# EVALUATION OF FUNGICIDES AS PROTECTIVE AND CURATIVE TREATMENTS AGAINST CANKER DISEASE OF *Eucalyptus urograndis* IN SARAWAK, MALAYSIA

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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**July 2024**

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**Faculty : Forestry and Environment**

Plantations of the genus Eucalyptus L'Hér have rapidly expanded in Sarawak, Malaysia, accounting for 19% of the total forest plantation area. In a routine forest health surveillance conducted in 2022 at Sarawak, Malaysia, tree stands of the hybrid between *Eucalyptus grandis x Eucalyptus urophylla* hybrid better known as *Eucalyptus urograndis* were detected with symptoms of stem canker disease caused by *Chrysoporthe* infection. Given the limited information on the chemical control of *Chrysoporth*e stem canker disease, there is a growing need to develop effective chemical control strategies to protect and cure *Chrysoporthe* infection on *E. urograndis* trees. Therefore, this study aimed to identify the causal pathogen of this stem canker disease in *E. urograndis* stands in Sarawak, Malaysia, and evaluate the efficacy of various fungicides as curative and protectant treatments on canker infection using artificial inoculation methods. All isolates were first collected and subjected to molecular identification and pathogenicity analysis. The inhabitation efficacy of five licensed fungicides: thiram, prochloraz manganese chloride, copper hydroxide, dimethomorph, and mancozeb against the disease pathogen was evaluated through an in vitro test. The performance of these fungicides was then assessed through protective and curative field experiments on 10-year-old *E. urograndis* trees using the artificial inoculation technique. The isolates were identified as *Chrysoporthe deuterocubensis* based on morphology and through comparisons of DNA sequence data from the ITS1/ITS4, β-tubulin 2 gene regions. Isolate CHRY18 recorded the highest virulence based on the pathogenicity test. The in-vitro efficacy tests showed that prochloraz manganese chloride achieved 100% inhibition against *C. deuterocubensis* at 1.0 mg/mL. Thiram significantly inhibited C. deuterocubensis infection in the protective experiment, yielding the shortest lesion length (19.40 mm) compared to the non-treated control (47.48 mm) at 20 weeks post-inoculation. In the curative experiment, a reduction of 54.7% in lesion length was observed in inoculated symptomatic trees after 20 weeks of post-fungicide treatment by copper hydroxide. In conclusion, this study demonstrated that the combination of prochloraz manganese chloride and copper hydroxide could be utilised an as effective chemical control of *C. deuterocubensis* stem canker on *E. urograndis*.

**Keywords:** Forest Plantation, Stem Canker Disease, Chrysoporthe deuterocubensis, Evaluation of Fungicides, Preventive and Curative

**SDG:** Goal 14: Life on Land

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

**PENILAIAN FUNGISID SEBAGAI RAWATAN PERLINDUNGAN DAN KURATIF TERHADAP PENYAKIT KANKER *Eucalyptus urograndis* DI SARAWAK, MALAYSIA**

Oleh

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Ladang hutan yang berasaskan tanaman pokok *Eucalyptus* telah berkembang pesat di Sarawak, Malaysia, mencakupi 19% daripada jumlah keseluruhan kawasan ladang hutan di Malaysia. Melalui kerja pemantauan tahap kesihatan ladang hutan yang dijalankan secara berkala pada tahun 2022 di Sarawak, Malaysia, didapati dirian pokok hybrid *Eucalyptus grandis x Eucalyptus urophylla* dikenali sebagai *Eucalyptus urograndis* telah dikesan dengan gejala penyakit kanker batang yang disebabkan oleh jangkitan kulat pathogen *Chrysoporthe*. Memandangkan maklumat terhad tentang pengurusan penyakit kanker batang *Chrysoporthe* melalui kawalan kimia, terdapat keperluan yang semakin meningkat untuk membangunkan strategi kawalan kimia yang berkesan untuk melindungi atau menyembuhkan jangkitan *Chrysoporthe* pada pokok *E. urograndis*. Maka, kajian ini bertujuan untuk mengenal pasti patogen penyebab penyakit kanker batang pada pokok *E. urograndis* yang berumur 7 tahun di Sarawak, Malaysia, dan menilai keberkesanan pelbagai racun kulat

