UNIVERSITI PUTRA MALAYSIA

BIOCONTROL OF Fusarium oxysporum of BANANA USING ANTAGONISTIC BACTERIA

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FP 2017 52

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BY

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

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ENDORSMENT

This project report entitled Biocontrol of *Fusarium oxysporum* f. sp. *cubense* on Banana Using Antagonistic Bacteria is prepared by Nur Syahirah Abd Halim and submitted to the faculty of Agriculture in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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ACKNOWLEDGMENT

Firstly, I want to express my gratitude to Allah S.W.T because of His bless and guidance to me from the beginning of the project until my thesis writing. I want to take this opportunity to express my appreciatiation to the people that have been involved directly or indirectly in the successful completion of this project.

I would like to express my thankfulness to my project supervisor, Assoc. Prof. Dr. Jugah Bin Kadir for his invaluable advice, ideas, expertise and encouragement, this project would not have happened. I would like to thank the laboratory staffs: Pn Asmalina, En Johari, En Mohd Nazri and En Razali for their guidance and help when run the project. Their help are giving me a good knowledge to work on the laboratory.

I would like to show my gratitude to my beloved family especially my parents, Abd Halim Md Yunus and Nooraidah Udin that have been support me through my project, for their prays and encouragement to complete the project. Last but not least, I want to say thank you to all my friends especially Nur Farihin, Nurul Izzati and Nur Farzana for their help and accompany during this project were conducted. With their support, pray and love, I will be able to finish this project in the given period.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BLH	*Bacillus licheniformis*
BS	*Bacillus subtilis*
℃	Degree celcius
cm	Centimeter
Foc	*Fusarium oxysporum* f. sp. *cubense*
ml	Millilter
NA	Nutrient Agar
PDA	Potato Dextrose Agar
PIRG	Percentage Inhibition of Radial Growth
μm	Micrometer

ABSTRACT

Fusarium oxysporum is an omnipresent pathogen and is known as one of the soil-borne pathogens that causes serious disease to the plant. *Fusarium oxysporum* f. sp. *cubense* causes the fusarium wilt or know as panama disease in banana and it causes great loss in banana production. Usually, the farmer controls it through the application of chemical fungicide however it is not very effective and it also causes problems to environment, humans and animals. The uses of the chemical to overcome the problem are also not effective as the misused of the fungicide has created resistant population of the pathogen. Many researches have been conducted and reported in controlling fusarium wilt using biocontrol. In Malaysia, few researches has been documented on biological control of *Fusarium* wilt therefore, the application of biocontrol agent is very limited. The objective of the study is to evaluate the effect of the five antagonistic bacteria which are *Bacillus subtilis* strain and *Bacillus licheniformis* on mycelia growth of the *Fusarium oxysporum* f. sp. *cubense.* In vitro screening of the biological control activities of the *Bacillus* is conducted using the dual culture test, cultural filtrate test, double plate test and bi-layer test. The average of the percentage of inhibition of the pathogen for all the method is 60%. This shows that *Bacillus* spp. has the ability to control *Fusarium oxysporum* f. sp. *cubense* to control *Fusarium* wilt.

ABSTRAK

Fusarium oxysporum merupakan patogen banyak dan dikenali sebagai salah satu daripada patogen bawaan tanah yang menyebabkan penyakit yang serius kepada tumbuhan. *Fusarium oxysporum* f. sp. *cubense* menyebabkan *Fusarium* layu atau dikenali sebagai penyakit panama dalam pisang dan ia menyebabkan kerugian besar dalam pengeluaran pisang. Biasanya, petani mengawalnya melalui penggunaan racun kulat kimia namun ia tidak begitu berkesan dan ia juga menyebabkan masalah kepada alam sekitar, manusia dan haiwan. Penggunaan bahan kimia untuk mengatasi masalah ini juga tidak berkesan kerana penyalahgunaan racun telah menyebabkan patogen mampu membina immunisasi terhadap bahan kimia. Banyak kajian telah dijalankan dan dilaporkan dalam mengawal *Fusarium* layu menggunakan kawalan biologi. Di Malaysia, hanya beberapa kajian yang telah dilaporkan tentang kawalan biologi *Fusarium* layu justeru, penggunaan agen kawalan biologi adalah sangat terhad di Malaysia. Objektif kajian ini adalah untuk menilai kesan empat jenis bakteria antagonis iaitu *Bacillus subtilis* dan *Bacillus licheniformis* terhadap pertumbuhan patogen *Fusarium oxysporum* f. sp. *cubense*. Kaedah yang digunakan untuk menguji bakteria antagonis ini ialah kaedah dual kultur, kaedah tapisan kultur, kaedah cantuman plate dan kaedah dwi lapisan. Purata pembantutan pertumbuhan patogen bagi semua kaedah 60%. *Bacillus* spp. berkemampuan digunakan sebagai agen biokontrol untuk mengawal *Fusarium oxysporum* f. sp. *cubense* di pisang.

