UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL CONTROL OF FUSARIUM WILT ON CHILLI (Capsicum annuum L.) CAUSED BY Fusarium oxysporum F.SP.CAPSICI USING Glomus mosseae AND ACTINOMYCETES

ANFAL MUAYAD JALALULDEEN

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By

ANFAL MUAYAD JALALULDEEN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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BIOLOGICAL CONTROL OF FUSARIUM WILT ON CHILLI (*Capsicum annuum* L.) CAUSED BY *Fusarium oxysporum* F.SP.CAPSICI USING *Glomus mosseae* AND ACTINOMYCETES

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Chairman : Associate Professor Kamaruzaman Sijam
Faculty : Agriculture

Chili (*Capsicum annuum*) is an important crop grown worldwide for its use as spices and vegetables. However, chili is highly susceptible to fungal and viral infection that cause considerable damage to the crop. Therefore,this study was initiated with the aim of assessing the effective Actinomyces and Arbuscular mycorrhizal fungi against the Fusarium wilt disease.

A total of twenty bacterial strains were isolated from the rhizosphere soil of healthy chili plants, collected from different locations in UPM, Serdang, Malaysia. Out of the 20 bacterial isolates, seven were selected based on their ability to inhibit the growth of *Fusarium oxysporum*. In dual culture test isolates At1,At5,At6,At8,At11,At17 and At18 showed 50.0,71.0,42.0,42.0,47.0,42.0 and 50.0% inhibitions, respectively. Selected isolates were showed that all these isolates were Gram positive and showed high growth on Yeast extract malt extract agar, Actinomycetes isolation medium and tryptone soya agar (TSA).

All seven isolates were identified as *Streptomyces spp.* based on the morphological and biochemical properties along with 16S rRNA sequence analysis that was compared with the related bacteria in the Gen-Bank. Isolate At5 (*Streptomycetes indiaensis*) was found to be the most effective actinomycetes against the pathogen, with an average percentage inhibition of 71%, Antimicrobial products extracted using ethyl acetate, was identified by Gas Chromatography-Mass Spectrometry, Seventy-seven compounds from this isolated were identified. Susceptibility testing by disc diffusion method showed that the fungus was sensitive to all compounds with high peak but varying in proportions. It was found to be 91.85% sensitive to Chloroxylenol and 88.7% to Pyrrolo {1,2-a} pyrazine-1,4 dione, hexahydro-3-(2methylpropyl) after 7 days of incubation. Tetradecane, 2,6,10-trimethyl also showed 50% sensitivity at seven days of incubation. The Dual treatments (*Streptomyces sp.* and AMF) was able to increase shoot length significantly (61%), shoot dry weight (47%) and flower number (59%) compared to the control plant at 8th

weeks of plant growth. The dual treatment alter root morphological characteristics such as root dry weight (50.5%), root tips (18.5%), root length (77%), root surface area (72%) and root volume (48%) compared with the control. The concentration of N(67%), P(35%), K(87%), Fe(80%), Ca(67%), Mg(82%) and Zn(53%) in chili shoots and roots were increased after the colonization of *Glomus*.

The extensive colonization by the dual treatments of Streptomycetes and AMF was the reason behind the high concentration of chlorophyll Ch (a) and (b), so in dual and AMF treated plants was significantly higher (2.57 and 2.18) mg/gm respectively compared to the control (1.80) mg/gm. The activities of defense enzymes reach a peak at ten days after inoculation with the pathogen. Analysis revealed the expression of additional isoforms of PPO and PO was observed in biocontrol agents treated seedlings due to induced systemic resistance (ISR) induction.The overall results concluded from these studies confirm that the use of microorganisms as biological control agents such as (AMF) and Actinomycetes, as rhizospheric microorganisms, play a significant role in promoting plant growth and protection against plant pathogen. These could participate direct or indirect in the enhancement of root colonization by developing their individual effects on plant growth promotion.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah.

