

**DELIVERY SYSTEMS OF *TRICHODERMA* INOCULANTS FOR THE  
CONTROL OF *RHIZOCTONIA* DISEASES IN *BRASSICA RAPA***

**By**

**IBRAHIM MOHAMED DAGHMAN**

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

**January 2005**

## **DEDICATION**

I dedicate this humble effort, the fruit of my thoughts and study, to my affectionate Mother, brothers and sisters, wife Suad and children Mohamed, Hanan and Sara who have inspired me to the higher ideals of life.

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

**DELIVERY SYSTEMS OF *TRICHODERMA* INOCULANTS FOR THE CONTROL OF *RHIZOCTONIA* DISEASES IN *BRASSICA RAPA***

By

**IBRAHIM MOHAMED DAGHMAN**

**January 2005**

**Chairman : Professor Sariah Meon, Ph.D**

**Faculty : Agriculture**

*Trichoderma* spp were tested as a biocontrol agent against *Rhizoctonia solani* Kuhn on *Brassica rapa*. *R. solani* was isolated from infected vegetables and confirmed the pathogenic to three cultivars of *Brassica* causing pre-and post emergence damping-off. Confrontation assay, based on the inhibition of mycelial growth and colony overgrowth, showed different degrees of antagonism by *Trichoderma* isolates to *R. solani* through competition and mycoparasitism. Two primers (OPC-11 and OPC-15), and RAPD-PCR analysis were used to establish the variability between the nine *Trichoderma* isolates. The results of which were used to construct a Dendrogram. OPC-11 generated 19 RAPD fragments ranging from 200 bp to 3000 bp and OPC-15 generated 18 RAPD fragments ranging from 300 bp to 4000 bp. They successfully grouped the *Trichoderma* isolates into two main clusters as species aggregates viz. *T. harzianum* and *T. virens*. The DNA polymorphism confirmed the identification based on cultural

and morphological characteristic. Two of the isolates *T. harzanium* (UPM40) and *T. virens* (TV3) were found to establish well on and colonize the roots and rhizospheres of *B. rapa*. The mixture substrate rice flour and molasses (1:4 RF: ML w/v), incubated three days under shaken culture (100 rpm), was the best for the inoculum production of both *Trichoderma* isolates. It produced the most conidia ( $30 \times 10^6$  and  $22.13 \times 10^6$  conidia / g dry mycelium) and mycelium (42.65 mg and 43.97 mg dry mycelium) for UPM40 and TV3, respectively, after only three days incubation. The biomass production was highest at 30 °C incubation temperature for both isolates. Further, desiccation for four days at this temperature still produced good spore viability. UPM40 incorporated on oil palm trunk and chicken dung (OPTCD) and stored at 28 °C and 20 % MC had a significantly longer ( $P \leq 0.05$ ) shelf life than other treatments. The shelf lives of the liquid antagonist preparations (UPM40 and TV3) on two food bases and carriers (distilled water and ¼ strength 1:4 RF: ML medium) kept at 10 °C and 28 °C were also evaluated. UPM40 incorporated in the ¼ strength medium and kept at 28 °C had a significantly longer shelf-life ( $P \leq 0.05$ ). The dry and liquid formulations of UPM40 could reduce the population of viable *R. solani* colonies in the infested soil. Both the applications were as effective as the fungicide (Brassicol® 200 mg / L) in protecting the *B. rapa* against *Rhizoctonia* pre- and post-emergence damping-off. Bio-coating the seeds of *Brassica* with similarly reduced damping-off by *R. solani*.

Abstrak tesis yang dikemukakan kepada Senate Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**SISTEM PENYAMPAIAN INOKULUM *TRICHODERMA* UNTUK  
PENGAWALAN PENYAKIT *RHIZOCTONIA* PADA *BRASSICA RAPA***

