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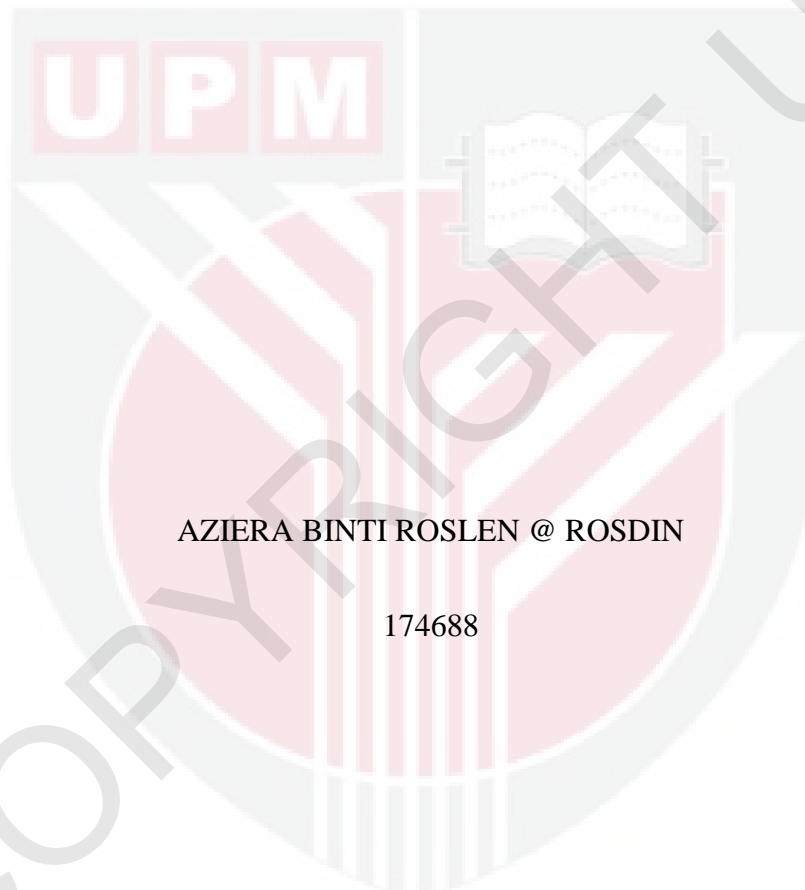
***MORPHOLOGICAL IDENTIFICATION AND MOLECULAR  
CHARACTERIZATION OF *Podosphaera xanthii* CAUSING  
POWDERY MILDEW DISEASE ON ZIGZAG PLANT  
(*Pedilanthus tithymaloides*)***

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**FP 2017 25**

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UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR DARUL EHSAN

2016/2017

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*(Pedilanthus tithymaloides)*

BY

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A project report submitted to the Faculty of Agriculture  
Universiti Putra Malaysia

In fulfilment of the requirement of PRT 4999 (Final Year Project)

For the award of the degree of  
Bachelor of Horticultural Science

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

2016/2017

This project report entitled Morphological Identification and Molecular Characterization of *Podosphaera xanthii* Causing Powdery Mildew Disease on *Pedilanthus tithymaloides* (Zigzag Plant) is prepared by Aziera Binti Roslen @ Rosdin and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT 4999 (FINAL YEAR PROJECT) for the award of the degree of Bachelor Horticultural Science.

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## ACKNOWLEDGEMENT

I would first like to thank my thesis advisor, Dr. Siti Izera binti Ismail, lecturer at the Department of Plant Protection, Faculty of Agriculture at University Putra Malaysia. The door to Dr. Siti Izera office was always open whenever I ran into problems or had a question about my research or writing. She consistently allowed this paper to be my own work, but steered me in the right the direction whenever he thought I needed it.

