



UNIVERSITI PUTRA MALAYSIA

**BIOLOGY OF TIGER MOTH (*ATTEVA SCIODOXA* MEYRICK)
INFESTING TONGKAT ALI (*EURYCOMA LONGIFOLIA* JACK) AND
ITS CONTROL BY *BEAUVERIA BASSIANA***

GHULAM ALI BAJWA

T FH 2009 11

**BIOLOGY OF TIGER MOTH (*ATTEVA SCIODOXA MEYRICK*) INFESTING
TONGKAT ALI (*EURYCOMA LONGIFOLIA* JACK) AND ITS CONTROL
BY *BEAUVERIA BASSIANA***

By

GHULAM ALI BAJWA

Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of
Philosophy

June 2009



DEDICATION

*I dedicate this humble effort, the fruit of my thoughts
and study to my spouse and children who always
inspired and supported me to achieve this goal*

Abstract of Thesis presented to the Senate of Universiti Putra Malaysia in
Fulfilment of the Requirement for the Degree of Doctor of Philosophy

**Biology of Tiger Moth (*Atteva sciodoxa* Meyrick) Infesting Tongkat Ali
(*Eurycoma longifolia* Jack) and its Control by *Beauveria bassiana***

By

GHULAM ALI BAJWA

June 2009

Chairperson: Faizah Abood, PhD

Faculty: Forestry

Eurycoma longifolia is a widely used medicinal plant in South East Asia. *Atteva sciodoxa*, the small golden brown moth, became serious pest with the onset of *E. longifolia* plantations. The widespread medicinal use of *E. longifolia* and deleterious effects of chemical insecticides led to search non-chemical control of *A. sciodoxa*. The research was undertaken to study some biological aspects of *A. sciodoxa* and to assess potential of entomopathogenic fungus *Beauveria bassiana* to control *A. sciodoxa*. *A. sciodoxa* feeds gregariously by building communal webs on the terminal shoots. The infestation ranged between $65.0 \pm 2.03\%$ and $92.6 \pm 1.13\%$. *A. sciodoxa* completed its five larval instars in 20.7 ± 0.2 days while lifecycle duration in 46.3 ± 0.49 days. The population rate of increase ranged between 0.33 and 1.39 female offsprings per female. The net reproductive rate, mean generation time and population doubling time were 42.03 female offsprings

per female, 11.41 days and 2.12 days respectively. The highest apparent, real and indispensable mortality were in first instar larvae. The lower threshold temperature was between 9.2°C and 14.7°C while the thermal constant ranged from 126.4 to 79.3 degree-days for different metamorphic stages. The mean food ingestibility, efficiency of conversion of ingested food, efficiency of conversion of digested food and approximate digestibility were 75.2±0.32%, 67.8±0.74%, 37.0±1.21% and 63.10.73%, respectively. The mean food consumption index was 0.23 mg dry leaf per mg larval body weight per day while relative growth rate was 0.08 mg body larval weight gain per mg larval body weight per day.

Seven *B. bassiana* isolates obtained from different sources were screened for pathogenicity. All the isolates were found to be pathogenic. The degree of pathogenicity varied significantly among the isolates. The earliest mortality was recorded on day three after inoculation in five isolates. The most virulent isolate was Bba-Pp with 100% mortality and median effective time of 3.6 days. The least infective isolate was Bba-SI3 with 24.9±2.10% mortality and the median effective time of 15.3 days. The median effective concentration was 9.89×10^5 and 3.85×10^6 conidia ml⁻¹ for Bba-Pp and FS-11, respectively. Mycosis time differed significantly among isolates. Isolate Bba-Pp appeared earliest on cadavers in 24 h. The conidial production ranged between $1.2 \pm 0.84 \times 10^6$ and $1.5 \pm 3.30 \times 10^7$ conidia per mg cadaver in the seven tested isolates. Isolate Bba-Pp decreased food consumption by 72.5% at concentration of 1×10^7 conidia ml⁻¹ as compared to the control. The age specific dose mortality response revealed high infectivity of *B. bassiana* Bba-

