THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Biotechnology)

DEGREE

Biotechnology   Biotechnology

FIELD   **DEPARTMENT**

TITLE: Entomopathogenic Fungi in the Southern Area of Cat Tien National Park, Vietnam and Cordycepin Production in Selected Species from Japan, Thailand and Vietnam

NAME: Mr. Le Tan Hung

THIS THESIS HAS BEEN ACCEPTED BY

_____ THESIS ADVISOR

(Assistant Professor Suttipun Keawsompong, Ph.D.   )

_____ THESIS CO-ADVISOR

(Mr. Somsak Sivichai, Ph.D.   )

_____ DEPARTMENT HEAD

(Associate Professor Sunee Nitisinprasert, D.Sc.   )
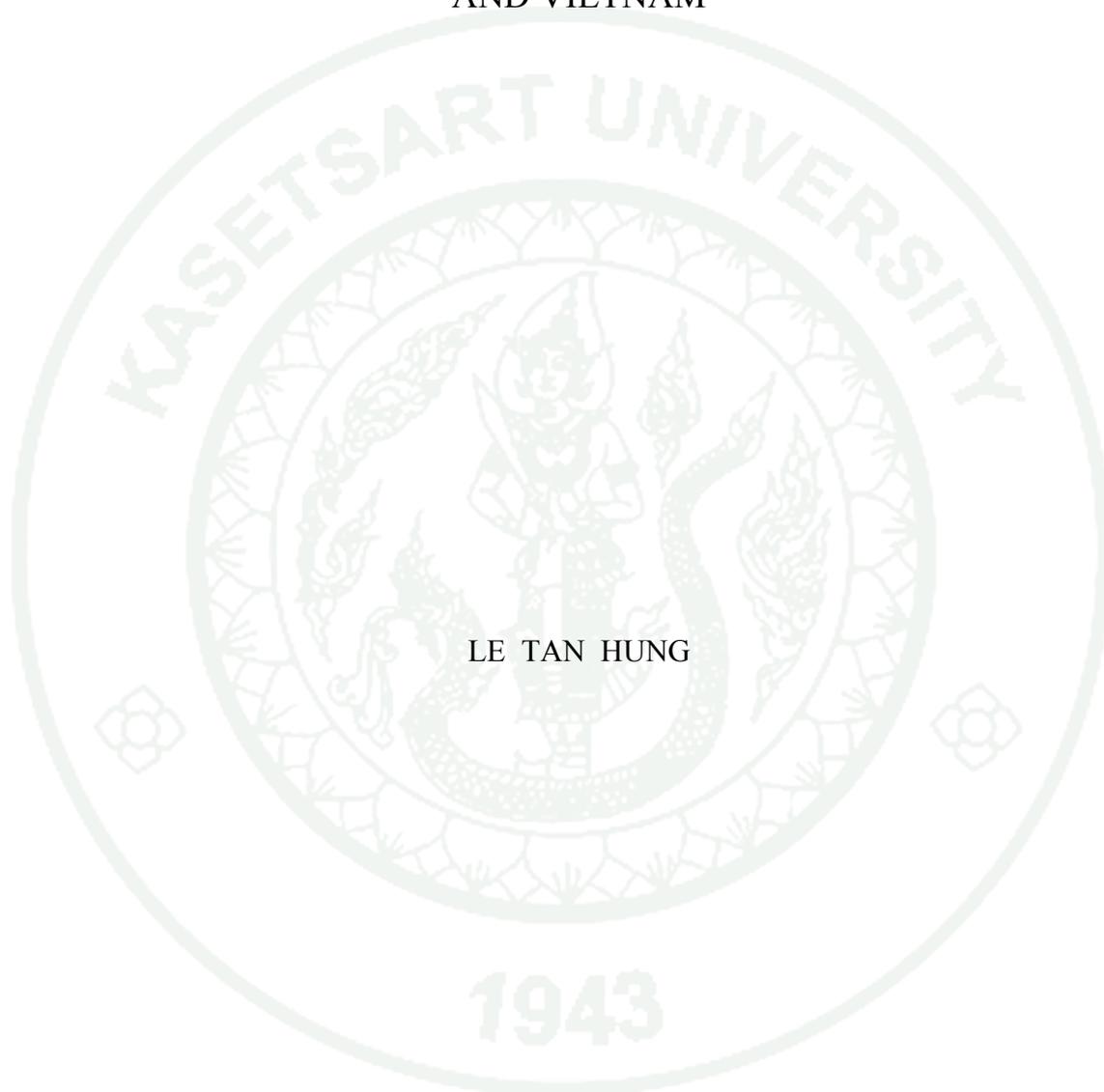

APPROVED BY THE GRADUATE SCHOOL ON _____

_____ DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

ENTOMOPATHOGENIC FUNGI IN THE SOUTHERN AREA OF
CAT TIEN NATIONAL PARK, VIETNAM AND CORDYCEPIN
PRODUCTION IN SELECTED SPECIES FROM JAPAN, THAILAND
AND VIETNAM

LE TAN HUNG

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Biotechnology)
Graduate School, Kasetsart University
2010

Le Tan Hung 2010: Entomopathogenic Fungi in the Southern Area of Cat Tien National Park, Vietnam and Cordycepin Production in Selected Species from Japan, Thailand and Vietnam. Master of Science (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Assistant Professor Suttipun Keawsompong, Ph.D. 200 pages.

Cat Tien National Park of Vietnam has members of entomopathogenic fungi, which are important for use in biological control of insect pests and production of bioactive compounds. A survey and collection was conducted in September 2007, 259 fungal specimens were collected and 41 species that belong to 17 genera were recorded. Regarding to fungal distribution, 84.9% specimens were on leaves of plants, 14.7% was on forest floor and only 0.4% was on tree twig. In addition, the availability of anamorphic (asexual) stage was higher that of teleomorphic (sexual) stage, and 8 arthropod Orders were recorded to be hosts of fungal entomopathogens. In laboratory, 149 cultures were successfully isolated and long-term preserved, which will be potential source for further applications. Cordycepin, a nucleoside analogue (3'-deoxyadenosine), is an important bioactive compound in *Cordyceps* products and has broad spectrum of biological activities. The current study on 35 cultures of 32 species origins of Japan, Thailand and Vietnam found that only Japanese strain, *Cordyceps militaris* BCC 2816, could secrete cordycepin into PDB and PG culture media. Further investigation on 15 *C. militaris* strains indicated that the highest cordycepin yields, 587.68 ± 16.82 mg/l and 544.82 ± 99.80 mg/l, were from two strains *C. militaris* BCC 2819 and *C. militaris* BCC 2816, respectively, at 25°C. Cordycepin quantities of other *C. militaris* strains were significant lower at the same condition. On the effect of temperature, fermentation temperature from 15°C to 20°C promoted growth however the suitable temperature for cordycepin production was 25°C and higher temperature at 30°C inhibited cordycepin production in consequence of growth inhibition. In conclusion, *C. militaris* BCC 2819 and *C. militaris* BCC 2816 are highly recommended in cordycepin production for commercial purpose.

_____   _____   ___ / ___ / ___
Student's signature   Thesis Advisor's signature

ACKNOWLEDGMENTS

First and foremost I offer my sincerest gratitude to my advisor, Assistant Professor Dr. Suttipun Kaewsompong, and my co-advisor, Dr. Somsak Sivichai, for their valuable advice, continuous guidance, helpful suggestions, kindness and encouragement throughout my study that help me to complete my Master thesis.

I owe my deepest gratitude to Dr. Nigel L. Hywel-Jones who created great ideas and provided valuable advices for my study. I also would like to show my gratitude to Professor Dr. Morakot Tanticharoen and Dr. Masahiko Isaka for their helpful comments and suggestions in my research.

I would like to thank the Board of Directors of National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand and the Board of Directors of Institute of Tropical Biology (ITB), Vietnam for financial supports.

Thanks are also given to all members and fellows in the Department of Biotechnology, Faculty of Agro-Industry for their distinguishable cheerfulness and cooperation.

I would like to thank Ms. Kanoksri Tasanatai, Mrs. Suchada Mongkolsamrit, Mrs. Rachada Promharn, Ms. Wiwantanee Chaygate, Mr. Boonchan Thongnuch, Dr**.** Janet Jennifer Luangsa-ard and staffs other in BIOTEC for their kindness, encouragement, support and friendship.

I am indebted to Dr. Vo Thi Hanh, Mrs. Le Bich Phuong and my colleagues to support me throughout many years. Lastly, but in no sense the least, I express my thanks and appreciation to my family for their understanding, motivation and patience.

Le Tan Hung
March, 2010

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

ANOVA	=	Analysis of variance
BBH	=	BIOTEC Bangkok Herbarium
BCC	=	BIOTEC Culture Collection
BIOTEC	=	National Center for Genetic Engineering and Biotechnology, Thailand
CE	=	Capillary Electrophoresis
CTNP	=	Cat Tien National Park
CZE	=	Capillary Zone Electrophoresis
DW	=	dry weight
HPLC	=	High Performance Liquid Chromatography
ITB	=	Institute of Tropical Biology
LC/ESI–MS	=	Liquid Chromatography Specifically Coupled with Mass Spectrometry
MIMS	=	Microbial Information Management System
PDA	=	Potato Dextrose Agar
PDB	=	Potato Dextrose Broth
pers. observ.	=	personal observation
PG	=	peptone glucose medium
SD	=	Standard Deviation
TLC	=	Thin Layer Chromatography

ENTOMOPATHOGENIC FUNGI IN THE SOUTHERN AREA OF CAT TIEN NATIONAL PARK, VIETNAM AND CORDYCEPIN PRODUCTION IN SELECTED SPECIES FROM JAPAN, THAILAND AND VIETNAM

INTRODUCTION

Entomopathogenic fungi belong to a particular fungal group that can infect healthy insects and are proven pathogen of insects or for which circumstantial evidence exists concerning their pathogenicity. These fungi usually attach to the external body surface of insects in the form of microscopic spores. Under permissive conditions of temperature and moisture, these spores germinate, grow as hyphae and colonize the insect's body cavity. Many species of entomopathogenic fungi are very important for biological control of insect and other arthropod pests (e.g. species of *Beauveria*, *Metarhizium*, *Paecilomyces* and others). *Ophiocordyceps sinensis* is a famous caterpillar fungus in traditional Chinese medicine, it has long been used in folk medicine throughout the Orient. Some other *Cordyceps* species are sources of biochemicals with interesting biological and pharmacological properties. The anamorph of *Cordyceps subsessilis* (*Tolypocladium inflatum*) was the source of cyclosporine, a drug helpful in human organ transplants, as it suppresses the immune system.

Over 750 species of entomopathogenic fungi are known to science (Hall and Menn, 1999), most of species are found from Asian countries. Thailand is reported to have highest number of entomopathogenic fungi with 321 species recorded (Hywel-Jones, 2002). The accounts of entomopathogenic fungal species from other nearby tropical countries (e.g. Vietnam) are poorly known. Preliminary survey in 2005 recorded that Cat Tien National Park of Vietnam has a diverse number of entomopathogenic fungal species. Because of the importance in the use of this fungal group, collection, taxonomy and culture collection of entomopathogenic fungi in Cat Tien National Park will provide useful information for further researches and applications.

Cordycepin (3'-deoxyadenosine) is a nucleoside analogue that has been considered importantly in the activity of *Ophiocordyceps sinensis* as an herbal medicine. Cordycepin was first described from the species *Cordyceps militaris* (Cunningham *et al.*, 1950) then later found to be presented in *Ophiocordyceps sinensis* (Hsu and Lo., 2002) and *Cordyceps kyushuensis* (Ling *et al.*, 2002). Cordycepin has a broad spectrum of biological activities including immunological stimulating (Zhou *et al.*, 2002), anti-cancer (Thomadaki *et al.*, 2005; Penman and Rosbach, 1970), anti-viral (Mueller *et al.*, 1991; de Julian-Ortiz *et al.*, 1999), anti-baterial (Ahn at al., 2000) anti-malarial (Trigg *et al.*, 1971), and anti-fungal activities (Sugar and McCaffrey, 1998). Recently, all studies on cordycepin production are concentrated only on two species, *Cordyceps militaris* and *Ophiocordyceps sinensis,* while the abilities of cordycepin production from other species members of entomopathogenic fungi have not yet been reported.

In this study, survey trip was made in Cat Tien National Park, Dong Nai Province of Vietnam to investigate the diversity and distribution of entomopathogenic fungi in this forest. Specimen samples were collected, isolated and identified to the genus and species levels basing on their morphologies. In addition, 35 entomopathogenic fungal cultures originated from Japan, Thailand and Vietnam were selected for screening cordycepin production. Fifteen *Cordyceps militaris* strains collected from Japan were also used to investigate the temperature effect on growth and cordycepin production. From this result, two *Cordyceps militaris* strains with highest capacities of cordycepin production are recommended for use in future research and application.

OBJECTIVES

The specific objectives of this project are as follows:

1. To collect, isolate and identify entomopathogenic fungi in the southern area of Cat Tien National Park, Dong Nai Province, Vietnam.

2. To study diversity of entomopathogenic fungi in the south of Cat Tien National Park.

3. To investigate the abilities of cordycepin production of 35 selected entomopathogenic fungal cultures originated from Japan, Thailand and Vietnam.

4. To investigate the effect of different temperature conditions on mycelium growth and cordycepin production of fifteen *Cordyceps militaris* strains. From the result, high capacity strains will be recommended for use in future research and application.

LITERATURE REVIEW

1. Entomopathogenic fungi

1.1 Definition of entomopathogenic fungi

In the nature, the association of fungi with insects and other arthropods can be either facultatively or obligatory. In this association, the relationship between fungi and arthropods ranges from symbiosis to pathogenesis. The term "Entomogenous Fungi" which used by scientists relates to fungi growing on or colonising in insect substrates. According to Evans (1988), entomogenous fungi can be divided into four groups: (1) the mutualistic symbionts are in the group of fungi that live on insect hosts and both of those gain benefits from each other in a mutualistic relationship. *Termitomyces* species are examples, these fungi grow on 'combs' which are excreta from the termites, dominated by tough woody fragments, absorb nutrients from the insect host but reciprocate some benefit functions; (2) endoparasites belong to a fungal group living confined in internal integuments of intestinal track of their insect hosts. These fungi absorb nutrients from the body fluids of a host organism and cause to the detriment of the host; (3) ectoparasites are within fungal group that usually develops on the exterior (cuticle) of insects and cause little damage to their host; and (4) entomopathogens are those which are directly or indirectly proven pathogens to their insect hosts.

Like most fungal pathogens of plants, entomopathogens infect their host through the external cuticle of a healthy host. Hence, the term "entomopathogenic fungi" has been used to correspond to the presence of fungal species within the insect bodies or grow on the surface of their exoskeleton within the insects or may be pathogenic to them. These include genera or species which are proven pathogens of insects or for which circumstantial evidence exists concerning their pathogenicity (Samson *et al.*, 1988). According to Samson (1988), the term "entomopathogenic fungi" is restricted to those fungal genera or species which are proven pathogens of insects with their pathogenicity. Therefore, the fungal species growing or colonising

on insect substrates, which are strictly non-pathogenic, are avoided in the entomopathogenic fungal group.

Entomopathogenic fungi can further be categorised into primary and secondary pathogens (Pu & Li, 1996). Primary pathogens are fungal genera or species that infect healthy insect hosts while secondary pathogens are those which can only infect weak or injured hosts. Secondary pathogens which parasitize aged or weak hosts are called facultative or opportunistic pathogens.

Apart from these, the obligate pathogens of spiders and other arthropods are included here in the treatments of the entomopathogenic fungi. Many of them are related to pathogens on insects and they exploit a similar ecological niche and hence face the same problems of host penetration, colonisation and preservation in addition to sporulation on and dissemination from the host. Therefore, the studies of entomopathogenic fungi will include the fungal pathogens of spiders.

1.2 Historical research perspective

Ophiocordyceps sinensis (Berk.) Sacc., known in Chinese as "Dong Chong Xia Cao" and Japanese as "Tosukacho" which can be translated literally as "winter-worm, summer-grass", is a famous Chinese medicinal fungus which has been discovered and used by Chinese since ancient time. The reviews of Steinhaus (1956, 1975) demonstrated the early history of entomopathogenic fungi about the problems of muscardine silkworm, the first observed insect disease by the Japanese Royal Family, infected by *Beauveria bassiana* in 900s A.D. Until 1726, the first publication which illustrated fungal pathogen on a Lepidoptera larva was published and probably predicted was *Ophiocordyceps sinensis* (the Chinese Plant Worm) (Steinhaus, 1956). Later in 1749, a catholic priest Josá Torrubia (1698-1761) recorded the presence of dead wasps in Cuba illustrating what is now recognized as *Ophiocordyceps sphecocephala* (Samson *et al.*, 1988). At that time, he made a compelling drawing and it is still considered worthy in some books. Moreover, there is little information collected of the interaction between fungi and their insect hosts.

During 16th to 17th centuries, silk production was important in France and Italy, however, it had been heavy loss of larval silkworms every year by "muscardine". It was important for insect mycology that in 1835, Agostino Bassi (1773-1856) discovered *Beauveria bassiana* (Bals.) Vuil. This fungus was named after the Italian bacteriologist who found it as the cause of the muscardine disease of domesticated silkworms. At that time, this fungus was causing serious economic losses to the silk industry in Italy. His pioneering study made the basis for the germ tube theory, which is very important in all pathology studies nowadays (Samson *et al.*, 1988). Later works of Elie (Ilya) Metchnikoff (1845 - 1916) published an account of a natural infection of the wheat cockchafer (*Anisoplia austriaca*) by the "green muscardine" (Zimmermann *et al.*, 1995), this was a fungus *Metarhizium anisopliae* Metchnikoff, and provided experimental methods for testing the possibility of controlling insects with fungi.

Entomopathogenic fungi are now well known in research by scientists in all over the world (Chen *et al.*, 2004; Evans and Samson, 1984; Griesch and Vilcinskas, 1998; Hajek and St. Leger, 1994; Hywel-Jones, 1994, 1995a, 1995b, 1996, 2002, and 2004; Main, 1958; McCoy, 1990; Samson *et al.*, 1989; Steinkraus, 1994; Vilcinskas, 1998; Zhu *et al.*, 1998a, b). Over 750 species of entomopathogenic fungi are known to science, many other new species are being described. However, most of the studies of entomopathogenic fungi were concentrated on the adverse effect of these fungi on the commercially important insects, for example, fungal pathogens on silkworm and honeybees (Hywel-Jones, 2002). Few hundred years ago, *Metarhizium anisopliae* which was used as a biological control agent against beetle larvae (*Anisoplia austriaca* Herbst) in Russia (Metschnikoff, 1879) became the potential for biological control of insect pests. Then, the researches on biology and taxonomy of many entomopathogenic fungal groups started to be considered. Recently, studies of entomopathogenic fungi have concentrated on molecular aspect to find out the relationships within insect fungal groups and its relationships with other fungal groups (Chen *et al.*, 2004; Spatafora *et al.*, 2007; Stensrud *et al.*, 2005; Sung *et al.*, 2001, 2007a,b). The results have elucidated significant characteristics that bring out helpfully for classification problems.

1.3 Taxonomy and classification of entomopathogenic fungi

For a long time, taxonomists considered fungi to the Plant Kingdom because they based on the "lifestyle" of fungi. Both fungi and plants have similarities in their general morphology and habitat. Moreover, the difference between Animal to those fungi and plant in presenting of cell wall (Shoji *et al.*, 2006), which character was considered to group fungi into Plant Kingdom (Bowman and Free, 2006). However, fungi were found to be different from Plant in an absent of chlorophylls (Wu *et al.*, 2007), therefore, fungi do not have photoautotrophic process as plants (Lomako *et al.*, 2004). Fungi are also non-motile, for this reason, it is distinct from Animal. The Fungi is now considered as a separate Kingdom away from Animal or Plant. So far, study identified several distinct morphology as well as genetic features of fungi and those results have clearly proved that fungi are belonged to their own Kingdom: Kingdom Fungi (Bruns, 2006).

According to Hawksworth *et al.* (1995), invertebrate-pathogenic fungi can be commensals or mutualists, through ectoparasites, which cause little damage or do not seriously affect their arthropod hosts, to pathogens that can infect healthy host through the cuticle and cause the lethal effect to all the major groups of fungi. As mentioned before, study in entomopathogenic fungi only concerns on fungal group which are completely pathogens of insect and arthropod hosts.

According to Hywel-Jones (2002), there are approximately 3000 species of invertebrate-pathogenic fungi which are associated with insects, spiders, and mites. However, there are only about 750 species considered as entomopathogenic fungi in the fungal kingdom. Most of these species are classified in the divisions Ascomycota and Zygomycota. The entomopathogenic fungal species within the division Ascomycota were formerly divided into two groups, the Ascomycota and the Deuteromycota. Deuteromycota latter were also known as the Fungi Imperfecti. All fungal species which produce asexually reproduction were classified in this group. However, later cultural and molecular studies have demonstrated that some of these "imperfect fungi," (formally class Hyphomycetes in the Deuteromycota) were

anamorphs (asexual forms) of the Ascomycota within the order Hypocreales, family Clavicipitaceae (Fukatzu *et al.*, 1997; Hodge, 2003; Krasnoff *et al.*, 1995; Shimazu *et al.*, 1998).

Within the Zygomycota most entomopathogenic species are in the order Entomophthorales. According to Hywel-Jones, fungal species of the Zygomycota are thought to be the most primitive of the terrestrial fungi (Hywel-Jones, 2002). The species of Zygomycota have the diagnostic feature that it produces thick-walled zygospores in sexual reproduction. Within the sub-division Zygomycota, there are two classes recognized, the Trichomycetes and Zygomycetes. These two classes have 15 genera that contain important invertebrate pathogens, i.e. *Entomophthora* species (Misra & Horn, 2001).

The taxonomic classification of ascomycetous entomopathogenic fungi have been considered in research of mycologists for many years. The taxonomy of entomopathogenic fungi of Ascomycota bases meanly on the macro- and microscopic features and these characters play important roles in their classification (Rogerson, 1970; Samson *et al.*, 1988). However, this method of classification is now becoming increasingly inappropriate when the molecular techniques are becoming more advantageous. Some of the entomopathogenic fungi within this division have lost their capacity to produce mitotic stage while others can produce anamorphs in their life cycles. Ascomycota contains more than 56 orders (Kirk *et al.*, 2001); within these, there are two important orders, the Laboulbeniales and the Hypocreales, which contain most entomopathogens. While for the anamorphic entomopathogens, e.g. *Aschersonia* (anamorph of *Hypocrella*), *Metarhizium* (anamorph of *Cordyceps*), *Paecilomyces* (anamorph of *Cordyceps*), etc., conidiomata, conidia, conidiogenous cell characteristics, and conidiogenesis are critical for identification (Tzean *et al.*, 1997).

Basidiomycota is one of two large phyla together with the Ascomycota. This Phylum includes fungi that involve the production of haploid basidiospores on basidia during sexual reproduction. There are over 30 orders of fungi recorded in this

group (Kirk *et al.*, 2001). However, for basidiomycetous entomopathogens, only two genera, *Septobasidium* and *Uredinella*, within the order Septobasidiales contain invertebrate pathogenic fungi. These genera are restricted to the immobile larval stages of homopteran scale insect (Hywel-Jones, 2002).

1.3.1 Morphological classification

Entomopathogenic fungi, likes other fungal groups, have complicated morphological characteristics. However, morphological concept is now a main and important way to identify entomopathogenic fungi. The morphological classification has been used for a long time to identify fungi based on their characteristics and features. Until in 17th century, the investment of microscope by Leeuwenhoek made an epoch to develop the taxonomy of entomopathogenic fungi and morphological classification of the entomopathogenic fungi has received increased interest since that time (Samson *et al.*, 1988). According to Samson *et al.* (1988), the majority of the fungi parasitic on arthropods can be readily recognized because the taxonomic criteria of separating the species are mostly based on morphological features.

Macroscopic examinations are very important for further step. For the macroscopic characterization, looking on the host is the necessary step; it can help to recognize the kind of host the way that the fungus develops from host. Some fungal species have wide range of hosts, however, many other species have specific host and it is an important feature to record information for further identification. Other features, such as size, shape and colour of fungus, are also keys to identify fungus. Fungal specimens can be observed either by eyes or by using a simple microscopic mount. By this way, we can observe the mode of reproduction; sexual or asexual reproduction is a key basic for classifying fungi. The genus *Cordyceps,* for example, has one or more rising stromatic clavae with erect and columnar shape, the fertile portion present on clava with flask-shaped ascomata immersed or superficial in/on stroma. It differs from *Cordyceps,* the genus *Torrubiella* almost does not have these characteristics, species of *Torrubiella* have flask-shaped ascomata arise directly

from mycelia covering host. In almost species, recognising the reproduction region (conidia or ascospores) directly on host helps to isolate of the fungi on artificial media and also be helpful for determination under microscopic approach.

Microscopic examinations can be made in order to identify fungi to different genera and/or species levels. With a compound microscope at a low or high magnification levels, many morphological features can be observed. Spore producing structures, spore size and shape can be recognized and measured. In some teleomorphic genera (such as *Cordyceps, Torrubiella, Hypocrella,* etc.), the examinations of asci and sexual forming structures are very important. Size, shape, or pattern of developing reproduction structures are varied from species to species within genus. Some species form ascospores in whole, whereas, other species have fragments on ascospores. According to Hywel-Jones (1994), two species *Cordyceps militaris* and *Cordyceps pseudomilitaris* have similar characteristics of host, development and colour of stromata when observing on specimens. However, when looking their ascospores under a microscope, *Cordyceps militaris* has whole-ascospores whereas *Cordyceps pseudomilitaris* has part-spores. This is also a basic morphological feature to separate two species. In anamorphic species, macroscopic observation is more difficult to recognize. Characterisations of conidia and other reproduction structures are the most important way to classify into different genera or species.

The asexually reproducing phase of the ascomycete was more or less ignored for many years in favour of teleomorph studies (Kendrick, 2000). However, it was considered that the anamorph is an important phenotypic expression of many ascomycete genotypes. Besides, when scientists can get DNA and RNA from anamorphs just as easily as from teleomorphs, so scientists are beginning to understand the relationships of anamorphs better. Of about 30,000 known ascomycetes, about 5,000 have so far been connected to their anamorphs (Kendrick, 2000).

1.3.2 Genetic classification

As discussed from previous section, morphological classification of entomopathogenic fungi is based on several characteristics. Host insect is also very important for classification basing on this concept. However, the applicability of hosts as a taxonomic character is complicated, due to the difficulty in identifying immature hosts (e.g., larvae and pupae) and insufficient host identification for many herbarium collections (Sung *et al.* 2007a). Several phylogenetic studies employing ribosomal DNA (Artjariyasripong *et al.* 2001, Sung *et al.*, 2001, Stensrud *et al.* 2005) have been conducted to test and refine the classification of *Cordyceps* and other genera of entomopathogenic fungi. According to Sung *et al.* (2007b), these phylogenetic studies were restricted by both limited taxon sampling and the inadequate resolution power of ribosomal DNA, resulting in limited conclusions regarding the systematics of the genus. Recent phylogenetic studies (Spatafora *et al.* 2007, Sung *et al.* 2007a,b) based on multiple independent loci provided a greater level of resolution and support, and revealed that neither *Cordyceps* nor the family *Clavicipitaceae* is monophyletic. Sung *et al.* (2007b) have successfully conducted the most extensive multi-gene phylogenetic analyses to provide a basis for the phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. This study provided the phylogenetic relationships of 162 taxa estimated based on analyses consisting of five to seven loci, including the nuclear ribosomal small and large subunits (*nrSSU* and *nrLSU*), the elongation factor 1α (*tef1*), the largest and the second largest subunits of RNA polymerase II (*rpb1* and *rpb2*), β-tubulin (*tub*), and mitochondrial ATP6 (*atp6*), these results strongly support the existence of three clavicipitaceous clades and reject the monophyly of both *Cordyceps* and *Clavicipitaceae* (Sung *et al.* 2007b). Two new species have been described, and lists of accepted names for species in *Cordyceps, Elaphocordyceps*, *Metacordyceps* and *Ophiocordyceps* have been provided. Therefore, a new classification of *Cordyceps* and the *Clavicipitaceae* is necessary to reflect the current hypotheses of phylogenetic relationships and to be predictive in nature.