I take this opportunity to express gratitude to all of the Department faculty staff members for their help and support. My sincere thanks to En. Mohamed Nazri bin Abdul Rahman, En Johari bin Mohd Sarikat, Pn Asmalina binti Abu Bakar for their assistances in getting materials used in this final year project.

I would also like to thank my parents for their wise counsel and sympathetic ear. You are always there for me. Finally, there are my friends. We were not only able to support each other by deliberating over our problems and findings, but also happily by talking about things other than just our final year project.

Thank you.

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## LIST OF ABBREVIATIONS

	ABBREVIATIONS	EXPLANATION
1.	µm	Micrometre
2.	%	Percentage
3.	µl	Microliter
4.	°C	Degree Celsius
5.	bp	Base pairs
6.	Mg	Milligrams
7.	kb	Kilobyte
8.	DNA	Deoxyribonucleic acid
9.	ITS	Internal transcribed spacer
10.	PCR	Polymerase chain reaction
11.	rDNA	Ribosomal deoxyribonucleic acid
12.	RNA	Ribonucleic acid
13.	TAE	Tris-acetate-EDTA

## ABSTRACT

*Pedilanthus tithymaloides* (Euphorbiaceae; known as 'Zigzag plant', 'Jacob's ladder,' 'Devil's Backbone') is a shrub perennial and succulent spurge. Powdery mildew is a common disease on ornamental plants and vegetables production such as cucurbits plants. These fungi have been reported to cause losses due to its important pathogen. It can reduce an ornamental value if Zigzag plant is grown in a garden or nursery. The identification of obligate parasite has not always been made accurately. Recently, taxonomic concepts for powdery mildew fungi use molecular DNA analyses for accurate identification at the species level. The fungus from symptomatic tissues was identified as *Podosphaera xanthii* based on morphological characteristics and rDNA Internal Transcribed Sequence (ITS) analysis. Leaves of Zigzag plant were covered with white fungal mycelia, abundant powdery mildew conidia, with the ultimate development of necrosis on symptomatic tissues. Conidia were hyaline, ellipsoid, produced in long chains (seven to eleven conidia per chain), and approximately 32.5 µm long and 20.1 µm wide. Conidiophores were erect, with a foot cell straight or slightly flexuous, approximately 240 µm in long. It had fibrosin body structures. However, chasmothecia were not observed on a surface of the infected leaves. Conidial DNA was subjected to polymerase chain reaction (PCR) using universal primers designed to amplify internal transcribed spacer (ITS) region of *Podosphaera xanthii*. ITS5-Forward and ITS4-Reverse were universal primers used in this study. The size of amplicons was approximately 580 bp. The ITS sequence of powdery mildew was sequenced and ITS sequence data indicated 100% homology to the ITS sequences of *Podosphaera xanthii* infecting *Cynanchum auriculatum* (KU711923) and *Kalanchoe blossfeldiana*

(KR049082). On the basis of morphological characteristics and ITS sequence analysis, pathogen infecting the leaves of *Pedilanthus tithymaloides* was identified as *Podosphaera xanthii*.



