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THE USE OF BACTERIAL ANTIBIOTICS EXTRACTED FROM *Stenotrophomonas maltophilia* TO CONTROL PLANT FUNGAL PATHOGENS *in vitro*

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THE USE OF BACTERIAL ANTIBIOTICS EXTRACTED FROM *Stenotrophomonas maltophilia* TO CONTROL PLANT FUNGAL PATHOGENS *in vitro.*

BY

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A project report submitted to the 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 Horticultural Science.

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ENDORSEMENT/CERTIFICATION

This project report entitled **The use of bacterial antibiotics extracted from *Stenotrophomonas maltophilia* to control plant fungal pathogens *in vitro*** is prepared by **Nor Intan Syazwani Binti Sazali** 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 Horticultural Science.**

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LIST OF ABBREVIATIONS

ILO	International Labor Organization
WHO	World Health Organization
DMRT	Duncan's multiple range test
CRD	Completely Randomized Design
ANOVA	Analysis of Variance
PIDG	Percent inhibition of diameter growth
FLOs	Fungi and fungal-like organisms
VOCs	Volatile organic compounds
S. maltophilia	*Stenotrophomonas maltophilia*
G. boninense	*Ganoderma boninense*
E. rostratum	*Exserohilum rostratum*
Foc	*Fusarium oxysporum* f. sp. *cubense*
R. solani	*Rhizoctonia solani*
BSR	Basal stem rot
BCAs	Biological control agents
NA	Nutrient Agar
PDA	Potato Dextrose Agar
NB	Nutrient Broth

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ABSTRACT

Fungal pathogens are known to cause important plant diseases which lead to significant lost in agricultural crops. The plant diseases need to be controlled to maintain the yield production and to avoid economic losses. Nowadays, people are aware of environmental issues caused by uncontrolled usage of synthetic fungicides. In response to this awareness, biological control using microbial antagonists is recommended instead of using synthetic chemicals for controlling plant diseases. *Stenotrophomonas maltophilia* can be used as a biological control agent against plant fungal pathogens. The bacterium can produce antibiotics that can inhibit pathogen growth and induce host resistance. In view of this, this research is conducted to 1) extract crude antibiotics from *S. maltophilia* and 2) study the effect of the crude antibiotics on the mycelial growth of selected plant fungal pathogens. This research used four different plant fungal pathogens which were *Ganoderma boninense, Fusarium oxysporum, Rhizoctonia solani* and *Exserohilum rostratum*. Crude antibiotics were extracted from bacterial culture in nutrient broth (NB) using centrifuge and lyophilization methods where the bacterial then was centrifuged to remove all bacterial cells and the supernatant was lyophilized in a vacuum evaporator. The four fungal pathogens were treated with five different concentrations of bacterial antibiotics which were 0.1, 0.2, 0.4, 0.8, 1.0 mg/L and control. The antagonism assay was done in five replications for each fungus and the experimental design was a Completely Randomized Design (CRD). Data were analysed using Analysis of Variance (ANOVA) from SAS version 9.4 and comparison of means with significant difference was further tested with Duncan's Multiple Range Test (DMRT). Each concentration significantly affected the percentage inhibition of diameter growth (PIDG) for all fungus (*G. boninense, E. rostratum, R. solani and* FOC). Extracted crude antibiotics from *S. maltophilia* was able to inhibit the mycelial growth of selected plant fungal pathogens at concentrations of 0.1, 0.2, 0.4, 0.8 and1.0 mg/L where concentration 1.0 mg/L gave the highest PIDG. *R. solani* showed the highest PIDG among all concentrations followed by *G. boninense*, *E. rostratum* and *F. oxysporum.*

ABSTRAK

Patogen kulat diketahui menyebabkan penyakit tumbuhan penting yang membawa kepada kerugian ketara dalam tanaman pertanian. Penyakit-penyakit tumbuhan perlu dikawal untuk mengekalkan pengeluaran hasil dan mengelakkan kerugian ekonomi. Pada masa kini, manusia mula sedar tentang isu-isu alam sekitar yang disebabkan oleh penggunaan racun kulat tiruan yang tidak terkawal. Sebagai tindak balas kepada kesedaran ini, kawalan biologi menggunakan antagonis mikrob adalah disyorkan untuk mengganti penggunaan bahan kimia sintetik bagi mengawal penyakit tumbuhan. *Stenotrophomonas maltophilia* boleh digunakan sebagai agen kawalan biologi terhadap patogen kulat tumbuhan. Bakteria ini boleh menghasilkan antibiotik yang boleh menghalang pertumbuhan patogen dan mendorong rintangan tanaman tuan perumah. Sehubungan itu, kajian ini dijalankan untuk 1) mengekstrak antibiotik daripada *S. maltophilia* dan 2) mengkaji kesan antibiotik ke atas pertumbuhan miselium patogen kulat tumbuhan yang terpilih. Kajian ini menggunakan empat patogen kulat tumbuhan yang berbeza iaitu *Ganoderma boninense, Fusarium oxysporum, Rhizoctonia solani dan Exserohilum rostratum*. Antibiotik diekstrak daripada kultur bakteria dalam "Nutrient Broth (NB)" menggunakan kaedah 'Centrifuge' dan penyejat vakum di mana bakteria dilakukan centrifuged untuk membuang semua sel-sel bakteria dan supernatant yang terhasil telah disejat beku dalam penyejat vakum. Empat patogen kulat telah dirawat dengan lima kepekatan antibiotik bakteria yang berbeza iaitu 0.1, 0.2, 0.4, 0.8, 1.0 mg / L dan kawalan. Cerakin antagonistik dilakukan dalam lima ulangan untuk setiap kulat dan reka bentuk eksperimen adalah Rekabentuk Penuh Rawak Lengkap (CRD). Data dianalisa dengan menggunakan analisis varian (ANOVA) dari SAS versi 9.4 dan perbandingan purata yang signifikan diuji pula dengan "Duncan's Multiple Range

