



**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**



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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

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*COLLETOTRICHUM TRUNCATUM* AND ITS CONTROL THROUGH  
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**MOST. MAHBUBA BEGUM**

**Chairman : Professor Sariah Meon, PhD**

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**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  $1.2 \times 10^9$  to  $5.1 \times 10^9$  seed<sup>-1</sup> 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)  $1.2 \times 10^9$  kepada  $5.1 \times 10^9$  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 menggalakkan 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 menggalakkan 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.

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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:

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## **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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**MOST. MAHBUBA BEGUM**

Date: 16 June 2008



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## LIST OF ABBREVIATIONS

|                                      |  |
|--------------------------------------|--|
| % N                                  | Percent Nitrogen                       |
| %                                    | Percent                                |
| μL                                   | Microlitre                             |
| μm                                   | Micrometer                             |
| μmol m <sup>-2</sup> h <sup>-1</sup> | Micromole per meter square per hour    |
| μS cm <sup>-1</sup> g <sup>-1</sup>  | 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 <sup>+3</sup>                     | 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 <sup>2</sup>                      | Millimeter square                      |
| MUFA                                 | Mono Unsaturated Fatty Acid            |
| NA                                   | Nutrient Agar                          |



|                   |                                     |
|-------------------|-------------------------------------|
| NaSO <sub>4</sub> | 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).