Pp in all metamorphic stages of *A. sciodoxa*. The highest egg infectivity was $22.6 \pm 1.60\%$ when 24 h-old eggs were inoculated at 1×10^8 conidia ml^{-1} while the highest delayed first instar larval mortality was $85.6 \pm 2.30\%$ when eggs were inoculated at 24 h before hatching at 1×10^8 conidia ml^{-1} . The third instar larva was most susceptible while the fifth instar larva was the least. The median effective concentration ranged between 9.87×10^5 and 21.3×10^5 conidia ml^{-1} for third to fifth instar larvae while the median effective time ranged from 3.3 to 8.2 days for three tested larval instars at different concentrations. There was a significant temperature effect on *B. bassiana* Bba-Pp infectivity with optimum range between 27°C and 30°C .

Infectivity, mycosis and sporulation were strongly affected when larva, leaf, or both, were inoculated by *B. bassiana* Bba-Pp. Larval mortality ranged between $38.1 \pm 3.21\%$ and $94.6 \pm 2.40\%$ in three exposure methods. Larval mortality of $54.1 \pm 1.74\%$ was recorded due to secondary acquisition of conidia from spray residues on the foliage of *E. longifolia*. The shortest mean mycosis time was 20.2 ± 0.37 h when both larvae and leaves were inoculated and cadavers incubated at 30°C while the longest was 28.0 ± 0.45 h when only leaves were inoculated and cadavers incubated at 21°C . The highest conidial production was $150.9 \pm 0.01 \times 10^5$ conidia per mg cadaver when both larvae and leaves were inoculated at a concentration of 1×10^8 conidia ml^{-1} and cadavers incubated at 27°C . The lowest conidial production was $46.6 \pm 0.02 \times 10^5$ conidia per mg cadaver when only leaves were inoculated at a concentration of 1×10^7 conidia ml^{-1} and cadavers incubated at 33°C . The highest conidial germination and the longest germ tube length was

99.2±0.37% and 45.6±0.84 µm at 27°C, respectively. The optimum temperature for mycosis, sporulation, conidial germination and germ tube growth rate was between 27°C and 30°C.

B. bassiana Bba-Pp was transmitted horizontally from exposed to unexposed larvae, via infective cadavers and contaminated faeces. The highest net transmitted mortality was 87.5±1.17% when exposed larvae were inoculated at 1×10^8 conidia ml⁻¹ and mixed with same number of unexposed larvae. There was significant effect of concentration and ratio on net transmitted mortality. The highest transmitted mortality via infective cadaver was 92.1±1.31% at a density of 0.12 cadaver cm⁻². Viable *B. bassiana* Bba-Pp was isolated from faeces when larvae were inoculated by different exposure methods and at different concentrations. The highest number of *B. bassiana* Bba-Pp colonies isolated was 15.5±0.12×10³ per mg faeces isolated on day one following inoculation of both larvae and leaves at concentration of 1×10^8 conidia ml⁻¹. The number of *B. bassiana* colonies was influenced by exposure method, concentration and the time following inoculation. Larval mortality of 42.2±1.36% was caused when larvae exposed to faeces with 15.5±0.12×10³ *B. bassiana* colonies per mg.

The findings biological studies indicated that *A. sciodoxa* has characteristics of a serious pest. The results also showed high infectivity, residual effect, horizontal transmission and recycling capacity of *B. bassiana* in *A. sciodoxa*. Based on these findings it is concluded that *B. bassiana* Bba-Pp has potential to control *A. sciodoxa*.

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

**BIOLOGI ULAT HARIMAU (*Atteva sciodoxa* Meyrick) YANG
MENYERANG TONGKAT ALI (*Eurycoma longifolia* Jack) SERTA
DIKAWAL OLEH *Beauveria bassiana***

