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EVALUATION of *Bacillus subtilis, Pseudomonas aeruginosa* AND *Pseudomonas fluorescens* AS BIOLOGICAL CONTROL AGENT OF *Fusarium oxysporum* f. sp. *Cubense*

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BY

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CERTIFICATION

This project report entitled Evaluation of *Bacillus subtilis, Pseudomonas aeruginosa and Pseudomonas fluorescens* as biological control agent of *Fusarium oxysporum* f. sp. *cubense* is prepared by Iznee Rehanna binti Hassan and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT4999 (Final Year Project) for the award of degree of Bachelor of Horticultural Science.

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ABSTRACT

In Malaysia, bananas are the second most important fruit crop and ranked as the 29th top world producer. However, production and export has declined mainly due to disease caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) especially on bananas of type Cavendish. The disease is known as Panama disease. Up to today, there is no definite solution towards Panama disease. Present methods are only able to delay the detrimental effect of Foc. The demand of organic food production by consumers is also increasing. These justifications lead to finding alternative method which is biological control. The objectives of this research were to determine the most effective bacterium as biological control agent of *F. oxysporum* f. sp. *cubense* and to identify presence of metabolites production of the bacteria that suppress Foc growth. Three different bacteria namely *Pseudomonas fluorescens* (UMB20), *Pseudomonas aeruginosa* (KMB25) and *Bacillus subtilis* (B40) strain were tested against Foc in PDA medium. The antagonistic metabolite production was evaluated using two different methods; double plate method and culture filtrate. The experimental design was completely randomized design and the treatment was replicated five times. Percentage inhibition of radial growth (PIRG), fungal radial growth and spore production were measured and enumerated for each treatment and compared to determine the most effective bacterium. The results from this experiment show that *P. aeruginosa* gave the highest PIRG of the Foc (27.86%) compared to the other two bacteria. *B. subtilis* and *P. fluorescens* gave PIRG of 9.274 and 26.01% respectively. The non-volatile metabolites showed more suppressive effect than volatile metabolites with PIRG of 76.53, 35.442 and 20.81% by *P. aeruginosa, B. subtilis* and *P. fluorescens* respectively.

ABSTRAK

Di Malaysia, pisang adalah tanaman buah-buahan kedua terpenting dan menjadi pengeluar utama ke-29 dunia. Namun, pengeluaran dan eksport telah menurun disebabkan oleh serangan penyakit oleh *Fusarium oxysporum* f. sp. *cubense* (Foc) terutamanya terhadap pisang jenis Cavendish. Penyakit tersebut dikenali sebagai penyakit Panama. Sehingga kini, tiada penyelesaian yang tetap bagi mengatasi penyakit ini. Kaedah masa kini hanya mampu melambatkan kesan buruk Foc. Permintaan pengeluaran makanan organik oleh pengguna juga meningkat.Justifikasi tersebut membawa kepada pencarian kaedah alternatif iaitu kawalan biologi. Objektif kajian ini adalah untuk menentukan bakteria yang paling berkesan sebagai agen kawalan biologi *Fusarium oxysporum* f. sp. *cubense* dan untuk mengenal pasti pengeluaran metabolit daripada bakteria yang menyekat pertumbuhan Foc.Tiga bakteria yang berbeza iaitu *Pseudomonas fluorescens* (UMB20), *Pseudomonas aeruginosa* (KMB25) dan *Bacillus subtilis* (B40) telah diuji terhadap Foc dalam media PDA. Pengeluaran metabolit antagonis dinilai menggunakan dua kaedah yang berbeza iaitu kaedah *double plate* dan *culture filtrate*. *Completely randomized design* ditetapkan sebagai reka bentuk eksperimen kajian ini dan replikasi rawatan diulang lima kali.Peratus menghalang pertumbuhan jejarian (PIRG), pertumbuhan jejarian dan pengeluaran spora kulat telah diukur bagi setiap rawatan dan dibandingkan untuk menentukan bakteria yang paling berkesan mengawal Foc. Hasil daripada kajian menunjukkan bahawa bakteria *P. aeruginosa* memberikan PIRG tertinggi (27.86%) berbanding bakteria lain. *B. subtilis* dan *P. fluorescens* memberikan PIRG sebanyak 26.01 dan 9.274% mengikut turutan.Jenis metabolit yang menghalang pertumbuhan Foc adalah metabolit tidak volatil yang

memberikan PIRG sebanyak 76.53, 35.442 dan 20.81% oleh *P. aeruginosa, B. subtilis* dan *P. fluorescens* mengikut turutan.

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CHAPTER 1

INTRODUCTION

Banana is one of the top 10 commodities exports of the world having export quantity of 18 million tonnes for the year 2011with Malaysia ranked 29th as the top producer of banana (FAOSTAT, FAO, 2013). In Malaysia, banana is the 10th top commodity production with the quantity of 335, 974 tonne (FAOSTAT, FAO 2013). There were fluctuations in quantity of banana produced from 2000 until 2012 (FAO, 2013). Varieties of banana grown are mainly of Cavendish type and Pisang Berangan. Other cultivars that are also planted and commercialized include Pisang Awak, Pisang Raja, Pisang Tanduk, Pisang Mas Pisang Abu, Pisang Nangka and Pisang Rastali. In the 1990s, a disease outbreak namely Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) race 4 which originated from Taiwan, hit Malaysia (Ong, 1996). The production of banana decreased mainly due the disease since the disease is hard to suppress. The type of banana that is susceptible to Foc is the Cavendish. The disease caused reduced production of banana and shorter life span of the plant.

Measures of controlling the Panama disease caused by Foc up to today includes quarantine to prevent spread, use of healthy or surviving plant materials, cultural practices such as sanitation control and rotation of non-susceptible plants, development of resistant cultivar, chemical control and biological control. Until present day, the methods used can only delay and a few give negative and unsatisfactory results (Vicente, 2014).

The demand of organic food production by consumers is also increasing. This is due to increasing awareness on the adverse effect of chemical residues in food. This leads to finding alternative method for disease control. One such method is the use of biological control agent to control plant disease. Biological control is environmentally friendly and safe to consumer as it does not leave any residual on the produce. Although research on the use of biological control agent for controlling Fusarium wilt of banana in Malaysia has been done, but the results are unsatisfactory and at times erratic. Thus, further research in finding the most effective bacterial isolates for controlling the Panama disease is required. The objectives of this research were to determine the most effective bacterium as biological control agent of *Fusarium oxysporum* f. sp. *cubense* and to identify presence of metabolites production of bacteria that suppress Foc growth.

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