UNIVERSITI PUTRA MALAYSIA

FORMULATION AND LABORATORY EFFICACY OF ISARIA FUMOSOROSEA AGAINST BAGWORMS (METISA PLANA WALKER AND PTEROMA PENDULA JOANNIS) (LEPIDOPTERA: PSYCHIDAE)

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By

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Relying solely on chemical insecticides for bagworm control in oil palm plantation could lead to many consequences to the ecosystem, environment and human. Thus, developing mycoinsecticide that able to control this pest could be the alternative to reduce chemical insecticides dependence. Entomopathogenic fungi might fulfil their potential with suitable choice of formulation and stable throughout the application and storage period either in biological, chemical, or physical aspect. Wettable powder is the formulation to be developed due to its easy and cheap production procedure. In this study, three fungi isolated from the infected *Pteroma pendula* collected from United Malacca Berhad (UMB) plantations in Pahang and Negeri Sembilan were found to be *Isaria fumosorosea* based on the morphological observations. Those three isolates were identified using molecular identification procedure along with six isolates obtained from Forest Entomology Laboratory, Faculty of Forestry Universiti Putra Malaysia (UPM). Six of the isolates were *I.fumosorosea* where isolate BSB01 isolated of infected bagworms from UMB Plantation in Kemayan, Pahang was the most virulent isolates against *P.pendula*. This isolate was then mass produced and formulated with surfactants and filler. The compatibility of dispersants and wetting agents with this isolate was determined earlier before being used in wettable powder formulation. Screening the surfactants against *I.fumosorosea* BSB01 conidia viability and fungal growth were conducted on the isolate, aerial conidia and submerged propagules. Surfactants found to be qualified were TERSPERSE® 2105, TERSPERSE® 2700, ELTESOL® SC, ELTESOL® SX, sodium naphthalenesulfonate, sodium lignosulfonate, sodium polyacrylates, and SIPERNAT® 22S. Among those qualified surfactants, two of them are wetting agents (ELTESOL® SC and ELTESOL® SX). Concentration of surfactant affects the conidia viability at 10% or higher. A surfactant system consisted of two dispersants and a wetting agent were prepared using pseudoternary phase diagram. Stable regions in each surfactant system were combined to find the mutual stable region. The single point was selected from the mutual stable region with the limit of wetting agents fixed at 15%. The stable region showed satisfying physical stability against kaolin. The best point was found to be at the ratio of 52:33:15 (Dispersant 1:Dispersant 2:Wetting agents). The surfactant ratio was then characterised with the presence of fungal aerial conidia and fungal submerged propagules. Submerged fungal propagules formulations showed better physical stability compared to the aerial conidia formulation. Although all formulations showed a steep decline of active colony counting from 9.0×10^8 to 1.0×10^8 cfu mL^{-1} within the first three days storage, these formulations maintained this viability until 90 days. Biological efficacy of the formulated submerged propagules were determined where all formulations showed no significant difference of LT_{50} values when compared with unformulated against *Metisa plana* and *P.pendula*.

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

FORMULASI DAN KEBERKESANAN MAKMAL *ISARIA FUMOSOROSEA* TERHADAP ULAT BUNGKUS (*METISA PLANA* WALKER DAN *PTEROMA PENDULA* JOANNIS) (LEPIDOPTERA: PSYCHIDAE)