Figure 1 New classification of *Cordyceps* and clavicipitaceous fungi based on
Bayesian consensus tree (Sung *et al.,* 2007b).

Base on morphological concept, *Torrubiella*, a genus of arthropod-pathogenic fungi that primarily attacks spiders and scale insects, was formerly classified in Clavicipitaceae (Hypocreales) of the old classification system and was also considered a close relative of *Cordyceps*, which was recently reclassified into three families (Clavicipitaceae, Cordycipitaceae, Ophiocordycipitaceae) and four genera (*Cordyceps*, *Elaphocordyceps*, *Metacordyceps*, and *Ophiocordyceps*) (Sung *et al.*, 2007). However, in the results of recently multi-gene phylogenetic classification, the monophyly of *Torrubiella* was rejected by the analyses with species of the genus present in Clavicipitaceae, Cordycipitaceae, and Ophiocordycipitaceae (Johnson *et al.,* 2009). The morphological characters that traditionally used to define the genus, therefore, not phylogenetically informative, with the stipitate stromata being gained and/or lost several times among clavicipitaceous fungi. From the results, two new genera (*Conoideocrella*, *Orbiocrella*) are proposed to accommodate two separate lineages of torrubielloid fungi in the Clavicipitaceae. In addition, one species is reclassified in *Cordyceps* and three are reclassified in *Ophiocordyceps*.

Similar to "*Cordyceps*" and "*Torrubiella*", the monophyly of the genus *Hypocrella* was also rejected. The genus Hypocrella (Ascomycota; Hypocreales; Clavicipitaceae) are insect pathogens characterised by their brightlycoloured stromata, filiform ascospores, and pycnidial to acervular anamorphs and are pathogens of scale insects (Coccidae and Lecaniidae, Homoptera) and whiteflies (Aleyrodidae, Homoptera). The recently phylogenetic analyses have revised *"Hypocrella"* into three genera by DNA sequences from nuclear ribosomal large subunit (28S), translation elongation factor 1-α (TEF 1-α), and RNA polymerase II subunit 1 (RPB1): *Hypocrella, Moelleriella,* and *Samuelsia* (Chaverri *et al.* 2008). Later work, Mongkolsamrit (2009) revealed new species of *Hypocrella* and *Aschersonia* (*Hypocrella siamensis* and *Aschersonia minutispora*) related to the type species *Hypocrella discoidea* from natural forest in Thailand by using a combined ITS and β-tubulin gene phylogeny.

1.4 Diversity and distribution of entomopathogenic fungi

Entomopathogenic fungi are common natural enemies of arthropods worldwide, attracting attention as potential biological control agents (Hajek and St. Leger, 1994; Shah and Pell, 2003). Fundamental research has focused on many theoretical and practical aspects of their biology, physiology, ecology, and epidemiology, but predominantly from the viewpoint of their potential in host population regulation.

The distribution and diversity of entomopathogenic fungi depend on environmental conditions, the opportunity (insect diversity) and the stage of the insect (Hajek and St. Leger, 1994). It was reported that the fungus is most effective at 20°C and 25°C, frequently present during autumn or in temperate weather, these climates also enhance the infectivity and germination of entomopathogenic fungi (Samson *et al.*, 1988). Fungal growth is favoured by moist conditions; humidity at relative range of 75 – 100 % is suitable for fungi to infect their host. Fungi also have resistant stages that maintain infection potential under dry condition.

The dense and diverse host populations are also favourable for their fungal pathogens. An entomopathogenic fungus will get more opportunities to infect its host in high-density host condition. Cool and humid weather in temperate countries also makes the dense and diverse species of insect. Therefore, there is no surprising when numbers of invertebrate pathogenic fungi are distributed in temperate countries (Mains, 1958; Tzean *et al.*, 1997).

Some insect species, including many pests, are particularly susceptible to infection by naturally occurring, these fungi are very specific to insects, often to particular species, and do not infect animals or plants (Hoffmann and Frodsham, 1993; Tanada *et al.*, 1993). There are many entomopathogenic fungi infect specific insect hosts. Some others can infect wide range of hosts and are usually found in man-made ecosystems such as *Metarhizium anisopliae* and *Beauveria bassiana*. *Metarhizium anisopliae* is reported to be capable of infecting more than 100 different

insect species belonging to a variety of insect orders (McCoy *et al.*, 1988; Mahr, 1997).

With these characteristics, entomopathogenic fungi may have wide geographical distribution around the world. However, little effort has been put in to describing of entomopathogenic fungi from natural habitats. Most numbers of entomopathogenic fungal species were found in Asia. Thailand has been known as the richest in terms of numbers of entomopathogenic fungal species. There were 321 species recorded in Thailand (Hywel-Jones, 2002). Until now, the number of entomopathogenic fungal species recorded in Thailand are over 400 in which many species are recorded as new and are in the process of description (unpubl.), but little records are available in nearby tropical countries, e.g. Malaysia or Vietnam. Recently, survey in Malaysia and Hong Kong recorded the presence of number of entomopathogenic fungal species (Kwong, 2003).

1.5 Association of entomopathogenic fungi and their arthropod hosts

Entomopathogenic fungi are associated with insects living in diverse habitats, including fresh water, soil, soil surfaces, and aerial locations (Hajek and St. Leger, 1994). Fungal pathogens of insects are common and widespread and it causes the decimation of the insect populations. Virtually, all insect orders can be susceptible to fungal diseases. Fungi can infect insects by breaching the host cuticle.

Even all insect orders are found to be associated with fungi, however, only some groups of arthropods found to be more susceptible than others, especially, the entomopathogenic fungi group. It is proved that some arthropod orders such as Coleoptera, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Orthoptera, Araneae, etc. are more susceptible than other groups. Insects infected by entomopathogenic fungi are suggested to be those with a close association with plants and that most of these fungi should be evolved from plant-pathogenic fungi (Hywel-Jones, 2002).

Until now, only little information concerns about the coevolution of entomopathogenic fungi and their insect hosts. It is reported that the evolution of species in the genus *Cordyceps* is related to the evolution of their hosts (Nikoh and Fukatsu, 2001). The term "coevolution" was first developed by Mode (Mode, 1958) to describe the influence interacting species have on one another through evolutionary time. Roy *et al.* (2006) described that the pathogen and host adapt to maximize their reproductive output and ultimate fitness by adopting physiological, ecological, and behavioural adaptations that are as diverse as the organisms themselves.

It is predicted that host-specific pathogens engage in a tight process of coevolution e.g. *Nomuraea rileyi* infects only lepidopteran larvae (Vimala Devi *et al.*, 1996), and *Hypocrella* (or *Aschersonia*) species infect scale insects (Homoptera). Changes in host behaviour reflect these diverse relationships and enable us to begin to address whether these are pathogen-induced, host-mediated, or incidental (Roy *et al.*, 2006). Poulin suggests that rigorous criteria are needed to decipher whether a behavioural modification is adaptive (Poulin, 1995). Changes of host behaviour are including the changes of reproductive behaviour, feeding behaviour, and social behaviour (Roy *et al.*, 2006). Fever is a common host response to many pathogens. Observations on fungal-infected grasshoppers and caterpillars demonstrated the benefits of raising body temperature because basking at elevated positions reduced pathogen-induced mortality (Carruthers *et al.*, 1992; Marikovsky, 1962). Watson and co-workers studied the behavioural fever infected with *Entomophthora muscae* and found that in the first few days of infection, house flies seek temperatures in excess of 40°C and benefit from this preference by suppressing the pathogen (Watson *et al.*, 1993).

It is demonstrated that survival time of infected house flies increased if they were exposed to high temperatures shortly after exposure to fungus inoculum. After insect is infected by entomopathogenic fungi, the reduction of feeding occurs. This is the change of insect behaviour by the infection of fungal pathogen. Most studies investigating insect species infected with *Metarhizium* sp. or *Beauveria* sp.,

have demonstrated a significant reduction in feeding as early as 1 to 4 days after inoculation (Tefera and Pringle, 2003).

1.6 Reproduction and life cycle of entomopathogenic fungi

Entomopathogenic fungi can attack a wide range of insects and spiders, however, individual species and strains of fungus are more specific. While some species appear to be generalists, such as *Beauveria bassiana*, other species have narrow host ranges, such as *Hirsutella thompsonii*. Entomopathogenic fungi produce spores, which infect their host insect by germinating on its surface and then growing into its body. The death takes between four to ten days, depends on the type of fungus and number of infecting spores. After death, the fungus produces thousands of new spores on the dead body, which disperse and continue their life cycle on new hosts.

According to Pu and Li (1996), there are three important elements required for the occurrence and development of pathogenesis of entomopathogenic fungi: (1) the ability for the pathogens to infect and cause disease to the insect hosts; (2) the capability of the insect itself to offer defence from the pathogens; and (3) the favourable environmental conditions for the infection to take place. The invasion process of insect pathogen was described by Taborsky (Figure 2) based on the *Metarhizium anisopliae* fungal life cycle. This process can be divided in to three following steps:

1.6.1 Adhesion of the spore to the cuticle

Adhesion appears to be a prerequisite for successful invasion as noted for hypovirulent strains of *Metarhizium anisopliae*. Dillon and Charnley (1989) showed that germination of *Metarhizium anisopliae* is initiated by water but progress to the first overt stage of germination (swelling) is depended on an exogenous nutrient. Prior exposure to water "soaking" synchronized and accelerated swelling, germ tube and appressorial formation when a nutrient was finally provided. It was proved that soak spores were significantly more pathogenic than the controls (Hassan

et al. 1989). Vegetative spores "conidia" are strong hydrophobic and keep their viability for more than two weeks after spraying on target insects (Taborsky, 1992). For germination of *Metarhizium anisopliae* conidia by the test of viability is possible, then put into drop on microscopy slides with special nutrient like orange juice (0.05%). Samson *et al.* (1988) described that the soak (wet) spores bind to the cuticle using the mucilaginous matrix surrounding them, while the dry spores employ a combination of electrostatic forces and chemical bonding agents (e.g. lipoproteins), which facilitate attachment to the hydrophobic, lipophilic epicuticle. Current evidence for *Metarhizium anisopliae* suggested that differentiation of appressoria is strictly governed by the concentration of low molecular weight nitrogen compounds on a conductive surface (Taborsky, 1992). The appressorium releases extracellular enzymes such as lipases which improve the attachment and provide nutrients for further enzyme (especially proteases and chitinases) production. The combination of enzymes together with the physical pressure induce the resultant appressorium bores through the cuticular layers, therefore the fungus can infect in to the insect body through its cuticle.

1.6.2 Penetration of the host cuticle

The successful germination of a germ-tube on the host epicuticle does not mean that it will bring infection. Germinated spore needs to be provided specific nutrients in order to continue the penetration process (St. Leger, 1995). Penetration of host exoskeleton appears to involve both mechanical and enzyme components (Goettel *et al.*, 1989). In soft cuticles, e.g. caterpillars, growth across the cuticle is more or less direct, in hard cuticles e.g. wireworms the fungus proceeds in a step-wise fashion (Taborsky, 1992). The invasion of the fungi to the host body through insect cuticle depends on the ability of the germ-tube to penetrate the epicuticle, endocuticle and procuticle. It was discovered that many entomopathogens can produce a range of cuticle-degrading enzymes corresponding to the diverse polymers in insect cuticle, including protein, chitin, and lipids (St. Leger *et al.*, 1986a; Charnley & St. Leger, 1991). The exocuticle of insect is composed of chitin, protein and phenols while the endocuticle is consisted of chitin and protein. Therefore, the

enzymatic dissolution of this complex laminated glycoprotein is more complicated and thus the penetration through the procuticle is relatively slower than penetration through the epicuticle (Evans, 1988).
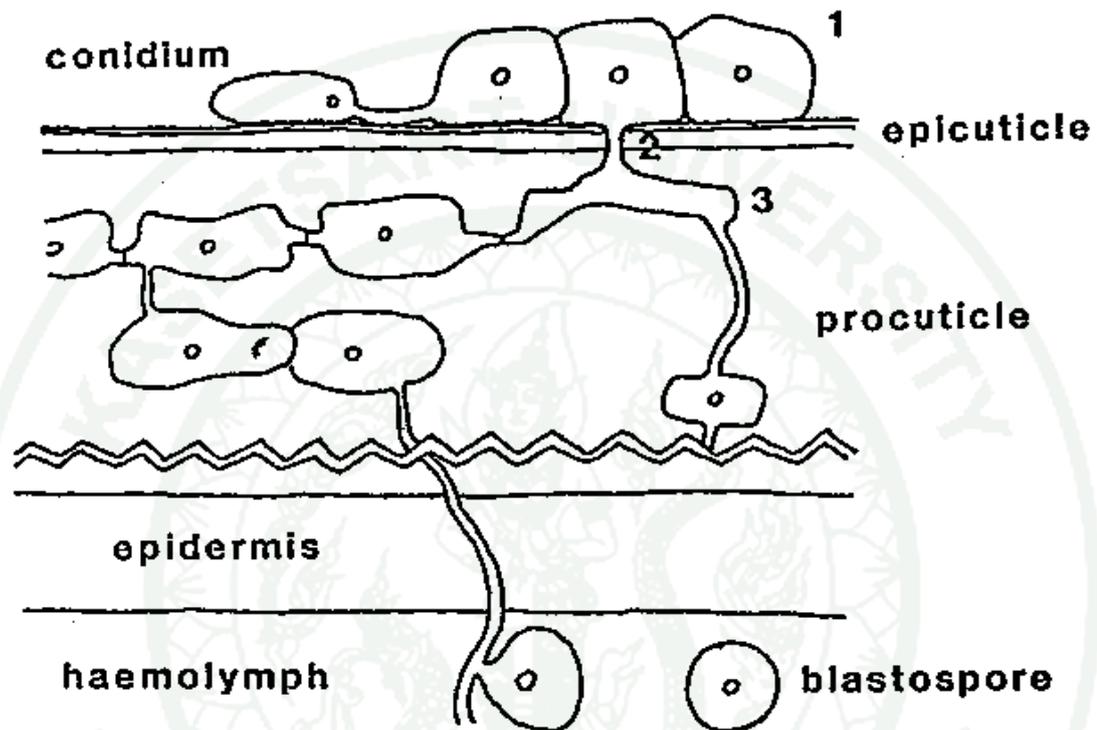
Figure 2 Penetration of host cuticle by an anamorphic entomopathogens (Taborsky, 1992).

Note: (1) appressorial complex, (2) penetration peg, and (3) penetration plate.

1.6.3 Development of fungus inside insect body and host defence

The development of fungus inside insect body occurs rapidly after fungus infected through the insect cuticle. The development of fungus is found to be in either fungal mycelia or blastospores, or both stages. *Conidiobolus coronatus* (Costantin) Batko after infected host, the mycelium continue to develop. Other fungal species, e.g. *Beauveria bassiana* or *Metarhizium anisopliae,* develop cell mass in their insect host by multiplying of yeast-like cells (blastospores). It was observed that the

blastospores spread throughout the haemocoel and invade the host tissues. The yeast-like blastospores, produced the insect haemolymph, reduces the effectiveness of the cellular defences by sheer weigh of numbers and not being as antigenic as the mycelium (Charnley, 1989).

Since an insect is infected by fungal cells, which can be recognized as an antigen of insect, the insect immune system will act to defence. Deposition of oxidized phenols (melanin) in cuticle by host phenoloxidase is the first overt response to infection. Antimicrobial effects of phenols are well established, but in insect cuticles, melanisation appears to be primarily an effective defence against more virulent pathogens. Protease inhibitors within the cuticle may serve to restrict pathogen enzyme activity (St. Leger *et al.*, 1986b,c). Within the haemocoel, the main cellular response of the insect is a multihaemocytic encapsulation of the fungal element following initial recognition of the fungus by the haemocytes. However, the fungus overcomes this by rapid reproduction of hyphal bodies so as to overwhelm the insect's immune system. Finally, the cyclodepsipeptide toxins, destruxin produced by *Metarhizium anisopliae* appear to interfere with haemocyte function, especially by suppressing prophenoloxidase activation (Huxham *et al.*, 1989). However, recent evidence is consistent with destruxins being a determinant of virulence for *Metarhizium anisopliae*. Destruxins are active in causing symptoms, principally by paralyzing muscles of caterpillars, while in other insect hosts of *Metarhizium anisopliae* such as Orthoptera, whose muscles are not susceptible to destruxins, and in susceptible insects infected with low destruxins producing strains, the toxin may act indirectly to assist the pathogen to overcome host defences perhaps as stated earlier by interfering with haemocyte activity (Samuels *et al.*, 1988).

After causing the death of insect host, fungus continues to grow inside insect body in saprophytic way. The development of hyphal body in host continues by consuming nutrients of insect tissue until all host tissue is replaced by fungal mycelium. Finally, the reproduction step occurs depending on surrounding environment. Fungus disperses spores in order to infect new hosts. In the presence of favourable environmental conditions, the fungus grows outwards through the insect

cuticle and produce reproductive structures. Under unfavourable climatic conditions, most fungi are able to produce resting spores (e.g. chlamydospores, zygospores or oospores) or produce sexual spores (e.g. ascospores) that allow the fungus to survive during winter season or withstand adverse conditions in the absence of the host (Latgé *et al.*, 1978; McCoy, 1981; Pendland, 1982).

1.7 Generic descriptions of common entomopathogenic fungal genera

Short generic descriptions of entomopathogenic fungal genera are given as below. These descriptions provide characteristic features of seventeen entomopathogenic fungal genera related to this study. The data source of each genus is also given, the descriptions were provided from both taxonomic and phylogenetic studies of various researches (Chaverri *et al.*, 2008; Johnson *et al.*, 2008; Luangsa-ard *et al.*, 2005; Samson *et al.*, 1988; Sung *et al.*, 2007; Sutton, 1980). In this descriptions, there are only six teleomorphic entomopathogenic fungal genera presented, these genera belong to the order Hypocreales of Ascomycota of the kingdom Fungi.

1.7.1 *Conoideocrella* D. Johnson, G.-H. Sung, Hywel-Jones and
           Spatafora 2008

Johnson, D., G.H. Sung, N.L. Hywel-Jones, J.J. Luangsa-Ard, J.F. Bischoff, R.M. Kepler, and J.W. Spatafora. 2009. Mycol. Res. 113(3): 286.

Classification: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Clavicipitaceae.

Stromata pulvinate to planar, not stipitate, variously coloured. Hypothallus hyaline. Perithecia produced on the stroma or hypothallus, elongated flask-shaped to conical, yellow-brown to purple-red. Asci cylindrical with thickened ascus apex. Ascospores filiform, multiseptate, disarticulating or non-disarticulating into part-spores. Anamorph: not known.

1.7.2 *Cordyceps* Freies 1833

Sung, G.H., N.L. Hywel-Jones, J.M. Sung, J.J. Luangsa-ard, B. Shrestha, and J.W. Spatafora. 2007. Stud. Mycol. 57.

Classifivcation: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Cordycipitaceae

Stromata or subiculum pallid or brightly pigmented, fleshy. Perithecia superficial to completly immersed, ordinal in arrangement. Asci hyaline, cylindrical with thickened ascus apex. Ascospores hyaline, cylindrical, multiseptate, disarticulating into part-spores or non-disarticulating, rarely possessing thread-like structure to connect fusiform ends. Anamorphs: *Beauveria, Isaria, Lecanicillium,* mariannaea-like*, Microhilum, Simplicillium*.

1.7.3 *Hypocrella* Saccardo 1878

Chaverri, P., M. Liu, K.T. Hodge. 2008. Stud. Mycol. 60: 53.

Classifivcation: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Clavicipitaceae.

Stromata generally pulvinate, with or without hypothallus, (0.5-)1-2(-3) mm diam; in various shades of whitish, yellow, orange, and green; stroma surface generally minutely pulverulent or pruinose; stromatic tissue generally changing colour in 3 % KOH, to reddish or brown; perithecia generally embedded in stroma or half-embedded; stroma outer tissue of textura epidermoidea, cell walls ca. (0.5-)1-2.5(-3) μm thick; stroma internal tissue generally of textura intricata, cell walls generally thin, (0.5-)1-2(-3.5) μm. Perithecia, obpyriform to subglobose, 250 - 50 × 110-200 μm. Asci mostly cylindrical or clavate, 110-180(-250) × 8-12(-20) μm, generally capitate. Ascospores hyaline, multiseptate, smooth, filiform to long fusiform, non-disarticulating, (50-)75-120(-170) × (1-)2-5(-7) μm. Conidiomata from

original substrata pycnidial generally flaskshaped; phialides formed in a thick compact palisade, in short conidiophores sometimes indistinct, branching once monochasial, twice monochasial, monoverticillate, or two level monochasial; phialides cylindrical, slightly tapering towards tip, (9-)12-20(-23) × 2-2.5 μm; conidial masses mucilaginous; conidia hyaline, smooth, unicellular, fusoid, generally with acute ends, (10-)12-25(-30) × (1.5-)2-5(-6) μm, l/w ratio 5-6(-7). Paraphyses generally present. Anamorph: *Aschersonia.*

1.7.4 *Moelleriella* Bresadola 1896.

Chaverri, P., M. Liu, K.T. Hodge. 2008. Stud. Mycol. 60: 31.

Classifivcation: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Clavicipitaceae.

Stromata mostly globose, thick pulvinate, convex, tuberculate, scutate, thin pulvinate almost effuse, (0.5-)1.5-2(-30) mm diam; whitish, yellowish, orange, brown, and black; stromatal surface glabrous, tomentose, pruinose, or roughened; stromatic tissue not changing colour in 3 % KOH; perithecia completely embedded in stroma, half-embedded, or forming strong tubercles; stromatal outer tissue of textura angularis, epidermoidea or intricata, cell walls (1-)2-2.3(-5) μm thick; stromatal internal tissue generally of textura epidermoidea or intricata, cell walls (0.8-)2.2-2.7(-4.7) μm thick. Perithecia, obpyriform to subglobose, (265-)325-375(-450) × (115-)135-200(-450) μm. Asci mostly cylindrical, (120-)180-200(-325) × (5.2-)9.5-11(-18) μm, capitate, cap thickness (0.8-)3.5-4.5(-6.5) μm. Ascospores hyaline, smooth, filiform, multiseptate, disarticulating at septa, part-ascospores fusoid, cylindrical, subcylindrical, or ventricose, (4.5-)11.5-12.5(-30) × (1.5-)3(-5.5) μm. Conidiomata from original substrate on host aschersonia-like; pycnidium-like conidiomata irregularly shaped; phialides formed in a thick compact palisade, in short conidiophores, sometimes indistinct, branching once monochasial, twice monochasial, monoverticillate, or two level monochasial; phialides flask-shaped to slender, (5.2-)11-12.5(-24.5) × (1.3-)2.2-2.5(-6.3) μm; conidia hyaline, smooth, unicellular, fusoid,

ventricose or ovoid, generally with acute ends, (5-)12-12.7(-25.5) × (1.5-)4-4.2(-7.8) μm, length/width ratio (2-)3.1-3.2(-7.7). Paraphyses sometimes present. Anamorph: *Aschersonia*-like.

1.7.5 *Ophiocordyceps* Petch 1931

Sung, G.H., N.L. Hywel-Jones, J.M. Sung, J.J. Luangsa-ard, B. Shrestha, and J.W. Spatafora. 2007. Stud. Mycol. 57.

Classifivcation: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Ophiocordycipitaceae.

Stromata or subiculum darkly pigmented or rarely brightly colored, tough, fibrous, pliant to wiry, rarely fleshy, often with aperithecial apices or lateral pads. Perithecia superficial to completely immersed, ordinal or oblique in arrangement. Asci hyaline, cylindrical, usually with thickened ascus apex, rarely fusoid to ellipsoid. Ascospores usually cylindrical, multiseptate, disarticulating into part-spores or non-disarticulating. Anamorphs: *Hirsutella*, *Hymenostilbe*, *Paraisaria*, *Syngliocladium*.

1.7.6 *Torrubiella* Boudier 1885

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Cordycipitaceae.

Stroma absent or poorly developed as light- to brightly coloured subiculum covering host; ascomata brightly coloured, often elongate, conical to pyriform, wall often covered with conidiogenous structures of the anamorph; asci long cylindrical, 8-spored, with prominent apical thickening penetrated by a fine

canal; ascospores filiform, multiseptate, disarticulating at maturity into one-celled partspores. Anamorphs: *Akanthomyces*, *Gibellula*.

1.7.7 *Akanthomyces* Lebert 1858

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification: Fungi, Ascomycota, Anamorphic fungi.

Conidiophores synnematous; synnemata often compact, cylindrical or clavate, covered with a hymenium-like layer of phialides. Phialides with inflated or cylindrical basal part and short neck, conidia one-celled, smooth, hyaline, in short fragile chains. Teleomorphs: *Torrubiella*.

1.7.8 *Aschersonia* Montagne 1848

Sutton, B.C. 1980, The Coelomycetes: 543.

Classification: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Clavicipitaceae.

Mycelium superficial, pruinose, fibrillose, membranous, hyaline, branched, septate, when present forming a hypothallus at the base of the stroma. Conidiomata stromatic, superficial, entomogenous on Lecaniidae and Aleyrodidae (scale insects), separate or aggregated, extremely variable in shape and pigmentation, subglobose, hemispherical or pulvinate, composed of very thick-walled, hyaline, branched, septate textura intricata which only rarely becomes more deeply pigmented at the periphery, multilocular, the locules tubular, flask-shaped, oval or globose, simple or convoluted, lacking any really distinct wall differentiating them from the stromatal tissue. Ostioles absent, dehiscence by wide, irregular openings in the upper stroma. Conidiophores septate, branched irregularly, cylindrical or tapered towards the apices, hyaline, smooth, formed from the inner tissue of the locular walls.

Conidiogenous cells enteroblastic, phialidic, integrated, determinate, hyaline, cylindrical, collarette and channel minute, periclinal wall thickened. Paraphyses often present, filiform, hyaline, aseptate, thin-walled, fusiform or narrowly oval, multiguttulate. Teleomorphs: *Hypocrella*, *Moelleriella*.

1.7.9 *Beauveria* Vuillemin 1912

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Cordycipitaceae.

Conidiophores consisting of whorls and dense clusters of conidiogenous cells, hyaline, smooth-walled; conidiogenous cells sympodial, short and globose or flask-shaped with an apical denticulate rachis giving a distinctly zig-zag appearance; conidia one-celled, hyaline, thin-walled, globose to ellipsoidal. Teleomorph: *Cordyceps*.

1.7.10 *Gibellula* Cavara 1894

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification: Fungi, Ascomycota, Anamorphic fungi.

Conidiophores occasionally mononematous but mostly distinctly synnematous, septate, rough-walled, with a small terminal vesicle on which phialides are born on metulae; synnemata often loosely arranged and not compact; phialides, mostly cylindrical or clavate with short necks; smooth- or rough-walled, producing conidia in chains; conidia one-celled, smooth walled. Granulomanus-synanamorph may occur ay base of synnemata or on hyphae covering host body. On spiders. Teleomorph: *Torrubiella*.

