UNIVERSITI PUTRA MALAYSIA

UTILIZATION OF AGRO-WASTE FOR MASS PRODUCTION OF *Trichoderma asperellum* TO PROMOTE PLANT GROWTH AND CONTROL FUSARIUM WILT INFECTION ON TOMATO

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By

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Chairman : Associate Professor Nur Ain Izzati Mohd Zainudin, PhD
Faculty : Science

Chemical pesticides are commonly used for managing Fusarium wilt disease of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. However, the use of chemicals can lead to ecological instabilities and the pathogen produces chlamydospores for survival in a dormant stage over a long period in the soil. Therefore, this present study was conducted to evaluate the *in vitro* and *in vivo* ability of *Trichoderma* spp. by formulating agro-waste to inhibit Fusarium wilt pathogen on a tomato plant. The screening of antagonistic *Trichoderma* isolates' potential against *F. oxysporum* f. sp. *lycopersici* was conducted under *in vitro*, while the efficacy of the selected isolate of *Trichoderma* to control the disease and promote growth was observed under *in vivo* conditions. Previously, *Trichoderma* species have been used as antagonists against different plant pathogens but recently, enhancing its efficacy becomes important. One-hundred and eighty-four isolates of *Trichoderma* were used in this study were originally isolated from soil. These isolates were obtained from the Mycology Laboratory, Faculty of Science, Universiti Putra Malaysia. Among 184 *Trichoderma* isolates tested to dual culture, six isolates showed a very high antagonistic activity based on the percentage inhibition of radial growth (PIRG) values against *F. oxysporum* f. sp. *lycopersici*, which were of *Trichoderma asperellum* isolates B1902 (81.23%), C1667 (80.27%), C1669 (79.41%), B2230 (78.35%) and T2007 (78.11%) as well as *T. harzianum* isolate C1675 (78.45%). Subsequently, three potential *Trichoderma* isolates (*T. asperellum* B1902, *T. asperellum* C1667 and *T. harzianum* C1675) were tested for their antibiosis properties through poison food agar assay. A maximum percentage of inhibition value (90.49%) was achieved at 80% culture filtrate concentration on metabolites of *T. asperellum* B1902 followed by *T. asperellum* C1667 and *T. harzianum* C1675, which gave 87.38% and 84.28%, respectively. This study continued on an evaluation of the best medium for *T. asperellum* mass and their micropropagule production. *Trichoderma* isolates were added to the seven types of media (topsoil, rice-bran,

compost, coconut husk fiber, oil palm empty fruit bunch and biochar). All the isolates survived at different levels. Based on the colony-forming units (cfu) evaluation among the tested media, coconut husk was most suitable in promoting the sporulation of *Trichoderma*. The highest cfu was found in coconut fiber with *T. asperellum* (B1902) based formulation ($9.053\times10^5 \pm 7.88$ cfu/g) followed by oil palm empty fruit brunch with *Trichoderma asperellum* (B1902) based formulation ($7.406\times10^5 \pm 9.03$ cfu/g). From this, the ability of the combination of *T. asperellum* B1092 with coconut fiber and oil palm empty fruit bunch to control the Fusarium wilt of a cherry tomato plant, as well as to promote plant growth was investigated in plant house and field conditions. *Trichoderma asperellum* B1092 reduced the severity of tomato Fusarium wilt and enhanced all plant growth parameters and quality of tomato. Total lycopene (122.30 g/kg), sugar (6.50%), K (4.22%), N (2.14%), Ca (0.14%), P (0.14%) and Mg (0.10%) contents in tomato fruits were significantly higher when inoculated with *Trichoderma* in coconut fiber. As a conclusion, *T. asperellum* B1902 may offer the potential for biologically controlling Fusarium wilt of tomato and increasing the quality of the fruit.

