CHARACTERIZATION OF *Paecilomyces dactyletromorphus* OF GUMMOSIS DISEASE ON *Albizia saman* AND ITS IN-VITRO FUNGICIDE EVALUATION

By

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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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Albizia saman (Jacq) Merr is a native species from northern South America and Central America, cultivated and naturalized throughout the tropics. The plant was initially planted in Malaysia and later introduced to urban areas in the 1900s. Over the past years, a problem gummosis disease followed by the gradual tree failure of the affected trees has been observed in various parts of the urban environment in Malaysia. *A. Saman* showed gummosis symptoms, which were observed and investigated. The obvious symptoms of the infected *A. saman* trees are trunk discoloration, exudation of gummosis from the infected area, crusty exudate when dry, sap-feeding beetle, abnormal cavities on the trunk, cracked, sunken, and splits on the bark. This study attempts to explore the causal agents (pathogens) that cause the gummosis disease of *A. saman*. The studies are conducted to assess the tree health of *A. saman* in urban areas by identifying the pathogen that causes the symptoms, testing out the pathogenicity, and determining the best fungicide to control the disease. Isolation of fungi from gummosis and pest samples of affected raintrees revealed one fungal isolate to be the most dominantly and consistently isolated fungus. The causal agent of gummosis disease in the rain trees was identified using a DNA sequence-based method (*ITS* and *β-tubulin*) and morphology identification by scanning electron microscope (SEM) microscope and a compound microscope. The results show that *Paecilomyces dactylethromorphus* was the causal agent and fulfilled Koch's postulates through a pathogenicity test. The result of the pathogenicity test revealed that there was a similarity in symptoms that arise between artificial inoculation and natural symptoms in the field. The effects of incorporating different concentrations (0.125%, 0.250%, 0.50%, 1.00%, and 2.00%) of each of the five fungicides (dimethomorph, thiram, copper hydroxide, mancozeb, and prochloraz manganese chloride) in 2% PDA medium were tested in vitro at the laboratory for 7 days and have shown that prochloraz manganese chloride fungicide with a concentration of 1.0% and 2.0% caused total inhibition of Paecilomyces growth. From this study, the susceptibility of fungal infections was due to injuries from anthropogenic and natural causes, and the application of fungicide of thiram mixture at 2.0 % can help control the disease at the field level.

Keywords: *Albizia saman*, Fungicide, Gummosis disease, Paecilomyces, Urban tree

SDG: GOAL 11: Sustainable Cities and Communities

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

CIRI-CIRI *Paecilomyces dactyletromorphus* PENYAKIT GUMMOSIS PADA *Albiza saman* DAN PENILAIAN IN-VITRO RACUN KULAT

Oleh

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Albizia saman (Jacq) Merr adalah spesies asli dari Amerika Selatan Utara dan Amerika Tengah, yang ditanam dan dinaturalisasikan di seluruh kawasan tropika. Tumbuhan ini mula ditanam di Malaysia dan kemudian diperkenalkan ke kawasan bandar pada tahun 1900-an. Sepanjang beberapa tahun kebelakangan ini, masalah penyakit gummosis diikuti dengan kematian pokok secara beransur-ansur telah diperhatikan di pelbagai bahagian kawasan bandar di Malaysia. *A. saman* menunjukkan gejala gummosis, yang telah diperhatikan dan dikaji. Gejala jelas pokok *A. saman* yang dijangkiti diikuti dengan perubahan warna batang, eksudasi gummosis dari kawasan yang dijangkiti, eksudat berkerak apabila kering, kumbang pemakan sap, rongga abnormal pada batang, retakan, lekukan, dan pecahan pada kulit kayu. Kajian ini bertujuan untuk meneroka agen penyebab (patogen) yang menyebabkan penyakit gummosis pada *A. saman*. Kajian dilakukan untuk menilai kesihatan pokok *A. saman* di kawasan bandar dengan mengenal pasti patogen yang menyebabkan gejala, menguji keupayaan patogen tersebut, dan menentukan racun kulat terbaik untuk mengawal penyakit ini. Pengasingan kulat dari sampel gummosis dan perosak pada pokok hujan yang terjejas mendedahkan satu isolat kulat yang paling dominan dan konsisten diasingkan. Agen penyebab penyakit gummosis pada pokok hujan dikenalpasti menggunakan kaedah berasaskan urutan DNA (*ITS* dan *β-tubulin*) serta pengecaman morfologi melalui mikroskop imbasan elektron (SEM) dan mikroskop kompaun. Hasil kajian menunjukkan bahawa *Paecilomyces dactylethromorphus* adalah agen penyebabnya dan memenuhi postulat Koch melalui ujian keupayaan patogen. Keputusan ujian keupayaan patogen menunjukkan persamaan gejala yang timbul antara inokulasi tiruan dan gejala semula jadi di lapangan. Kesan penggabungan kepekatan berbeza (0.125%, 0.250%, 0.50%, 1.00%, dan 2.00%) daripada setiap lima racun kulat (dimethomorph, thiram, copper hydroxide, mancozeb, dan prochloraz manganese chloride) dalam medium PDA 2% diuji secara in vitro di makmal selama 7 hari dan menunjukkan bahawa racun kulat prochloraz manganese chloride dengan kepekatan 1.0% dan 2.0% menyebabkan perencatan sepenuhnya terhadap pertumbuhan Paecilomyces. Daripada kajian ini, kerentanan terhadap jangkitan kulat adalah disebabkan oleh kecederaan daripada sebab antropogenik dan semula jadi, dan aplikasi campuran racun kulat thiram pada 2.0% boleh membantu mengawal penyakit ini di peringkat lapangan.

