



UNIVERSITI PUTRA MALAYSIA

**BIOLOGY OF TIGER MOTH (*ATTEVA SCIDOXIA* 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

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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±0.12x10³ 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±0.12x10³ *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

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Jun 2009

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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 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 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 kosenterasi 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 konsentrasi 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 konsentrasi 1×10^8 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 $42.2 \pm 1.36\%$ 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*.

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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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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:

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