Oleh

GHULAM ALI BAJWA

Jun 2009

Pengerusi: **Faizah Abood, PhD**

Fakulti: **Perhutanan**

Eurycoma longifolia adalah spesies tumbuhan ubatan yang digunakan secara meluas di Asia Tenggara. *Atteva sciodoxa*, kupu-kupu bersaiz kecil berwarna coklat keemasan telah menjadi serangga perosak serius sejak ladang *E. longifolia* mula diperkenalkan. Penggunaan secara meluas *E. longifolia* sebagai tumbuhan ubatan dan risiko kesan kimia pada racun serangga terhadap nya telah membawa kepada pencarian penggunaan bahan bukan kimia untuk mengawal serangga *A. sciodoxa*. Projek ini untuk mengkaji aspek biologi *A. sciodoxa* dan menilai potensi *B. bassiana* untuk mengawal *A. sciodoxa*. *A. sciodoxa* memakan secara berkelompok dengan membina jaringan pada bahagian terminal pucuk. Kesan kerosakan berjulat diantara $65.0 \pm 2.03\%$ dan $92.6 \pm 1.13\%$. *A. sciodoxa* melengkapkan lima peringkat larva dalam masa 20.7 ± 0.2 hari dimana kitaran hidupnya di dalam masa 46.3 ± 0.49 hari. Kadar peningkatan populasi berjulat antara 0.33 hingga

1.39 betina muda bagi setiap betina manakala kadar bersih pembiakan, masa purata generasi dan masa berganda adalah 42.03 betina muda pada setiap dan 2.12 hari. Kadar kematian yang nyata berlaku adalah pada larva peringkat pertama. Julat suhu yang paling rendah adalah diantara 9.2°C dan 14.7°C manakala haba yang seimbang diantara 126.4 and 379.3 darjah-hari pada peringkat metamorfic yang berbeza. Purata penghadaman makanan, kecekapan penukaran pengambilan makanan, kecekapan penukaran makanan yang dihadam dan anggaran makanan yang dicernakan masing-masing adalah $75\pm 0.32\%$, $67.8\pm 0.74\%$, $37.0\pm 1.21\%$ dan $63.1\pm 0.73\%$. Purata index pemakanan adalah 0.23 mg untuk daun kering bagi setiap berat larva pada setiap hari manakala kadar pertumbuhan bandingan adalah 0.08 mg berat larval bertambah bagi setiap mg berat larval untuk setiap hari.

Tujuh isolat kulat *B. bassiana* disaring untuk tahap patogenik. Kesemuanya telah didapati memberi kesan jangkitan. Walaubagaimana pun, darjah jangkitannya berbeza keertian diantara pengasingan tersebut. Peringkat awal kematian direkod selepas tiga hari inokulasi. Isolat yang virulen adalah Bba-Pp dengan 100% kematian dimana masa median adalah 3.6 hari, dalam pada itu, isolat yang kurang berkesan adalah Bba-SI3 dengan $24.9\pm 2.10\%$ kematian dalam masa 15.3 hari ET_{50} . Kekurangan median kosentrasi yang berkesan adalah 9.89×10^5 dan 3.85×10^6 konidia ml^{-1} bagi Bba-Pp dan FS-11. Masa mykosis berbeza dan keertian dengan pergasingan. Isolat Bba-Pp menampilkan yang terawal pada kadaver dalam masa 24 jam. Konidia yang dihasilkan diantara $1.2\pm 0.84\times 10^6$ dan $1.5\pm 3.30\times 10^7$ konidia per mg kadaver didalam tujuh pergasingan. Isolat Bba-Pp berkurangan dengan penggunaan

makanan adalah 72.5% pada 1×10^7 konidia ml^{-1} dibandingkan dengan pengawalan. Kajian pada tingkat umur tertentu dan dos kematian menunjukkan signifikan jangkitan pada *B. bassiana* Bba-Pp dalam semua tahap metamorฟิก *A. sciodoxa*. Kadar paling tinggi telur yang dijangkiti adalah $22.6 \pm 1.60\%$ ketika telur berumur 24 jam setelah di inokulat pada 1×10^8 konidia ml^{-1} sementara penanguhan yang tinggi kepada larva peringkat pertama dengan kadar kematian adalah $85.6 \pm 3.30\%$. Larva peringkat ketiga kebanyakan mudah dijangkiti berbanding dengan larva peringkat kelima yang susah dijangkiti. Median kosentrasi yang berkesan adalah di antara 9.87×10^5 dan 21.3×10^5 konidia ml^{-1} untuk larva peringkat ketiga kepada larva peringkat kelima dimana median yang efektif pada masa antara 3.3 hari dan 8.2 hari untuk tiga ujian larva pada konsiterasi yang berbeza. Terdapat keertian kesan suhu kepada jangkitan *B. bassiana* Bba-Pp pada suhu optimum diantara 27°C and 30°C .

