UNIVERSITI PUTRA MALAYSIA

ISOLATION, IDENTIFICATION & PATHOGENICITY TEST OF CAUSAL AGENT OF DIEBACK-LIKE SYMPTOM OF PLUMERIA SPP.

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By

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DEPARTMENT OF PLANT PROTECTION

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ENDORSEMENT

This project entitled " Isolation, identification and pathogenicity test of causal agent of dieback-like symptom of *Plumeria* spp." is prepared by Norshahadib Bin Anuar and submitted to Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science.

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LIST OF ABBREVIATION

PDA	Potato Dextrose Agar
NA	Nutrient Agar
LCB	Lactophenol Cotton Blue
BLAST	Basic Local Search Alignment Tools
APHA	American Public Health Association
MUG	4-methylumbelliferyl-β-D-glucuronide
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
rDNA	ribosomal Deoxyribonucleic Acid
rRNA	ribosomal Ribonucleic Acid
°C	Degree Celcius
ITS	Internal Transcribed Spacer
EF1-α	Elongation factor 1-alpha
IGS	Intergenic Spacer Region
CTAB	Cetyl trimethylammonium bromide

EDTA	Ethylenediaminetetraacetic Acid
TE	Tris EDTA
PVP	Polyvinylpyrrolidone
NaCl	Sodium Chloride
rpm	Revolution per minute
UV	Ultra-violet
kb	kilobase
Rep	Replications
MP	Maximum Parsimony
Cm	Centimeter
m	meter
g	grams
ml	mililiter
inch	inches
M	Molar
mM	milimolar
µl	microliter
%	Percentage

ABSTRACT

Plumeria spp. is an ornamental tree in the family Apocynaceae that can grow up to 9m tall and it is natively found in tropical America. The strong scent of their flowers is the main attraction in the landscape area. However, this ornamental tree can be infected by several diseases such as the stem rot, black tip rot, leave rusts and dieback. Based on some related reports, dieback may be caused by fungus from Botryosphaeriaceae family. In Malaysia, there is no report found about the causal agent of dieback on *Plumeria* spp. even though the symptom has been observed and less case reported in other countries. In this research, the objectives were 1) to identify the causal agent of dieback on *Plumeria* spp and 2) to conduct pathogenicity test to verify the causal agent. The infected samples were collected from landscape area in Tropicana Height in Kajang, Selangor on May 2016 to July 2017. The pathogen was isolated and cultured on Potato Dextrose Agar (PDA) medium. The causal agent of dieback on *Plumeria* spp. was confirmed by Koch's Postulate method using 15 healthy stem cuttings of *Plumeria* spp at 4-6 weeks old by re-isolating the pathogen in PDA media. For objective 1, the fungal pathogen was identified through morphological and molecular characteristics. From morphological analysis, the fungus was grayish to black in color and turned out to be dimorphic in culture. The conidia were ellipsoid, translucent and mix with paraphyses ranging from 3.5-5 x 6.5-12 µm in size and arranged in arthric chains. Meanwhile, for molecular analysis the culture samples were successfully extracted and amplified by PCR using ITS1 and ITS4 primers to produce about 1000 basepairs (bp). The PCR products were sent for sequencing and identified by using

Basic Local Alignment Tool (BLAST). From BLAST analysis, it has about 971 nucleotide sequences for ITS1. The ITS1 gene has 99% of identity and 95% of the query covered for the sequence. Based on morphological characteristics and ITS sequence region, the pathogen was identified as *Neoscytalidium dimidiatum*. For objective 2, pathogenicity test was successfully done and shows that both plant species of *P. rubra* and *P. obtusa* shows the same severity when inoculated with this pathogen in duration of two weeks. It can be concluded that this fungus is the main pathogen that cause dieback-like symptom on this plant species. Generally, this is the first report of dieback disease on *Plumeria* spp. caused by *Neoscytalidium dimidiatum* in Malaysia.

