UNIVERSITI PUTRA MALAYSIA

EFFECTS OF TRICHODERMA-INDUCED SUPPRESSIVE SOIL ON FUSARIUM WILT OF TOMATO

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By

ROZLIANAH FITRI BTE SAID

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Agricultural Science

May 2005

DEDICATION

Special dedication to:

My dearest mother, brothers and sisters for their
endless and boundless love, understanding and encouragement throughout my study.
Not forgetting, my beloved late father, thank you for endless hours you spent with me.
I really missed you...

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Agricultural Science

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Chairman : Professor Sariah Meon, PhD

Faculty : Agriculture

Twenty-two isolates of *Fusarium* spp. were isolated from stems and roots of tomato plants showing symptoms of foliar wilting and brown discoloration of the vascular systems. Differentiation of the isolates based on cultural and morphological characteristics had identified twelve isolates of *F. oxysporum*, six isolates of *F. solani*, two isolates of *F. moniliforme*, one isolate of *F. chlamydosporum* and one isolate of *F. lateritium*. However, the colonies of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) appearance are not easy to distinguish from those of the non-pathogenic *F. oxysporum* even though they can be differentiated from other species. Species aggregates of *Fusarium* were further distinguished based on the DNA polymorphism. Twenty 10-mer primers were used in the initial screening of the fungal DNA and three (OPC-11, OPC-15 and OPC-18) were selected. Based on UPGMA clustering, two main clusters were defined. *F. oxysporum* were grouped in Cluster I and *F. solani* were

grouped in Cluster II. The other isolates of *F. moniliforme*, *F. chlamydosporum* and *F. lateritium* were distinctly isolated from these two main clusters. Pathogenicity testing was carried out on tomato cultivars Baccarat 322 and Cherry to further confirmed the differentiation between FOL and other forms of *F. oxysporum*. Isolate M1 produced symptoms of Fusarium wilt on Baccarat 322 and Cherry, and therefore identified as *Fusarium oxysporum* f. sp. *lycopersici* (FOL). However, percentage of disease incidence was higher on Baccarat 322 variety (65.55%) compared to Cherry (29.44%). Histopathological studies of infected stems of tomato inoculated by isolate M1 further confirmed the presence of fungal mass in the xylem vessels. Tomato plants with *Trichoderma*-induced suppressive soil (UPM 40 and UPM 23) individually and as mixture (UPM 2340) gave increased in plant height, fresh weight and dry weight of leaf and root, early flower initiation and increase in yield compared to control. Disease incidence of Fusarium wilt was significantly lower at week 12 (12%) when treated with UPM 2340, followed by UPM 40 (21%), UPM 23 (29.5%) and compost alone (59.5%). Control gave the highest value of disease incidence of 100% at week 12. The experiment carried out in this study indicated that treatments with UPM 2340, UPM 40 and UPM 23 improved vigor of tomato plants and was effective in inducing suppressiveness against Fusarium wilt development, suggesting their potential role as biological control in the management of Fusarium wilt.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

KESAN TANAH PENINDAS ARUHAN TRICHODERMA TERHADAP LAYU FUSARIUM PADA TANAMAN TOMATO

Oleh

ROZLIANAH FITRI BTE SAID

Mei 2005

Pengerusi : Professor Sariah Meon, PhD

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Dua puluh dua isolat *Fusarium* spp. telah dipencilkan daripada batang dan akar tanaman tomato yang menunjukkan gejala layu pada daun dan pewarnaan coklat kemerahan pada sistem vaskularnya. Perbezaan isolat-isolat tersebut berdasarkan ciri-ciri kultur dan morfologinya telah dapat mengenalpasti dua belas isolat *F. oxysporum*, enam isolat *F. solani*, dua isolat *F. moniliforme*, satu isolat *F. chlamydosporum* dan satu isolat *F. lateritium*. Walau bagaimanapun, kemunculan koloni *Fusarium oxysporum* f. sp. *lycopersici* (FOL) adalah sukar untuk dibezakan daripada *F. oxysporum* lain yang tidak patogenik, walaupun, ia boleh dibezakan daripada spesies-spesies lain. Spesies agregat *Fusarium* seterusnya dibezakan berdasarkan polimorfisme DNA. Dua puluh jenis primer telah digunakan untuk saringan pada peringkat awal DNA kulat dan tiga daripadanya (OPC-11, OPC-15 dan OPC-18) telah dipilih. Berdasarkan pada pengumpulan UPGMA, dua kumpulan utama telah dikenalpasti sebagai *F.*

