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EFFECT OF MICROBIAL ANTAGONISTS ON THE DEVELOPMENT OF BACTERIAL WILT ON TOMATO

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By

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Specially Dedicated

To

My Family

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

EFFECT OF MICROBIAL ANTAGONIST ON THE DEVELOPMENT OF BACTERIAL WILT ON TOMATO

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Bacterial wilt caused by *Ralstonia solanacearum* is the major constraint to the production of tomato in Malaysia. In this study, the effect of application methods of biological control agents to control bacterial wilt of MT11, a moderately resistant variety of tomato was evaluated under greenhouse and field condition. Six antagonistic strains of *Pseudomonas aeruginosa* showed *in vitro* antagonism towards *R. solanacearum*. Seed bio-priming with these strains of *P. aeruginosa* either individually or in combination increased the percentage of seed germination. Whereas, biomass of plants was increased only after seeds were bio-primed with the combined strain. Combination of strains KT8+72PTT was used as seeds bio-prime agent in greenhouse and field experiments.

Penicillium sp. showed *in vitro* antagonism towards *R. solanacearum*. Greenhouse experiment also showed that the antagonist could reduce the incidence of bacterial wilt on MT11. An experiment was carried out on the effect of the application methods and levels of concentration of the antagonist

on the biomass of plants. Results showed that soil incorporated with *Penicillium* sp. just before transplanting significantly reduced the biomass of top part of six true leaves seedlings. Direct drenching of antagonist also significantly reduced the biomass of four-week-old seedlings, either at high or low concentration. However, no significant reduction was observed in the biomass of root system and dry weight of top when the antagonist was incorporated into soil one week prior to transplanting.

Two greenhouse trials were conducted to evaluate the integrated control using combination of bacterial and fungal antagonist and calcium nitrate, on MT11. Both the experiments showed reduced incidence of bacterial wilt on plants, which were treated with the integrated control measures. Combined treatment of seed bio-priming and carrier application of *Penicillium* sp. at high concentration recorded the lowest Disease Severity Index of bacterial wilt in the second greenhouse trial. Carrier application of *Penicillium* sp. provided better protection to plants compared to direct drenching regardless of inoculum concentration.

In the field experiment, disease incidence on MT11 was reduced after combined treatment of seed bio-priming and application of *Penicillium* sp. directly or in carrier with calcium nitrate as supplement. Subsequently, tomato yield also increased by 129.6% and 133.4% as compared to the control after five harvests when treated with combined treatment of seed bio-priming with direct application of *Penicillium* sp. or in carrier application, respectively. However, the same integrated control measures did not provide sufficient protection to the susceptible variety, Pearl, which recorded 100% infection at

the end of experiment. With the susceptible variety, the integrated control measures significantly reduced disease incidence only at the third week after transplanting.

In conclusion, bio-priming of tomato seeds with *P. aeruginosa*, strains KT8 and 72PTT, with application of *Penicillium* sp. at transplanting stage was able to control bacterial wilt and thus increased the yield. This study indicated that timing and application method for microbial antagonists as biocontrol agents were critical.

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

KESAN ANTAGONIS MIKOB TERHADAP PERTUMBUHAN PENYAKIT LAYU PADA TOMATO

OLEH

CHOO EE FONG

March 2003

Pengerusi : Prof. Madya Dr. Hiryati Abdullah

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Penyakit layu bakteria yang disebabkan oleh *Ralstonia solanacearum* merupakan halangan utama kepada pengeluaran tomato di Malaysia. Dalam kajian ini, kesan penggunaan kaedah kawalan biologi untuk mengawal layu bakteria pada tomato MT11, varieti sederhana resistan terhadap penyakit ini telah dinilai di rumah kaca dan ladang. Enam *Pseudomonas aeruginosa* strain menunjukkan sifat antagonis terhadap *R. solanacearum* secara *in vitro*. Rawatan bijibenih secara biologi dengan *P. aeruginosa* strain ini secara tunggal atau dalam kombinasi, telah meningkatkan peratus percambahan bijibenih. Manakala, biojisim tumbuhan hanya dapat dipertingkatkan selepas rawatan biologi dengan kombinasi strain. Kombinasi strain KT8 + 72PTT dipilih sebagai agen rawatan biologi dalam kajian rumah kaca dan ladang.

