

UNIVERSITI PUTRA MALAYSIA

PERFORMANCE OF THREE GENERA OF ENTOMOPATHOGENIC FUNGI AS POTENTIAL MICROBIAL CONTROL AGENTS AGAINST THE FLEA BEETLE PHYLLOTRETA STRIOLATA F. (COLEOPTERA : CHRYSOMELIDAE)

TRI PUJI PRIYATNO

FP 2001 23

PERFORMANCE OF THREE GENERA OF ENTOMOPATHOGENIC FUNGI AS POTENTIAL MICROBIAL CONTROL AGENTS AGAINST THE FLEA BEETLE PHYLLOTRETA STRIOLATA F. (COLEOPTERA : CHRYSOMELIDAE)

By

ΤRI PUJI PRIYATNO

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

August 2001



DEDICATION

To my wife, Yaya Suciati and my daugther, Izdihar Afaf



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

PERFORMANCE OF THREE GENERA OF ENTOMOPATHOGENIC FUNGI AS POTENTIAL MICROBIAL CONTROL AGENTS AGAINST THE FLEA BEETLE PHYLLOTRETA STRIOLATA F. (COLEOPTERA : CHRYSOMELIDAE)

By

TRI PUJI PRIYATNO

August 2001

Chairman : Assoc. Prof. Dr. Yusof Ibrahim

Faculty : Agriculture

The striped flea beetle (FB), *Phyllotreta striolata* F. (Coleoptera : Chrysomelidae), is not only a serious pest of canola and mustard but also feed on a wide range of other brassicas. Entomopathogenic fungi (EF) are promising agent for biological control of FB and are gaining increasing attention worldwide as mycoinsecticide. The potential of three genera of EF, *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus*, has been studied in the laboratory and the field against the striped FB, *Phyllotreta striolata* F.

Surveys for FB naturally infected with EF indicated that *M. anisopliae* v. *manus* and *B. bassiana* were the potential EF in the populations of FB sampled from vegetable area at UPM's Research Park, Serdang. However, the incidence of infection was very low. Therefore, introduction of virulent isolates into a temporary habitat must be done.



Test for pathogenicity of 16 isolates of EF against adult FB found only one isolate of *M. anisopliae* (MPs) causing mortality in excess of 50%. Four isolates were tested for pathogenicity against the eggs and larvae of the FB. Two isolates of *M. anisopliae* (MPs and Cy3), one *B. bassiana* (Wls) and one *P. fumosoroseus* (Pf) were found to be highly pathogenic against the FB larvae while both isolates of *M. anisopliae* were infective against the FB eggs.

The resistance of FB adults against EF was caused by the existence of fungistatic compounds on the integument. Five straight chain fatty acids (C4, C6, C7, C8, and C9) suspected as fungistatic compounds based on analysis using Gas Chromatography were proven to inhibit conidial germination.

Two media, rice flour and sponge-rice flour medium, examined for conidial massproduction of *M. anisopliae* v. *majus* and *P. fumosoroseus* indicated that the spongerice flour medium was shown to be potentially efficient for mass-production of fungal spores.

Three formulations of microbial control agent (MCA), namely liquid, dust and granule, were prepared for this study using oil and glycerine, kaolin, and peat soil as carriers, respectively. The oil, glycerine, and kaolin-formulated conidia were equally significant causing higher infection on adult beetles compared to that of the control. Granules consisted of peat-formulated mycelia showed good sporulation on peat and thus have high potential as soil inoculum. However, its effectiveness was dependent on insect



mobility to make contact with the conidia on peat since the peat-formulated mycelia is not an infective agent.

The conidial viability in MCA formulation was observed during storage at room temperature and under refrigeration. Propagules viability in all the formulations of MCA was very dependent on storage condition. Room termperature was detrimental to conidial and mycelial viability. In the refrigerator (4°C), conidia in glycerine and kaolin formulation still showed good viability up to 32 weeks after storage. The viability and conidiation of mycelia in granular formulation were also good when kept under refrigeration up to 32 weeks.

The most virulent *M. anisopliae* (MPs) isolate did not provide adequate protection against the FB on Chinese mustard. However, peat-formulated mycelia as soil inoculum sporulated well and survived for a long time. In the current study, it would be highly probable that *M. anisopliae* could establish well if the plots were to be continuously inoculated with the peat-formulated mycelia, thus affording an additional suppressing agent in an integrated pest management programme.