Kaedah pendedahan *B. bassiana* Bba-pp sangat memberi kesan kepada jangkitan, micokis dan sporulasi. Kadar kematian berjulat diantara $38.1 \pm 3.21\%$ dan $94.6 \pm 2.40\%$ didalam tiga kaedah pendedahan. Kadar kematian paling tinggi adalah $54.1 \pm 1.74\%$ disebabkan kepada penerimaan konidia sekunder yang diperolehi daripada baki semburan pada daun *E. Longifolia*. Purata mykosis yang paling singkat adalah 20.2 ± 0.37 jam apabila larva dan daun di inokulasi dan kadaver di inkubator pada 30°C dimana masa paling lama adalah 28.0 ± 0.45 jam apabila hanya daun sahaja di inokulat dan kadaver di inkubator pada 21°C . Konidia dihasilkan paling banyak iaitu $150.9 \pm 0.01 \times 10^5$ konidia per mg kadaver apabila larva dan daun

di inokulat pada kosenterasi 1×10^8 konidia ml^{-1} dan kadaver di inkubator pada 27°C menghasilkan paling rendah iaitu $46.6 \pm 0.02 \times 10^5$ konidia per mg kadaver apabila hanya daun sahaja di inokulat pada konsenterasi 1×10^7 konidia ml^{-1} dan kadaver di inkubator pada 33°C . Pada suhu 27°C , konidia didapati paling tinggi bercambah iaitu pada kadar $99.2 \pm 0.37\%$ dan menghasilkan salur paling panjang iaitu $45.6 \pm 0.84 \mu\text{m}$. Suhu optimum bagi mykosis, sporulasi, percambahan konidia, geminasi jenis konidia dan kadar pertumbuhan salur adalah diantara 27°C dan 30°C .

B. bassiana Bba-Pp dipindahkan secara mendatar daripada larva yang telah didedahkan kepada *B. bassiana* Bba-Pp serta kadaver yang telah dijangkiti dan najis larva yang telah tecemar dipindahkan secara horizontal kepada larva yang belum dijangkiti. Larva yang belum didedahkan dan bebas daripada *B. bassiana* Bba-Pp diletakkan bersama larva yang telah didedahkan dan dijangkiti *B. bassiana* Bba-Pp yang di inokulat pada 1×10^8 konidia ml^{-1} menunjukkan kadar kematian paling tinggi iaitu $87.5 \pm 1.17\%$. Kadar kematian tertinggi melalui permindahan kadaver yang telah dijangkiti adalah $92.1 \pm 1.13\%$ pada ketumpatan kadaver 0.12 cm^{-2} . *B. bassiana* Bba-Pp yang masih aktif pada najis diasingkan dan larva di inokulat dan didedahkan secara berbeza serta di kenakan konsentrasinya yang berbeza. Bilangan koloni *B. bassiana* Bba-Pp (BbaCs) paling banyak adalah $15.5 \pm 0.12 \times 10^3$ per mg najis yang telah pada hari pertama di ikuti inokulasi kepada larva dan daun pada konsentrasinya 1×10^8 konidia ml^{-1} . Bilangan BbaCs bergantung kepada kaedah pendedahan, konsentrasinya dan masa yang diambil untuk inokulasi. Kadar kematian disebabkan oleh *B. bassiana* Bba-Pp yang masih aktif dalam

najis adalah $42.2 \pm 1.36\%$ apabila larva didedahkan kepada najis dengan $15.5 \pm 0.12 \times 10^3$ BbaCs per mg najis. Pada keseluruhannya kajian berbeza yang dilakukan mendapati potensi *B. bassiana* Bba-Pp untuk mengawal *A. sciodoxa*.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Merciful and Most Benevolent

I bow my head before Allah Almighty Who blessed me with good health and vision to accomplish this endeavour. These research investigations were supervised by Dr (s) Faizah Abood (Assoc. Prof.), Yusof Bin Ibrahim (Prof.) and Ab Ghani Ab Rasip.