ABSTRAK

Plumeria spp. adalah sejenis pokok hiasan yang tergolong di dalam keluarga Apocynaceae yang boleh membesar setinggi 9m dan ia secara asalnya dijumpai di kawasan tropikal Amerika. Bau bunganya yang kuat menjadi tarikan utama di kawasan landskap. Walaubagaimanapun, pokok hiasan ini boleh dijangkiti oleh beberapa penyakit seperti reput batang, reput pangkal hitam, mati rosot dan karat daun. Berdasarkan beberapa laporan yang berkaitan, penyakit mati rosot (*dieback*) mungkin disebabkan oleh kulat daripada keluarga *Botryosphaeriaceae*. Di Malaysia, tiada laporan yang menyatakan ejen penyebab penyakit dieback pada *Plumeria* spp. walaupun simptom telah banyak diperhatikan dan kurang kes dilaporkan di negara-negara lain. Dalam kajian ini, objektif utama adalah 1) untuk mengenalpasti ejen penyebab *dieback* pada *Plumeria* spp. dan 2) menjalankan ujian patogenisiti untuk mengesahkan ejen penyebab tersebut. Sampel yang ada simptom jangkitan telah dikumpulkan di kawasan taman lanskap sekitar Tropicana Heights di Kajang, Selangor pada Mei 2016 – Julai 2017. Kulat pathogen dipencil dan dibiakkan di atas medium Potato Dextrose Agar (PDA). Agen penyebab *dieback* pada *Plumeria* spp. telah disahkan dengan kaedah Koch's Postulate menggunakan 15 keratan batang *Plumeria* spp di usia 4-6 minggu dengan mengasingkan semula pathogen di atas medium PDA. Untuk mencapai objektif 1, patogen telah dikenalpasti dengan kaedah morfologi dan molecular. Melalui analisis secara morfologi, kulat ini adalah berwarna kelabu kehitaman dan adalah dimorfik dalam kultur. Konidia berbentuk ellipsoid, lutsinar dan bercampur dengan paraphyses yang bersaiz antara 3,5-5 x 6,5-12 µm dan tersusun

secara dalam rantaian *arthric*. Sementara itu, untuk analisis molekular, sampel telah berjaya diekstrak dan diamplifikasi oleh PCR menggunakan ITS1 dan ITS4 primers dan menghasilkan kira-kira 1000 basepairs (bp). Produk PCR telah dihantar untuk penjujukan dan dikenalpasti dengan menggunakan *Basic Local Alignment Tool* (BLAST). Menurut analisis BLAST, ia mempunyai kira-kira 971 urutan nukleotida untuk ITS1. Gen ITS1 mempunyai 99% daripada identiti dan 95% daripada pertanyaan meliputi untuk urutan nukleotida. Berdasarkan ciri-ciri morfologi dan rantauan urutan ITS, pathogen ini telah dikenalpasti sebagai *Neoscytalidium dimidiatum.* Untuk objektif ke-2, ujian patogenisiti telah berjaya dilaksanakan dan menunjukkan bahawa kedua-dua spesis pokok, *P. rubra dan P. obtusa* terjangkit dengan serius dalam masa dua minggu. Kesimpulanya, kulat ini adalah merupakan patogen utama yang menyebabkan simptom seakan *dieback* pada spesis pokok ini. Umumnya, ini adalah laporan pertama penyakit *dieback* pada *Plumeria* spp. yang disebabkan oleh *Neoscytalidium dimidiatum* di Malaysia.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Plumeria spp. has a wide contribution and importance to economy as it's provides medicinal values and culturally can be used to create drums, bowls and furniture from its wood (Eggenberger and Eggenberger 2005). For example, the latex produced by the tree is mix with coconut oil can be utilized to treat itch, rheumatism, and gum troubles (Farooque et al. 2012). However, this plant species can also infected by several diseases that can affect the quality of the plant itself such as stem rot, black tip rot, leave rusts and dieback.

During May 2016 to July 2016, stem cutting samples of *Plumeria spp*. with dieback-like symptoms were randomly sampled in Tropicana Heights, Kajang located in Selangor, Malaysia with disease incidence exceeding 90% in some severely affected landscape areas reported by Nerissa Chu, staff of the landscape area. The dieback symptom was observed in the infected plants including the rusts of leaves but, in this study dieback of *Plumeria* spp. was observed as primary observation. Since there is no research has been done regarding the causal of dieback on *Plumeria* spp. in Malaysia,

therefore, a pathogenicity test and observation for dieback of *Plumeria* spp. were conducted in the laboratory by taking infected samples to be examined.

1.2 Problem statement

In Malaysia, there is no report found about the causal agent of dieback on *Plumeria* spp. even though the symptom has been observed and less case reported in other countries.

1.3 Hypothesis

1. The dieback of *Plumeria* spp. may be caused by fungus in family of Botryosphaeriaceae because many of these fungi cause canker and dieback diseases on wide range of plant hosts over the world (von Arx, 1987; Burgess et al.,2006; Slippers and Wingfield, 2007).

1.4 Objectives

1. To identify the causal agent of dieback on *Plumeria* spp.
2. To conduct pathogenicity test to verify the causal agent.

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