UNIVERSITI PUTRA MALAYSIA

SUPPRESSION OF BIOFILM FORMATION ON SELECTED PLANT PATHOGENIC FUNGI USING COMMERCIAL FUNGICIDE

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FP 2017 69

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BY

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A Project Report Submitted to Faculty of Agriculture, Universiti Putra Malaysia, in Fullfilment of the Requirement of PRT 4999 (Final Year Project) for the Award of the Degree of Bachelor of Agricultural Science

FACULTY OF AGRICULTURE
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ENDROSEMENT

This project report entitled "Suppression of Biofilm Formation on Selected Plant Pathogenic Fungi using Commercial Fungicide" is prepared by Sudamma Soh Chien Hsien and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 2009 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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ACKNOWLEDGEMENT

First and foremost, I would like to express my greatest gratitude to God for granting me the strength, knowledge, wisdom and patience to complete this final year project successfully. I would also like to express my gratitude to my supervisor, Dr. Khairulmazmi Bin Ahmad, for his guidance and advise during this whole process of conducting this final year project. I would not be able to finish my final year project without his guidance and encouragement.

Secondly, I would like to take this opportunity to thank everyone that assisted me during the whole process of this final year project. This includes all the staffs in the Department of Plant Protection especially Puan Junaina Jaafar for granting me the permission to use the Bacteriology Lab as my main working space and Puan Asmalina Abu Bakar that taught me a lot about the fungus *Phytophthora palmivora*. I would also like to extend my gratitude to master students including Puan Joranisah Hamid for guiding me throughout the whole process, Mr. Ali Abdul Ameeridan for providing me with the *Pyricularia oryzae* culture and Miss Hazirah Mohd Din for teaching me some technical stuffs. In addition, I would also like to thank my lab partner, Bernice Andrew for her effort and willingness to help me throughout this project.

Last but not least, I would like to thank my beloved parents, Soh Poh Soon and Victoria Joy Thambyraja for their moral support and encouragement throughout this project.

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LIST OF ABBREVIATIONS

AhR	Aryl hydrocarbon receptor
ANOVA	Analysis of Variance
º C	Celcius
cm	Centimetre
CRD	Completely Randomized Design
CMA	Corn Meal Agar
et al	et alia 'and others'
EC	Effective Concentration
EPS	Extracellular polymeric substance matrix
F. oxysporum	*Fusarium oxysporum*
F. solani	*Fusarium solani*
g/mol	Gram per mol
HSD	Tukey's Studentised
mRNA	Messenger RNA
µl	Microlitre
µm	Micrometre
mg	Milligram
ml	Millilitre
m.p.	Melting Point
P. palmivora	*Phytophthora palmivora*
ppm	Parts per million

PIRG	Percentage Inhibition of Radial Growth
PBS	Phosphate Buffered Saline
P. aeruginosa	*Pseudomonas aeruginosa*
PDA	Potato Dextrose Agar
P. oryzae	*Pyricularia oryzae*
nm	Nanometre
QSMs	Quorum sensing molecules
UV	Ultraviolet
XTT	2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide

ABSTRACT

Plant pathogenic fungi are the fungus that brings harm to many plant species by reducing quality and yield of plant produces. *Fusarium* spp. are toxin producers, endophytes and saprotrophs as well as biofilm producers. Biofilm formation is the coordination action of multiple strains that helps and protects each other. Thus, the objective of this study is to assess efficacy of commercial fungicide to suppress formation of biofilm on selected plant pathogenic fungi. In this study, Carbendazim was used to suppress biofilm formation and mycelial growth of *Fusarium oxysporum*, *F. solani*, *Pyricularia oryzae* and *Phytophthora palmivora*. Microtiter plate and poison media methods were used to evaluate the efficacy of the tested fungicide on the fungi *in vitro*. Five concentrations i.e. 0 ppm (control), 10 ppm, 50 ppm, 100 ppm and 200 ppm were assigned for the poison media method. Results from poison media method showed that Carbendazim promoted significant ($P \leq 0.05$) inhibition on mycelial growth of the fungi. Carbendazim displayed 100% mycelial inhibition on *F. oxysporum* and *P. oryzae* at concentration of 200 ppm. However, for microtiter plate method, Carbendazim was found to be not effective in suppressing the growth of biofilm in fungi *F. oxysporum* and *F. solani* due to the strong natural resistance formed by fungal biofilm when there was presence of fungicide. Therefore, in this study, Carbendazim was only effective in disrupting the mycelial growth of fungi and not the fungal biofilm.