KAWALAN BIOLOGIKAL BAGI FUSARIUM LAYU KE ATAS CILI (*Capsicum annuum* L.) DISEBABKAN OLEH *Fusarium oxysporum F.SP.CAPSICI MENGGUNALAN Glomus mosseae* DAN AKTINOMISETE

Oleh

ANFAL MUAYAD JALALULDEEN

Januari 2016

Pengerusi : Professor Madya Kamaruzaman Sijam
Fakulti : Pertanian

Cili (Capsicum annuum) adalah tanaman penting yang ditanam di seluruh dunia disebabkan penggunaannya sebagai rempah dan sayur-sayuran. Walau bagaimanapun, cili sangat mudah terdedah kepada kulat dan jangkitan virus yang menyebabkan kerosakan besar kepada tanaman.

Oleh itu, kajian ini telah dimulakan dengan tujuan untuk menilai keberkesanan Aktinomiset dan kulat mikoriza arbuskel terhadap penyakit layu Fusarium . Sebanyak dua puluh strain bakteria telah diasingkan daripada tanah rhizosphere oleh tumbuhan cili yang sihat , yang dikumpul dari lokasi yang berbeza di UPM , Serdang , Malaysia. Daripada 20 pencilan bakteria, tujuh dipilih berdasarkan kepada keupayaan mereka untuk menghalang pertumbuhan Fusarium oxysporum . Dalam ujian dwi budaya mengasingkan AT1 , AT5 , At6 , At8 , At11 , At17 dan At18 menunjukkan 50.0,71.0,42.0,42.0,47.0,42.0 dan 50.0 % kekangan masing-masing. pencilan terpilih telah menunjukkan bahawa semua pencilan ini adalah Gram positif dan menunjukkan pertumbuhan yang tinggi pada ekstrak Yis agar ekstrak malt, medium Aktinomiset pengasingan dan agar tryptone soya (TSA).

Semua tujuh pencilan telah dikenal pasti sebagai Streptomyces spp. berdasarkan sifat-sifat morfologi dan biokimia bersama-sama dengan 16S rRNA analisis urutan yang telah dibandingkan dengan bakteria yang berkaitan dalam Gen- Bank. Pengasingan AT5 (Streptomycetes indiaensis) didapati menjadi aktinomiset paling berkesan terhadap patogen ,dengan purata perencatan peratusan 71 % , produk Antimicrobial diekstrak menggunakan etil asetat, telah dikenal pasti oleh Gas Chromatography -Mass spektrometri , Tujuh puluh tujuh sebatian daripada ekstrak tersebut telah dikenal pasti. Ujian kecenderungan dengan kaedah cakera penyebaran menunjukkan bahawa kulat adalah sensitif kepada semua sebatian dengan puncak tertinggi tetapi variasi dalam perkadaran . Ia didapati menjadi 91,85 % sensitif kepada

Chloroxylenol dan 88.7 % kepada Pyrrolo { 1,2 -a } Pyrazine - 1,4 Dione , hexahydro -3- (2methylpropyl) selepas 7 hari pengeraman . Tetradecane , 2,6,10 - trimethyl juga menunjukkan sensitiviti 50 % pada tujuh hari pengeraman.

Dwi Rawatan (*Streptomyces* sp. Dan AMF) dapat meningkatkan kepanjangan tembakan dengan ketara (61 %) , tembakan berat kering (47%) dan bilangan bunga (59 %) berbanding tumbuhan kawalan di minggu ke-8 pertumbuhan tumbuhan. Dwi Rawatan mengubah akar ciri-ciri morfologi seperti berat akar kering (50.5 %) , tips akar (18.5%), panjang akar (77%) , kawasan permukaan akar (72 %) dan jumlah akar (48 %) berbanding dengan kawalan. Kepekatan N (67%) , P (35 %), K (87 %), Fe (80%), Ca (67 %), Mg (82 %) dan Zn (53%) dalam pucuk cili dan akar adalah meningkat selepas penjajahan Glomus.

