



**UNIVERSITI PUTRA MALAYSIA**

***WETTABLE POWDER FORMULATIONS OF *Metarhizium anisopliae*  
(METCHNIKOFF) SOROKIN AND THEIR EFFECTIVENESS AGAINST  
*Bemisia tabaci* GENNADIUS AND *Aphis gossypii* GLOVER***

**NORHELINA LATIFF**

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**By**

**NORHELINA LATIFF**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Philosophy**

**May 2019**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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**May 2019**

**Chair : Professor Dzolkhifli Omar, PhD**  
**Faculty : Agriculture**

Sap sucking insect *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and *Aphis gossypii* Glover (Hemiptera: Aphididae) have become important pests infesting economic important crops and worsened by their resistance to a multiple classes of chemical pesticide and among them are organophosphates, pyrethroids and neonicotinoid. These have prompted potential of utilizing biological control as an alternative to chemical insecticide. Of these, Entomopathogenic fungi (EPF) are the most suitable due to their ability infecting through the cuticle and displaying a great prospective for controlling sap-sucking pest. One of these EPF is *Metarhizium anisopliae*. There is little information on the effect of *M. anisopliae* on the sap-sucking insect in Malaysia. The objectives of this study were to screen the most virulent isolate of *M. anisopliae* against *B. tabaci* and *A. gossypii*, prepare the wettable powder (WP) and evaluate the effect of UV and storage on the formulations, and assess the WP formulations effectiveness in the field. Five isolates of *M. anisopliae* (PR1, GT2, TFFH3, GJ4 and HSAH5) were highly pathogenic against first instar of *B. tabaci* and *A. gossypii* and the two most superior were PR1 and GJ4. The *B. tabaci* was invulnerable on brinjal than chilli and vice versa on *A. gossypii*. The LC<sub>50</sub>'s of PR1 isolate against *B. tabaci* and *A. gossypii* were lower than GJ4 isolate on both brinjal and chilli host plant. Both PR1 and GJ4 isolates were highly infective to all developmental stages of *B. tabaci* and *A. gossypii*; with the first instar was prone to fungal disease than other life stage decreasing through the next stages. In the preparation of the wettable powder (WP) formulation, the compatibility of inert ingredients on conidial viability was never lower than 80% and most was between 90–96% in all tests. The ingredients of the WP formulation comprised of active ingredient of fungal conidia and inert ingredients. The inert ingredients consisted of diluent (glycerin, distilled water), three components of surfactant system (combination of three of the following: sodium naphthalene sulphonates, sodium lignosulfonate, kaolin, silica powder, skimmed milk powder), UV protectant (lignin, skimmed milk powder, molasses) and additive (sodium glutamate, sodium alginate). The 22 prepared WPs from both isolates gave germination ranging from 65.0 to 93.6%. Wettable powder-based kaolin and WP-based skimmed

milk powder enhanced the UV tolerance of conidia and gave significantly better germination than WP based silica powder. The WPs were able to maintain conidia viability up to four months storage at temperature 4°C, 16°C and 25°C. Nevertheless, at 30°C the viability was only maintain for a maximum of four weeks. In the small-scale field trial, selected WP of PR1 and GJ4 based kaolin were more effective compared to unformulated isolates conidia and untreated control. These formulations also gave significantly better yield compared to unformulated isolates and untreated control. *M. anisopliae* isolates PR1 and GJ4 effectively control sap sucking insect both in laboratory and in the field against all stages of *B. tabaci* and *A. gossypii* and able to improve the yield.



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

**FORMULA SERBUK BASAH *Metarhizium anisopliae* (METCHNIKOFF)  
SOROKIN DAN KEBERKESANANNYA TERHADAP *Bemisia tabaci*  
GENNADIUS DAN *Aphis gossypii* GLOVER**