oxysporum yang dikumpulkan dalam kumpulan I dan *F. solani* yang dikumpulkan dalam kumpulan II. Isolat-isolat lain seperti *F. moniliforme*, *F. chlamydosporum* dan *F. lateritium* adalah dengan jelas terasing daripada dua kumpulan utama tersebut. Ujian patogenisiti telah dilakukan pada kultivar tomato Baccarat 322 dan Cherry untuk pengesahan selanjutnya terhadap perbezaan di antara FOL dan *F. oxysporum* yang lain. Isolat M1 menghasilkan gejala layu Fusarium pada Baccarat 322 dan Cherry, dan oleh itu ia telah dikenalpasti sebagai *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Walau bagaimanapun, peratusan insiden penyakit adalah lebih tinggi pada varieti Baccarat 322 (65.55%) berbanding Cherry (29.44%). Kajian histologi terhadap jangkitan pada batang tanaman tomato yang telah diinokulat dengan isolat M1 seterusnya mengesahkan kehadiran kulat di dalam saluran xilem. Tanaman tomato dengan tanah penindas aruhan *Trichoderma* (UPM 40 dan UPM 23) secara individu dan campuran (UPM 2340) telah memberikan peningkatan terhadap tinggi pokok, berat segar dan berat kering daun dan akar, inisiasi bunga dan peningkatan hasil berbanding kawalan. Insiden penyakit layu Fusarium adalah lebih rendah secara signifikan pada minggu ke-12 (12%) apabila dirawat dengan UPM 2340, diikuti oleh UPM 40 (21%), UPM 23 (29.5%) dan kompos sahaja (59.5%). Kawalan memberikan nilai insiden penyakit yang paling tinggi iaitu 100% pada minggu ke-12. Eksperimen yang dilakukan dalam kajian ini menunjukkan bahawa rawatan dengan UPM 2340, UPM 40 dan UPM 23 boleh meningkatkan ketegaran tanaman tomato dan berkesan dalam merangsang penindasan terhadap kejadian layu Fusarium, mencadangkan

potensinya yang berperanan sebagai kawalan biologi dalam pengurusan layu Fusarium.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AUDPC	Area Under Disease Progress Curve
bp	base pair
cfu	colony forming unit
CRD	Complete Randomized Design
CTAB	Cetyltrimethyl ammonium bromide
DI	Disease Incidence
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra-acetic acid
FAO	Food and Agriculture Organization
FOL	*Fusarium oxysporum* f. sp. *lycopersici*
FSM	*Fusarium* Selective Medium
IPM	Integrated Pest Management
kb	kilo-base pair
LCB	Lactophenol Cotton Blue
MBC	Methyl bromide chloropicrin
M	Molar
mM	millimolar
mtDNA	mitochondrial DNA
ng	nanogram
NPK	Nitrogen, Phosphorus, Potassium
OPC	Oligo-nucleotide Purification Column Primers
%	percent
°C	degree Celsius
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PIRG	Percentage Inhibition of Radial Growth
PSM	Phosphate Solubilizing Microorganisms
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SDS	Sudden Death Syndrome
spp	spesies
Taq	Thermus aquaticus
TME	*Trichoderma* Selective Medium
TSS	Total Soluble Solids
UPM	Universiti Putra Malaysia
UPM 23	*Trichoderma virens*
UPM 40	*Trichoderma harzianum*
UPM 2340	Mixture of UPM 23 and UPM 40
µm	micrometer
µl	microliter
v/v	volume per volume
w/w	weight per weight
wt	weight

CHAPTER 1

INTRODUCTION

Tomato is the second most commonly grown vegetable crop in the world, potato being number one. Per capita fresh market tomato consumption continue to increase in much of the world. A long–term medical study has revealed that individuals who consume either fresh tomato or processed tomato products on a regular basis are less likely to have some forms of cancer than those who do not (Giovannucci, 1999; Agarwal and Rao, 2000). Tomato is rich in vitamin A and C and contains an antioxidant, lycopene. However, the demands for high quality and safe produce (pesticide-free) posses major challenges for agricultural research.

Nowadays, demand for tomatoes has increased substantially. Based on the Food and Agriculture Organization (FAO) of the United Nations in 1994, tomato fruit for the fresh market and processing is produced worldwide on approximately 2.8 million hectares (ha). In Malaysia, the production of tomato was reported as 10, 000 million ton in 2001.

The tomato plant is widely adapted to diverse environments. It is a soil–exhausting feeder and unless the soil is well supplied with plant food the plant will not yield satisfactorily. Tomatoes should be planted in fertile well–drained

soil that is high in organic matter. They also require plenty of water but not excessive because tomato roots will not function under waterlogged (anaerobic) conditions. When the moisture level surrounding the roots is too high, epinasty, poor growth, fewer flowers and lower fruit set occur. Fruit disorders such as fruit cracking will occur when water availability is inconsistent (Peets and Willits, 1995).