Katakunci: *Albizia saman*, Paecilomyces, Penyakit gummosis, Pokok bandar, Racun kulat

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AQ	Automated Quantitative
β-tub/BT2	Beta tubulin
BDB	Bacterial Dieback Disease
BMP	Best Management Practice
BLASTN	Basic Local Alignment Search Tool Nucleiotide
CAA	Carboxylic Acid Amide
ClO_2	Chlorine Dioxide
CO_2	Carbon Dioxide
DBH	Diameter Breast Height
DI	Disease Incidence
DNA	Deoxyribonucleic Acid
EBI	Ethylene bisisothiocyanate
EBDC	Ethylene Bisdithiocarbamate
EBIS	Ethylene bisisothiocyanate sulphide
EC50	Effective Concentration 50%
EFSA	The European Food Safety Authority
EPA	Environmental Protection Agency's
FRAC	Fungicide Resistance Action Committee
GPS	Global Positioning System
ISA	International Society of Arboriculture
ITS	Internal transcribed spacer
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National Center for Biotechnology Information
NTP	National Transformation Program
PDA	Potato Dextrose Agar

PCR	Polymerase Chain Reaction
QTRA	Quantified Tree Risk Assessment
RGI	Radial Growth Inhibition
SEM	Scanning electron microscope
SSR	Sclerotinia Stem Rot
UPM	Universiti Putra Malaysia
USDA	United States Department of Agriculture
°C	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Albizia saman (Jacq) Merr is an exotic or introduced species in Malaysia. The species originated from South America and was introduced to Malaya, now Peninsula Malaysia, in 1876 (Mohmod et al., 2020). According to (Mohmod et al., 2020), the species belongs to the Fabaceae family and has the synonym names *Samanea saman*, *Enterolobium saman*, and *Inga saman*, as well as the common names in Malaysia called "Hujan-Hujan" and "Pokok Pukul Lima". Additional information from the same publication stated that it has dark green bipinnate compound leaves that will contract at dusk or evening, pod-shaped fruits, and pinkish flowers (Figure 1).

Figure 1.1: Pinkish flowers of Albizia saman with dark green bipinnate compound leaves

Figure 1.2: A healthy rain tree (*Albizia saman*) with an elegant crown, big trunk, and branches.

Other characteristics would be that it can grow up to 25 meters in height and have a broad umbrella and dome-shaped crown, making it a preferred species for urban planting as a shade tree, landscaping tree, roadside tree, heritage tree, avenue tree, ornamental tree, and amenity tree. It is also preferable because of its fast growth in challenging environments such as urban ones at the roadside, parks, parking lots, private property, and walkways (Figure 2). Other than being planted in an urban environment, several authors reported that *A. saman* has a wide range of economic importance, including prospective manufacturers of tannins for industrial use, fodder for wild cattle, timber and fuel wood, and soil improvement with nitrogen fixation (Mukuma, 2016).

In Malaysia, *A. saman* has been reported to be infected by *Fusarium semitectum*, weakening the infected parts (Terhem et al., 2021). A weakened part or parts, if severe, may cause tree failure. Based on a thesis submitted in India, there are reported diseases found for *A. saman* (Hossain, 2004). Damping-off disease caused by *Pythium spp*., *Fusarium spp*., *Rhizoctonia solani*, and other soil-dwelling fungi (Figure 3). Collar rot disease caused by *Rhizoctonia bataticola*, leaf spot caused by *Colletotrichum sp*., stem canker and gummosis caused by *F. semitectum*, seedlings powdery mildew caused by *Erysiphe communis*, wilt disease caused by *Hendersonula toruloidea.*

Figure 1.3: Damping off disease caused by A) *Phytium spp.* B) *Fusarium spp.* C) *Rhizoctonia solani.*
(Source: Matny, 2012; Kilany et al, 2015; Keinath, 2022)

Plant pathological assessment of urban trees in terms of tree health in Malaysia is extensive but only limited studies reported on diseases associated with the insect pest of *A. saman*. This could be due to the vast number of urban trees planted with native and exotic (then localized) species such as *Hopea odorata*, *Syzygium sp*., *Tabebuia argentia*, and *Swietenia macrophylla*. The lack of reported diseases indicates the knowledge gap about *A. saman* diseases in comparison to other urban tree species, however, the need for the identification of insect-borne diseases particularly fungal attacks and infestation remains important (Ye et al., 2021).