## ABSTRAK

*Pedilanthus tithymaloides* (Euphorbiaceae; dikenali sebagai pokok Zigzag, pokok 'Jacobs ladder', pokok 'Backbone Devil'). Kulapuk berdebu merupakan pokok tumbuhan jenis renek dan sejenis pokok herba yang bergetah. Kulapuk berdebu adalah penyakit biasa pada tanaman hiasan dan sayur-sayuran pengeluaran seperti tumbuhan di dalam kelas *Cucurbit*. Kulat ini menyebabkan kerugian dan merupakan patogen tumbuhan penting kerana boleh mengurangkan nilai hiasan pokok apabila Pokok Zigzag ini ditanam dalam taman atau nurseri. Pengenalpastian bagi obligat parasit kulapuk berdebu selalunya tidak dibuat dengan tepat. Kini, konsep taksonomi untuk kulat menggunakan kaedah molikul DNA untuk ketepatan pengenalpastian pada spesies kulat obligat parasit. Berdasarkan pada ciri-ciri morfologi kulat dan juga turutan rDNA Internal Transcribed Spacer (ITS) analisa, tisu simptomatik daripada gejala kulat telah dikenalpasti sebagai *Podosphaera xanthii*. Daun pada pokok Zigzag diselaputi dengan miselia kulat putih, konidia kulapuk berdebu. Oleh disebabkan penyakit kulapuk berdebu, pokok mengalami pertumbuhan nekrosis pada tisu yang bersimptom. Konidia adalah hialin, ellipsoid, terbentuk dalam rantai panjang (tujuh hingga sebelas konidia setiap rantai) dan mempunyai kepanjangan sebanyak 32.5  $\mu\text{m}$  dan lebar pada 20.1  $\mu\text{m}$ . Konidiopora adalah tegak, dengan sel kaki lurus atau sedikit bengkok, anggaran 240  $\mu\text{m}$  kepanjangan. Kulat ini mempunyai badan fibrosis. Bagaimanapun, kasmothecia tidak diperhatikan pada permukaan daun simptom penyakit kulapuk berdebu. Tindakan rantai polimerase telah dilakukan pada DNA konidia kulapuk berdebu dengan menggunakan primer universal untuk meningkatkan bahagian internal transcribed spacer (ITS) untuk *Podosphaera*. Dalam kajian ini, ITS5-Forward dan ITS4-Reverse merupakan primer yang digunakan.

Saiz bagi amplicon dianggarkan dalam 580 bp. Keputusan amplicon diunjukkan and dikenalpasti 100% mempunyai persamaan kepada jujukan ITS bagi *Podosphaera xanthii* yang menjangkiti *Cynanchum auriculatum* (KU711923) dan *Kalanchoe blossfeldiana* (KR049082). Berdasarkan ciri-ciri morfologi dan jujukan analisa ITS, patogen menjangkiti daun *P. tithymaloides* dikenalpasti sebagai *Podosphaera xanthii*.



## CHAPTER 1

### INTRODUCTION

*Pedilanthus tithymaloides* known as 'Jacob's ladder,' 'Devil's Backbone' is a shrubs perennial, succulent spurge, grown primarily as a border plant in ornamental landscape. *Pedilanthus tithymaloides* also is being planted in landscapes to remediate toxic soils.

Micheal (2010) described that morphological characteristics of their leaves are alternate, simple, lanceolate, glabrous, and undulate to entire margins; leaf color varies from green to green flushed red on the species type, besides the cultivar 'Variegated' that has combination of green and white on colour of leaves. But, sometimes the pink variegated leaves is randomly found in Malaysia.

Unfortunately, the plants are regularly subjected to infection by fungal pathogens. Important of this unique plants are as shrub borders and potted accent. Moreover, this plant can be propagated easily and is low cost (Micheal, 2010). In Malaysia, it is typically used as a potted plant. Traditionally, this plant can cure some injury from stings, relieve some headache and joint pains. This plant has a milky sap on stems and leaves can cause stomach distress to human if we swallowed it. It is may lead humans to infect by disease of dermatitis. Also, there have been some studies in using this *P. tithymaloides* as a renewable fuel source.

Powdery mildew brought a local necrosis to *P. tithymaloides*. Hosagoudar *et al.* (2013) stated that powdery mildew is the most widespread and destructive disease of



worldwide. Powdery mildew is one of diseases caused by fungus. It is a spot of white and powdery on infected surfaces of leaves.

Numerous conidia on the leaves that bring whitish and dusty appearance (Figure 1). Most of the part of leaves can be infected by this fungus (Figure 2). The stem also showed a dark color. They are easily recognized, but specific identifications gave difficult condition or even impossible depending on the reproductive stage of the fungus (Braun and Cook, 2012). The infection is favored by the high humidity. Normally, individual species of powdery mildew fungi typically have a very narrow host range. Powdery mildew will give side effect towards that *P. tithymaloides* which is plant ultimately die.

