



UNIVERSITI PUTRA MALAYSIA

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

LEE KAM LOONG

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By

LEE KAM LOONG

**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
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April 2003

Chairman: Associate Professor Hiriyati 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 1×10^8 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 KALSIMUM NITRAT DAN
PSEUDOMONADS BERPENDAFLUOR PADA
PENYAKIT LAYU BAKTERIA YANG DISEBABKAN OLEH
*RALSTONIA SOLANACEARUM***

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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 aplikasi 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 aplikasi larutan kalsium nitrat pada kepekatan 0.5% sebelum tempoh pengubahan, bersepadu dengan aplikasi 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 1×10^8 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, aplikasi kalsium nitrat pada jarak waktu yang tertentu sebelum dan selepas pengubahan, dan aplikasi 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), aplikasi kalsium nitrat, dan dengan atau tanpa larutan separuh kepekatan medium Stonier telah diuji untuk pengawalan penyakit layu bakteria ke atas dua jenis varieti tomat, 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 koresistensi dalam varieti yang terpilih adalah penting demi pengawalan penyakit. Kajian ini telah menunjukkan bahawa rawatan kombinasi kalsium nitrat dan antagonis dapat meningkatkan koresistensi pada varieti yang mempunyai koresistensi 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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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

	Page
ABSTRACT	2
ABSTRAK	5
ACKNOWLEDGEMENTS	8
APPROVAL	10
DECLARATION	12
LIST OF TABLES	16
LIST OF FIGURES	19
 CHAPTER	
I INTRODUCTION	22
II LITERATURE REVIEW	25
The Economic Importance of Tomato	25
Bacterial Wilt of Tomato	27
Causal Organism	27
Symptomatology	30
Disease Cycle	32
Control Measures	33
Bio-priming Seed Treatment for Disease Management	41
Fluorescent Pseudomonads in Biological Control	46
Effects of Calcium on Plant Disease Incidence	51
III METHODOLOGY	55
Effect of Calcium Nitrate Concentrations on Growth of Tomato Seedlings	55
Calcium nitrate - $5\text{Ca}(\text{NO}_3)_2 \cdot \text{NH}_4\text{NO}_3 \cdot 10\text{H}_2\text{O}$	55
Plant Propagation	55
Experimental Design	56
Data Assessment and Analysis	56
Effect of Calcium Nitrate on Bacterial Wilt Incidence on Tomato Seedlings in The Greenhouse	58
Bacterial Culture of <i>R. solanacearum</i>	58
Plant Propagation	59
Experimental Design	60
Data Assessment and Analysis	61
Antagonism of Selected Bacterial Strains from Fluorescent and Non-Fluorescent Pseudomonads on <i>Ralstonia solanacearum</i>	64
Bacterial Culture of Antagonistic Pseudomonads	64
Bacterial Culture of <i>Ralstonia solanacearum</i>	64
Dual Culture Agar Plates	65
Experimental Design	65
Data Assessment and Observation	65



	Effect of Antagonistic Bacteria in Bio-priming on Germination of Tomato Seeds	66
	Bio-priming Seed Treatment	66
	Experimental Design	66
	Data Assessment and Analysis	67
	Effect of Antagonistic Bacteria in Bio-priming on Biomass of Tomato Seedlings	68
	Bio-priming Seed Treatment	68
	Plant Propagation	68
	Experimental Design	68
	Data Assessment and Analysis	69
	Effect of Antagonist and Soil Nutrient Amendment on Bacterial Wilt Incidence in the Greenhouse	70
	Bacterial Culture	70
	Plant Propagation	70
	Experimental Design	72
	Data Assessment and Analysis	72
	Second Trial	74
	Combined Effect of Antagonist and Soil Nutrient Amendment on Bacterial Wilt Incidence on Tomato in The Field	80
	Preparation of Field Plot	80
	Bacterial Soil Inoculation	80
	Plant Propagation	81
	Experimental Design	82
	Data Assessment and Analysis	83
IV	RESULTS	84
	Effect of Calcium Nitrate Concentrations on Growth of Tomato Seedlings	84
	Effect of Calcium Nitrate on Bacterial Wilt Incidence on Tomato Seedlings in The Greenhouse	88
	Antagonism of Selected Strains of Fluorescent and Non-Fluorescent Pseudomonads on <i>R. solanacearum</i>	91
	Effect of Antagonistic Bacteria in Bio-priming on Germination of Tomato Seeds	95
	Effect of Antagonistic Bacteria in Bio-priming on Tomato Seedlings	97
	Effect of Antagonist and Soil Nutrient Amendment on Bacterial Wilt Incidence in the Greenhouse	99
	Combined Effect of Antagonist and Soil Nutrient Amendment on Bacterial Wilt Incidence on Tomato in The Field	104
V	DISCUSSIONS	119
	Effect of Calcium Nitrate Concentrations on Growth of Tomato seedlings	119
	Effect of Calcium Nitrate on Bacterial Wilt Incidence of Tomato in The Greenhouse	121
	Effect of Antagonistic Bacteria in Bio-priming on Germination of Tomato Seeds	124



