



**UNIVERSITI PUTRA MALAYSIA**

**BIO-PELLETING OF BRASSICA RAPA SEEDS USING  
TRICHODERMA INOCULANTS FOR IMPROVED KEEPING QUALITY  
AND CONTROL OF PYTHIUM DAMPING-OFF**

**KHOR SIEW EIM**

**FP 2002 35**

**BIO-PELLETING OF *BRASSICA RAPA* SEEDS USING TRICHODERMA  
INOCULANTS FOR IMPROVED KEEPING QUALITY AND CONTROL OF  
PYTHIUM DAMPING-OFF**

**By**

**KHOR SIEW EIM**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of Requirements for the Degree of Master of Agriculture Science**

**September 2002**



**Special dedication to my parents, Khor Ah Hock and Ng Choon Thor, my sisters and brothers, Cheu Houg, Siew Muai, Hu Ming and Hu Thye, my sisters and brothers-in-law, and my nieces.**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agriculture Science

**BIO-PELLETING OF *BRASSICA RAPA* SEEDS USING TRICHODERMA INOCULANTS FOR IMPROVED KEEPING QUALITY AND CONTROL OF *PYTHIUM* DAMPING-OFF**

By

**KHOR SIEW EIM**

**September 2002**

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

**Faculty: Agriculture**

An attempt was made to study the effect of biological seed treatment on the viability and keeping quality of *B. rapa* and incidence of *Pythium* damping-off. *Trichoderma virens* (UPM 23) and *Trichoderma harzianum* (UPM 29) were used as the microbial inoculants in bio-pelleting of the seeds. UPM 23 produced greater antagonistic activity against *Pythium splendens*, the causal pathogen of damping-off of *B. rapa*, based on the *in vitro* dual culture and colony degradation tests. Percentage inhibition of radial growth (PIRG) of *P. splendens* was 83.8% and 94.0% when co-inoculated on Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA) respectively. Parasitized hyphae failed to regenerate when plated on fresh CMA medium. UPM 23 was as effective as Captan® in protecting the *B. rapa* seeds against *Pythium* pre-emergence damping-off, due to its ability to colonize the germinating seed, compete for sites and nutrients and directly parasitizing *Pythium*. Percentage reduction in seedling emergence was 7.0%, 14 days after

sowing, as compared to the control (80.6%). There was no significant difference in percentage post-emergence damping-off of seedlings between bio-pelleted seeds or control. However, number of surviving plants at harvest was higher for UPM 23 bio-pelleted seeds relative to the other treatments. Measurement of seed viability based on germination test, electrical conductivity test, accelerated aging test and tetrazolium staining, showed that *B. rapa* seeds either *Trichoderma* bio-pelleted or rice flour pelleted with 10% moisture content has better keeping quality when stored at  $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , until week 24 of storage. Seed viability and quality was further improved by reducing the moisture content to 4%. Incorporation of biocontrol agents such as UPM 29 and UPM 23 in bio-pelleting, regardless of seed moisture content required low storage temperature to ensure the survival and proliferation of these inoculum on the seed surface.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

**SADURAN BIOLOGI BIJI BENIH *BRASSICA RAPA* DENGAN  
INOKULUM *TRICHODERMA* UNTUK MEMPERBAIKI KUALITI  
PENYIMPANAN DAN PENGAWALAN PENYAKIT LECUH *PYTHIUM***

Oleh

**KHOR SIEW EIM**

**September 2002**

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

**Fakulti: Pertanian**

Satu percubaan telah dijalankan untuk mengkaji kesan rawatan biologi biji benih ke atas kebolehhidupan dan kualiti penyimpanan *Brassica rapa* serta kejadian penyakit lecu yang disebabkan oleh *Pythium*. *Trichoderma virens* (UPM 23) and *Trichoderma harzianum* (UPM 29) telah digunakan sebagai inokulum mikroba di dalam saduran biologi biji benih. UPM 23 menghasilkan aktiviti keantagonisan yang lebih kuat ke atas *P. splendens*, patogen penyebab penyakit lecu *B. rapa*, berdasarkan ujian *in vitro* "Dual Culture" dan "Colony degradation". Peratusan perencatan pertumbuhan jejari miselium *P. splendens* di atas media PDA dan CMA adalah sebanyak 83.8% dan 94.0% berurutan. Hifa yang diparasit gagal untuk tumbuh semula apabila dipindahkan ke atas media CMA yang baru. Keberkesanan UPM 23 adalah bersamaan dengan Captan® di dalam memberi perlindungan kepada biji benih daripada lecu pra-cambah. Ini berdasarkan kepada keupayaan UPM 23 untuk mengkoloni biji benih yang bercambah, dan