Oleh

**IBRAHIM MOHAMED DAGHMAN**

**January 2005**

**Pengerusi : Profesor Sariah Meon, Ph.D**

**Fakulti : Pertanian**

*Trichoderma* spp telah di uji sebagai agen kawalan biologi terhadap *Rhizoctonia solani* Kuhn pada tanaman *Brassica rapa*. *R. solani* telah dipencilkan daripada sayuran yang dijangkiti dan disahkan kepatogenannya pada tiga kultivar *Brassica* menyebabkan lecu pra- dan pos- kemunculan. Pencerakinan bersemuka, berdasarkan perencatan pertumbuhan miselium dan langkau pertumbuhan koloni, menunjukkan tahap keantagonisan isolat-isolat *Trichoderma* yang berbeza terhadap *R. Solani* melalui mekanisme persaingan dan mikoparasitisme. Analysis RAPD-PCR dan dua primer (OPC11 dan OPC15) digunakan untuk menentukan kebolehubahan di antara sembilan isolat *Trichoderma* tersebut. Hasil analisis digunakan untuk penyediaan dendogram. OPC-11 memberi 19 pecahan RAPD diantara 200bp dan 3000bp dan OPC-15 memberi 18 pecahan RAPD diatara 300bp sehingga 4000bp. Ia mengkelaskan isolat *Trichoderma* kepada dua kumpulan/aggregat species viz. *T. harzianum*

and *T. virens*. Persamaan DNA mengesahkan pengenalpastian berdasarkan ciri-ciri kultur dan morfologi. Dua daripada isolat, *T. harzianum* (UPM40) dan *T. virens* (TV3), didapati menetap dengan baik di atas dan menakluki akar dan rizosfera *B. rapa*. Campuran substrat hasil pertanian, seperti tepung beras dan molasses (1:4 RF:ML w/v), dieramkan selama tiga hari sebagai kultur goncang (100 rpm), adalah yang paling baik untuk penghasilan kedua-dua isolat *Trichoderma* yang di uji. Ia menghasilkan konidia ( $30 \times 10^6$  dan  $22 \times 10^6$  konidia / g miselium kering) dan miselium yang optima (42.65 mg dan 43.97 mg miselium kering) untuk UPM40 dan TV3, masing-masing selepas hanya tiga hari pengeringan. Pengeringan biomas adalah tinggi pada suhu pengeringan 30 °C untuk kedua-dua isolat. Selanjutnya, pengeringan selama empat hari pada tahap suhu ini masih memberi keberhasilan spora yang baik. Penambahan UPM40 kepada OPTCD dan disimpan pada 28 °C dan 20 % kandungan air mempunyai usia rak yang lebih tinggi dibandingkan dengan yang lain. Usia rak persediaan cecair antagonis ( UPM40 dan TV3) diatas dua makanan asas dan pembawa (air suling dan ¼ kepekatan media 1:4 RF: ML), disimpan pada 10 °C dan 28 °C juga dinilai. UPM40 ditambah kepada ¼ kepekatan media dan disimpan pada 28 °C mempunyai usia rak ( $P \leq 0.05$ ) yang lebih panjang daripada yang lain. Persediaan kering dan cecair UPM40 mengurangkan populasi koloni *Rhizoctonia* yang bernas di dalam tanah yang dikerumuni. Kedua-dua rawatan adalah sama berkesan dengan rawatan racun kulat (Brassicol® 200 mg / L) untuk melindungi *B. rapa* daripada jangkitan pra- dan pos- lecu *Rhizoctonia*. Saduran biologi biji benih *Brassica* juga mengurangkan kejadian lecu oleh *R. solani*.

## **ACKNOWLEDGEMENTS**

All praises and thanks are to Allah, the beneficent and merciful. The author invokes Allah's blessings of peace for the Holy Prophet Mohammad (peace be on him), the messenger of Allah, who advised us that education is to be imbibed from cradle to grave.

I avail myself of this opportunity to record my sincerest thanks and appreciation to Professor Dr. Sariah Meon, chairman of my supervisory committee, for her dedicated efforts, support, invaluable advice, intellectual guidance and encouragement in the conduct of my research and in the preparation of this thesis.

Grateful thanks are also due to my supervisory committee members, Associate Professor Dr. Zainal Abidin Bin Mior Ahmad, Associate Professor Dr. Jugah Kadir and Associate Professor Dr. Rosenani Abu Bakar, for their constructive comments, advice and help throughout my studies and in the preparation of this final manuscript.

I am exceedingly grateful to the Libyan Government and Libyan Embassy in KL for their financial support without which this project would have been well nigh impossible.