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

PENAMPILAN TIGA GINERA KULAT PATOGEN SERANGGA SEBAGAI EJEN KAWALAN MIKROBAL YANG POTENSIAL UNTUK MENGAWAL KUMBANG LENTING *PHYLLOTRETA STRIOLATA* F. (COLEOPTERA : CHRYSOMELIDAE)

Oleh

TRI PUJI PRIYATNO

Ogos 2001

Pengerusi : Profesor Madya Dr. Yusof Ibrahim

Fakulti : Pertanian

Kumbang lenting berjalur (KL), *Phyllotreta striolata* F. (Coleoptera : Chrysomelidae), merupakan perosak yang penting bukan sahaja pada tanaman canola dan mustard tetapi juga pada lain-lain tanaman jenis brassicas. Kulat entomopatogen (KE) merupakan satu agen kawalan biologi kepada KL dan semalain mendapat perhatian di seluruh dunia sebagai "mycoinseticide". Potensi tiga genera KE yaitu *Metarhizium anisopliae*, *Beauveria bassiana* dan *Paecilomyces fumosoroseus*, telah diselidiki keberkesanannya di makmal dan di lapang terhadap KL, *Phyllotreta striolata* F.

Hasil survei ke atas KL yang dijangkiti secara asli oleh KE mendapati *M. anisopliae* v. *manus* dan *B. bassiana* adalah KE yang berpotensi terhadap populasi KL yang telah disampel dari kawasan tanaman sayur di Pusat Taman Penyelidikan UPM, Serdang. Walau bagaimanapun kejadian jangkitan adalah sangat rendah. Oleh yang demikian pengenalan pencilan yang virulen pada habitat sementara perlu dilakukan. Ujian kepatogenan 16 pencilan KE terhadap KL dewasa mendapati hanya satu pencilan *M. anisopliae* (MPs) yang menyebabkan kematian melebihi 50%. Ujian kepatogenan empat pencilan telah dijalankan terhadap telur dan larva KL. Didapati dua isolat *M. anisopliae* (MPs dan Cy3), satu *B. bassiana* (Wls) dan satu *P. fumosoroseus* (Pf) menunjukkan kepatogenan yang tinggi terhadap larva, manakala kedua-dua pencilan *M. anisopliae* berupaya menjangkiti telur KL.

Kekebalan KL dewasa terhadap KE adalah disebabkan oleh kewujudan sebatian fungistatik pada integumen. Lima asid lemak berantai lurus (C4, C6, C7, C8 & C9) yang disyaki sebagai sebatian fungistatik berdasarkan analisis kromatografi gas telah terbukti merencatkan percambahan konidium *M. anisopliae*, *B. bassiana* dan *P. fumosoroseus.*

Ujian penentuan penghasilan konidium *M. anisopliae* v. majus dan *P. fumosoroseus* ke atas dua jenis media yaitu media tepung beras dan media sponge-tepung beras menunjukkan media span-tepung beras berpotensi sebagai penghasil konidium yang cekap.

Tiga formulasi agen kawalan mikrobial (AKM), yaitu cecair, debu dan granul, telah sediakan masing-masing menggunakan minyak dan gliserin, kaolin, dan tanah gambut sebagai pembawa. Formulasi konidium dalam minyak, gliserin, dan kaolin didapati sama-sama meningkatkan jangkitan kulat ke atas KL dewasa berbanding kawalan. Granul yang terdiri dari miselium yang dibalut tanah gambut menunjukkan pembentukan konidium yang baik dan oleh itu mempunyai potensi tinggi sebagai



inokulum tanah. Walau bagaimanapun, keberkesanannya bergantung kepada mobiliti serangga untuk bersentuhan dengan konidium pada tanah gambut karena formulasi miselium dalam tanah gambut bukanlah agen jangkitan.

