



**UNIVERSITI PUTRA MALAYSIA**

**SEED-BORNE INFECTION COLLETOTRICHUM (SCHW.) ANDRUS  
AND MOORE IN SOYBEAN SEEDS AND ITS CONTROL**

**SOMBAT SRICHUWONG**

**FP 1992 2**

SEED-BORNE INFECTION OF COLLETOTRICHUM TRUNCATUM (SCHW.)  
ANDRUS AND MOORE IN SOYBEAN SEEDS AND ITS CONTROL

SOMBAT SRICHUWONG

DOCTOR OF PHILOSOPHY  
UNIVERSITI PERTANIAN MALAYSIA

1992



SEED-BORNE INFECTION OF COLLETOTRICHUM TRUNCATUM (SCHW.)  
ANDRUS AND MOORE IN SOYBEAN SEEDS AND ITS CONTROL

By

SOMBAT SRICHUWONG

Thesis Submitted in Fulfilment of the Requirement for  
the Degree of Doctor of Philosophy in the Faculty of  
Agriculture, Universiti Pertanian Malaysia

February 1992



## ACKNOWLEDGEMENT

I would like to express my deepest and sincere gratitude to my supervisor, Associate Professor Dr. Sariah Meon for her dedicated efforts, suggestions, invaluable guidance, constructive criticisms and constant encouragement throughout the planning and execution of this research and in the preparation of the final manuscript.

I wish also to acknowledge my gratitude to SEARCA for providing the necessary research funding throughout my study.

Thanks are also extended to the staff members of the Department of Plant Protection, UPM for their help and cooperation, Mr. Ho Oi Kuan for his technical assistance for the preparation of the electron micrograph, Mr. Alias Awang and Dr. Isabelo del Pilar Palacio for assisting in the statistical analysis.

Last but not least, I am greatly indebted and wish to thank my beloved parents, family members, wife and sons for their understanding and encouragement throughout the course of this study.



## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES .....	vi
LIST OF FIGURES .....	viii
LIST OF PLATES .....	x
LIST OF ABBREVIATIONS.....	xiv
ABSTRACT .....	xv
ABSTRAK .....	xix
 CHAPTER	
I    INTRODUCTION .....	1
II   LITERATURE REVIEW .....	6
Anthracnose Disease of Soybean .....	6
Symptomatology of Anthracnose .....	7
Host Range and Pathogenicity .....	9
Epidemiology .....	11
Colletotrichum truncatum as Seed-borne Pathogen .....	13
Infection and Transmission of Seed-borne C. truncatum .....	14
Control of Anthracnose Seed-borne Disease .....	16
III  EFFECT OF COLLETOTRICHUM TRUNCATUM ON SOYBEAN SEED QUALITY.....	20
Introduction .....	20
Materials and Methods .....	22



	PAGE
Detection and Isolation of <i>C. truncatum</i> in Naturally Infected Seeds .....	22
Effect of <i>C. truncatum</i> on Seed Germination, Seedling Emergence and Seedling Vigour .....	23
Effect of <i>C. truncatum</i> on the Physicochemical Properties of Soybean Seeds .....	27
Results .....	32
Detection and Isolation of <i>C. truncatum</i> in Naturally Infected Seeds .....	32
Effect of <i>C. truncatum</i> on Seed Germination, Seedling Emergence and Seedling Vigour .....	39
Effect of <i>C. truncatum</i> on the Physicochemical Properties of Soybean Seeds .....	49
Discussion .....	64
IV TRANSMISSION STUDIES .....	76
Introduction .....	76
Materials and Methods .....	77
Transmission through Mother Plants .....	77
Transmission through Infected Seeds to Seedlings .....	81
Site of Infection within Infected Seeds .....	83
Results .....	86
Transmission through Mother Plants .....	86
Transmission through Infected Seeds to Seedlings .....	93