sebagai rawatan penyembuhan dan atau pelindung terhadap penyakit kanker *Chrysoporthe* ini melalui kaedah inokulasi buatan. Pertamanya, kerja persampelan kulat penyebab penyakit dilapangan, pemencilan kulat, pengecaman dan pengenalpastian kulat dilakukan melalui pemerhatian pada struktur morfologi dan teknik molecular dan dipastikan secara analisis patogenik. Kemudian, ujian penyaringan lima racun kulat berlesen: thiram, prochloraz manganese chloride, copper hydroxide, dimethomorph, dan mancozeb terhadap pencilan kulat *Chrysoporthe* dijalankan dalam persekitaran makmal (in vitro). Selepas itu, kajian prestasi racun kulat untuk pencegahan dan penyembuhan pada pokok *E. urograndis* berusia 10 tahun telah dijalankan menggunakan teknik inokulasi buatan. Berdasarkan struktur morfologi dan analisis filogenetik ITS1/ITS4, β-tubulin 2 (BT2), dan gabungan jujukan ITS1/ITS4 dan BT2 yang diekstrak daripada 20 pencilan kulat maka *Chrysoporthe deuterocubensis* dikenal pasti sebagai patogen penyebab penyakit kanker, dengan kulat CHRY18 merekodkan virulen tertinggi. Ujian keberkesanan in vitro menunjukkan bahawa prochloraz manganese chloride mencapai perencatan 100% terhadap *C. deuterocubensis* pada 1.0 mg/mL. Dalam eksperimen secara pencegahan, thiram menghalang jangkitan *C. deuterocubensis* dengan ketara, menunjukkan saiz lesi terkecil (19.40 mm) berbanding kawalan tidak dirawat (47.48 mm) pada 20 minggu selepas inokulasi. Dalam eksperimen penyembuhan, pengurangan ketara sebanyak 54.7% dalam saiz panjang lesi diperhatikan dalam pokok bersimptom yang diinokulasi selepas 20 minggu rawatan selepas racun kulat dengan copper hydroxide. Kesimpulannya, kajian ini menunjukkan *prochloraz manganese chloride* dan *copper hydroxide* boleh digabung pengunaannya dan berpotensi sebagai kawalan kimia yang berkesan dalam mengawal penyakit kanker batang *C. deuterocubensis* pada *E. urograndis*.

**Kata Kunci:** Ladang Hutan, Penyakit Kanker Batang, *Chrysoporthe deuterocubensis*, Penilaian Racun Kulat, Pencegahan dan Pemulihan

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| BLAST | Basic Local Alignment Search Tool |
| CABI | Centre for Agriculture and Bioscience International |
| CFPP | Compensatory Forest Plantation Project |
| CHRY | *Chrysoporthe* |
| CRD | Completely Randomised Design |
| CWDE | Cell Wall Degradative Enzymes |
| DBH | Diameter Breast Height |
| DNA | Deoxyribonucleic acid |
| FDPM | Forestry Department of Peninsular Malaysia |
| FRIM | Forest Research Institute Malaysia |
| ha | Hectares |
| IDM | Integrated Disease Management |
| IFRC | Industrial Forest Research Centre |
| ITS | Internal Transcribed Spacer |
| LPF | License Planted Forest |
| MEA | Malt Extract Agar |
| ML | Maximum-likelihood |
| MPIC | Ministry of Plantation and Commodities |
| MTIB | Malaysian Timber Industry Board |
| MUSCLE | Multiple Sequence Comparison by Log Expectation |
| NA | North America |
| NCBI | National Center for Biotechnology Information |
| PCR | Polymerase Chain Reaction |

| | |
|---|---|
| PRFs | Permanent Reserve Forests |
| rDNA | Ribosomal DNA |
| RGI | Radial Growth Inhibition |
| RMG | Relative Mycelial Growth |
| SE | Standard Error |
| SFM | Sustainable Forest Management |
| UPM | Universiti Putra Malaysia |

# CHAPTER 1

# INTRODUCTION

## 1.1 General Introduction

The region of Sarawak, located in East Malaysia at the northwest of Borneo has a landmass of approximately 12.4 million hectares of which 7.7 million hectares or 62 per cent is under forest cover. Considering the decline of natural forest resources, since 1997 Sarawak State government has been developing industrial forest plantations that are governed by the Planted Forests Rules, 1997; Forests (Nursery) Rules, 2017; and Revised Policy Direction on Industrial Forest Plantation 2020, targeting 1 million hectares to be planted by 2025 (Abd Latif et al., 2018; Ratnasingam et al., 2020) aim to fulfil the increasing demands for timber and to further reduce pressure and dependency on the natural forests.

Sarawak has successfully expanded its industrial tree plantations to 566,083 hectares at current. The planters in Sarawak, known as the License Planted Forest (LPF), prefer to plant three exotic tree species, namely *Acacia mangium* Willd., *Falcataria moluccana* (Miq.) Barneby & J.W. Grimes (batai), and *Eucalyptus pellita* F. Muell (Ambrose et al., 2022). These three tree generas are preferred because they can produce seeds easily and can be stored for years without losing their viability. Additionally, the trees have fast-growing traits, which means they can be harvested between 5 to 8 years for wood chips, pulp and paper, veneer and even sawn timber.