Pemerhatian viabiliti conidium di dalam formulasi AKM telah dilakukan semasa penyimpanan pada suhu bilik dan di dalam peti sejuk. Viabiliti propagul pada semua formulasi AKM didapati bergantung kepada keadaan dalam simpanan. Suhu bilik didapati boleh merosakan viabiliti konidium dan mycelium. Di dalam peti sejuk (4°C), konidium di dalam formulasi gliserin dan kaolin masih menunjukkan viabiliti yang baik sehingga minggu ke 32 tempoh simpanan. Viabiliti dan konidiasi miselium dalam formulasi tanah gambut juga baik apabila disimpan di dalam peti sejuk selama 32 minggu.

Formulasi *M. anisopliae* (MPs) yang paling virulen tidak memberi perlindungan ke atas Chinese mustard terhadap KL di lapangan. Walau bagaimanapun, formulasi miselium dalam tanah gambut berupaya bersporulan dengan baik dan mandiri untuk jangka masa yang lama. Kajian masa kini menunjukkan *M. anisopliae* berkemungkinan tinggi untuk berkembang dengan berkesan jika plot diinokulasikan secara berterusan dengan granul, oleh yang demikian bertindak sebagai penambahan agen kawalan di dalam sesuatu program pengurusan perosak bersepadu.



ACKNOWLEDGEMENTS

Above all, I would like to thank Allah S.W.T., Most Gracious, Most Merciful, for his Compassion and Mercy.

I would like to express my deep sense of gratitude and appreciation to my supervisor, Associate Professor Dr. Yusof Bin Ibrahim, for his advice, guidance, and constructive criticisms in connection with the research and preparation and revisions of this manuscript. I also like to thank my other committee members ; Associate Professor Dr. Ahmad Said Sajap and Associate Professor Dr. Dzolkhifli Omar, thank you for your helpful insight and suggestions in this study.

I gratefully acknowledge Agriculture Research Management Project (ARMP-II) in Department of Agriculture in Indonesia for providing me the financial support which enables me to complete this degree.

A special note of thanks goes to my friends, colleagues, and staff members of Department of Plant Protection, Faculty of Agriculture, UPM.

I would like to express my deepest thanks and appreciation to my father, Miso and mother, Minah, for their encouragement, support and endless prayers during my study in Malaysia. This endeavour would not have been feasible without the sacrifice, patience, understanding and encouragement of my dearest wife Yaya Suciati and my daughter Izdihar Afaf.



TABLE OF CONTENTS

DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL SHEETS	x
DECLARATION FORM	xii
LIST OF TABLES	XV
LIST OF FIGURES	xvii
LIST OF PLATES	xviii

CHAPTER

1.	INTRO	DUCTION	1
2.	LITER	ATURE REVIEW	5
	2.1.	Bionomic of Flea Beetle	5
	2.2.	Biology of Phyllotreta	7
	2.3.	Management of Flea Beetle	11
		2.3.1. Cultural Control	11
		2.3.2. Chemical Control	13
		2.3.3. Biological Control	16
	2.4.	Biology of M. anisopliae, B. bassiana and P. fumosoroseus	18
	2.5.	Development of Mycoinsecticide	23
		2.5.1. Strains Selection	23
		2.5.2. Mass Production	25
		2.5.3. Basic Concepts of Microbial Control Agent Formulation	27
		2.5.4. Oil-Formulated Conidia	29
		2.5.5. Glycerine-Formulated Conidia	35
		2.5.6. Kaolin-Formulated Conidia	36
		2.5.7. Peat-Formulated Mycelia	38
3.	METH	ODOLOGY	41
	3.1.	Experiment Set-Up	41
	3.2.	Survey for Flea Beetle Naturally Infected by Entomopathogenic	
		Fungi	41
	3.3.	Test for Pathogenicity	42
		3.3.1. Insects	42
		3.3.2. Fungus	43
		3.3.3. Bioefficacy Procedure	44
	3.4.	Toxin Analysis of Adults of The Flea Beetle Integument	45
	3.5.	Test for Toxicity of Fatty Acids to Conidial Germination	47
	3.6.	Mass-Production of Conidia	48
		3.6.1. Rice Flour Medium	48



