UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ENTOMOPATHOGENIC FUNGUS (metarhizium anisopliate) ON THE TERMITE (coptotermes curvignathus)

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By

HOE PIK KHENG

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

September 2010

Dedicated to

My grandma and my family

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

EFFECTS OF ENTOMOPATHOGENIC FUNGI (*Metarhizium anisopliae)* ON THE TERMITE (*Coptotermes curvignathus)*

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HOE PIK KHENG

September 2010

Chairman: Joseph Bong Choon Fah, Ph.D

Faculty: Agriculture and Food Sciences, Bintulu

Coptotermes curvignathus (BLATTODEA: Rhinotermitidae) is a major termite pest in the oil palm plantations on peat as it attacks the living tissues of the plants. Biological control using *Metarhizium anisopliae* in the peat area has potential for termite control due to its advantages such as environmental friendly and non-hazardous to human health compared to chemical measures. In this study, several isolates of *M. anisopliae* was obtained from both peat and mineral soils and screened for their pathogenic effect on *C. curvignathus*. Five potential isolates (TA, LR2, MG, CI and AR3) had been further characterised and evaluated for their pathogenicity activity and investigated for their mode of action. There was also no repellent effect of

the termites towards the isolates. Among the five isolates isolate TA had the most potential, followed by isolate LR2 and the least potential was isolate CI. Isolate TA which was obtained from peat soil, had the highest virulence and exhibited the quickest sporulation at 2 days post inoculation. The ultra structural studies revealed that isolate TA was able to have a higher virulence level compared to other isolates was due to its quick germination and penetration (within 3 hours) of the mycelia into the termite body, where the colonization of mycelia happened subcutaneously. Between isolate TA and LR2, isolate TA that germinated and penetrated into the termite more quickly. Isolate CI was the least virulent as this was the only isolate that did not spread subcutaneously; hence, it was very likely that the mycelia would have been groomed off before they were able to penetrate. The total mortality of the termites was not only due to the infection of the isolates, but also due to the induced alarm behaviour by the isolates among the termites causing them to be buried or cannibalized the weakened or infected member. Direct applications, like drenching and topical application were very effective but not practical for field application, unless the termite nests or colony can be identified in the peat area. Hence, with a potential isolate like isolate TA and LR2, successful biological control for termites, *C. curvignathus*, for oil palms on peat can be achieved. However, enhancement of the potential isolates and a suitable application method can augment the effect of the isolates during the field applications.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN KULAT PATOGENIK SERANGGA (*Metarhizium anisopliae)* TERHADAP ANAI-ANAI (*Coptotermes curvignathus)*

Oleh

HOE PIK KHENG

September 2010

Pengerusi: Joseph Bong Choon Fah, Ph.D

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Coptotermes curvignathus (BLATTODEA: Rhinotermitidae) ialah perosak utama di ladang kelapa sawit di kawasan tanah gambut. Ini adalah kerana jenis anai-anai ini menyerang tisu-tisu hidup pada pokok kelapa sawit. Kawalan biologi dengan menggunakan *Metarhizium anisopliae* mempunyai potensi untuk kawalan anai-anai di kawasan tanah gambut. Ini adalah disebabkan oleh beberapa faedahnya seperti kesan baik kepada alam sekitar dan tidak berbahaya kepada kesihatan manusi jika dibandingkan kepada kawalan secara kimia. Dalam kajian ini, beberapa isolat *M. anisopliae* diperolehi daripada tanah gambut dan tanah mineral dan ditapis untuk kesan patogeniknya terhadap *C. curvignathus*. Lima isolat yang

