalam tesis ini menumpu kepada kesa-kesan inokulasi buatan pada pokok karas muda yang dicucuk dengan pelbagai jenis kulat daripada pokok gaharu yang dijangkit secara semulajadi dan penurutan *Fusarium solani* dalam pelbagai sampel pokok yang dilukakan dengan menggunakan teknik PCR masa nyata. Selain itu, penghasilan bahan kimia minyak gaharu dalam kayu yang cedera dan sampel komersial telah dijalankan melalui analisis menggunakan kromatografi gas – spesifikasi jisim (GC-MS).

Apabila mengesan jumlah inokulum *F. solani* dalam kedua-dua pokok yang dicederakan dalam hutan, kulat didapati kepadatan menjajah kecederaan karas paling kerap di peringkat awal, paling banyak dalam tempoh 2 minggu pertama. Jumlah tertinggi salinan DNA sasaran adalah pada minggu kedua pada kedua-dua pokok yang dikaji. Ini menunjukkan bahawa jangkitan kulat mungkin berlaku pada peringkat awal proses lukaan. Penurunan dalam bilangan salinan gen yang dikesan di peringkat yang kemudian, 6 - 12 bulan menunjukkan bahawa tidak ada bukti yang menyatakan bahawa kulat dijajah lebih banyak pada kayu gelap. Hal ini adalah disebabkan penghasilan resin yang lebih banyak pada masa itu. Bahan kimia yang dikenali sebagai terpenes berperanan dalam mengawal jangkitan mikroorganisma pada pokok. Kehadiran *F. solani* yang konsisten pada setiap masa, mendedahkan penyatuan mikrob ini kepada pembentukan gaharu. Walau bagaimanapun, peranan kulat yang sebenar dalam pembentukan gaharu perlu lagi dikaji, mungkin dalam persekitaran yang lebih terkawal.

Kajian mengenai inokulasi kulat telah dijalankan ke atas pokok berumur 4-tahun-muda dalam keadaan rumah teduh. Pokok dituai pada 1, 3 dan 6 bulan selepas inokulasi. Perubahan warna pada luka dan intensiti warna diukur. Analisis ANOVA menyimpulkan bahawa lima spesies inocula kulat diuji tidak memberikan perbezaan yang ketara dari segi panjang perubahan dan intensiti warna. Walau bagaimanapun, panjang perubahan warna dan intensiti warna meningkat secara beransur-ansur dari kuning pucat ke perang gelap sepanjang tempoh masa yang diuji. Ini menunjukkan bahawa masa memainkan peranan yang penting dalam penghasilan gaharu.

Apabila menganalisis sebatian gaharu dengan menggunakan GC-MS, sejumlah 39 jenis kompaun terutamanya daripada kumpulan monoterpenes, sesquiterpenes, sesquiterpenes oksigen, derivatik chromone dan asid lemak telah dikenal pasti. Sampel yang dianalisis adalah daripada gaharu komersial, inokulasi buatan dan kayu cedera secara mekanikal. Daripada profil GC-MS, semua lima komersial gaharu, gred SA, A, B, C dan D mempunyai sebatian penting yang sama. Antaranya ialah (-)-Aristolene, Palustrol (derivatif guaiene), γ. Eudesmol, (-)-Aristolene, α-Elemol, Anisylacetone, Benzaldehyde, Benzylacetone, 6 - (Benzyloxy) -4,4-dimetil-2-chromaone dan Agarospirol. Apabila dibandingkan dengan kayu tiruan yang disuntik, terdapat beberapa sebatian penting wangi dijumpai seperti Guaiene, asid Benzenepropanoic,2,5-dimethoxy, Palustrol, Benzylacetone, Benzaldehyde, 6 - (Benzyloxy) -4,4-dimetil-2-chromaone. Satu sebatian yang baru iaitu 6 - (Benzyloxy) -4,4-dimetil-2-chromaone dikesan dalam sampel kayu dan juga dikenali sebagai 4,4-dimetil-6-phenylmethoxy-3H-chromen-2-one. Kewujudan sebatian ini membuktikan bahawa gaharu telah dihasilkan pada kayu yang disuntik kulat. Kajian ini menunjukkan bahawa inokulasi kulat boleh menghasilkan gaharu dalam pokok-pokok muda hanya dalam masa 6 bulan. Serangan kulat boleh menjadi satu kaedah untuk memanjangkan pencerobohan kerana ia secara tidak langsung dapat meningkatkan pengeluaran resin. Walau bagaimanapun, kajian terhadap inokulasi kulat perlu diuji pada skala yang lebih besar contohnya di kebun tanaman yang lebih luas.

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I certify that a Thesis Examination Committee has met on 26 April 2012 To conduct the final examination of Jong Phai Lee on her Master Thesis entitled "Effects of Mechanical Wounding and Infection Patterns of *Fusarium solani* on Gaharu Formation in *Aquilaria malaccensis* Lam.*"* 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 Master of Science.
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowleged. 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.

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JONG PHAI LEE

Date:

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LIST OF ABBREVIATIONS

Symbol	Description
ANOVA	analysis of variance
ASST	Allergic Stress Strain Technology
BLAST	Basic Local Alignment Research Tool
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
Ct	threshold cycle
DNA	deoxyribonucleic acid
dw	distilled water
EDTA	ethylenediaminetetraacetic acid
FID	flame ionization detection
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
ITS	Internal Transcribed Spacer
IUCN	International Union for Conservation of Nature
NIST	National Institute of Standards and Technology
NTC	non template control
PCR	polymerase chain reaction
PDA	potato dextrose agar
qPCR	quantitative polymerase chain reaction
RCBD	randomized complete block design
RNA	ribonucleic acid
ROX	6-Carboxyl-X-Rhodamine
SYBR	Sybregreen
TAE	tri acetate EDTA
UPM	Universiti Putra Malaysia
UV	ultraviolet

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