I am extremely thankful to my supervisor, Assoc. Prof. Dr Faizah Abood for her inspiring encouragement and full support from the initial phase of implementation to completion and for her diligence in reviewing the draft and final copies of the manuscripts. Special thanks for her all out support to ensure timely availability of equipment and other research materials to complete this project timely. I wish to express my sincere thanks to worthy members of my supervisory committee for their consistent guidance, support and encouragement throughout the study period. I am also highly indebted to my supervisor, worthy members and Prof. Dr. Hamami Bin Sahri (former Dean, Faculty of Forestry) for their valuable assistance in their own capacities to upgrade my MS Programme to PhD. The help and guidance provided by Prof. Dr Ahmad Said Sajap and Dr Rozi Mohamad, initially member of my committee and Advisor in first semester, respectively are particularly acknowledged. Special thanks to the teaching faculty and staff of the faculty who provided me the advance knowledge and training in forestry and related fields. I am grateful to Dr (s) Victor Neto and Mohamad Roslan

Mohamad Kasim for assistance in analysis of data. The technical expertise provided by Dr Gisbert Zimmermann, Institute for Biological Control, Darmstadt, Germany is also acknowledged. My special thanks due also to Mr Ramle bin Muslim, Malaysian Palm Oil Research Board and Prof Dr. Ahmad Said Sajap for providing some of the *Beauveria bassiana* isolates. The cooperation and help extended by Messieurs Razak Bin Terhem, Norhisham Bin Ahmad Razi and Muhammad Shahman Bin Muhammad Azmi especially to set the photographs and Bahasa Melayu translation of abstract is acknowledged. My special thanks are extended to Dr (s) Zahid Rizwan, Amanullah Akhtar and Mr Ammad-ud-Din for their encouragement and assistance during this period. The financial assistance by the Government of Pakistan for some of this study programme is acknowledged.

To those individuals and agencies not mentioned, but who in one way or another contributed in the completion of this research work, thank you for your cooperation.

Finally I wish to express my gratitude to my father for his prayers, love, continuous support and encouragement. I would like to acknowledge that all these endeavours and achievements are endowed to my wife, Rizwana Ali, daughters, Ayesha Ali, Asma Ali and sons, Hannan Ali and Huzaiifa Ali for their love, patience and understanding they showed throughout this period.



I certify that a Thesis Examination Committee has met on 14 August 2009 to conduct the final examination of Ghulam Ali Bajwa on his thesis entitled "Biology of tiger moth (*Atteva sciodoxa* Meyrick) infesting tongkat Ali (*Eurycoma longifolia* Jack) and its infectivity by *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Kamaruzaman Sijam, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Dzolkhifli Omar, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Rita Muhammad Awang, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Idris Abd. Ghani, PhD

Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Faizah Abood, PhD
Associate Professor
Faculty of Forestry
Universiti Putra Malaysia
(Chairperson)

Yusof Bin Ibrahim, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Ab Ghani Ab Rasip, PhD
Senior Research Officer
Biotechnology Division
Agro-Forestry Programme
Forest Research Institute Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 16 October 2009

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

GHULAM ALI BAJWA

Date:

TABLE OF CONTENTS

DEDICATION	ii
ABSTRACT	iii
ABSTRAK	viii
ACKNOWLEDGEMENTS	xiii
APPROVAL	xv
DECLARATION	xvii
LIST OF TABLES	xxiii
LIST OF FIGURES	xxvii
LIST OF APPENDICES	xxx
LIST OF ABBREVIATIONS	xxxvi

CHAPTER	Page
1 INTRODUCTION	1
2 LITERATURE REVIEW	7
2.1 <i>E. longifolia</i>	7
2.1.1 Distribution	8
2.1.2 Silvicultural characteristics	13
2.1.3 Uses	11
2.1.4 Insect Pests	12
2.2 <i>A. sciodoxa</i>	13
2.2.1 Morphological Characteristics and Taxonomy	14
2.2.2 Distribution	16
2.2.3 Nature and Extent of Damage	16
2.2.4 Control of <i>A. sciodoxa</i>	17
2.3 <i>B. bassiana</i>	21
2.3.1 History	21
2.3.2 Morphological Characteristics	22
2.3.3 Natural Occurrence and Geographical Distribution	23
2.3.4 Host Range	24
2.3.5 Infection Mechanism	25
2.3.6 Enzymes, Metabolites and Toxins	30
2.3.7 Pathogenicity	33