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Serangga yang menghisap sap iaitu *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) dan *Aphis gossypii* Glover (Hemiptera: Aphididae) telah menyebabkan masalah ekonomi kepada tanaman dan kebal kepada beberapa kelas racun serangga perosak antaranya adalah organophosphates, pyrethroids and neonicotinoid. Hal ini memberi peluang kepada potensi penggunaan kawalan biologi sebagai alternatif kepada racun serangga kimia. Fungi penyebab penyakit serangga (EPF) adalah yang paling sesuai berbanding kawalan biologi lain kerana kebolehannya untuk menjangkiti melalui lapisan kulit luar dan menunjukkan prospek untuk kawalan serangga penghisap sap. Salah satu daripada EPF ini adalah *Metarhizium anisopliae*. Maklumat berkenaan kesan penggunaan *M. anisopliae* terhadap serangga penghisap sap di Malaysia adalah sangat sedikit. Objektif kajian ini adalah untuk memilih isolat *M. anisopliae* yang paling beracun terhadap *B. tabaci* dan *A. gossypii*, persediaan formula serbuk basah (WP) dan menilai kesan UV dan penyimpanan terhadap formula, dan mengkaji keberkesanan formula WP di ladang. Lima isolat *M. anisopliae* (PR1, GT2, TFFH3, GJ4 dan HSAH5) mempunyai kadar patogenik yang tinggi terhadap instar pertama *B. tabaci* dan *A. gossypii* dan antaranya dua isolat paling tinggi kadar patogenik adalah PR1 dan GJ4. *B. tabaci* yang membiak pada tanaman terung lebih kebal berbanding cili dan sebaliknya pula kepada *A. gossypii*. Isolat PR1 mempunyai LC<sub>50</sub> yang lebih rendah berbanding GJ4 apabila dikaji terhadap *B. tabaci* dan *A. gossypii* ke atas tanaman terung dan cili. Kedua-dua isolat PR1 dan GJ4 mempunyai kadar keberkesanan yang tinggi terhadap semua peringkat pembesaran *B. tabaci* dan *A. gossypii*; dengan instar pertama lebih mudah diserang penyakit kulat berbanding peringkat hidup lain yang semakin menurun apabila ke peringkat seterusnya. Semasa penyediaan formula serbuk basah, keserasian bahan-bahan lengai ke atas keupayaan hidup konidia didapati tidak pernah berada di bawah 80% dan dalam semua ujian kebanyakannya adalah di antara 90–96%. Bahan-bahan untuk formula WP termasuklah bahan aktif iaitu kulat konidia dan bahan lengai. Bahan-bahan lengai termasuklah pencair (glycerin, air suling), tiga komponen sistem surfactant sodium naphthalene sulphonates, sodium lignosulfonate,

kaolin, serbuk silica, serbuk susu skim), pelindung UV (lignin, serbuk susu skim, molasses) dan pengawet (sodium glutamate, sodium alginate). 22 WP daripada kedua isolate menghasilkan kadar germinasi daripada 64.95 kepada 93.62%. WP berasaskan kaolin dan WP serbuk susu skim menghasilkan germinasi yang ketara lebih baik untuk meningkatkan toleransi konidia terhadap UV berbanding dengan WP berasaskan serbuk silica. Kesemua WP berupaya mengekalkan keupayaan hidup konidia dalam empat bulan penyimpanan pada suhu 4°C, 16°C and 25°C. Manakala, pada suhu 30°C keupayaan konidia untuk hidup hanya berjaya dikekalkan pada kadar maksimum empat minggu sahaja. Kajian di ladang berskala kecil mendapati WP terpilih PR1 dan GJ4 adalah lebih berkesan berbanding dengan konidia isolat yang tidak diformulasikan dan kawalan yang tidak dirawat. Formula ini juga memberikan kesan hasil yang lebih baik berbanding dengan isolat tidak diformulasikan dan kawalan yang tidak dirawat. Isolat PR1 dan GJ4 *M. anisopliae* berkesan untuk mengawal serangga penghisap sap di makmal dan di ladang kepada semua peringkat *B. tabaci* dan *A. gossypii* dan memperbaiki hasil di ladang.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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## TABLE OF CONTENTS