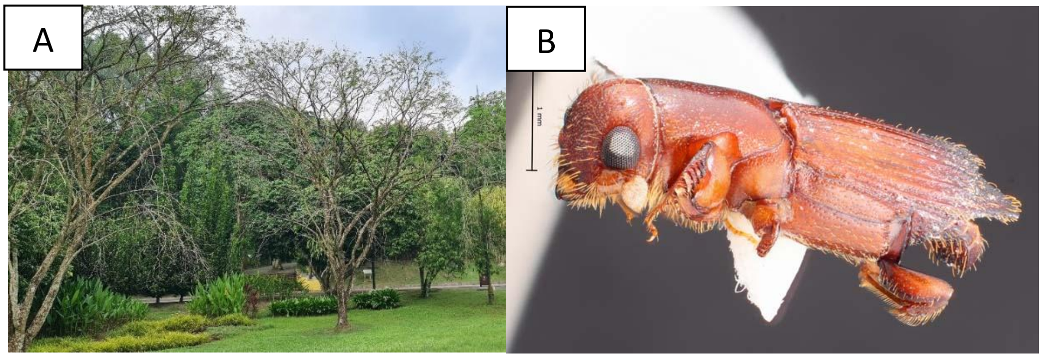

Figure 1.4: Pterocarpus indicus attacked by A) *Fusarium oxysporum* B) *Euplatypus paralellus*
(Source: Atkinson, 2024)

Malaysia's urban tree diseases are susceptible to diseases, and multiple reports have identified tree diseases as being caused by fungus pathogens. According to Philip (1999) Angsana tree (*Pterocarpus indicus*) has been infected with vascular wilt disease caused by the pathogen of *Fusarium oxysporum* (Ascomycota; Hypocreales) with symptoms of wilting leaf and root rot and eventually, the dead trees are invaded by insect pest *Euplatypus paralellus* (Coleoptera; Curculionidae) (Figure 4). Other diseases caused by fungus pathogens have also been reported in urban tree parks where *Alternaria sp.* (Ascomycota; Pleosporales), *Fusarium sp*. (Ascomycota; Hypocreales), and *Penicillium sp.* (Ascomycota; Eurotiales) extensively infected *Michelia alba*, *Lagerstroemia speciosa*, *Shorea multiflora*, and *Hopea odorata* causing leaf blotch and leaf scorch (Wei Chen et al., 2020). According to Terhem et al (2021), *Fusarium semitectum* was identified as a pathogen causing canker disease on a raintree which may cause structural damage (Figure 5). The genus Fusarium is known to be an extensive plant pathogen, while some can be host-specific.

Figure 1.5: ***Albizia* saman was infected by *Fusarium semitectum***
(Source: Terhem et al, 2021)

In terms of insect-borne fungus pathogens, the Ambrosia beetle (*Xylosandrus sp*.) is known to be a strict Fusarium fungus farmer (Figure 6). The beetles usually infest vulnerable trees by boring holes into the stems of the trees thus allowing the Fusarium fungus to spread and grow on the walls of the *Xylosandrus sp*. galleries (Agnello et al., 2017). Studies conducted have shown that fungal isolates from these *Xylosandrus spp*. are plant pathogens of the genus Ophiostoma, Fusarium, Phomopsis, Ambrosiella, and Raffaelea, however, their pathogenicity remains untested (Contarini et al., 2020; Gugliuzzo et al., 2021).

Figure 1.6: ***Xylosandrus sp*** **lateral view (top), dorsal habitus (bottom)**
(Source: Marchioro et al, 2022)

1.2 Problem statement

Albizia saman is a tree species planted in Malaysia's urban areas for years for its function and the aesthetic value it gives. The tree’s health is expected to suffer because of a disease that attacked most of the trunks and barks of the trees. The failure of trees will cause harm to its surroundings and facilities such as pedestrian areas, parking lots, and buildings. The spreading of the diseases infected will also destroy biodiversity and the area’s aesthetic value. Therefore, it is considered necessary to know the causative factors of the disease and the biology of the causative pathogen so that further attempts can be made to prevent and/or control the disease from further spreading. Furthermore, by offering the necessary knowledge to combat the diseases, this study would help extend the lifespan of already-existing rain trees in addition to guaranteeing the safety of the trees themselves, as well as the people and property beneath these trees. It is possible to build customized solutions to guarantee *A. saman*’s survival and continuous resilience by exchanging research and practices. These trees need a very high standard of arboricultural care, along with a thorough awareness of their existing circumstances, to prevent insufficient and ill-informed mismanagement that could endanger the trees. Thus, the outcomes might offer a thorough and impartial foundation to support management and decision-making. Recognizing their potential for development and benefit could yield trustworthy data to advise decisions about their restoration or conservation.

1.3 Research objectives

Thus, the general objective of this study was to investigate the development and extent of *A. saman's* gummosis disease.

The specific objectives of this research were:

i. To identify the causal agents (pathogens) that cause the gummosis disease of *Albizia saman*.
ii. To perform the pathogenicity test of isolated pathogens.
iii. To determine the in-vitro response of the pathogen to various fungicides.

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