berpotensi (TA, LR2, MG, CI dan AR3) telah dikaji dan dinilai ciri-ciri dan kegiatan patogennya. Kaedah-kaedah tindakan lima isolat tersebut juga diselidik. Anai-anai tidak mempunyai aktiviti penolak terhadap kelima-lima isolat tersebut. Di antara lima isolat tersebut, isolat TA mempunyai paling potensi yang paling tinggi, diikuti oleh isolat LR2 dan isolat CI mempunyai potensi yang paling rendah Keputusan kajian ultra-struktur menunjukkan bahawa isolat TA memiliki kesan patogenik yang lebih tinggi jika dibandingkan dengan isolat-isolat yang lain kerana percambahan dan penembusan miselianya yang cepat (dalam masa 3 jam) ke dalam tubuh anai-anai, di mana kejadian penjajahan miselia lepas itu berlaku di bawah kulit anai-anai. Di antara isolat TA dan LR2, isolat TA yang bercambah dan menembusi ke dalam badan anai-anai dengan lebih cepat. Oleh kerana demikian, semakin lama isolat tersebut untuk menembus ke dalam badan anai-anai, kemungkinan konidia-konidia isolat untuk dibersihkan dari kulit anai-anai menjadi semakin. Isolat CI adalah isoalt yang paling tidak efektif kerana ia merupakan satu-satu isolat yang mempunyai miselia yang menjajah di atas kulit anai-anai. Oleh kerana demikian miselia tersebut mungkin akan dibersihkan oleh anai-anai yang lain sebelum miselia tersebut dapat menembus ke dalam badan anai-anai. Jumlah kematian anai-anai tidak semestinya disebabkan oleh jangkitan penyakit daripada isolat-isolat. Ia juga disebabkan oleh tingkah laku anai-anai yang disebabkan oleh isolat-isolat tersebut di mana anai-anai tersebut akan menanam dan menguburkan atau memakan ahli-ahli yang lemah atau dijangkiti penyakit. Kaedah applikasi secara langsung, seperti ‘drenching’ dan aplikasi topikal adalah sangat efektif tetapi ianya adalah tidak praktikal kecuali semburan dapat

dilakukan pada sarang atau koloni jajahan anai-anai dapat dikenalpasti di kawasan tanah gambut. Oleh itu, dengan menggunkan satu isolat yang berpotensi seperti isolat TA dan LR2, kawalan biologi yang berjaya untuk anai-anai, *C. curvignathus*, pada tanaman kelapa sawit di kawasan tanah gambut boleh dicapai. Namun demikian, peningkatan potensi isolat tersebut dan kaedah applikasi yang sesuai untuk meningkatkan kesan isolat ketika penggunaannya dalam ladang

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I certify that an Examination Committee met on _____ to conduct the final examination of Hoe Pik Kheng on her Master thesis entitled “Effect of Entomopathogenic Fungus, *Metarhizium anisopliae* on *Coptotermes curvignathus* (Blattodea: Rhinotermitidae)” in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Pertanian (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 9 December 2010

DECLARATIONS

I hereby declare that the thesis is based on my original work except for quotations and citation, which have been duly acknowledged. I also declare that it has not been previously or concurrent submitted for any degree at Universiti Putra Malaysia or other institutions.

HOE PIK KHENG

Date: 3 September 2010

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ha	Hectare
e.g.	Example
ODA	Oatmeal Dodine Agar
DTA	Doberski and Tribe Agar
VFA	Veen and Ferron Agar
MAA	*Metarhizium anisopliae* selective media
K_2HPO_4	Dipotassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
MgSO4. $7H_2O$	Magnesium sulphate hydrate
CTAB	hexadecyl tri-methyl ammonium bromide
cm	centimetre
ml	millilitre
^oC	degree Celcius
PDA	Potato Dextrose Agar
PDAY	Potato Dextrose Agar with 1% yeast extract
SDA	Sabaroud Dextrose Agar
SDAY	Sabaroud Dextrose Agar with 1% yeast extract
mm	mililiter
ANOVA	Analysis of Variance
DNA	Deoxy ribonucleic acid
PCR	Polymerase Chain Reaction
$MgCl_2$	Magnesium chloride
dNTP	Deoxy nucleotide triphosphate

µl	Microlitre
TBE	Tris-borate EDTA
EDTA	Ethylenediaminetetraacetic acid
UV	Ultra-violet
BLAST	Basic Local Alignment Search Tool
RH	Relative humidity
DNMRT	Duncan New Multiple Range Test
Var.	variety
v/v	Volume per volume

CHAPTER 1

INTRODUCTION

Coptotermes curvignathus Holmgren, a serious termite pest classified under the subfamily of Rhinotermitidae, causes serious damage in oil palm plantations especially in Malaysia and Indonesia (Cheng *et al*., 2008; Bong and King, 2006; Lim and Silek, 2001; Zulkefli *et al*., 2000; Sudharto *et al*., 1991; Wood, 1968). Termite infestation is a major concern in oil palm plantations especially those that are located in peat areas. This is because the planting of oil palm is extended to the peat areas. In Sarawak, the expansion of oil palm increased from 460,000 ha to nearly 800,000 ha within these five years. This termite species attacks the living tissues and eventually kill the palms. Currently, the control measures of *C. curvignathus* infestation in oil palms is still limited to conventional methods, such as drenching and trunk injection of insecticides (Lim and Silek, 2001; Sudharto *et al*., 1991). These forms of control are not only very costly; but bring adverse effects to the environment as most of the insecticides (chloropyrifos, fipronil, imidacloprid) used are highly soluble in water and are highly toxic. Hence, alternative control strategies are needed to curb the termite infestation problem in oil palm plantations on peat.