Thanks are also extended to all the staff in the Pathology, Microbiology and Nematology Laboratories - Mr. Nazri, Mr. Johari, Mr. Samsuddin, Mr. Khir, Mr. Yusof and Mr. Ghani - for their assistance.

Finally, I also take this opportunity to express my deepest gratitude to my Mother, brothers, sisters, wife Suad Mustafa, son Mohamed and daughters Hanan and Sara. I thank them for all their love, support and encouragement throughout my study in UPM and my whole life.



I certify that an Examination Committee met on 12/1/2005 to conduct the final examination of Ibrahim Mohamed Daghman on his degree in Doctor of Philosophy thesis entitled “Delivery Systems of *Trichoderma* Inoculants for the Control of *Rhizoctonia* Diseases in *Brassica rapa*” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Examiner 1, Ph.D

Examiner 2, Ph.D

Examiner 3, Ph.D

---

**GULAM RUSUL RAHMAT ALI, Ph.D.**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follow:

**SARIAH MEON, Ph.D**

Professor  
Faculty of Agriculture  
University Putra Malaysia  
(Chairman)

**Zainal Abidin Bin Mior Ahmad, Ph.D**

Associate Professor  
Faculty of Agriculture  
University Putra Malaysia  
(Member)

**Jugah Kadir, Ph.D**

Associate Professor  
Faculty of Agriculture  
University Putra Malaysia  
(Member)

**Rosenani Abu Bakar, Ph.D**

Associate Professor  
Faculty of Agriculture  
University Putra Malaysia  
(Member)

---

**AINI IDERIS, Ph.D.**

Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

---

**IBRAHIM MOHAMED DAGHMAN**

Date:

## TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	ix
DECLARATION	xii
LIST OF TABLES	xvii
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxiii
<b>Chapter</b>	
1 INTRODUCTION	1.1
1.1 <i>Rhizoctonia solani</i>	1.1
1.2 Biological Control	1.3
1.3 Objectives of This Study	1.6
2 LITERATURE REVIEW	2.1
2.1 Soil-Borne Pathogens	2.1
2.2 Biological Control of Plant Pathogens	2.3
2.3 <i>Trichoderma</i> spp	2.7
2.3.1 Temperature	2.10
2.3.2 Light	2.12
2.3.3 Carbon dioxide (CO <sub>2</sub> )	2.12
2.3.4 Aeration	2.13
2.3.5 pH	2.13
2.3.6 Moisture	2.14
2.3.7 Substrate	2.14
2.4 <i>Trichoderma</i> as Biological Control Agent of Plant Pathogens	2.15
2.5 <i>Trichoderma</i> as Plant Growth Promoting Fungi	2.18
2.6 Rhizosphere Competency	2.19
2.7 Mass Culturing of <i>Trichoderma</i>	2.21
2.8 Formulation	2.25
2.9 Application Methods	2.31
2.10 Soil Augmentation	2.34
2.11 Seed Treatment	2.36
2.12 Integrated Biological Control	2.39
3 ANTAGONISTIC ACTIVITY OF <i>TRICHODERMA</i> AGAINST <i>RHIZOCTONIA SOLANI</i>	3.1
3.1 Introduction	3.1
3.2 Materials and Methods	3.3