However, the expansion of *A. mangium* tree plantations in Malaysia especially in Sabah has been declining over the years following the attack of *Ceratocystis* spp that caused stem-wilt canker disease which rapidly reduced the overall volume production of *A. mangium* (Tarigan et al., 2010) This development has prompted the replacement of *A.mangium* with *Eucalyptus pellita* and its hybrid namely *Eucalyptus urograndis* (a hybrid of *Eucalyptus urophylla* x *Eucalyptus grandis*), and *Acacia crassicarpa* A. Cunn. ex Benth (Lee, 2018) as the main species planted in Sabah and Sarawak as these species are reported to be more tolerant and/or resistant traits to *Ceratocystis* spp. disease pathogens.

However, in Sarawak, a study undertaken by (Ambrose et al., 2022) deduced that the incidence of Ceratocystis wilt and canker disease remains relatively low at 7.5 %, indicating poor virulence but the risk of virulence may increase over successive rotations (Syazwan et al., 2021), which might have reflected the scares numbers of study in evaluating the incidences of this disease nationwide, especially in a vast area of Sarawak.

*Eucalyptus* is a genus belonging to the myrtle family of Myrtaceae and is native to Australia. The interest in growing *Eucalyptus* as one of the fast-growing timber species is apparent in the equatorial region, subtropical, and temperate regions, with over 22.57 million ha of planted areas across 95 countries, equivalent to 30% of the global forest plantation area (Naidoo et al., 2014;

Zhang & Wang, 2021). The hybrid of *Eucalyptus grandis* × *E. urophylla* is a successful interspecific hybridisation of *Eucalyptus* that was first initiated in Brazil back in the 1980s, one of the pioneer nations that propelled the *Eucalyptus* breeding strategies of clonal forest production to a global height (Colodette et al., 2014; de Oliveira Castro et al., 2021; Rezende et al., 2014) ). *Eucalyptus urograndis* is one of the most preferred hybrids to be planted in Malaysia (Zuhaidi Yahya, 2020) for various end-product utilisations, such as solid wood, charcoal, pulp and paper, given its advantageous properties, including rapid growth and yield that can exceed an average of >40 $m^3$/ha/year, greater resistance to pest and disease attacks, exceptional rooting ability, and excellent wood quality (de Oliveira Castro et al., 2021; Rezende et al., 2014).

Despite the future outlook of *Eucalyptus* as a promising alternative plantation tree in Malaysia, there were concerns raised regarding their susceptibility and vulnerability against disease threats globally, ranging from the most common leaf diseases caused by *Mychosphaerella* species and *Teratosphaeria* species (Hunter al., 2011; Mitchell et al., 2011; Silva & Asiegbu, 2023), Ceratocystis wilt disease caused by *Ceratocystis* species (Chen et al., 2013; Roux et al., 2020), Myrtle rust (*Puccinia psidii*) disease (Carnegie, 2015; Yong et al., 2021), and stem canker disease caused by *Cryphonectriaceae* (Gryzenhout et al., 2004; Pegg et al., 2010; Rauf et al., 2020) and *Botryosphaericeae* (Chen et al., 2013; Li et al., 2022)

*Chrysoporthe* stem canker is an infection caused by the genus *Chrysoporthe* Gryzenh. and M. J. Wingf, a vital genus in the family *Cryphonectriaceae*

(Diaporthales, Ascomycota) (Chen et al., 2010; Gryzenhout et al., 2004; Soares et al., 2018). The degree of pathogenicity of this disease has ranged from the ability to cause mortality in *Eucalyptus* tree stands less than 2 years old to altering the stem structures making it weaker, deformed, and cracked and subsequently reducing the volume and vitality of overall tree stands (Chen et al., 2010).

Recently, *Chrysoporthe deuterocubens*is and *Chrysoporthe cubensis* were found to inflict stem canker disease in *E. urograndis* in Malaysia and based on a pathogenicity test conducted by (Rauf et al., 2020), *C. deuterocubensis* can infect *E. grandis* and *E. deglupta.* (Ambrose et al., 2022) described this canker pathogen exhibits symptoms of swelling and cracking of the stems from the basal area that could spread up to the upper branches of the infected trees, and able to inflict mortality on juvenile trees.