		Page
<b>ABSTRACT</b>		i
<b>ABSTRAK</b>		iii
<b>ACKNOWLEDGEMENTS</b>		v
<b>APPROVAL</b>		vi
<b>DECLARATION</b>		viii
<b>LIST OF TABLES</b>		xiii
<b>LIST OF FIGURES</b>		xv
<b>LIST OF ABBREVIATIONS</b>		xviii
<b>CHAPTER</b>		
<b>1</b>	<b>INTRODUCTION</b>	1
	1.1 Introduction	1
	1.2 Objectives	2
<b>2</b>	<b>LITERATURE REVIEW</b>	3
	2.1 Biological Control	3
	2.2 Entomopathogenic Fungi (EPF)	4
	2.2.1 Mass Production	5
	2.2.2 Effect on UV, Humidity and Temperature	6
	2.3 <i>Metarhizium anisopliae</i>	7
	2.3.1 Background of <i>M. anisopliae</i> as Mycoinsecticides	8
	2.3.2 Application of <i>M. anisopliae</i> as Mycoinsecticide	8
	2.3.3 Formulation of <i>M. anisopliae</i>	9
	2.4 Overview of <i>Bemisia tabaci</i> (Gennadius)	10
	2.4.1 Distribution and Background	11
	2.4.2 Morphology of <i>Bemisia tabaci</i>	11
	2.4.3 Biology and Life Cycle of <i>Bemisia tabaci</i>	12
	2.4.4 EPF against <i>Bemisia tabaci</i>	13
	2.4.5 Damage and Economic Important of <i>Bemisia     tabaci</i>	13
	2.5 Overview <i>Aphis gossypii</i>	15
	2.5.1 Distribution and Background	15
	2.5.2 Morphology of <i>Aphis gossypii</i>	16
	2.5.3 Biology and Life Cycle of <i>Aphis gossypii</i>	16
	2.5.4 EPF against <i>Aphis gossypii</i>	17
	2.5.5 Damage and Economic Important of <i>Aphis     gossypii</i>	17
<b>3</b>	<b>SCREENING AND SELECTION OF <i>Metarhizium anisopliae</i> AGAINST <i>Bemisia tabaci</i> AND <i>Aphis gossypii</i></b>	20
	3.1 Introduction	20
	3.2 Materials and Methods	22

	3.2.1	Location	22
	3.2.2	Plant	22
	3.2.3	Insect	22
	3.2.4	Fungi	22
	3.2.5	Virulent evaluation of isolates against <i>B. tabaci</i> and <i>A. gossypii</i>	23
3.3		Results	25
	3.3.1	Screening toxicity of <i>B. tabaci</i> on <i>S. melongena</i>	25
	3.3.2	Screening toxicity of <i>B. tabaci</i> on <i>C. annuum</i>	27
	3.3.3	Screening toxicity of <i>A. gossypii</i> on <i>S. melongena</i>	29
	3.3.4	Screening toxicity of <i>A. gossypii</i> on <i>C. annuum</i>	31
	3.3.5	Mortality of <i>B. tabaci</i> and <i>A. gossypii</i> on two host plant	33
	3.3.6	Concentration Response Assay	34
	3.3.7	Influence of Larval Instars Life Stages and Conidia Concentration on Larval Mortality	40
3.4		Discussion	43
3.5		Conclusion	48
<b>4</b>		<b>FUNGAL PRODUCTION AND PREPARATION OF WETTABLE POWDER FORMULATIONS OF THE <i>Metarhizium anisopliae</i></b>	
	4.1	Introduction	49
	4.2	Material and Methods	50
	4.2.1	Fungal Production	50
	4.2.2	Preparation of Wettable Powder of <i>M. anisopliae</i>	53
	4.2.3	Performance of Newly Constructed WPs	55
4.3		Results	57
	4.3.1	Fungal Production	57
	4.3.2	Preparation of Wettable Powder of <i>M. anisopliae</i>	59
	4.3.3	Performance of Newly Constructed WPs	64
4.4		Discussion	67
4.5		Conclusion	73
<b>5</b>		<b>EVALUATION OF WETTABLE POWDER OF <i>Metarhizium</i> <i>anisopliae</i> SHELF-LIFE AND STORAGE STABILITY</b>	
	5.1	Introduction	74
	5.2	Materials and Methods	74
	5.2.1	Source of Conidia	75
	5.2.2	Storage Stability	75
	5.2.3	Viability of Conidia after Storage	75
5.3		Result	76
	5.3.1	Storage of WPs-PR1	76
	5.3.2	Storage of WPs-GJ4	79
5.4		Discussion	82
5.5		Conclusion	84

<b>6</b>	<b>FIELD EVALUATION OF WETTABLE POWDER <i>Metarhizium anisopliae</i> FOR CONTROL OF <i>Bemisia tabaci</i> AND <i>Aphis gossypii</i></b>	85
6.1	Introduction	85
6.2	Materials and Methods	86
6.2.1	Site Study	86
6.2.2	Sources of plant	86
6.2.3	Source of insect	86
6.2.4	Source of Conidia	87
6.2.5	Field Plot and Treatment	87
6.3	Result	89
6.3.1	WPs Treatments on <i>B. tabaci</i>	89
6.3.2	WPs Treatment on <i>A. gossypii</i>	91
6.4	Discussion	92
6.4.1	<i>Bemisia tabaci</i>	94
6.4.2	<i>Aphis gossypii</i>	95
6.5	Conclusion	96
<b>7</b>	<b>SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION</b>	97
7.1	Summary	97
7.2	General Conclusion	98
7.3	Recommendation for Future Research	99
	<b>REFERENCES</b>	100
	<b>APPENDICES</b>	127
	<b>BIODATA OF STUDENT</b>	148
	<b>LIST OF PUBLICATIONS</b>	149

