



UNIVERSITI PUTRA MALAYSIA

**DETERIORATION OF SOYBEAN [*GLYCINE MAX* (L.) MERR.] SEED BY
COLLETOTRICHUM TRUNCATUM AND ITS CONTROL THROUGH
BIO-PRIMING**

MOST. MAHBUBA BEGUM

FP 2008 6

**DETERIORATION OF SOYBEAN [*GLYCINE MAX* (L.) MERR.] SEED BY
COLLETOTRICHUM TRUNCATUM AND ITS CONTROL THROUGH
BIO-PRIMING**

By

MOST. MAHBUBA BEGUM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

April 2008



DEDICATION

I dedicate this humble effort to my beloved parents, sisters, affectionate husband and sons, without their inspiration and help this ambition could have not been achieved in Universiti Putra Malaysia.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**DETERIORATION OF SOYBEAN [*GLYCINE MAX* (L.) MERR.] SEED BY
COLLETOTRICHUM TRUNCATUM AND ITS CONTROL THROUGH
BIO-PRIMING**

By

MOST. MAHBUBA BEGUM

Chairman : Professor Sariah Meon, PhD

Faculty : Agriculture

ABSTRACT

A study was conducted to evaluate the effect of *Colletotrichum truncatum* infection on soybean seed quality and its control through bio-priming. A total of 11 genera comprising of 17 species of seed-borne fungi were found to be associated with soybean var. Palmetto. The prominent fungus isolated externally and internally was *C. truncatum* with the frequency values of 12.75 and 9.75%, respectively, followed by *Fusarium oxysporum* f. sp. *glycines* and *Diaporthe phaseolorum* var. *sojae* based on moist blotter and agar plate methods. The typical symptoms of *C. truncatum* on the infected seeds appeared as brown to black speckled lesions, producing numerous acervuli with black setae and conidia over the seed surface. Seed infection by *C. truncatum* in soybean seed caused pre and post-emergence damping-off, resulting in reduced seed germination and seedling survivability by 62.35 and 88.24%, respectively.



Histopathological studies of naturally infected soybean seeds confirmed the presence of *C. truncatum* predominantly both intra- and inter-cellularly in the seed coat, cotyledon and embryonic axes of seed. The fungi were also detected on and in the seed coat, cotyledon and embryonic axes of artificially infected seeds. Seed viability and vigour were also reduced in *C. truncatum* infected seeds as determined by tetrazolium (TZ) and electrical conductivity (EC) tests. Seed volume of infected seeds was reduced, with an increase in soluble protein and oleic acid and a decrease in linoleic acid content as compared with healthy seeds. Two fungal biocontrol agents (BCAs), *Trichoderma virens* (UPM23) and *T. harzianum* (UPM40) were found to inhibit strongly the growth of *C. truncatum* through mycoparasitism, competition and antibiosis based on PIRG (Percent Inhibition of Radial Growth) values. However, one bacterial BCA, *Pseudomonas aeruginosa* (UPM13B8) gave the highest PIRG values of 100% in the culture filtrate test, suggesting that antibiosis could be the main mechanism of antagonism. No phytotoxic effect was observed on soybean seeds and seedlings, when treated with suspensions of UPM23, UPM40 and UPM13B8. Therefore, the efficacy of bio-priming was conducted for controlling *C. truncatum* infection in soybean seeds using UPM23, UPM40 and UPM13B8. Artificially infected seeds by *C. truncatum* were bio-primed for 12 hours as this was determined as the safe time limit for soybean. Treatments included were chemo-primed, Benlate® (T1); bio-primed, UPM13B8 (T2); bio-primed, UPM40 (T3); bio-primed, UPM23 (T4); bio-primed, UPM23+40 (T5) and the controls as hydro- primed (T6) and non- primed seeds (T7). *Trichoderma* isolates used either singly (UPM 23 and UPM40) or as a mixture (UPM23+40) colonized the seed surface with germinating hyphae after 12 hours of bio-priming. Bacterial isolate, *P. aeruginosa* was also detected to colonize the seed surface with increase in the colony