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

PENGGUNAAN BAHAN BUANGAN PERTANIAN DALAM PENGHASILAN PUKAL *Trichoderma asperellum* UNTUK MENINGKATKAN PERTUMBUHAN DAN PENGAWALAN JANGKITAN LAYU FUSARIUM PADA TOMATO

Oleh

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Januari 2020

Pengerusi : Profesor Madya Nur Ain Izzati Mohd Zainudin, PhD
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Racun perosak berasaskan kimia biasanya digunakan untuk mengawal penyakit layu Fusarium pada tomato yang disebabkan oleh *Fusarium oxysporum* f. sp. *lycopersici*. Walau bagaimanapun, penggunaan bahan kimia ini boleh mengakibatkan ketidakseimbangan ekologi dan patogen tersebut menghasilkan klamidospora yang boleh bertahan dalam jangka masa panjang di dalam tanah. Oleh itu, kajian ini dijalankan bagi menilai secara *in vitro* dan *in vivo* keupayaan *Trichoderma* spp. dengan formulasi bahan buangan pertanian bagi merencatkan penyakit layu Fusarium pada tomato. Pemilihan *Trichoderma* yang berpotensi sebagai perencat *F. oxysporum* f. sp. *lycopersici* dijalankan secara *in vitro* manakala keberkesanan isolat terpilih *Trichoderma* untuk mengawal dan merangsang pertumbuhan tumbuhan diperhatikan di bawah persekitaran *in vivo*. Terdahulu, spesies *Trichoderma* digunakan sebagai agen perencat melawan patogen tumbuhan yang berbeza-beza tetapi kini, meningkatkan keberkesanannya adalah penting. Seratus lapan puluh empat isolat *Trichoderma* digunakan dalam kajian ini yang asalnya dipencilkan dari tanah. Isolat-isolat ini diperolehi dari Makmal Mikologi, Fakulti Sains, Universiti Putra Malaysia. Antara 184 isolat *Trichoderma* yang diuji secara dwi-kultur, enam isolat menunjukkan aktiviti perencatan yang sangat tinggi berdasarkan peratus perencatan pertumbuhan radius (PIRG) melawan *F. oxysporum* f. sp. *lycopersici*, iaitu isolat *Trichoderma asperellum* B1902 (81.23%), C1667 (80.27%), C1669 (79.41%), B2230 (78.35%), T2007 (78.11%) dan isolat *T. harzianum* C1675 (78.45%). Seterusnya, tiga isolat yang berpotensi (*T. asperellum* B1902, *T. asperellum* C1667 dan *T. harzianum* C1675) diuji aktiviti antibiosis mereka melalui ujian agar beracun. Kadar perencatan maksima (90.49%) direkodkan pada 80% kepekatan filtrasi metabolit kultur yang dihasilkan oleh *T. asperellum* B1902 diikuti oleh *T. asperellum* C1667 dan *T. harzianum* C1675, yang mana masing-masing pada 87.38% dan 84.28%. Kajian ini diteruskan ke atas penilaian media yang terbaik dalam penghasilan

mikropropagul. Isolat *Trichoderma* diinokulasi ke dalam tujuh jenis media (tanah atas, sekam padi, kompos, sabut kelapa, tandan buah sawit kosong dan Biochar). Kesemua isolat mampu hidup pada peringkat yang berbeza. Berdasarkan kepada penilaian unit pembentukan koloni (*cfu*) di antara media yang diuji, sabut kelapa adalah paling sesuai dalam meningkatkan sporulasi *Trichoderma.* Cfu tertinggi direkodkan pada sabut kelapa yang telah diformulasi dengan *Trichoderma asperellum* (B1902) ($9.053\times10^5 \pm 7.88$ cfu/g) diikuti oleh tandan buah sawit kosong diformulasi dengan *Trichoderma asperellum* (B1902) ($7.406\times10^5 \pm 9.03$ cfu/g). Melalui ujikaji ini, keupayaan kombinasi *T. asperellum* B1092 dengan sabut kelapa dan tandan buah kelapa sawit dalam mengawal penyakit layu Fusarium pada tomato ceri serta meningkatkan pertumbuhan tumbuhan seperti yang dikaji di bawah persekitaran rumah tumbuhan dan lapangan. *Trichoderma asperellum* B1092 mengurangkan keseriusan Fusarium layu pada tomato dan meningkatkan semua parameter pertumbuhan tumbuhan dan kualiti tomato. Kandungan likopen (122.30 g/kg), gula (6.50%), K (4.22%), N (2.14%), Ca (0.14%), P (0.14%) dan Mg (0.10%), dalam buah tomato adalah lebih tinggi apabila ditambah dengan *Trichoderma* yang dicampurkan bersama sabut kelapa. Kesimpulannya, *T. asperellum* B1902 ini berpotensi mengawal layu Fusarium pada tomato secara biologi dan meningkatkan kualiti buah.