Penicillium sp. menunjukkan sifat antagonisnya terhadap *R. solanacaerum* secara *in vitro*. Eksperimen rumah kaca juga menunjukkan antagonis ini dapat mengurangkan insiden layu bakteria pada MT11. Eksperimen telah dijalankan untuk mengaji kesan kaedah aplikasi dan tahap

kepekatan bagi antagonis pada biojisim tumbuhan. Keputusan menunjukkan bahawa pengaulan tanah dengan *Penicillium* sp. sebaik sebelum pemindahan tumbuhan telah mengurangkan biojisim bagi anak benih yang mempunyai enam daun sebenar. Pencurahan *Penicillium* sp. secara terus juga menurunkan biojisim bahagian atas bagi anak benih yang berumur empat minggu, sama ada pada kepekatan yang tinggi atau rendah. Walaubagaimanapun, tiada pengurangan yang bererti diperhatikan dalam biojisim sistem akar dan jisim kering bahagian atas anak benih, apabila antagonis dimasukkan dalam tanah seminggu sebelum pemindahan tumbuhan.

Dua ujian rumah kaca telah dijalankan untuk mengaji kawalan gabungan dengan kombinasi antagonis bakteria dan kulat serta kalsium nitrat ke atas MT11. Keuda-dua ujian ini telah menunjukkan pengurangan insiden layu bakteria dengan rawatan gabungan tersebut. Kombinasi rawatan bijibenih secara biologi, serta aplikasi *Penicillium* sp. oleh pembawa dalam kepekatan yang tinggi mencatatkan indeks keterukan penyakit yang terendah bagi layu bakteria dalam ujian rumah kaca yang kedua. Aplikasi *Penicillium* sp. dalam pembawa memberi perlindungan yang lebih baik kepada tumbuhan berbanding dengan cara pencurahan terus tanpa mengira kepekatan inokulum.

Eksperimen di ladang menunjukkan insiden penyakit pada MT11 berkurangan setelah dirawat dengan kombinasi rawatan bijibenih secara biologi serta aplikasi *Penicillium* sp. secara pencurahan atau dalam pembawa, dengan kasium nitrat sebagai tambahan. Seterusnya, hasil tomato

juga telah meningkat sebanyak 129.6% dan 133.4% berbanding kepada kawalan selepas lima pungutan, apabila dirawat dengan kombinasi rawatan bijibenih secara biologi serta aplikasi *Penicillium* sp. secara terus atau dalam pembawa, masing-masing. Walaubagaimanapun, ukuran kawalan gabungan yang sama tidak memberi perlindungan yang cukup kepada varieti rentan, Pearl, yang telah mencatatkan 100% jangkitan pada akhir eksperimen. Dengan varieti rentan, ukuran kawalan itu mengurangkan insiden penyakit dengan bererti hanya pada minggu ketiga selepas pemindahan tumbuhan.

Kesimpulannya, rawatan bijibenih secara biologi dengan *P. aeruginosa*, strain KT8 dan 72PTT dengan aplikasi *Penicillium* sp. pada peringkat pemindahan tumbuhan dapat mengawal layu bakteria dan turut menambahkan hasil. Kajian ini menunjukkan bahawa masa dan kaedah aplikasi adalah kritikal untuk antagonis mikrob berfungsi sebagai agen kawalan biologi.