forming unit (CFU) from 1.2×10^9 to 5.1×10^9 seed⁻¹ after the bio-priming period. Bio-priming was effective to control pre and post-emergence damping-off and promote seed germination, seedling establishment and growth in the presence of *C. truncatum* in soybean seeds. Under the glass house conditions, *Trichoderma* isolates however, gave better control of pre and post-emergence damping-off and enhancement of growth followed by bio-priming with UPM13B8 and chemo-priming with Benlate®. Under the field conditions, UPM13B8 was better in controlling pre and post-emergence damping-off ranging from 48.64 to 51.85% and 65.0 to 97.20%, respectively and also enhanced seed germination, final seedling stand and increase in shoot length and dry weight of seedling. However, the biocontrol efficacy and subsequent growth enhancement of UPM13B8 were not significantly ($P \leq 0.05$) different from UPM40 or UPM23+40 or the fungicide 'Benlate®'.

Bio-priming with Malaysian isolates of *P. aeruginosa* and *T. harzianum* offered an effective biological seed treatment system and an alternative to chemo-priming with Benlate® to control seed-borne infection by *C. truncatum* in seeds and seedlings of soybean. Besides, they also improve seed germination, seedling establishment and vegetative growth. This study has explored up new dimension of biological control for preventive as well as remedial of seed-borne infection by *C. truncatum*. Thus, bio-priming can be exploited by seed companies and organic farmers in the sustainable agriculture, which would be more economical and environmental friendly.

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

**KEMEROSOTAN BIJI BENIH KACANG SOYA [*GLYCINE MAX (L.) MERR.*]
OLEH JANGKITAN *COLLETOTRICHUM TRUNCATUM* DAN KAWALAN
SECARA BIO-PRIMING**

Oleh

MOST. MAHBUBA BEGUM

Pengerusi : Profesor Sariah Meon, PhD

Fakulti : Pertanian

Satu percubaan telah dijalankan untuk menilai kesan kemerosotan yang disebabkan oleh *Colletotrichum truncatum* pada kualiti biji benih kacang soya yang digunakan sebagai bahan penanaman dan makanan dan mengawalinya secara bio-priming. Sejumlah 11 genera yang terdiri daripada 17 spesis kulat bawaan biji benih telah dijumpai mempunyai kaitan dengan kacang soya var. Palmetto. Kulat yang paling kerap dipencilkan ialah *C. truncatum* dengan nilai kekerapan iaitu 12.75% dan 9.75% diikuti oleh *Fusarium oxysporum* f. sp. *glycines* dan *Diaporthe phaseolorum* var. *sojae*, berdasarkan kaedah kertas serap lembap dan plat agar. Simtom utama *C. truncatum* pada biji benih yang dijangkiti kelihatan sebagai lesion berwarna perang atau hitam, menghasilkan banyak acervuli, dengan seta berwarna hitam dan konidia pada permukaan biji benih. Biji benih kacang soya yang dijangkiti oleh *C. truncatum* akan menyebabkan pre dan pra-lecuh, mengakibatkan pengurangan percambahan dan kebolehan hidup biji benih dengan nilai masing-masing 62.35% dan 88.24%. Kajian



