UNIVERSITI PUTRA MALAYSIA

ASSESSMENT of Metarhizium anisopliae (Metchnikoff) FROM NATURAL FOREST TO CONTROL TIGER MOTH Atteva sciodoxa (MEYRICK)

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By

WAN MUHAMMAD AZRUL BIN WAN AZHAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillments of the Requirements for the Degree of Master of Science

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DEDICATION

Specially dedicated to my family members,
My dad, Wan Azhar Wan Ibrahim, mom, Rosnah Jamaluddin and wife Che Nurul Fariza, for your patient and courage given. Also not forgotten my siblings and friends.

Special thanks to the supervisor committee and laboratory members at Mycology and Pathology, Forest Research Institute of Malaysia that always support and help me during the progress of this project and thesis.

Wan Muhammad Azrul Bin Wan Azhar

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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July 2017

Chairman : Professor Ahmad Said Bin Sajap, PhD
Faculty : Forestry

Eurycoma longifolia or 'tongkat ali' is a well-known tropical medicinal plant used widely to cure human illness. However, this plant is highly vulnerable to several insect pests, especially *Atteva sciodoxa* larvae. In the present study, a total of 28 *E. longifolia* forest and cultivation areas in Peninsular Malaysia were surveyed. Results of the surveys showed that *A. sciodoxa* larvae infestation was major and common in *E. longifolia*'s plantations, followed by scale insect, *Zeuzera* stem borer, animal damage, *Coptotermes* sp. and spider mites. Several diseases were also observed such as algal leaf spots, sooty mould, Sudden Death Syndrome (SDS) and *Colletotrichum* leaf spot. Twenty five *Metarhizium anisopliae* isolates obtained from natural forest soils of Peninsular Malaysia were identified and screened for pathogenicity against *A. sciodoxa* larvae. Morphology of the fungus was studied and a phylogenetic tree of the fungal ITS region sequences was developed. The study revealed that cultures of the fungal isolates were flat with a yellowish mycelial mat bearing brownish to dark green masses of conidia. Conidia were single celled, cylindrical with rounded tips and varied in size, ranging from 5.7 ± 0.5µm × 2.5 ± 0.4 µm to 7.4 ± 1.0 µm × 3.3 ± 1.0 µm. They were then identified as *M. anisopliae* var. *anisopliae* due to short shaped conidia. Phylogenetic analysis found that all the isolates were closely related within species regardless of their geographic origins. The species was also found to be closely related with *M. anisopliae* var. *majus* supported with 99% bootstrap value. Growth rate test showed that SDA is a media supported fastest fungus growth followed by PDA, MEA, CMA and WA at temperatures 25ºC, 28 ºC and 31 ºC. Screening tests revealed that all the isolates were pathogenic against the inoculated *A. sciodoxa* larvae with mortality as early as 3 days after inoculation. The highest larval mortality recorded by FRIM858, followed by FRIM880, FRIM873, FRIM859, FRIM862, FRIM871 and the least virulent was FRIM878. None of the larvae in the inoculated control treatment died. Further study was conducted on isolates FRIM880, FRIM871, FRIM859 and FRIM858 to determine the median effective conidia concentration (EC_{50}) and median effective time (ET_{50}) against the pest larvae. The study revealed that FRIM589 was the most effective and aggressive against the pest larvae. The EC_{50} recorded was

$1.1x10^6$ conidia ml^{-1} and ET_{50} was 2.9 days. Field trials in KESEDAR Gua Musang and Sg. Menyala Forest Reserve show that FRIM859 significantly reduce population of *A. sciodoxa* larvae with 57.0% and 63.4% larvae reduction, respectively. The study suggests that FRIM589 was the best candidate to be developed as biological control agent against *A. sciodoxa* larvae.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENILAIAN *Metarhizium anisopliae* (METCHNIKOFF) DARI HUTAN SEMULAJADI SEBAGAI KAWALAN ULAT HARIMAU *Atteva sciodoxa* (MEYRICK)