	PAGE
Site of Infection within Infected Seeds .....	101
Discussion .....	108
 CHAPTER	
V    CONTROL OF SEED-BORNE COLLETOTRICHUM TRUNCATUM .....	119
Introduction .....	119
Materials and Methods .....	120
In vitro Screening of Microorganisms as Potential Biocontrol agents.....	120
Seed Treatment .....	121
Results .....	126
In vitro Screening of Microorganisms as Potential Biocontrol agents.....	126
Seed Treatment .....	130
Discussion .....	135
VI    GENERAL DISCUSSION AND CONCLUSION .....	142
BIBLIOGRAPHY .....	149
APPENDICES .....	160
VITAE .....	173



## LIST OF TABLES

Table		Page
1	World Soybean Production by Major Countries for the Year 1960, 1970, and 1980 .....	2
2	Percentage Isolation of <i>C. truncatum</i> and Other Seed-borne Fungi from 5 Samples of Soybean Seeds Tested by Blotter Method .....	33
3	Percentage Isolation of <i>C. truncatum</i> and Other Seed-borne Fungi from 5 Samples of Soybean Seeds Tested by Agar Method .....	34
4	The Effect of Seed Inoculation with Conidial Suspension of <i>C. truncatum</i> on Germination, Emergence and Seedling Vigour .....	44
5	The Effect of Seed Inoculation with Culture Filtrate of <i>C. truncatum</i> on Germination, Emergence and Seedling Vigour .....	46
6	The Effect of Soil Inoculation with Culture Suspension of <i>C. truncatum</i> on Emergence and Seedling Vigour .....	48
7	Comparison on the Effect of Seed Inoculation with Conidial Suspension and Culture Filtrate and the Effect of Soil Inoculation with Culture Suspension of <i>C. truncatum</i> on Germination, Emergence, Seedling Height, Fresh and Dry Weight of Seedling .....	50
8	Concentration of Total Soluble Protein and Carbohydrate from <i>C. truncatum</i> Infected and Healthy Soybean Seeds .....	58
9	Effect of <i>C. truncatum</i> Infection During Different Growth Stages of Soybean on the Seed Quality .....	90
10	Percentage Recovery of <i>C. truncatum</i> from Different Seed Components of Soybean .....	92





Table	Page
11    Hundred Seed Weight and Seed Yield of Seeds from Plants Inoculated with <i>C. truncatum</i> at Different Growth Stages .....	94
12    Percentage of Seed Transmission of <i>C. truncatum</i> from Infected Seeds to Seedlings as Tested by the Seedling Symptom Test .....	100
13    Fungicides and Antagonists Used as Seed Treatment Against Seed-borne <i>C. truncatum</i> .....	124
14    Antagonistic Effects of Associated Seed-borne Microorganisms of Soybean to <i>C. truncatum</i> in Dual Culture .....	127
15    The Effect of Seed Treatment with Fungicides and Antagonists on Incidence of <i>C. truncatum</i> , Seed Germination and Seedling Emergence of Soybean .....	131
16    The Effect of Seed Treatment with Fungicides and Antagonists on Height, Fresh and Dry Weight of Soybean Seedlings .....	133