histopatologi keatas biji benih kacang soya yang dijangkiti secara semulajadi menggunakan mickroskop cahaya (LM) dan mikroskop pengimbas elektron (SEM) telah membuktikan kehadiran *C. truncatum* secara intra dan inter-selular dalam lapisan kulit, kotiledon dan embrio kacang soya. *C. truncatum* juga dikesan dalam lapisan kulit, kotiledon dan embrio kacang soya yang dijangkiti secara buatan. Kebolehan hidup dan kebernasan bijih benih kacang soya yang dijangkiti *C. truncatum* juga telah dipengaruhi seperti ditunjukkan oleh ujian tetrazolium (TZ) dan ujian konduktiviti elektrik (EC). Isipadu biji benih kacang soya yang dijangkiti berkurangan dengan peningkatan protein larut dan asid oleik, tetapi penurunan dalam kandungan asid linoleik berbanding dengan biji benih kacang soya yang tidak dijangkiti. Dua isolat kawalan biologi (BCAs) kulat *Trichoderma virens* (UPM23) dan *T. harzianum* (UPM40) telah didapati boleh merencat pertumbuhan *C. truncatum* melalui aktiviti mikoparasitisme persaingan dan antibiosis berdasarkan nilai PIRG (peratus perencatan pertumbuhan). Walaubagaimanapun, isolat bacteria, *P. aeruginosa* (UPM13B8) memberikan nilai PIRG 100% dalam filtrat kultur, mencadangkan antibiosis sebagai mekanisme keantagonisan yang utama. Tiada kesan fitotoksikan dilihat pada biji benih dan anak benih kacang soya yang dirawat dengan UPM23, UPM40 atau UPM13B8. Oleh itu, keberkesanan bio-priming telah diuji untuk mengawal jangkitan *C. truncatum* pada kacang soya menggunakan UPM23, UPM40 atau UPM13B8. Kacang soya yang dijangkiti oleh *C. truncatum* telah dirawat secara bio-priming untuk 12 jam dan tempoh ini telah ditentukan sebagai tempoh yang selamat untuk kacang soya. Rawatan biji benih, chemo-primed menggunakan Benlate® (T1); bio-primed, UPM13B8 (T2); bio-primed, UPM40 (T3); bio-primed, UPM23 (T4); bio-primed, UPM23+40 (T5) dan kawalan hidro-primed (T6) dan tanpa-primed (T7). Isolat *Trichoderma* sama ada secara individu (UPM 23 dan UPM 40) atau secara campuran

(UPM23+40) mengkoloni dengan pertumbuhan hifa yang nyata pada permukaan kacang soya selepas 12 jam bio-priming. Isolat bakteria *P. aeruginosa* juga dikesan mengkoloni seluruh permukaan biji soya dengan peningkatan unit pembentuk koloni (CFU) 1.2×10^9 kepada 5.1×10^9 per biji benih kacang soya selepas tempoh bio-priming. Bio-priming telah berkesan untuk mengawal pra- dan pos lecu serta mengalakkan pertumbuhan biji benih. Di rumah kaca, rawatan *Trichoderma* sama ada secara individu atau campuran telah menunjukkan pengurangan jangkitan lecu secara signifikan dan mengalakkan percambahan dan pertumbuhan vegetatif ikuti oleh UPM13B8 dan racun kulat Benlate®. Manakala, di ladang, UPM13B8 pula adalah lebih baik dalam mengawal pre dan pos lecu pada julat 48.64 to 51.85% dan 65.0 to 97.20% dan juga mengalakkan percambahan biji benih, pertumbuhan anak banih, sarta peningkatan berat kering daun. Walaubagaimanapun, keberkesanan kawalan dan pengalakkan pertumbuhan oleh UPM13B8 adalah tidak signifikan berbanding dengan UPM40, UPM23+40 dan juga racun Benlate®. Bio-priming menggunakan *P. aeruginosa* (UPM13B8) dan *T. harzianum* (UPM40) telah memberikan satu kaedah pengawalan yang berkesan dan alternatif kepada penggunaan racun kulat untuk mengawal jangkitan *C. truncatum* pada peringkat biji benih dan anak pokok. Disamping itu, agen kawalan biologi juga mengalakkan percambahan biji benih dan pertumbuhan anak pokok yang sihat. Kajian ini telah membuka dimensi baru dalam penggunaan agen kawalan biologi untuk pengawalan jangkitan biji benih. Oleh itu, bio-priming boleh disyorkan kepada syarikat biji benih dan petani yang mengamalkan pengeluaran secara organik, dimana ia lebih ekonomi dan mesra alam.