1.7.11 *Hirsutella* Patouillard 1892

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Ophiocordycipitaceae.

Conidiophores mononematous or synnematous; synnemata erect, cylindrical or slightly tapered, varying from short and verrucose to long and hair-like, sometimes branched; conidiogenous cells phialidic, solitary or crowded in hymenium-like layer along synnemata or born directly on hyphae emerging from host body, mostly with inflated basal portion with one or more slender, thin necks; conidia one- or two-celled, hyaline, variously shaped, often with distinct mucus covering; chlamydospores sometimes present, large, flattened, disc-like, thick-walled; sclerotia produced by some species. Teleomorphs: *Cordyceps*, *Torrubiella* and '*Colonectria*'.

1.7.12 *Hymenostilbe* Petch 1931

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Ophiocordycipitaceae.

Synnemata simple, rarely branched, white to dark-brown. Conidiophores in dense hymenium-like layers along synnema or on stromata of teleomorph. Conidiogenous cells polyblastic, cylindrical to clavate, apically crowded with distinct denticles. Conidia solitary often apiculate, one-celled, smooth- or rough-walled, hyaline. Teleomorph: *Ophiocordyceps*.

1.7.13 *Isaria* Persoon 1794

Luangsa-ard, J.J., N.L. Hywel-Jones, L. Manoch and R.A. Samson. Mycol. Res. 2005. 109 (5): 581.

Classification**:**   Fungi,   Ascomycota,   Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Cordycipitaceae.

Conidiophores mono- or synnematous, usually consisting of several verticillate branches, each bearing a dense whorl of phialides. Synnemata often branched with apical sporulating structures. Phialides consisting of a cylindrical or swollen basal portion, terminating in a thin often long neck, producing divergent conidial chains. Conidia one- or rarely two-celled, smoothwalled, hyaline. Colonies bright coloured, white, yellow, pale green, pink, red or purple. Hyphae hyaline to slightly pigmented, rough- or smooth-walled. Chlamydospores present in some species. Teleomorph: *Cordyceps*.

1.7.14 *Metarhizium* Sorokin 1879

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification**:**   Fungi,   Ascomycota,   Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Clavicipitaceae.

Conidiophores in compact to nearly stromatic patches, mostly mononematous but also synnematous when occurring on arthropods buried in soil or other substrates; conidiogenous cells phialides in whorls, often arranged in a candle-like fashion, clavate to cylindrical; conidia one-celled, smooth-walled, hyaline to slightly coloured, forming long chains often aggregated into prismatic columns. Teleomorph: *Metacordyceps*.

1.7.15 *Nomuraea* Maublanc 1903

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification**:** Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Clavicipitaceae.

Conidiophores mononematous or synnematous, verticillate, bearing dense whorls of short branches with clusters of phialidic conidiogenous cells; phialides short, more or less flask-shaped to cylindrical without a distinct neck; conidia one-celled, smooth walled, green to purple, in dry divergent chains. Teleomorph: *Cordyceps*.

1.7.16 *Paecilomyces* Bainier 1907

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification**:** Fungi, Ascomycota, Anamorphic fungi.

Conidiophores erect, mononematous, but species on insect often synnematous, verticillate, bearing whorls of divergent branches and phialides; phialides flask-shaped or with swollen basal part, abruptly tapering into a distinct neck; conidia one-celled, hyaline to slightly pigmented, produced in dry divergent chains, smooth-walled or occasionally spiny. Teleomorphs: not known.

1.7.17 *Verticillium* Nees 1817

Samson, R.A., H.C. Evans & J.P. Latgé, 1988.

Classification**:** Fungi, Ascomycota, Anamorphic fungi.

Conidiophores erect or not distinctly differentiated from vegetative hyphae, mostly verticillate with loose whorls of phialidic conidiogenous cells; phialides mostly awl-shaped, sometimes slightly inflated at the base; conidia, one-celled, hyaline, smooth-walled, formed in slimy heads of occasionally in chains. Teleomorph: *Cordyceps*, *Torrubiella*.

2. Introduction of Cat Tien National Park

Cat Tien National Park is one of the important national parks in the south of Vietnam. The park is located in approximately 150 km north of Ho Chi Minh City. It protects one of the largest areas of lowland tropical rainforests left in Vietnam. Cat Tien National Park has the geographic coordinates of $11^{\circ}21'$-$11^{\circ}48'$N and $107^{\circ}10'$-$107^{\circ}34'$E. This National Park consists of three sectors: Nam Cat Tien (Southern Cat Tien) in Dong Nai province, with an area of 38,100 ha; Tay Cat Tien (Western Cat Tien) in Binh Phuoc province, with an area of 5,143 ha; and Cat Loc in Lam Dong province, with an area of 30,635 ha. Hence, the total area of the national park is currently 73,878 ha (CTNP, 2003b). Cat Tien National Park is part of the wet tropical forest complex and one of the specious natural forests remaining in Vietnam. That is why this area is significant not only in Vietnam but in the world as well. The diversity of the Park has been recognized by World Wildlife Fund (WWF) when this international organization selected the Park as one of 200 global ecological zones including the land and its water area with its well-known biological diversity. In 2001 Cat Tien National Park has been listed by the United Nations Educational, Scientific and Cultural Organization (UNESCO) as the 411th biosphere reserve zone in the world.

Cat Tien National Park supports a variety of habitat types, including primary and secondary lowland evergreen forest dominated by species in the Dipterocarpaceae; primary and secondary lowland semi-evergreen forest, dominated by *Lagerstroemia* spp.; freshwater wetlands with open lakes and seasonally inundated grasslands, and a range of secondary habitat types, including grassland and areas

dominated by bamboo (FIPI, 1993). During Second Indochina War, Cat Tien National Park was sprayed intensively with herbicides, therefore, in areas of dense bamboo and grass cover, natural re-growth of trees hardly occurs. Only 50% of the total area of the national park is classified as evergreen forest, semi-evergreen forest or mixed forest. Bamboo forest accounts for 40% of total land cover.

The flora of Cat Tien National Park includes more than 1,300 species of vascular plants, among which are 34 species listed in the *Red Data Book of Vietnam* (Anon. 1996) and many valuable timber species, such as *Afzelia xylocarpa*, *Dialium cochinchinensis*, *Dalbergia oliveri* and *Pterocarpus macrocarpus* (Vu Van Dung *in litt*. 2000). For the diversity of animal, to date, 76 mammal, 322 bird, 73 reptile, 35 amphibian, 99 fish and 435 butterfly species have been confirmed to occur at the national park; and there are unconfirmed records of an additional 32 mammal, 18 bird, eleven reptile, four amphibian, 31 fish and four butterfly species. The species confirmed to occur include 16 mammals, 15 birds and 8 reptiles that are globally threatened (Polet and Ling, 2004).

Table 1 Species totals recorded to date in Cat Tien National Park, updated after Polet and Ling (2004).

Taxa	All species		IUCN Red List 2000
	Total	**% of VN total**	
Mammals	76 (108)	30 (43)	16 (27)
Birds	322 (340)	37 (40)	15 (16)
Reptiles	73 (84)	27 (31)	8 (8)
Amphibians	35 (39)	29 (38)	0
Fresh water fish	99 (130)	21 (28)	1
Butterflies	435 (439)	43 (44)	Data not available

Note: Non-bracketed data are confirmed records, bracketed data in include possible.
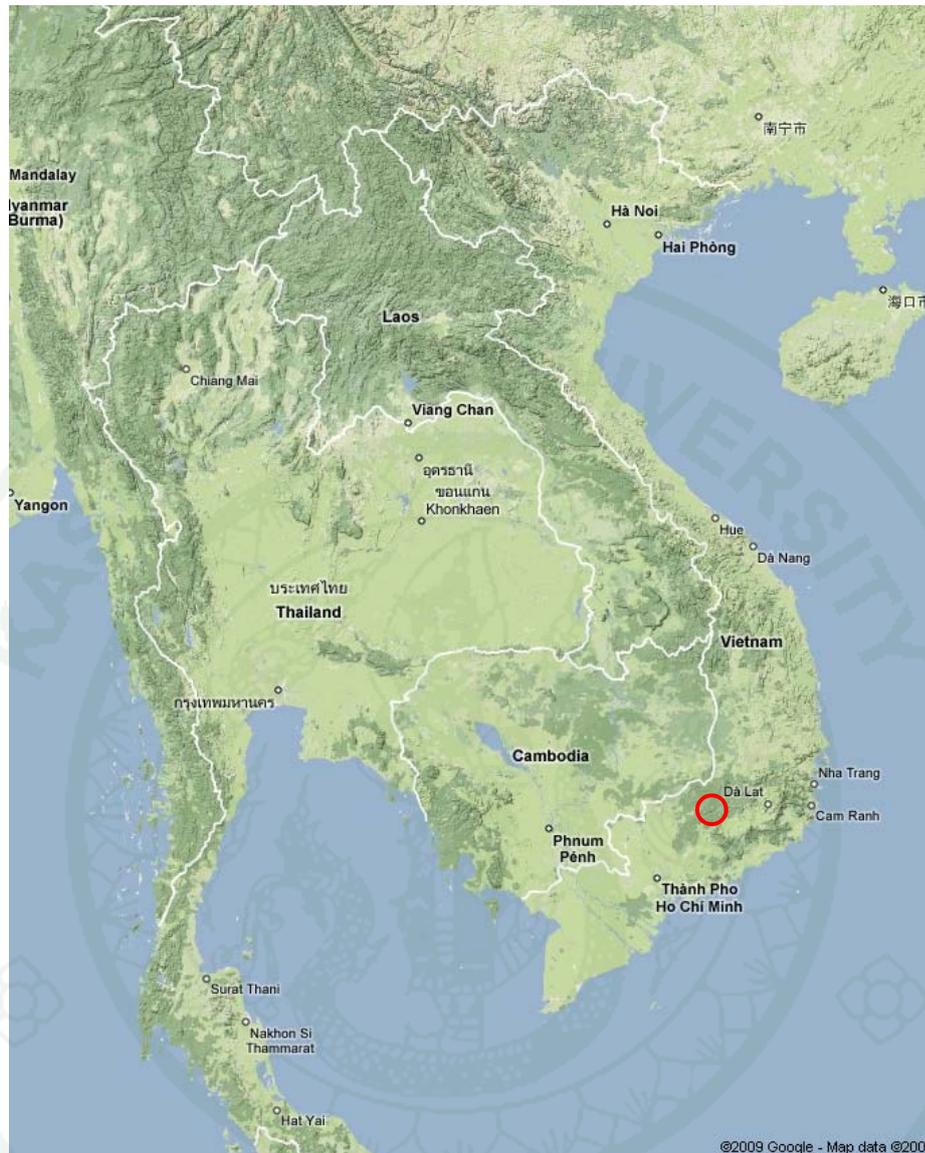
Figure 3 Location of Cat Tien National Park in the cycle.

Note: Picture captured from Google Maps.

However, lack of information about diversity of microorganisms has been published in Cat Tien National Park. Some attempts have been made to survey the diversity of mushroom and yeast species, however, No data of these have been reported. Since entomopathogenic fungi have been reported for use in many purposes, the research on entomopathogenic fungi have put in the plan of the Institute of Tropical Biology, which belongs to Vietnamese Academy of Science and Technology. Preliminary survey of entomopathogenic fungi in Cat Tien National Park from 2005 found that this National Park has a diverse numbers of entomopathogenic fungal species (unpubl.). Studies on collection and taxonomy of Entomopathogenic fungi in Cat Tien National Park are necessary to provide information for further research and application of this fungal group.

3. Applications of Entomopathogenic Fungi

3.1 Entomopathogenic fungi for biological control of insect pests

Several anamorphic fungal entomopathogens showed potential as microbial control agents for commercial development including *Beauveria bassiana, Metarhizium anisopliae* and *Normurea rileyi*. They were used commercially in some countries outside the United States (McCoy, 1990). All these species could be produced on various artificial and natural media in large enough quantities for large-scale field tests. According to McCoy (1990), the characteristics that are needed before an entomogenous fungus could be considered as a potential microbial insecticide include (1) high virulence, (2) rapid mode of action, (3) a broad host range, (4) stability in culture and storage, (5) amenability to submerged fermentation, (6) amenability to quantitative bioassay, and (7) safety to workers.

Several companies in the United States and abroad are engaged in commercial production of new mycoinsecticides, which will be marketed for control of various insects in the near future. Most formulations are based on recently isolated, highly virulent strains of *Beauveria bassiana, Metarhizium anisopliae,* and *Verticillium lecanii* and are formulated for greenhouse and field crop pests.

According to Taborsky the first attempt in using *Metarhizium anisopliae* for biological control was by Krassilstchik (1888), he was successful in destroying 55-80% of Clones punctiventris insects in small areas (Taborsky, 1992). Varied results have also been obtained when *Metarhizium anisopliae* was used against other insects (Picles, 1945; James, 1946). Presently, *Metarhizium anisopliae* were applied successfully against insect pests of several crops (Ferron, 1981), including pests of rice in the tropics (Rombach *et al.*, 1987) and in this region annually treated about several hundred thousand hectares, and using around 2.5 x 10^{12} viable spores/ha.

Biological control experiments with entomopathogenic fungi were numerous and have been directed particularly to the Colorado beetle and other Coleoptera and Lepidoptera. Various species of mosquito larvae could be killed by dusting conidia on the water surface (Clark *et al.* 1968); conversely, bees larvae were not susceptible while adult bees succumbed (Toumanoff, 1931). It has also been isolated from the lungs of giant tortoises and box turtles affected wit a pulmonary disease (Georg *et al.*, 1962); an increase in the air spore had also led to allergic responses in man (Roberts, 1973). *Beauveria bassiana* was employed on a large scale in the People's Republic of China to control pine caterpillars *(Dendrolimus punctatus)*, green leafhoppers (*Nephotettix* spp.) and corn borer (*Ostrinia nubilalis*). In the Russia mycopesticide made from *Beauveria bassiana* like "Boverin" was produced for the control of the Colorado beetle (*Leptinotarsa decemlineata*) and the codling moth (*Laspeyresia pomonella*), "Boverol" was also used to control against Colorado beetles, Whiteflies (*Trialeurodes vaporariorum*) and black vine beetle (*Otiorhynchus sulcatus)*.

3.2 Traditional herbal medicines

Cordyceps, one of the well-known traditional Chinese medicines, is an interesting little fungus which resembles a worm and has been used for about 2000 years in the Far East. These products consist of the dried fungus *Ophiocordyceps sinensis* growing on the larva of the caterpillar. It is commonly used in China for the replenishment of general body health. *Cordyceps* has a broad range of

pharmacological and biological actions on the liver, kidneys, heart, and immune system. One of the known pharmacological effects is its anti-oxidation activity.

Ophiocordyceps sinensis known to the Chinese as "Dong Chong Xia Cao" and to the Japanese as "Tochukaso" has been used in medicine for a very long time. The first known written record of this herbal medicine was in the "Ben-Cao-Cong-Xin" (*New Compilation of Materia Medica*) by the author Wu-Yiluo. Written around the year 1757 AD during the Qing Dynasty (1644 – 1912), this early medical text listed the traditional usage of *Cordyceps* as going to the Lung and Kidney meridian and being useful as a "Lung Protectorate", for "Kidney Improvement" and as a "Yin/Yang double invigorator". *Cordyceps* in traditional Chinese medicine was prepared and usually still is prepared by cooking the whole caterpillar and fruiting body combination in chicken or duck soup. (Chen *et al.*, 2005; Gao *et al.*, 2000; Gong *et al.*, 2000; Hsu *et al.*, 2003; Huang *et al.*, 2004; Koh *et al.*, 2003; Liu and Shen, 2003).

3.3 Drug production

Cyclosporine is a famous immunosuppressant drug. It is widely used in post-allergenic organ transplant to reduce the activity of the patient's immune system and so the risk of organ rejection. It has been studied in transplants of skin, heart, kidney, lung, pancreas, bone marrow and small intestine (Lichtiger *et al.*, 1994; Starzl *et al.*, 1981; Sullivan *et al.*, 2000). Cyclosporine is a cyclic nonribosomal peptide of 11 amino acids (an undecapeptide) produced by the fungus *Tolyplocadium inflatum* (the anamorph state of *Cordyceps subsessilis*), initially isolated from a Norwegian soil sample (Borel *et al.*, 2002).
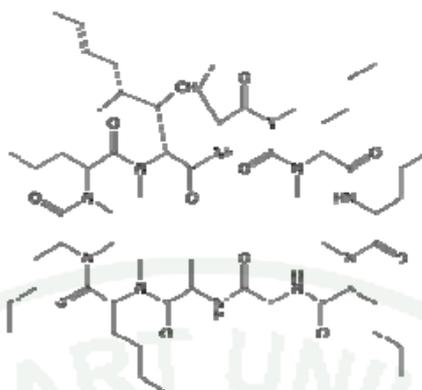
Figure 4 Molecular structure of cyclosporine.

3.4 Enzyme and secondary metabolite productions

There were several researches have been successfully reported in the development of bioactive compounds from several entomopathogenic fungal species. Six bioactive naphthoquinone derivatives (erythrostominone, deoxyerythrostominone, 4-*O*-methyl erythrostominone, epierythrostominol, deoxyerythrostominol and 3,5,8-trihydroxy-6-methoxy-2-(5-oxohexa-1,3-dienyl)-1,4-naphthoquinone) were isolated from the entomopathogenic fungus *Ophiocordyceps unilateralis* BCC 1869. While the latter is synthetically known, both it and 4-*O*-methyl erythrostominone are products of fungus strain *Ophiocordyceps unilateralis* BCC1869 (Kittakoop *et al*, 1999). Cordyanhydrides A and B, two new alkenoic acids bearing two and three maleic anhydride moieties in the linear acid chain, were isolated and identified from a culture broth of the entomopathogenic fungi fungus *Cordyceps pseudomilitaris* BCC 1620 (Isaka *et al.*, 2001).

It was also reported that the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* produce several chitinases which may have a variety of different functions. Some of these chitinases are important cuticle-degrading enzymes and act synergistically with proteases to hydrolyze insect cuticle. However, chitinase involvement in insect fungus pathogenesis has not been fully characterized. Endochitinase, had been purified to homogeneity from liquid cultures

of *Beauveria bassiana* grown in a medium containing colloidal chitin (Weiguo *et al.*, 2005).

4. Cordycepin

4.1 General information

Cordycepin, 3'-deoxyadenosine (Table 2), is a nucleoside analogue, which the molecular is a combination of adenine and 3'-deoxyribose differed from adenosine by the absence of oxygen in the 3' position of its ribose part (Figure 5).

Table 2 General information and characteristics of cordycepin.

Names	Properties
IUPAC name	9-(3-Deoxy-β-D-ribofuranosyl)adenine
Other names	Cordycepin 3'-Deoxyadenosine
CAS number	73-03-3
SMILES	O[C@@H]1C[C@@H](CO)O[C@H]1N2C(N=CN=C3)=C3N=C2
Molecular formula	$C_{10}H_{13}N_5O_3$
Molar mass	251.24 g/mol
Melting point	225.5 °C
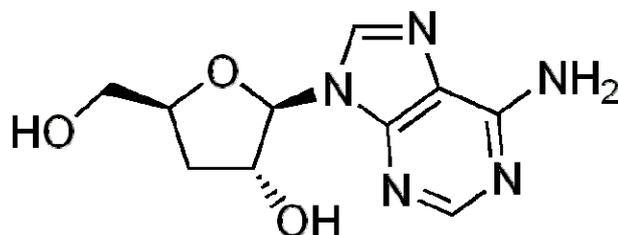
Figure 5 Molecular structure of cordycepin.

Cordycepin was first discovered from the insect-pathogenic fungus *Cordyceps militaris* in 1950 by Cunningham (Cunningham *et al.*, 1950). Recently, it was found to be presented in the stroma of *Ophiocordyceps sinensis* (Hsu and Lo, 2002) and *Cordyceps kyushuensis* (Ling *et al.*, 2002).

4.2 Production of Cordycepin

4.2.1 Chemical synthesis

A numerous methods for the chemical synthesis of cordycepin exist in the literature. These routes for cordycepin chemical synthesis can be classified as belonging to one of three types: total synthesis from nonnucleoside derivatives (McDonald and Gleason, 1995, 1996; Ito *et al.*, 1981), metal hydride reduction of the 2',3'-anhydro derivative (Hansske and Robins, 1985; Bazin and Chattopadhyaya, 1985; Herdewijn, 1989) and reduction of the 3'-halo derivatives using both noble metal catalysts (Russell *et al.*, 1973; Jain *et al.*, 1974). However, these methods of cordycepin synthesis indicated several disadvantages, the yield of cordycepin obtained was not high and it required a complicated process for separation, in addition, a large amount of organic solvent was discharged causing problem for environment. Aman and co-workers developed the manufacturing process suitable for the production of 3'-deoxyadenosine (cordycepin) in 20% yield from adenosine (Aman *et al.*, 2000). However the larger scale synthesis has not yet been developed due to its costly process.

4.2.2 Biological synthesis

Ophiocordyceps sinensis and *Cordyceps militaris* were reported as cordycepin producers (Hsu and Lo., 2002; Cunningham *et al.*, 1950, 1951). However the *Cordyceps militaris* species (Figure 6) has been widely use in the research by scientists due to its fast growing and easily cultivation and maintenance.

As mentioned, cordycepin was first extracted from *Cordyceps militaris* by Cunningham in 1950. Recently, there were many research demonstrated the ability of cordycepin production on this type species. In 2006, Masuda and co-workers developed the method to produce cordycepin by surface culture of *Cordyceps militaris* NBRC 9787 using liquid medium. By adding various compounds such as purine biosynthesis related compounds, coenzymes and surfactant to the basal medium for enhancing the cordycepin production, glycine, L-aspartic acid, L-glutamine, adenine and adenosine were shown to be effective additives. This research obtained that about 97% of the cordycepin synthesized by *Cordyceps militaris* was excreted into the culture medium, and the production of cordycepin and guanine might be linked to each other (Masuda *et al.*, 2006). Mao and co-workers also successfully produced cordycepin by submersed culture of *Cordyceps militaris* with the optimum glucose and peptone concentrations were 42.0 and 15.8 g/l in the medium, and the cultivation on 5-L and 30-L bioreactors showed the promised results for scaling up and industrial production (Mao and Zhong, 2004, 2006; Mao *et al.*, 2005). Recently, Shih and co-workers did a research on *Cordyceps militaris* CCRC 32219 and reported that the optimum conditions to produce cordycepin were at pH 6, yeast extract concentration of 45 g/l and 8.0 day of the shake culture followed by 16 days of the static culture. Under the optimized conditions, the maximum production was 2214.5 mg/l (Shih *et al.*, 2007).

In order to find the process of cordycepin synthesis by the fungus *Cordyceps militaris,* the biosynthesis of 3'-deoxyadenosine (cordycepin) *Cordyceps militaris* had been investigated using [U-C^{14}] adenosine and [3-H^3] ribose. Crystallization of the resulting radioactive 3'-deoxyadenosine to a constant specific

activity showed incorporation of both labeled compounds. A control showed that the H^3: C^{14} ratio of the AMP isolated from the RNA was the same as the H^3: C^{14} ratio in the 3'-deoxyadenosine. The C^{14} ratio in the adenine: ribose of the [U-C^{14}] adenosine added to the 3'-deoxyadenosine producing cultures of *C. militaris* and of the isolated 3'-deoxyadenosine was the same, e.g. 50:50. These data provide strong evidence that adenosine in converted to 3'-deoxyadenosine without hydrolysis of the N-riboside bond. Degradation of the 3-deoxyribose from 3'-deoxyadenosine showed that the H^3 was retained on carbon-3. These results suggest that the formation of 3'-deoxyadenosine may proceed by a reductive mechanism similar to that for the formation of 2'-deoxynucleotides (Lennon and Suhadolnik, 1976).

Figure 6 *Cordyceps militaris* fruiting body arising from Lepidoptera larva in soil.

Kredich and Guarino (1961) in their study on the biosynthesis of cordycepin discussed that the biosynthesis of cordycepin by the *Cordyceps militaris* may be thought of to involve three individual problems: the synthesis of adenine, the synthesis of cordycepose, and the synthesis of the N-glycosidic bond. The results of this study give some indication on the first two aspects of this problem, but at present

give little information as to how, or at what stage in the overall synthesis the N-glycosidic bond is established.

The communication of Suhadolnik *et al.* reported the results of studies on incorporation of formate-C^{14} and adenosine-U-C^{14} into cordycepin and the distribution of C^{14} in the cordycepin. These results found that adenosine-U-C^{14} and formate-C^{14} were both incorporated into cordycepin. These findings ruled out the hydrolysis of adenosine prior to the formation of cordycepin and demonstrate that none of the carbon atoms of ribose is lost (Suhadolnik *et al.*, 1964).

4.3 Bioactivities of Cordycepin.

Cordycepin was considered as an active component (Cunningham *et al.* 1950). It was illustrated that cordycepin has effects altering cytokines secretion (Zhou *et al.* 2002), improving lung function, increasing energy levels and sex drive (Cui, 1999), and having antitumor effect on mouse melanoma and lung carcinoma cells (Nakamura *et al.* 2006). It has also been demonstrated that cordycepin could inhibit polyadenylate polymerase (Thomadaki *et al.* 2005, 2008a,b) or inactivate mRNA polyadenylation (Lallas *et al.* 2004) to induce tumour cell apoptosis.

Cordycepin has been reported to have many bioactivities. It inhibited viral RNA synthesis in the replication of western equine encephalitis virus, thereby causing a reduction of virus production (Hasjiimoto and Simizu, 1976) while at a concentration of 20 µg/ml, it has a marked effect on Semliki Forest virus multiplication. It had been investigated to be a naturally occurring antibacterial agent. Cordycepin could be useful as a new preventive agent against various diseases caused by clostridia (Ahn *et al.*, 2000). This compound revealed potent growth inhibiting activity toward *Clostridium paraputrificum* and *C. perfringens* at 10 µg/disk without adverse effects on the growth of useful lactic acid bacteria such as *Bifidobacterium bifidum*, *B. breve*, *B. longum*, *B. adolescentis*, *Lactobacillus acidophilus*, and *L. casei* (Ahn *et al.*, 2000).

In 1983, when testing on mouse L929 cells and cultured human fibroblasts, it was found that cordycepin can inhibit protein synthesis but was not cytotoxic to mammalian cells in culture (Lee *et al.*, 1983). Cordycepin increased interleukin-10 production and the expression of interleukin-10 mRNA, interleukin-10 is considered as an immunoregulator for controlling autoimmune diseases. Moreover, it simultaneously reduced the production of interleukin-2, the proliferation of peripheral blood mononuclear cells and the expression of surface markers indicative for activation of T lymphocytes (Zhou *et al.*, 2002). The results of testing showed that the antitumour effect of orally administered cordycepin was examined in mice inoculated with B16 melanoma (B16-BL6) cells (Yoshikawa *et al.*, 2004). Recent study reported that cordycepin inhibited dose-dependently collagen-induced platelet aggregation in the presence of various concentrations of exogenous $CaCl_2$ (Cho *et al.*, 2007).

MATERIALS AND METHODS

1. Collection, isolation and identification of Entomopathogenic fungi

1.1 Collection of entomopathogenic fungi

The collecting trip was conducted in Cat Tien National Park (Southern Cat Tien sector), Dong Nai Province of Vietnam from 6th to 8th September, 2007. The collection was done with the cooperation of Mr. Tran Thanh Phong and Mr. Nguyen Duy Long, and the instruction of Dr. Somsak Sivichai, Ms. Kanoksri Tasanathai and Mrs. Suchada Mongkongsamrit.