## LIST OF TABLES

Table		Page
2.1	Hypocreales fungi (Wraight et al., 2007)	5
2.2	Commercially <i>Metarhizium</i> developed for the biological control of pests (adapted from Wraight et al., 2001)	9
2.3	Classification of <i>Bemisia tabaci</i> (Gennadius) (Gillott, 2005)	10
2.4	Classification of <i>Aphis gossypii</i> (Glover) (Blackman & Eastop, 2007)	15
3.1	<i>Metarhizium anisopliae</i> isolates from Faculty of Forestry, Universiti Putra Malaysia stock culture	23
3.2	Analysis of probit mortality and log-concentration of bioassay with the most effective <i>M. anisopliae</i> against first instar nymphs of <i>B. tabaci</i> and <i>A. gossypii</i>	39
4.1	The combination components of active ingredients and inert ingredient (Feng & Wu, 2011)	55
4.2	Test of conidia interaction with inert ingredients	59
4.3	Ingredients of Wettable Powder formulation	61
4.4	Surfactant system with filler (Kaolin or Silica Powder or Skimmed Milk Powder) and dispersant (Sodium lignosulphonate)	63
4.5	Surfactant system with filler (Kaolin or Silica Powder or Skimmed Milk Powder) and dispersant (Gum arabica)	64
4.6	Effect of WPs on conidia <i>M. anisopliae</i>	65
A1	Combination of active ingredient and inert ingredients with kaolin	127
A2	Combination of active ingredient and inert ingredients with silica	132
A3	Combination of active ingredient and inert ingredients with skimmed milk powder	138
A4	Summary of ANOVA for screening of <i>B. tabaci</i> on solanaceae	143
A5	Summary of ANOVA for screening of <i>A. gossypii</i> on solanaceae	143
A6	Summary of ANOVA for effect of selected <i>M. anisopliae</i> on life stage of <i>B. tabaci</i> on brinjal	143
A7	Summary of ANOVA for Effect of selected <i>M. anisopliae</i> on life stage of <i>B. tabaci</i> on chilli	143
A8	Summary of ANOVA for Effect of selected <i>M. anisopliae</i> on life stage of <i>A. gossypii</i> on brinjal	143
A9	Summary of ANOVA for Effect of selected <i>M. anisopliae</i> on life stage of <i>A. gossypii</i> on chilli	144
A10	Summary of ANOVA for Effect of Drying Biomass	144
A11	Summary of ANOVA for Effect of Rice Medium	144
A12	Summary of ANOVA for Effect of Inert Ingredient on Conidia GJ4	144
A13	Summary of ANOVA for Effect of Inert Ingredient on Conidia PR1	144
A14	Summary of ANOVA for Effect of WPs on Germination	144
A15	Summary of ANOVA for Effect of UVB radiation on WPs-PR1	145

A16	Summary of ANOVA for Effect of UVB radiation on WPs-GJ4	145
A17	Summary of ANOVA for Storage of WPs-PR1 at 4°C	145
A18	Summary of ANOVA for Storage of WPs-PR1 at 16°C	145
A19	Summary of ANOVA for Storage of WPs-PR1 at 25°C	145
A20	Summary of ANOVA for Storage of WPs-PR1 at 30°C	145
A21	Summary of ANOVA for Storage of WPs-GJ4 at 4°C	146
A22	Summary of ANOVA for Storage of WPs-GJ4 at 16°C	146
A23	Summary of ANOVA for Storage of WPs-GJ4 at 25°C	146
A24	Summary of ANOVA for Storage of WPs-GJ4 at 30°C	146
A25	Summary of ANOVA for Field WPs <i>B. tabaci</i>	146
A26	Summary of ANOVA for Field WPs <i>B. tabaci</i> (Brinjal Yield)	146
A27	Summary of ANOVA for Field WPs <i>A. gossypii</i>	147
A28	Summary of ANOVA for Field WPs <i>A. gossypii</i> (Chilli Yield)	147