ACKNOWLEDGEMENTS

All praises and appreciations to the Almighty Allah SWT, the most merciful, who blessed me with good health and enabled me to complete this work within the specified time.

I wish to express my profound gratitude to my reverend supervisor, Professor Dr. Sariah Meon, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia (UPM) for her keen interest, scholastic guidance, precious suggestions, encouragement, patience and constructive criticisms from the beginning to the end of the research work. I express my heartfelt indebtedness to her for offering valuable suggestions for the improvement of the thesis writing and editing.

Grateful thanks are also extended to my supervisory committee members, Associate Professor Dr. Zainal Abidin Bin Mior Ahmad, Department of Plant Protection, Faculty of Agriculture, UPM and Senior Lecturer Dr. Adam Puteh, Department of Crop Science, Faculty of Agriculture, UPM for rendering all possible guidance and constructive comments in carrying out the research work.

Thanks are also extended to all the staff-members in the Plant Pathology, Microbiology and Nematology Laboratories for their kind assistance. I would like to thank Dr. Nayan Kanwal and Dr. Yasmeen Siddique Warsi for carefully editing the thesis. Special thanks are also extended to Dr. Sanda, Dr. Asgar Ali Warsi, Zaiton, Fitri, Ujey, Niza and Ila for their help and moral support towards the completion of this study.



I also take this opportunity to express my deepest and sincere gratitude to TWOWS (Third World Organization for Women in Science), Triesty, Italy for their financial support without which this study would have not been possible in UPM.

With deepest emotion I would like to express my enormous appreciation and gratefulness to my beloved husband ‘Md. Atiqur Rahman’ for his painstaking service, continuous guidance, generous help and assistance during my study period. I express also my cordial feelings and affectionate love to my elder son ‘Mohammad Jubaer Rahman’ and my younger son ‘Mohammad Jarif Rahman’. Thanks a lot to all of them for willing to share my sadness and happiness and absorb the weight of anxieties throughout the study period that we had been together in UPM.

Finally, this acknowledgement will not be complete if I do not explicit my sincere thanks to my honourable parents, ‘Mohammad Moqim Uddin’ and ‘Mosammat Noor Golap Banu’, my elder sister ‘Mosammat Nurus Sabah’ and younger sister ‘Mosammat Mahmuda Begum’ for their patience, inspiration, encouragement and endless love to complete my higher study. I will never forget the greatest love that you gave me from the day I was born till the day I die.



I certify that an Examination Committee has met on **30 April 2008** to conduct the final examination of **Most. Mahbuba Begum** on her **Doctor of Philosophy** thesis entitled “**Deterioration of Soybean [*Glycine max* (L.) Merr.] Seed by *Colletotrichum truncatum* and its Control through Bio-priming**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

Name of Chairperson, PhD

Dr. Kamaruzaman Sijam
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Name of Internal Examiner 1, PhD

Dr. Jugah Kadir
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia

Name of Internal Examiner 2, PhD

Dr. Mohammad Mohd. Lassim
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia

Name of External Examiner, PhD

Dr. Jwu-Guh Tsay
Department of Microbiology and Immunology
National Chiayi University
Taiwan

HASANAH MOHD. GHAZALI, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



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

SARIAH MEON, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Adam Puteh, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Zainal Abidin Bin Mior Ahmad, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 June 2008



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.

