PATHOGENICITY STUDY OF *Ceratocystis acaiivora* and *Ceratocystis manginecans* ASSOCIATED WITH WILTING & DIEBACK DISEASES ON *Acacia mangium* AT ACACIA FOREST INDUSTRY (AFI), SABAH

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DECLARATION

I hereby declare that the materials in this thesis is my own except for quotations, citations, equations, summaries and references, which have been duly acknowledged.

06 April 2019

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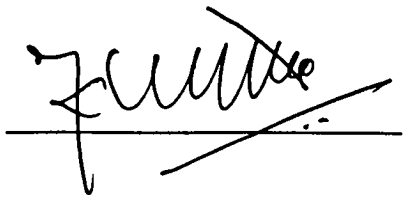

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ABSTRACT

A radical rise posting in the incidence of wilting and dieback diseases induced by plant pathogenic fungi *Ceratocystis* sp. in commercially planted *Acacia mangium* are major problem faced by industrial plantations such as Acacia Forest Industry (AFI) Plantation. Two types of *Ceratocystis* isolates; *Ceratocystis manginecans* (AFI/CM/001) and *Ceratocystis acaciivora* (AFI/CA/001) are recognized and collected from AFI Plantation in Pitas, Sabah. The general aim is to scientifically determine the immensity of *Ceratocystis* isolates to induce disease on plantation grown *A. mangium* using pathogenicity and interaction study. On this objective, field inoculation is done in the plantation nursery and interaction behavior is to be observed in-vitro. In field inoculation, 100 *A. mangium* seedlings were inoculated with *C. manginecans* and *C. acaciivora* to observe the ability of both fungi to induce diseases for 6 weeks. The efficacy study shows both fungi have capability to enhance lesion on *A. mangium* seedlings. *C. manginecans* isolate produced longer lesions (mean=47.103) compared to *C. acaciivora* isolate (mean=42.940), the difference is not significant (p=0.148, F=2.099). Yet, *C. acaciivora* showed a more linear growth in lesion increment compared to *C. manginecans* by weekly observation. Dual culture method was used to assess the ability of 6 test isolates which were identified during the isolation of infected *A. mangium* sample to control *C. manginecans* and *C. acaciivora*, which causes the wilting and dieback diseases. The highest percentage inhibition of radical growth (PIRG) values was observed with isolate KHES/AFI-UMS/D001; *Trichoderma* sp (60.00%; C. *manginecans* and 53.33%; *C. acaciivora*) and the lowest recorded (15.55%; C. *manginecans* and 21.67%; *C. acaciivora*) were observed with isolate KHES/AFI-UMS/E001; *Verticillium* sp. This study showed that isolate *Trichoderma* sp. has a good antagonistic effect on *C.manginecans and C. acaciivora* mycelial growth. Diseases developments from *C.manginecans and C. acaciivora* inoculated samples which suggesting that this fungus is the primary cause of the wilting of trees under natural conditions.

ABSTRAK

KAJIAN INTERAKSI & PATHOGENICITI Ceratocystis sp. BERHUBUNG DENGAN PENYAKIT LAYU & 'DIEBACK' PADA Acacia mangium DALAM PERLADANGAN INDUSTRI DI PITAS, SABAH