Penjajahan yang meluas oleh rawatan dwi Streptomycetes dan AMF adalah punca di sebalik kepekatan klorofil yang tinggi Ch (a) dan (b), jadi dalam dua dan AMF dirawat tumbuhan adalah jauh lebih tinggi (2.57 dan 2.18) mg / gm masing-masing berbanding kawalan (1.80) mg / gm. Aktiviti enzim pertahanan mencapai puncak pada sepuluh hari selepas inokulasi dengan patogen. Analisis mendedahkan ungkapan isoforms tambahan PPO dan PO diperhatikan dalam agen kawalan biologi rawatan benih kerana disebabkan oleh induksi rintangan sistemik (ISR) .Satu keputusan keseluruhan disimpulkan dari kajian ini mengesahkan bahawa penggunaan mikroorganisma sebagai agen kawalan biologi seperti (AMF) dan Actinomycetes , kerana mikroorganisma rhizospheric , memainkan peranan penting dalam menggalakkan pertumbuhan tumbuhan dan perlindungan terhadap patogen tumbuhan. Penggunaan Ini boleh mengambil bahagian secara langsung atau tidak langsung dalam peningkatan penjajahan akar dengan membangunkan kesan individu mereka kepada mempromosikan pertumbuhan tumbuhan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Kamaruzaman Sijam, PhD.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Radziah Othman, PhD.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Zainal Abidin Mior Ahmad, PhD.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature: _____
Name of Chairman of Supervisory Committee: Dr. Kamaruzaman Bin Sijam

Signature: _____
Name of Member of Supervisory Committee: Dr. Radziah Othman

Signature: _____
Name of Member of Supervisory Committee: Dr. Zainal Abidin Mior Ahmad

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LIST OF ABBREVIATIONS

%	Percent
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
bp	Base Pairs
CAS	Chromo Azural S
CFU	Colony Forming Units
CRD	Completely randomized design
EDTA	Ethylenediaminetetraacetic acid
et al.	and others
FAO	Food Agriculture Organization
HCN	Hydrogen Cyanide
IAA	Indole acetic acid
IAR	Intrinsic Antibiotic Resistance
ISR	Induced Systemic Resistance
LSD	Least Significant Difference
mg	Milligram
ML	Microliter
mL	Milliliter
mm	Millimeter
MR	Methyl red
nm	Nanometer
^{o}C	Degree Celsius
p.	Page
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PGPR	Plant Growth Promoting Rhizobacteria
PO	Peroxidase
ppm	Parts per million
PPO	Polypynol oxidase
rpm	Rotation per minute
SAS	Statistical Analysis System
sp.	Species (singular)

spp.	Species (plural)
TSA	Trypticase Soya Agar
TSB	Trypticase Soya Broth
w/v	Weight per volume
w/w	Weight per weight

CHAPTER 1

INTRODUCTION

Chili (*Capsicum annuum* L.) is one of the most popular vegetables, originated from South and Central America (Bahurupe *et al*., 2013). It is the second most important solanaceous vegetable after tomato grown worldwide, both as a spice or vegetable crop (Hasan *et al*., 2014). It genetically self-pollinated and chasmogamous crop whose flowers open only after pollination (Lemma, 1998). Chili is used as spice, condiment, vegetable, culinary supplement, medicine and as ornamental plant (Berke and Shieh 2001). The pungent chemical principle of Capsicum is fruit specific capsaicin which is found useful in a wide range of pain problems, including urticaria, psoriasis, diabetic neuropathy, arthritis, pruritis, contact allergy, post-surgical neuromas etc (Palevitch and Craker 1995).

Capsicum (hot pepper) grown worldwide in an area of 17, 03, 486 hectares with a production of 2, 60, 56,900 tones (FAO, 2009). China is the largest capsicum producing country in the world. India stands fourth on world production of capsicum with an average annual production of 0.9 million tons from an area of 0.885 million hectares with a productivity of 1266 kg per hectare (Sreedhara *et al*., 2013).