MOST. MAHBUBA BEGUM

Date: 16 June 2008



TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xviii
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	01
2 LITERATURE REVIEW	06
2.1 Characteristics of soybean	06
2.1.1 Taxonomy	06
2.1.2 Structural composition of the soybean seeds	07
2.2 Uses of soybean	07
2.3 The nutritional value of the soybean seeds	08
2.4 Production of soybean	09
2.5 Major fungal seed-borne diseases of soybean	10
2.6 Anthracnose disease of soybean	10
2.6.1 Economic importance	10
2.6.2 Causal organism	11
2.6.3 Taxonomy and morphology of <i>C. truncatum</i>	11
2.6.4 Symptoms of anthracnose	12
2.6.5 Host range and pathogenicity	13
2.6.6 Favourable conditions for disease	13
2.6.7 Disease cycle and epidemiology	14
2.7 Enzyme and toxin production	14
2.8 Deterioration of soybean seed	15
2.8.1 Decrease in the seed germinability	16
2.8.2 Discolorations and abnormalities of seeds	16
2.8.3 Spoilage of nutritional value of seeds	16
2.9 Management of the anthracnose disease of soybean	17
2.10 Biological control	18
2.10.1 Types of biological control	19
2.10.2 Mechanisms of biological control	20
2.11 Biocontrol agents (BCAs)	21
2.11.1 Fungal BCAs - <i>Trichoderma</i> spp.	22
2.11.2 Bacterial BCAs	23
2.12 Biological seed treatments	24
2.12.1 Formulations of BCAs for seed treatment	26



2.13	Seed priming	27
2.13.1	Advantages of seed priming	28
2.13.2	Disadvantages of seed priming	28
2.13.3	Methods of seed priming	29
2.14	Bio-priming	30
2.15	Biocontrol safety	33
3	ISOLATION, IDENTIFICATION AND SITE OF INFECTION OF <i>COLLETOTRICHUM TRUNCATUM</i> IN SOYBEAN SEEDS	
3.1	Introduction	35
3.2	Materials and methods	37
3.2.1	Isolation and identification of <i>C. truncatum</i> in naturally infected soybean seeds	37
3.2.2	Pathogenicity testing	38
3.2.3	Site of infection of <i>C. truncatum</i> in naturally infected soybean seeds	40
3.2.4	Statistical analysis	42
3.3	Results	
3.3.1	Isolation and identification of <i>C. truncatum</i> in naturally infected soybean seeds	43
3.3.2	Pathogenicity testing	46
3.3.3	Site of infection of <i>C. truncatum</i> in naturally infected soybean seeds	49
3.4	Discussion	54
4	THE EFFECT OF <i>COLLETOTRICHUM TRUNCATUM</i> INFECTION ON SOYBEAN SEED QUALITY	
4.1	Introduction	58
4.2	Materials and methods	60
4.2.1	Infection frequency of <i>C. truncatum</i> on seed components	60
4.2.2	Effect of <i>C. truncatum</i> infection on the physical structure, physiological and chemical changes of soybean seeds	61
4.2.3	Statistical Analysis	70
4.3	Results	71
4.3.1	Effect of incubation time on the infection frequency of <i>C. truncatum</i> on seed components	71
4.3.2	Effect of <i>C. truncatum</i> on the physical structure and physiological changes of soybean seeds	72
4.3.3	Effect of <i>C. truncatum</i> on chemical change of soybean seeds	74
4.4	Discussion	76