Collection was done in five locations in the park (Figure 8) including (1) track from behind the Headquarter to Bang Lang trail, (2) Left area along the main road to Crocodile swamp, (3) Cay Go Bac Dong trail, (4) Bang Lang trail, and (5) area behind the guesthouse. These trails and locations are located in the lowland part of Southern Cat Tien sector (Figure 7).

Search of entomopathogenic fungi was made on several microhabitats in the forest such as underside of leaves, trunk of forest plants, and leaf litter of forest floor.

Different specimens were separated and stored in clear plastic boxes. Collected specimens then were numbered in ordinal number by the ITB codes provided by the Institute of Tropical Biology of Vietnam.
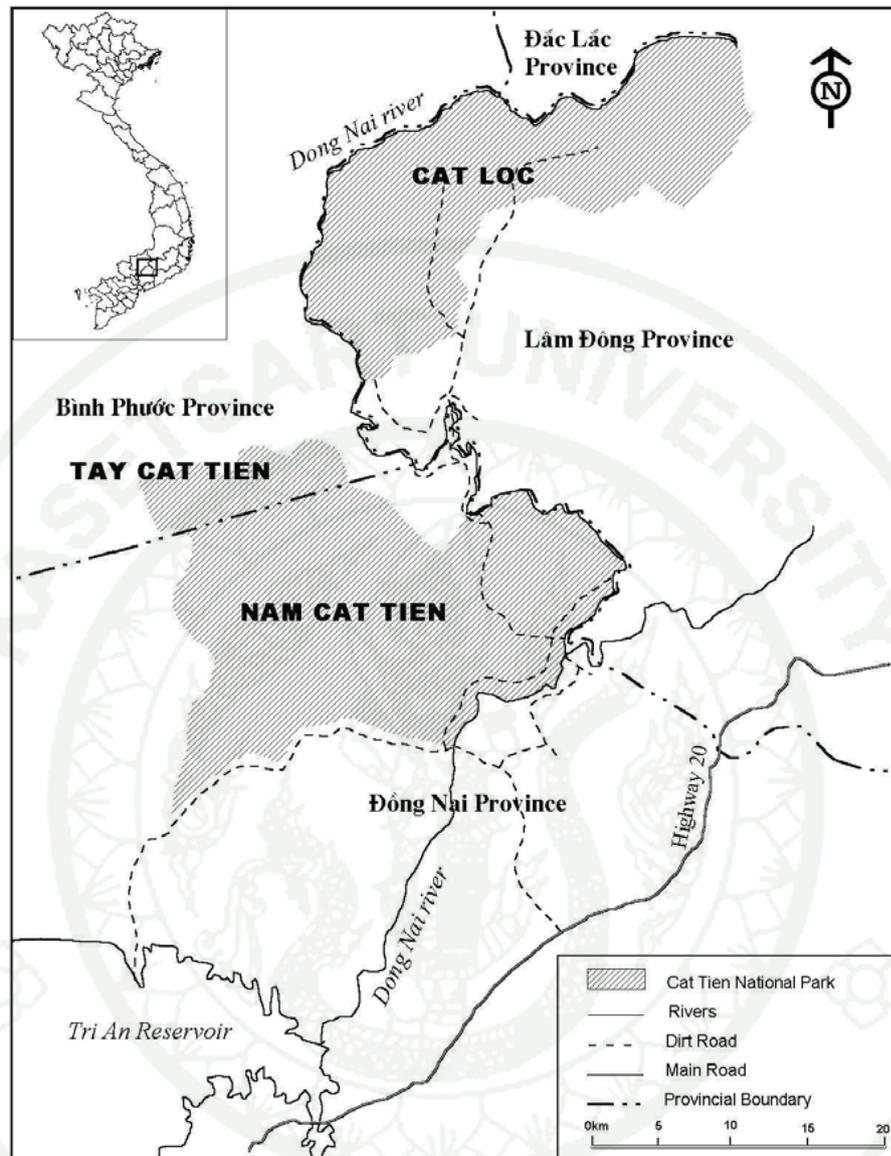
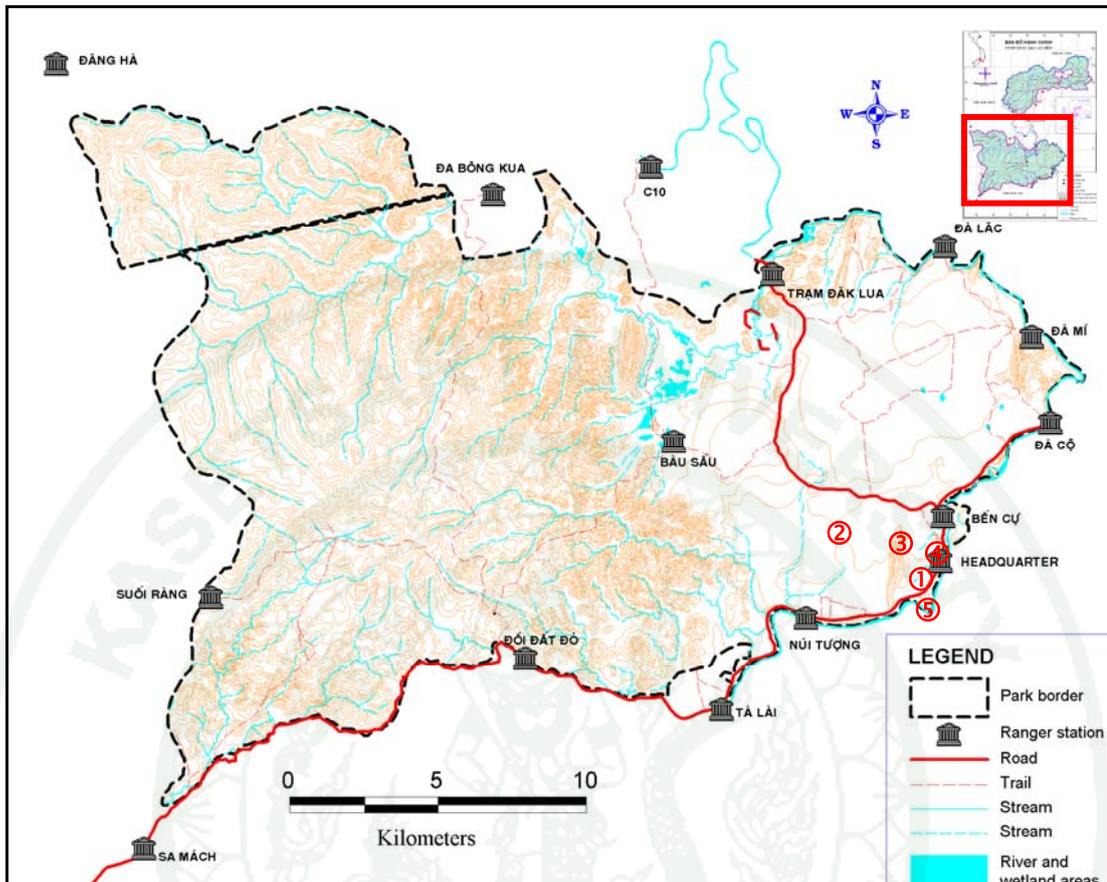**Figure 7** Map of Cat Tien National Park showing three sectors.

Figure 8 Location of five trails and colleting sites in Cat Tien National Park.

Note: (1) Track from behind the Headquarter to Bang Lang trail, (2) Area along the main road to Crocodile swamp, (3) Cay Go Bac Dong trail, (4) Bang Lang trail, and (5) area behind the guesthouse.

1.2 Isolation of entomopathogenic fungi

Primary isolation was conducted in the same day of collection at guesthouse of the National Park. Specimens were processed under a stereo microscope Olympus SZ60 (Olympus, Japan), conidia or ascospores were picked out from fertile part of specimen using a needle and stretched out in a drop of sterile water on a microscope slide. Fungal spores on microscope slide then were transferred on to surface of plastic Petri plate (60 mm diameter) containing manual Potato Dextrose Agar (PDA) medium supplemented with antibiotics (see Appendix A) using an experimental loop. One drop of lactophenol was poured on the microscope slide containing spores. The slide was covered with a microscope lamella, then sealed and kept in a storage box for later examination on identification.

Some teleomorphic specimens such as *Cordyceps, Conoideocrella, Hypocrella, Moelleriella, Ophiocordyceps* and *Torrubiella* were isolated by naturally discharging ascospores on to PDA surface overnight. The fertile part of specimen was placed above the PDA surface near the top cover of Petri plate. This set was incubated in a moist incubator chamber in room condition overnight. In next-day morning, the Petri plates with discharged ascospores were observed under the stereo microscope. Single or group of germinated ascospores, with germ-tubes, were picked out using a needle stick and transferred to the surface of PDA plate with several replicates.

Other un-isolated specimens were kept in plastic boxes and placed in a carton box in cool temperature and carried back to laboratory for further isolation and other experimental process. These specimens were stored at 4^oC in a refrigerator in order to keep it fresh and to avoid being contaminated by mites and other microorganisms.

All collected specimens and primary isolated cultures later were carried to Mycology Laboratory of the National Center for Genetic Engineering and Biotechnology (BIOTEC) of Thailand for further isolation, purification, identification and preservation. PDA plates containing purified spores after primary isolation were

sealed using parafilm (Whatman, Germany) and incubated in the Low Temperature Incubator 815 (Precision Scientific Inc., USA) at 20°C in Mycology Laboratory. Growth of fungal mycelia was observed daily under the stereo microscope to check for the growth and contamination of spider mites and microorganisms such as fungi, yeast or bacteria.

1.3 Growth and long-term preservation of fungal cultures

Purified cultures in Petri plates were incubated at 20°C in an incubator for colony development. Observation under stereo microscope was done everyday to check for mycelial growth and contamination. When these cultures were surely purified, BIOTEC Culture Collection (BCC) code was given for each culture in strain order. When colonies on PDA surface reached 5-20 mm in diameter, they were cut into small pieces, about 1x1 mm^{2} sizes, and divided into 6 caped Eppendorf tubes 2 ml in volume containing 1ml of 10% v/v sterile glycerol. Six tubes containing purified culture in glycerol then were maintained in freezer model Jouan VXE570 (Thermo Electron Corporation) at -80 ^{o}C for long-term preservation. All freezing cultures were stored in BCC. These cultures later were used for the cordycepin production experiments. Cutting mycelium also were transferred on to 5 slope PDA test tubes and incubated in room temperature condition (25°C to 28°C) and then were checked monthly for mycelial growth and contamination by other microorganisms.

1.4 Preservation of specimens herbarium

After isolated, fungal specimens were dried under fresh air. After dried, a piece of tissue paper and a drop of 95% ethanol were placed in each specimen box. These specimens then were sealed with clear sticking plaster and preserved in BIOTEC Bangkok Herbarium (BBH) at 4°C in a cool preservation room.

1.5 Database collection and processing

A Lab note book was prepared for writing details of collection, characteristics of specimens and cultures, details of specimen such as ordinal code, predicted scientific name of genera or species (if available), collecting site, trip condition, characteristics of specimen and host insect, etc.. Hand drawing of specimens was also made for each collected specimens for later reorganization when dealing with a large number of specimens. All recorded data were posted on to Excel document in order to use for the Microbial Information Management System (MIMS) database for specimens and cultures management.

2. Determination of cordycepin production from selected entomopathogenic fungal species

2.1 Microorganisms and culture

The entomopathogenic fungal species were selected from Japanese, Thai and Vietnamese sources fermentation and screen for cordycepin production. Total 35 fungal strains belonging to 32 species were used to determine the availabilities of cordycepin production (Table 3). These cultures were maintained in the condition of -80°C in freezer before using for experiment. Before the experiment, activated mycelium in PDA Petri plate was prepared by cultivation of fungal colonies on PDA at 25°C for one to three weeks depending on different growth rates of these fungal species.

2.2 Preparation of stock inoculum

Fungal mycelia were taken from activated PDA plates for stock inoculum preparation. When fungal colonies on PDA plates reached 5 – 10 mm in diameter, mycelium of each strains was cut to small pieces (about 1 mm^2 per piece) using a experimental knife. Cutting mycelium of each fungal strain was transferred into a 250 ml Erlenmeyer flask (16 pieces per flask) containing 100 ml Potato Dextrose Broth

(PDB, Difco Laboratories, USA) (see Appendix A). All cultures were inoculated in shaking condition at 150 rpm using a Bigger Bill M49230-26 shaker (Thermolyne, USA). Incubation were conducted in a Walked-in incubator room model TCR-2P (Nippon Medical and Chemical Instrument Co. Ltd., Japan) at 25°C for 7 days. After 7 days inoculation, flasks containing stock inoculum were then used in fermentation.

2.3 Fermentation

250 ml Erlenmeyer flasks (Pyrex, USA) were used in fermentation for cordycepin production of selected entomopathogenic fungal species. Each flask contained 45 ml fermentation medium. The fermentation media used in this experiment were PG and Potato Dextrose Broth (PDB). The PG medium (modified from study of Mao *et al.*, 2005) consisted of 40 g/l glucose (VWR International Ltd., England), 15 g/l Bacto Peptone (Difco Laboratories, USA), 0.5 g/l KH_2PO_4 (Asia Pacific Specially Chemical Ltd., Australia), 0.5 g/l K_2HPO_4 (Sigma-Aldrich Laborchemika, GmbH, Germany) and 0.5 g/l $MgSO_4.7H_2O$ (Sigma-Aldrich Laborchemika, GmbH, Germany) (See Appendix A). The PDB medium (Difco, Gemany) consisted of 4 g/l potato starch and 20 g/l glucose (See Appendix A). Fermentation media were sterilized at 110°C for 15 minutes using a Tomy SS325 autoclave (Tomy, Japan). After the sterilization, 5 ml stock inoculum of each strain was taken and transferred into fermentation flasks containing fermentation medium. The fermentation in each treatment was conducted in two replicated flasks (in duplicate). The neck of each flask was filled in with a cotton plug during fermentation. The fermentation was set in a Low Temperature Incubator 815 (Precision Scientific Inc., USA) and maintained at the temperature of 20 ± 1°C for 15 days. After fermentation, all cultures were taken and filtrated through filter papers (Whatman, USA) to separate mycelium and filtrates. Mycelium on filter papers then were washed twice with distilled water and dried for the determination of dry biomass. The liquid filtrates were poured into caped test tubes and stored at -20 ^{o}C in a freezer for later determination of cordycepin concentration using High Performance Liquid Chromatography (HPLC).

Table 3 List of fungal strains selected form Japanese, Thai and Vietnamese sources for determination of growth and cordycepin production.

No.	Fungal strains	Sources
1	*Akanthomyces arachnophilus* BCC 22900	Doi Inthanon, Thailand
2	*Akanthomyces cinereus* BCC 14289	Khao Yai , Thailand
3	*Akanthomyces* sp. ITB 360	Cat Tien, Vietnam
4	*Aschersonia badia* ITB 486.02	Cat Tien , Vietnam
5	*Aschersonia confluens* ITB 262	Cat Tien , Vietnam
6	*Aschersonia luteola* ITB 278	Cat Tien , Vietnam
7	*Aschersonia oxystoma* ITB 259	Cat Tien , Vietnam
8	*Aschersonia placenta* ITB 269	Cat Tien , Vietnam
9	*Aschersonia placenta* ITB 414.03	Cat Tien , Vietnam
10	*Aschersonia samoensis* ITB 261	Cat Tien , Vietnam
11	*Beauveria bassiana* BCC 22355	Mae Wong , Thailand
12	*Conoideocrella luteorostrata* ITB 300	Cat Tien , Vietnam
13	*Cordyceps militaris* BCC 2816	Appi Highlands (Ahiro), Japan
14	*Cordyceps ninchukispora* BCC 22834	Doi Inthanon , Thailand
15	*Cordyceps nipponica* BCC 19924	Khlong Nakha , Thailand
16	*Cordyceps pseudomilitaris* BCC 27802	Namtok Samlan , Thailand
17	*Gibellula leiopus* BCC 22177	Phu Khiao , Thailand
18	*Gibellula pulchra* ITB 371	Cat Tien , Vietnam
19	*Gibellula* sp. ITB 367	Cat Tien , Vietnam
20	*Hyperdermium* sp. BCC 27812	Khao Chong, Thailand
21	*Hypocrella calendulina* ITB 128	Cat Tien , Vietnam
22	*Hypocrella calendulina* ITB 425.01	Cat Tien , Vietnam
23	*Isaria javanica* ITB 218	Cat Tien , Vietnam
24	*Isaria tenuipes* ITB 301	Cat Tien , Vietnam
25	*Isaria tenuipes* ITB 363	Cat Tien , Vietnam

Table 3 (Continued).

No.	Fungal strains	Sources
26	*Metarhizium anisopliae* BCC 22353	Sakaerat, Thailand
27	*Metarhizium flavoviride* BCC 2335	Unknown or missing data
28	*Metarhizium* sp. ITB 258	Cat Tien , Vietnam
29	*Moelleriella raciborskii* ITB 304	Cat Tien , Vietnam
30	*Nomuraea atypicola* ITB 323	Cat Tien , Vietnam
31	*Ophiocordyceps* cf. *brunneipunctata* ITB 328	Cat Tien , Vietnam
32	*Ophiocordyceps communis* BCC 27807	Khao Yai , Thailand
33	*Ophiocordyceps sphecocephala* BCC 27811	Khao Chong , Thailand
34	*Paecilomyces lilacinus* ITB 454	Cat Tien , Vietnam
35	*Torrubiella hemipterigena* ITB 169	Cat Tien , Vietnam

3. Effect of strains and fermentation temperature on growth and cordycepin production of *Cordyceps militaris*

Fifteen *Cordyceps militaris* strains from different sources of collection were selected to investigate the effect of different fungal strains and cultivation temperatures on the growth and cordycepin production. The list of cultures, source of collection and maintenance conditions are showed on the Table 4.

To investigate effect of different fermentation temperatures on mycelial growth and cordycepin production, four fermentation temperature conditions (15°C, 20°C, 25°C and 30°C) were designed. The stock inoculum of each strain (seed cultures) were prepared as the description from Section 2.2. The medium for fermentation was PG medium, which was described from previous section. The experiment was carried out in 250 ml Erlenmeyer flasks (Pyrex, USA) containing 45 ml fermentation medium. After sterilization and cooling, 5 ml stock inoculum of each strain was added into fermentation medium. Each treatment was carried out in two flasks (in duplicates). The treatments at the temperatures of 15°C and 20°C were incubated in low temperature incubators model 815 (Precision Scientific Inc., USA),

other two treatments at the temperatures of 25°C and 30°C were incubated in a walked-in incubator rooms model TCR-2P (Nippon Medical and Chemical Instrument Co. Ltd., Japan). The fermentation was set in dark and static condition for 15 days. After fermentation, all samples were processed to determine mycelial dry weight and cordycepin concentration as in the previous description.

Table 4 List of fifteen *C. militaris* cultures, sources and date of collection.

Strains	Collecting source	Collecting date
Cordyceps militaris BCC 1974	n/a	n/a
C. militaris BCC 1975	n/a	1998
C. militaris BCC 2790	Nyutu Spa, Lake Tazawa, Tazawako Town, Japan	July 19, 2000
C. militaris BCC 2814	Tsuchiyu Hot Spring, Fukushima City, Japan	July 21, 2000
C. militaris BCC 2815	Appi Highlands, Ahiro Town, Japan	July 18, 2000
C. militaris BCC 2816	Appi Highlands, Ahiro Town, Japan	July 18, 2000
C. militaris BCC 2817	Appi Highlands, Ahiro Town, Japan	July 18, 2000
C. militaris BCC 2818	Appi Highlands, Ahiro Town, Japan	July 18, 2000
C. militaris BCC 2819	Appi Highlands, Ahiro Town, Japan	July 18, 2000
C. militaris BCC 2824	Nyutu Spa, Lake Tazawa, Tazawako Town, Japan	July 19, 2000
C. militaris BCC 2826	Nyutu Spa, Lake Tazawa, Tazawako Town, Japan	July 19, 2000
C. militaris BCC 2838	Nyutu Spa, Lake Tazawa, Tazawako Town, Japan	July 19, 2000
C. militaris NBRC 5298	n/a	n/a
C. militaris NBRC 8797	Ohdaigaharayama, Nara Pref., Japan	n/a
C. militaris NBRC 100741	Hioki, Miyazu, Kyoto, Japan	n/a

Note: (n/a) data missing or not available for this strain.

4. Analytical methods

4.1 Determination of mycelial dry weight

After fermentation, samples collected from various Erlenmeyer flasks were filtrated through pre-weighed filter papers (8 cm in diameter) (Whatman, Germany). Filter papers containing wet mycelium were washed twice with distilled water to remove residue sugar and other dissolved components then dried in a Memmert UNE 400 dryer oven (Memmert, Germany). Dry biomasses in filter papers were weighed daily using the Mettler AT261 analytical balance (Mettler Toledo, USA) until the dry weights reached constantly in two continuing days. Five ml stock inoculum of each strain was also taken and filtrated through pre-weighed filter paper then was washed twice with distilled water and dried in dryer oven to determine the mycelial initial dry weights.

The maximal and initial dry weights (g/l) were calculated as: (mycelium and filter paper dry weight – filter paper weight).

Mycelial dry weight (g/l) was calculated as: [(maximal dry weight – initial dry weight)*1000/50].

4.2 Measurement of cordycepin from culture filtrates

An accurate amount of (2 mg) standard cordycepin (Fluka BioChemika AG, Germany) was weighed using an analytical balance (AT261, Mettler Toledo, USA) and dissolved in total 2 ml nano-pure water. The cordycepin solution (1mg/ml) was then diluted in various gradient concentrations for standard calibration. The standard curve was made according to the practical standard cordycepin concentrations and the peak area obtained from HPLC analysis.

Frozen samples obtained as in the description from section 2.3 were rapidly defrosted under water tap. One ml of liquid filtrate from each sample was

pulled using an one-millilitre syringe (Nipro, Thailand) and force injected into a filter containing a cellulose acetate filter paper (13 mm in diameter, 0.2 µm pore size) (Sartorious AG, Germany). After filtrated, samples were kept in 1.5 ml Eppendorf tubes for analysing in HPLC.

Cordycepin concentration of samples (mg/l) was determined by HPLC. A C_{18} column (150 x 4.6 mm, 4 µm particle size, Phenonemex, USA) was used for sample separation in HPLC. A filter guard, 4 µm pore size, (Nova-Pak C_{18}, Waters, USA) was applied to protect the column. The mobile phase consisted of 10 mM KH_2PO_4 dissolved in mixture of methanol and water (in a ratio 15:85) (Mao *et al.*, 2005) and was driven by a double pump Waters 626 (Millipore, USA) controlled by a Waters 600S Controller (Millipore, USA). The running process was performed at a flow rate of 1 ml/min. The column temperature was $25^{o}C \pm 1$ according to the temperature in air condition room. The UV wavelength was monitored at 254 nm using a Photodiode Array Detector (Waters 996, Millipore, USA). An amount 50 µl of sample was manually injected using a 100 µl micro syringe.

Cordycepin concentration (mg/l) was calculated by subjecting peak area obtained in HPLC to the standard equation.

The cordycepin yield on biomass (mg/g) was calculated as: (cordycepin concentration)/(mycelial dry weight).

Specific cordycepin formation rate (mg/g/day) was calculated as: (cordycepin concentration)/(mycelium dry weight)/(cultivation time).

Cordycepin productivity (mg/l/day) was calculated as: (cordycepin concentration)/(cultivation time).

5. Data processing and statistical analysis.

The results of mycelial dry weight (DW), cordycepin concentration, cordycepin productivity, cordycepin yield on biomass and specific cordycepin formation rate were expressed as means ± standard deviation (SD) of two replicated experiments. The analysis of variance (ANOVA) was applied to estimate the significant difference of means at P=0.05. For multiple comparison, The Tukey's test was applied at P=0.05.

For regression of different sources of *C. militaris* strains, five strain samples from Appi Highlands source and four strain samples form Nyutu Spa source were selected to estimate the pattern of growth and cordycepin production of strains in different locations.

For regression the dependent of growth and cordycepin production on fermentation temperatures, the General Linear Model multivariate procedure was performed. Total 15 samples (15 *C. militaris* strains) were applied in the model on affected of four fermentation temperatures (15°C, 20°C, 25°C and 30°C). The following profile plots (interaction plots) were used for comparing marginal means in these models. A profile plot is a line plot in which each point indicates the estimated marginal mean of a dependent variable (adjusted for any covariates) at one level of a factor. For multivariate analyses, profile plots are created for each dependent variable.

Data analysis was performed statistically using SPSS Version 13.0 software program.

RESULTS AND DISCUSSION

1. Diversity of entomopathogenic fungi in Cat Tien National Park

1.1 Condition of Cat Tien National Park

The rainy season occurs in the South of Vietnam from May until the end of October of the year. During this season, the weather is cool and high humidity which creates the favourable condition for the development of insect population. These factors will be the co-evolutionary effects for the development of the entomopathogenic pathogenic fungi. It is possible to believe that with the higher density and diversity of the insect population will create more chance for the entomopathogenic fungi to contact and infect to insect bodies. In this study, one collection trip was created in Cat Tien National Park from September 6th to 8th, 2007, when it was in the middle of rainy season so heavy rain occurred everyday, especially in the afternoon and the evening. By personal observation, the difference between Cat Tien National Park with other parks in Thailand (i.e. Khao Yai National Park) was the characteristics of forest floor. Cat Tien National Park has a low and flat land floor which consisted of black soil and very little of falling leaves on the leaf litter layer. These conditions, therefore, may affect on the distribution of fungi and their insect hosts as well.

As described in literature, the diversities of plants and animals (including insect) are well-known in Cat Tien National Park, however, diversity and distribution information of microorganisms, such as bacteria, yeast and other fungi (especially entomopathogenic fungi), are less been in consideration by scientists. Since 2005, few primary surveys of entomopathogenic fungi were made under the cooperative research program between the Institute of Tropical Biology of Vietnam and the National Centre for Genetic Engineering and Biotechnology of Thailand, it was found that Cat Tien National Park has a number of yeast and entomopathogenic fungal species presented (unpubl.). Therefore, survey and record the presence of

entomopathogenic fungi in Cat Tien National Park is very important to provide primary information about this fungal group for further researches and applications.

1.2 Collection of entomopathogenic fungal specimens

In this study, three-day survey was done in Cat Tien National Park from September 6th to 8th, 2007. Survey and collection were made in five locations: (1) Track behind the Headquarter to Bang Lang trail, (2) left area along the main road to Crocodile swamp, (3) Cay Go Bac Dong trail, (4) Bang Lang trail, and (5) area behind bungalow. The details of specimen number, fungal name, insect and spider hosts, habitat distribution and collecting location of specimens are shown in Appendix Table B1.

There were six collectors in the conducted trip. During three days of collection, there were total 259 specimens collected. The distribution of fungal specimens in five locations is described in Figure 10 in which 71 specimens were collected along the track from behind the Headquarter to Bang Lang trail, 14 specimens were collected along the main road to Crocodile Swamp, 16 specimens were collected in Cay Go Bac Dong trail, 156 specimens were collected in Bang Lang trail, and only two specimens were collected in the area behind the bungalow (guest house).

The numbers of specimens collected from different area were different however, these information did not express the biological diversity or distribution features of entomopathogenic fungi in those locations. There are several factors that affects on the quantity of specimens from each location, such as the geographical condition of each location, collecting season, number and skill of collectors, the time to spend in each area, etc. In this study, higher number of specimens was found from Bang Lang trail and from the track from behind the Headquarter to Bang Lang trail than others because it was easy for travelling, and the collecting time spending there was longer than that in other area. In some other cases, the conditions of these area are also important for the quantity of samples as well as the diverse of genera.