		3.6.2. Sponge-Rice Flour Medium	49
	3.7.	Bioefficacy of Microbial Control Agent Formulation	49
		3.7.1. Conidial Production	51
		3.7.2. Mycelial Production	51
		3.7.3. Formulation	52
		3.7.4. Bioefficacy Procedure for Liquid and Dust	
		Formulation	52
		3.7.5. Bioefficacy Procedure for Peat Formulation	53
	3.8.	Effect of Oil Formulation on Conidial Viability	54
		3.8.1. Conidial Production	54
		3.8.2. Formulation	55
		3.8.3. Viability Determination	55
	3.9.	Effects of Glycerine and Kaolin Formulation on Conidial	
		Viability	55
		3.9.1. Conidial Production	56
		3.9.2. Formulation	56
		3.9.3. Viability Determination	56
	3.10.	Determination of Mycelial Viability in Peat Formulation	57
	3.11.	Utilisation of Microbial Control Agent Formulation in the Field	58
4.	RESU	LTS AND DISCUSSION	60
	4.1.	Occurrence of Entomopathogenic Fungi on Flea Beetle	
		Population	60
	4.2.	Pathogenicity of Entomopathogenic Fungi to Flea Beetle	68
	4.3.	Fungistatic Compound on Adult of the Flea Beetle Integument	77
	4.4.	Potential of Mass-Production Media	82
	4.5.	Effect Formulation on Virulence	89
	4.6.	Conidial Viability in Oil Formulation	96
	4.7.	Conidial Viability in Glycerine Formulation	101
	4.8.	Conidial Viability in Kaolin Formulation	105
	4 .9.	Mycelial Viability in Peat Formulation	106
	4.10.	Studies on Field Efficacy	110
5.	CONC	LUSION	116
RE	FEREN	CES	117
AP	PENDIC	CES	138
BIC	DDATA	OF THE AUTHOR	150



LIST OF TABLES

Table		Page
1.	Morphological and biochemical characters of the genus Beauveria.	22
2.	Isolates of <i>M. anisopliae</i> , <i>B. bassiana</i> , and <i>P. fumosoroseus</i> , their original hosts and countries of origin.	43
3.	Total number of flea beetles collected and the proportions infected entomopathogenic fungi.	61
4.	Average conidial size of M. anisopliae and B. bassiana.	62
5.	Pathogenicity of M. anisopliae, M. flavoviridae, B. bassiana and P. fumosoroseus isolates on adult P. striolata.	69
6.	Effect of <i>M. anisopliae</i> (MPs) on <i>P. striolata</i> at different doses $(LT_{50} \text{ in days})$.	70
7.	Mean percent infection $(\pm SD)$ of first instar larvae of <i>P. striolata</i> upon treatments with varying doses of <i>M. anisopliae</i> , <i>B. bassiana</i> and <i>P. fumosoroseus</i> .	71
8 .	ED_{50} of effect of isolates of <i>M. anisopliae</i> (MPs, Cy3), <i>B. bassiana</i> (W1s) and <i>P. fumosoroseus</i> (Pf) on first instar larvae of <i>P. striolata</i> .	71
9.	LT_{50} of effect of isolates of <i>M. anisopliae</i> (MPs, Cy3), <i>B. bassiana</i> (Wls) and <i>P. fumosoroseus</i> (Pf) on first instar larvae of <i>P. striolata</i> .	72
10.	Mean percent infection of P . striolata eggs against doses used in assays of isolates of M ansiopliae, B . bassiana and P . fumosoroseus.	73
11.	Effect of M. anisopliae (Cy3, MPs) on the eggs of P. striolata.	73
12.	Efficacy of three formulations of P . fumosoroseus, M . anisopliae and B . bassiana to flea beetle.	90
13.	Means percent mortality of flea beetle larvae, C. curvignathus and M. gilvus treated with peat-formulated mycelia of M. anisopliae, M. flavoviridae, B. bassiana and P. fumosoroseus.	95



14.	Efficacy of peat-formulated mycelia of P. fumosoroseus and M. anisopliae on first instar larvae P. striolata.	95
15.	Colony forming unit of kaolin-formulated conidia of P. fumosorosues (PF), B. bassiana (BPs), and M. anisopliae (Cy3).	106
16.	Mean percentage of plant survival, plant damage, and total number of flea beetles on Chinese mustard planted with seeds and seedling after application of three formulation conidia of <i>M. anisopliae</i> and cypermethrin.	111
17.	Mean percentage of plant number, plant survival, and total number of flea beetles on Chinese mustard applied by three formulation conidia of <i>M. anisopliae</i> and cypermethrin.	111
18.	Mean percentage of plant survival, plant damage, total number of flea beetles, and mean percentage of mealworm infected on Chinese mustard after application with peat-formulated mycelia of <i>M. anisopliae</i> .	113
19.	Mean percentage of plant survival, plant damage, total number of flea beetles and mean percentage of mealworm infected on Chinese mustard after application with three conidial formulation of <i>M. anisopliae</i> and cypermethrin.	113