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Kebergantungan kepada racun serangga kimia untuk kawalan ulat bungkus di ladang kelapa sawit boleh membawa kepada keburukan kepada ekosistem, alam sekitar dan juga manusia. Maka, membina mikoinsektisida yang boleh mengawal perosak ini boleh menjadi alternatif untuk mengurangkan kebergantungan kepada racun serangga kimia. Kulat entomopatogenik mampu memenuhi potensi dengan pilihan formulasi yang sesuai dan stabil sepanjang penggunaan dan tempoh penyimpanan samaada dalam aspek biologi, kimia atau fizikal. Serbuk boleh basah adalah formulasi yang akan dibangunkan kerana prosedur penghasilan yang mudah dan murah. Dalam kajian ini. Tiga kulat yang diasingkan dari *Pteroma pendula* terjangkit yang dikumpulkan dari ladang-ladang United Malacca Berhad (UMB) di Pahang dan Negeri Sembilan dijumpai adalah *Isaria fumosorosea* berdasarkan pemerhatian secara morfologi. Tiga isolat tersebut telah dikenal pasti s molekular bersama dengan enam isolat yang diperolehi daripada Makmal Entomologi Hutan, Fakulti Perhutanan, Universiti Putra Malaysia (UPM). Enam isolat adalah *I.fumosorosea* di mana isolat BSB01 diasingkan dari ulat bungkus terjangkit daripada ladang UMB di Kemayan, Pahang adalah isolat paling viral terhadap *P.pendula*. Isolat ini seterusnya dihasil jisim dan diformulasi dangan surfaktant dan pemenuh. Keserasian penyerak dan agen pembasah dengan isolat ini telah ditentukan lebih awal sebelum digunakan di dalam serbuk boleh basah. Penyaringan surfaktant terhadap percambahan konidia dan perkembangan kulat *I.fumosorosea* BSB01 telah dijalankan ke atas konidia arial dan propagul tenggelam. Surfaktant didapati terlibat adalah TERSPERSE® 2105, TERSPERSE® 2700, ELTESOL® SC, ELTESOL® SX, sodium NAPHTHALENESULPHONATE sodium lignosulfonate, sodium polyacrylates, and SIPERNAT® 22S. Di antara surfactant-surfaktant yang layak, dua daripadanya adalah agen pembasah (ELTESOL® SC and ELTESOL® SX).

Kepekatan surfaktant mempengaruhi percambahan konidia pada kepekatan 10% atau lebih. Satu sistem surfaktant terdiri daripada dua penyerak dan satu agen pembasah disediakan menggunakan Rajah Fasa Pseudoternary. Rantau stabil dalam setiap sistem surfaktant digabungkan untuk mencari rantau stabil bersama. Satu titik dipilih daripada rantau stabil bersama dengan agen pembasah dihadkan kepada 15%. Rantau stabil menunjukkan ujian kestabilan fizikal yang memuaskan terhadap kaolin. Titik terbaik dijumpai pada nisbah 52:33:15 (Penyerak 1:Penyerak 2:Agen Pembasah) dan digunakan dalam penyediaan formulasi sebuk boleh basah isolat BSB01. Nisbah surfaktant ini kemudiannya dikarekterkan dengan kehadiran bahan aktif, konidia kulat udara dan propagul kulat tenggelam. Formulasi propagul kulat tenggelam menunjukkan kestabilan fizikal lebih baik berbanding dengan formulasi konidia. Walaupun semua formulasi menunjukkan kejatuhan mendadak dalam kiraan koloni aktif daripada 9.0×10^8 ke 1.0×10^8 cfu mL^{-1} pada tiga hari pertama penyimpanan, formulasi-formulasi ini mengekalkan percambahan ini sehingga 90 hari. Keberkesanan biologi formulasi propagul kulat tenggelam telah ditentukan di mana semua formulasi menunjukkan tiada perbezaaan signifikan dalam LT_{50} dibandingkan dengan propagul tidak diformulasi terhadap *Metisa plana* dan *P.pendula*.