## LIST OF FIGURES

Figure		Page
2.1	Nymph of <i>B. tabaci</i> suck phloem sap from minor veins underside of the foliar as illustrated diagrammatically by (Cohen et al., 1998)	14
2.2	Aphid feeding on plant adapted from (Gullan & Cranston, 2005)	18
3.1	Mean cumulative mortality of <i>B. tabaci</i> on <i>S. melongena</i> for seven DAT	25
3.2	Percentage Mortality ( $\pm$ SEM) of first instar nymphs of <i>B. tabaci</i> on <i>S. melongena</i> four DAT (blue bars and lowercase) and of seven DAT (red bars and capital) by conidial suspensions of several <i>M. anisopliae</i> isolates ( $1 \times 10^7$ conidia/ml). For each <i>B. tabaci</i> nymph, bars with the same letter are not significantly different	26
3.3	Mean cumulative mortality of <i>B. tabaci</i> on chilli for seven DAT	27
3.4	Percentage Mortality ( $\pm$ SEM) of first instar nymphs of <i>B. tabaci</i> on <i>C. annum</i> four DAT (blue bars and lowercase) and of seven DAT (red bars and capital) by conidial suspensions of several <i>M. anisopliae</i> isolates ( $1 \times 10^7$ conidia/ml). For each <i>B. tabaci</i> nymph, bars with the same letter are not significantly different	28
3.5	Mean cumulative mortality of <i>A. gossypii</i> on <i>S. melongena</i> seven DAT	29
3.6	Percentage Mortality ( $\pm$ SEM) of first instar nymphs of <i>A. gossypii</i> on <i>S. melongena</i> four DAT (blue bars and lowercase) and of seven DAT (red bars and capital) by conidial suspensions of several <i>M. anisopliae</i> isolates ( $1 \times 10^7$ conidia/ml). For each <i>A. gossypii</i> nymph, bars with the same letter are not significantly different	30
3.7	Mean cumulative mortality of <i>A. gossypii</i> on <i>C. annum</i> seven DAT	31
3.8	Percentage Mortality ( $\pm$ SEM) of first instar nymphs of <i>A. gossypii</i> on <i>C. annum</i> four DAT (blue bars and lowercase) and of seven DAT (red bars and capital) by conidial suspensions of several <i>M. anisopliae</i> isolates ( $1 \times 10^7$ conidia/ml). For each <i>A. gossypii</i> nymph, bars with the same letter are not significantly different	32
3.9	Mortality rate ( $\pm$ SEM) of first instar nymphs of <i>B. tabaci</i> on <i>S. melongena</i> seven DAT (blue bars) and on <i>C. annum</i> seven DAT (red bars) by conidial suspensions of several <i>M. anisopliae</i> isolates ( $1 \times 10^7$ conidia/ml). For each <i>B. tabaci</i> nymph, bars with the same letter are not significantly different	33
3.10	Percentage Mortality ( $\pm$ SEM) of first instar nymphs of <i>A. gossypii</i> on <i>S. melongena</i> seven DAT (blue bars) and on <i>C. annum</i> seven DAT (red bars) by conidial suspensions of several <i>M. anisopliae</i> isolates ( $1 \times 10^7$ conidia/ml). For each <i>A. gossypii</i> nymph, bars with the same letter are not significantly different	34
3.11	PR1 Mean cumulative mortality of <i>B. tabaci</i> on <i>S. melongena</i>	35
3.12	PR1 Mean cumulative mortality of <i>B. tabaci</i> on <i>C. annum</i>	35
3.13	GJ4 Mean cumulative mortality of <i>B. tabaci</i> on <i>S. melongena</i>	36
3.14	GJ4 Mean cumulative mortality of <i>B. tabaci</i> on <i>C. annum</i>	36
3.15	PR1 Mean cumulative mortality of <i>A. gossypii</i> on <i>S. melongena</i>	37
3.16	PR1 Mean cumulative mortality of <i>A. gossypii</i> on <i>C. annum</i>	37
3.17	GJ4 Mean cumulative mortality of <i>A. gossypii</i> on <i>S. melongena</i>	38
3.18	GJ4 Mean cumulative mortality of <i>A. gossypii</i> on <i>C. annum</i>	38