Oleh

WAN MUHAMMAD AZRUL BIN WAN AZHAR

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Eurycoma longifolia or "tongkat ali" merupakan tumbuhan ubatan tropika yang sangat dikenali digunakan secara meluas untuk menyembuhkan penyakit manusia. Walau bagaimanapun, tumbuhan ini dilaporkan terdedah kepada serangan serangga perosak terutamanya larva *Atteva sciodoxa*. Dalam kajian ini, sejumlah 28 hutan dan ladang *E. longifolia* di Semenanjung Malaysia telah di survei. Keputusan survei menunjukkan serangan larva *A. sciodoxa* adalah yang masalah utama dan biasa didapati di ladang-ladang tongkat ali, diikuti teritip, ulat pengorek batang *Zeuzera*, kerosakan oleh haiwan, *Coptotermes sp*. dan hama lelabah. Beberapa penyakit juga diperhatikan, seperti karat alga, jeragat, sindrom mati mengejut (SDS) dan bintik daun *Colletotrichum*. Dua puluh lima isolat *Metarhizium anisopliae* diperoleh daripada sampel tanah hutan semulajadi Semenanjung Malaysia telah dikenalpasti dan diperiksa untuk ujian patogenik ke atas larva *A. sciodoxa*. Morfologi kulat-kulat tersebut telah dikaji dan pokok filogenetik bagi penjujukan ITS telah dibina. Kajian menunjukkan kultur isolat kulat adalah rata dengan hamparan miselia kekuningan mengalas konidia berwarna perang ke hijau gelap yang padat. Media SDA merupakan media yang mencatatkan pertumbuhan kulat ini paling cepat diikuti media PDA, MEA, CMA dan WA pada suhu 25^{o}C, 28 ^{o}C and 31 ^{o}C. Konidia adalah sel tunggal, berbentuk silinder dengan hujungnya membulat dan saiznya berbeza, antara 5.72 ± 0.51µm × 2.51 ± 0.38 µm to 7.36 ± 0.96 µm × 3.30 ± 1.00 µm. Kulat-kulat ini dikenali sebagai *M. anisopliae var. anisopliae* berdasarkan konidia yang bersaiz kecil. Analisis fiologenetik menemui kesemua isolat-isolat adalah berkaitan rapat dalam lingkungan spesies tanpa mengira geografi asal. Spesies ini ditemui berkait rapat dengan *M. anisopliea* var. *majus* disokong nilai bootsrap 99%. Walau bagaimanapun, kedua-dua *M. anisopliae* var. *anisopliae* dan *M. anisopliae* var. *majus* kurang berkaitan dengan *M. acridum*. Ujian saringan menunjukkan kesemua isolat adalah patogenik ke atas larva *A. sciodoxa* dengan kematian seawal selepas 3 hari selepas inokulasi. Isolat yang merekodkan kematian larva paling tinggi adalah FRIM858, diikuti FRIM880, FRIM873, FRIM859, FRIM862, FRIM871 dan yang paling kurang berkesan adalah

FRIM878. Tiada kematian larva pada rawatan kawalan. Kajian lanjutan telah dijalankan ke atas FRIM880, FRIM871, FRIM859 dan FRIM858 untuk menentukan median kepekatan efektif (EC_{50}) dan media masa efektif (ET_{50}) konidia. Kajian menunjukkan isolat FRIM859 adalah paling efektif dan agresif ke atas larva perosak ini. EC_{50} direkodkan adalah $1.1x10^{6}$ konidia ml^{-1} dan ET_{50} adalah 2.9 hari. Ujian lapangan di KESEDAR Gua Musang dan Hutan Simpan Sungai Menyala menunjukkan isolat FRIM859 pengurangan bererti populasi larva *A. sciodoxa* dengan mencatatkan 57.0% dan 63.4% pengurangan larva bagi kedua-dua lokasi. Kajian mencadangkan FRIM589 adalah calon terbaik untuk dijadikan sebagai agen kawalan biologi bagi mengawal larva *A. scoidoxa.*

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I certify that a Thesis Examination Committee has met on 13 July 2017 to conduct the final examination of Wan Muhammad Azrul bin Wan Azhar on his thesis entitled "Assessment of *Metarhizium anisopliae* (Metchnikoff) from Natural Forest to Control Tiger Moth *Atteva sciodoxa* (Meyrick)" 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 Master of Science.

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

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CEC	Cation Exchange Capability
CMA	Corn Meal Agar
DNA	Deoxy Ribonucleic Acid
DSI	Damage Severity Index
EC_{50}	Median Effective Concentration
ET_{50}	Median Effective Time
FELDA	Federal Land Development Authority
FRIM	Forest Research Institute of Malaysia
HSD	Honestly Significant Difference
IPM	Intergrated pest management
ITS	Internal Transcribe Region
KESEDAR	South Kelantan Development Authority
KETENGAH	Middle Terengganu Development Authority
MARDI	Malaysia Agriculture Research Department
MEA	Malt Extract Agar
MPOB	Malaysia Palm Oil Board
NaOCl	Sodium Hypochloride
NAP	Third National Agricultural Policy
PDA	Potato Dextrose Agar
rDNA	nuclear ribosomal Deoxy Ribonucleic Acid
SDA	Saubaouraud Dextrose Agar
SDS	Sudden Death Syndrome
UV	Ultra Violet

UVB	Ultra Violet B
WA	Water Agar

CHAPTER 1

INTRODUCTION

Eurycoma longifolia Jack or locally known as 'tongkat ali' is a famous and highly demanded herb used as supplement to improve human health due to its medicinal properties. Traditionally this plant is used as medicine against illnesses such as mouth ulcer, fever and as after birth treatment (Burkhill 1966). Nowadays, many products based on *E. longifolia* have been developed and marketed locally and internationally whether in form of energy drink, capsule or soluble extract. In Malaysia, products from *E. longifolia* generate revenue estimated at RM2 billion per annum (Mohd Azmi et al. 2003). However, the raw material is limited as the supple comes from natural forest. Mass harvesting of *E. longifolia* directly from natural forest could put pressure on this species. Planting *E. longifolia* in large scales as in plantations is seen as an alternative to overcome these problems.

