UNIVERSITI PUTRA MALAYSIA

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

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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

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Special dedication to my parents, Khor Ah Hock and Ng Choon Thor, my sisters and brothers, Cheu Houng, 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

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

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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°C ± 2°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

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September 2002

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

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Satu percubaan telah dijalankan untuk mengkaji kesan rawatan biologi biji benih ke atas kebolehhidupan dan kualiti penyimpanan *Brassica rapa* serta kejadian penyakit lecuh yang disebabkan oleh *Pythium*. *Trichoderma virens* (UPM 23) and *Trichoderma harzianum* (UPM 29) telah digunakan sebagai inokulum mikrob di dalam saduran biologi biji benih. UPM 23 menghasilkan aktiviti keantagonisan yang lebih kuat ke atas *P. splendens*, patogen penyebab penyakit lecuh *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 kapada biji benih daripada lecuh 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 simtom lecuh 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°C ± 2°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.

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LIST OF ABBREVIATION

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

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 *Brassicas*, *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.