Peningkatan radikal dalam kejadian penyakit layu dan 'dieback' berleluasa yang disebabkan oleh kulat patogenik Ceratocystis sp. dalam Acacia mangium yang ditanam secara komersil adalah masalah utama yang dihadapi oleh perladangan industri seperti Acacia Forest Industries (AFI). Dua jenis isolat Ceratocystis; Ceratocystis manginecans (AFI / CM / 001) dan Ceratocystis acaciivora (AFI / CA / 001) dikenali dan dikutip dari ladang AFI di Pitas, Sabah. Objektif umum adalah secara saintifik menentukan potensi isolat Ceratocystis untuk menimbulkan penyakit pada pokok A. mangium yang menggunakan kajian patogenik dan interaksi. Untuk mengecapi objektif ini, inokulasi dilakukan di tapak semaian ladang dan tingkah laku interaksi diperhatikan 'in-vitro'. Semasa inokulasi, 100 anak pokok A. mangium telah diinokulasi dengan C. manginecans dan C. acaciivora untuk melihat keupayaan kedua-dua kulat untuk menggalakkan penyakit selama 6 minggu. Kajian menunjukkan kedua-dua kulat mempunyai keupayaan untuk meningkatkan 'lesion' pada anak pokok A. mangium. C. manginecans telah menghasilkan 'lesion' yang lebih panjang (min = 47.103) berbanding dengan isolat C. acaciivora (min = 42.940), perbezaannya tidak signifikan (p=0.148, F=2.099). Walau bagaimanapun, C. acaciivora menunjukkan pertumbuhan yang lebih nyata dalam peningkatan 'lesion' berbanding dengan C. manginecans oleh pemerhatian mingguan. Kaedah 'dual culture' digunakan untuk menilai keupayaan 6 'test isolate' yang telah diisolat daripada sampel A. mangium yang telah jangkitan untuk mengawal C. manginecans dan C. acaciivora, yang menyebabkan tanda-tanda penyakit berleluasa. Kadar 'percentage inhibition of radical growth' (PIRG) tertinggi diperhatikan dengan isolat KHES / AFI-UMS / D001; Trichoderma sp. (60.00%; C. manginecans dan 53.33%; C. acaciivora) dan rekod terendah (15.55%; C. manginecans dan 21.67 %; C. acaciivora) diperhatikan dengan isolat KHES / AFI-UMS / E001; Verticillium sp. Kajian ini menunjukkan bahawa isolat Trichoderma sp. mempunyai kesan antagonistik yang baik terhadap pertumbuhan mycelial C. manginecans dan C. acaciivora. Perkembangan penyakit daripada isolat C.

manginecans dan C. acaciivora mencadangkan bahawa kulat ini adalah penyebab utama pokok-pokok di bawah keadaan semula jadi.

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LIST OF SYMBOLS

%	Percentage
n	number of
m	meter
mm	millimeter
g	gram
ml	milliliter
µm	micrometer
°C	degree Celsius
e.g	for example
etc.	Et cetera/ and so forth

LIST OF ABBREVIATIONS

A. mangium	*Acacia mangium*
A. auriculiformis	*Acacia auriculiformis*
C. manginecans	*Ceratocystis manginecans*
C. acaciivora	*Ceratocystis acaciivora*
AFI	Acacia Forest Industries Sdn. Bhd.
SAFODA	Sabah Forest Development Authority
SSSB	Sabah Softwoods Sdn. Bhd.
SFI	Sabah Forest Industry
SPF	Sarawak Planted Forest Sdn. Bhd.
SFC	Sarawak Forest Cooperation
BCA	Biological Control Agent
MEA	Malt Extract Agar

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CHAPTER 1

INTRODUCTION

1.1 Background Study

Plantation forestry is being the backbone to the world's future timber supply and it is important that they are managed well from production, environmental, social and economic perspectives. Forest plantation is established because timber from natural forest are depleting due to over exploitation, illegal logging and deforestation (Intachat *et al.*, 1999). Natural forest need to be protected as it is playing important role in balancing ecosystem, serves as habitat for flora and fauna and act as water catchment area.

The importance of forest plantation is to supply timber or raw material continuously for wood manufacturing industry such as furniture, saw timber, pulp and paper. Forest plantations meet demand for timber and timber products in the country or outside the country for industrial uses is increasing. Apparently in Malaysia alongside with the government sectors, private organizations also interested in forest plantation management such as IOI Group, Sime Darby Berhad, Genting Plantations Berhad, Sarawak Planted Forest Sdn. Bhd. (SPF), Sarawak Forest Cooperation (SFC), Sabah Forest Development Authority (SAFODA), Sabah Softwoods Sdn.Bhd. (SSSB), Forestry Department and Sabah Forest Industry (SFI).

There are several tree species are planted widely in plantation industry in Malaysia such as *Azadirachta excelsa*, *Hevea brasiliensis*, *Eucalyptus* sp., and also *Acacia mangium*. The selection of the species for example *A. mangium* was due to the characteristics such as it can grow well in tropical region, easily adapt to the environment and also can be harvested in short period (Krisnawati *et al.*, 2011).

Acacia mangium is a single-stemmed evergreen tree or shrub that grows to 25-35 m in height. Young trees have smooth, greenish bark that fissures begin to develop at 2-3 years. Bark in older trees is rough, hard, fissured near the base, greyish-brown to dark brown, inner bark pale brown. *Acacia mangium* starts to flower and produce seeds 18-20 months after planting. Mature fruits occur 3-4 months after planting period. The density of *A. mangium* is one of the factors the tree chosen in plantation which ranges from 290 kg m^{-3} to as high as 675 kg m^{-3} at 14 years- old trees and 15% moisture content (Lim *et al.*, 2011). These light hardwood properties makes the wood suitable for wide range of products.