## LIST OF FIGURES

Figure		Page
1	Effect of <i>C. truncatum</i> on the Germination of Soybean Seeds ( $t_{0.01} = 3.499$ ) .....	51
2	Effect of <i>C. truncatum</i> on the Emergence of Soybean Seedlings ( $t_{0.01} = 3.499$ ) .....	52
3	Effect of <i>C. truncatum</i> on the Plant Height of Soybean Seedlings ( $t_{0.01} = 3.499$ ) .....	53
4	Effect of <i>C. truncatum</i> on the Fresh Weight of Soybean Seedlings ( $t_{0.01} = 3.499$ ) .....	54
5	Effect of <i>C. truncatum</i> on the Dry Weight of Soybean Seedlings ( $t_{0.01} = 3.499$ ) .....	55
6	Bovine Serum Albumin Protein Standard Curve .....	56
7	Carbohydrate Standard Curve with Glucose as the Substrate .....	57
8	Diagrammatic Representation of Electrophoretic Patterns of Buffer Soluble Protein of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds .....	60
9	Molecular Weight of Individual Protein Bands from Healthy and <i>C. truncatum</i> Infected Seed as Compared with Standard Curve .....	61
10	Diagrammatic Representation of Electrophoretic Esterase Patterns of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds .....	62
11	Diagrammatic Representation of Electrophoretic Peroxidase Patterns of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds .....	63
12	Diagrammatic Representation of Electrophoretic Acid Phosphatase Patterns of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds .....	65
13	Diagrammatic Representation of Electrophoretic Alkaline Phosphatase Patterns of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds .....	66



Figure		Page
14	Parameters Used to Evaluate the Mode of Inhibition Between Test Antagonists (T) and <i>C. truncatum</i> (P) .....	122
15	Effect of Different Seed Treatments on the Incidence of <i>C. truncatum</i> . Seed Germination and Emergence of Soybean Seedlings .....	132
16	Effect of Different Seed Treatments on the Height, Fresh and Dry Weight of Soybean Seedlings .....	134



## LIST OF PLATES

Plate		Page
1	Soybean Seeds Infected by <i>C. truncatum</i> Incubated on Moist Blotter; A. Acervuli (arrow) on Seed Coat B. Close-up of Acervuli (arrow) Showing Numerous Long Black Setae .....	35
2	Colonies of <i>C. truncatum</i> (arrow) Developing from Infected Soybean Seeds Incubated on PDA .....	36
3	A. Typical Colony of <i>C. truncatum</i> on PDA, 10 Days after Incubation B. Micrograph of Acervulus of the Anthracnose Fungi, <i>C. truncatum</i> .....	38
4	<i>C. truncatum</i> Infection on Soybean Seedlings; A. Cotyledons and Hypocotyl of Seedlings B. Cotyledonary Leaf C. Advance Infection on Cotyledonary Leaves .....	41
5	Seedling Blight Caused by <i>C. truncatum</i> on Soybean Seedlings .....	42
6	Damping off of Soybean Seedling (arrow) Caused by <i>C. truncatum</i> .....	43
7	Electrophoretic Patterns of Buffer Soluble Protein of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds Compared with Molecular Weight Marker (M) .....	60
8	Electrophoretic Patterns of Esterases of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds .....	62
9	Electrophoretic Patterns of Peroxidase of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds .....	63
10	Electrophoretic Patterns of Acid Phosphatase of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds .....	65



Plate	Page
11	Electrophoretic Patterns of Alkaline Phosphatase of <i>C. truncatum</i> Infected (I) and Healthy (H) Soybean Seeds ..... 66
12	Layout of Field Experiment to Study the Transmission of <i>C. truncatum</i> through Mother Plant at Different Stages of Growth ..... 78
13	Plating of Seed Components of Soybean Seeds for Detection of <i>C. truncatum</i> Infection a = seed coat b = cotyledon c = hypocotyl-radical axis ..... 82
14	Seedling Symptom Test to Study the Transmission of <i>C. truncatum</i> from Infected Soybean Seeds to Seedlings a = healthy seedlings b = infected seedlings ..... 84
15	Anthracoze Symptoms on Inoculated Soybean at R7 Stage; A. Symptom on the Pod B. Symptom on the Stem ..... 87
16	Healthy Seeds (a) and <i>C. truncatum</i> Infected Seeds (b) of Soybean ..... 88
17	Seed Rot or Pre-emergence Damping off of Soybean Caused by <i>C. truncatum</i> ..... 95
18	<i>C. truncatum</i> Transmitted from Infected Seed to Seedling Causing Typical Anthracnose Lesion on Cotyledonary Leaves ..... 96
19	Typical Anthracnose Lesions (arrow) on Hypocotyl (A), Cotyledonary Leaves (B) and Epicotyl (C) Leading to Seedling Blight ..... 97
20	Soybean Seedling from <i>C. truncatum</i> Infected Seed Showing Severe Stunting and Thriftness (a) Compared with Seedling from Healthy Seed (b) ..... 99