5	SCREENING OF POTENTIAL ANTAGONISTIC BIOCONTROL AGENTS (BCAs) AGAINST <i>COLLETOTRICHUM TRUNCATUM</i>	
5.1	Introduction	81
5.2	Materials and Methods	83
5.2.1	Screening of fungal and bacterial BCAs against <i>C. truncatum in vitro</i>	83
5.2.2	Techniques to study the mechanism of antagonism	87
5.2.3	Effect of seed inoculation with UPM40, UPM23 and UPM13B8 on seed germination, seedling establishment and growth of soybean	88
5.2.4	Statistical analysis	90
5.3	Results	91
5.3.1	Dual culture test of <i>Trichoderma</i> isolates	91
5.3.2	Dual culture test of bacterial isolates	92
5.3.3	Mechanisms of antagonism	94
5.3.3.1	Mycoparasitism	94
5.3.3.2	Antibiosis	96
5.3.4	Effect of seed inoculation by UPM40, UPM23 and UPM13B8 on seed germination, seedling establishment and growth of soybean	97
5.4	Discussion	99
6	THE EFFECT OF BIO-PRIMING IN CONTROLLING <i>COLLETOTRICHUM TRUNCATUM</i> INFECTION IN SOYBEAN SEEDS UNDER GLASS HOUSE AND FIELD CONDITIONS	
6.1	Introduction	102
6.2	Materials and Methods	104
6.2.1	Determination of the safe limit time for the hydro-priming of soybean seeds	104
6.2.2	Bio-priming of soybean seeds	105
6.2.3	Colonization and proliferation of BCAs on bio-primed soybean seeds	106
6.2.4	Glass house evaluation of bio-primed seeds	107
6.2.5	Field evaluations of bio-primed seeds	109
6.2.6	Statistical analysis	110
6.3	Results	
6.3.1	The effect of hydro-priming duration on seed Germination and seedling emergence, uniformity and growth of soybean	111
6.3.2	The effect of bio-priming on colonization and proliferation of BCAs over the seed surface of soybean	113
6.3.3	The effect of bio-priming in controlling <i>C. truncatum</i>	

	infection in soybean seeds and the growth performance under the glass house conditions	115
	6.3.4 The effect of bio-priming in controlling <i>C. truncatum</i> infection in soybean seeds and the growth performance under the field conditions	120
6.4	Discussion	128
7	SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	136
	7.1 Summary	136
	7.2 Conclusion	140
	7.3 Recommendations for future research	140
	REFERENCES	142
	APPENDICES	163
	BIODATA OF STUDENT	172
	LIST OF PUBLICATIONS	174



LIST OF TABLES

Table		Page
2.1	Common fatty acid composition of soybean oil.	09
3.1	Frequency of isolates occurrence of seed-borne fungi in soybean var. Palmetto using blotter and agar plate methods.	44
3.2	Pathogenicity of <i>C. truncatum</i> on soybean seeds and seedlings at 14 days after sowing (DAS).	47
4.1	Effect of <i>C. truncatum</i> on physical structure change of soybean seeds.	72
4.2	Effect of <i>C. truncatum</i> on physiological change of soybean seeds.	73
4.3	Effect of <i>C. truncatum</i> on chemical change of soybean seeds.	75
5.1	Isolates of different species of <i>Trichoderma</i> and bacteria.	84
5.2	Antagonistic effect of <i>Trichoderma</i> isolates on the radial growth and time needed for overgrowth of <i>C. truncatum</i> in the dual culture test.	91
5.3	Antagonistic effect of bacterial isolates on the radial growth and inhibition zone against <i>C. truncatum</i> in the dual culture test.	93
5.4	Antagonistic effect of UPM40, UPM23 and UPM13B8 on the radial growth of <i>C. truncatum</i> in the culture filtrate test.	96
5.5	Effect of seed inoculation by UPM40, UPM23 and UPM13B8 on seed germination, seedling establishment and growth of soybean.	98
6.1	Treatments for evaluating the effect of different bio-priming methods on the infection of <i>C. truncatum</i> of soybean seeds under glass house and field conditions.	107
6.2	The effect of hydro-priming duration on the growth performance of soybean seedling at 7 DAS.	113
6.3	The effect of bio-priming in controlling pre and post-emergence damping-off caused by <i>C. truncatum</i> in soybean seeds under the glass house conditions.	115
6.4	The effect of bio-priming on seedling vigour and dry weight of soybean under the glass house conditions at 14 DAS.	118



6.5	The effect of bio-priming in controlling pre-emergence damping-off caused by <i>C. truncatum</i> infection in soybean seeds under the field conditions.	121
6.6	The effect of bio-priming in controlling post-emergence damping-off caused by <i>C. truncatum</i> infection in soybean seeds under the field conditions.	122
6.7	The effect of bio-priming on the shoot height and dry weight of soybean seedling under the field conditions at 21 DAS.	126



