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EFFECT OF ANTIBIOTICS EXTRACTED FROM *Pseudomonas aerugimosa* AGAINST PLANT FUNGAL PATHOGENS*E*

MAISARAH MAYUDIN

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BY

MAISARAH BINTI MAYUDIN

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

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MAISARAH BINTI MAYUDIN

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ENDORSEMENT

This project report entitled "The Effect of Antibiotics Extracted from *Pseudomonas aeruginosa* Against Plant Fungal Pathogen" is prepared by Maisarah binti Mayudin 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.

Student's name: Student's signature:

MAISARAH BINTI MAYUDIN ……

Certified by:

……

Assoc. Prof. Dr. Wong Mui Yun

Department of Plant Protection,

Faculty of Agriculture,

University Putra Malaysia.

Date: …….

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Fungi are among the most important plant pathogens that cause severe yield loss of crops such as rice, maize, fruits and vegetables. This problem may be effectively controlled using fungicides but somehow it will trigger the emergent of new resistant fungi. Furthermore, the usage of chemical control is not the best solution as the environmental pollution is critically high. However, *Pseudomonas aeruginosa* is one of the *Pseudomonas spp* that was shown could be used as a biological control agent against plant pathogenic fungi. This research is conducted to extract crude antibiotics from *Pseudomonas aeruginosa* UPM P3 and to determine the effect of different concentrations of the extracted crude antibiotics on the mycelial growth of selected plant fungal pathogens. Four types of important fungi culture will be maintained for screening. Bacterial suspension will be prepared using Luria Bertani (LB) broth. By using cell-free supernatant, the antibiotics will be extracted and tested against the selected fungal pathogens using poison food technique with six different concentrations (0, 0.1, 0.2, 0.4, 0.8 and 1.0 mg/ml). This experiment was conducted in a completely randomized design (**CRD**). The assessment of mycelial growth will be done by measuring the diameter of the mycelium for each concentration used and compare with the control treatment. Antibiotics produced by *Pseudomonas aeruginosa* UPMP3 can be extracted and are expected to inhibit the growth of plant fungal pathogens *in vitro*.

ABSTRAK

Kulat merupakan pathogen tanaman yang penting kerana mampu mengakibatkan kerugian hasil tanaman penting yang serius seperti padi, jagung, buah dan sayur. Masalah ini boleh dikawal secara efektif menggunakan kaedah kawalan kimia iaitu racun kulat namun ia akan mengakibatkan risiko kulat menjadi rintang terhadap racun kulat. Tambahan pula, penggunaan kawalan kimia bukan penyelesaian terbaik kerana mempertimbangkan keadaan persekitaran yang kian tercemar. Bagaimanapun, bakteria *Pseudomonas aeruginosa* terbukti boleh digunakan sebagai satu agen kawalan biologi terhadap kulat penyebab penyakit tumbuhan. Penyelidikan ini dijalankan untuk mengekstrak antibiotik dari *Pseudomonas aeruginosa* UPMP3 dan menentukan kesan kepekatan antibiotik yang berbeza kepada pertumbuhan miselium patogen-patogen kulat yang terpilih. Empat jenis kulat penting yang digunakan dalam penyelidikan ini. Kultur cecair bacteria akan disediakan menggunakan penyediaan medium Luria Bertani (LB). Dengan menggunakan supernatan tanpa sel, antibiotik akan diekstrak dan diuji menentang pathogen-patogen kulat terpilih menggunakan teknik media beracun dengan enam kepekatan berbeza (0, 0.1, 0.2, 0.4, 0.8, 1.0 mg/ml). Eksperimen ini dijalankan menggunakan rekabentuk Completely Randomized Design (CRD). Penilaian pertumbuhan miselium akan dibuat dengan mengukur diameter miselium untuk setiap kepekatanbdan dibandingkan dengan rawatan terkawal (tanpa rawatan). Antibiotik yang dihasilkan oleh *Pseudomonas aeruginosa* UPMP3 boleh diekstrak dan dijangka dapat menghalang pertumbuhan patogen-patogen kulat tumbuhan secara *in vitro*.

CHAPTER 1

INTRODUCTION

Fungi are among the most important group of plant pathogens that cause severe yield loss of crops such as rice, maize, fruits and vegetables (Carris *et al*, 2012). Diseases caused by fungi can easily spread long distances because of their microscopic structures that can easily attach to the host plants or flown by the wind or harbor in soil (Moore, 2011). For example a group of fungi that infect various stage of rice including species of *Fusarium, Rhizoctonia* and *Pyricularia* and Panama disease of banana is one of the most famous vascular wilt caused by fungi *Fusarium oxysporum* f. sp *cubense (*Carris *et al*, 2012*)*.

This problem may be effectively controlled using fungicides but somehow it will trigger the emergent of new resistant fungi. Moreover, the usage of chemical control is not the best solution as the environmental pollution is critically high (Gerhadson, 2002). The use of biocontrol bacteria isolated from the rhizosphere may present an alternative for plant disease prevention (Compant *et al*., 2005; Fernando *et al*., 2006; Fatima *et al*., 2009). One of the most popular bacteria studied and exploited as biocontrol agent is the *Pseudomonas* species (Ahmad *et al*., 2008).

One particular approach for the control of plant diseases has been to inoculate (or ‘‘bacterise’’) the seeds with particular strains of bacteria which produce inhibitory factors to the particular pathogenic fungi. These bacteria then proliferate on the plant root systems and prevent fungal diseases. Such biological control method has been

claimed to be a realistic alternative to the problems of chemical pesticides (Kerr, 1999). *Pseudomonas* spp. has widely been used for induction of systemic resistance against plant pests and diseases (Fishal *et al*, 2010). Some researchers had found that the presence of antimicrobial substances produced by *Pseudomonas aeruginosa* could inhibit the mycelial growth of fungi (Sindhu *et al*, 1997).

Pseudomonas aeruginosa is one of the *Pseudomonas* spp that was shown could be used as a biological control agent against plant pathogenic fungi (Fishal*et al*, 2010). It is an aerobic motile Gram-negative bacterium, widely distributed in water, soil and sewage, and one of the biotechnologically valuable microorganisms (Joshua *et al*, 2000). Most of the identified *Pseudomonas* biocontrol strains produce antifungal metabolites such phenazines, pyrrolnitrin, pyoluteorin and cyclic lipopeptides like viscosinamide. It was demonstrated that viscosinamide prevents the infection of sugar beet by *Pythium ultimum* (Bloemberg and Lugtenberg, 2001). These bacterial strains beside the antagonistic effect also influence the defense system of plants (Maksimov *et al*., 2011).

Many pseudomonads inhibit fungal growth. *P. aeruginosa* produces three antifungal factors; dihydroaeruginoic acid, which inhibits phytopathogenic fungi, and pyocyanin and 1-hydroxyphenazine which inhibit growth of various *Candida* spp. and *Aspergillus fumigatus*; pyocyanin may also inhibit yeast-mycelial transformation in *C. albicans (*Kerr, 1999). Pyocynin is one of the redox-active phenazine compound that responsible for the blue-green colour characteristic produced by *Pseudomonas* spp (Jayaseelan *et al*, 2013).

The objective of this study was to extract crude antibiotics from *Pseudomonas aeruginosa* and also to determine the effect of different concentrations of crude antibiotics on the mycelial growth of selected plant fungal pathogens.

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