2.3.8	Age Specific Dose Mortality Response	34
2.3.9	Exposure Methods	37
2.3.10	Horizontal Transmission	39
3	FIELD EXPERIMENTS AND LIFE TABLE STUDY	40
3.1	Introduction	40
3.1.1	Feeding Behaviour and Infestation	40
3.1.2	Food Utilization and Consumption	41
3.1.3	Life Table and Demographic Parameters	42
3.2	Material and Methods	43
3.2.1	Feeding Behaviour	43
3.2.2	Infestation and Larval Intensity	43
3.2.3	Food Consumption and Utilization	46
3.2.4	Life Table and Demographic Parameters	48
3.3	Results	50
3.3.1	Feeding Behaviour	50
3.3.2	Infestation and Larval Intensity	52
3.3.3	Food consumption and Utilization	54
3.3.4	Life Table and Demographic Parameters	62
3.4	Discussion	69
3.4.1	Feeding Behaviour	69
3.4.2	Food Consumption and Utilization	70
3.4.3	Life Table and Demographic Parameters	74
3.5	Conclusion	75
4	DEVELOPMENTAL BIOLOGY	77
4.1	Introduction	77
4.1.1	Developmental Biology	77
4.1.2	Effect of Temperature on Developmental Biology	77
4.1.3	Effect of Humidity on Developmental Biology	79
4.2	Materials and Methods	80
4.2.1	Developmental Biology	80
4.2.2	Effect of Temperature on Developmental Biology	82
4.2.3	Effect of Relative Humidity on Developmental Biology	85

4.3	Results	87
4.3.1	Developmental Biology	87
4.3.2	Effect of Temperature on Developmental Biology	98
4.3.3	Effect of Relative Humidity on Developmental Biology	119
4.4	Discussion	127
4.4.1	Effect of Temperature on Developmental Biology	127
4.4.2	Effect of Relative Humidity on Development Biology	134
4.5	Conclusion	136
5	PATHOGENICITY OF <i>B. BASSIANA</i>	138
5.1	Introduction	138
5.2	Materials and Methods	139
5.2.1	Fungal Isolates	139
5.2.2	Fungal Cultures	140
5.2.3	Screening of the Isolates	141
5.2.4	Mycosis of the Isolates	143
5.2.5	Sporulation and Conidial Viability of the Isolates	143
5.2.6	Effect of Fungal Infection on Food Consumption	144
5.3	Results	145
5.3.1	Screening of the Isolates	145
5.3.2	Mycosis of the Isolates	151
5.3.3	Sporulation and Viability of the Isolates	152
5.3.4	Effect of Fungal Infection on Food Consumption	153
5.4	Discussion	156
5.5	Conclusion	163
6	AGE SPECIFIC DOSE MORTALITY RESPONSE	164
6.1	Introduction	164
6.2	Materials and Methods	165
6.2.1	Fungal Culture	165
6.2.2	Insect Culture	166
6.2.3	Egg Infectivity	167

6.2.4	Delayed First Instar Mortality	168
6.2.5	Larval Infectivity	169
6.2.6	Pupal Infectivity	170
6.2.7	Effect of Temperature on Bba-Pp Infectivity	170
6.3	Results	172
6.3.1	Egg Infectivity	172
6.3.2	Delayed First Instar Mortality	176
6.3.3	Larval Infectivity	180
6.3.4	Pupal Infectivity	185
6.3.5	Effect of Temperature on <i>B. bassiana</i> Bba-Pp Infectivity	187
6.4	Discussion	189
6.5	Conclusion	198
7	EFFECT OF EXPOSURE METHOD ON <i>B. BASSIANA</i> Bba-Pp INFECTIVITY, MYCOSIS AND SPORULATION	200
7.1	Introduction	200
7.2	Materials and Methods	202
7.2.1	Fungal Culture	202
7.2.2	Insect Culture	203
7.2.3	Effect of Exposure Method on Infectivity	204
7.2.4	Effect of Exposure Method and Temperature on Mycosis	205
7.2.5	Effect of Exposure Method, Concentration and Temperature on Sporulation	206
7.2.6	Effect of Temperature on Conidial Viability	207
7.2.7	Effect of Temperature on Conidial Mode of Germination and Germ Tube Growth Rate	208
7.3	Results	208
7.3.1	Effect of Exposure Method on Infectivity	208
7.3.2	Effect of Exposure Method and Temperature on Mycosis	214
7.3.3	Effect of Exposure Method, Concentration and Temperature on Sporulation	218
7.3.4	Effect of Temperature on Conidial Viability	227
7.3.5	Effect of Temperature on Conidial Mode of Germination and Germ Tube Growth Rate	229
7.4	Discussion	231
7.5	Conclusion	243