LIST OF FIGURES

Figure		Page
3.1	Photomicrographs showing (A) asymptomatic (healthy) soybean seed and (B) <i>C. truncatum</i> infected soybean seed with numerous black acervuli.	45
3.2	Photomicrographs showing the cultural and morphological characteristics of <i>C. truncatum</i> on PDA after 20 days of incubation.	46
3.3	Photomicrographs showing the pathogenicity of <i>C. truncatum</i> on soybean seeds and seedlings at 9 DAS.	48
3.4	Photomicrographs showing the typical symptoms of post-emergence damping-off.	49
3.5	Photomicrographs showing transverse sections of seed components of asymptomatic (healthy) soybean seeds.	51
3.6	Light microscopy photomicrographs showing transverse sections of seed components in <i>C. truncatum</i> infected soybean seeds.	52
3.7	Scanning electron microscopy photomicrographs showing transverse sections of seed components in <i>C. truncatum</i> infected soybean seeds.	53
4.1	Representative GC Chromatogram of fatty acid methyl ester from standard samples.	68
4.2	Infection frequencies of <i>C. truncatum</i> on seed components of inoculated seeds within six days of incubation period.	71
4.3	Staining patterns of the viable seeds of soybean by TZ test.	73
4.4	Staining patterns of the non-viable seeds of soybean by TZ test.	74
5.1	Photomicrograph showing the cultural characteristics of <i>Trichoderma</i> isolates on PDA at 7 days of incubation.	83
5.2	Photomicrograph showing the cultural characteristics of bacterial isolates on Nutrient Agar (NA) at 48 hours of incubation.	84
5.3	Measurement of radial growth of <i>C. truncatum</i> in the control and dual culture plates using <i>Trichoderma</i> isolates.	85



5.4	Measurement of radial growth of <i>C. truncatum</i> in the control and dual culture plates using bacteria.	86
5.5	Antagonistic effect of <i>Trichoderma</i> isolates on the radial growth of <i>C. truncatum</i> in the dual culture test at 5 days after incubation.	92
5.6	Antagonistic effect of bacterial isolates on the radial growth and inhibition zone against <i>C. truncatum</i> in the dual culture test on PDA (at five days incubation).	94
5.7	Photomicrographs showing the hyphal morphology of parasitized mycelia of <i>C. truncatum</i> by UPM40 and UPM13B8.	95
5.8	Effect of UPM40, UPM23 and UPM13B8 on the radial growth of <i>C. truncatum</i> in the culture filtrate test (at 7 days of incubation).	97
6.1	Disease symptoms developing on soybean seeds and seedlings infected by <i>C. truncatum</i> .	108
6.2	Field layout for evaluating the efficacy of seed bio-priming in controlling <i>C. truncatum</i> infection in soybean seeds.	110
6.3	The effect of hydro-priming duration on seed germination at 3, 5 and 7 DAS of soybean.	111
6.4	The effect of hydro-priming duration on seedling emergence and uniformity at 7 DAS of soybean.	112
6.5	SEM photomicrographs showing the hyphal growth and colonization of <i>T. harzianum</i> on the bio-primed seed of soybean.	114
6.6	SEM photomicrographs showing the colonization of <i>P. aeruginosa</i> on the bio-primed seed of soybean.	114
6.7	The effect of bio-priming on seed germination and final seedling stand of soybean under the glass house conditions at 14 DAS.	117
6.8	The effect of bio-priming on final seedling stand and growth of soybean under the glass house conditions at 14 DAS.	119
6.9	The effect of bio-priming on seed germination and final seedling stand of soybean in the field at 21 DAS.	124
6.10	The effect of bio-priming on the final seedling stand and the growth performance of soybean at 21 DAS.	125