bersaing untuk tapak dan nutrien serta tindakannya dalam memparasit *Pythium*. Peratus penurunan dalam kemunculan anak benih adalah 7.0%, 14 hari selepas penyemaian, berbanding dengan kawalan (80.6%). Walaubagaimanapun, tiada perbezaan bererti di dalam peratusan anak benih yang menunjukkan simptom lecut pos-cambah di antara biji benih saduran biologi dan biji benih kawalan. Tetapi bilangan pokok yang mandiri pada waktu penuaian adalah tinggi untuk biji benih *B. rapa* yang disadur dengan UPM 23 relatif kepada rawatan lain. Penilaian keatas kebolehhidupan biji benih *B. rapa*, berdasarkan ujian-ujian percambahan, pengkonduksian elektrik, penuaan dan pewarnaan tetrazolium, menunjukkan biji benih sama ada saduran biologi ataupun tidak, dengan peratus kelembapan 10%, mempunyai kualiti penyimpanan yang lebih baik apabila disimpan pada suhu  $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , sehingga minggu ke-24 penyimpanan. Kebolehhidupan dan kualiti biji benih boleh dikekalkan dengan menurunkan kandungan kelembapan kepada 4%. Penggabungan agen kawalan biologi seperti UPM 29 dan UPM 23 di dalam saduran biologi, tanpa mengambilkira kandungan kelembapan biji benih, memerlukan suhu simpanan yang rendah untuk kemandirian dan perkembangan inokulum di atas permukaan biji benih.

## ACKNOWLEDGEMENTS

I wish to express my deepest and sincere gratitude and appreciation to my supervisor, Professor Dr. Sariah Meon for her dedicated efforts, support, invaluable advice, intellectual guidance and encouragement in conducting my research and in preparation of this dissertation.

Grateful thanks also to my supervisory committee members, Dr. Jugah Kadir and Dr. Uma Rani for their constructive comments, advice and help throughout my studies and in the preparation of the final manuscript.

Thanks are extended to Dr. Anuar Abdul Rahim, from Department of Soil Science, for his valuable advice in the statistical analysis, to Puan Norma and also to the staff members of the Department of Plant Protection, especially Mr. Khir, Mr. Lim, Mr. Nazri, Mr. Johari, Mr. Gani, and Madam Junainah, for their help and co-operation.

Special thanks also to my friends Kam Loong, Ee Fong, Mueen, Long Chang, Sek Yee, Ganesan, Adeline, Leng Choo, Hooi Ling, Kak Nor, Chia Ying, Chee Ngan and Khairul for their help, support, and encouragement throughout my study period at Universiti Putra Malaysia.





To my parents, Khor Ah Hock and Ng Choon Thor, my brothers-Hu Ming and Hu Thye, my sisters- Cheu Houng and Siew Muai, my sisters-in-law, Siew Theng and Mei Peng, my brothers-in-law, Jun Siew and Aik Guan, my aunts, uncles, my nieces- Ching Yee, Ching Wei, Eng Yee and Su Jun, my nephews and cousins. I thank them for their love, support and encouragement throughout my study in UPM and my whole life.

## TABLE OF CONTENTS

	<b>Page</b>
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxiii
 <b>CHAPTER</b>	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Leafy Vegetables	5
2.2 Seedling Diseases	8
2.3 Methods of Control	11
2.3.1 Cultural Control Methods	11
2.3.2 Physical Control Methods	14
2.3.3 Chemical Control Methods	16
2.3.4 Biological Seed Treatment	18
2.3.5 Advantages of Biological Seed Treatment	18
2.4 Application of Biological Control Agents in Seed Treatment	22
2.4.1 Seed Coating	23
2.4.2 Dry Powdery Formulation	23
2.4.3 Slurry Formulation	24
2.4.4 Film-coating	24
2.4.5 Seed Pelleting	25
2.5 Physiological Seed Treatment	29
2.5.1 Fluid Drilling	29
2.5.2 Seed Priming	30
3 MATERIALS AND METHODS	34
3.1 Characterization of <i>Pythium</i>	34
3.1.1 Isolation of Fungal Pathogen	34
3.1.2 Identification of <i>Pythium</i>	34
3.1.3 Pathogenicity of <i>Pythium</i> on <i>Brassica</i>	34