Figure 9 Preparation for entomopathogenic fungi collection in Cat Tien National
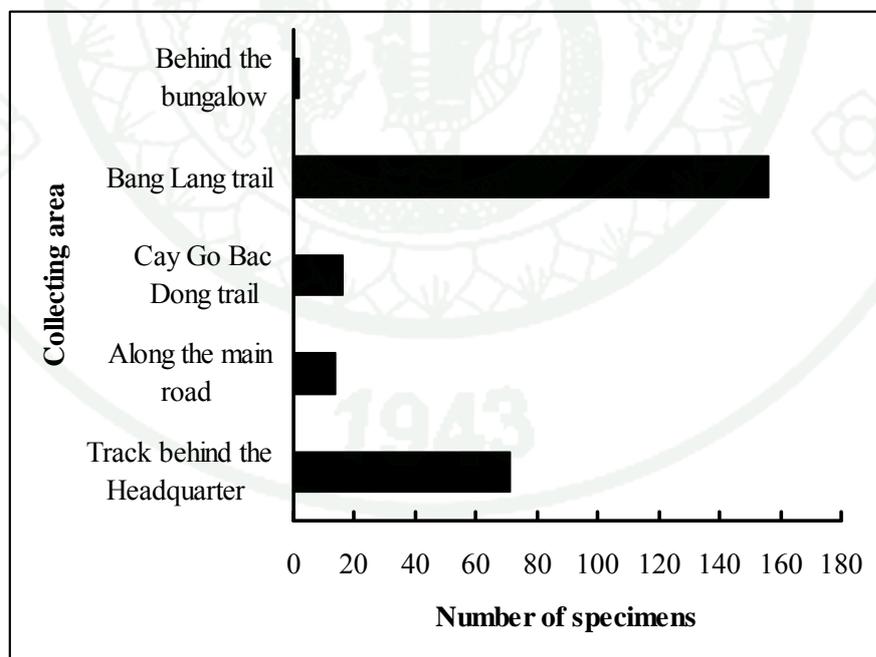Park.

Figure 10 Distribution of fungal specimens from five locations in Cat Tien National
Park.

1.3 Presence of entomopathogenic fungal genera in Cat Tien National Park

Among total 259 specimens collected from five trails and locations of Cat Tien National Park, it was observed that the numbers of genera were diverse. There were 17 genera of entomopathogenic fungi had been recorded, which include *Akanthomyces*, *Aschersonia*, *Beauveria*, *Conoideocrella*, *Cordyceps*, *Gibellula*, *Hirsutella*, *Hymenostilbe*, *Hypocrella*, *Isaria*, *Metarhizium*, *Moelleriella*, *Nomuraea*, *Ophiocordyceps*, *Paecilomyces*, *Torrubiella* and *Verticillium*. All of those genera belong to the Phylum Ascomycota, Order Hypocreales of the kingdom Fungi. The number of specimens collected in each genus is described in Figure 11 and the relative occurrence frequency of each genus (percentage of specimens in each genus) is shown in Figure 12. The genus *Aschersonia* was found in the highest number of specimens with total 72 specimens (with relative occurrence frequency of 27.8%). Two teleomorphic genera of *Aschersonia*, *Hypocrella* and *Moelleriella*, had the second highest number with total 53 specimens in total (with relative occurrence frequency of 20.4% (10.0% and 10.4% respectively). Both *Aschersonia* and its teleomorphic genera *Hypocrella* and *Moelleriella* have the same habitat on leaves of forest plants, and same insect host (scale insects Homoptera). The genus Isaria also indicated with high number of specimens with 33 samples (12.7% of total specimens) following by *Ophiocordyceps*, one of the most important teleomorphic genera of the Order Hypocrales, family Ophiocordycipitaceae that previously classified in the family Clavicipitaceae and genus *Cordyceps*. There were 29 *Ophiocordyceps* specimens collected (11.2%), whereas only one specimen of the genus *Cordyceps* could be found. Comparing with total number of *Aschersonia* and its anamorphs, *Moelleriella* and *Hypocrella*, specimens, the availabilities of *Cordyceps* and *Ophiocordyceps* were much lower. Two well-known genera using for biological control of insect pests, *Beauveria* and *Metarhizium*, were also recorded in the collection, however, there were only one specimen of *Beauveria* and three specimens of *Metarhizium* (relative occurrence frequencies were 0.4% and 1.2% respectively). Beside, there were six specimens could not be identified because they were fungal parasites on entomopathogenic fungus *Hirsutella* (Hyperparasites).
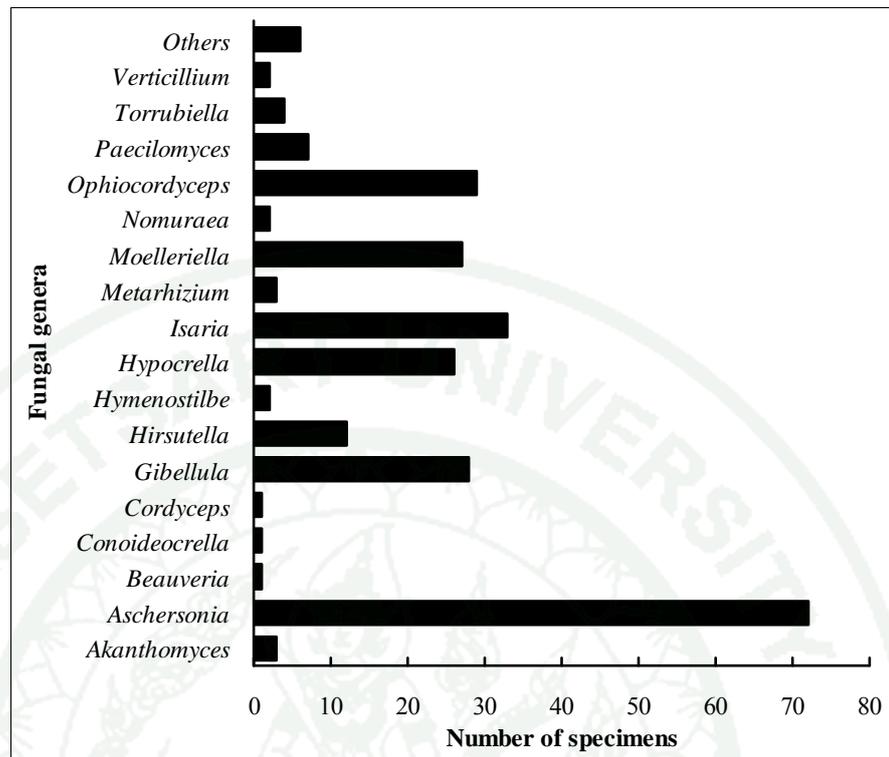
Figure 11 Comparison the number of collected specimens among different
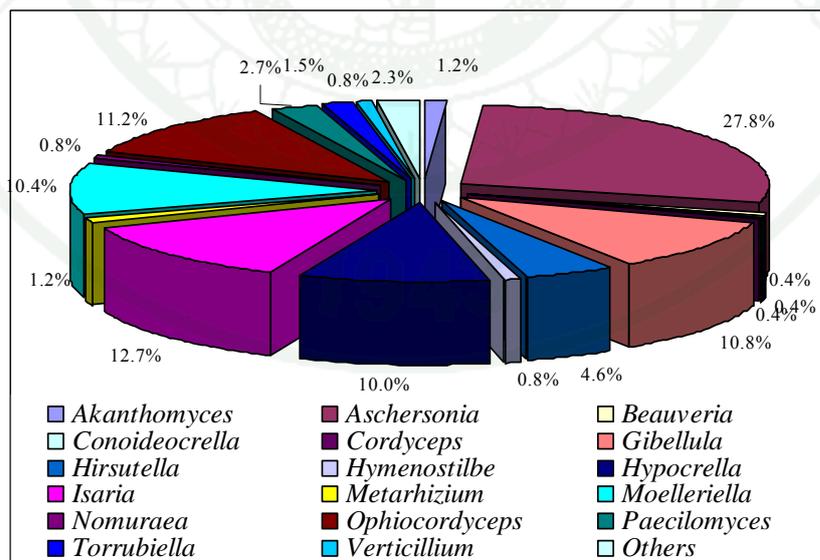entomopathogenic fungal genera.

Figure 12 Relative occurrence frequency of collected specimens among different
entomopathogenic fungal genera.

When comparing with overall entomopathogenic fungal genera represented from two forest sites of Hong Kong and Pasoh forest, Ulu Gompak forest and Genting Highlands of Peninsula, Malaysia (Kwong, 2003), it was found that the recorded numbers of entomopathogenic fungal genera in Cat Tien National Park were higher even this data was collected from only one trip in rainy season. The comparison of the presence of entomopathogenic fungi in Cat Tien National Park and other locations in Hong Kong and Malaysia is shown in Table 5. According to the observation of Kwong (2003) in her studies from Hong Kong and Malaysia, although *Cordyceps*, *Ophiocordyceps* and their anamorphs (*Hirsutella* and *Hymenostilbe*) species are more frequently presence in rainy season, the numbers of collected specimens of *Cordyceps, Ophiocordyceps, Hirsutella* and *Hymenostilbe* were far less than total number of *Aschersonia* and its teleomorphs *Hypocrella* and *Moelleriella.* The current study in Cat Tien National Park also recorded that it was similar to those of Kwong's record. *Aschersonia, Hypocrella* and *Moelleriella* species infect scale insect and develop fungal stromata under side of leaves of forest plants. The development of *Aschersonia, Hypocrella* and *Moelleriella* occurs during the year.

Because of limitations of time and travelling condition, survey trip of this study was carried out once only in rainy season and the trip was done only in Southern area of Cat Tien National Park (Nam Cat Tien sector). It can be expected that more entomopathogenic fungal genera would be found if collections were done regularly throughout the year in both rainy and dry seasons, and studies from other locations would be conducted in the forest. This is also the target for our further research in the future.

Table 5 Comparison the presence of entomopathogenic fungal genera in Cat Tien
National Park with other represented data in Malaysia and Hong Kong.

Fungal genera	Cat Tien National Park	Peninsula Malaysia [*]	Two forest sites, Hong Kong [*]
Akanthomyces	+	+	-
Aschersonia	+	+	+
Beauveria	+	-	+
Cordyceps	+	+	+
Conoideocrella	+	-	-
Gibellula	+	+	+
Hirsutella	+	+	-
Hymenostilbe	+	-	+
Hypocrella	+	+	+
Isaria	+	+	+
Metarhizium	+	-	+
Moelleriella	+	+	+
Nomuraea	+	-	+
Ophiocordyceps	+	+	+
Paecilomyces	+	-	-
Polycephalomyces	-	-	+
Stilbella	-	+	-
Torrubiella	+	+	-
Verticillium	+	-	-

Note: (*) Data recorded by Kwong (2003).

1.4 Presence of entomopathogenic fungal species in Cat Tien National Park

Among 259 specimens of 17 entomopathogenic fungal genera recorded, there were 41 species identified. The list of entomopathogenic fungal species recorded from Cat Tien National Park are listed in Table 7. The comparison data of species numbers between fungal genera and their relative occurrence frequency are shown in Figure 13 and Figure 14. These results showed that the highest number of species found in the genus *Aschersonia* with seven species recorded, which includes *A. badia, A. confluens, A. marginata, A. oxystoma, A. placenta, A. samoensis* and *Aschersonia* sp.. The relative occurrence frequency of *Aschersonia* species was 17.1%. The teleomorphs of *Aschersonia*, *Hypocrella* and *Moelleriella*, were also found in this study. There were two species of *Moelleriella* and three species of *Hypocrella* identified (4.9% and 7.3% respectively). It was said that the presence of a teleomorphic species would come together with one or more than one anamorphic species, for an example of the well-known species *Ophiocordyceps sinensis*, there were several anamorphic stages of this species, which have been reported in several reports. In this study, anamorphs and their related teleomorphs of *Aschersonia* and *Hypocrella* and *Moelleriella* species showing in Table 6 indicated that not all *Aschersonia* and *Hypocrella* or *Moelleriella* species have their relative to teleomorphs and anamorphs, *A. confluens, A. marginata* and *A. oxystoma* were the examples, their teleomorphic stages were absent from our collection. This study also recorded that Aschersonia anamorph has two teleomorphs (*Hypocrella* and *Moelleriella*). Other entomopathogenic fungal genera were also recorded with high species number in Cat Tien National Park. Numbers of species of *Paecilomyces* together with *Isaria*, which previously named as *Paecilomyces*, were five (*Paecilomyces cinnamomeus, P. lilacinus, Isaria javanica, Isaria* sp. and *I. tenuipes)*. The relative occurrence frequency of *Paecilomyces* and *Isaria* was 12.2% based on their total species number in Cat Tien National Park. Whereas, the total species numbers of other genera such as *Hirsutella* were five (12.2%); *Gibellula, Metarhizium* and *Ophiocordyceps* were three (7.3%); *Akanthomyces* and *Torrubiella* were two (4.9%), however, *Conoideocrella, Cordyceps*, *Beauveria, Hymenostilbe*, *Nomuraea* and *Verticillium* had only one species in each genus.

It was also observed from previous study (Kwong, 2003) that both teleomorphic and the related anamorphic stages might or might not appear at the same time. In her collection, several *Aschersonia* species lacked the presence of their *Hypocrella* stages. It can be discussed that there have several conditions affected on the development of sexual or asexual forms of a fungus, for example season (weather) which is very an important factor. Missing anamorphs or teleomorphs would be found if more surveys were made frequently during the year, and the locations for colleting in the forest would be varied. In the pass, the relationship between anamorphs and teleomorphs in fungal life cycle was more or less ignored for many years of fungal studies. Less researches and information have been done on the condition of development of the sexual and asexual stages of entomopathogenic fungi and the relationship between teleomorphs and anamorphs with fungal evolution. Only until the development of molecular technology, scientists can get DNA and RNA from anamorphs just as easily as from teleomorphs, so scientists are beginning to understand the relationships of anamorphs and teleomorphs better. Furthermore, recent researches have proved that many anamorphic species have close relationship with teleomorphic species in the phylogenetic classification of entomopathogenic fungi (Sung *et al.*, 2006, 2007).
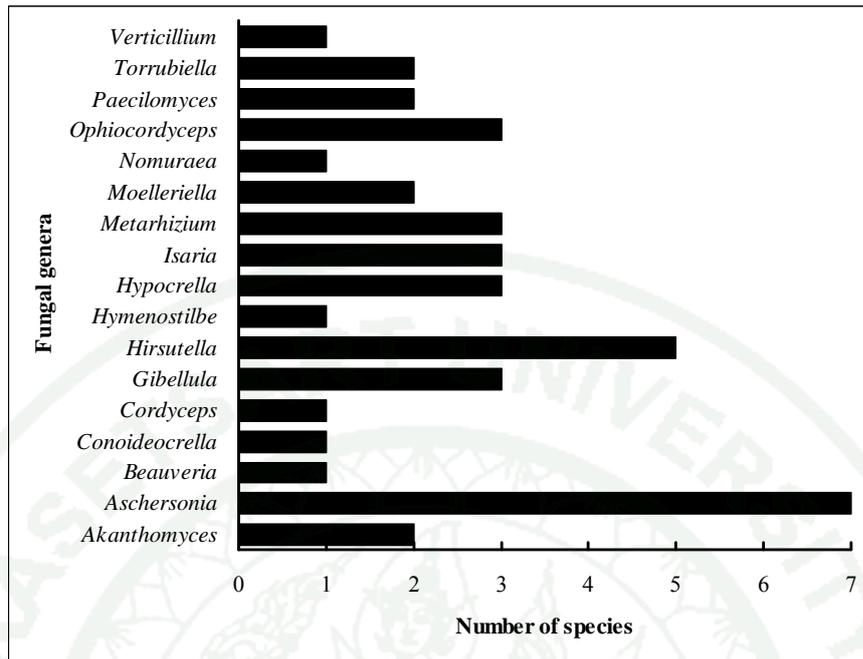
Figure 13 Comparison the number of recorded species in 15 entomopathogenic
fungal genera in Cat Tien National Park.
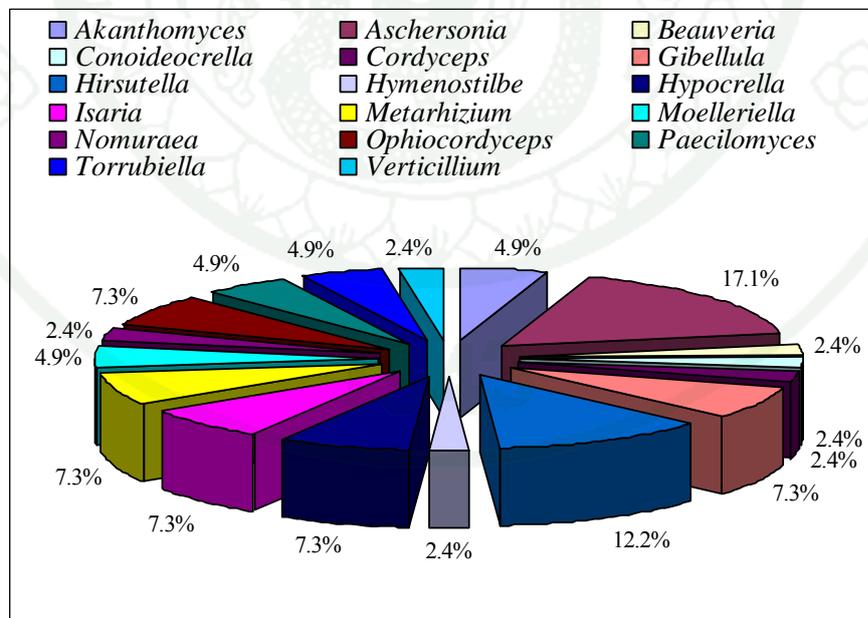
Figure 14 The species relative occurrence frequencies of 15 entomopathogenic
fungal genera in Cat Tien National Park.

Table 6 Presence of *Aschersonia* and their teleomorphic species in Cat Tien National
Park.

***Aschersonia* species**	**Relative teleomorphic species**
Aschersonia confluens	*n/a*
Aschersonia badia	*Hypocrella siamensis*
Aschersonia marginata	*n/a*
Aschersonia oxystoma	*n/a*
Aschersonia placenta	*Moelleriella raciborskii*
Aschersonia samoensis	*Hypocrella calendulina*
n/a	*Hypocrella luteola*
n/a	*Moelleriella* sp.

Note: n/a: related anamorphic or teleomorphic species were not found.

Table 7 List of entomopathogenic fungal species recorded in Cat Tien National Park.

No.	Name	Epithet	Authors
1	*Akanthomyces*	*pistillariiformis*	(Pat.) Samson & H.C. Evans 1974
2	*Akanthomyces*	sp.	
3	*Aschersonia*	*badia*	Pat. 1897
4	*Aschersonia*	*confluens*	Henn. 1899
5	*Aschersonia*	*marginata*	Ellis & Everh. 1895
6	*Aschersonia*	*oxystoma*	Berk. 1854
7	*Aschersonia*	*placenta*	Berk. 1875
8	*Aschersonia*	*samoensis*	Henn. 1896
9	*Aschersonia*	sp.	
10	*Beauveria*	sp.	
11	*Conoideocrella*	*tenuis*	(Petch) D. Johnson, G.H. Sung, Hywel-Jones & Spatafora 2008
12	*Cordyceps*	sp.	
13	*Gibellula*	*pulchra*	Cavara 1894
14	*Gibellula*	sp.1	
15	*Gibellula*	sp.2	
16	*Hirsutella*	cf. *brunneipunctata*	
17	*Hirsutella*	*citriformis*	Speare 1920
18	*Hirsutella*	*formicarum*	Petch 1935
19	*Hirsutella*	*saussurei*	(Cooke) Speare 1920
20	*Hirsutella*	sp.	
21	*Hymenostilbe*	*aurantiaca*	Hywel-Jones 1996
22	*Hypocrella*	*calendulina*	Hywel-Jones & Mongkolsamrit 2009
23	*Hypocrella*	*luteola*	Hywel-Jones & Mongkolsamrit 2009
24	*Hypocrella*	*siamensis*	Hywel-Jones & Mongkolsamrit 2009
25	*Isaria*	*javanica*	(Friedrichs & Bally) Samson & Hywel-Jones 2005

Table 7 (Continued).

No.	Name	Epithet	Authors
26	*Isaria*	sp.	
27	*Isaria*	*tenuipes*	Peck 1879
28	*Metarhizium*	sp.1	
29	*Metarhizium*	sp.2	
30	*Metarhizium*	sp.3	
31	*Moelleriella*	*raciborskii*	(Zimm) Chaverri & K.T. Hodge (2009)
32	*Moelleriella*	sp.	
33	*Nomuraea*	*atypicola*	(Yasuda) Samson 1974
34	*Ophiocordyceps*	cf. *brunneipunctata*	(Hywel-Jones) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora 2007
35	*Ophiocordyceps*	*irangiensis/ myrmecophila*	(Moureau) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora 2007/ (Ces.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
36	*Ophiocordyceps*	*unilateralis*	(Tul.) Petch 1931
37	*Paecilomyces*	*cinnamomeus*	(Petch) Samson & W. Gams 1974
38	*Paecilomyces*	*lilacinus*	(Thom) Samson 1974
39	*Torrubiella*	sp.1	
40	*Torrubiella*	sp.2	
41	*Verticillium*	sp.	

1.5 Presence of anamorphic and teleomorphic stages

In overall 41 entomopathogenic fungal species of 17 genera, the numbers of teleomorphs and anamorphs were different. In the genus level, the numbers of anamorphic genera were much higher than that of teleomorphic genera. There were total 11 anamorphic genera, while only six teleomorphic genera could be recorded. Similar to the genus level, a difference between the numbers of anamorphic and

teleomorphic species could be recorded. There were 29 species of anamorphs recorded, whereas only 12 species of teleomorphs could be found in the collection of Cat Tien National Park (Figure 16). When comparing total number of specimens of anamorphs with teleomorphs in overall 259 specimens, the number of anamorphic specimens was much higher (Figure 15). There were 174 anamorphic and 85 teleomorphic specimens in the record, the percentages of anamorphic and teleomorphic specimens were 67.18% and 32.82% respectively. *Conoideocrella*, *Cordyceps*, *Hypocrella*, *Moelleriella*, *Ophiocordyceps* and *Torrubiella* are known as six main teleomorphic genera in the Order Hypocreales of the Phylum Ascomycota. In this study, all of these teleomorphic genera could also be observed from Cat Tien National Park even the numbers of collected specimens and species were less than those of their anamorphs. In this study, the detail of anamorph-teleomorph connection and the relationship of individual anamorph-teleomorph species were not discussed, however, many anamorphic species of entomopathogenic fungi have been connected to their teleomorph in both morphological and phylogenetic classifications (Sung *et al.*, 2007). The hypothesis proved that one anamorphic species has only one related teleomorph, however, one teleomorphic species may have one or more than one anamorphic species even these anamorphic stages are classified in different genera (Hywel-Jones *et al.*, 1998; Sivichai *et al.*, 2000). The term synanamorph is used to describe a fungus that produces more than one anatomic forms, or anamorphs. For those reasons, it was found from previous research and survey on entomopathogenic fungi that the numbers of anamorphic species are always higher than that of teleomorphic species (Kwong, 2003). There were proofs that some anamorphic fungal species may have lost their Teleomorphs or evolved along separate lines (Hywel-Jones, 2002), therefore, many anamorphs still have not yet been connected to teleomorphs, and these species were previously grouped in a distinct fungal group Deuteromycetes in the former fungal classification.
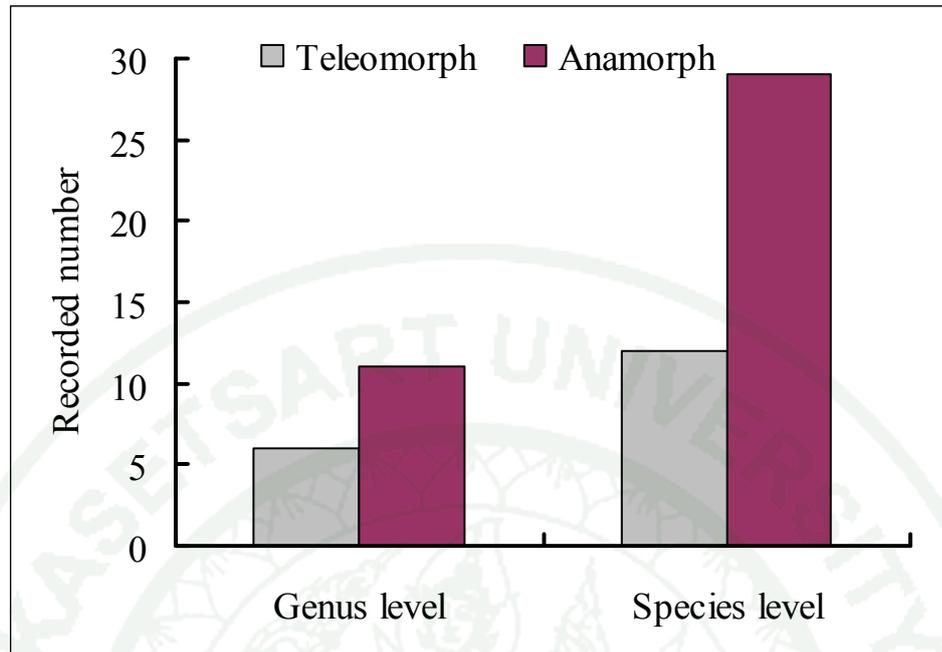
Figure 15 Comparison the numbers of teleomorphic and anamorphic stages in genus
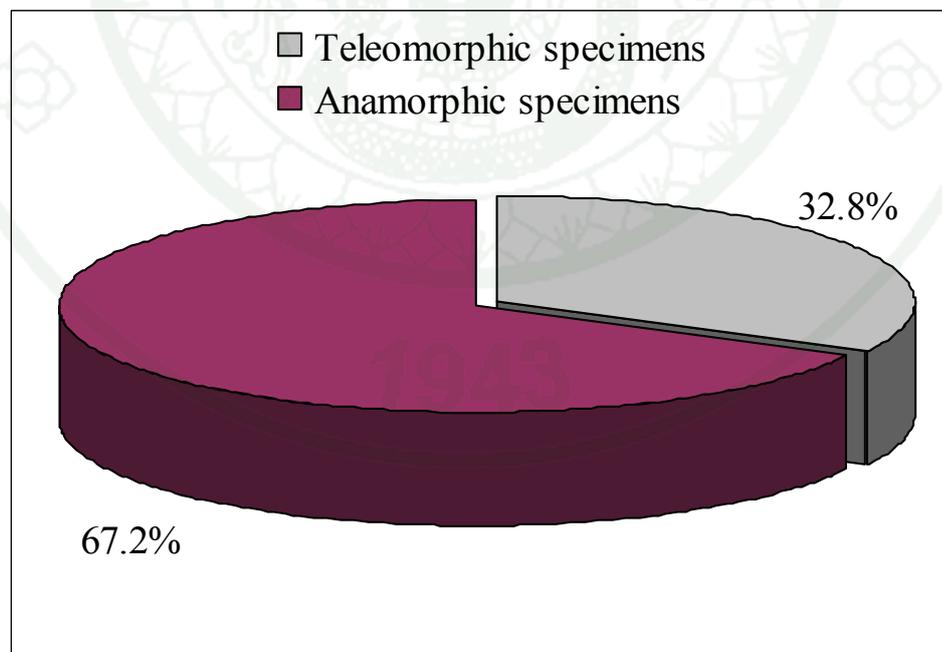and species levels

Figure 16 The relative occurrence of collected anamorphic and teleomorphic
specimens.