Effect of Antagonistic Bacteria in Bio-priming on Tomato Seedlings	126
Effect of Antagonist and Soil Nutrient Amendment on Bacterial Wilt Incidence in The Greenhouse	128
Combined Effect of Antagonist and Soil Nutrient Amendment on Bacterial Wilt Incidence on Tomato in the Field	131
VI CONCLUSION	137
BIBLIOGRAPHY	138
APPENDICES	162
VITA	177



LIST OF TABLES

Table		Page
1	Biovars of <i>Ralstonia solanacearum</i>	29
2	Effect of calcium nitrate applications on the percentage of survival of tomato seedlings planted in soil artificially infested with <i>R. solanacearum</i> at the density of 10^4 and 10^6 cfu/ g oven-dry soil, six weeks after inoculation	89
3	Analysis of Variance (ANOVA) for Factorial Experiment showing the effects of 'Pathogen density' and 'Treatment application' on the survival of tomato seedlings.	90
4	The diameter of the growth of antagonists and the Inhibition Zone of <i>R. solanacearum</i> on Dual Culture Plate test incubated at 30 ⁰ C.	94
5	Effect of bio-priming with antagonist on the percentage of germination of tomato seeds.	96
6	Effect of <i>Pseudomonas putida</i> strains KTS26 and AC1 in bio-priming on the fresh and dry weight of tomato seedlings at 30 days after planting in the greenhouse.	98
7	Effect of antagonist and soil nutrient amendment on the incidence of bacterial wilt of MT11 at six weeks after transplanting, in the greenhouse.	101
8	Combined effect of calcium nitrate and <i>Pseudomonas putida</i> on bacterial wilt disease severity in the field, on tomato plants on the eighth weeks after transplanting.	107
9	Analysis of variance (ANOVA) for factorial experiment showing the effects of 'Variety' and 'Treatment application' on the percentage of bacterial wilt disease incidence in the field.	108
10	Postulation on the yield of field-planted MT11 tomato.	117
11	ANOVA table of the effect of different concentrations of calcium nitrate solution on fresh weight of tomato seedlings at 30 days.	165
12	ANOVA table of the effect of different concentrations of calcium nitrate solution on dry weight of tomato seedlings at 30 days.	165

13	ANOVA table of the effect of calcium nitrate on survival of tomato seedlings planted in soil artificially infested with <i>R. solanacearum</i> at the density of 10^4 cfu/ g oven-dry soil.	166
14	ANOVA table of the effect of calcium nitrate on survival of tomato seedlings planted in soil artificially infested with <i>R. solanacearum</i> at the density of 10^6 cfu/ g oven-dry soil.	166
15	ANOVA table for factorial experiment on the effect of 'pathogen density' and 'treatment application' on the survival of tomato seedlings.	167
16	ANOVA table of the effect of antagonist in bio-priming on the germination of tomato seeds.	167
17	ANOVA table of the effect of <i>Pseudomonas putida</i> strains KTS26 and AC1 in bio-priming on the fresh weight of tomato seedlings planted under greenhouse condition.	168
18	ANOVA table of the effect of <i>Pseudomonas putida</i> strains KTS26 and AC1 in bio-priming on the dry weight of tomato seedlings planted under greenhouse condition.	168
19	ANOVA table of the effect of antagonists and soil nutrient amendment on the incidence of bacterial wilt under greenhouse condition (first trial).	169
20	ANOVA table of the effect of antagonists and nutrient soil amendment on the disease severity index of bacterial wilt under greenhouse condition (first trial).	169
21	ANOVA table of the effect of antagonists and nutrient soil amendment on the incidence of bacterial wilt under greenhouse condition (second trial).	170
22	ANOVA table of the effect of antagonists and nutrient soil amendment on the disease severity index of bacterial wilt under greenhouse condition (second trial).	170
23	ANOVA table of the combined effect of calcium nitrate and <i>P. putida</i> on the percentage of bacterial wilt disease incidence on tomato in the field.	171
24	ANOVA table of the combined effect of calcium nitrate and <i>P. putida</i> on the disease severity index of bacterial wilt on tomato in the field.	171