3.2.1	Isolation of <i>Rhizoctonia solani</i>	3.3
3.2.2	Infectivity of <i>Rhizoctonia solani</i> on <i>Brassica</i>	3.3
3.2.3	Screening of <i>Trichoderma</i> Isolates Against <i>Rhizoctonia solani</i>	3.4
3.2.3.1	Dual Culture Test	3.5
3.2.3.2	Colony Degradation Test	3.7
3.2.3.3	Hyphal Interaction Test (mycoparasitism)	3.8
	Light Microscopy	3.8
	Scanning Electron Microscopy	3.9
3.2.4	DNA Polymorphism of <i>Trichoderma</i> Isolates	3.9
3.2.4.1	Preparation of Freeze-Dried Mycelium	3.9
3.2.4.2	DNA Extraction	3.10
3.2.4.3	Quantification and Purity of DNA	3.11
3.2.4.4	RAPD-PCR Amplification	3.11
3.2.4.5	Electrophoresis	3.12
3.2.4.6	Data Analysis	3.13
3.2.5	Characteristics of <i>Trichoderma</i>	3.13
3.2.5.1	Cultural and Morphological	3.13
	Light Microscopy	3.14
	Scanning Electron Microscopy	3.14
3.2.6	Colonization and Establishment of <i>Trichoderma</i> (UPM40 and TV3) on Roots and Rhizosphere of <i>Brassica rapa</i>	3.15
3.3	Results and Discussion	3.17
3.3.1	Isolation and Characterization of <i>R. solani</i>	3.17
3.3.2	Infectivity of <i>R. solani</i> on <i>Brassica</i>	3.19
3.3.3	Antagonistic Activity of <i>Trichoderma</i> Isolates Against <i>R. solani</i>	3.22
3.3.4	Characterization of <i>Trichoderma</i> Species Aggregates as Expressed by DNA Polymorphism	3.28
3.3.5	Cultural and Morphological Characteristics of UPM40 and TV3	3.32
3.3.6	Colonization and Establishment of <i>Trichoderma</i> (UPM40 and TV3) on Roots and Rhizosphere of <i>Brassica rapa</i>	3.35
4	MASS CULTURE OF <i>TRICHODERMA</i>	4.1
4.1	Introduction	4.1
4.2	Materials and Methods	4.3
4.2.1	Preparation of <i>Trichoderma</i> Inoculum	4.3
4.2.2	Effect of Rice Flour and Molasses on Mycelial Growth and Sporulation of <i>Trichoderma</i>	4.3
4.2.3	Effect of Incubation Temperature on Mycelial Growth and Sporulation of <i>Trichoderma</i>	4.6
4.2.4	Optimizing the Biomass Production	4.8
4.3	Results and Discussion	4.9
4.3.1	Effect of Rice Flour and Molasses on Mycelial Growth	

	and Sporulation of <i>Trichoderma</i>	4.9
4.3.2	Effect of Incubation Temperature on Mycelial Growth and Sporulation of <i>Trichoderma</i>	4.30
4.3.3	Optimization the Biomass Production	4.35
5	FORMULATION OF <i>TRICHODERMA</i> INOCULANTS	5.1
5.1	Introduction	5.1
5.2	Materials and Methods	5.4
5.2.1	Preparation of the Food-Base Carrier for Dry Formulation	5.4
5.2.1.1	Rice Flour	5.4
5.2.1.2	Compost	5.4
	Bulk Density	5.4
	Compost Maturity	5.5
	Water Holding Capacity	5.6
	Nutrient Contents	5.7
	Total N	5.7
	Total Carbon	5.8
	Microbial Properties	5.8
5.2.2	Statistical Analysis	5.9
5.2.3	Dry Formulation of <i>Trichoderma</i>	5.10
5.2.4	Liquid Formulation of <i>Trichoderma</i>	5.12
5.2.5	Statistical Analysis	5.13
5.3	Results and Discussion	5.15
5.3.1	Physical, Chemical and Microbiological Properties of Compost	5.15
5.3.1.1	Bulk Density	5.15
5.3.1.2	Compost Maturity	5.17
5.3.1.3	Water Holding Capacity	5.18
5.3.1.4	Nutrient Contents	5.19
5.3.1.5	Microbial Properties	5.21
5.3.2	Dry Formulation of <i>Trichoderma</i>	5.25
5.3.3	Liquid Formulation of <i>Trichoderma</i>	5.33
6	POTENTIAL OF <i>TRICHODERMA HARZIANUM</i> (UPM40) IN CONTROLLING RHIZOCTONIA DAMPING-OFF IN <i>BRASSICA RAPA</i>	6.1
6.1	Introduction	6.1
6.2	Materials and Methods	6.4
6.2.1	Efficacy of Dry Formulation of <i>T.harzianum</i> (UPM40)	6.4
6.2.2	Efficacy of Seed Coating	6.7
6.2.3	Statistical Analysis	6.10
6.3	Results and Discussion	6.11
6.3.1	Efficacy of Dry Formulation of <i>T. harzianum</i> (UPM40)	6.11
6.3.2	Efficacy of Seed Coating	6.25
7	GENERAL DISCUSSION AND CONCLUSION	7.1