In West Malaysia, *Acacia* trees are not widely planted as in East Malaysia. In Sabah, *A. mangium* was introduced to plantation forestry in 1966 by D. I. Nicholson. In 1967, *A. mangium* grown trees were planted as a fire break (Ito & Nanis, 1997). An agency called Sabah Forest Development Authority was then inaugurated in 1976 to establish forest plantations on land degraded by shifting cultivation and logging with *A. mangium*. The plantation occurred in the interior Keningau and also northern part of Sabah (Bengkoka, Pitas). Then, many organizations were very interested in cultivating *A. mangium* on their own for the production of timber, chips and pulp.

Acacia Forest Industries (AFI) Sdn. Bhd., is a joint venture company signed in 2003 between Serisar Forest Plantation and Products Sdn. Bhd. (SFPP) and SAFODA (Chong, 2011). This venture was formed to plant and replant an area of 25, 000 ha with *Acacia* forests in Bengkoka, Pitas, Sabah, which was initially filled with *Imperata cylindrical*. The seeds of *A. mangium* were bought from Queensland. In order to replant after harvesting, they had set up a nursery to grow their seedlings. There were approximately 0.3 million seedlings grown in the nursery AFI (pers. comm., Pn. Najjah Salfinas) for each rotation.

Past few years have been unpropitious times for *Acacia* forest plantation in Malaysia due to diseases outbreak of *Ceratocystis* wilting and dieback, in the same way, cases also had been reported in all over forest plantation world-wide such as Brazil, Vietnam, Indonesia and Myanmar (Roux & Wingfield, 2009). This situation

creates concern to forest plantation companies as it gives both international and local wood market an alternative to the dwindling supply of tropical hardwood from natural forest and rubber wood.

The increasing demand of *Acacia* wood in the manufacturing industry contributes to the company's turnover. Unfortunately, AFI management had to face economic losses in the past few years due to infection by *Ceratocystis* sp. on its *A. mangium* plantation. 90% of the planted *A. mangium* tree showed wilting and dieback symptoms and more than 50% mortality was recorded on the infected *A. mangium* trees (pers. comm., Pn. Najjah). Therefore, it had serious impact on raw material supply.

The preliminary results showed that wilting and dieback symptoms in *Acacia* trees were caused by a fungi *Ceratocystis* sp. The fungi, *Ceratocystis* act as the primary pathogen in *Acacia* tree symptoms which eventually causes mortality. According to Taringan *et al.*, 2011, *Ceratocystis* fungi enable to enter the tree via the pinhole made by the nitiludid beetle, the insect vector. Then, the pathogen colonizes the non-living xylem vessels and form propagules and metabolites that are transported up in the transpiration stream. The pathogen and its metabolites occupy the host xylem tissues. It is only when the plants become moribund that the pathogens invade the xylem parenchyma, medullary rays, phloem and cortex (Chandniwala, 2010). Moreover, dieback symptoms become visible due to presence of the fungal metabolites.

This study was done to show a scientific proof of the ability of *Ceratocystis* fungi to produce diseases on *A. mangium* seedlings. This study will allow AFI to produce good seedlings and it also helps in addressing the problem faced. Besides that, interaction study was done to identify antagonistic effect between *Ceratocystis* fungi and other fungi. Fungi tend to extend its growth to capture its substrate for its resources. This study also opens a pathway to find a solution to overcome the mortality of young seedlings caused by fungi and pests.

1.2 Problem Statement

The economic importance of the disease was significant. When *A. mangium* stand population was reduced, yield directly reduced. Furthermore, replanting and recruiting *A. mangium* increased the cost of the plantation management. Fungus infection that caused plant diseases was a major problem for plantation industry which effected from the nursery. In AFI, they needed a vast supply of seedlings for its transplanting and reforestation programs.

Therefore, maintaining a nursery involved high cost which included buying the seeds, maintaining the seedlings, paying labours, maintenance cost for nursery and etc. It costed about RM 7.00 to raise a seedling and there are 300, 000 seedlings in that plantation. About 90% of the yield of *A. mangium* trees was affected by wilting and dieback symptoms. Therefore, approximately 270, 000 trees were infected and need to be eradicated, that is about RM 2 million loss of revenue.