1.6 Distribution of entomopathogenic fungi in their habitats

In the studies of the distribution of entomopathogenic fungi in their natural ecosystem, fungal specimens can be searched in their several micro-habitats. Their habitats are often related to host habitats (ecological niches). In survey of entomopathogenic fungi in natural habitats, searching is often made in two main habitats: on forest floor and on leaves of forest plants. However, specimens can also be found from other habitats such as tree branches, stems or small caves in rock sites, etc. In this study, most specimens were found from two main micro-habitats: on leaves of forest plants and on forest floor (including in soil and on leaf litter). The numbers of collected specimens found from these two habitats were significantly different, there were 220 specimens collected on leaves but only 38 specimens were found on forest floor whereas only one specimen of *Isaria* sp. ITB372 was recorded its niche on a small tree twig of a Dicot plant.

In a comparison of the relative occurrence frequencies (the frequencies of ecological distribution), the leaf surfaces of plants were most prevalent for searching of entomopathogenic fungal specimens with about 84.9% specimens had been collected from leaf surfaces. However, only 14.7% specimens could be found on leaf litter and in the soil (on forest floor) (Figure 17). From the list of collected specimens shown on Appendix Table B1, it is easy to observe that all specimens of *Aschersonia* species and their teleomorphs *Hypocrella* and *Moelleriella* species, *Gibellula* species and *Ophiocordyceps unilateralis* were located on leaves. These species made up a large number of specimens in the collection.

It can be discussed that the infecting generation of an entomopathogenic fungus to its host will be ruled by many factors. The abiotic factors, which include humidity, temperature and solar radiation, and the biotic factors, which includes pathogen population (i.e. diverse numbers of fungal spores), insect population and other microorganisms. Therefore, there have some possible reasons which caused the less number of entomopathogenic fungal specimens collected from forest floor. First, it might be because of the geographical and climate conditions of the forest, the

collecting period was in the middle of rainy season and in lowland areas, the forest floor was wet and low dense of leaf litter. Comparing with Khao Yai National Park of Thailand, these conditions were found to be different from our observation in which Khao Yai National Park always contents a thick layer of decayed leaves (pers. observ.). These unfavourable conditions of forest floor of Cat Tien National Park therefore could not protect fungal spores from heavy raindrops or sunray.
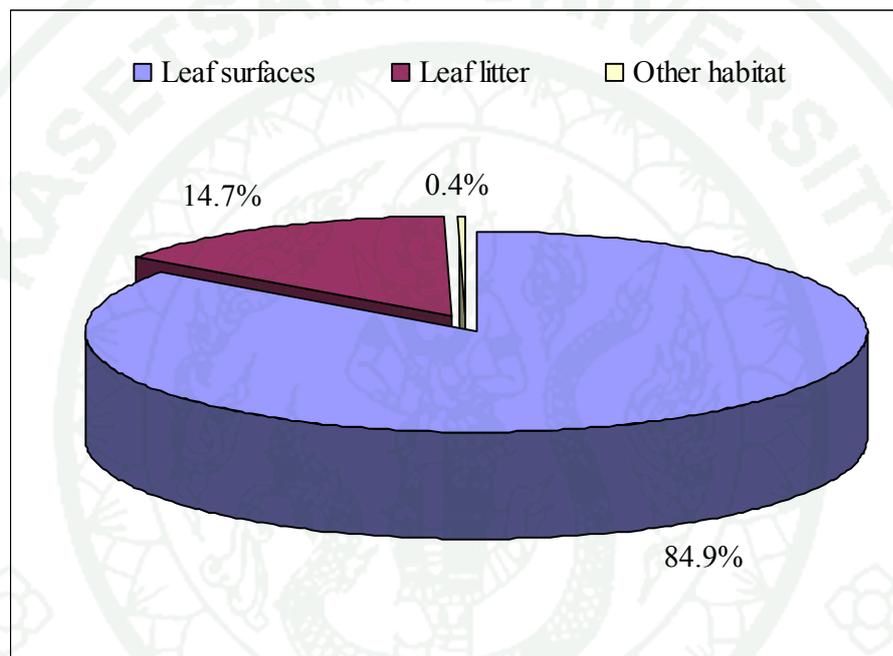
Figure 17 The relative occurrence frequency of fungal specimens in 3 different micro-habitats.

Other biotic and abiotic factors might also affect to the diversity and distribution of microorganisms in their natural habitats. The hypothesis has proved that moisture or high relative humidity is the most important abiotic factor for the growth of entomopathogenic fungi, especially for the germination of fungal spores. McCoy (1990) demonstrated that high relative humidity of more than 90% is essential for the infection cycle including germination and sporulation. However, Griffin (1963) concluded that the survival, germination and growth of soil fungi are greatly influenced by water potential, but high moisture reduces oxygen availability and

increases carbon dioxide level, it may not be a favourable condition for the germination and development of spores.

The texture of soil also plays an important role in microbial activity. It may function as a protectant against biodeterioration thus may increase the stability and the longevity of conidia or blastospores (Fargues *et al.*, 1983). In other case, dead leaves fallen from forest plants may be also important because it will become niches for various insect species to develop and it become a co-evolution factor enhances the opportunity for fungal spores contact with their host on leaf litter. Low leaf-litter surface with a muddy land of Cat Tien National Park therefore created unfavourable condition for the development of entomopathogenic fungi on the ground. As the results, searching of entomopathogenic fungi in Cat Tien National Park in other trials and locations is highly recommended. The survey would be regularly during several times of years.

When the environment on the forest floor was not favourable for entomopathogenic fungi, the leaf surfaces became the suitable habitat for the development of this insect's pathogens. Leaf surfaces, especially lower surface, were the safe niches for numerous insects and other arthropods such as scale insects, white flies, ants, spiders, etc. because it is well-protected from heavy rain fall and sunray. It was reported that free water on leaf surface might enhance the infection of fungal spores (Meekes, 2001). Other research also reported that *Hypocrella* and *Aschersonia* species could be found throughout the year even when the relative humidity of the atmosphere was low in winter (45%-80%) (Kwong, 2003). The surface of leaves is dry at daytime during sunlight and wet at night when the temperature reduces and some drops of dew still remain on leaf (pers. observ.), thus the spores of *Aschersonia, Hypocrella* and *Moelleriella* could be discharged in this condition and the running-off of drops and drew might carry fungal spores along the leaf and contact with healthy hosts locating on leaf. Drew or drops of water locating in the leaf surfaces therefore play an important role for discharging, germinating and infecting host on leaf. Other entomopathogenic fungal species such as *Gibellula* spp., *Ophiocordyceps unilateralis* may have different way of sporulation and infection when comparing with the

generation of *Aschersonia, Hypocrella* or *Moelleriella*. Ascomata of *Ophiocordyceps unilateralis* and synemata of *Gibellula* spp. are begotten on stalks, which is a thickness of hyphal body. After matured and in appropriate condition, their ascospores or conidia will be discharged and fallen into the lower leave surfaces, or might be carried by wind and deposited on other leaves around an area. These spores will be in "sit and wait" status to get change to infect when it contacts to a living host. Until now, no detailed report has been done to demonstrate the pattern of sporulation of these species, however, the discussions above are possible to think and put in the research consideration.

1.7 Relationship between fungi and arthropod hosts

In order to find out the relationship between fungal pathogens and their hosts, arthropod orders, which associated with entomopathogenic fungi, had been recorded in the collection. There were eight arthropod Orders of the Phylum Athropoda found to be associated with entomopathogenic fungi including seven orders of the class Insecta (Blattaria, Coleoptera, Homoptera, Hemiptera, Hymenoptera, Lepidoptera and Mantodea) and only one order of the class Arachnida (Araneida).

The recorded numbers of specimens and its relative occurrence frequency entomopathogenic fungi in the association with their arthropod Orders are shown in Table 8. When comparing the number of collected specimens among eight arthropod Orders, it was found that the highest number of specimens was fungal pathogens on the Order Homoptera. Total 131 specimens had Homopteran host (52.4%). Specimens on other arthropod Orders such as Araneida, Hymenoptera and Lepidoptera were also frequently found during three-day collecting trip with the relative occurrence frequencies of 15.2%, 15.6% and 11.2%, respectively. However, specimens which have the hosts belonging to the Orders Coleoptera, Blattaria, Hemiptera and Mantodea were rarely found in Cat Tien National Park. Nine specimens could not be connected with their hosts because whether their hosts were lost or they were unidentified fungi.

When looking on host specificity of each entomopathogenic genus, it could be observed that *Aschersonia* and its teleomorphs (*Hypocrella* and *Moelleriella*) and *Gibellula* are host specific. It was found that *Aschersonia, Moelleriella* and *Hypocrella* could be colonized only on scale insect (Homoptera), while *Gibellula* species were only the pathogen of spiders (Araneida). It was reported that other fugal genera such as *Cordyceps, Hirsutella, Metarhizium, Paecilomyces* and *Torrubiella* have broad host range and these entomopathogenic fungi associate with many arthropod groups including invertebrates from order Coleoptera, Diptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera and Orthoptera. Many entomopathogenic fungal species have wide host spectrum (Inglis *et al.*, 2001) even on several insect orders, e.g. *Metarhizium* spp., *Beauveria* spp. and *Paecilomyces* spp.. Species of the genus *Paecilomyces* and *Isaria* have been recorded from infected host of Coleoptera, Homoptera, Lepidoptera, Orthoptera, etc. (Kwong, 2003), however, this research found that lapidopteran larvae or pupae (larvae and pupae of moths and butterflies) were the preferred hosts for *Paecilomyces* and *Isaria*.

Table 8 Number of specimens and its relative occurrence frequencies of fungal genera in association with their arthropod Orders.

Fungal genera	The presence of arthropod host							
	Araneida	**Blattaria**	**Coleoptera**	**Hemiptera**	**Homoptera**	**Hymenoptera**	**Lepidoptera**	**Mantodea**
Akanthomyces	2 (0.8%)	-	-	-	-	-	1 (0.4%)	-
Aschersonia	-	-	-	-	72 (28.8%)	-	-	-
Beauveria	-	-	-	-	-	1 (0.4%)	-	-
Conoideocrella	-	-	-	-	1 (0.4%)	-	-	-
Cordyceps	-	-	-	-	-	-	-	1 (0.4%)
Gibellula	28 (11.2%)	-	-	-	-	-	-	-
Hirsutella	-	-	1 (0.4%)	1 (0.4%)	-	9 (3.6%)	-	-
Hymenostilbe	-	-	-	-	-	2 (0.8%)	-	-
Hypocrella	-	-	-	-	26 (10.4%)	-	-	-
Isaria	4 (1.6%)	-	-	-	-	-	27 (10.8%)	-
Metarhizium	-	1 (0.4%)	-	1 (0.4%)	1 (0.4%)	-	-	-
Moelleriella	-	-	-	-	27 (10.8%)	-	-	-
Nomuraea	2 (0.8%)	-	-	-	-	-	-	-
Ophiocordyceps	-	-	4 (1.6%)	-	-	25 (10.0%)	-	-
Paecilomyces	-	-	-	5 (2.0%)	2 (0.8%)	-	-	-
Torrubiella	2 (0.8%)	-	-	-	-	2 (0.8%)	-	-
Verticillium	-	-	-	-	2 (0.8%)	-	-	-
Total	38 (15.2%)	1 (0.4%)	5 (2.0%)	7 (2.8%)	131 (52.4%)	39 (15.6%)	28 (11.2%)	1 (0.4%)

1.8 Cultures and maintenance

Isolation was made within or after the day of collection. In total 259 collected entomopathogenic fungal specimens, the numbers of successfully isolated cultures were 149 with the relative successfully isolation was 57.5%. All successfully isolated cultures were processed and deposited in BIOTEC Culture Collection (BCC) with BCC ordinal numbers. These cultures were maintained in cryotubes containing 10% v/v glycerol and stored in freezer under -80°C for long-term preservation. The list of specimen numbers, BCC numbers, and fungal names of 149 deposited cultures is shown in Appendix Table B2.

Among 17 entomopathogenic fungal genera recorded, only 12 fungal genera had been succeeded to get into cultures (Table 9). The result shown in Figure 18 indicated that fungal species of the genera *Aschersonia*, *Gibellula*, *Hypocrella*, *Isaria*, *Moelleriella*, *Nomuraea*, *Paecilomyces* and *Torrubiella* got high number of cultures, species of these genera were easy growing even some species (*Gibellula*) grew very slowly on the surface of PDA medium in laboratory condition. Other entomopathogenic fungal species such as *Beauveria*, *Cordyceps*, *Ophiocordyceps*, *Hirsutella*, *Hymenostilbe* and *Verticillium* indicated very poor results of successful isolation. *Beauveria* species are known as fast growing and is an easily cultivating fungus, however there was no culture of *Beauveria* which have successfully isolated because there was only one specimen of *Beauveria* had been collected, as the result, during the culture purification, it was contaminated.

Among collected *Ophiocordyceps* and *Cordyceps* species, *Ophiocordyceps unilateralis* and its anamorph *Hirsutella formicarum* resulted as uncultivable species. Total 22 specimens of *Ophiocordyceps unilateralis* and 8 specimens of *Hirsutella formicarum* had been collected; however, none of them could be successfully isolated into culture. Their ascospores and conidia germinated on PDA, ascospores produced tiny microspores, and after that, these cultures were no longer grown.

It was reported that specimens of *Ophiocordyceps unilateralis* are very difficult to isolate and grow on common artificial media in laboratory, only the complex and expensive Grace's insect cell culture medium can be used for *in vitro* cultivation (as blastospores and mycelium) of this fungus (Kocharin and Wongsa, 2006; Wongsa *et al.*, 2005). It was also suggested that high glucose concentration and in suitable C:N ratio in the semi-defined medium from Grace's insect cell medium should be applied in order to achieve blastospore formation and mycelium growth (Kocharin, 2006). The development of blastospores in submersed cultures of several entomopathogenic species have been suggested as an alternative method for isolation, cultivation and fermentation for enzyme and metabolite production (Le, unpubl.).

Table 9 Numbers of specimens and isolated cultures of entomopathogenic fungi.

Fungal genera	No. of cultures	No. of specimens
Akanthomyces	1	3
Aschersonia	52	72
Beauveria	0	1
Conoideocrella	0	1
Cordyceps	0	1
Gibellula	22	28
Hirsutella	0	12
Hymenostilbe	1	2
Hypocrella	14	26
Isaria	24	33
Metarhizium	2	3
Moelleriella	18	27
Nomuraea	2	2
Ophiocordyceps	3	29
Paecilomyces	6	7
Torrubiella	4	4
Verticillium	0	2
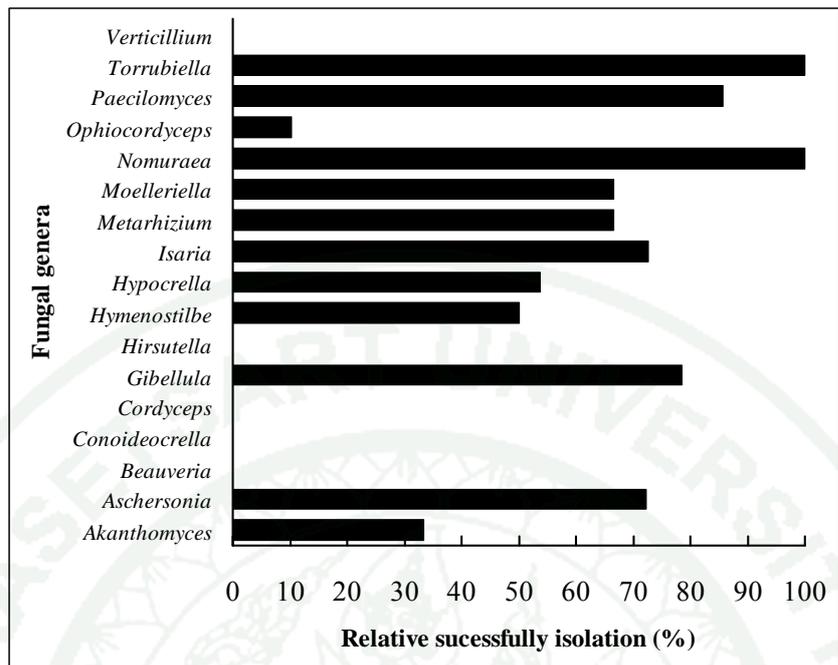
Figure 18 The relative successfully isolation of 17 entomopathogenic fungal genera.
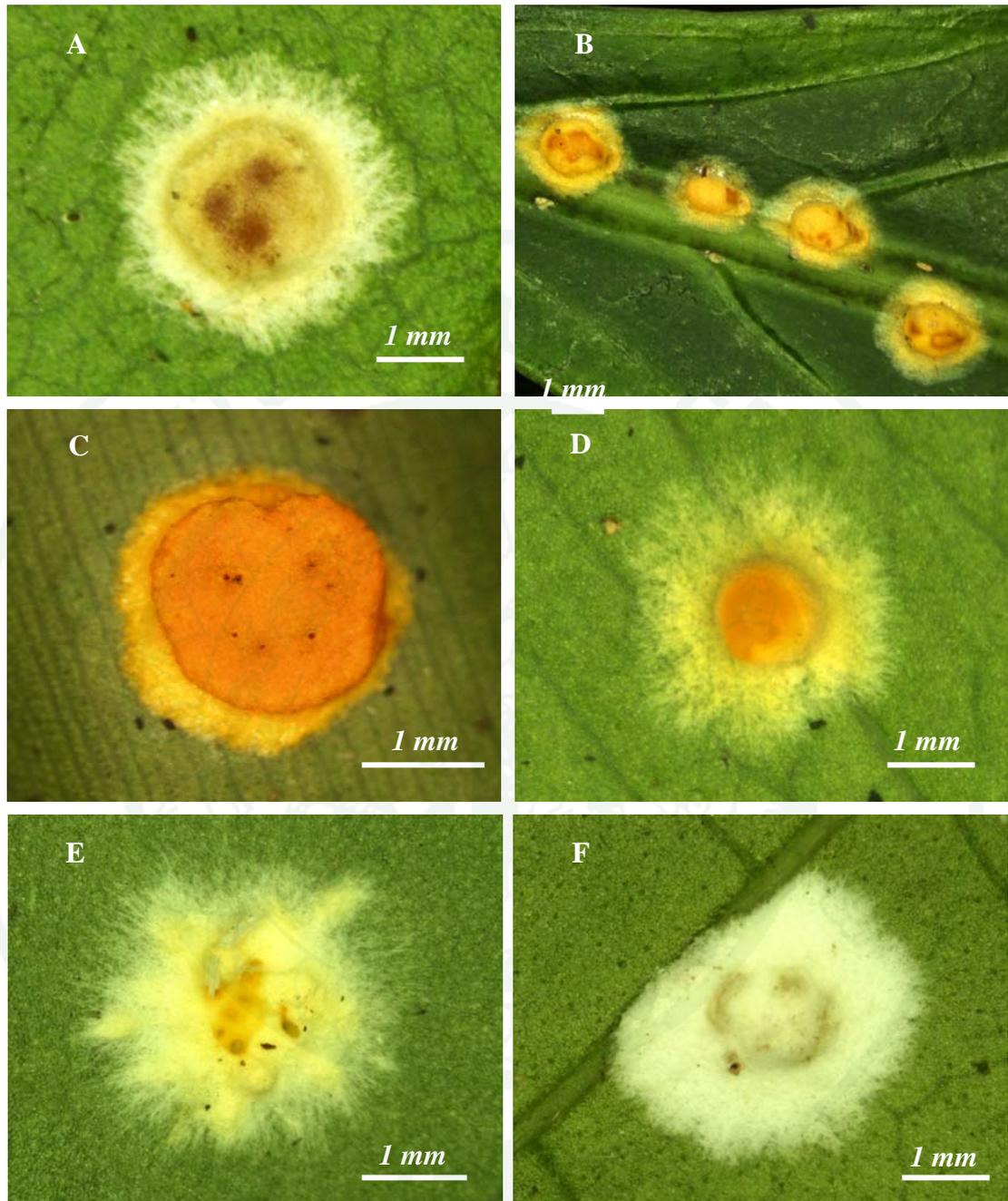
Figure 19 *Aschersonia* species infected scale insect (Homoptera) on leaves.

Note: (A) *A. badia*, (B) *A. marginata*, (C) *A. samoensis*, (D): *A. oxystoma*, (E) *A. placenta*, and (F): *A. confluens.*
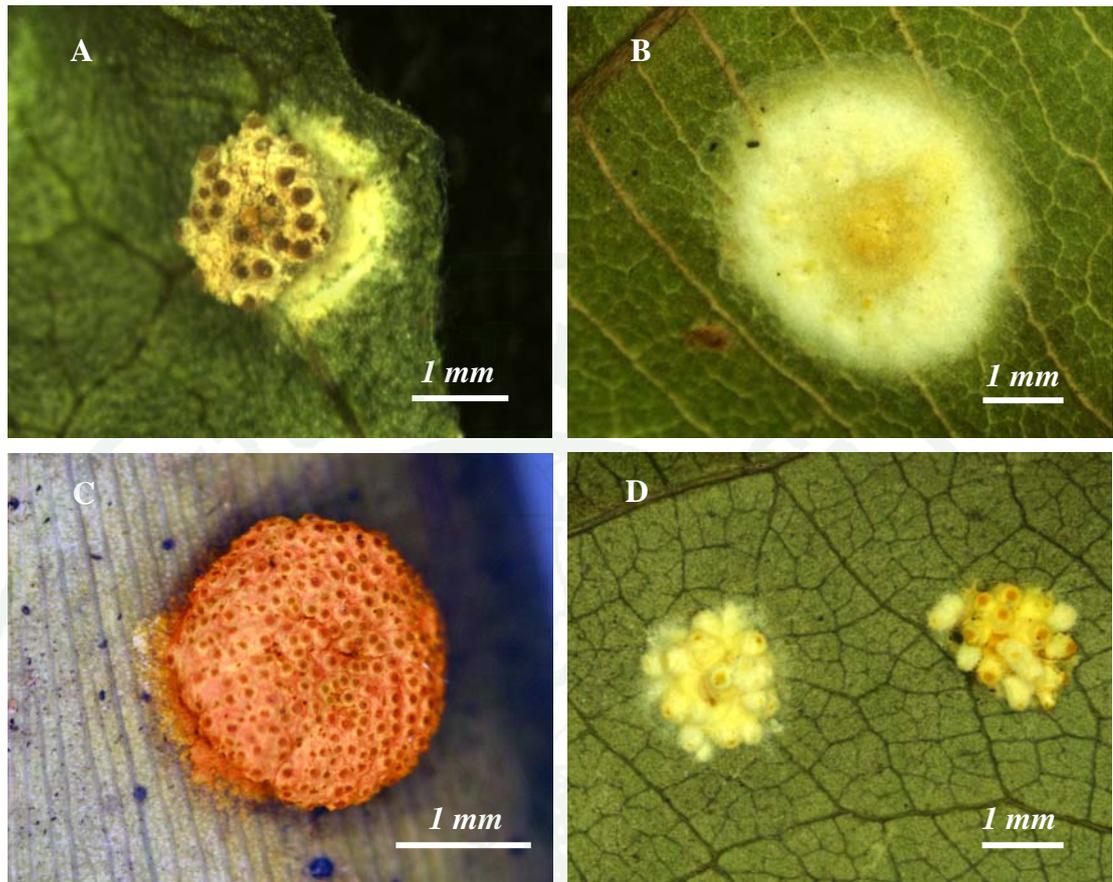
Figure 20 *Hypocrella* and *Moelleriella* species infected scale insect (Homoptera) on leaves.

Note: (A) *H. siamensis*, (B) *Moelleriella* sp., (C) *H. calendulina*, and (D) *M. raciborskii.*
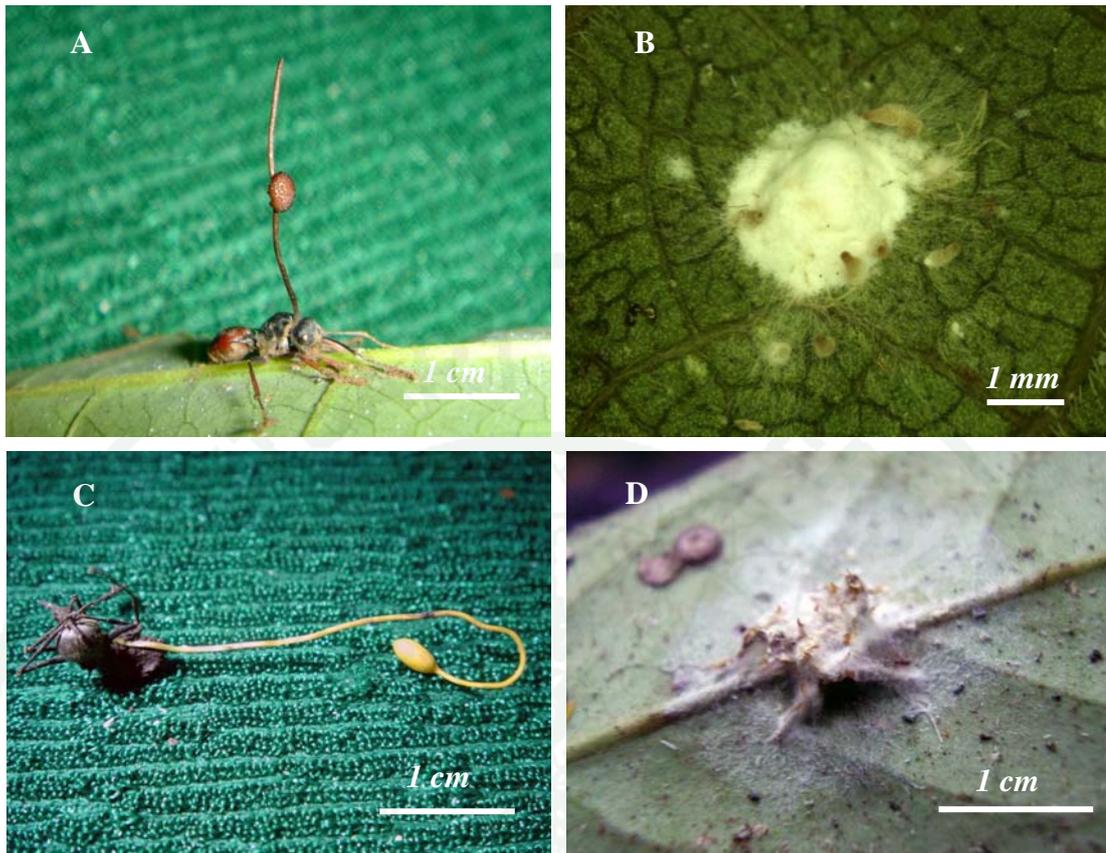
Figure 21 *Conoideocrella, Ophiocordyceps* and *Torrubiella* entomopathogenic
fungal species.