Plate	Page
21	Transverse Sections of Healthy Soybean Seed; A. Seed Coat Showing Cuticle (Cu), Palisade Cell (Pl), Hourglass Cell (Hg), Parenchyma Cell (Pr) and Aleurone Layer (Al) B. Close-up of Palisade Cells of the Cotyledon ..... 102
22	Transverse Sections of <i>C. truncatum</i> Infected Seed Coat Showing the Development of Fruiting Structure; A. Acervulus Primodia (arrow) B. Matured Acervulus with Setae (arrow) ..... 103
23	Hilar Region of the Infected Seed Showing Development of Acervulus (a) on the Hilar Area and Hyphae (arrow) in the Hilar Tracheids ..... 104
24	Development of Acervulus (a) in the Hypocotyl-radical Axis Area of <i>C. truncatum</i> Infected Soybean Seed ..... 105
25	Transverse Sections of <i>C. truncatum</i> Infected Cotyledon Showing Production of Acervulus (a) on the; A. Abaxial Site of Cotyledon B. Adaxial Site of Cotyledon ..... 106
26	Acervulus Primodia (ap) Developing in the; A. Hourglass Layer B. Inner Endodermis Layer ..... 107
27	Transverse Section of Seed Coat Showing Mycelium (arrow) of <i>C. truncatum</i> in the Hourglass Layer (Hg) ..... 109
28	SEM Micrograph of Soybean Seed Coat Infected by <i>C. truncatum</i> : A. Inter- and Intra-cellular Hyphae (arrow) in the Infected Seed Coat B. Section of Mature Acervulus on the Seed Coat (arrow) ..... 110
29	SEM Micrograph of Soybean Seed Infected by <i>C. truncatum</i> : A. Whole Mount of Mature Acervulus B. Setae and Conidia within the Acervulus ..... 111



Plate	Page
30	Experimental Layout in the Glasshouse to Study the Effect of Different Seed Treatments on Seedling Emergence and Seedling Vigour of Soybean ..... 125
31	Inhibition of Mycelial Growth of <i>C. truncatum</i> (Ct) in Dual Culture (distance) by; A. <i>Fusarium oxysporum</i> B. <i>Bacillus</i> sp. .... 128
32	Inhibition of Mycelial Growth of <i>C. truncatum</i> (Ct) in Dual Culture (contact) by <i>Chaetomium globosum</i> ..... 129
33	Comparison of Seedling Growth between the Different Seed Treatments three Weeks after Sowing. Seeds Treated with Tilt (arrow) Give a Stunting Effect ..... 136



## LIST OF ABBREVIATIONS

°C	degree (s) Celsius
%	percent (percentage)
viz.	namely
g	gram
g/pt	gram per plant
h	hour
ha	hectare
cm	centimetre
m	metre
m	square metre
mm	millimetre
ml	millilitre
mg	milligram
mt	metric ton
ma	milliampere
min	minute
nm	nanometre
NA	nutrient agar
PDA	potato dextrose agar
M	molar
N	normal
pH	hydrogen ion concentration
rpm	round per minute
i.e.	that is
kg	kilogram
μ	micrometre
μl	microlitre
μg	microgram
HgCl	magnesium chloride
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
H <sub>2</sub> SO <sub>4</sub>	sulfuric acid
MW	molecular weight
NaOH	sodium hydroxide
NaOCl	sodium hypochloride
NUV	near ultraviolet
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis





Abstract of thesis submitted to the Senate of Universiti  
Pertanian Malaysia in fulfilment of the requirements for the  
Degree of Doctor of Philosophy.

