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COMBINED EFFECT OF CALCIUM NITRATE AND FLUORESCENT PSEUDOMONADS ON BACTERIAL WILT CAUSED BY RALSTONIA SOLANACEARUM

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By

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

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

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Chairman: **Associate Professor Hiryati Abdullah, Ph.D.**

Faculty: **Agriculture**

A study on the combined effect of calcium nitrate and fluorescent Pseudomonads on bacterial wilt caused by *Ralstonia solanacearum* on tomato was carried out in the greenhouse and field. Greenhouse studies showed that application of calcium nitrate solution at 0.1% and 0.5% on tomato seedlings prior to transplanting gave significant increase ($p \leq 0.01$) on the fresh and dry weight of the treated seedlings compared to the 1% treatment and the non-treated control. Application of 0.5% calcium nitrate solution prior to transplanting, complemented with application of granular calcium nitrate after transplanting gave significant reduction ($p \leq 0.01$) on bacterial wilt incidence on tomato seedlings planted in soil artificially infested with *R. solanacearum* at approximately 10^6 cfu/g oven-dry soil.

Four strains of antagonistic Pseudomonads, that inhibited the growth of *R. solanacearum* on dual-culture plates, were tested individually in bio-priming (approximately 1 X 10^8 cfu/ml in 1.5% methylcellulose) on their

effects towards seed germination and biomass of the treated tomato seedlings. *Pseudomonas putida* strains KTS26 and AC1 individually gave significant ($p \leq 0.01$) increase on germination (%) compared to when seeds were coated with methylcellulose only. Fresh weight of 30-days-old seedlings was significantly increased ($p \leq 0.01$) by bio-priming with combination of KTS26 and AC1, and with KTS26 alone. However, dry weight of seedlings was not affected by the treatments.

Greenhouse studies were conducted to evaluate the combined effects of seed bio-priming with *Pseudomonas putida* strains KTS26 and AC1, either individually or in combination; application of calcium nitrate, at regular intervals, prior to and after transplanting; and application of Stonier's medium (half-strength) in controlling bacterial wilt on MT11 up to 6 weeks after transplanting. All combination of treatments significantly ($p \leq 0.01$) reduced the disease, compared to the non-treated control. However, no significant difference was observed between the treatments.

Subsequently, the combined effects of seed bio-priming with combination of KTS26 and AC1, application of calcium nitrate and with or without half-strength of Stonier's medium in controlling bacterial wilt of two tomato varieties, MT 11 and Pearl, was evaluated under field condition. The highly susceptible 'Pearl' variety was included in the field trial as a comparison for MT11. Results showed that all treatments significantly reduced ($p \leq 0.01$) bacterial wilt on treated MT11, compared to the non-treated control. However, no significant difference was observed between

the treatments. Thus, incorporation of Stonier's medium to enhance antibiosis by the antagonists did not give additive effect on disease suppression. The control measures applied did not have any effect on the Pearl variety, which recorded 100% mortality. This indicated that some level of disease resistance in the variety used was essential. This study indicated that the combined treatments of calcium nitrate and antagonists could increase the resistance of moderately resistant variety. Thus, it could be used in the management of the disease in infested areas.

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

KESAN KOMBINASI KALSIUM NITRAT DAN PSEUDOMONADS BERPENDAFLUOR PADA PENYAKIT LAYU BAKTERIA YANG DISEBABKAN OLEH *RALSTONIA SOLANACEARUM*

Oleh

LEE KAM LOONG

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Pengerusi: **Profesor Madya Hiryati Abdullah, Ph.D.**

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Kesan kombinasi kalsium nitrat dan Pseudomonads berpendafluor pada penyakit layu bakteria yang disebabkan oleh *Ralstonia solanacearum* pada pokok tomato telah dikaji dalam rumah kaca dan di ladang. Keputusan yang diperolehi dari kajian dalam rumah kaca menunjukkan bahawa applikasi larutan kalsium nitrat dengan kepekatan 0.1% dan 0.5% ke atas anak benih tomato sebelum tempoh pengubahan telah meningkatkan nilai berat basah dan kering anak benih tersebut secara bererti ($p \leq 0.01$), berbanding dengan anak benih yang dirawat dengan 1% kalsium nitrat ataupun kawalan yang tidak dirawat. Rawatan dengan applikasi larutan kalsium nitrat pada kepekatan 0.5% sebelum tempoh pengubahan, bersepadu dengan applikasi butiran kalsium nitrat selepas tempoh pengubahan telah mengurangkan kejadian layu bakteria ($p \leq 0.01$) pada anak benih tomato yang ditanam dalam tanah campuran yang diinfestasi dengan *R. solanacearum* secara buatan pada kepekatan 10^6 sel/ g tanah yang dikeringkan dalam ketuhar.