Note: (A) *Ophiocordyceps unilateralis*, (B) *Conoideocrella tenuis*, (C)
Ophiocordyceps irangiensis/myrmecophila, (D) *Torrubiella* sp.2.
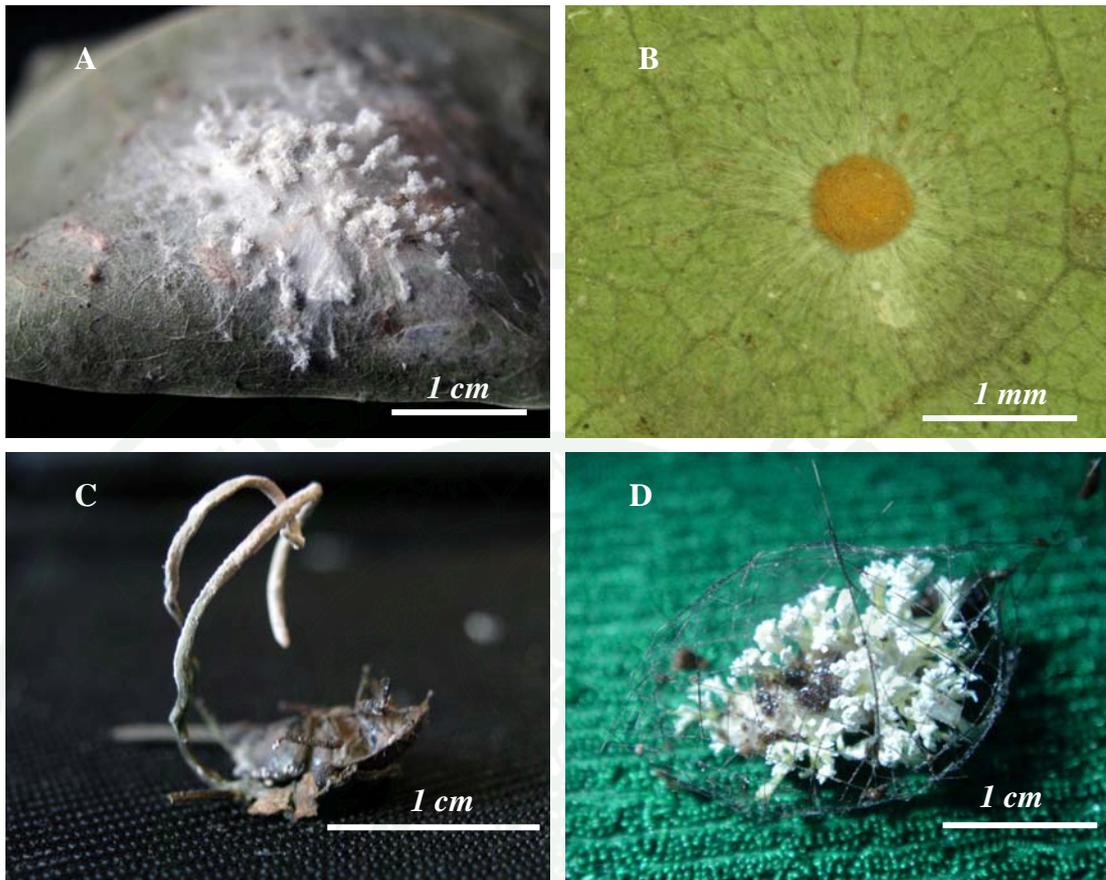
Figure 22 *Isaria* and *Paecilomyces* species.

Note: (A) *Isaria* sp*.,* (B) *Paecilomyces cinnamomeus,* (C) *P. lilacinus,* and (D) *I. tenuipes.*
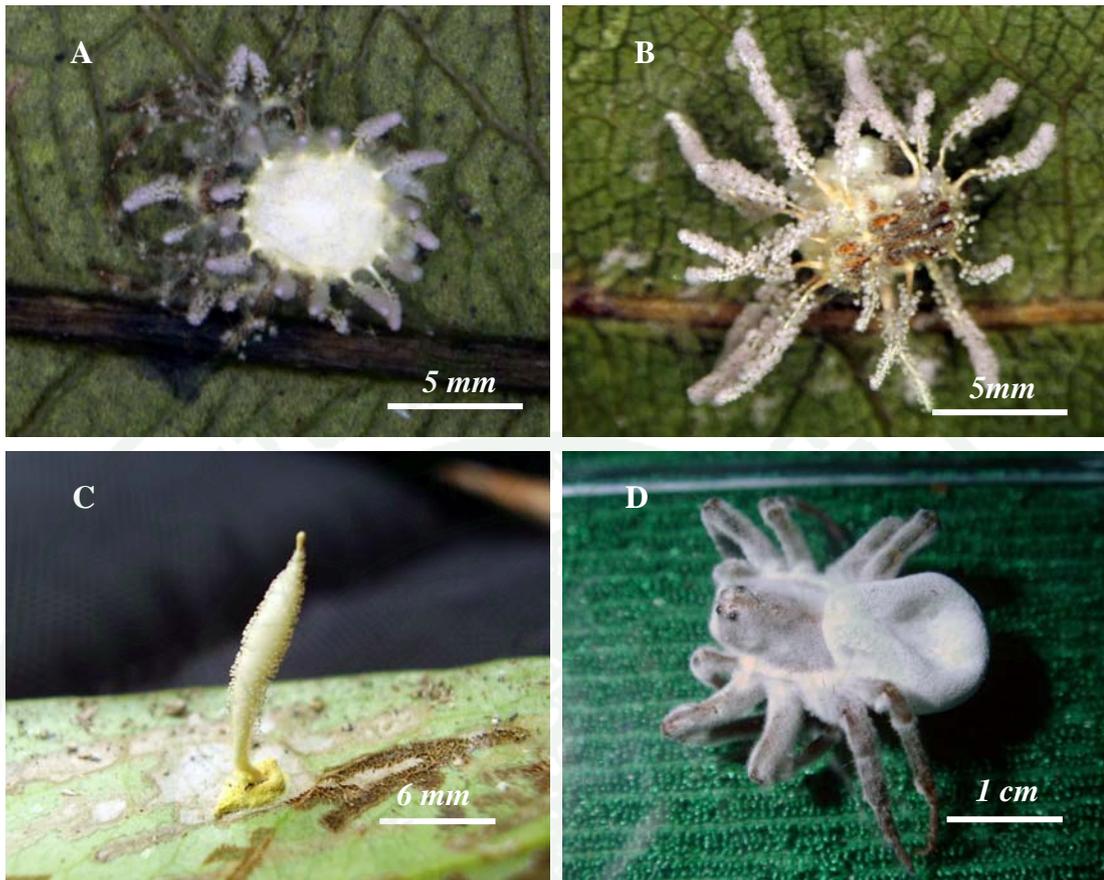
Figure 23 *Gibellula* and *Nomuraea* species infected spiders.

Note: (A) *Gibellula* sp.2, (B) *G. pulchra,* (C) *Gibellula* sp.1, and (D) *Nomuraea atypicola.*

Figure 24 Other anamorphic entomopathogenic fungi.

Note: (A) *Hirsutella formicarum*, (B) *Metarhizium* sp.1, (C) *Akanthomyces pistillariiformis*, (D) *Hirsutella citriformis*.
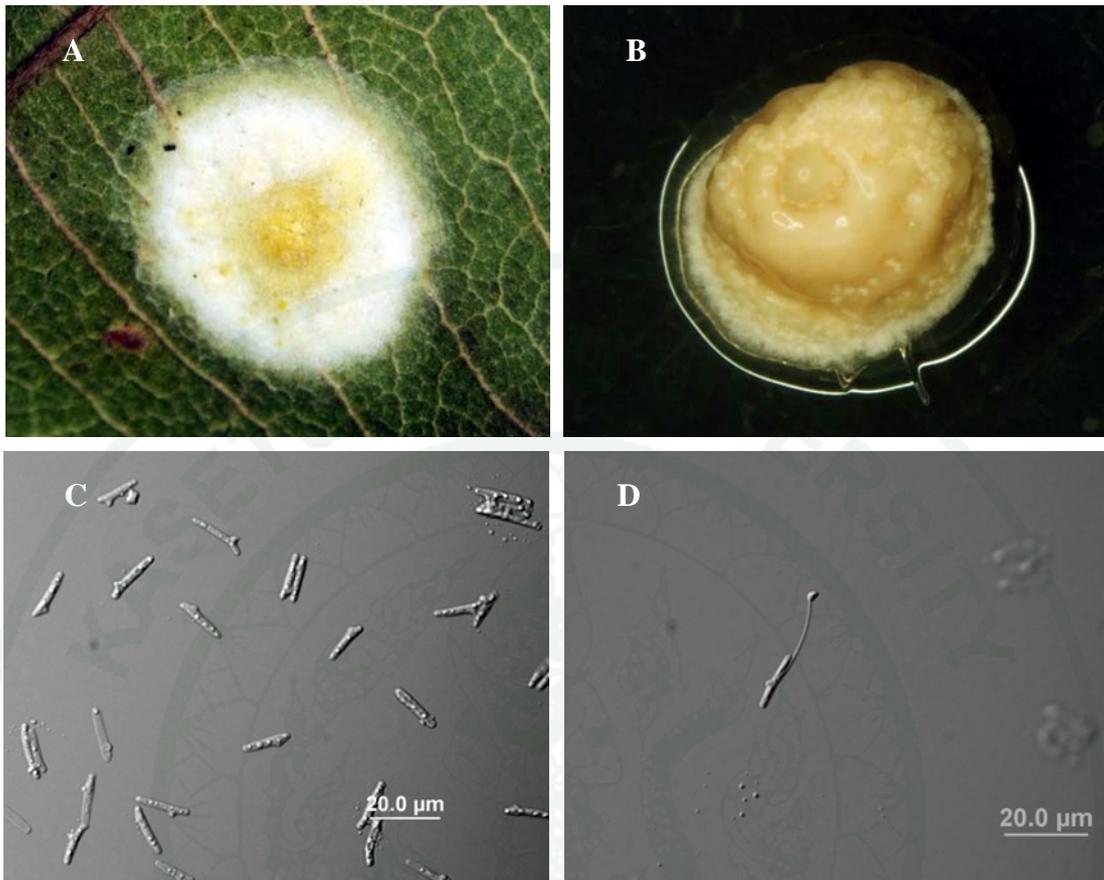
Figure 25 Development of entomopathogenic fungus *Moelleriella* sp.

Note: (A) *Moelleriella* sp. colonising scale insect on leaf, (B) Culture of *Moelleriella* sp. on PDA surface, (C) Ascospores and germinating ascospores, and (D) Germinated ascospore producing microspore on a stalk.

2. Growth and cordycepin production of selected entomopathogenic fungi

2.1 HPLC profile of cordycepin

Previous study has proved that about 98% cordycepin produced from surface culture mycelium of *C. militaris* was excreted into fermentation broth (Masuda *et al.*, 2007). In this study, cordycepin from culture broths were determined by using HPLC. Initially, the standard curve was established as the dependence of peak area on the cordycepin concentrations. As shown in Figure 26, the linear calibration equation for cordycepin quantification was y = 139592x (R^2 = 0.9997). In this equation, x-axis showed cordycepin concentration and y-axis showed the dependent cordycepin peak area obtained from HPLC. The intercept was set to the value of zero. The coefficient of determination, R^2, obtained in the linear equation was 0.9997 (~1.00), it indicated that the regression line well approximates the real data points because an R^2 of 1.0 indicates that the regression line perfectly fits the data.
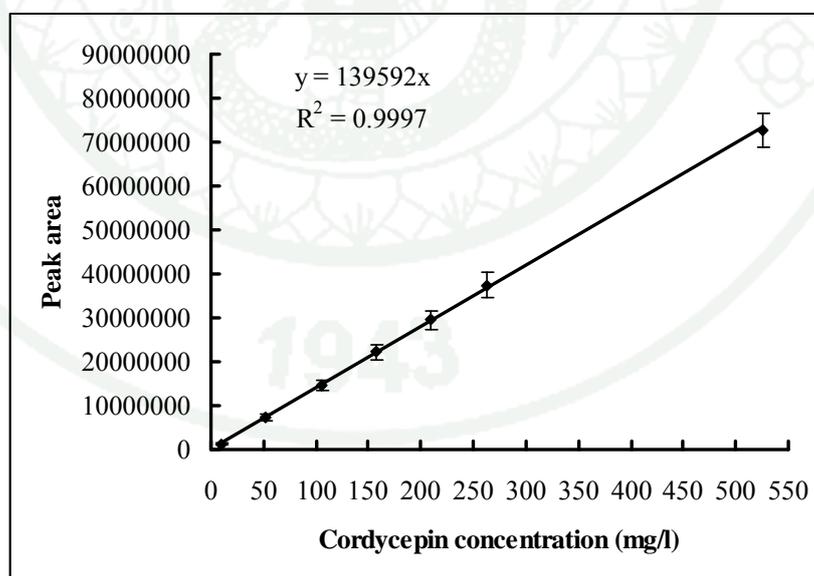
Figure 26 Standard curve of cordycepin.

Note: Error bars showing errors of peak area obtained in 3-time injection of standard.
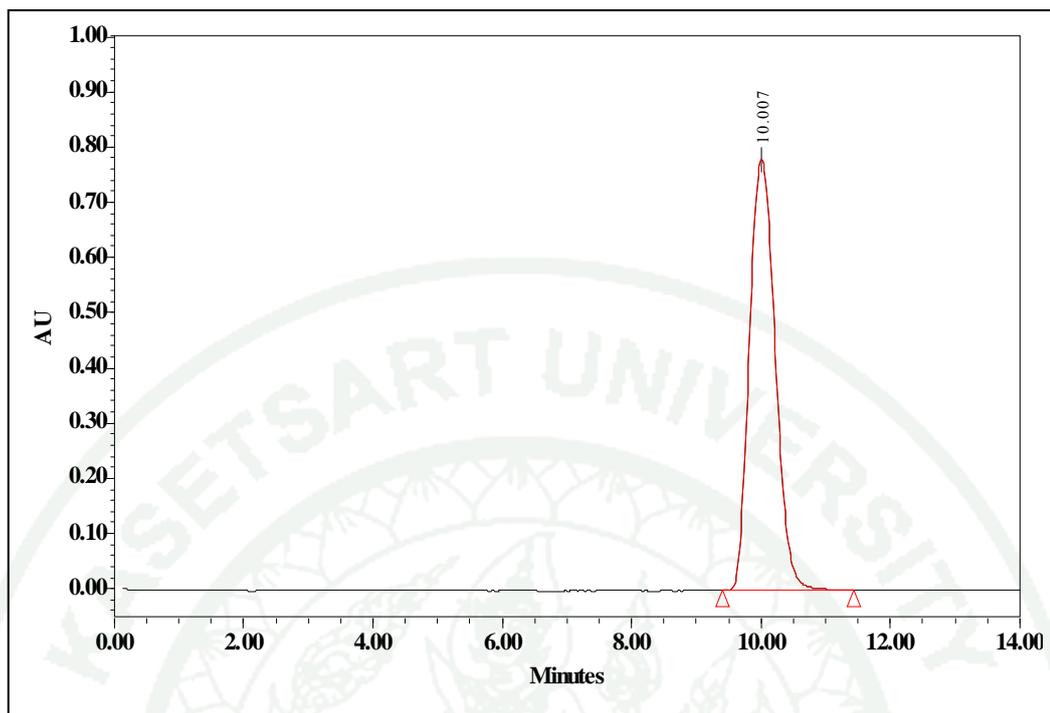
Figure 27 HPLC profile of standard cordycepin.
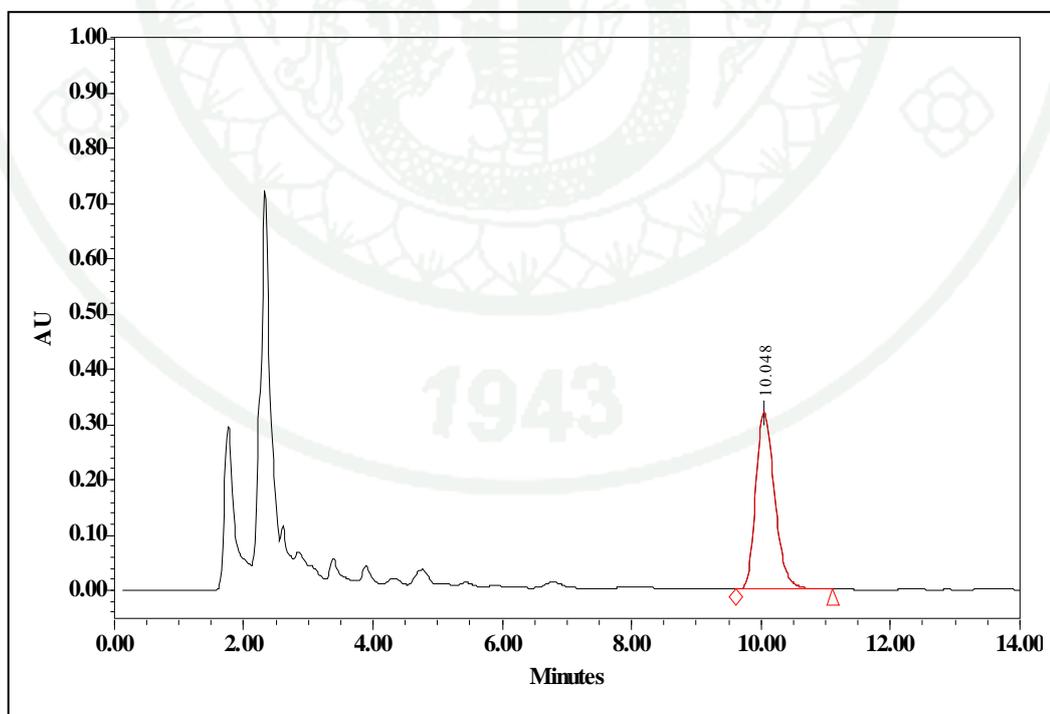
Figure 28 HPLC profile of fermented broth of *C. militaris* BCC 2816.

The HPLC profile in Figure 27 showed migration time and cordycepin peak of standard cordycepin and Figure 28 shoed migration time and cordycepin peak of the fermented broth of *C. militaris* BCC 2816. Peaks were identified by comparing the migration times of the unknown peak with those of the standard cordycepin eluted with the same conditions. It was observed from analysis that cordycepin peak presented in high resolution and can be easily to identify. There was no impurity could be found in cordycepin peak.

In this study, however, the migration time was changed occasionally when analyses were not in the same condition (data not shown), especially when the analyses were conducted in different days. Change of migration time also occurred due to the change of room temperature. Temperature change directly affected to column elements, when the room temperature was increased, it was observed that the column pressure was also increased, as the result, it also affected to the flow. To avoid mistaking in identifying cordycepin peak, standard cordycepin 10.5 mg/l was injected after five samples.

From previous studies, there were several methods for determining cordycepin in the culture of *C. militaris*. Analyses of cordycepin content in *C. militaris* by different techniques have been described such as thin layer chromatography (TLC) (Wu *et al.*, 2000; Cheng and Zhang, 1995), high performance liquid chromatography (HPLC) (Guo *et al.*, 1998), liquid chromatography specifically coupled with mass spectrometry (LC/ESI–MS) (Huang *et al.*, 2003), and capillary zone electrophoresis (CZE) (Ling *et al.*, 2002). A new capillary electrophoresis (CE) procedure with UV detection at 254 nm for determination of cordycepin was also developed and optimized (Rao *et al.*, 2006). Each method showed its own advantages and disadvantages. HPLC is one of the suitable methods because it gives a very detailed characterization of the investigated nucleosides with regard not only to the qualitative but also to the quantitative analysis.

2.2 Selective species for mycelium growth and cordycepin production

2.2.1 Development of mycelial biomass in culture medium

In this study, there were 35 strains belonging to 32 entomopathogenic fungal species selected for screening of cordycepin production in laboratory condition. In total 35 fungal strains, 13 strains were selected from the collection of Thailand (with BCC codes) and 22 strains were selected from Vietnamese collection (with ITB codes). The ITB's strains included strains that collected from this study and other strains, which had been collected before. All investigated strains were recognized to be located in three main Clades of the phylogenetic tree according to the recent classification of Sung *et al.* (2007). The results of mycelial dry weights of 35 fungal strains, which calculated after 15 days cultivation in two culture media (PDB and PG), is shown in Table 10.

Among 35 strains, mycelial dry weights (growths) were different. Some strains appeared fast growing which achieved high biomass in liquid culture media (more than 10 g/l). Other strains indicated low biomass production in culture media. Growths of fungal strains were dependent on the species characteristics.

In an observation when being cultivated on Petri plates in laboratory with Potato Dextrose Agar medium, *Gibellula* species, most *Aschersonia, Hypocrella* and *Moelleriella* species, and some *Ophiocordyceps* species grew very slowly, it took few weeks to few months for the colonies on agar surface to reach 10 mm in diameter. This characteristic was observed in the result of mycelium growths from both PDB and PG media in static culture condition, mycelium of these species grew slowly, it was submersed in culture broths. A dense mycelium aggregated together in round balls in cultures. In contrast, cultures of other entomopathogenic fungal species appeared as quickly as described fast growing species, i.e. *C. militaris, C. pseudomilitaris, C. ninchukispora, Isaria tenuipes, Nomuraea atypicola, Metarhizium* sp. etc. Biomass of these species developed quickly in culture media,

and a density of mycelial mass developed quickly on the top surface of culture media to cover medium surface in part or complete.

Table 10 Development of mycelial dry weights of investigated strains in PDB and PG culture media.

Fungal strains	Mycelial dry weights	
	PDB	PG
Akanthomyces arachnophilus BCC 22900	2.66 ± 0.08	6.06 ± 0.60 *
Akanthomyces cinereus BCC 14289	11.04 ± 0.76	14.48 ± 0.04 *
Akanthomyces sp. ITB 360	4.24 ± 0.30	5.54 ± 0.17 *
Aschersonia badia ITB 486.02	4.40 ± 0.30	5.66 ± 0.47 ns
Aschersonia confluens ITB 262	7.15 ± 0.63	15.00 ± 0.04 *
Aschersonia luteola ITB 278	3.08 ± 0.32	6.13 ± 0.40 *
Aschersonia oxystoma ITB 259	2.09 ± 1.17	2.59 ± 0.34 ns
Aschersonia placenta ITB 269	2.34 ± 0.04	5.73 ± 0.51 *
Aschersonia placenta ITB 414.03	6.57 ± 1.28	7.89 ± 0.09 ns
Aschersonia samoensis ITB 261	5.39 ± 1.31	4.36 ± 0.44 ns
Beauveria bassiana BCC 22355	3.58 ± 0.28	6.22 ± 1.23 ns
Conoideocrella luteorostrata ITB 300	8.63 ± 0.27	11.92 ± 0.15 *
Cordyceps militaris BCC 2816	9.66 ± 0.89	11.01 ± 0.51 ns
Cordyceps ninchukispora BCC 22834	7.75 ± 0.41	13.67 ± 0.27 *
Cordyceps nipponica BCC 19924	6.52 ± 0.76	6.74 ± 0.12 ns
Cordyceps pseudomilitaris BCC 27802	8.30 ± 1.81	10.37 ± 1.99 ns
Gibellula leiopus BCC 22177	3.12 ± 1.02	5.11 ± 0.71 ns
Gibellula pulchra ITB 371	4.51 ± 0.07	5.21 ± 0.65 ns
Gibellula sp. ITB 367	4.05 ± 0.05	6.70 ± 0.40 *
Hyperdermium sp. BCC 27812	7.27 ± 1.10	8.74 ± 0.75 ns
Hypocrella calendulina ITB 128	5.41 ± 2.10	4.84 ± 0.32 ns
Hypocrella calendulina ITB 425.01	5.73 ± 0.32	4.88 ± 0.96 ns

Table 10 (Continued).

Fungal strains	Mycelial dry weights	
	PDB	PG
Isaria javanica ITB 218	4.24 ± 0.40	7.61 ± 0.28 *
Isaria tenuipes ITB 301	8.19 ± 0.21	17.56 ± 2.98 *
Isaria tenuipes ITB 363	7.75 ± 0.41	13.67 ± 0.29 *
Metarhizium anisopliae BCC 22353	6.41 ± 0.55	7.73 ± 0.29 ns
Metarhizium flavoviride BCC 2335	8.64 ± 1.26	9.60 ± 0.15 ns
Metarhizium sp. ITB 258	12.55 ± 1.00	19.36 ± 0.51 *
Moelleriella raciborskii ITB 304	4.73 ± 0.19	8.66 ± 0.32 *
Nomuraea atypicola ITB 323	8.35 ± 1.43	10.95 ± 0.03 ns
Ophiocordyceps cf. *brunneipunctata* ITB 328	3.70 ± 0.50	4.81 ± 0.66 ns
Ophiocordyceps communis BCC 27807	4.27 ± 0.05	4.85 ± 1.26 ns
Ophiocordyceps sphecocephala BCC 27811	1.32 ± 0.11	3.70 ± 0.33 *
Paecilomyces lilacinus ITB 454	6.22 ± 0.16	7.41 ± 0.61 ns
Torrubiella hemipterigena ITB 169	9.22 ± 0.92	5.55 ± 0.20 *

Note: Data followed with (*) indicated the significant difference, and followed with (ns) indicated no significant difference between treatments at P=0.05.

The result showed in table 10 indicated that DWs of most strains (except *Torrubiella hemipterigena* ITB 169) in PG medium were similar of significantly higher than that in PDB medium. The strain *Metarhizium* sp. ITB 258, which is pathogen of cockroach, produced highest yield of DW in PG medium (19.36 ± 0.51 g/l), however, it was found to be significantly lower in PDB medium (12.55 ± 1.00 g/l). Similarly, DWs of 14 strains in PG medium were also significantly higher than that in PDB medium (*Akanthomyces arachnophilus* BCC 22900, *Akanthomyces cinereus* BCC 14289, *Akanthomyces* sp. ITB 360, *Aschersonia luteola* ITB 278, *Aschersonia confluens* ITB 262, *Aschersonia placenta* ITB 269, *C. ninchukispora* BCC 22834, *Gibellula* sp. ITB 367, *Moelleriella raciborskii* ITB 304, *Isaria tenuipes* ITB 301, *Isaria tenuipes* ITB 363, *Ophiocordyceps sphecocephala* BCC 27811, *Isaria*

javanica ITB 218, and *Conoideocrella luteorostrata* ITB 300). It was also observed that growths of other 19 fungal strains, which include *Aschersonia badia* ITB 486.02, *Aschersonia oxystoma* ITB 259, *Aschersonia placenta* ITB 414.03, *Aschersonia samoensis* ITB 261, *Beauveria bassiana* BCC 22355, *C. militaris* BCC 2816, *C. nipponica* BCC 19924, *C. pseudomilitaris* BCC 27802, *Gibellula pulchra* ITB 371, *Gibellula leiopus* BCC 22177, *Hyperdermium* sp. BCC 27812, *Hypocrella calendulina* ITB 128, *Hypocrella calendulina* ITB 425.01, *Metarhizium anisopliae* BCC 22353, *Metarhizium flavoviride* BCC 2335, *Nomuraea atypicola* ITB 323, *Ophiocordyceps* cf. *brunneipunctata* ITB 328, *Ophiocordyceps communis* BCC 27807 and *Paecilomyces lilacinus* ITB 454, were not significantly different in both PG and PDB culture media. The well-known fungus *C. militaris* BCC 2816 had been reported to produce 10-membered macrolides including cordycepin when being cultivated in PDB (Rukachaisirikul *et al.*, 2004). *Torrubiella hemipterigena* ITB 169 was an exception, the mycelial biomass of this strain obtained in PDB medium was significantly higher than this in PG medium (9.22 ± 0.92 g/l compares to 5.55 ± 0.20 g/l).

PG medium which consists of glucose and peptone as main carbon and nitrogen sources was reported as a basal medium for growth and cordycepin production of *C. militaris*. This medium have been used in several studies (Mao *et al.*, 2005; Mao and Zhong, 2006). PDB is the extracted potato supplemented with glucose, which is suitable for cultivation of all filamentous fungi in laboratory. Comparing DW of all strains in two fermentation media of this study, DWs achieved in PG medium were similar or even better than that in PDB. It can be explained that nitrogen source derived from peptone might be suitable for these fungi. It is also possible to believe that metabolite production of fungi will be enhanced together with mycelium growth in some cases. Indeed, the growth and exobiopolymer production in shake flask culture of *Ophiocordyceps sphecocephala* J-201 and the production of mycelia and polysaccharide in submerged cultivation of *C. jiangxiensis* were increased together (Oh *et al.*, 2007; Xiao *et al.*, 2006).