3.19	Percentage Mortality ( $\pm$ SEM) of life stages of <i>B. tabaci</i> on <i>S. melongena</i> with PR1 (blue bars) and of GJ4 (red bars) by conidial suspensions of <i>M. anisopliae</i> isolates. For each <i>B. tabaci</i> nymph, bars with the same letter are not significantly different	40
3.20	Percentage Mortality ( $\pm$ SEM) of life stages of <i>B. tabaci</i> on <i>C. annuum</i> with PR1 (blue bars) and of GJ4 (red bars) by conidial suspensions of <i>M. anisopliae</i> isolates. For each <i>B. tabaci</i> nymph, bars with the same letter are not significantly different	41
3.21	Percentage Mortality ( $\pm$ SEM) of life stages of <i>A. gossypii</i> on <i>S. melongena</i> with PR1 (blue bars) and of GJ4 (red bars) by conidial suspensions of <i>M. anisopliae</i> isolates. For each <i>A. gossypii</i> nymph, bars with the same letter are not significantly different	42
3.22	Percentage Mortality ( $\pm$ SEM) of life stages of <i>A. gossypii</i> on <i>C. annuum</i> with PR1 (blue bars) and of GJ4 (red bars) by conidial suspensions of <i>M. anisopliae</i> isolates. For each <i>A. gossypii</i> nymph, bars with the same letter are not significantly different	43
4.1	Mean fungal mass in gram ( $\pm$ SEM) for the four drying methods of PR1 (blue bars) and GJ4 (red bars) with D <sub>0</sub> no drying, D <sub>1</sub> air-drying, D <sub>2</sub> two times and D <sub>3</sub> three times drying. Means sharing the same letter do not differ significantly at the 95% confidence level	57
4.2	Mean fungal mass in gram ( $\pm$ SEM) of <i>M. anisopliae</i> with PR1 (blue bars) and GJ4 (red bars) for the four rice grain medium with R <sub>0</sub> 0%, R <sub>1</sub> 100%, R <sub>2</sub> 50% and R <sub>3</sub> 30% broken rice grain. Means sharing the same letter do not differ significantly at the 95% confidence level	58
4.3	Three types of surfactant system (A, B and C) for construction of WP formula	63
4.4	Germination rate ( $\pm$ SEM) for the WPs-PR1 of <i>M. anisopliae</i> exposure to UV treatments with 10 minute (blue bars), 20 minutes (red bars), 30 minutes (green bars) and 60 minutes (purple bars). Means sharing the same letter do not differ significantly at the 95% confidence level	66
4.5	Germination rate ( $\pm$ SEM) for the WPs-GJ4 of <i>M. anisopliae</i> exposure to UV treatments with 10 minute (blue bars), 20 minutes (red bars), 30 minutes (green bars) and 60 minutes (purple bars). Means sharing the same letter do not differ significantly at the 95% confidence level	67
5.1	Germination rate ( $\pm$ SEM) for the WPs-PR1 of <i>M. anisopliae</i> at 4°C time of storage with production day (blue bars), two weeks (red bars), four weeks (green bars), eight weeks (purple bars) and 16 weeks (cyan bars). Means sharing the same letter do not differ significantly at the 95% confidence level	77
5.2	Germination rate ( $\pm$ SEM) for the WPs-PR1 of <i>M. anisopliae</i> at 16°C time of storage with production day (blue bars), two weeks (red bars), four weeks (green bars), eight weeks (purple bars) and 16 weeks (cyan bars). Means sharing the same letter do not differ significantly at the 95% confidence level	77
5.3	Germination rate ( $\pm$ SEM) for the WPs-PR1 of <i>M. anisopliae</i> at 25°C time of storage with production day (blue bars), two weeks (red bars), four weeks (green bars), eight weeks (purple bars) and 16 weeks (cyan bars). Means sharing the same letter do not differ significantly at the 95% confidence level	78
5.4	Germination rate ( $\pm$ SEM) for the WPs-PR1 of <i>M. anisopliae</i> at 30°C time of storage with production day (blue bars), two weeks (red bars),	78

