UNIVERSITI PUTRA MALAYSIA

EFFECTS OF *BACILLUS LICHENIFORMIS* BLI008 ON *RHIZOCTONIA* ROOT ROT DISEASE AND PLANT GROWTH IN CHILLI

KANDANGAMUWA PATHIRANNAHALAGE SOMACHANDRA

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By

KANDANGAMUWA PATHIRANNAHALAGE SOMACHANDRA

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

June 2011

DEDICATION

Special dedication to:

"JANAKIE"

My ever-loving wife. I live far away from you but I realize how much you sacrificed.

"DINITHI, MIHIRI, and DINIDU"

My loving daughters and son. Your endless love keeps me alive.

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

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June 2011

Chair: **Associate Professor Jugah b. Kadir, PhD**

Faculty: **Agriculture**

Rhizoctonia root rot disease caused by *Rhizoctonia solani* Kühn is economically important on chilli (*Capsicum annuum* L.). Due to the increasing public concerns of fungicide usage, unavailability of resistant varieties and the limitations of cultural methods, biological control is emerging as a promising alternative to control *R. solani* in an environmentally-friendly way. Locally isolated *Bacillus* strain, *Bacillus licheniformis* BLI008, was tested against *R. solani in-vitro* to find out the antagonistic activity and the mechanism of antagonism. Strong mycelial growth reduction (74.79±0.48%) associated with frequent induction of a variety of morphological abnormalities and changes in mycelial color was observed in dual culture assay. Similar growth reduction (44.99±1.65%) and delaying of

sclerotial germination occurred with the volatile compounds produced by the bacterium. Several methods of seed bacterization were tested under pot culture conditions in the presence of *R. solani*. A two days old nutrient agar culture of the bacterium applied as slurry on sterilized chilli seeds subsequently coated with $CaCO_3$ provided best protection against damping-off. This method of seed bacterization significantly (P=0.05) increased seed germination (86%) and reduced post emergence damping-off (22.78%) over untreated seeds, and performed equally as chemical seed treatment. The control of root disease and bacterium mediated plant growth promotion was observed in greenhouse pot experiment. Measurements on root disease severity and growth parameters were taken 30 and 75 days after transplantation. The highest disease suppression was achieved when *B. licheniformis* BLI008 was inoculated ($1x10^9$ CFUs/mL) at the time of seed sowing as a drench to the nursery medium. *Bacillus licheniformis* BLI008 inoculation significantly increased shoot length (26.26%), root length (22.98%) total biomass (173.53%), and leaf chlorophyll content (13.64 mg/g of fresh weight) of chilli seedlings over the control at 30 days after transplantation. Those plants flowered earlier producing higher number (27.25) of flowers. In the presence of *R. solani*, bacterium mediated growth promotion was observed only on plants raised in bacterized nursery medium. They showed 12.46% increase in shoot length, 17.77% in root length and 99.00% in total biomass compared to untreated control. However, the responses varied with time, and were not

consistent up to maturity. Among bacterized treatments challenged with *R. solani*, the highest (11.21 mg/g fresh weight) and lowest (8.98mg/g fresh weight) leaf chlorophyll values were reported in plants raised in bacterized medium and bacterized after four days of planting, respectively. The time taken for flower initiation and the number of flowers produced by bacterized treatments challenged with *R. solani*, except plants inoculated after four days of transplantation were similar to the control. The results of this study showed that the bacterial biocontrol agent tested has a great potential in controlling Rhizoctonia damping-off and root rot of chilli. Its plant growth enhancement can be considered as an added advantage in crop production.