LIST OF ABBREVIATIONS

% N	Percent Nitrogen
%	Percent
μL	Microlitre
μm	Micrometer
μmol m ⁻² h ⁻¹	Micromole per meter square per hour
μS cm ⁻¹ g ⁻¹	Microsiemens per Centimeter per Gram
ANOVA	Analysis of Variance
BCAs	Biocontrol Agents
BHT	Butylated Hydroxy Toluene
CFU	Colony Forming Unit
cm	Centimeter
CPD	Critical Point Drying
CRD	Completely Randomized Design
DAS	Days after Sowing
DAIP	Days after Incubation Period
DI	Disease Incidence
DR	Disease Reduction
EC	Electrical Conductivity
EFAs	Essential Fatty Acids
etc	Etcetera
FAME	Fatty Acid Methyl Ester
Fe ⁺³	Ferric Iron
FID	Flame Ionization Detector
g	Gram
GC	Gas Chromatography
GMOs	Genetically Modified Organisms
HCN	Hydrogen Cyanide
HSD	Tukey's Studentized Range Test
i.e.	That is
IF	Infection Frequency
ISTA	International Seed Testing Association
Kg	Kilogram
L	Liter
LCB	Lactophenol Cotton Blue
LM	Light Microscopy
m	Meter
M	Molar
mg	Milligram
mL	Millilitre
mm	Millimeter
mm ²	Millimeter square
MUFA	Mono Unsaturated Fatty Acid
NA	Nutrient Agar



NaSO ₄	Sodium Sulphate
NB	Nutrient Broth
nm	Nanometer
NUV	Near-ultra Violet
°C	Degree Celcius
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PEG	Poly Ethylene Glycol
PIRG	Percent Inhibition of Radial Growth
pH	Hydrogen ion concentration
PUFA	Poly Unsaturated Fatty Acid
RCBD	Randomized Complete Block Design
RH	Relative Humidity
rpm	Rotation per minute
Rt	Retention time
SAS	Statistical Analysis System
SEM	Scanning Electron Microscopy
SMP	Solid Matrix Priming
Spp	Species
syn	Synonym
t	Tonnes
TZ	Tetrazolium test
UK	United Kingdom
UPM	Universiti Putra Malaysia
USA	United States of America
viz.	Namely
v/v	Volume per volume
v/v/v	Volume per volume per volume
var	Variety
VI	Vigour Index
wp	Wettable powder
wt	Weight
w/v	Weight per volume
w/w	Weight per weight



CHAPTER 1

INTRODUCTION

The soybean [*Glycine max* (L.) Merrill] is one of the most economically important legume crops in the world (Liu, 2000; Olguin *et al.*, 2003). It is grown for an excellent and cheaper source of good quality protein and vegetable oil for human and livestock nutrition (Wilcox, 1987; Liu, 1997). Soybean seed has a wide range of uses including soy food, soy sauce, soy milk, animal feed and dietary supplements in the industry; thus, the position of soybean among legumes is unique all over the world (ASA, 2005).

The production of soybean in the tropics is less than that of the temperate regions due to high humidity and rainfall patterns which affect the distribution and prevalence of different seed-borne diseases. Fungi causing seed-borne diseases such as anthracnose, Phomopsis seed decay, frog-eye leaf spot and purple seed stain, are important in tropical environments (Hartman and Sinclair, 1992; Hartman *et al.*, 1999). Among these, anthracnose is the most destructive and widespread seed-borne disease which frequently occurs in soybean, especially under warm and humid conditions in the tropics (Hepperly, 1985; Sinclair and Backman, 1989; Ploper and Backman, 1992). Several species of *Colletotrichum* are associated with anthracnose, but the most common and prevalent species recorded on soybean is *C. truncatum*. The fungus causes pre and post-emergence damping-off and infected plants are shorter and tend to senesce earlier than other healthy plants in the field (Sinclair and Backman, 1989; Hartman and Sinclair, 1992; Ploper and Backman, 1992).