	<b>Page</b>
3.2 Antagonistic Activity of <i>Trichoderma</i> Isolates against <i>Pythium</i> sp.	36
3.2.1 Dual Culture Test	36
3.2.2 Colony Degradation Test	38
3.3 The effect of Bio-pelleting on Seed Viability and Keeping Quality of <i>Brassica</i> Seed	39
3.3.1 Preparation of <i>Trichoderma</i> Inoculum	39
3.3.2 Bio-pelleting of <i>Brassica</i> Seeds with <i>Trichoderma</i> Inoculum	39
3.4 Assessment of Seed Viability and Keeping Quality	41
3.4.1 Seed Germination Test	42
3.4.2 Tetrazolium Test	42
3.4.3 Electrical Conductivity Test	45
3.4.4 Accelerated Aging Test	45
3.4.5 Survival and Proliferation of <i>Trichoderma</i> on Seed Surface	46
3.5 The effect of Seed Moisture on Seed Vability, Keeping Quality, and Survival and Proliferation of UPM 23 on Seed Surface	46
3.6 The effect of Bio-pelleting on Incidence of Seedling Disease Caused by <i>Pythium</i>	47
3.7 Statistical Analysis	48
<b>4 RESULTS AND DISCUSSIONS</b>	<b>49</b>
4.1 Characterization of <i>Pythium</i>	49
4.2 <i>Pythium</i> Infection on <i>Brassica</i>	51
4.3 Antagonistic Activity of <i>Trichoderma</i> Isolates against <i>Pythium</i>	56
4.4 The effect of Bio-pelleting and Storage Temperature on Seed Viability, and Keeping Quality of <i>Brassica</i> Seeds	65
4.5 The effect of Bio-pelleting and Storage Temperature on Survival and Establishment of <i>Trichoderma</i> on Seed Surface	83
4.6 The effect of Bio-pelleting, Seed Moisture and Storage Temperature on Seed Viability and Keeping Quality of <i>Brassica</i> Seeds	88
4.7 The effect of Seed Moisture and Storage Temperature on Survival and Proliferation of <i>Trichoderma virens</i> (UPM 23) on Seed Surface	98
4.8 The effect of Biological Seed Treatment on Damping-off Caused by <i>P. splendens</i> on <i>B. rapa</i>	101

	<b>Page</b>
5      GENERAL DISCUSSION AND CONCLUSION	114
REFERENCES	120
APPENDICES	145
BIODATA	202



## LIST OF TABLES

<b>Table</b>		<b>Page</b>
1	Staining patterns used for interpreting tetrazolium test results for <i>B. rapa</i> seeds stored at 28°C and 10°C	44
2	Percentage emergence of <i>Brassica</i> in <i>Pythium</i> -infested and non- infested soil	55
3	Antagonistic effect of UPM 23 and UPM 29 on <i>Pythium</i> in dual culture test on PDA and CMA, four days after co-inoculation	60
4	The effect of seed moisture on germination percentage of UPM 23 bio-pelleted <i>B. rapa</i> seeds stored at 28 and 10±2°C, over a period of 24 weeks	94
5	The effect of seed moisture on survival and proliferation of UPM 23 on <i>B. rapa</i> seeds stored at 28 and 10±2°C, over a period of 24 weeks	99
6	The effect of bio-pelleting on percentage of seedling emergence of <i>B. rapa</i> seeds in <i>Pythium</i> -infested and non-infested soil, 14 days after sowing	102
7	The effect of bio-pelleting on percentage post-emergence damping-off and survival of <i>B. rapa</i> seedlings in <i>Pythium</i> -infested soil, 35 days after sowing	106
8	The effect of bio-pelleting on plant dry weight of <i>B. rapa rapa</i> seeds in <i>Pythium</i> -infested and non-infested soil, 35 days after sowing	109
9	The effect of bio-pelleting on colony forming units of <i>Trichoderma</i> recovered from <i>B. rapa</i> roots, rhizosphere soil and and bulk soil, 35 days after sowing	111
10	ANOVA table of interaction between soil treatment and <i>Brassica</i> variety used in pathogenicity test	146
11	ANOVA Table for percentage reduction of emergence in <i>Pythium</i> pathogenicity test	146