LIST OF FIGURES

Figure		Page
1.	GC tracing of hexane extract of adult flea beetle (P. striolata) integument.	79
2.	Effect of fatty acids on percentage conidial germination of <i>M. anisopliae</i> , <i>B. bassiana</i> and <i>P. fumosoroseus</i> on water agar.	80
3.	Effect of baking soda on conidial production of P . fumosoroseus and M . anisopliae at 0, 0.5, 1.0, 1.5, and 2.0% of yeast extract on rice flour medium.	83
4.	Effect of concentration of rice flour and percent dose of media on conidial production of M anisopliae and P . fumosoroseus using sponge-rice flour medium.	87
5.	Correlation between size of sponge and conidial production of <i>P. fumosoroseus</i> on sponge-rice flour medium.	88
6.	Viability of oil-formulated conidia of <i>P. fumosoroseus</i> with moisture content of 10.0, 6.4, and 3.3% in red palm oil, palm oil, and control (unformulated).	98
7.	Viability of oil-formulated conidia of <i>B. bassiana</i> with moisture content of 8.7, 4.4, and 1.6% in red palm oil, palm oil, and control (unformulated).	99
8.	Viability of oil-formulated conidia of M anisopliae with moisture content of 11.2, 8.3, and 4.8% in red palm oil, palm oil, and control (unformulated).	100
9.	Conidial viability of <i>P. furnosoroseus</i> , <i>B. bassiana</i> and <i>M. anisopliae</i> in 10% and 20% glycerine formulation at room temperature.	103
10.	Conidial viability of <i>P. fumosoroseus</i> , <i>B. bassiana</i> , and <i>M. anisopliae</i> in 10% and 20% glycerine formulation at 4°C.	104
11.	Colony forming unit (CFU) of peat-formulated mycelia of P . fumosoroseus, B . bassiana, and M . anisopliae stored at room temperature and 4°C	108
12.	Percentage sporulation of peat-formulated mycelia sprout of P . fumosoroseus, B . bassiana, and M anisopliae (Cy3 & GmC) stored at room temperature and 4°C	109



LIST OF PLATES

Plate		Page
1.	Adult mating (a) and eggs (b) of P. striolata on Chinese mustard.	9
2.	Larvae of P. striolata on Chinese mustard	10
3.	Formulation of microbial control agents (granules, dust, and liquid) (a) and granules of peat-formulated mycelia (b)	50
4.	A flea beetle (a) infected with M . anisopliae and mealworm $(T.molitor)$ (b) contaminated with M . anisopliae in soil sample from field experiment.	64
5.	A culture of M . anisopliae on PDA (a) and cylindrical conidia borne on chains from cylindrical phialides of conidiophore of M . anisopliae (b)	65
6.	A flea beetle infected with B bassiana (a) and conidia of B . bassiana arranged alternately on short pedicels on the sterigmata (b)	66
7.	Mealworm attacked by entomopathogenic nematode (a) and entomopathogenic nematode attacking mealworm (b)	67
8.	An adult flea beetles infected with <i>M. anisopliae</i> (a) and flea beetle larva infected with <i>M. anisopliae</i> (b)	74
9.	A flea beetle larva infected with P . fumosoroseus (a) and flea beetle larva infected with B . bassiana (b)	75
10.	Early stage of mycosis of <i>M.anisopliae</i> on Flea beetle eggs (a and b) and sporulation of <i>M. anisopliae</i> on flea beetle eggs (b)	76
11.	Sporulation of M . anisopliae (a) and P . fumosoroseus (b) spongerice flour medium.	86
12.	Sporulation of peat-formulated mycelia of M . anisopliae (a) and B . bassiana (b) on sterile sand with 6.6% MC 5 days after inoculation.	93
13.	Sporulation of peat-formulated mycelia of <i>P. fumosoroseus</i> on sterile sand with 6.6% MC 5 days after inoculation.	94
14.	Peat-formulated mycelial sporulation (a) and mealworms infected with <i>M. ansiopliae</i> in the field experiment (b)	115