8	HORIZONTAL TRANSMISSION OF <i>B. BASSIANA</i>	244
8.1	Introduction	244
8.2	Materials and Methods	246
8.2.1	Fungal Culture	246
8.2.2	Insect Culture	247
8.2.3	Horizontal Transmission between Exposed and Unexposed Larvae	247
8.2.4	Horizontal Transmission via Infective Cadavers	248
8.2.5	Isolation of <i>B. bassiana</i> Bba-Pp from Faeces	250
8.2.6	Horizontal Transmission of Infectivity via Contaminated Faeces	252
8.3	Results	253
8.3.1	Horizontal Transmission between Exposed and Unexposed Larvae	253
8.3.2	Horizontal Transmission via Infective Cadavers	257
8.3.3	Isolation of <i>B. bassiana</i> Bba-Pp from Faeces	258
8.3.4	Horizontal Transmission of Infectivity via Contaminated Faeces	266
8.4	Discussion	267
8.5	Conclusion	275
9	SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	276
9.1	Summary	276
9.2	General Conclusion	281
9.3	Recommendations for Future Research	284
	REFERENCES	285
	APPENDICES	313
	BIO DATA OF STUDENT	342
	LIST OF PUBLICATIONS	343



LIST OF TABLES

Table		Page
3.2.1	<i>E. longifolia</i> plot details, Setiu, Kuala Terengganu	64
3.3.1	Mean plant height, extent of infestation and larval intensity of <i>A. sciodoxa</i> at different sites, Setiu, Kuala Terengganu	53
3.3.2	Chronological food consumption and utilization (\pm SE) by third instar <i>A. sciodoxa</i> larva	55
3.3.3	Chronological food consumption and utilization (\pm SE) by fourth instar <i>A. sciodoxa</i> larva	57
3.3.4	Chronological food consumption and utilization (\pm SE) by fifth instar <i>A. sciodoxa</i> larva	59
3.3.5	Food consumption and utilization (\pm SE) by different <i>A. sciodoxa</i> larval instars	61
3.3.6.	Pooled age specific mortality of <i>A. sciodoxa</i> at $27\pm 2^{\circ}\text{C}$ and $75\pm 5\%$ relative humidity with a 12 h photoperiod	62
3.3.7	Demographic attributes of <i>A. sciodoxa</i> at $27\pm 2^{\circ}\text{C}$ and $75\pm 5\%$ relative humidity with 12 h photoperiod	67
4.2.1	Salts used to maintain different levels of relative humidity	86
4.3.1	Wing span of <i>A. sciodoxa</i> moths	88
4.3.2	Reproductive parameters of <i>A. sciodoxa</i> under laboratory conditions	90
4.3.3	Egg and pupal dimensions of <i>A. sciodoxa</i> under laboratory conditions	91
4.3.4	Dimensions of larval instars of <i>A. sciodoxa</i> and application of Dyar's Rule on head capsule	93
4.3.5	Development periods of different metamorphic stages of <i>A. sciodoxa</i> at $27\pm 2^{\circ}\text{C}$ and $80\pm 5\%$ relative humidity with 12 h photoperiod	94
4.3.6	Mean egg developmental time, range of variation and coefficient of variation of <i>A. sciodoxa</i> at different temperatures	99