Test (DMRT)". Setiap kepekatan mempengaruhi peratusan perencatan diameter pertumbuhan miselium (PIDG) dengan signifikan untuk semua kulat yang diuji (*G. boninense, E. rostratum, R. solani dan* FOC) Antibiotik yang diekstrak daripada *S. maltophilia* dapat menghalang pertumbuhan miselium patogen kulat tumbuhan yang terpilih dalam kepekatan 0.1, 0.2, 0.4, 0.8, dan1.0 mg/L di mana kepekatan 1.0 mg/L memberikan peratusan PIDG tertinggi. *R. solan*i menunjukkan peratusan tertinggi PIDG di kalangan semua kepekatan diikuti oleh *G. boninense, E. rostratum* dan *F. oxysporum*.

CHAPTER 1

INTRODUCTION

Fungal pathogens are known as the most destructive pathogens that cause plant diseases which affect plant yield or quality. About 85% which was an estimated two-third of infectious plant diseases were caused by fungal pathogens. Majority of diseases caused by fungal pathogens are rusts, smuts, needle casts, leaf curls, mildew, sooty molds, anthracnoses, cankers, blights, wilts and galls (Pelczar, 2016). Infectious plant diseases caused by fungi are not new problems and they had been discovered many years before. In fact, they are recognized to be a worldwide threat to food security as they also cause yield reductions of almost 20% of crops worldwide (Dias, 2012). Thus, these became serious problems as they involved crops that being food supply for human in life. The crops that being attacked by the fungal pathogens broadly are cereals, oilseeds, fruits and vegetables, tree crops, and animal feeds (Wareing, 2010).

Mostly, the control of plant diseases relies on chemical control using pesticides. To solve fungal disease problems in plantation, fungicide have been widely used. However, the use of fungicides tends to cause many problems. It increased the agricultural costs, contaminate the environment with very toxic substances, results in prolonged human health and environmental hazard (Dias, 2012). The International Labor Organization (ILO) estimates that as much as 14 per cent of all occupational injuries are due to exposure to pesticides and other agrochemical constituents while The World Health Organization (WHO) and the United Nations Environment Programme estimates that each year, three million

workers in agriculture in developing world experience severe poisoning from pesticides, about 18,000 of whom died (Suprapta, 2012).

Nowadays people are more concern about their health and environment. Thus, they tends to avoid relying on the use of fungicides instead they are trying more to use biological control methods. The biological control methods are intended to replace chemical pesticides as an alternative environmentally-friendly strategy for the protection of agricultural and horticultural crops against pathogens. Instead of using chemicals, antibiotic extracts from bacteria could be used in control plant fungal pathogens (Pal, 2006). Thus, environmental problems could be minimized. Microbial antagonists that have been reported to demonstrate antagonistic activities against plant fungal pathogens are *Pseudomonas fluorescens, Pseudomonas trivialis, Agrobacterium radiobacter, Bacillus subtilis, B. cereus, B. amyloliquefaciens, Trichoderma virens, Trichoderma harzianum, Burkholderia cepacia, Saccharomyces* sp., *Gliocadium* sp., *Serratia plymuthica, Serratia odorifera, Stenotrophomonas maltophilia,* and *Stenotrophomonas rhizophila* (Suprapta, 2012; Kai, 2007).

These bacterial antagonists isolated from rhizosphere of plants showed negatively affect the growth of other organisms. They inhibit the growth of fungi by various mechanisms, for example, secretion of lytic enzymes, siderophores and antibiotics, emit small organic volatile compounds (VOCs) (Kai, 2007; Vespermann, 2007; Pal, 2006). In view of this, this research was conducted firstly to extract crude antibiotics from bacterial *S. maltophilia* and secondly to study the effect of the extracted crude antibiotics on the mycelial growth of selected plant fungal pathogens. The crude antibiotics were expected to give effects on the mycelial growth which inhibit the mycelial growth of selected plant fungal pathogens.

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