UNIVERSITI PUTRA MALAYSIA

SUPPRESSION OF BIOFILM FORMATION ON SELECTED PLANT PATHOGENIC FUNGI USING TEA TREE OIL

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BY

BERNICE ANDREW

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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ENDORSEMENT

This project report entitled "Suppression of Biofilm Formation On Selected Plant Pathogenic Fungi Using Tea Tree Oil" is prepared by Bernice Andrew and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
℃	Celcius
cm	Centimetre
CRD	Completely Randomized Design
CMA	Corn Meal Agar
et al	et alia 'and others'
EPS	Extracellular polymeric substance matrix / Exopolysaccharides
eDNA	Extracellular DNA
F. oxysporum	*Fusarium oxysporum*
F. solani	*Fusarium solani*
HSD	Tukey's Studentised
hr	Hours
M. alternifolia	*Melaleuca alternifolia*
µl	Microlitre
µm	Micrometer
mg	Milligram
ml	Millilitre
ppm	Parts per million
PIRG	Percentage Inhibition of Radial Growth
PBS	Phosphate Buffered Saline
P. palmivora	*Phytophthora palmivora*

PDA	Potato Dextrose Agar
P. oryzae	*Pyricularia oryzae*
TTO	Tea Tree Oil
XTT	2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide

Abstract

Fusarium solani, *F. oxysporum*, *Phytophthora palmivora* and *Pyricularia oryzae* are best examples of plant pathogenic fungi that have the ability to cause enormous economic losses on crops worldwide annually. Formation of biofilm is one of the survival strategies for bacteria and fungi to adapt to their surrounding environment, especially when exposed to the hostile environment. Thus, the objective of this study was to assess efficacy of tea tree oil to suppress formation of biofilm in fungi. *Fusarium solani*, *F. oxysporum* and *P. oryzae* were cultured in PDA medium while *P. palmivora* was cultured in CMA medium. Microtiter plate method and poisoned food technique were used in this study. It was revealed that tea tree oil (TTO) showed 100% mycelial inhibition on *F. oxysporum* and *F. solani* at concentration of 1000 ppm. TTO was found to be less effective against *P. oryzae* and *P. palmivora*. For 96-well plates, TTO was able to suppress the growth of biofilm at concentration of 50,000 ppm and 100,000 ppm. Biofilm quantification was determined based on the reduction of XTT (a tetrazolium salt) by metabolically active fungal biofilm cells.

Abstrak

Fusarium solani, *F. oxysporum*, *Phytophthora palmivora* dan *Pyricularia oryzae* adalah contoh terbaik bagi kategori kulat patogenik tumbuhan yang mempunyai keupayaan untuk menyebabkan kerugian ekonomi yang besar kepada tanaman di seluruh dunia setiap tahun. Pembentukan 'biofilm' adalah salah satu strategi untuk bakteria dan kulat bagi menyesuaikan diri dengan persekitaran disekelilingnya, terutamanya apabila terdedah kepada persekitaran yang tidak sesuai untuk pertumbuhan kulat tersebut. Pembentukan ciri-ciri fenotip unik dalam mikroorganisma tersebut mempunyai keupayaan untuk menyebabkan jangkitan. *Fusarium* adalah penyakit kulat bawaan tanah dan kulat ini boleh menyebabkan jangkitan serius jika terbentuk 'biofilm'. Oleh itu, objektif kajian ini adalah untuk menilai keberkesanan racun pokok teh untuk menghalang pembentukan 'biofilm' pada kulat. *Fusarium solani*, *F. oxysporum* dan *P. oryzae* dikultur dalam media PDA dan *P.palmivora* dikultur dalam media CMA. Ujian 96 plat lubang dan teknik racun media telah digunakan dalam kajian ini. Kajian ini telah membuktikan bahawa minyak pokok teh (TTO) menunjukkan 100% perencatan miselium untuk *F. oxysporum* dan*F.solani* hanya pada kepekatan 1000 ppm. TTO kurang berkesan untuk mengawal pertumbuhan miselium bagi *P. oryzae* dan *P. palmivora*. Bagi ujian 96 plat lubang, TTO mampu menghalang pertumbuhan 'biofilm' pada kepekatan 50,000 ppm dan 100,000 ppm. Kuantifikasi 'biofilm' ditentukan berdasarkan pengurangan XTT (sejenis garam tetrazolium) oleh sel 'biofilm' kulat yang mempunyai metabolisma yang aktif.