<b>Table</b>	<b>Page</b>
12 ANOVA table for percentage post-emergence damping-off off in <i>Pythium</i> pathogenicity test	146
13 ANOVA and mean comparison table for dual culture test on CMA media	147
14 ANOVA and mean comparison table for dual culture test on PDA media	147
15 Percentage germination of <i>B. rapa</i> seeds stored at 28±2°C and 10±2°C over 24 weeks of storage.	148
16 The effect of bio-pelleting on electrical conductivity leakage of <i>B. rapa</i> seeds stored at 10±2°C over a period of 24 weeks	149
17 The effect of bio-pelleting on electrical conductivity leakage of <i>B. rapa</i> seeds stored at 28±2°C over a period of 24 weeks	149
18 The effect of bio-pelleting on percentage germinable seeds obtained from tetrazolium test of <i>B. rapa</i> seeds stored at 28°C and at 10°C over 24 weeks of storage, relative to 50 seeds tested	150
19 The effect of bio-pelleting on percentage germination after aging of <i>B. rapa</i> seeds stored at 28±2°C over a period of 24 weeks	150
20 The effect of bio-pelleting on percentage germination after aging aging of <i>B. rapa</i> seeds stored at 10±2°C over a period of 24 weeks	151
21 The effect of bio-pelleting on survival and proliferation of UPM 23 and UPM 29 on <i>B. rapa</i> seeds stored at 28°C and 10°C over 24 weeks of storage	151
22 The effect of seed moisture on percentage germination of <i>B. rapa</i> seeds bio-pelleted with UPM 23 stored at 28 and 10±2°C, over a period of 24 weeks	152
23 The effect of seed moisture on percentage germination after aging of UPM 23 bio-pelleted <i>B. rapa</i> seeds stored at 28 and 10±2°C over a period of 24 weeks	152

<b>Table</b>	<b>Page</b>
24 The effect of seed moisture on electrical conductivity leakage of UPM 23 bio-pelleted <i>B. rapa</i> seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	153
25 Sorting by Week and Bio-pelleting: The effect of bio-pelleting on percentage germination of <i>B. rapa</i> seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	154
26 Sorting by Week and Bio-pelleting: The effect of bio-pelleting on percentage germination after aging of <i>B. rapa</i> seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	155
27 Sorting by Week and Bio-pelleting: The effect of bio-pelleting on electrical conductivity leakage of <i>B. rapa</i> seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	156
28 Sorting by Week and Bio-pelleting: The effect of bio-pelleting on Survival and proliferation of <i>Trichoderma</i> of <i>B. rapa</i> seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	157
29 Sorting by Week and Bio-pelleting: The effect of seed moisture on percentage germination of UPM 23 bio-pelleted seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	158
30 Sorting by Week and Bio-pelleting: The effect of seed moisture on percentage germination after aging of UPM 23 bio-pelleted seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	159
31 Sorting by Week and Bio-pelleting: The effect of seed moisture on electrical conductivity of UPM 23 bio-pelleted seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	160
32 Sorting by Week and Bio-pelleting: The effect of seed moisture on survival and proliferation of UPM 23 bio-pelleted seeds stored at 28±2°C and 10±2°C over 24 weeks of storage	161
33 ANOVA table of interaction between treatment, week, and storage temperature for all test	162
34 ANOVA table of interaction between seed moisture, week, and storage temperature for all test	164

<b>Table</b>	<b>Page</b>
35 ANOVA table for treatment and storage temperature effect according to week and storage temperature	166
36 ANOVA table for germination test according to treatment and storage temperature	170
37 ANOVA table for aging germination test according to treatment and storage temperature	171
38 ANOVA table for electrical conductivity test according to treatment and storage temperature	172
39 ANOVA table for <i>Trichoderma</i> colony count according to treatment and storage temperature	173
40 ANOVA table for seed moisture and storage temperature effect according to week and storage temperature	174
41 ANOVA table for germination test according to seed moisture and storage temperature	178
42 ANOVA table for aging germination test according to seed moisture and storage temperature	178
43 ANOVA table for electrical conductivity test according to seed moisture and storage temperature	179
44 ANOVA table for <i>Trichoderma</i> colony count according to seed moisture and storage temperature	179
45 ANOVA table for percentage emergence in glasshouse test	180
46 ANOVA table for percentage post-emergence damping-off	180
47 ANOVA table for plant dry weight in glasshouse test	180
48 ANOVA table for percentage survival in glasshouse test	180
49 ANOVA table for <i>Trichoderma</i> colony count in glasshouse test	181





