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

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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±2.03% and 92.6±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-Sl3 with 24.9±2.10% mortality and the median effective time of 15.3 days. The median effective concentration was $9.89x10^5$ and $3.85x10^6$ 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 $1.2±0.84x10^6$ and $1.5±3.30x10^7$ conidia per mg cadaver in the seven tested isolates. Isolate Bba-Pp decreased food consumption by 72.5% at concentration of $1x10^7$ 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±1.60% when 24 h-old eggs were inoculated at $1x10^8$ conidia ml^{-1} while the highest delayed first instar larval mortality was 85.6±2.30% when eggs were inoculated at 24 h before hatching at $1x10^8$ conidia ml^{-1}. The third instar larva was most susceptible while the fifth instar larva was the least. The median effective concentration ranged between $9.87x10^5$ and $21.3x10^5$ 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±3.21% and 94.6±2.40% in three exposure methods. Larval mortality of 54.1±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 $150.9±0.01x10^5$ conidia per mg cadaver when both larvae and leaves were inoculated at a concentration of $1x10^8$ conidia ml^{-1} and cadavers incubated at 27ºC. The lowest conidial production was $46.6±0.02x10^5$ conidia per mg cadaver when only leaves were inoculated at a concentration of $1x10^7$ 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 27ºC, respectively. The optimum temperature for mycosis, sporulation, conidial germination and germ tube growth rate was between 27ºC and 30ºC.

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 $1x10^8$ 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 $15.5±0.12x10^3$ per mg faeces isolated on day one following inoculation of both larvae and leaves at concentration of $1x10^8$ 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 $15.5±0.12x10^3$ *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 spesis tumbuhan ubatan yang digunakan secara meluas di Asia Tenggara. *Atteva sciodoxa*, kupu-kupu bersaiz kecil berwarma 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 pengunaan 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±2.03% dan 92.6±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 pengahadaman makanan, kecekapan penukaran pengambilan makanan, kecekapan penukaran makanan yang dihadam dan anggaran makanan yang dicernakan masing-masing adalah 75±0.32%, 67.8±0.74%, 37.0±1.21% dan 63.1±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±2.10% kematian dalam masa 15.3 hari ET_{50}. Kekerapan median kosentrasi yang berkesan adalah $9.89x10^5$ dan $3.85x10^6$ konidia ml^{-1} bagi Bba-Pp dan FS-11. Masa mykosis berbeza dan keertian dengan pergasingan. Isolat Bba-Pp menampakan yang terawal pada kadaver dalam masa 24 jam. Konidia yang dihasilkan diantara $1.2±0.84x10^6$ dan $1.5±3.30x10^7$ konidia per mg kadaver didalam tujuh pergasingan. Isolat Bba-Pp berkurangan dengan penggunaan

makanan adalah 72.5% pada $1x10^7$ konidia ml^{-1} dibandingkan dengan pengawalan. Kajian pada tingkat umur tertentu dan dos kematian menunjukan siginifikan jangkitan pada *B. bassiana* Bba-Pp dalam semua tahap metamorpik *A. sciodoxa*. Kadar paling tinggi telur yang dijangkiti adalah 22.6±1.60% ketika telur berumur 24 jam setelah di inokulat pada $1x10^8$ konidia ml^{-1} sementara penanguhan yang tinggi kepada larva peringkat pertama dengan kadar kematian adalah 85.6±3.30%. Larva peringkat ketiga kebanyakan mudah dijangkiti berbanding dengan larva peringkat kelima yang susah dijangkiti. Median kosentrasi yang berkesan adalah di antara $9.87x10^5$ dan $21.3x10^5$ konidia ml^{-1} untuk larva peringkat ketiga kepada larva peringkat kelima dimana median yang effectif 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 sporalasi. Kadar kematian berjulat diantara 38.1±3.21% dan 94.6±2.40% didalam tiga kaedah pendedahan. Kadar kematian paling tinggi adalah 54.1±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 $150.9±0.01x10^5$ konidia per mg kadaver apabila larva dan daun

di inokulat pada kosenterasi $1x10^8$ konidia ml^{-1} dan kadaver di inkubator pada 27°C menghasilkan paling rendah iaitu $46.6\pm0.02x10^5$ konidia per mg kadever apabila hanya daun sahaja di inokulat pada konsenterasi $1x10^7$ konidia ml^{-1} dan kadaver di inkubator pada 33°C. Pada suhu 27°C, konidia didapati paling tinggi bercambah iaitu pada kadar 99.2±0.37% dan menghasilkan salur paling panjang iaitu 45.6±0.84 µm. 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 didedah dan dijangkiti *B. bassiana* Bba-Pp yang di inokulat pada $1x10^8$ konidia ml^{-1} menunjukkan kadar kematian paling tinggi iaitu 87.5±1.17%. Kadar kematian tertingi melalui permindahan kadaver yang telah dijangikiti adalah 92.1±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 konsentrasi yang berbeza. Bilangan koloni *B. bassiana* Bba-Pp (BbaCs) paling banyak adalah $15.5\pm0.12x10^3$ per mg najis yang telah pada hari pertama di ikuti inokulasi kepada larva dan daun pada konsentrasi $1x10^8$ konidia ml^{-1}. Bilangan BbaCs bergantung kepada kaedah pendedahan, konsentrasi dan masa yang diambil untuk inokulasi. Kadar kematian disebabkan oleh *B. bassiana* Bba-Pp yang masih aktif dalam

najis adalah 42.2±1.36% apabila larva didedahkan kepada najis dengan $15.5\pm0.12x10^3$ 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 Huzaifa 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.

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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:

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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.

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GHULAM ALI BAJWA

Date:

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