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TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xii
LIST OF FIGURES		xiv
LIST OF ABBREVIATIONS		xv
CHAPTER		
1	**GENERAL INTRODUCTION**	1
2	**LITERATURES REVIEW**	3
	2.1 Entomopathogenic Fungi	3
	2.1.1 *Isaria fumosorosea* Wize	4
	2.2 Mycoinsecticides Formulations	5
	2.3 Wettable Powder Formulations	6
	2.3.1 Fungal Propagules Mass Production	6
	2.3.1.1Conidia Diphasic Fermentation	8
	2.3.1.2 Submerged Propagules Liquid Fermentation	9
	2.3.2 Formulation Components	10
	2.3.3 Dispersants and Polymeric Dispersants	12
	2.3.4 Pseudoternary Phase Diagram	13
	2.4 Formulating Process	13
	2.5 Physical Characteristics	14
	2.5.1 Wettability	14
	2.5.2 Suspensibility	15
	2.5.3 Dispersability	16
	2.5.4 Storage Stability	16
	2.6 Bagworms in Malaysia	17
	2.6.1 *Metisa plana* Walker	18
	2.6.2 *Pteroma pendula* Joannis	19
3	**COLLECTION, ISOLATION, IDENTIFICATION AND SCREENING OF ISARIA FUMOSOROSEA ISOLATES**	20
	3.1 Introduction	20
	3.2 Material and Methods	20
	3.2.1 Fungal collection and isolation	20
	3.2.2 *Pteroma pendula* rearing	22

	3.2.3 Molecular Identification	22
	3.2.3.1 DNA Extraction	22
	3.2.3.2 DNA Amplification	22
	3.2.3.3 DNA Sequencing	23
	3.2.4 Screening for virulence of *I.fumosorosea* isolates	23
	3.2.4.1 Preparation of fungal suspensions	23
	3.2.4.2Bioassay screening for *I. fumosorosea* isolates	24
	3.3 Results	24
	3.3.1 Isolation and identification of *I.fumosorosea*	24
	3.3.2 Screening of *I.fumosorosea* virulence against *P.pendula*	27
	3.4 Discussion	28
	3.5 Conclusion	28
4	**PREPARATION AND CHARECTERIZATION OF WETTABLE POWDER (WP) FORMULATION OF ISARIA FUMOSOROSEA WIZE**	30
	4.1 Introduction	31
	4.2 Material and Methods	31
	4.2.1 Mass Production of Fungal Propagules	31
	4.2.1.1 *I.fumosorosea* Mother Culture	31
	4.2.1.2 Conidia mass production	31
	4.2.1.3 Submerged propagules mass production	31
	4.2.2 Surfactants	32
	4.2.2.1 Screening of Surfactant effect on the viability of fungal conidia	34
	4.2.2.2 Screening of surfactants effect on the fungal growth	34
	4.2.3 Wettable Powder Formulation of Entomopathogenic	35
	4.2.3.1 Constructing Dispersants-Wetting Agent System	35
	4.2.3.2 Mixing Formulation	35
	4.2.4 Physical test of powder formulation	35
	4.2.4.1 Wetting time test for wettability	36
	4.2.4.2 Suspensibility	36
	4.2.4.3 Foam and Sedimentation	37
	4.2.4.4 Dispersibility	38
	4.2.4.5 Calculating physical test evaluation	40
	4.2.5 Storage stability	41
	4.3 Results	41

4.3.1 Mass Production of Fungal Propagules 41
4.3.2 Screening Additives against Entomopathogenic Fungi 43
4.3.2.1 Screening additives against fungal conidia germination 43
4.3.2.2 Screening additives against fungal growth 44
4.3.3 Wettable Powder Formulation 45
4.3.4 Physical Test of Wettable Poweder Formulations 49
4.3.5 Storage Stability 51
4.4 Discussion 51
4.5 Conclusion 56

5 LABORATORY BIOEFFICACY EVALUATION OF THE WETTABLE POWDER FORMULATIONS 57
5.1 Introduction 57
5.2 Material and Methods 57
5.2.1 Bagworm Rearing 57
5.2.2 Preparation of treatment solutions 57
5.2.3 Bioassay 58
5.3 Results 59
5.4 Discussion 62
5.5 Conclusion 63