Plantation of *E. longifolia* blooms in the late 1990s with government agencies, private sectors companies and personal planters play active role at developing new plantations. However, it did not proceed as planned when the plantations were reported attacked by insect pests such as *Atteva sciodoxa* (Lepidotera), scale insect (Hemiptera) and *Zeuzera* stem borer. Sudden Dead Syndrome (SDS) disease, which can be fatal to *E. longifolia* plants was also reported (Patahayah et al. 2011). Of these pests and diseases, *A. sciodoxa* larvae has been claimed as most the destructive and hard to control. Outbreak of *A. sciodoxa* was first reported in 2000, when it caused severe damages to several *E. longifolia* plantations, mostly affecting young saplings (Mohd Noh & Mohd Ilham 2002). The infestation continues as Patahayah et al. (2011) also reported the incidence of this pest in plantations. Failure in controlling this pest infestation can bring downfall to *E. longifolia* industries in this country. Besides, most of *E. longifolia* plantations in Peninsular Malaysia have poor pest management and some have been abandoned. Therefore, survey on infestation of *A. sciodoxa* in forests and plantations are crucial as the current status of infestation can be assessed. The information will be useful in developing a pest management strategy for *A. sciodoxa.*

Although the use of chemical pesticides is effective to control *A. sciodoxa* larvae the potential hazardous effects from chemical residues to human, animal, beneficial insect rendered it non-desirable. Numerous cases have been reported due to pesticides poisoning among agricultural workers worldwide which lead to more serious diseases like cancers, asthma, foetal defect and leukaemia (Owens et al. 2010). Moreover, toxic from pesticides are easily spread via air, water or foods. Insect pest can also develop an immune response to pesticide, which makes them resistance (Naqqash et al. 2016).

Biological control is considers the best approaches to control this problem replacing application of chemical pesticide. Uses of biological agents to control insect pests have been promoted decades ago with most beneficial effects. Biological agents do not give

harm to human, environment and non-target species. Bacteria, fungi, nematodes and viruses among the agents used in biological control approaches. Some commercial products based on bacteria like *Bacillus thriguienesis,* and entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana,* are examples of biological control products used widely in plantations and nurseries to control insect pests (Nishi et al. 2011).

At present, mycoinsecticides from entomopathogenic fungi receives a lot of attention for developing biological insecticides because of their high virulence and wide host range characteristic. Entomopathogenic fungi can infect many orders of arthropods including Lepidoptera, Hemiptera and Scarabaeidae (Sajap et al. 2014; Islam et al. 2016; Goble et al. 2014). Numerous studies on entomopathogenic fungi like *Metarhizium* spp., *Beauveria* spp., *Paecilomyces* spp. and *Ventricillium* spp. have been conducted as agents to control insect pests (Keller & Zimmerman 1989). These fungi can be isolated from soils or dead insect. Mass propagation of these fungi is easy to achieve using low technology, thus the production is not costly.

Metarhizium anisopliae is reported as the most reliable entomopathogenic fungus as it is easily isolated, widely distributed in soils, and have wide host range of arthropods compared to others fungi. Undisturbed area is reported to have high occurrence and distribution of entomopathogenic fungi compared to agricultural land, which may be due to human cultivation activities (Meyling and Eilenberg 2006). Thus, rainforest areas in this country could offer better candidates of effective entomopathogens with robust strains to be developed into biocontrol agents compared to agroecosystems. Furthermore, isolation of entomopathogenic fungus from different forest areas can give information on relation between types and nutrients in soils with the occurrence of certain entomopathogenic fungal species. This information can be used to specify sampling site to find new fungus candidate in future.

The capability to control *A. sciodoxa*, the major threat of *E. longifolia* plantations using biological agents such as *M. anisopliae* is attractive as it could offer a safer cultivation practice, enhance the value of *E. longifolia* and avoid chemical contaminants in *E. longifolia* based products. Even in the same species, different strains of *M. anisopliae* posses different level of virulence and host specificity. Therefore, screening for a strain that is high virulence and robust is a mandatory step before it can be used as an active ingredient in mycoinsecticide to control *A. sciodoxa* larvae.

This study focused on the severity of *A. sciodoxa* infestation on *E. longifolia* cultivation and screening for virulent *M. anisopliae* strains with potential to be developed as biological control agent. Hence, the specific objectives of this study were as below:

1. To asses current incidence and severity of *A. sciodoxa* infestation and other pest and diseases on *E. longifolia* plantations in Peninsular Malaysia.
2. To isolate and characterize *M. anisopliae* harboring natural forest soils.

3. To identify *M. anisopliae* isolates with high virulency against *A. sciodoxa* larvae in laboratory and field.

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