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LIST OF ABBREVIATIONS

%	Percentage
µL	Microliter
ANOVA	Analysis of variance
C	Degree Celsius
C	Control
C/N	Carbon and nitrogen ratio
Ca	Calcium
Cfu	Colony formint unit
Cm	Centimeter
cm2	Centimeter square
CRD	Complete randomized design
Dpi	Day post inoculation
DSI	Disease severity index
EFB	oil palm empty fruit bunch
f. sp.	Forma speciales
Fm	Maximum fluorescence
Fo	Minimal fluorescence
Fv	Variable fluorescence
Fv/Fm	Maximum efficiency of photosystem II
Fv/Fo	Maximum yield of photosystem II
G	Gram
H_2O	Water

Ha	Hectare
Hr	Hour
K	potassium
Kg	Kilogram
Mg	miligram
Mg	Magnesium
Min	Minute
mL	Milliliter
Mm	milimeter
O_2	Oxygen
P	phosphorus
PDA	Potato dextrose agar
PI	Performance index
PIRG	percentage inhibition of radial growth
PSII	Photosystem II
RC/ABS	Density of reaction centres per PSII antenna chlorophyll
RCBD	randomized complete block design
ROS	Reactive oxygen species
S	Second
SD	Standard deviation
TSM	Tricoderma selective media
ΦPSII	Relative quantum efficiency of PSII

CHAPTER 1

INTRODUCTION

1.1 Background of study

In agriculture, tomato plant has a great significance as the world's most popular vegetable second after potato (Georgé et al., 2011). The major source of commercially cultivated tomatoes in Malaysia is from the Cameron Highlands. Nowadays, the tomato crop is exposed to Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici*, which is a fungal pathogen traditionally linked to wilt symptom. This disease has caused very significant economic losses (Khan et al., 2017). The utilisation of varieties with high resistance is the most strategically effective approach in controlling the disease (Silva & Bettiol, 2005; Sheu & Wang, 2006). However, in Malaysia, no available variety of tomato so far has been proven fully and permanently resistant towards Fusarium wilt disease. Nevertheless, *Fusarium oxysporum* f. sp. *lycopersici* has been discovered to develop a resistance to chemical fungicides. This phenomenon makes it urgent to seek other approaches for controlling the disease. Since years ago, various research funding has been provided to identify the suitable biocontrol agents among fungi or bacteria to decrease the use of fungicides and lower the production costs.

Since tomato is a common vegetable crop among customers owing to its nutritional value and financial significance as a major contributor to Malaysia's agricultural sector, there appears limited information available on how it can be protected from tomato wilt caused by the soil-borne, *F. oxysporum* f. sp. *lycopersici*. Specifically, several studies have investigated the treatment of tomato wilt with *Trichoderma* spp., while other studies investigated the defense system of tomato plants following treatment with *Trichoderma* spp. after infected by Fusarium wilt in Malaysia. *Trichoderma* species is a filamentous fungus that holds a great promise as an effective antagonist to certain pathogenic fungi in soil such as *Rhizoctonia solani*, *Pythium ultimum* and *Sclerotinia trifoliorum* (Kandula et al., 2015). Furthermore, it possesses a stimulatory impact on the growth of different plants as well as their distribution, survival and proliferation in soil (Khan et al., 2017).

The fungi acting as biocontrol agents exhibit beneficial attributes and show specific mechanisms and associations such as mycoparasitism, substrate competition antibiotic activity and induced resistance that prevents or reduces infections of pathogenic organisms (Harman et al., 2004a). Biocontrol agents can act as a living barrier to subsequent pathogen invasion and mobilese nutrients for a target plant (Brimner & Boland, 2003). In addition, it is known that

fungal biological control agents can affect microbial communities and constrain root pathogens for a long duration (Harman et al., 2004a).