ABSTRAK

Kulat patogenik tumbuhan adalah kulat yang mendatangkan bahaya kepada spesies tumbuhan dengan mengurangkan kualiti dan hasil tanaman. *Fusarium* spp. adalah pengeluar toksin, endofitik, saprofit dan pengeluar 'biofilm'. Pembentukan 'biofilm' ialah tindakan penyelarasan beberapa sel yang menolong dan melindungi antara satu sama lain. Oleh itu, objektif kajian ini adalah untuk menilai keberkesanan racun kulat komersil untuk menghalang pembentukan 'biofilm' terhadap beberapa kulat yang telah dipilih. Dalam kajian ini, Carbendazim telah digunakan untuk menghalang pembentukan 'biofilm' dan pertumbuhan 'mycelial' pada *F. oxysporum*, *F. solani*, *Pyricularia oryzae* dan *Phytophthora palmivora***.** Kaedah 96 plat lubang dan kaedah media beracun telah digunakan untuk menilai keberkesanan Carbendazim terhadap kulat-kulat secara *in vitro*. Lima kepekatan berbeza; 0 ppm (kawalan), 10 ppm, 50 ppm, 100 ppm dan 200 ppm telah ditentukan untuk menjalankan kaedah media beracun. Keputusan daripada kaedah ini menunjukkan bahawa Carbendazim menyebabkan perencatan 'mycelial' yang ketara ($P \leq 0.05$) pada semua kulat. Carbendazim berjaya menyebabkan 100% perencatan 'mycelial' pada *F. oxysporum* dan *P. oryzae* pada kepekatan 200 ppm. Walau bagaimanapun, dalam kaedah 96 plat lubang, Carbendazim didapati tidak berkesan dalam menghalang pertumbuhan 'biofilm' bagi kulat *F. oxysporum* dan *F. solani*. Hal ini disebabkan oleh ketahanan semula jadi yang kuat yang telah dibentuk oleh 'biofilm' kulat apabila terdapat kehadiran racun kulat. Oleh itu, dalam kajian ini, Carbendazim hanya berkesan dalam menghalang pertumbuhan miselium kulat dan tidak berkesan bagi 'biofilm' kulat.

CHAPTER 1

INTRODUCTION

1.1 Background

In recent years, biofilm formation in bacteria, fungi and other types of microbes has raised some heads in many sectors especially in the agriculture sector. At first, no one thought that biofilm is that serious of a problem until now (Patel *et al.*, 2014). Biofilms are condensed, much hydrated clumps of cells that conclusively attached to a surface or each other and they self-produce a gelatinous matrix that contains extracellular poly metric substances (EPS) (Singh *et al.*, 2011). As the biofilm gets older by time, the extracellular matrix in it most likely have an effect related to cohesion (Fanning and Mitchell, 2012).

The biofilms containing cells contain a changed phenotype that can resist and survive under harsh conditions (Singh *et al.*, 2011). This most basic defined phenotype is related to biofilm is resistance increment against chemical or antibiotic treatments (Harding *et al*., 2010). Besides, biofilm may be a like structure or containing several species societies (Eberl *et al.*, 2007). It is also a 'group behavior' (Kostakioti, *et al.*, 2013), whereby they facilitate survival in adverse environment. According to Harding *et al*., (2010), many crucial discoveries in environmental, medical and industrial microbiology have been possible because of biofilm research. However, biofilm

formation in fungus is still new and there are very few studies has been done on them and this is probably because fungal biofilms is not 100% identical as compared to bacteria and yeast related biofilm formation (Peiqian *et al.*, 2014).

In this study, four types of phytopathogenic fungi will be tested on the effect of their biofilm formation against a commercial fungicide. The four fungi are *Fusarium solani*, *F. oxysporum*, *Pyricularia oryzae* and *Phytophthora palmivora*. *Fusarium oxysporum* is one of the major enemies in cucumber. It is a soil-borne pathogen that can cause Fusarium wilt disease and this is a major disaster for the cucumber and other economic importance crops worldwide (Peiqian *et al.*, 2014). *Fusarium* spp. usually grows as a biofilm and this is the reason why they are normally very hard to be suppressed in field.

The ability of fungi to form biofilms in various environments has made its impact not only in the agriculture sector but in ecology, medicine, and industrial sectors. This has attracted considerable attention by many researchers (Patel *et al.*, 2014). According to Patel *et al.*, (2014), when fungi are under stress, they will team up and form a community called biofilm. This will cause them to have elevated resistance to many antifungal agents.

1.2 Objective

The objective of this study was to assess efficacy of fungicide Carbendazim to suppress formation of biofilm in fungi *in vitro*.

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