CHAPTER 1

INTRODUCTION

The flea beetle belonging to the family Chrysomelidae (Coleoptera) has been reported as serious pest of canola and mustard. The two abundant species, *Phyllotreta* cruciferae (Goez) and P. striolata F., have been reported as the most common and prevalent insect pests distributed around the world (Varma, 1961; Elsawaf et al., 1965; Bonnemaison, 1965; Wylie, 1979; Burgess, 1982; Lamb and Turnock, 1982; Elliot, 1992). The most severe damage usually occurs when adult beetles emerge from the soil. They feed voraciously not only the leaves but also the seed pods of canola, mustard, and several other species of cruciferous plants. Seedlings are especially susceptible to flea beetle attack, and extensive flea beetle feeding on seedlings may destroy a crop (Lamb, 1984). They can kill plant directly by severing the hypocotyl or by eating the newly emerged meristem, or they may decrease leaf area by inflicting small, round, shot-hole wounds on cotyledons, leaves and stems (Soroka and Pritchard, 1987)). Their larvae may significantly reduce yield by feeding on the roots (Wylie, 1979). The economic impact of flea beetles on crop production varies with population densities. Yield losses of about 10% are common where flea beetles are abundant even when the crops are protected with insecticides (Bracken and Bucher, 1986).

Limited control of this pest is achieved by using cultural practices and various biological control measures (Wylie, 1984; Hazzard and Ferro, 1991; Burgess, 1982). Majority of the growers preferred applying chemical insecticides by spray, seed treatment, or in-furrow granules treatment against insects pests at seedling stage (Elliot, 1992). However, the use of chemical insecticides on crucifers presents a number of problems that may assume greater importance in the future, such as the development of resistance and environmental contamination that can dangerously affect non-target organisms (Lamb, 1989). As such, dependency on the use of chemical insecticides must be curtailed and some suitable safe alternative control methods must be found.

The adoption of integrated pest management (IPM) systems has placed biological control in a much more important role. Predators, parasitoids, and diseases have become important factors in regulating insect population. To date the effect of biological control agents on flea beetles seems to be limited (Wylie *et al.*, 1981). Lacewing larvae (*Chrysoperla carnae*), big-eyed bugs (*Geocoris bullatus*), the two-lined collops (*Collops vittatus*), the western damsel bug (*Nabis alternatus*) and the northern field cricket(*Gryllus pennsylvanicus*) are a few of the insects known to prey on flea beetles (Gerber and Osgood, 1975). The braconid wasp, *Microctonus vittatae*, parasitizes flea beetle adults; however, its overall effect on flea beetle populations is unknown (Wylie *et al.*, 1981). Unfortunately, flea beetles emerge in large number during relatively short period of time and tend to overwhelm the parasites and predators.

Biological control of flea beetles using fungal pathogens has not much been investigated. Also there are no records, of natural infections of flea beetles by fungal pathogens. Recently, however, Butt *et al.*, (1994) identified isolates of *Metarhizium anisopliae* which were highly pathogenic for the cabbage stem flea beetle (*Psylloides chrysocephala*), however, its infectivity was low against the closely related chrysomelid



Phaedon cochleariae (mustard beetle). Miranpuri and Khachatourians (1994) reported the effectiveness of *Beauveria bassiana* against flea beetle. They reported the LT_{50} values for *B. bassiana* ranged from a low of 1.9 to a high of 16.6 days, and 50-90% of the cadavers showed fungal mummification within seven days, depending upon the isolates tested.

Metarhizium anisopliae and B. bassiana are virulent pathogens of very wide range of soil-inhabiting Coleoptera such as Phyllotreta larvae that feed on the roots of plants, shrubs, and trees, because the soil ecosystems can provide favourable conditions for fungal survival, i.e. protection from solar radiation. So, they have successfully persisted in the hosts environment (Carruther and Haynes, 1986). Fungi have some advantages that make them unique among the entomopathogens. Rather than killing their host by toxigenic action following oral ingestion, they usually invade their host directly through the integument via the germ tube of a germinating spore (Steinhaus, 1963; Tanada and Kaya, 1993). The infection is not only limited to chewing insects, but also occurs in Homoptera and other arthropods with piercing-sucking mouth-parts and all stages of development of insect. In addition, they are able to persist in some soils for long periods and infect soil-inhabiting coleopteran larvae of all stages given them a distinct advantage over most chemical pesticides that do not persist in soil and frequently contaminate the environment. Therefore fungal pathogens have potentials to be developed as a suitable and safe alternative control agents in IPM programme of flea beetles.