SUMMARY OF THE STUDY 64
FUTURE RECOMMENDATIONS 65

REFERENCES 66
BIODATA OF STUDENT 83

LIST OF TABLES

Table		Page
2.1	Previous study recommended percentage on every wettable powder formulation components	12
3.1	List of ITS primers and sequences (White *et al*, 1990) used in fungi identification	23
3.2	Cycling conditions for PCR program ITS	23
3.3	Fungal isolates name and molecular result after BLAST	26
4.1	Surfactants used in the experiment	33
4.2	Criteria evaluation of dispersion state. Point I determined through observation	39
4.3	Criteria for evaluation of dispersion state time factor (f) in the evaluation of the dispersion state is that, in the case of practical products, the dispersion state after the lapse of time is more important than that immediately after dispersion.	40
4.4	Component percentage after 12% water loss from submerged propagules based wettable powder formulation	47
4.5	Physical test evaluation measurements for submerged propagules formulations	49
4.6	Physical test evaluation measurements for conidia formulations	50
5.1	Concentration equality in Wettable powder formulation after air drying process	58
5.2	Bioefficacy test of three formulations against *Pteroma pendula*	61
3.2	Bioefficacy test of three formulations against *Metisa plana*	60

LIST OF FIGURES

Figure		Page
3.1	Pink shades colour of the sporulated *I.fumosorosea* BSB01 culture	24
3.2	(a) Globose to ellipsoidal shape of *I.fumosorosea* conidia under microscope (40x magnifications), (b) conidia formed in chain	25
3.3	Bagworm mortality pattern against different *I.fumosorosea* isolates where 25 bagworms represent 50% *P.pendula* mortality	27
4.1	Rice media culture colonized with *I.fumosorosea* BSB01fungus after 14 days incubation	42
4.2	Creamy brown aqueous formed after 4 days incubation on rotary shaker	42
4.3	Germination percentage of *I.fumosorosea* BSB01 conidia against formulation surfactants	43
4.4	Additive concentration effect on *Isaria fumosorosea* germination rate	44
4.5	Surfactant effect on *I.fumosorosea* BSB01 growth on PDA plate via Poison Food Technique after 7 days incubation	45
4.6	Point selected from the mutual stable region for all surfactant system tested	46
4.7	Surfactants systems and regions used to pick the mutual point of stable formulation	48
4.8	Conidia Formulation and Submerged Propagules Formulation physical characterization cumulative score with different surfactant systems	50
4.9	*I.fumosorosea* BSB01 submerged propagules formulations viability within three-month storage period	51
5.1	Experiment setup for bioefficacy test	59
5.2	Mycotized *P.pendula* was observable after 4 days formulation spraying	60

LIST OF ABBREVIATIONS

ANOVA	Universiti Putra Malaysia
BLAST	Basic Local Alignment Search Tool
bp/kbp	Base pair/kilo base pair
cfu	Colony forming unit
cfu mL^{-1}	Colony forming ynit per millilitre
CTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribonucleic acid
g	Gram
IPM	Integrated Pests Management
ITS	Internal Transcribe Spacer
LT_{50}	Lethal time 50%
mm	millimetre
mmHg	Millimetre Hargentum
MT	Method Technical
PCR	Polymerase Chain Reaction
PCV	Pressure control valve
PDA	Potato dextrose agar
ppm	Parts per million
rpm	Revolution per minute
SAS	Statistical Analysis System
sec	Second
TAE	Tris Acetate EDTA
TE	Tris EDTA
UMB	United Malacca Berhad
UPM	Universiti Putra Malaysia
ULV	Ultra-low volume
UV	Ultra Violet
WHO	World Health organization
µL	Microlitre
µg L^{-1}	Microgram per litre
®	Reserved
°C	Degree Celsius
%	Percent
∞	Infinity

CHAPTER 1

GENERAL INTRODUCTION

Environmental concerns remain eternal issue involving chemical pesticides and more eco-friendly approaches have been integrated to prevent economic losses caused by pests and diseases. Biological control approaches have always been highlighted on their ability to play major roles in pest and diseases management system. Recent studies on biological control have shown promising results as an alternative to chemical control. The ability to manipulate living things including microbes as biological agent has highlighted the importance of biological pesticide usage in agriculture industries today. Biopesticides are types of pesticide derived from such natural materials as animals, plants, bacteria, and certain minerals. Integrated Pest Management (IPM) program to maintain pest population below economic threshold could minimize the use of chemical pesticide. Microorganisms could give infection naturally with nature's assistance but need to continuously present to keep the pest population below economic threshold.