SEED-BORNE INFECTION OF COLLETOTRICHUM TRUNCATUM (SCHW.)  
ANDRUS AND MOORE IN SOYBEAN SEED AND ITS CONTROL

By

SOMBAT SRICHUWONG

FEBRUARY 1991

Supervisor : Assoc. Prof. Dr Sariah Meon

Faculty : Agriculture

*C. truncatum* have been recognized as a soybean seed pathogen for over 40 years, causing pre-and post-emergence damping-off of seedlings and seedling blight. Although much is known of the effects of this pathogen on soybean seed quality and vigour, fundamental studies on the pathogen, its seed-borne nature, seed transmission and control are rather limited. Present studies are thus focused on their issues.

Detection and isolation of this pathogen was carried out in order to understand the nature of the seed-borne pathogen. In the examination of seed-borne microorganisms of soybean (*Glycine max* (L.) Merrill cultivar Palmetto, SJ4 and SJ5 collected from Malaysia and Thailand, nine genera comprising of 13 species of fungi were found to be associated with the seeds. Among these fungi, *Colletotrichum truncatum* incidence



was recorded in the range of 2.5-6.5%. The blotter method proved more suitable for detecting *C. truncatum* than agar plate method.

It has been confirmed that *C. truncatum* infection reduced seed quality and seedling vigour. Infestation of soybean seeds with conidial suspension or culture filtrate and infestation of soil with culture suspension significantly reduced percentage of seed germination, seedling emergence and seedling height in in vitro and glasshouse studies. The fungus caused seed rot, pre- and post-emergence damping-off and lesions on the hypocotyls and cotyledons of young seedlings. Seeds infected by *C. truncatum* contained more soluble protein but less soluble carbohydrate than that in the healthy seeds. Eventhough the electrophoretic patterns are identical qualitatively between infected and healthy seeds, the relative amount still varies quantitatively. This further supported the observation that *C. truncatum* resulted in seed deterioration and reduced germibility. In addition, infected seeds have lower activity of soluble isozymes: esterase, peroxidase, acid phosphatase and alkaline phosphatase than healthy seeds.

Soybean plants were found to be more susceptible to *C. truncatum* at or during the maturing stage. Inoculation at different stages (R1, R3, R5 and R7) of plant growth showed that seed-borne *C. truncatum* was recovered in the highest percentage at R7 (17.56%) followed by R5 (10.38%), R3 (9.19%),



R1 (7.69%) and uninoculated control (3.44%), respectively. Component plating of individual seed parts confirmed that the fungus was internally-borne and well-established within the seed coat. Therefore, this study positively showed that *C. truncatum* can be transmitted from mother plant to developing seeds. Transmission of the pathogen also occurred from infected seeds to seedlings. *C. truncatum* produced reddish brown to light brown lesions on infected cotyledonary leaves, hypocotyl and epicotyl regions of germinating seeds. Lesions became dark brown to black as they progressed producing numerous black acervuli in the centre. Severe infection resulted in pre- and post-emergence mortality and stunting of young seedlings. Seed transmission was assessed to be 83% and 59% by the seedling symptom tests on soil and sand, respectively.

Light and scanning electron microscopy confirmed that the fungus was internally-borne within infected soybean seed. Abundant inter- and intra-cellular hyphae were observed in all the three layers of the seed coat (palisade cell, hourglass cell and parenchyma cell layers) and in the hilar tracheids of the seed. Acervuli primordia were produced in the hourglass cell layer and endodermis cell. Fruiting structures consisting of mature setose acervuli were later produced on the seed surface. Parenchyma cells appeared collapsed and macerated.