1.2 Problem statement and research questions

There is limited information about the relationship between the impact of *Trichoderma* species on plant health, growth, their establishment and proliferation in the root system (Hohaman, 2010). The focus of this research is on biological control agent of *Trichoderma* species against *Fusarium oxysporum* f. sp. *lycopersici*, and to a lesser extent, the interaction between the agent and plant. As a result, the routine use of *Trichoderma*-based bio-inoculants for yielding improvement in commercial agriculture remains challenging (Verma et al., 2007).

To understand more on the mechanisms, this study was conducted to answer the following research questions: i) how to control the growth of *Fusarium oxysporum* f. sp. *lycopersici* using *Trichoderma* spp. in various media as supplemented substrate?; ii) can *Trichoderma* species survive in different kinds of soil media?; and iii) how effective is the use of *Trichoderma* species, survival, growth and proliferation of the antagonist in the soil and rhizosphere. These entire research questions need to be answered.

Although many researchers have investigated the prevalence, dissemination and isolation of *Trichoderma* in its natural habitats, only a few have attempted on quantitatively studying its population, survival and proliferation in soil and other environments. The scant details of the antagonist survival rate in soil are due to the absence of techniques and proper culture media to isolate and record relevant details. Quantitative estimation on *Trichoderma* spp. in soil is frequently difficult due to the fast growth of other fungi in conventional agar media. Therefore, this study was conducted to address the issues related to the research questions.

1.3 Aims and importance of study

The aims of this study are to evaluate how effective *Trichoderma* species would be in suppressing the spread of *Fusarium oxysporum* f. sp. *lycopersici* in tomatoes under *in vivo* conditions for selecting microbial isolates to be used in agriculture, optimizing the use of new beneficial isolates and enhancing their practical use.

It is essential and worth for considering the phenomenon of antagonistic institution and proliferation in soil as a biological control. Besides, it is necessary to examine and associate the survival capacity of biocontrol agents such as

population size, survival duration and spread in or on plants. Whereas it is essential to define and assess the kinds of media and natural habitats that affect the establishment and population dynamics of introduced *Trichoderma* strains, which can provide more predictable and efficient crop disease biocontrol.

Adding *Trichoderma* species as microbial growth and biocontrol agents to the roots of the plants is an efficient and inexpensive means to enhance the growth of the plants with disease protection when planted in the field. In addition, it is useful to address the issue of whether or not the *Trichoderma* species has the capability to prevent the progress of *Fusarium oxysporum* f. sp. *lycopersici* and increase the growth of tomatoes. Furthermore, using these microorganisms as biocontrol agents and biofertilizers is a main step away from chemicals used to promote growth and control plant diseases. The demand for biocontrol agents is increasing dramatically due to concerns on safety and environmental impact of chemicals.

In this study, the effectiveness of the native isolates of the *Trichoderma* species in promoting the development and crop factors of tomato and the management of Fusarium wilt disease under *in vitro* and *in vivo* settings was explored. This study was endeavored to verify the ability of native *Trichoderma* spp. as a possible biocontrol to suppress *Fusarium oxysporum* f. sp. *lycopersici*, which would be of great importance to tomato farmers not only in Malaysia, but globally in solving the *Fusarium* issue that plagues plants all over the world.

1.4 Objectives of study

This study was designed to achieve the following main objectives:

i. To examine the antagonistic effect of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici* under *in vitro* condition,
ii. To determine the survival and proliferation of *Trichoderma* sp. in different types of media, and
iii. To evaluate the effects of *Trichoderma asperellum* inoculant on growth performance of tomatoes and quality of fruits in Fusarium wilt-infected tomato plants under *in vivo* condition.

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LIST OF PUBLICATIONS

Zainap Ab Easa Hasan, Nur Ain Izzati Mohd Zainudin, Asma Aris, Mohd Hafiz Ibrahim, Mohd Termizi Yusof (2020). Biocontrol efficacy of *Trichoderma asperellum*-enriched coconut fiber against Fusarium wilts of cherry tomato. *Journal of Applied Microbiology*. DOI: 10.1111/jam.14674. In Press.

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