## 1.2 Problem Statement

*Eucalyptus* species have gained significant interest from tree planters in Malaysia due to their potential to be a valuable resource for both timber and fibre industries (Seng Hua et al., 2022). It has a desirable trait of fast-growing characteristics, the ability to grow and varying degrees of adapting to different and harsh environments; from extreme drought to cold temperature tolerance to drought and high wood density (Gallo et al., 2018; Mphahlele et al., 2020). However, the sustainability of this plantation tree species in Malaysia is under threat from various disease pathogens, notably the stem canker disease caused by *C. deutrocubensis*.

This pathogen attacks the *E. urograndis* tree by forming cankers, which will girdle the stem destroying the cambium tissue entirely, stem to decay, wilting and death of the infected trees, especially juvenile trees (Guimarães et al., 2010). The presence of the pathogen in Sarawak is particularly a concern across the forest plantation industry, where it has the potential to significantly impact productivity with the wood quality of infected trees lower in density and poorer strength to the point where it is unusable for lumber and timber-based products (Dahali et al., 2022). In this study, there will be an evaluation of the impact of this disease on the overall plantation especially on the trees affected where this disease is a new incidence that emerged in Sarawak but was detected earlier at Sabah.

Relative aggressiveness or the virulence trait among *C. deuterocubensis* isolates could pose a major challenge in managing this stem canker disease. Highly pathogenic isolates can lead to severe disease outbreaks, decreasing wood quality, tree mortality and economic loss (Tarigan et al., 2024). The variation in virulence in different isolates may respond differently to fungicides and other management strategies, as well as to different types of tree clones. Therefore, the study of pathogenicity could underline a more targeted and effective treatment in optimising disease management strategies to mitigate the disease impact.

The current management procedures for controlling *Chrysoporthe* stem canker include excising the canker, cutting down and burning the trees, or simply leaving it alone, depending on the severity of the disease. Thus, the sustainability of *Eucalyptus* spp. and the overall forest plantation sector hinges on recognising emerging infectious diseases and incorporating effective chemical management strategies with advanced silvicultural practices. To date, there is limited information on the chemical control of *C. deuterocubensis* stem canker disease incorporating field experiments on the efficacy of fungicides through protectant and curative modes against these species, with most of the available research mainly focusing on characterising the species, such as taxonomy, host range, and geographic distribution (Awing et al., 2023; Gryzenhout et al., 2004; Li et al., 2022; Rauf et al., 2020).

Given the lack of study on chemical control and the need to develop chemical strategies for protection and wound healing of *E. urograndis* trees from *Chrysoporthe* infection, this study aimed to determine the canker disease-causing pathogens and evaluate the efficacy of a range of fungicide products through curative and protectant treatments on canker infection of *C. deuterocubensis* using artificial inoculation methods.

Hypothetically, at least one of the various currently available fungicides in Malaysia is significantly more effective than the others in inhibiting the growth of *C. deuterocubensis* on *E. urograndis* and could be applied in the long-term Integrated Disease Management (IDM) strategies of *Eucalyptus* and its hybrids in Malaysia.

## 1.3 Research Objectives

The objectives of this research are aimed:

a) To identify the causal pathogen associated with stem canker diseases *of Eucalyptus urograndis* trees in Sarawak;

b) To assess the pathogenicity among the isolates of the identified causal pathogen;

c) To conduct an in vitro evaluation of a range of fungicides available in Malaysia against the identified causal pathogen and;

d) To evaluate the efficacy of a range of fungicides available in Malaysia through curative and protectant treatments on canker infection of the causal pathogen using the artificial inoculation method.

## 1.4 Significance of Study

The establishment of *Eucalyptus* plantations in Malaysia back in the 1980s was halted as the areas were wiped out due to attacks of fungal diseases but the interest in planting this species is growing again due to the development of successful plantation areas in China and Vietnam (Zaiton et al., 2020). However, with the increasing numbers of disease pathogens infection and insect pest infestation that have contributed to the failure of plantation forestry globally, it is important to monitor, observe and examine potential outbreaks that can relapse the failure of this species again in future.

Evaluation of the impact of this disease also needs to be conducted and needs to be discussed at all levels with the stakeholders, policymakers, timber players, researchers and planters as this will prompt the strategies to control the issues for future management. Thus, it is imperative to conduct intensive studies to understand the problems and to provide key data for the policyholder in catering to new management strategies to overcome any possible factors to prevent any upcoming disease pathogens outbreak.

In this study, *Chrysoporthe* spp which has been widely actively infected and caused canker disease in *Eucalyptus* trees stands in plantations forestry in Sarawak, Malaysia was identified. In response to that, disease management which will specifically focus on fungicides that can act as a protection against the pathogen, and to cure or reduce the severity of the disease was established in this study.

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