It was a serious problem to AFI as raw material supply is decreasing and economic deficiency occurs. This urged its research team to study on the symptoms. Due to the huge loss of revenue, the causal agents of the diseases need to be identified. Two types of *Ceratocystis* fungi were procured in plantation trees which caused mortality was assessed through experimental inoculations in seedlings to learn the vigor of both fungi.

This study was essential for development of the *A. mangium* plantation industry towards its treatment. Identifying the relationship and behavior was important for future research in AFI to develop a potential treatment to overcome the disease outbreak in their future timber plantation industry. Therefore, a suitable isolate be identified in order to reduce the pest and disease problem which caused a huge loss in revenue.

1.3 Research objective

The research was conducted with the following objectives:

i. to assess the causal organism that causing wilting and dieback symptoms of *A. mangium*

ii. to identify the pathogenicity activity of *Ceratocystis* fungi on *A. mangium* in the plantation industry and

iii. to examine *Ceratocystis* bio-interaction with other fungi isolates as biological control.

CHAPTER 2

LITERATURE REVIEW

2.1 Plantation Forestry

There are two types of plantation forestry namely traditional and exotic plantation. The traditional plantation forestry plants indigenous species. It is commonly found in countries such as Europe, Japan, and North America. Currently, plantation forest involves planting exotic species. This type of plantation forest is commonly found subtropical regions of South America, Africa, and majorly in Asia. These are fast-growing commercial species such as pines from North America or eucalyptus from Australia (Sedjo, 1999).

In 1966, D.I. Nicholson was the first to introduce *A. mangium* into Sabah from Queensland and planted in the Ulu Kukut and Gum-Gum areas. Its rapid growth rate, high yielding capacity and adaptability to degraded grassland were noticed during the 70s. This led to extensive planting of *A. mangium* in Sabah and subsequently in Peninsular Malaysia as well. In 1976 the Sabah Forestry Development Authority (SAFODA), a constitutional body, was set up. SAFODA's task was to reforest the nearly 200,000 ha of degraded lands that resulted from bad shifting cultivation activity (Sudin *at el.*, 1993).

In the process SAFODA was further aiming at providing additional employment in the rural areas. In the 1980s more commercial forest plantation ventures were established. The first pulp and paper mill was set up in Sipitang by the Sabah Forest Industries (SFI). SFI expects to reforest up to 130,000 ha with industrial forest plantations, mainly with *Acacia mangium.*

In Sabah, although the reforestation and tree planting started much later than in Peninsular Malaysia, overall they have performed better in total hectares

planted. In addition, the private sector has been instrumental in leading the planting of timber trees.

Initially, many plantations involved simply the planting of seedlings with minimal subsequent management beyond protection. In later rotations, however, more intensive management has often been applied during the growing period. In the southern United States, for example, recent studies have shown that the productivity and financial returns to additional management can be substantial (Yin, 2001).

Major sources of increased productivity and returns in forestry have been breeding programs and vegetative control at the time of planting. Although planted forests are not new, the transition of forestry from foraging to an agricultural cropping mode has been underway on a truly global scale only within the past half century or less (Sedjo, 1999).

2.2 *Acacia mangium*

Acacia species are among the most common species in the plantation industry in Malaysia. *Acacia* species such as *Acacia mangium, A. auriculiformis* and *Acacia* hybrid are fast growing plantation species. There are used for greenery purposes other than timber production in the tropical countries (Krisnawati *et al.*, 2011). The properties of the plantation species seem to vary much more than that of naturally grown species. The commercially planted species, *Acacia* is marginally durable when especially exposed to extreme condition (Wahab, *et al.*, 2011).

The current name is *Acacia mangium*. It is authorities as wild from family Fabaceae – Mimosoideae. Common names that are found are; (English) : black wattle, brown salwood, hickory wattle, mangium, sabah salwood; (Filipino) : maber; (Indonesian) : mangge hutan, nak, tongke hutan; (Malay) : mangium; (Polynesia) : arr; (Spanish) : zamorano; (Thai) : kra thin tepa, krathin-theph (Awang & Taylor, 1993).

Acacia mangium is a single-stemmed evergreen tree or shrub that grows to 25-35 m in height (Krisnawati *et al.*, 2011). Young trees have smooth, greenish

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