CHAPTER 1

INTRODUCTION

The existence of biofilms in microorganisms especially in bacteria, yeasts and fungi have gained attention recently as it was believed that biofilm is a structure formed to cause harmful infections. Generally, over the years, fungal biofilms have become a significant economic problem due to the persistence in fungal infections (Martinez and Bettina, 2010). Harding *et al*., (2010) stated that the research on biofilms formed by bacteria and yeasts were much known but there were very few descriptions of biofilms formed by filamentous fungi. Biofilm research had been conducted in many fields which include environmental, medical and industrial microbiology but in agriculture, biofilms have not been well investigated. Besides, it is also estimated that 80% of the bacteria in environment exist as biofilm communities and are able to survive in hostile environments due to its self-protective layer of enclosure (Saini *et al*., 2011).

Biofilms are defined as microbial communities attached on surface or aggregated micro-colonies surrounded by thick extracellular polymeric substances (EPS) that include nucleic acids, proteins, lipids and polysaccharides (Harding *et al*., 2009; Tan *et al*., 2014). Studies by Harding *et al.*, (2010) revealed that these extracellular polymeric substances (EPS) are self-produced by the biofilm and it acts as a protective shielding from the environment. He also described that microbial biofilms attached to a host by growing on a biotic or abiotic surface. After successfully attached on the surface, biofilms undergo gene expression and phenotypic changes and as a result, it may become resistant to any kinds of treatment

or stress conditions. In addition, from his research, he reported that most of the plant diseases are caused by filamentous fungi as it can survive in a harsh environment. Apart from that, according to Peiqian *et al.,* (2013), these surface-attached communities of biofilm are able to form pathogenesis and in fungi, biofilms can represent much more than a mere biological coating.

The formation of biofilm is a dynamic process which comprises of attachment, micro colony formation and maturation as well as dispersal (Kostakioti *et al.,* 2013; Tan *et al*., 2014). Biofilm easily developed within 24 or 48 hours depending on the types of fungus. According to Wongsuk *et al.,* (2016), during biofilm development, it involves quorum-sensing molecules which allow cell to cell communication so that biological activities and behaviours of the microorganisms are being controlled. In the attachment of fungal cells on biotic or abiotic surfaces, quorum-sensing molecules also regulate defence against fungal invasion during infection. These quorum-sensing molecules play a role in fungal pathogenicity, morphogenesis and are important for infectious process too.

In this study, *Fusarium solani*, *F. oxysporum*, *Phytophthora palmivora* and *Pyricularia oryzae* are being tested *in vitro*. These fungi are plant pathogenic fungi that harm worldwide crops over the years. Besides causing infection in plants, it also causes a huge loss in the yield of crops among farmers by spreading destructive plant diseases. According to Di e*t al.,* (2016), *Fusarium oxysporum* species complex consist of soil fungi that cause infection and disease in over 120 different plant species in the world.

There was very few research carried out to investigate the biofilm formation in *Fusarium solani*, *F. oxysporum*, *Phytophthora palmivora* and *Pyricularia oryzae*. Due to its self-protective structure to defend against the invasion of pathogens, any biological or chemical treatments hardly overcome this infectious biofilm structure. Saini *et al.*, (2011) reported that biofilms display unique characteristics that increase resistance to host immune mechanisms. Thus, in this study, a commercial biofungicide or antifungal agent containing tea tree oil is used to test to inhibit the growth of biofilm in fungi. This natural product is a plant-derived compound (tea tree (*Melaleuca alternifolia)* oil) that has the potential in killing the conidia and preventing germination of the spore in fungi (Rogawansamy *et al.*, 2015). Liquid application of essential oils such as tea tree oil (TTO) is used to control a broad spectrum of fungal and bacterial plant diseases. Based on Carson *et al.*, (2006), TTO disrupts the function of fungal membranes. Besides, it was reported also that tea tree oil (TTO) has the potential to exert its yeast-killing effect by inhibiting fungi's ability to replicate. Therefore, the objective of this study was to assess efficacy of tea tree oil to suppress mycelial growth and formation of biofilm in fungi *in vitro*.

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