The Plant Protection Department of UPM has a collection of isolates of *Metarhizium anisopliae* var. *majus*, *Beauveria bassiana*, and *Paecilomyces fumosoroseus* which are currently being tested on cabbage caterpillars. They are promising agent for biological control and are gaining increasing attention worldwide as mycoinsecticide. Thus, it is of prime importance to examine their usefulness as microbial control agents against the flea beetle (*P. striolata*). The aim of this study are to survey for flea beetles naturally infected with entomopathogenic fungi and investigate the effect of oil, kaolin, glycerine, and peat soil as carrier in formulation of mycoinsecticide on viability and virulence of those fungi. The study also aimed at elucidating the efficacy of these entomopathogenic fungi when applied against the flea beetles in the field.



CHAPTER 2

LITERATURE REVIEW

2.1. Bionomic of Flea Beetle

Flea beetles feed on plants belonging to the mustard family (Cruciferae) grown throughout the world (Varma, 1961; Bonnemaison, 1964; Lamb, 1980). Eight species of flea beetles are known to attack canola, mustard, and rape seed (Jones and Jones, 1977). Of these, only the crucifer flea beetle (*P. cruciferae* Goeze) and the striped flea beetle (*P. striolata* F) are significant pests (Burgess, 1977; Kinoshita *et al.*, 1979).

The economic impact of flea beetles on crop production varies with population densities (Bracken and Bucher, 1986). Yield losses of about 10 percent are common where flea beetles are abundant even when the crop is protected with insecticides. Annual crop losses in North America from flea beetles probably exceed US\$300 million.

Flea beetles feed on the cotyledons, leaves, apical bud tissue, petioles, stems, roots and seed pods of crucifers (Kinoshita *et al.*, 1979; Lamb, 1984). The effect of the feeding activity upon crop development varies with the part of the plant fed on, crop development, growing conditions and the intensity of the attack (Lamb, 1984). Adult beetles feed on the surface of leaves, stems and seed pods and produce small pits (Kinoshita *et al.*, 1979). The tissue underneath the injury eventually withers and dies. On leaves and cotyledons, the damaged tissues break up and fall out producing a shot



hole appearance (Wesdal and Romanow, 1972; Burges, 1977). Heavy infestations may severely damage cotyledons, the first leaves, petioles, and stems (Putman, 1977; Lamb and Turnock, 1982; Bracken and Bucher, 1986). The crop can usually compensate for the destruction of the individual plants, provided large portions of the crop are not totally destroyed (Bracken and bUcher, 1986). Feeding damage is most severe when beetles attack the growing point (meristem) because it limits the ability of the plant to compensate (Putman, 1977; Lamb, 1984).

Light to moderate infestations delay plant development and cause uneven maturity (Lamb, 1984). Delayed maturity may expose the crop to adverse temperatures during flowering or before the plants have matured. Uneven maturity at harvest reduces seed quality or yield. Delaying harvest to allow immature pods to ripen contributes to yield loss when over-ripe seed pods shatter during harvest. Harvesting too early produces a crop with many immature seeds containing high chlorophyll levels, affecting seed quality and yield. Most of this damage can be prevented if canola is protected from flea beetle injury during two to three weeks following emergence.

Flea beetle may also compound crop damage indirectly, by virtue of their ability to transmit diseases. *Phyllotreta* sp. have been reported to transmit turnip yellow and turnip mosaic viruses (Finch and Thompson, 1992), thus reducing the plant stands and affecting the appearance and market ability of the cruciferus crops (Kinoshita *et al.*, 1979). The corn flea beetle (*Chaetocnema pulicaria*) transmits *Erwinia stewartii*, the causal pathogen of stewart's wilt disease in maize (Munkvold *et al.*, 1996), and transmission of broom mosaic virus in barley is by *Phyllotreta vittula*. Additionally,