Empat strain bakteria antagonistik dari kumpulan Pseudomonads yang dapat menghalang pertumbuhan *R. solanacearum* di atas piring dwi-kultur telahpun diuji kesannya secara individu melalui kaedah rawatan biji benih secara biologi (lebih kurang 1 x 10^8 sel/ ml dalam 1.5% metilselulosa) ke atas kesan percambahan biji benih dan biojisim anak benih tomato. *Pseudomonas putida* strain KTS26 dan AC1, secara individu meningkatkan peratusan percambahan secara bererti ($P \leq 0.01$) berbanding dengan biji benih yang dirawat dengan metilselulosa sahaja. Nilai berat-basah anak benih yang berusia 30 hari telah meningkat secara bererti ($p \leq 0.01$) selepas dirawat dengan bakteria KTS26 dan AC1, samada secara kombinasi, ataupun dengan KTS26 sahaja. Walau bagaimanapun, nilai berat-kering anak benih tersebut tidak dipengaruhi oleh rawatan itu.

Kajian telah dijalankan dalam rumah kaca untuk menilai kesan kombinasi yang terdiri dari rawatan biji benih secara biologi dengan *P. putida* strain KTS26 dan AC1, samada secara individu ataupun dalam kombinasi, applikasi kalsium nitrat pada jarak waktu yang tertentu sebelum dan selepas pengubahan, dan applikasi larutan separuh kepekatan medium Stonier untuk pengawalan penyakit layu bakteria pada pokok MT11 hingga ke minggu ke-6 selepas pengubahan. Kesemua rawatan dapat mengurangkan kejadian layu bakteria secara bererti ($p \leq 0.01$), berbanding dengan pokok kawalan yang tidak dirawat. Walau bagaimanapun, tiada sebarang perbezaan yang bererti di antara jenis-jenis kombinasi rawatan.

Justeru itu, kesan kombinasi rawatan yang terdiri dari rawatan biji benih secara biologi (dengan kombinasi KTS26 dan AC1), applikasi kalsium nitrat, dan dengan atau tanpa larutan separuh kepekatan medium Stonier telah diuji untuk pengawalan penyakit layu bakteria ke atas dua jenis varieti tomoto, iaitu MT11 dan Pearl, di ladang. Varieti Pearl yang sangat rentan dimasukkan dalam kajian ladang tersebut sebagai perbandingan untuk varieti MT11. Keputusan menunjukkan bahawa kesemua jenis rawatan mengurangkan kejadian layu bakteria secara bererti ($p \leq 0.01$) pada pokok MT11 yang dirawat, berbanding dengan pokok kawalan yang tidak dirawat. Walau bagaimanapun, tiada perbezaan yang bererti antara jenis-jenis kombinasi rawatan. Dengan itu, pergabungan larutan medium Stonier dalam kombinasi rawatan yang bertujuan untuk mempertingkatkan proses antibiosis bagi antagonis yang digunakan, tidak memberi kesan tambahan ke atas pengawalan layu bakteria. Rawatan yang diberi ke atas varieti Pearl tidak memberi sebarang kesan, malahan ia mencatatkan kematian 100% di ladang. Ini menunjukkan bahawa tahap keresistanan dalam varieti yang terpilih adalah penting demi pengawalan penyakit. Kajian ini telah menunjukkan bahawa rawatan kombinasi kalsium nitrat dan antagonis dapat meningkatkan keresistanan pada varieti yang mempunyai keresistanan sederhana. Justeru itu, ia boleh diguna untuk pengurusan penyakit ini di kawasan yang terdapat infestasi.