Chili pepper is thought to be the most popular spice with over 20% of the
r d r
2010). However in addition to culinary use (as spice, cooked vegetable, food ingredient, colorant etc.), Capsicum species are also used in cosmetics (Bosland PW & Votava EJ. 2003) and medicine (Lahbib K, F Bnejdi & El-Gazzah M. 2015). Capsicum spp. contain a range of essential nutrients and bioactive compounds which are known to exhibit antioxidant, antimicrobial, antiviral, anti-inflammatory and anticancer properties (Khan MA *et al* .,2014). Some of the known chemicals contained in Capsicum fruit include steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, proteins, fiber and mineral elements (Bosland & Votava, 2003). Capsicums are an excellent source of Vitamins A, B, C, E and P. Fresh green chili peppers contain more vitamin C than citrus fruits and fresh red chili has more vitamin A than carrots (Marin A *et al* .,2004).

In worldwide the yield of chili is now decreased up to 50%. There are many biotic and abiotic factors which are decreasing chili production, among these factors is the most devastating fungal diseases which lower the yield per acre annually. Chili wilt has been found as the most frequently encountered disease problems (Siddiqui & Akhtar, 2007). Wilt disease caused by *Fusarium oxysporum* F.Sp.capsici is a major problem of chili pepper production worldwide that calls for a better understanding of defensive mechanisms in the chili plant (Wongpia, Aphinya, and Khemika Lomthaisong, 2010). This fungus

enters the vascular systems via the root tissues and subsequently uses the xylem vessels as a venue to rapidly colonize the plant, leading to the characteristic wilt symptoms (Roncero et al., 2003).

In order to reduce the economic loss and to protect the crop from pathogen, different control methods are used. One very common method is to use a resistant cultivar. Besides, soil fumigants and solarization are practiced to minimize pathogens in soil. Carbendazim and Bordeaux mixture are also the most commonly used chemicals, but increasing use of pesticides in the past has led to several problems, such as environmental degradation, health hazards, pest resistance and decrease in population of beneficial insects (Groenewald, 2005). Thus, it is a universal requisite to implement the practice of sustainable agriculture, using strategies that are environmentally-friendly and less damaging to soil and water resources. Biological and environmental innocuous substitutes, such as biocontrol agents, natural plant metabolites, and cultural methods, are being explored for possible use in integrated disease management platforms and to improve the foreign exchange on the basis of the efficacy the biological factors (Javaid & Rauf, 2015). Biological control is considered an important approach of agricultural biotechnology in recent years for controlling many fungal plant pathogens (Zaher *et al*.,2013 and Abada and Eid,2014).

r r r r (M
groups of soil-borne r r r
in agricultural sustainability after forming a symbiotic relationship with roots of most plant species (Jeffries *et al*., 2003). (AMF) can provide numerous benefits to their host plants, including improved nutrient uptake, drought resistance, and disease resistance (Sharma *et al*., 2014).

Plant growth promoting rhizobacteria (PGPR) are usually in contact with the root surface or rhizoplane, and increase plant growth and yield by one or more mechanisms such as improved mineral nutrition, disease suppression and phytohormone production (Défago and Keel, 1995; Gusain *et al*., 2015b). An additional possibility is that the beneficial effects of some PGPR are due to their interactions with AMF (Bianciotto et al., 2001).

Actinomycetes are one of the major components of the microbial populations present in soil. In addition, these bacteria are known for their economic importance as producers of biologically active substances, such as antibiotics, vitamins and enzymes (De Boer *et al*., 2005). Actinomycetes are also an important source of diverse antimicrobial metabolites (Terkina *et al*., 2006). Actinobacteria were often found to be associated with AMF spores. Mugnier and Mosse (1987) reported that *G. mosseae* spores germinated in vitro only in the presence of microorganisms, including *Streptomyces orientalis* Actinomycetes found from the rhizosphere of field grown plants Trifolium repens L. (Franco-Correa *et al*., 2010) was able to improve plant growth and nutrition, and benefit root colonization by AM fungi. Therefore the study aims to:

I. Characterize actinomycetes for antagonistic activity against *Fusarium oxysporum* F.Sp.capsici.
II. Identify and determine the present of secondary metabolites from selected actinomycetes associated with resistance mechanism of chili pepper plants against FOC.
III. Evaluate the interaction between *Glomus mosseae* and selected Actinomycetes on growth of chili pepper plants infected with *FOC* under greenhouse conditions.

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