<b>Table</b>	<b>Page</b>
50 Mean comparison table for germination test according to treatment and temperature of storage	181
51 Mean comparison table for aging germination test according to week and temperature of storage	184
52 Mean comparison table for electrical conductivity test according to week and temperature of storage	186
53 Mean comparison table for c.f.u. test according to week and temperature of storage	189
54 Mean comparison table for germination test according to treatment and temperature of storage	191
55 Mean comparison table for aging germination test according to week and temperature of storage	193
56 Mean comparison table for electrical conductivity test according to week and temperature of storage	195
57 Mean comparison table for c.f.u. test according to week and temperature of storage	197
58 ANOVA and mean comparison table for the effect of bio-pelleting on percentage emergence of <i>B. rapa</i> seeds in glasshouse assay	199
59 ANOVA and mean comparison table for the effect of bio-pelleting on percentage survival of <i>B. rapa</i> in glasshouse assay	199
60 ANOVA and mean comparison table of c.f.u of <i>Trichoderma</i> inoculant in <i>Pythium</i> -infested soil in glasshouse assay	200

## LIST OF FIGURES

Figure		Page
1	Measurement of radial growth of <i>Pythium</i> in dual culture test	37
2	Measurement of the growth of <i>Pythium</i> in colony degradation test	39
3	Bio-pelleted <i>Brassica rapa</i> seeds	41
4	Vegetative structure of <i>P. splendens</i> , as seen under the light microscopy (100x)	50
5	Scanning electron micrograph (SEM) of <i>P. splendens</i> oogonium and antheridium	50
6	<i>P. splendens</i> antheridium, oospore and sporangium, as seen under the light microscope (400x)	51
7	Sporangium of <i>P. splendens</i> under scanning electron microscope	52
8	Chlamydospore of <i>P. splendens</i> produced at 28 day-old of CMA culture (1000x)	52
9	Infected seed of <i>B. chinensis</i> var. <i>pekinensis</i> showing showing symptom of pre-emergence damping-off	53
10	Seven-day old seedling of hybrid variety "Sawi Dwarf" which was infected by <i>P. splendens</i> (A), and healthy seedling (B)	54
11	<i>Pythium</i> colonies re-isolated from infected <i>Brassica</i> seeds and plants parts	55
12	The effect of UPM 23 and UPM 29 on radial growth of <i>P. splendens</i> on CMA and PDA media after 4 and 12 days of co-incubation	59

<b>Figure</b>	<b>Page</b>
13 Mycelial plugs taken at 0, 1 cm, and the opposite edge of inhibition zone to test for <i>Pythium</i> regenerative ability and antagonistic efficacy of <i>Trichoderma</i>	61
14 Electron micrograph of <i>T. virens</i> produced appressoria-like structure and attached itself to the oogonium prior to penetration	63
15 Electron micrograph of parasitism of oogonium of <i>P. splendens</i> by <i>T. harzianum</i>	63
16 Electron micrograph of mycoparasitism of <i>Pythium</i> hypha: <i>T. harzianum</i> growing towards the <i>Pythium</i> hypha producing appressoria-like structure (A), penetration and degradation of <i>Pythium</i> hypha (B)	64
17 The effect of bio-pelleting on percentage germination of <i>B. rapa</i> seeds stored at $28\pm 2^{\circ}\text{C}$ and $10\pm 2^{\circ}\text{C}$ over a period of 24 weeks	67-68
18 The effect of bio-pelleting on electrical conductivity leakage of <i>B. rapa</i> seeds stored at $28\pm 2^{\circ}\text{C}$ and $10\pm 2^{\circ}\text{C}$ over a period of 24 weeks	72-73
19 Staining patterns obtained in tetrazolium test. (A), (B), and (C)-non-germinable seed (D) and (E)-germinable seed	76
20 The effect of bio-pelleting on germination percentage of <i>B. rapa</i> seeds stored at 28 and $10\pm 2^{\circ}\text{C}$ , over a period of 24 weeks in tetrazolium test	77-78
21 The effect of bio-pelleting on percentage germination of <i>B. rapa</i> seeds stored at 28 and $10\pm 2^{\circ}\text{C}$ over a period of 24 weeks	81-82
22 The effect of bio-pelleting on survival and proliferation of UPM 23 and UPM 29 on <i>B. rapa</i> seeds stored at $28^{\circ}\text{C}$	85
23 The effect of bio-pelleting on survival and proliferation of UPM 23 and UPM 29 on <i>B. rapa</i> seeds stored at $10^{\circ}\text{C}$	85