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ANOVA	=	Analysis of Variance
$Ca(NO_3)_2$	=	Calcium nitrate
cfu	=	colony forming unit
CPG	=	casamino acid peptone glucose
CYA	=	Czapek's yeast autolysate
DMRT	=	Duncan's Multiple Range Test
DSI	=	Disease Severity Index
FAMA	=	Federal Agriculture and Marketing Authority
FAO	=	Food and Agriculture Organization
ISR	=	Induced Systemic Resistance
K	=	potassium
KB	=	King's B
LSD	=	Least Significant Difference
MARDI	=	Malaysian Agricultural Research and Development Institute
MEA	=	malt extract agar
N	=	nitrogen
OD	=	Optical density
P	=	phosphorus
PDA	=	potato dextrose agar
PGPR	=	Plant Growth-Promoting Rhizobacteria
pH	=	Hydrogen ion concentration
TZC	=	tetrazolium chloride
Vol.	=	volume

CHAPTER I

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the economically important vegetables throughout the world. In Malaysia, the planting hectareage and production of tomatoes has rapidly increased during 1989 to 1999 (FAO, 2000). It is the third important vegetable of the export commodities of Malaysia. It has become a potential source of cash income for the farmers. Recent interest has been directed towards the red pigment in the ripe tomato fruit, lycopene, one of the world's most powerful antioxidants (Jones, 1999). In Malaysia, production of tomato tends to be more successful in highland area, primarily because of the mild temperature. Disease and pests often hamper the tomato production. Occurrence of tomato disease can cause the reduction in the quality and quantity of the yield.

The bacterial wilt disease caused by *Ralstonia solanacearum* (syn. *Pseudomonas solanacearum*)(Yabuuchi *et al.*, 1992) was considered as the most serious factor limiting commercial production in the lowlands (Graham *et al.*, 1977). Therefore large-scale tomato cultivation was presently carried out in Cameron Highlands. In Malaysia, this soil-borne pathogen had been reported on 43 hosts distributed over a wide range of families which, includes crop plants, ornamentals and weeds (Abdullah, 1992). It also includes some major economic hosts, such as brinjal, chilli, ginger, groundnut, potato, tobacco and tomato. Losses due to this disease had been reported from cultivated or non-cultivated plants from many countries of the world.

However, the estimates of losses in tomato production in Malaysia due to bacterial wilt disease are not available.

Control measures commonly employed to control bacterial wilt include resistant or tolerant varieties or cultivars, crop sanitation, crop rotation and other cultural practices. Greenhouse experiments showed that chemical control measures were able to significantly reduce the incidence of bacterial wilt in chilli and tomato plant (Abdullah, 1998). The use of resistant varieties is known as the most effective, popular and easiest strategy for disease control. However, for controlling bacterial wilt disease, resistance is not expressed under certain environmental conditions (Sequeira and Rowe, 1969) and the breakdown of resistance due to high temperature has been known to occur (Krausz and Thurstan, 1975; French and De Lindo, 1982).

Biological control, a promising control strategy has provided an alternative for the management of bacterial wilt. Many researchers have investigated the introduction of antagonistic microbial agents in biological control during the past few years (Chen and Echandi, 1984; Ciampi *et al.*, 1989; Trigalet and Demery, 1990; Hara and Ono, 1991). Strains of fluorescent pseudomonad have been reported as beneficial microbial agent to increase growth and control soilborne plant pathogens (Schroth and Hancook, 1982; Suslow, 1982; Burr and Caesar, 1984; Leeman *et al.*, 1995). Some of them are known as plant-growth promoting rhizobacteria (Kloepper *et al.*, 1980b; Ongena *et. al.*, 1999). Identification of these fluorescent pseudomonads is readily made by the production of pigmented siderophores, which fluoresces under ultra violet and blue light (Kloepper *et al.*. 1980a).

Even though there was no report of the control of bacterial wilt with *Penicillium* sp., it has been reported as a biocontrol agent to control other diseases (Windels, 1981; Kaiser and Hannan, 1984; Fang and Tsao, 1995; De Cal *et al.*, 1995, 2000). To date, there is no effective control measure to control bacterial wilt disease in heavily infested areas in Malaysia. A new approach in the management of this disease using moderately susceptible variety coupled with microbial antagonists and calcium nutrient amendment was investigated for their effectiveness. The objectives of the study were:

1. To determine the effect of bio-priming with *Pseudomonas aeruginosa* on tomato seed germination and the biomass of plant.
2. To evaluate the effect of application methods of the microbial antagonists, in controlling bacterial wilt of tomato under greenhouse and field conditions.