4.3.7	Lower threshold temperatures and thermal constants of different developmental stages of <i>A. sciodoxa</i>	100
4.3.8	Mean larval and pupal developmental time, variation range and coefficient of variation (\pm SE) of <i>A. sciodoxa</i> at different temperatures	101
4.3.9	Mean male and female moth lifespan, variation range and coefficient of variation of <i>A. sciodoxa</i> at different temperatures	104
4.3.10	Adult longevity, fecundity and demographic parameters of <i>A. sciodoxa</i> at different temperatures	106
4.3.11	Ovipositional parameters (\pm SE) of <i>A. sciodoxa</i> at different temperatures	112
4.3.12	Mathematical expression of effect of temperature on ovipositional parameters of <i>A. sciodoxa</i>	112
4.3.13	Mathematical expression of effect of temperature on fertility parameters of <i>A. sciodoxa</i>	116
4.3.14	Fertility parameters of <i>A. sciodoxa</i> at different temperatures	117
4.3.15	Effect of relative humidity on different immature developmental stages of <i>A. sciodoxa</i>	120
4.3.16	Mathematical expression of effect of relative humidity on different immature developmental stages of <i>A. sciodoxa</i>	121
4.3.17	Effect of relative humidity on adult moths and lifecycle (\pm SE) of <i>A. sciodoxa</i>	122
4.3.18	Mathematical expression of effect of relative humidity on adult moths, lifespan and fecundity parameters of <i>A. sciodoxa</i>	123
4.3.19	Effect of relative humidity on ovipositional parameters (\pm SE) of <i>A. sciodoxa</i>	125
5.2.1	Isolates of <i>B. bassiana</i> screened against <i>A. sciodoxa</i>	140
5.3.1	Mean mortality (\pm SE) and median effective time of <i>B. bassiana</i> isolates against 3 rd instar <i>A. sciodoxa</i> at seven day after inoculation	146
5.3.2	Median effective concentration (10^5 conidia ml ⁻¹) of Bba-Pp and FS-11 against third instar <i>A. sciodoxa</i> larva	148



5.3.3	Median effective time for different concentrations (conidia ml ⁻¹) of Bba-Pp and FS-11 against third instar <i>A. sciodoxa</i> larva	150
5.3.4	Time to mycelial appearance (h) (\pm SE) of <i>B. bassiana</i> isolates on third instar <i>A. sciodoxa</i> larval cadavers	152
5.3.5	Mean number (1×10^7 conidia per mg cadaver) of conidia (\pm SE) and their viability (\pm SE) on <i>A. sciodoxa</i>	153
6.3.1	Mathematical expression of effect of Bba-Pp concentration on pooled infectivity of egg, delayed 1 st instar mortality and larval mortality	173
6.3.2	Effect of egg age and <i>B. bassiana</i> Bba-Pp concentration on egg infectivity (\pm SE) of <i>A. sciodoxa</i>	175
6.3.3	Mathematical expression of effect of Bba-Pp concentration on egg infectivity, delayed 1 st instar mortality and larval infectivity of <i>A. sciodoxa</i>	176
6.3.4	Effect of egg age and Bba-Pp concentration on delayed 1 st instar mortality of <i>A. sciodoxa</i>	179
6.3.5	Effect of larval instar and Bba-Pp concentration on larval infectivity (\pm SE) of <i>A. sciodoxa</i>	182
6.3.6	Median effective concentration (10^5 conidia ml ⁻¹) for three larval instars and pupa of <i>A. sciodoxa</i> on day seven after inoculation	183
6.3.7	Median effective time (days) for three <i>A. sciodoxa</i> larval instars and pupa at different concentrations (conidia ml ⁻¹) of Bba-Pp	185
6.3.8	Effect of temperature on Bba-Pp infectivity of third instar <i>A. sciodoxa</i> larva at $75 \pm 5\%$ relative humidity with 12 h photoperiod	188
7.3.1	Mathematical expression of effect of concentration on <i>B. bassiana</i> Bba-Pp infectivity in different exposure methods	211
7.3.2	Effect of exposure method and concentration on <i>A. sciodoxa</i> larval mortality (\pm SE)	212
7.3.3	Median effective concentration (1×10^5 conidia ml ⁻¹) of <i>B. bassiana</i> Bba-Pp in three exposure methods	212