Entomopathogenic fungi are one of the biological agents used as biopesticides due to their epizootic nature to insects. The term used for a biopesticides utilizing entomopathogenic fungi to control insect pest is called mycoinsecticides. The mycoinsecticides highlight the concept of mass production entomopathogenic fungus formulating as active ingredient in insecticides and applying as conventional pesticides to the target insect pests. In order to successfully delivering the fungus to the target hosts, physically stable formulation has to be developed without causing much effect to the its viability and growth that might reduce the infection. A stable formulation at optimum dosage would ensure the fungus to spread at optimum dosage throughout the target host's population in the treated area.

Entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauvaria bassiana*, and *Isaria fumosorosea* are ubiquitous microbes known for their epizootic to insect pests and possess desirable traits favourable for their development as microbial control agents (Lacey *et al*., 2015). Wettable powder formulation has been associated with the early introduction of mycoinsecticides. This type of formulation has always available in pesticides market despite several limitations. Fungus could not be delivered to its optimum due to the physical instability of the suspension formed. In order to form stable spraying suspension, formulation should consist of favourable dispersants and wetting agents to ensure good delivery of active ingredient to the target. According to Alves *et al*., (1996), biological agents as active ingredient in biopesticide may suffer degradation in terms of germination and viability due to both biotic (microorganism antagonistic factors) and abiotic (temperature and humidity) factors. This is the main reason on why formulating mycoinsecticides should be done meticulously considering all possible factors affecting the efficacy of the fungal active ingredient.

Wettable powder formulation is the most common and simplest form of formulation in pesticide industry. In recent years, formulators need to review the composition of wettable powder formulation for greater adaptation and more practical application. The introduction of improvised formulations such as suspension concentrates, water dispersible granule, capsule suspension and suspo-emulsion arguably improved the efficacy of pesticides delivery. Despite the emergence of new improvised formulation, this formulation is still getting much attention due to their long storage stability, good miscibility with water and convenient application using conventional spraying equipment (Brar, 2006). Knowles (2005) reported that wettable powder formulation has the advantage on having low production cost, low phytotoxicity, good persistence on porous substrates, and versatile. Developing a wettable powder formulation of mycoinsecticides requires the art of balancing the composition of epizootic fungus and inert ingredient to achieve physically stable formulation and fungal spore's viability during storage period and cause significant mortality to the target hosts.

Surfactant is probably the most important additives in the formulation (Knowles, 2005). Surfactants were more preferred be included inside the formulation. Tank mix surfactants are not recommended as compared to formulation build in surfactant for two reasons, simpler usage and proportion wise advantage where all the ingredients are in the right amount (Jones & Burges, 1998). There are two types of surfactants recommended in wettable powder formulation which are dispersants and wetting agents. These surfactants combination affects formulation stability performance in wettable powder suspension. Every formulation component should be at its optimum amount to make a good suspension and well-perform on infecting target insect pests. The amount of surfactant used in the formulation should be optimised to achieve suspensible formulation as the amount of surfactant could greatly affect the production cost. This type of study could give basic knowledge to the importance of achieving good formulation stability for commercialization. Thus, this study sets several objectives to be accomplished to overcome the all challenges faced during formulating process.

The objectives for this study were as follows:

1.To evaluate the compatibility of ingredients for development of Wettable Powder (WP) formulation of mycoinsecticides
2.To prepare and characterize the Wettable Powder (WP) formulation of entomopathogenic fungus
3.To evaluate the biological performance of the Wettable Powder formulations against bagworms

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