*Pedilanthus tithymaloides* is an ornamental plant commonly planted in Malaysia. Unfortunately, this plant is susceptible to pathogenic infection. The infection causes undesirable physiological growth and decreases the ornamental value of *P. tithymaloides*. Therefore, the objectives of this study is to characterize the morphology of powdery mildew fungus infecting *P. tithymaloides* and to analyze its rDNA and ITS sequence.



Figure 1. Characteristic powdery mildew disease signs caused by *Podosphaera xanthii* on susceptible *P. tithymaloides* leaves.



Figure 2. Severe infected leaves of *P. tithymaloides* powdery mildew infection and defoliation.

In Table 1, powdery mildew genera are now grouped into five family which are Phyllactineae, Erysipheae, Blumeriae, Golovinomyceteae and Cystotheceae.

Table 1. A classification of five family powdery mildew associated with the common host. The previous teleomorphic names (and less commonly used anamorphic names) are given to aid in reference to the older literature.

Tribe	New holomorphic genus	Anamorphic genus	Former teleomorphic genus	Common hosts
Phyllactineae	<i>Phyllactinia</i>	<i>Ovulariopsis</i>	<i>Phyllactinia</i>	Trees and shrubs
	<i>Leveillula</i>	<i>Oidiopsis</i>	<i>Leveillula</i>	Solanaceae
Erysipheae	<i>Erysiphe section erysiphe</i>	<i>Oidium</i>	<i>Erysiphe section erysiphe</i>	Legume
	<i>Erysiphe section microsphaera</i>	<i>Oidium</i>	<i>Microsphaera</i>	Trees and shrubs
	<i>Erysiphe section uncinula</i>	<i>Oidium</i>	<i>Uncinula</i>	Trees and shrubs
Blumeriae	<i>Blumeria</i>	<i>Oidium</i>	<i>Blumeria/Erysiphe</i>	Grasses
Golovinomyceteae	<i>Golovinomyces</i>	<i>Oidium</i>	<i>Erysiphe section Golovinomyces</i>	Cucurbits and composites
Cystotheceae	<i>Podosphaera section Podosphaera</i>	<i>Oidium</i>	<i>Podosphaera</i>	Rosaceae
	<i>Podosphaera section sphaerotheca</i>	<i>Oidium</i>	<i>Sphaerotheca</i>	

Vincelli *et al.* (2008) concluded that molecular biology techniques, especially those involving the polymerase chain reaction (PCR), have provided an alternative and sensitive approach for the detection and identification of plant pathogens and many soilborne pathogenic fungi.

By using the rDNA internal transcribed spacer (ITS) region is very useful in order to study species level of fungi (Cook *et al.* 2006; Cunnington *et al.* 2004; Samuels and Seifert 1995; and Bruns *et al.* 1991;). It could represent a useful region in linking anamorphic specimens with their respective teleomorphs. Hirata and Takamatsu (1996) examined the ITS regions from several powdery mildew specimens also obtained more 99% similarity within each species but only 75% similarity between species in different genera. Also, the universal ITS primers used by Hirata and Takamatsu (1996) worked with conidia collected from fresh material, the use of these primers is limited with dried herbarium specimens.

The study of morphological and molecular characterization of fungus should be done in order to know phylogenetic analysis of fungal species. Plus, reliable identification and detection of the pathogen required due to implement of appropriate disease management and measures needed in prevent or control the powdery mildew disease. When the identification species detected, then further management step can be taken in order to conserve and maintain *P. tithymaloides* as ornamental plants in Malaysia.

Based on this study, the morphological characteristic of powdery mildew fungus that infecting *P. tithymaloides* was observed and identified. Besides, the rDNA ITS sequences of the specimens was analyzed by using molecular characterization.

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