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

KESAN *BACILLUS LICHENIFORMIS* BLI008 KEATAS REPUT AKAR *RHIZOCTONIA* DAN PERTUMBUHAN POKOK CILI

Oleh

KANDANGAMUWA PATHIRANNAHALAGE SOMACHANDRA

Jun 2011

Pengerusi: **Profesor Madya Jugah b. Kadir, PhD**

Fakulti: **Pertanian**

Penyakit reput akar Rhizoctonia yang berpunca daripada *Rhizoctonia solani* Kuhn merupakan penyakit yang penting daripada aspek ekonomi kepada tanaman cili (*Capsicum annuum* L.). Faktor-faktor seperti peningkatan kesedaran orang ramai terhadap penggunaan racun kulat, ketiadaan varieti yang tahan penyakit dan kekangan teknik kebiasaan penanaman menjadikan, kaedah pengawalan biologi muncul sebagai alternatif yang berpotensi untuk mengawal *R. solani* secara mesra alam. Strain Bacillus tempatan, *Bacillus licheniformis* BLI008, telah diuji terhadap *R. solani* secara *in-vitro* bagi mengkaji aktiviti antagonistik dan mekanisme antagonistik. Pengurangan pertumbuhan miselia secara mendadak (74.79±0.48%) dikaitkan dengan kekerapan ransangan menyebabkan kecacatan morfologi dan perubahan warna miselia dapat diperhatihan selepas ujian diwikultur. Komponen volatil yang dihasilhan oleh *B. licheniformis* menyebabkan pengurangan pertumbuhan yang serupa pada kadar (44.99±1.65%) serta

memperlahankan percambahan sklerotia. Beberapa teknik pembakteriaan benih telah diuji dalam rumah kaca terhadap *R. solani*. Bakterium berusia 2 hari yang ditumbuhkan pada agar nutrien telah dicairkan dan diaplikasikan kepada benih cili yang sudah disteril kemudian diselaputi dengan $CaCO_3$ untuk melindungi daripada penyakit "lecuh". Teknik pembakteriaan benih ini signifikan pada (P=0.05) dalam meningkathan percambahan benih (86%) dan mengurangkan kemunculan "lecuh"(22.78%) berbanding benih tidak dirawat, serta menghasilkan keputusan sama seperti penggunaan racun kimia. Pengawalan terhadap penyakit akar dan ransangan pertumbuhan kepada tanaman yang dirawat dengan penggunaan bakteria dapat diperhatikan pada ujian di rumah hijau. Bacaan untuk kerosakan penyakit dan parameter pertumbuhan diambil 30 dan 75 hari selepas pemindahan tanaman. Pengawalan penyakit pada kadar tertinggi terhasil apabila *B. licheni*formis BLI008 diinokulasi ($1x10^9$ CFUs/mL) pada media tanaman ketika proses penyulaman benih sebagai rawatan kepada media tanaman. Penginokulatan *B. licheniformis* BLI008 memberi kesan signifikan dengan meningkathan pertumbuhan pucuk (26.26%) pertumbuhan akar (22.98%), berat keseluruhan (173.53%) dan kandungan kolorofil pada daun (13.64 mg/g daripada berat segar) terhadap anak pokok cili berbanding anak pokok cili yang tidak dirawat selepas 30 hari pemindahan tanaman. Pokok cili yang dirawat berbunga lebih awal dan menghasilkan lebih jumlah bunga pada keadaan kehadiran *R. solani*. Ransangan pertumbuhan hanya diperhatikan pada pokok yang ditanam di media penanaman yang dirawat dengan bakteria. Tanaman menunjukkan peningkatan 12.46% pertumbuhan pucuk, 17.77% pertumbuhan akar dan 99% berat keseluruhan berbanding tanaman tidak dirawat. Walaubagaimanapun, tindakbalas dihasilkan berbeza mengikut tempoh, dan tidak

konsisten sehingga proses kematangan. Di antara rawatan bakteria terhadap *R. solani*, nilai klorofil yang tertinggi (11.21 mg/g berat segar) dan terendah (8.98 mg/g berat segar) dikatakan pada tanaman yang ditanam pada media yang dirawat dengan bakteria dan dirawat dengan bakteria selepas empat hari ditanam. Pokok yang dirawat dengan bakteria bagi mencegah jagkitan *R. solani* menghasilkan jumlah bunga serta tempoh pembungaan yang sama dengan pokok yang tidak dirawat kecuali pokok yang diinokulasi selepas 4 hari. Keputusan kajian menunjukkan bahawa penggunaan bakteria sebagai agen kawalan biologi terbukti berpotensi dalam mengawal "lecuh" Rhizoctonia dan kerosakan akar pada pokok cili. Pertambahan kadar pertumbuhan pada tanaman boleh dijadikan sebagai nilai tambah kepada pengeluaran tanaman ini.

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I certify that a Thesis Examination Committee has met on 7th June 2011 to conduct the final examination of Kandangamuwa Pathirannahalage Somachandra on his thesis entitled "**Effect of *Bacillus licheniformis* BLI008 on Rhizoctonia root rot disease and plant growth of chilli"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science degree.
Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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KANADANGAMUWA PATHIRANNAHALAGE SOMACHANDRA

Date: 7 June 2011

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