	four weeks (green bars), eight weeks (purple bars) and 16 weeks (cyan bars). Means sharing the same letter do not differ significantly at the 95% confidence level	
5.5	Germination rate ( $\pm$ SEM) for the WPs-GJ4 of <i>M. anisopliae</i> at 4°C time of storage with production day (blue bars), two weeks (red bars), four weeks (green bars), eight weeks (purple bars) and 16 weeks (cyan bars). Means sharing the same letter do not differ significantly at the 95% confidence level	80
5.6	Germination rate ( $\pm$ SEM) for the WPs-GJ4 of <i>M. anisopliae</i> at 16°C time of storage with production day (blue bars), two weeks (red bars), four weeks (green bars), eight weeks (purple bars) and 16 weeks (cyan bars). Means sharing the same letter do not differ significantly at the 95% confidence level	80
5.7	Germination rate ( $\pm$ SEM) for the WPs-GJ4 of <i>M. anisopliae</i> at 25°C time of storage with production day (blue bars), two weeks (red bars), four weeks (green bars), eight weeks (purple bars) and 16 weeks (cyan bars). Means sharing the same letter do not differ significantly at the 95% confidence level	81
5.8	Germination rate ( $\pm$ SEM) for the WPs-GJ4 of <i>M. anisopliae</i> at 30°C time of storage with production day (blue bars), two weeks (red bars), four weeks (green bars), eight weeks (purple bars) and 16 weeks (cyan bars). Means sharing the same letter do not differ significantly at the 95% confidence level	81
6.1	Mortality rate ( $\pm$ SEM) for the treatments of WPs of <i>M. anisopliae</i> against <i>B. tabaci</i> on <i>S. melongena</i> . Means sharing the same letter do not differ significantly at the 95% confidence level	89
6.2	Yield/kg ( $\pm$ SEM) for the treatments of WPs of <i>M. anisopliae</i> against <i>B. tabaci</i> on <i>S. melongena</i> . Means sharing the same letter do not differ significantly at the 95% confidence level	90
6.3	Mortality rate ( $\pm$ SEM) for the treatments of WPs of <i>M. anisopliae</i> against <i>A. gossypii</i> on <i>C. annum</i> . Means sharing the same letter do not differ significantly at the 95% confidence level	91
6.4	Yield/kg ( $\pm$ SEM) for the treatments of WPs of <i>M. anisopliae</i> against <i>A. gossypii</i> on <i>C. annum</i> . Means sharing the same letter do not differ significantly at the 95% confidence level	92

## LIST OF ABBREVIATIONS

±SEM	±Standard Error Mean
µl	Microliter
µm	Micrometre
DAT	Days after treatment
EPF	Entomopathogenic fungi
L : D	Light : Dark
LC <sub>50</sub>	50% Lethal Concentration
LSF	Submerged fermentation/ Diphasic fermentation
LT <sub>50</sub>	50% Lethal Time
ml	Millilitre
°C	Degrees Celsius
RH	Relative Humidity
SDA	Sabouraud Dextrose Agar
SDAY	Sabouraud Dextrose Agar with Yeast
SSF	Solid substrate fermentation
UPM	University Putra Malaysia
UV	Ultraviolet
UVB	Ultraviolet-B
WP	Wettable Powder

# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction

Solanaceae such as brinjal, *Solanum melongena* L. and chilli, *Capsicum annum* L. are important crops that are cultivated in tropical and subtropical region and highly value in Southeast Asia (Aied *et al.*, 2017; Sujayanand *et al.* 2015; Rahman *et al.* 2010). *Solanum melongena* has become increasing appreciate and demand due to its considerable dietary value of high moisture content and low calorie with highly content of antioxidants and proteins (Kandoliya *et al.*, 2015; Adamczewska-Sowinska and Krygier, 2013; Lo Scalzo, *et al.*, 2010). However, *S. melongena* facing serious exposed to the attacked of more than a dozen insect-pest species even with much preventive care due to long cropping season (Khandekar *et al.* 1982; Dhandapani *et al.*, 2003). *Solanum melongena* is one of the most common fruit vegetables sell in Malaysia's market yet the outbreak of *Bemisia tabaci* commonly known as whitefly causing the farmer losing their interest in growing them and thus cause limited supply of *S. melongena* in local market (Mohd Rasdi *et al.*, 2009). Population of *B. tabaci* on *S. melongena* reaches the highest peak due to the food availability and Malaysia's tropical climate that favourable to their development all year round (Mohd Rasdi *et al.*, 2009).

*Capsicum annum* is an important crop due to their economic, nutritional and medicinal value contains an appreciable amount of essential micro and macronutrients and healthy ingredients to promote health (Emmanuel-Ikpeme *et al.*, 2014; Olatunji and Afolayan, 2018). In Malaysia, *Aphis gossypii* is a major pest of *C. annum* and heavily infested by colonizing the crop for direct feeding and transmits virus diseases (Rahman *et al.*, 2010). Although, chemical pesticides proved to control pests of brinjal and chili however the used of chemical pesticides regularly result in development of resistant strains of *B. tabaci* and *A. gossypii* (Basit *et al.*, 2013; Yuan *et al.*, 2012; Basit *et al.*, 2011; Rahman *et al.*, 2010; Palumbo *et al.*, 2001; Morales, 2001).