Biological control of subterranean termites with pathogenic fungi is a promising alternative to chemical control as it is generally perceived as providing both long-lasting insect control and having less potential for damage to the environment or non-target organisms than chemical

interventions (Castrillo *et al*., 2005; Lord, 2005; Sun *et al*., 2002; Khetan, 2001; Grace, 1997; Hokkanen and Lynch, 1995; Howarth, 1991). Entomopathogenic fungi, such as *Metarhizium anisopliae* (Metchnikoff) Sorokin, have the ability to invade a host upon landing of the fungal conidia on the host by penetrating its cuticle, followed by a series of infection processes (Castrillo *et al*., 2005; Moino Jr. *et al*., 2002; St. Leger *et al*., 1993; St. Leger *et al*., 1991; Goettel *et al*., 1989; Hanel, 1982).

Termites are social insects where individual interacts with one another through several social behaviours like grooming, feeding and other forms of contact communications. These social interactions are important factors for considerations when a biological control agent, such as an entomopathogenic fungus, is being used on the termites. It is during these activities that horizontal transmission of fungal conidia happen, thus creating an epizootic within the colony (Sun *et al*., 2002; Rosengaus and Tranillo; 1997; Grace and Zoberi, 1992; Kramm *et al*., 1982). However, termites also have defensive mechanisms such as allogrooming and necrophagy that halt the spread of conidia and disease within the colony (Yanagawa and Shimizu, 2007; Zoberi, 1995; Logan *et al*., 1990; Rosengaus *et al*., 1988b; Su *et al*., 1982). Termite excretions, like fecal pellets or sheeting, and volatiles are also suggested to reduce the fungal growth (Wright *et al*., 2000; Chen *et al*., 1998; Rosengaus *et al*., 1998a).

During the application of a biological control agent, the termites' social behaviours like grooming and trophallaxis were needed. However, some of the termites' behaviours like allogrooming, necrophagy and several cellular

defence mechanisms will curb the spread of fungal disease (Chouvenc *et al*., 2009a; Chouvenc *et al*., 2009b; Yanagawa *et al*., 2009; Yanagawa *et al*., 2008; Yanagawa and Shimizu, 2007; Wang and Powell, 2004; Myles, 2002a; Rosengaus *et al*., 1998a). Hence, the search for an entomopathogen that can withstand the harsh local environment of the peat soil and cope with the defence mechanisms of the termites in the field colony and thus initiate disease epizootics is essential and must be taken into account. The best fungal pathogens for termite control must be adapted for the living environment and have characteristics like quick and high sporulation (Sun *et al*., 2002; Goettel *et al*., 2000; Fuxa, 1989). Most species or isolates within species of the fungal pathogens like entomopathogenic fungi behave very differently and vary in terms of insect host range, infection levels, conidial germination rates and temperature requirements (Shah and Pell, 2003; Shaw *et al*., 2002; Pell *et al*., 2001; Sierotzki *et al*., 2000). The infection process of each entomopathogen varies and it is essential to recognize the process as it contributes to the virulence of an isolate. It is also essential to find a suitable application technique for the entomopathogen, *M. anisopliae* in the field to enhance the effect of the potential isolates.

Thus, the main objective of this research was to find a suitable *M. anisopliae* isolate as an alternative method to control *C. curvignathus* in oil palm plantations on peat. This objectives can be achieved by the following specific objectives 1) to isolate and characterize of local isolates of *M. anisopliae,* 2) to evaluate the virulence of locally isolated *M. anisopliae* against *C. curvignathus*, 3) to study the defence behaviour of *C. curvignathus* against

locally isolated *M. anisopliae, 4)* to study the infection process that contribute to the virulence of potential isolates of *M. anisopliae* and 5) to evaluate several delivery methods of locally isolated *M. anisopliae* in laboratory trials.

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