UNIVERSITI PUTRA MALAYSIA

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

MOST. MAHBUBA BEGUM

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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

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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 bji benih kacang soya yang digunakan sebagai bahan penanaman dan makanan dan mengawalnya 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 warna 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 mekanisma 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 mengunakan 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 pembentukkan koloni (CFU) 1.2 $x10^9$ kepada 5.1 x 10^9 per biji benih kacang soya selepas tempoh bio-priming. Bio-priming telah berkesan untuk mengawal pra- dan pos lecuh serta mengalak pertumbuhan biji benih. Di rumah kaca, rawatan *Trichoderma* sama ada secara individu atau campuran telah menunjukkan pengurangan jangkitan lecuh secara signifikan dan mengalakan percambahan dan pertumbuhan vegetatif ikuti oleh UPM13B8 dan racun kulat Benlate®. Manakala, di ladang, UPM13B8 pula adalah lebih baik dalam mengawal pre dan pos lecuh 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 singifikan 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 menggalakan 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 menggamalkan pengeluaran secara organik, dimana ia lebih ekonomi dan mesra alam.

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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:

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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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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.

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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^{-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
ºC	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, frogeye 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).