REFERENCES	R.1
APPENDICES	A.1
BIODATA OF THE AUTHOR	B.1

## LIST OF TABLES

Table		Page
2.1	Methods of application of <i>Trichoderma</i> inoculants.	2.33
3.1	Isolates of <i>Trichoderma</i> screened for antagonistic activity against <i>R. solani</i> .	3.5
3.2.	Percentage emergence of <i>Brassica</i> spp. in <i>R. solani</i> infested and non-infested soil.	3.21
3.3	Antagonistic effect of <i>Trichoderma</i> isolates against <i>R. solani</i> in the dual culture and colony degradation tests.	3.24
3.4	Cultural and morphological characteristics of <i>T. harzianum</i> (UPM40) and <i>T. virens</i> (TV3) on PDA after 5 days' incubation.	3.33
4.1	Composition of Rice flour (Tepung Beras Cap Teratai) used in the study.	4.4
4.2	Composition of sugar cane molasses used in the study.	4.5
4.3	Production of dry mycelia (mg) by UPM40 on various substrates of Rice Flour and Molasses in static culture.	4.11
4.4	Production of dry mycelia (mg) by TV3 on various substrates of Rice Flour and Molasses in static culture.	4.11
4.5	Production of chlamydospores ( $\times 10^5$ ) by UPM40 on various substrates of Rice Flour and Molasses in shaken culture.	4.24
4.6	Production of chlamydospores ( $\times 10^5$ ) by TV3 on various substrates of Rice Flour and Molasses in shaken culture.	4.25
4.7	Mean biomass production of <i>Trichoderma</i> isolates after 3 days at 30 °C in a Bench-Top Fermentor.	4.36
5.1	Treatments in dry formulations stored at ( $10 \pm 2$ °C).	5.11
5.2	Treatments in dry formulations stored at ( $28 \pm 2$ °C).	5.12
5.3	Treatments in liquid formulation stored at $10 \pm 2$ °C.	5.14
5.4	Treatments in liquid formulation stored at $28 \pm 2$ °C.	5.14
5.5	Bulk densities of Composts.	5.17



5.6	Maturity of the composts used on germination test.	5.18
5.7	Moisture contents of the composts at different suctions and their water holding capacity (%).	5.19
5.8	Macronutrient contents of the composts (% dry weight).	5.21
5.9	Micronutrient contents of the composts (% dry weight).	5.21
5.10	Microbial populations in OPTCD (cfu / g dry weight).	5.24
5.11	Microbial populations in MCD (cfu / g dry weight).	5.24
5.12	Viability of <i>Trichoderma</i> inoculants as dry formulation with time of storage at 10 °C.	5.27
5.13	Viability of <i>Trichoderma</i> inoculum as dry formulation with time of storage at 28 °C.	5.30
5.14	Viability of <i>Trichoderma</i> in the liquid formulation with storage time at 10 ± 2 °C.	5.35
5.15	Viability of <i>Trichoderma</i> in the liquid formulation with time of storage at 28 °C.	5.37
6.1	Treatments for testing the effectiveness of dry formulations of <i>Trichoderma</i> (UPM40) in controlling <i>Rhizoctonia</i> diseases of <i>B. rapa</i> .	6.7
6.2	Treatments for testing the effect of seed coating in controlling <i>Rhizoctonia</i> diseases of <i>B. rapa</i> .	6.10
6.3	Numbers of viable <i>R. solani</i> propagules in soil infested by <i>R. solani</i> initially and at 36 days after sowing.	6.12
6.4	Seedling emergence (%) of <i>B. rapa</i> seeds in <i>Rhizoctonia</i> -infested and non-infested soils at 4 days after sowing.	6.13
6.5	Epidemic rates and AUDPC for <i>Rhizoctonia</i> under the different treatments at 36 days after sowing.	6.16
6.6	Total plant dry weights produced in the different treatments in <i>Rhizoctonia</i> -infested and non-infested soils at 36 days after sowing.	6.21
6.7	Effect of the different treatments (T1, T2 and T3) on the total	