25	ANOVA table of factorial experiment on the effect of 'variety' and 'treatment application' against the bacterial wilt incidence in the field.	172
26	ANOVA table of the combined effect of calcium nitrate and <i>P. putida</i> on the yield of MT11 in the field.	172

LIST OF FIGURES

Figure		Page
1	Commercial potting mixture (TROPIGRO®).	57
2	Forty-eight hour culture of <i>R. solanacearum</i> on casamino acid-peptone-glucose agar plate.	62
3	Forty-eight hour culture of <i>R. solanacearum</i> on Triphenyltetrazolium chloride agar plate.	62
4	Tomato seedlings in pots placed on the benches in the greenhouse.	63
5	Bio-primed seedlings planted in the jiffy pots and placed on benches in the greenhouse.	69
6	Tomato seedlings in polyethylene bags on benches in the greenhouse.	71
7	Healthy tomato plant without wilt symptom. (rating scale = 0)	74
8	Tomato plant infected by bacterial wilt, with one or two leaves wilted. (rating scale = 1)	75
9	Tomato plant infected by bacterial wilt, with all leaves wilted except the top two or three leaves. (rating scale = 2)	76
10	Tomato plant infected with bacterial wilt, with all leaves Wilted. (rating scale = 3)	77
11	Dead tomato plant infected by bacterial wilt. (rating scale = 4)	78
12	Bacterial ooze exuded from the vascular bundles of infected plant, when the stem was cut and immersed in water.	79
13	Tomato seedlings in the field with 20 plants planted on a planting bed.	83
14	Effect of different concentrations of calcium nitrate solution on fresh weight of tomato seedlings at 30 days.	85
15	Effect of different concentrations of calcium nitrate solution on dry weight of tomato seedlings at 30 days.	86

16	Forty days old tomato seedlings treated with 0, 0.1, 0.5 and 1% concentration of calcium nitrate solutions.	87
17	Inhibition of the growth of <i>R. solanacearum</i> on King's B agar media in Dual Culture Test. The antagonist was spot-inoculated at the center of the plate. Formation of inhibition zone indicated antibiosis, A: <i>Pseudomonas aeruginosa</i> strain 72PTT; B: <i>Pseudomonas cepacia</i> strain 85PMS.	92
18	Inhibition of the growth of <i>R. solanacearum</i> on King's B agar media in Dual Culture Test. The antagonist was spot-inoculated at the center of the plate. Formation of inhibition zone indicated antibiosis, A: <i>Pseudomonas putida</i> strain KTS26; B: <i>Pseudomonas putida</i> strain AC1.	93
19	Effect of antagonists and soil nutrient amendment on the incidence of bacterial wilt of MT11 at six weeks after transplanting, in the greenhouse.	100
20	Percentage of bacterial wilt disease incidence on MT11 in the greenhouse recorded at weekly intervals after transplanting.	103
21	Severity of bacterial wilt on tomato plants in the field at the seventh weeks after transplanting. All the Pearl tomato plants succumbed to bacterial wilt disease.	105
22	The combined effect of calcium nitrate and <i>Pseudomonas putida</i> on the percentage of bacterial wilt disease incidence on tomato in the field, on the eighth week after transplanting.	106
23	Percentage of bacterial wilt disease incidence of tomato in the field, recorded at weekly intervals after transplanting.	110
24	Healthy tomato plant of moderately resistant variety, MT11 bearing fruits cluster in the sixth week after transplanting.	111
25	Formation of adventitious roots at the lower end of the stem of tomato plants at the early stages of bacterial wilt infection.	111
26	Comparison between healthy and infected root system of tomato plants in the field, showing A: fresh looking healthy roots with well-established rooting system; B: decayed roots system of tomato plants infected by bacterial wilt.	112
27	Comparison on vascular system at the base of the stem, between healthy and infected tomato plants in the field, showing A: healthy stem with non-discolored normal vascular system; B: infected plants with discolored and decayed vascular system.	113

28	Blossom end rots are prominent on fruits of the non-treated MT11 plants, attributed to calcium deficiency.	114
29	Ripened tomato MT11 fruits infected by bacterial wilt with signs of shrinkage on the flesh as a result of water loss.	114
30	Averaged yield (kg) of tomato per planting bed as a result of the respective treatments.	116
31	Ripe tomato fruits harvested from healthy moderately resistant variety, MT11 plants, with variable shape and size.	118
32	The occurrence of 'radial cracking' on the MT11 tomato fruits as a result of the hot weather.	118

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 possess 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.