<b>Figure</b>	<b>Page</b>
24 The effect of seed moisture on germination percentage of <i>B. rapa</i> seeds bio-pelleted with UPM 23, stored at 28 and 10°C, over a period of 24 weeks	90-91
25 The effect of seed moisture on electrical conductivity leakage of <i>B. rapa</i> seeds bio-pelleted with UPM 23, stored at 28°C, over a period of 24 weeks	92
27 The effect of seed moisture on electrical conductivity leakage of <i>B. rapa</i> seeds bio-pelleted with UPM 23, stored at 10°C, over a period of 24 weeks	94
28 The effect of bio-pelleting on percentage germination after aging of <i>B. rapa</i> seeds stored at 28±2°C and 10±2°C over a period of 24 weeks	96-97
29 The effect of seed moisture on survival and proliferation (c.f.u.) of UPM 23 on <i>B. rapa</i> seeds stored at 28±2°C, over a period of 24 weeks	99
30 Symptoms of post-emergence damping-off of <i>B. rapa</i> seedling in <i>Pythium</i> -infested soil; wilted seedling (A); seedling with dark brown lesion on the hypocotyl (B); dead seedling (C); and healthy seedling (D)	105

## LIST OF ABBREVIATION

<b>AOSA</b>	<b>Association of Official Seed Analysts</b>
<b>CMA</b>	<b>Corn Meal Agar</b>
<b>CMI</b>	<b>Commonwealth Mycological Institute</b>
<b>DOA</b>	<b>Department of Agriculture</b>
<b>FAMA</b>	<b>Federal Agriculture Marketing Authority (FAMA)</b>
<b>FAO</b>	<b>Food and Agriculture Organization</b>
<b>IBPGR</b>	<b>International Board for Plant Genetic Resources</b>
<b>IPGRI</b>	<b>International Plant Genetic Resources Institute</b>
<b>IPM</b>	<b>Integrated Pest Management</b>
<b>ISTA</b>	<b>International Seed Testing Association</b>
<b>MADI</b>	<b>Malaysia Agricultural Directory &amp; Index</b>
<b>MAFF</b>	<b>Ministry of Agriculture, Fisheries and Food</b>
<b>NRC</b>	<b>National Research Council</b>
<b>PDA</b>	<b>Potato Dextrose Agar</b>
<b>SMP</b>	<b>Solid Matrix Priming</b>
<b>WHO</b>	<b>World Health Organization</b>



## CHAPTER I

### INTRODUCTION

Leafy vegetables, such as *Brassica* contribute significant amount of nutrients that are required for human growth and health (Rice *et al.*, 1986). The genus *Brassica* is not only consumed by man but are also used as fodder and oil crops, such examples are *Brassica campestris* (syn. *rapa*), *Brassica oleracea*, and *Brassica juncea* (Griffiths *et al.*, 1998). Among these three species of *Brassic*as, *B. campestris* has the widest distribution, with secondary centres of diversity in Europe, Western Russia, Central Asia and the Near East (Varilov, 1949; Mizushima and Tsunoda, 1967; Zeven and Zhukovsky, 1975).

In Peninsular Malaysia, *Brassica rapa*, L. (leaf mustard) is the most widely cultivated leafy vegetable that provides relatively quick returns. As of 1999, about 2,402 hectares (highest proportion) of agriculture land in Peninsular Malaysia was planted with this crop (Anon, 1999), showing the high market potential of this crop compared to other vegetables (FAMA, 1991). Therefore, high populations of *B. rapa* are grown intensively and continuously (Ding and Vimala, 1986) on limited planting area to meet the demand for leafy vegetable production, thus reducing their importation. In 1999, Malaysian importation of fresh and chilled vegetables had increased from 506 599 t (RM 655.124 million) in the year 1998, to 551 949 t (RM 683.758 million) (Department of Statistics Malaysian, 1999) which represents an increase of 4.4% in terms of monetary value.