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I certify that an Examination Committee met on 10th April 2003 to conduct the final examination of Lee Kam Loong on his Master of Agricultural Science thesis entitled "Combined Effect of Calcium Nitrate and Fluorescent Pseudomonads on Bacterial Wilt Caused by *Ralstonia solanacearum*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Agricultural Science. The members of the Supervisory Committee are as follow:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

錦隆.

(LEE KAM LOONG)

Date: 21 MAR 2003

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The bacterial wilt disease caused by *Ralstonia solanacearum* has been reported as the most devastating bacterial plant disease that threatened the production of tomato in the tropics, subtropics and warm temperate regions worldwide (Kishun, 1981; Hayward, 1991). In Malaysia, the entire crop from a susceptible variety can be wiped out by the disease (Yang, 1979). Control of the disease through chemical application, soil fumigation and crop rotation are practically ineffective, or are not adapted to low-income farming systems (Enfinger *et al.*, 1979). Sterilization of soil by solarization or with chemicals such as methyl bromide or chloropicrin have been demonstrated, but neither of these were highly effective since the chemicals were absorbed by soil particles and degraded by microorganisms (Monma and Sakata, 1993). In addition, methyl bromide is scheduled to be phased out by 2005 under the Montreal Protocol (Anon, 1998), because of its negative impacts on the environment.

The use of resistant cultivars has been reported to be the most effective method of disease control (Mew and Ho, 1976; Hayward, 1991; Monma *et al.*, 1993; Thoquet *et al.*, 1996; Monma *et al.*, 1997; Wang *et al.*, 1998). In Malaysia, several bacterial wilt-tolerant tomato varieties have been released for cultivation in the lowlands (Ho, 1988; MARDI, 1990). However, none of these tomato varieties posses both high resistance and high fruit quality. Among these varieties, variety MT11 was stated to be

moderately resistant to bacterial wilt disease (80% survival) and produced acceptable fruit size (40 – 80g) compared to the other variety with smaller fruit size (MARDI, 1990). In spite of that, resistance may often breaks down or may not be expressed under certain environmental conditions, and is insufficient for use in heavily infested fields (Sequeira and Rowe, 1969; Thoquet *et al.*, 1996; Monma *et al.*, 1997; Wang *et al.*, 1998). Thus, incorporation of other control measures such as biological control and nutrient soil amendment into disease management programme may have better potential in reducing the disease. However, local study on this aspect is lacking.

Many studies have been done to reveal the role of fluorescent Pseudomonads in promoting plant growth, increasing crop yield and suppressing disease caused by soil-borne plant pathogens (Xu and Gross, 1986; Hsu *et al.*, 1992; Powell *et al.*, 2000). Research done on *Pseudomonas fluorescens* (Aspiras and Cruz, 1986; Hsu *et al.*, 1992), *P. aeruginosa* (Furuya *et al.*, 1997) and *P. cepacia* (Elphinstone and Aley, 1992) have shown some promise in the control of bacterial wilt by these antagonists. Several reports suggest that these pseudomonads colonize the root surface and inhibit infection by producing antibiotics, or siderophores, or volatile substances (Buysens *et al.*, 1996; Mulya *et al.*, 1996).

Applications of calcium nitrate as soil amendment in controlling some fungal and bacterial disease incidence have been reported (Corden,

1965; Spiegel *et al.*, 1987; Sitterly, 1962; Bateman and Lumsden, 1965; Muchovej *et al.*, 1980; Sariah *et al.*, 1997; Rahman and Abdullah, 1997; Yamazaki *et al.*, 1999). Previous finding indicated that calcium improves plant cell wall integrity and inhibits enzymatic degradation of tissues by pathogens, thus resulting in the enhancement of plant resistance (Demarty *et al.*, 1984).

This study was therefore carried out with the following objectives: 1) to evaluate the effect of calcium nitrate on growth of tomato seedlings and on the incidence of bacterial wilt on tomato; 2) to evaluate the effect of bacterial antagonists in seed bio-priming on tomato germination and seedlings biomass; 3) to evaluate the effect of antagonists and nutrient soil amendment on the incidence of bacterial wilt under greenhouse and field conditions.