CHAPTER 1

INTRODUCTION

Fusarium wilt or Panama wilt is a typical vascular infection or disease. It causes massive destruction and loss in plantation. Jeger et. al (1995) state that 100 000 acres of bananas plantations were destroyed and abandoned because of the disease. *Fusarium* wilt caused by the fungal *Fusarium oxysporum* f. sp. *cubense. Fusarium* wilts actually have been present for a long time. The first recorded of *fusarium* wilt from Australia in 1874 (Pegg and Langdon, 1987). According to Jeger et. al (1995) the disease also recorded early days from Hawaii, South America and Asia and also from West Africa. These diseases give the big impact to the Gros Michel cultivars (AAA) and Silk (AAB) as these cultivars are susceptible to race 1 of Foc.

This disease rapidly spread in America and Africa due to the monocultures of the banana and plantation with sucker that were infected with Foc were established, therefore it hastened the disease to developed (Stover, 1962). Gros Michel cultivars were replaced by Cavendish cultivars as it is resistant towards race 1. After a long time, Cavendish cultivars also developed with *Fusarium* wilt. Race 4 of Foc only affect banana in subtropics area but early 1990s in Southeast Asia, *Fusarium* wilt were developed in Cavendish varieties in tropics area with a new race of Foc; tropical race 4 (TR4) (Ploetz, 2015). Currently, Panama wilt still causes losses in banana plantation as there is no ultimate solution to overcome this problem.

There are so many methods to control *Fusarium* wilt which include chemical control, cultural control, plant resistance, biological control and others. Mostly farmers will use chemical control to overcome *Fusarium* wilt without further thinking. The most environmental friendly control is biological control. In controlling *Fusarium* wilt, the method that suitable is using beneficial or antagonistic bacteria such as *Bacillus*.

Bacillus are very common bacteria were used to control plant pathogenic pathogen. *Bacillus subtilis* and *Bacillus thuringiensis* were the usual *Bacillus* spp. used as biocontrol to suppress the pathogen. *Bacillu*s spp. has the special characteristic such as *Bacillus subtilis,* it possess characteristic that heighten its survival in the rhizosphere (Cawoy et al., 2011). Cawoy et al., (2011) added *B. subtilis* is a motile bacteria that can move towards the root which help enhance the colonization to the new area. Active substance produces during growth by *B. subtilis* such as subtilisin, polymyxin, nystatin, gramicidin and others are the substance that can inhibit growth of plant pathogenic pathogen (Yang, 2001, Muxiang et al., 2014).

Bacillus licheniformis also can be used as antagonistic bacterial to control the plant pathogenic pathogen as it produce antibiotic such as bacillomycin, bacitracin, licheniformon and protein (Neyra et al., 1996, Katz and Demain, 1997). Neyra et al. (1996) stated that the ability of the *B. licheniformis* grow anaerobically at high temperature and form desiccation-resistant endospores enable to be used as biocontrol in safe way. Neyra et al. (1996) also added the antifungal *B. licheniformis* strains is stable for a long time and the ease of the purification are the advantage of

this bacterium to act as biological control in controlling the plant pathogenic pathogen.

OBJECTIVES

1. To determine the antagonisms activity of the *Bacillus subtilis* and *Bacillus lichenifromis* strain to inhibit the growth of the pathogen *Fusarium oxysporum* f. sp. *cubense*
2. To observe the production of the volatile antibiotic and diffusible antibiotic by the *Bacillus* spp. strains

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