Six of the 12 isolates of fungi and bacteria which were detected and isolated from soybean seeds were found promising as biocontrol agents against *C. truncatum* in dual culture. These were *Chaetomium globosum*, *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum*, and *Bacillus* sp.. *Chaetomium globosum*, *F. oxysporum* and *Bacillus* sp. were selected for further study as seed treatment in comparison with six other commonly used fungicides. All seed treatments significantly reduced the incidence of *C. truncatum* in the infected soybean seeds in in vitro test. However, in glasshouse studies, results showed that only the fungicidal seed treatment: Delsene, Benlate, Captan and Thiram improved the rate of seed germination and seedling emergence compared to the untreated control.



Abstrak tesis yang di kemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat keperluan untuk ijazah Doktor Falsafah

JANGKITAN BAWAAN BIJI BENIH OLEH COLLETOTRICHUM TRUNCATUM (SCHW.) ANDRUS AND MOORE PADA BIJI BENIH KAJANG SOYA DAN KAWALANNYA.

oleh

SOMBAT SRICHUWONG

FEBRUARI 1992

Penyelia : Prof. Madya Dr. Sariah Meon

Fakulti : Pertanian

C. truncatum (Schw.) Andrus and Moore telah dikenali sebagai patogen bawaan biji benih kacang soya selama lebih daripada 40 tahun, menyebabkan pelecuhan pra- dan pos- cambah dan hawar anak benih. Walaupun banyak telah diketahui berkenaan kaitan patogen ini dengan kualiti dan kebernasan biji kacang soya, kajian-kajian asas tentang patogen dan cara bawaan biji benih, penyebaran melalui biji benih dan pengawalan masih lagi terhad. Oleh itu, kajian-kajian semasa ini ditumpukan kepada isu-isu tersebut.

Pengesanan dan pemencilan patogen dijalankan untuk memberi kefahaman tentang keadaan bawaan penyakit biji. Dalam pencerapan mikroorganisma-mikroorganisma bawaan biji benih., kacang soya (*Glycine max* (L.) Merrill), var Palmetto, SJ4 dan SJ5, yang dikutip dari Malaysia dan Thailand, 9 genera kulat



terdiri daripada 13 spesies didapati bersekutu dengan biji benih. Di antara kulat-kulat ini, kejadian *C. truncatum* dicatatkan dalam julat 2.5-6.5%. Kaedah kertas serap ditunjukkan lebih sesuai untuk pengesanan *C. truncatum* daripada kaedah piring agar.

Telah dibuktikan bahawa jangkitan *C. truncatum* mengurangkan kualiti biji benih dan kecergasan anak benih. Penjangkitan biji benih kacang soya dengan ampaiian konidia atau filtrat kultur dan penjangkitan tanah dengan ampaiian kultur mengurangkan dengan signifikannya peratus percambahan biji benih, penjelmaan dan ketinggian anak benih dalam kajian *in vitro* dan rumah kaca. Kulat menyebabkan pereputan biji benih, pelecuhan pra- dan pos- penjelmaan dan lesion pada hipokotil dan kotilidon anak benih muda. Biji benih yang dijangkiti oleh *C. truncatum* mengandungi lebih banyak protein terlarut tetapi kurang karbohidrat terlarut dibandingkan dengan biji benih yang sihat. Walaupun corak elektroforesis protein bersamaan secara kualitatif di antara biji benih yang dijangkiti dan biji benih yang sihat, namun relatifnya masih berbeza secara kuantitatif. Ini menyokong pencerapan yang menunjukkan bahawa *C. truncatum* menyebabkan kemerosotan mutu biji benih dan mengurangkan kadar percambahan. Di samping itu, biji benih yang dijangkiti mempunyai aktiviti isozim terlarut yang rendah; esterase, peroksidase, fosfatase asid dan fosfatase beralkali daripada biji benih yang sihat.