In Malaysia, production of tomato tends to be more successful in highland area, primarily because of the mild temperature. Therefore, large-scale tomato cultivation was presently carried out in Cameron Highlands. A satisfactory crop of top–grade tomatoes can only be obtained from well–nourished plants that are free from diseases. This has prompted the utilization of soilless culture systems using non-soil materials or agro-wastes compost preparation for optimization of nutrients and plant growth. Utilization of these potting mixes are considered environmentally sustainable, and has attracted interest among research scientists and horticulturists.

The production of tomato is also being threatened by the wide spread of different *Fusarium*-associated diseases. *Fusarium oxysporum* f. sp. *lycopersici* is a fungal pathogen commonly associated with wilt of tomato. Other *Fusarium* related diseases on tomatoes are *Fusarium* crown rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* and foot rot of tomato caused by *Fusarium solani* (Nunez and Davis, 2000). *Fusarium*-associated diseases of tomato has not been extensively studied in this country. Correct identification of the causal

pathogen should be carried out to enable formulation of effective strategy for disease management.

Methods are lacking to differentiate among strains within *Fusarium* species or to determine variability and abundance of strains in natural ecosystem. Different species or strains may vary in their ability to cause diseases on tomatoes. It is not always possible to get an accurate and reliable identification of fungi by using morphological characters. Identification of intraspecific elements are difficult and more often impossible (Mills, 1994). Recently a number of techniques comprised biochemical and molecular methods have been developed. These include intracellular isozyme and DNA-base method: Restriction Fragment Length Polymorphism (RFLP) analysis, DNA fingerprinting, Polymerase Chain Reaction (PCR) and DNA sequence analysis. The most rapidly used DNA-based method is PCR, meanwhile Random amplified polymorphic DNA (RAPD) technique incorporates PCR technique. It is a method based on incorporation of single arbitrary primers and proved to be able to distinguish variations within species.

Resistant cultivars have been the most effective means of controlling Fusarium wilt (Beckman, 1987). However, new races of the pathogen have appeared that overcome resistance in grown cultivars (Tello – Marquina and Lacasa, 1988). Methyl bromide chloropicrin (MBC) has also been used as chemical control of Fusarium wilt. However, the implication in soil and water pollution proved that

MBC is an ozone depletor (Ristaino and Thomas, 1997). Therefore, new methods have to be developed to control Fusarium wilt on tomato.

One of the alternatives is through the use of suppressive soil. Suppressive soil is referred to soils in which disease development is suppressed, even when the pathogen is present with a susceptible host (Schneider, 1984). Soil suppressiveness could be due to soil physical and chemical characteristics and/or microbial activity. Soil suppressiveness can further be 'induced' by inoculating antagonistic microorganisms into the soil, which inhibits the sporulation and disease development of the pathogen.

The manipulation of the microbial communities in the rhizosphere of crop plants for increasing yields and the biological control of diseases has been extensively studied in field crops and greenhouse crops (Menzies and Ehret, 1997). Introduction of *Trichoderma harzianum* strains as granules and wettable powder has shown significant results, both as plant growth promoter in several crops, increased in the development of the root system (Ismail, 2001; Franklin, 2002) and in the prevention against certain root diseases of greenhouse crops (Heemart and Veenstra, 1997; Jinantana and Sariah, 1998; Ibrahim, 2005). Combination treatment of *Gliocladium virens (T. virens)* and *Burkholderia cepacia* resulted in improvement in disease severity and fresh weight for pepper and fruit yield for tomato in the field (Fravel and Larkin, 1997).

This research was undertaken with the following objectives:

1. To establish the causal pathogens of *Fusarium*-associated disease of tomato.
2. To study the effects of *Trichoderma*-induced suppressive soil for the control of Fusarium wilt of tomato.

The hypothesis of this experiment is that Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* can be controlled by using *Trichoderma*-induced suppressive soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Tomato Plant

Tomato belongs to the genus *Lycopersicon L. esculentum* (Mill.) that is grown for its edible fruits. Tomato is classified as below:

Division : Anthophyta

Class : Dicotyledons

Family : Solanaceae

Genus : *Lycopersicon esculentum* Mill (Jones, 1999)

Tomato, in the past known as 'Golden Apple', is a valuable raw material for a wide range of processed foods including canned tomato juice, canned tomato whole or slice and tomato puree. It is believed to have originated in the coastal strip of the western South America, from the equator to about 30° latitude south (Taylor, 1986).

The botanical classification of tomato had an interesting history, first being placed in the genus *Solanum lycopersicon*. However, this designation was changed to *Lycopersicon esculentum*, in which *Lycopersicon* is derived from the