In addition to the resistant, there is increasing awareness from people for food's safety due to the chemical residual that may enters food they consume which may pose risk to their health (Butt *et al.* 2001; Kaur and Kaur, 2007). Most of agriculture nowadays still depends heavily on the chemical pesticide that leads to deterioration of environment, food contamination and increasing resistance to insect pests (Zafar *et al.*, 2016; Kranthi *et al.* 2001; Faria and Wraight, 2001). The utilization of naturally occurring organisms such as bacteria, entomopathogenic fungi (EPF) and viruses in order to control crop pests, weeds and diseases application as an alternative for pest management in agriculture also encouraging more research and offer environmentally friendly alternative to chemical pesticides (Hidalgo *et al.*, 1998; Faria and Wraight, 2001). Given these concerns, using biological control approach as alternative has been

considered economically and environmentally worthwhile (Wang *et al.*, 2002; Kaur and Kaur, 2007).

This study was justified in order to provide an alternative and environmental friendly strategy for control sap sucking insect particularly pest that infesting on vegetable crops *S. melongena* and *C. annuum* in Malaysia. In addition to the increase of insect resistance due to the use of chemical, the actual problem for the local farmer is due to the highly cost of cultivating the crops as common chemical pesticides used as coping strategy to manage the pest infestation are expensive. Considerable amounts of literature have published on biological control using fungi as active ingredient in their formulation. However, research has consistently shown that these study showing varied result depends on the types of microbial control used including the geographically sources which may differ in temperature and origin. However, much uncertainty still exists about the varied result of the EPF on pest insects and potential of applying EPF against sap sucking insect due to limited sources of information available.

This study seeks to remedy these problems by analyzing the literature of EPF *M. anisopliae* against selected sap sucking insect. So far, there has been little discussion about using *M. anisopliae* against sap sucking pest such as *B. tabaci* and *A. gossypii*. Although there are studies of using *M. anisopliae* in laboratory, however limited information is available on comparative study of formulated and unformulated conidia of EPF for the control of *B. tabaci* and *A. gossypii* in the field. Therefore, in Malaysia, to the best of our knowledge, this is the first study of using EPF *M. anisopliae* for the control of sap sucking insect (*B. tabaci* and *A. gossypii*) in both laboratory and field. It is postulated that isolate of *M. anisopliae*, as wettable powder formulations is effective against *B. tabaci* and *A. gossypii*.

## 1.2 Objectives

The objectives of this study are:

1. to screen the most virulent isolate of *M. anisopliae* for the control of whitefly (*B. tabaci*) and aphid (*A. gossypii*)
2. to prepare wettable powder formulations of *M. anisopliae*
3. to evaluate the effect of WP formulations of *M. anisopliae* to UVB radiation and shelflife in storage
4. to evaluate the effectiveness of WP formulations of *M. anisopliae* against *B. tabaci* and *A. gossypii* in the field

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The student Norhelina Latiff was born in Sepang, Selangor. She studied high school at Sekolah Menengah Kebangsaan Sungai Pelek and MARA College Seremban for Matriculation Program. She graduated with a Bachelor of Science Honor in Conservation Biology on 2008 from Universiti Malaysia Sabah (UMS). During her study, she also joined research expedition and inventory at Lumaku Mountain, Sipitang, Tawau Hill Park and Klias, Beaufort, Sabah. She got research training from Prof. Dr. Ahmad Said Sajap at Forest Entomology Laboratory, University Putra Malaysia (UPM) assisting research project (Extractive of *Vitex pinnata* to control subterranean termites, *Coptotermes curvignathus*). She employed as a microbiologist at NOVA Pharmaceutical Research Laboratory. She then graduated with Master of Science in Entomology on 2010 from Universiti Kebangsaan Malaysia (UKM).

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## LIST OF PUBLICATIONS

- Norhelina, L., Sajap, A. S., Mansour, S. A., and Idris, A. B., 2013. Infectivity of Five *Metarhizium anisopliae* (Deuteromycota: Hyphomycetales) Strains on Whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) Infesting Brinjal, *Solanum melongena* (Solanaceae). *Academic Journal of Entomology* 6 (3): 127-132
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- Norhelina L., Sajap A.S., Omar D. and Radhiah R. 2013. Control of Aphid, *Aphis gossypii* (Glover) using *Metarhizium anisopliae* (Metch.), 2nd Global Conference on Entomology (GCE-2), Sarawak, Malaysia, 8-12 November 2013, P200



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