Tanaman kacang soya didapati lebih rentan kepada jangkitan *C. truncatum* pada atau semasa peringkat kematangan. Penginokulatan pada peringkat pertumbuhan yang berbeza (R1, R3, R5 dan R7) menunjukkan *C. truncatum* bawaan biji benih dapat dikesan pada peratus yang tinggi pada peringkat pertumbuhan R7 (17.56%) diikuti oleh R5 (10.38%), R3 (9.19%), R1 (7.69%) dan kawalan (3.44%) berturutan. Pengasingan daripada bahagian-bahagian individu biji benih membuktikan yang kulat ini didapati dan menetap di dalam lapisan kulit biji benih. Oleh itu kajian ini memastikan secara positif yang *C. truncatum* boleh disebarkan daripada tanaman induk kepada biji benih yang sedang terbentuk. Penyebaran patogen juga berlaku daripada biji benih yang dijangkiti kepada anak benih. Jangkitan *C. truncatum* menghasilkan lesion berwarna perang kemerahan kepada perang muda pada daun-daun kotilidon yang dijangkiti, hipokotil dan bahagian-bahagian epikotil biji benih yang bercambah. Lesion kemudian bertukar kepada perang pekat hingga hitam dengan pengeluaran aservulus berwarna hitam dengan banyaknya di tengah-tengah lesion. Jangkitan serius mengakibatkan kematian pra- dan pos-cambah dan kebantutan anak-anak benih yang muda. Penyebaran melalui biji benih telah dinilai sehingga 83% dan 59% melalui ujian simptom anak benih dalam tanah dan pasir berturutan.

Kajian-kajian mikroskop cahaya dan SEM membuktikan bahawa kulat bertempat di dalam biji kacang soya yang dijangkiti. Hifa



inter- dan intra- sel didapati dengan banyaknya dalam ketiga-tiga lapisan kulit biji benih (lapisan sel palisad, sel hourglass dan sel parenkima) dan dalam tracheid hilum biji benih. Primordia acervulus terbentuk dalam lapisan "hourglass" dan endodermis. Struktur-struktur berbuah kemudian dikeluarkan di atas permukaan biji benih. Sel-sel parenkima kelihatan runtuh dan maserat.

Enam daripada 12 pencilan kulat dan bakteria yang dikesan dan dipencilkan daripada biji benih kacang soya didapati berpotensi sebagai agen biologi terhadap *C. truncatum* dalam kultur saingan. Mereka adalah *Chaetomium globosum*, *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum* dan *Bacillus* sp.. *C. globosum*, *F. oxysporum* dan *Bacillus* sp. dipilih untuk kajian seterusnya sebagai rawatan biji benih dibandingkan dengan 6 racun kulat yang biasa digunakan. Kesemua rawatan biji benih mengurangkan kejadian *C. truncatum* dengan signifikan dalam biji benih secara *in vitro*. Walau bagaimanapun, ujian di rumah kaca, menunjukkan hanya rawatan racun kulat, Delsene, Benlate, Captan dan Thiram membaikelokan kadar percambahan dan penjelmaan anak benih dibandingkan dengan kawalan.





## CHAPTER I

### INTRODUCTION

Soybean, *Glycine max* (L.) Merrill is a subtropical annual short day legume. It originated in the northeastern provinces of China and Manchuria (Probst and Judd, 1973; Hymowitz, 1976). The crop is now the most important grain legume and is widely cultivated throughout the world due to the strong demand for edible oils and protein feed supplements. Table 1 shows the production trend around the world during the past 20 year period (between 1960-1980). Major increases in world production were in the United States followed by several countries of the Americas, mostly Brazil and Argentina, whereas production in Asia remained nearly static (Hinson and Hartwig, 1982). Increased production in most countries have been contributed by an increase in areas planted and the adoption of improved cultivars and management practices. However, yield was relatively quite low in developing countries especially in Asia. At present, United States is the biggest producer and exporter of soybean, producing about 51.8 million mt, followed by Brazil, China and Argentina, producing 18.5, 11.8, and 8.5 million mt, respectively (Sinclair and Backman, 1989).

Soybean is an important field crop as a major source of protein and edible oil. The approximate composition of soybean