	dry weights of roots produced in the non-infested soil at 36 days after sowing.	6.22
6.8	Average cfu's of UPM40 recovered from <i>B. rapa</i> roots 36 days after sowing in infested and non-infested soils.	6.24
6.9	Numbers of viable <i>R. solani</i> propagules in soil infested by <i>R. solani</i> initially and at 36 days after sowing.	6.26
6.10	Seedling emergence (%) of <i>B. rapa</i> seeds in <i>Rhizoctonia</i> -infested and non-infested soils at 4 days after sowing.	6.27
6.11	Epidemic rates and AUDPC for <i>Rhizoctonia</i> under the different treatments at 36 days after sowing.	6.29
6.12	Total plant dry weights produced in the different treatments in <i>Rhizoctonia</i> -infested and non-infested soils at 36 days after sowing.	6.30
6.13	Effect of the different treatments (T1, T2 and T3) on the total dry weights of roots produced in the <i>Rhizoctonia</i> non-infested soil at 36 days after sowing.	6.31

## LIST OF FIGURES

Figure		Page
3.1	Measurement of radial growth of <i>R. solani</i> in the dual culture test.	3.7
3.2	Antagonistic activity of <i>Trichoderma</i> in the colony degradation test.	3.8
3.3	Micrographs of <i>R. solani</i> hyphae showing the typical right angle branching (arrow) Light microscopy (A), and Scanning electron microscopy (B).	3.18
3.4	5-day old pure culture of <i>R. solani</i> on PDA with sclerotia at the colony margin.	3.18
3.5	Pre-emergence damping-off in <i>Brassica rapa</i> caused by <i>R. solani</i> 4 days after sowing. The seeds infected by <i>R. solani</i> failed to germinate.	3.20
3.6	Typical symptom of post-emergence damping-off in <i>Brassica rapa</i> - 'water-soaked' lesion (arrow) on the hypocotyl.	3.21
3.7	Effect of <i>Trichoderma</i> isolates on radial growth of <i>R. solani</i> in the dual culture test (4 days' incubation).	3.23
3.8	Micrographs showing <i>Trichoderma</i> (UPM40) growing along the hyphae of <i>Rhizoctonia</i> and producing hooks Light microscopy (A) and Scanning electrone microscopy (B).	3.27
3.9	Micrographs showing <i>Trichoderma</i> (TV3) growing along the hyphae of <i>Rhizoctonia</i> and producing hooks Light microscopy (A) and Scanning electrone microscopy (B).	3.28
3.10	RAPD Fingerprints of <i>Trichoderma</i> isolates obtained by OPC-11	3.29
3.11	RAPD Fingerprints of <i>Trichoderma</i> isolates obtained by OPC-15	3.30
3.12	UPGMA dendogram of RAPD markers amplified for DNA using combinations of primers OPC-11 and OPC-15.	3.31
3.13	4-day culture of <i>Trichoderma</i> on PDA plates. <i>T. virens</i> (TV3) (A), and <i>T. harzianum</i> (UPM40) (B).	3.34
3.14	SEM Micrograph of <i>T. harzianum</i> (UPM40) showing the production of conidia (A) on verticillate phialides(B).	3.34

3.15	SEM Micrograph of <i>T. virens</i> (TV3) showing the production of conidia (A) on ampulliform phialides (B).	3.35
3.16	Populations (cfu / g) of UPM40 and TV3 in the rhizosphere of <i>Brassica</i> plants.	3.37
3.17	Population (cfu / g) of UPM40 (A) and TV3 (B) on root tips and mature roots of <i>Brassica</i> .	3.38
4.1	Effects of rice flour and molasses on the production of dry mycelia by UPM40 in static culture.	4.12
4.2	Effects of rice flour and molasses on the production of dry mycelia by TV3 in static culture.	4.13
4.3	Effects of rice flour and molasses on the production of conidia by UPM40 in static culture.	4.16
4.4	Effects of rice flour and molasses on the production of conidia by TV3 in static culture.	4.17
4.5	Effect of incubation period on conidia production by UPM40 and TV3 on the 1:4 w/v RF: ML mixed substrate.	4.18
4.6	Effects of rice flour and molasses on the production of chlamyospores by UPM40 in static culture.	4.20
4.7	Effects of rice flour and molasses on the production of chlamyospores by TV3 in static culture.	4.21
4.8	Effects of rice flour and molasses on the production of conidia by UPM40 and TV3 in shaken culture.	4.23
4.9	Effects of rice flour and molasses on the production of chlamyospores by UPM40 and TV3 in shaken culture.	4.26
4.10	Effects of rice flour and molasses on the production of dry mycelia by UPM40 and TV3 in shaken culture.	4.28
4.11	Morphology of conidia and chlamyospore of <i>Trichoderma</i> .	4.29
4.12	Effect of incubation temperature on the production of conidia, chlamyospores and mycelia by <i>Trichoderma</i> UPM40, and viability of the conidia.	4.31
4.13	Effect of incubation temperature on the production of conidia,	

	chlamyospores and mycelia by <i>Trichoderma</i> TV3, and viability of the conidia.	4.33
5.1	Viability of <i>Trichoderma</i> as dry formulation after seven months' storage at $10 \pm 2$ °C.	5.26
5.2	Viability of <i>Trichoderma</i> as dry formulation after seven months' storage at $28 \pm 2$ °C.	5.29
5.3	Effect of storage temperature ( $10 \pm 2$ °C) on the viability of <i>Trichoderma</i> in the liquid formulation.	5.35
5.4	Effect of storage temperature ( $28 \pm 2$ °C) on the viability of <i>Trichoderma</i> in liquid formulation.	5.36
6.1	Symptoms of post-emergence damping-off in <i>B. rapa</i> seedlings in <i>Rhizoctonia</i> -infested soil.	6.15
6.2	Effect of the treatments (T1, T2 and T3) on the disease progression of <i>Rhizoctonia</i> in <i>B. rapa</i> seedlings planted in infested soil.	6.16
6.3	Growth of <i>Brassica</i> seedlings in <i>Rhizoctonia</i> -infested soil.	6.18
6.4	Growth of <i>Brassica</i> seedlings in non-infested soil.	6.20
6.5	Roots of <i>Brassica</i> seedlings in the non-infested soil at 36 days after sowing.	6.23
6.6	Effect of the treatments (T1, T2 and T3) on the disease progression of <i>Rhizoctonia</i> in <i>B. rapa</i> seedlings planted in infested soils.	6.28

## LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celcius
1:4, RF:ML w/v	1g Rice flour: 4 mL Molasses in 100 mL distil water
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
bp	base pair
BSR	Basal Stem Rot
c.f.u	Colony Forming Units
cm	Centimeter
cm <sup>2</sup>	Centimeter Square
CPD	Critical Point Drying
CRD	Completely Randomized Design
DI	Disease Incidence
DNA	Deoxy-ribo-nucleic Acid
EDTA	Ethylene Diaminetetra Acetic Acid
EtBr	Ethidium Bromide
g	Gram
HCL	hydrochloric acid
IPM	Integrated Pest Management
Kb	Kilo-base pair
Kg / ha	Kilogram per Hectare
LSD	Least Significant Difference
M	Molar
M-CSL	Molasses-Corn Steep Liquor
mg	Milligram
mL	Milliliter
mm	Milimeter
mM	Millimolar
MCD	Mesocarp and Chicken Dung
NaOAc	Sodium Acetate
nm	Nanometer
NPK	Nitrogen, Phosphorous, Potassium
NUV	Near Ultra Violet
OA	Oat meal Agar
OPC	Oligo-nucleotide Purification Column primers
OPTCD	Oil Palm Trunk and Chicken Dung
PCNB	Penta-Chloro-Nitro-Benzene
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PGPF	Plant Growth Promoting Fungi
PGPR	Plant Growth Promoting Rhizobacteria
PIRG	Percent Inhibition of Radial Growth
POME	Palm Oil Mill Effluent
RAPD	Random Amplified Polymorphic DNA

RH	Relative Humidity
SEM	Scanning Electron Microscopy
spp	Species
TAE	Tris-HCL- glacial acetic acid-EDTA
Taq	Thermal aquatius
TE	Tris-EDTA
Th	<i>Trichoderma harzianum</i>
TME	<i>Trichoderma</i> selective media
Tv	<i>Trichoderma virens</i>
UPM	Universiti Putra Malaysia
UV	Ultra violet
v/v	Volume per volume
WA	water Agar
μl	Micro liter