2.2.2 Cordycepin production of selected species

The liquid filtrates of culture broths in two culture media (PDB and PG) were analyzed for their cordycepin contents using HPLC. The HPLC procedure was performed as shown from previous section of this book. In HPLC procedure, liquid filtrates from culture of each strain were analysed in order to detect the presence and measure the concentration of cordycepin from two different culture media. The result of cordycepin concentrations in broth cultures from 35 entomopathogenic fungal strains is shown in Table 11. The indices from these analyses indicated that except *C. militaris* BCC 2816, all other entomopathogenic fungal strains were not able to produce cordycepin in both PDB and PG culture media. The HPLC results showed that no cordycepin peak could be detected from the cultures of these strains. Only *C. militaris* BCC 2816 showed the positive result of cordycepin production in the analysis.

Comparing cordycepin productions of *C. militaris* BCC 2816 between two different culture media, it indicated that PG medium could yield significantly higher amount of cordycepin than PDB could, cordycepin concentration in PG culture was more than three-fold higher than that in PDB. The quantities of cordycepin measured in PDB and PG media were 82.11 ± 2.70 mg/l and 272.25 ± 0.07 mg/l respectively. As described before, mycelial biomasses of *C. militaris* BCC 2816 obtained from both culture media were not significantly different, however, a significant difference was found from the ability of cordycepin production, it can be discussed that the nutrient components in PG was favourable for this fungus in case of cordycepin production.

Table 11 Cordycepin productions of investigated strains in PDB and PG culture
media.

Strain	Cordycepin production	
	PDB	**PG**
Akanthomyces arachnophilus BCC 22900	-	-
Akanthomyces cinereus BCC 14289	-	-
Akanthomyces sp. ITB 360	-	-
Aschersonia badia ITB 486.02	-	-
Aschersonia confluens ITB 262	-	-
Aschersonia luteola ITB 278	-	-
Aschersonia oxystoma ITB 259	-	-
Aschersonia placenta ITB 269	-	-
Aschersonia placenta ITB 414.03	-	-
Aschersonia samoensis ITB 261	-	-
Beauveria bassiana BCC 22355	-	-
Conoideocrella luteorostrata ITB 300	-	-
Cordyceps militaris BCC 2816	82.11 ± 2.70	272.25 ± 0.07 *
Cordyceps ninchukispora BCC 22834	-	-
Cordyceps nipponica BCC 19924	-	-
Cordyceps pseudomilitaris BCC 27802	-	-
Gibellula leiopus BCC 22177	-	-
Gibellula pulchra ITB 371	-	-
Gibellula sp. ITB 367	-	-
Hyperdermium sp. BCC 27812	-	-
Hypocrella calendulina ITB 128	-	-
Hypocrella calendulina ITB 425.01	-	-

Table 11 (Continued).

Fungal strains	Cordycepin production	
	PDB	**PG**
Isaria javanica ITB 218	-	-
Isaria tenuipes ITB 301	-	-
Isaria tenuipes ITB 363	-	-
Metarhizium anisopliae BCC 22353	-	-
Metarhizium flavoviride BCC 2335	-	-
Metarhizium sp. ITB 258	-	-
Moelleriella raciborskii ITB 304	-	-
Nomuraea atypicola ITB 323	-	-
Ophiocordyceps cf. *brunneipunctata* ITB 328	-	-
Ophiocordyceps communis BCC 27807	-	-
Ophiocordyceps sphecocephala BCC 27811	-	-
Paecilomyces lilacinus ITB 454	-	-
Torrubiella hemipterigena ITB 169	-	-

Note: Data followed with (*) indicated the significant difference between treatments at P=0.05; minus sign (-) indicated that cordycepin could not be detected by HPLC.

The recent phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi of Sung *et al.* (2007) supported the existence of three clavicipitaceous clades and rejected the monophyly of both *Cordyceps* and *Clavicipitaceae*. According to the new revision, the "historically" taxonomic classification of *Cordyceps* and the related *Clavicipitaceae* were separated into three major families according to their multi-gene phylogeny: *Cordycipitaceae*, *Ophiocordycipitaceae* and *Clavicipitaceae*. The Cordycipitaceae clade contents well-known fungus *C. militaris*, other closely related *Cordyceps* species that possess brightly coloured, fleshy stromata, and their anamorphic fungi. The *Ophiocordycipitaceae* clade contents formerly *Cordyceps* species which produce

darkly pigmented, tough to pliant stromata that often possess aperithecial apices and the related anamorphs. The well-known fungus, *Ophiocordyceps sinensis* (formerly named *Cordyceps sinensis*), is identified within this clade. The Clavicipitaceae clade includes the core clade of grass symbionts (e.g., *Balansia*, *Claviceps*, etc.), and the entomopathogenic genus *Hypocrella* and relatives. Until now, only three entomopathogenic fungal species have been recognized as cordycepin producers (*C. militaris*, *C. kyushuensis* and *Ophiocordyceps sinensis*). In this study, *C. militaris* and closely related species including *C. pseudomilitaris*, *C. ninchukispora*, and other anamorphic species (Table 12) is located in Cordycipitaceae clade. In our result, however, cordycepin could not be detected from other fungal species which are identified in the same clade with *C. militaris*. The similar results were also recorded from investigated strains that belong to *Ophiocordycipitaceae* and *Clavicipitaceae*. *Ophiocordyceps* strains, including *Ophiocordyceps* cf. *brunneipunctata* ITB 328, *Ophiocordyceps communis* BCC 27807, *Ophiocordyceps sphecocephala* BCC 27811, and other anamorphs that are belonging to *Ophiocordycipitaceae* in the phylogenetic classification, could not produce cordycepin *in vitro*. The negative result of cordycepin production was also observed from other fungal species that belong to the family *Clavicipitaceae* in our experiment.

From the result of current study, it can be proved that the abilities of cordycepin production of investigated entomopathogenic fungi are not correlated with their multi-gene phylogenetic classification. Even *C. pseudomilitaris*, which has very similar morphological characteristics and is classified in the same clade with *C. militaris*, however, the result of cordycepin production appeared to be different. It can be recognized that three cordycepin-enabled species have hosts in the same arthropod Order, the Lepidoptera, but in this study, other entomopathogenic fungi species that have Lepidoptera host, i.e. *C. pseudomilitaris, Isaria tenuipes,* did not give the possibility of cordycepin production even in trace amount. The possible evidence is that *C. militaris*, *C. kyushuensis* and *Ophiocordyceps sinensis* are temperate derived species that have been found only from temperate countries with year-round low temperature. Therefore, it can be suggested that entomopathogenic

fungal species, which is collected from temperate, should be used to screen for cordycepin production in future studies.

In conclusion, among 35 strains of 32 entomopathogenic fungal species investigated, only *C. militaris* BCC 2816 is able to produce cordycepin. Cordycepin production of entomopathogenic fungi does not correlate with the represented multi-gene phylogenetic classification of this fungal group. The modified (PG) medium which consists of 15 g/l peptone, 40 g/l glucose, 0.5 g/l KH_2PO_4, 0.5 g/l K_2HPO_4 and 0.5 g/l $MgSO_4.7H_2O$ indicated significantly higher cordycepin production than PDB medium. For this reason, PG medium was used as a basal medium for growth and cordycepin production of *C. militaris* in later experiments in this study.

Table 12 Distribution of investigated fungal strains on three clades of multi-gene phylogeny.

Cordycipitaceae Clade	Ophiocordycipitaceae Clade	Clavicipitaceae Clade
Akanthomyces arachnophilus BCC 22900	*Nomuraea atypicola* ITB 323	*Aschersonia luteola* ITB 278
Akanthomyces cinereus BCC 14289	*Ophiocordyceps* cf. *brunneipunctata* ITB 328	*Aschersonia badia* ITB 486.02
Akanthomyces sp. ITB 360	*Ophiocordyceps communis* BCC 27807	*Aschersonia confluens* ITB 262
Beauveria bassiana BCC 22355	*Ophiocordyceps sphecocephala* BCC 27811	*Aschersonia oxystoma* ITB 259
Cordyceps militaris BCC 2816	*Paecilomyces lilacinus* ITB 454	*Aschersonia placenta* ITB 414.03
Cordyceps ninchukispora BCC 22834		*Aschersonia placenta* ITB 269
Cordyceps nipponica BCC 19924		*Aschersonia samoensis* ITB 261
Cordyceps pseudomilitaris BCC 27802		*Conoideocrella luteorostrata* ITB 300
Gibellula leiopus BCC 22177		*Hyperdermium* sp. BCC 27812
Gibellula pulchra ITB 371		*Hypocrella calendulina* ITB 128
Gibellula sp. ITB 367		*Hypocrella calendulina* ITB 425.01
Isaria javanica ITB 218		*Metarhizium anisopliae* BCC 22353
Isaria tenuipes ITB 301		*Metarhizium flavoviride* BCC 2335
Isaria tenuipes ITB 363		*Metarhizium* sp. ITB 258
Torrubiella hemipterigena ITB 169		*Moelleriella raciborskii* ITB 304

3. Growths and cordycepin productions of different *C. militaris* strains

3.1 Mycelium biomass production of *C. militaris* strains

Temperature is a very important factor for mycelium growth and metabolite production of *C. militaris in vitro*. In nature, this fungus infects host and grows only in temperate climate. In laboratory condition, the effect of fermentation temperatures on mycelium growth was investigated. Based on the results from previous experiment of this study, the PG medium, which resulted growth and cordycepin promoting effects, was used as a basal medium. The result of mycelial dry weights of 15 *C. militaris* strains in four temperature conditions is shown in Table 13. It was observed that the growths were different between 15 *C. militaris* strains. In general, the growths of 15 *C. militaris* strains were higher in 15 and 20°C, lower at 25°C, and very low at 30°C. Particularly, the results of DWs were statically analysed, a multiple comparison was conducted to compare the significant difference between variables (DWs).
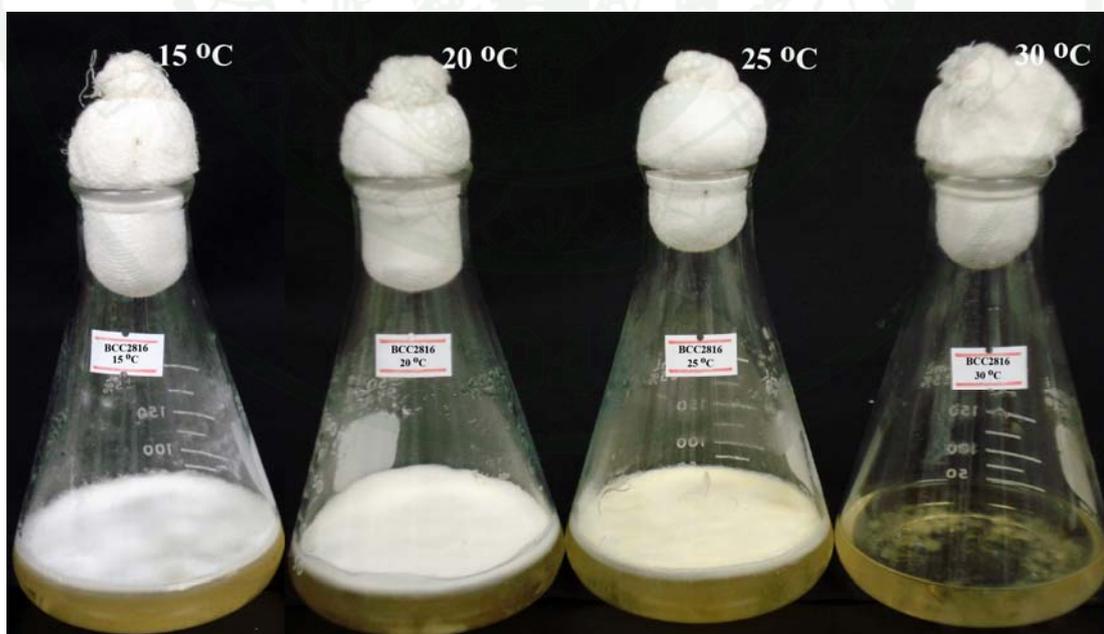
Figure 29 Mycelium growths of *C. militaris* BCC 2816 in different fermentation
temperature conditions.

Table 13 Effect of different cultivation temperatures on mycelium growths (g /l) of 15 *C. militaris* strains.

Fungal strains	Cultivation Temperatures			
	15°C	20°C	25°C	30°C
Cordyceps militaris BCC 1974	8.71 ± 2.14ab	9.53 ± 0.47b	7.82 ± 0.86ab	3.80 ± 0.25a
C. militaris BCC 1975	11.56 ± 2.05b	11.16 ± 0.52b	9.69 ± 0.63b	3.75 ± 0.27a
C. militaris BCC 2790	16.59 ± 0.29b	7.28 ± 2.53a	6.66 ± 0.13a	5.06 ± 0.66a
C. militaris BCC 2814	8.69 ± 1.27a	9.47 ± 3.11a	6.46 ± 1.02a	3.48 ± 0.48a
C. militaris BCC 2815	5.25 ± 0.26a	6.33 ± 0.60a	4.96 ± 1.12a	3.63 ± 0.97a
C. militaris BCC 2816	3.88 ± 1.69a	11.27 ± 1.90b	8.40 ± 1.01ab	4.46 ± 0.78a
C. militaris BCC 2817	6.51 ± 1.64ab	9.19 ± 0.55b	7.81 ± 0.20ab	4.18 ± 1.12a
C. militaris BCC 2818	3.63 ± 0.55a	7.70 ± 0.54c	6.50 ± 1.06bc	4.72 ± 0.11ab
C. militaris BCC 2819	4.99 ± 1.78a	10.37 ± 0.17b	10.19 ± 0.16b	3.50 ± 0.41a
C. militaris BCC 2824	5.42 ± 0.98a	6.60 ± 0.04a	6.72 ± 0.27a	4.88 ± 0.50a
C. militaris BCC 2826	8.90 ± 0.06bc	10.36 ± 1.39c	6.47 ± 0.06ab	4.02 ± 0.51a
C. militaris BCC 2838	6.25 ± 1.03a	5.43 ± 1.25a	4.88 ± 0.23a	3.15 ± 0.69a
C. militaris NBRC 5298	9.20 ± 0.94b	7.50 ± 1.50ab	4.74 ± 0.36a	5.48 ± 0.52ab
C. militaris NBRC 9787	13.53 ± 3.92b	7.08 ± 0.06ab	6.63 ± 1.77ab	4.61 ± 0.09a
C. militaris NBRC 100741	7.55 ± 0.87a	6.67 ± 0.26a	5.32 ± 0.62a	4.09 ± 1.32a

Note: Values are means of duplicate experiments ± SD. Values with same letters within a rows are not significantly different at P=0.05.

Table 14 ANOVA result on effect of different cultivation temperatures on growth of 15 *C. militaris* strains.

Strains		Sum of Squares	df	Mean Square	F	P
Cordyceps militaris BCC 1974	Between Groups	38.760	3	12.920	9.254	0.028
	Within Groups	5.585	4	1.396		
	Total	44.345	7			
C. militaris BCC 1975	Between Groups	78.478	3	26.159	21.163	0.006
	Within Groups	4.944	4	1.236		
	Total	83.423	7			
C. militaris BCC 2790	Between Groups	163.120	3	54.373	31.364	0.003
	Within Groups	6.935	4	1.734		
	Total	170.055	7			
C. militaris BCC 2814	Between Groups	43.293	3	14.431	4.595	0.087
	Within Groups	12.563	4	3.141		
	Total	55.856	7			
C. militaris BCC 2815	Between Groups	7.383	3	2.461	3.744	0.117
	Within Groups	2.629	4	0.657		
	Total	10.012	7			

Table 14 (Continued).

Strains		Sum of Squares	df	Mean Square	F	P
C. militaris BCC 2816	Between Groups	72.703	3	24.234	12.002	0.018
	Within Groups	8.077	4	2.019		
	Total	80.779	7			
C. militaris BCC 2817	Between Groups	27.302	3	9.101	8.501	0.033
	Within Groups	4.282	4	1.071		
	Total	31.584	7			
C. militaris BCC 2818	Between Groups	19.694	3	6.565	15.132	0.012
	Within Groups	1.735	4	0.434		
	Total	21.429	7			
C. militaris BCC 2819	Between Groups	75.051	3	25.017	29.359	0.003
	Within Groups	3.408	4	0.852		
	Total	78.459	7			
C. militaris BCC 2824	Between Groups	4.863	3	1.621	5.056	0.076
	Within Groups	1.282	4	0.321		
	Total	6.145	7			

Table 14 (Continued).

Strains		Sum of Squares	df	Mean Square	F	P
C. militaris BCC 2826	Between Groups	46.572	3	15.524	28.227	0.004
	Within Groups	2.200	4	0.550		
	Total	48.772	7			
C. militaris BCC 2838	Between Groups	10.311	3	3.437	4.356	0.095
	Within Groups	3.156	4	0.789		
	Total	13.466	7			
C. militaris NBRC 5298	Between Groups	24.386	3	8.129	9.204	0.029
	Within Groups	3.533	4	0.883		
	Total	27.919	7			
C. militaris NBRC 9787	Between Groups	89.534	3	29.845	6.452	0.052
	Within Groups	18.501	4	4.625		
	Total	108.035	7			
C. militaris NBRC 100741	Between Groups	13.880	3	4.627	6.271	0.054
	Within Groups	2.951	4	0.738		
	Total	16.832	7			

Table 15 ANOVA result on effect of different *C. militaris* strains on mycelial dry weight in four temperature conditions.

Temperatures		Sum of Squares	df	Mean Square	F	P
15°C	Between Groups	370.112	14	26.437	10.281	0.000
	Within Groups	38.569	15	2.571		
	Total	408.681	29			
20°C	Between Groups	100.537	14	7.181	3.991	0.006
	Within Groups	26.990	15	1.799		
	Total	127.527	29			
25°C	Between Groups	76.487	14	5.463	8.678	0.000
	Within Groups	9.444	15	0.630		
	Total	85.931	29			
30°C	Between Groups	12.332	14	0.881	1.949	0.106
	Within Groups	6.779	15	0.452		
	Total	19.111	29			

Among different cultivation temperatures, the analysis of variance (ANOVA) result (P=0.05) showed in Table 14 indicated that DWs obtained from nine strains (BCC 1974, BCC 1975, BCC 2790, BCC 2816, BCC 2817, BCC 2818, BCC 2819, BCC 2826 and NBRC 5298) were significantly different. Meanwhile, other *C. militaris* strains (BCC 2814, BCC 2815, BCC 2824, BCC 2838, NBRC 9787 and NBRC 100741) indicated lower biomass production abilities, and DWs measured in four temperatures were not significantly different in these strains. The highest DWs were archived in two strains *C. militaris* BCC 2790 and *C. militaris* NBRC 9787 at fermentation temperature of 15°C (16.59 ± 0.29 g/l and 13.53 ± 3.92 g/l, respectively). However, DWs of other seven *C. militaris* strains (BCC 1974, BCC 1975, BCC 2816, BCC 2817, BCC 2818, BCC 2819 and BCC 2826) at 20°C of fermentation temperature were significantly higher than those at other temperatures. In fact, it was observed in laboratory that white-coloured mycelial masses of all 15 *C. militaris* strains completely covered top surface of culture medium in three treatments at 15, 20 and 25°C, meanwhile, only little submersed mycelium could be found from the treatment at 30°C during cultivation (Figure 29).

When comparing among strains, ANOVA result (P=0.05) shown in Table 15 indicated that DWs of different strains were significantly different in three fermentation temperature condition (15, 20 and 25°C). However, in the fermentation temperature of 30°C, DWs of 15 strains were not significantly different due to the inhibitory effect of high temperature on growth of *C. militaris*. By our observation during cultivation process, mycelium development in culture medium of 30°C treatment was found in the first few days, then it might be stopped afterward. Mycelium in culture was remained in submersion while surface mycelium could not be found on top of cultures.

It is reported that *in vitro* culture of *Cordyceps* spp. has been employed increasingly for food and medicines. Some researches supported that the natural related *Cordyceps* fruiting bodies (i.e. *Ophiocordyceps sinensis* and *C. militaris*) are costly and are not available because of host specificity and rarity in nature; they grow extremely slowly in nature, their growth is restricted to a specific area and their sizes

are very small. Therefore, collection of sufficient quantities of natural *Cordyceps* fruiting bodies for extensive use as a drug remedy is prohibited.

Because solid culture of mushrooms takes long time to complete a fruiting body, many attempts have been made to obtain useful and potent intracellular or extracellular substances from a mycelial culture for use in the formulation of nutraceuticals and functional foods. Thus, the growing numbers of so-called *Cordyceps* products that derive from mycelial cultures of the asexual forms of these fungi have become commercially available (Hamburger, 2007). Mycelium cultivation resulted in establishing a number of cultures derived from holomorphic species of *Ophiocordyceps sinensis*, these include *Paecilomyces hepiali* and *Cephalosporum sinensis* (Russell and Paterson, 2008). In addition, mycelia from *Cordyceps* have been reported to activate the immune system (Kuo *et al.*, 1994; Koh *et al.*, 2001; Koh *et al.*, 2002). Moreover, several strains have been isolated from natural *Cordyceps* and produced in large quantities by fermentation, and have been found to have pharmacological activities similar to the natural product (Li *et al.*, 2001). Mass productions of several *Cordyceps* species have been allowed to be supplied for public demands and employed as a target to search for a new anticancer and immunomodulating drug (Kiho *et al.*, 1996; Kim *et al.*, 2001). Feng *et al.* (1987) reported the vasodilating effect of cultured mycelia of *Ophiocordyceps sinensis* in an investigation of the cardiovascular system of dogs. Zhou *et al.* (1990) also reported thirty-three cases of chronic hepatitis B patients treated with cultured *Ophiocordyceps sinensis* mycelia have shown that the drug improves liver function, promotes negative transfer HBsAg, markedly helps to raise plasma albumin, helps patients resist high gamma globulin and adjusts body immunocompetence. It was also found that cultivated *Cordyceps* mycelia inhibited humoral immune hyperfunction and increase the serum complement level in patients with post-hepatic cirrhosis, and improved liver function (Dai *et al.*, 2001; Zhu and Liu, 1992). Yang *et al.* (2006) mentioned that mycelium could inhibit tumour growth and induce tumour cell apoptosis. However, the anti-tumour mechanisms are not fully understood so it is believed that several enzymes and metabolites are induced together with the induction of mycelia.

Recently, the type species *C. militaris* has been put under the consideration of many investigators in mycelial mass and metabolite production. Different with *Ophiocordyceps sinensis*, *C. militaris* is the fast growing species in normal PDB and other artificial media. Many researches have attempted to obtain optimal culture conditions for mycelial biomass and exopolysaccharides.

There are several factors affected to growth and metabolite production *in vitro* of *C. militaris*, such as medium nutritional requirements, pH, aeration, cultivation temperature, etc. Several carbon sources have been suggested for the production of mycelia, exopolysaccharide and cordycepin, including glucose, sucrose, corn steep powder (Kim *et al.*, 2002; Kim and Yun, 2005). It was also found that yeast extract and/or peptone are the favourable nitrogen sources for biomass, exopolysaccharide and cordycepin production (Kim *et al.*, 2002; Shih *et al.*, 2007 and Masuda *et al.*, 2006). In this study, the medium compositions were the mixture of glucose and peptone as the carbon and nitrogen sources for mycelium growth and cordycepin production of various *C. militaris* strains. These component was in a basal quantities, therefore, in order to have better biomass and cordycepin production, fermentation medium should be optimized. Even though culture mycelia of *C. militaris* act as medicinal material for human and have various pharmaceutical activities, only trace amount of intracellular cordycepin can be found from mycelial culture of this fungus. Masuda *et al.* (2006, 2007) reported that by surface culture using *C. militaris* NBRC 9787, about 98% of the cordycepin synthesized by *C. militaris* was secreted into the culture medium, while only about 2% of synthesized cordycepin was found in mycelium. Other nucleic acid-related compounds except for cordycepin and guanine were only slightly observed in the medium. Therefore, our study did not concern the further use of mycelial biomass of 15 *C. militaris* strains. The main purpose of this study was cordycepin production, however, extraction of intracellular cordycepin requires a complicated process and it needs large amount of biomass in the process to get only small amount of cordycepin in mycelium. Furthermore, our experiments carried out only in using flask surface culture, the harvested mycelial biomasses were in small amount. For those reasons, cordycepin

production in this work was only based on the analysis of cordycepin concentration in the culture filtrates.

To date, only three *Cordyceps* species have been reported to have cordycepin production ability. The *in vitro* cultivation of *Ophiocordyceps sinensis* for cordycepin production is hardly to apply because of the difficulty in growing of this fungus in laboratory condition. In addition, culture of *C. kyushuensis* is rare and the information regarding to the cultivation of this fungus is not available. So far, further researches of cordycepin production are concerning only on the fungus *C. militaris*. Even though hundreds of cultures of this species are available from culture collections around the world, all recent researches have been done only on single strain while many other strains with high capacity of cordycepin production are waiting to be discovered. Therefore, selection of suitable strains that showed the ability of fast growing and high cordycepin production is very important for commercial purpose.

3.2 The abilities of cordycepin production of different *C. militaris* strains

Until now, several researches have been done on cordycepin production from *C. militaris*. Some kinds of culture media as well as nutrient sources have been applied for fermentation. However, no information related to the difference of cordycepin productions in different *C. militaris* strains has been reported. As discussed before, selection of excellent *C. militaris* strains with high cordycepin yield from multiple strains is very important for further step for optimization of production. In this study, the abilities of cordycepin production by 15 different *C. militaris* strains were successfully investigated.

Different fermentation temperatures were also tested in order to find the suitable temperature for cordycepin production. An analysis of variance (ANOVA) following by Tukey's HSD Post-hoc test was applied in order to analyse the statically significantly differences between group variables (among strains and temperatures).

The ANOVA result shown in Table 17 indicate that there were significant differences (P=0.05) of cordycepin concentrations among different *C. militaris* strains. The detail of total cordycepin productions (mg/l) of multiple *C. militaris* strains is shown in Table 16, and its corresponding cordycepin productivities is shown in Figure 30. Among 15 *C. militaris* strains, *C. militaris* NBRC 5298 indicated very poor result of cordycepin production. Cordycepin could not be detected at the fermentation temperature of $15^{o}C$, at 20, 25 and $30^{o}C$, cordycepin peaks could be detected by HPLC, however, only trace amounts of cordycepin were found in these treatments (0.55 ± 0.02, 0.55 ± 0.03, and 0.42 ± 0.39 mg/l/d respectively). Other *C. militaris* strains indicated the possibilities of cordycepin production, however, cordycepin quantities in the same temperature condition were different from strain to strain. In details, two strains *C. militaris* BCC 2819 and *C. militaris* BCC 2816 presented very high cordycepin production abilities, the highest cordycepin concentrations of these strains were found in the $25^{o}C$ fermentation condition with total amounts of 587.68 ± 16.82 mg/l and 558.60 ± 102.58 mg/l, which correspond to 39.18 ± 1.12 mg/l/d and 36.32 ± 6.65 mg/l/d respectively. At the same that temperature condition, other nine *C. militaris* strains (BCC 2814, BCC 2815, BCC 2817, BCC 2818, BCC 2790, BCC 1974, BCC 2826, NBRC 9787 and NBRC 100741) also showed high productions. Cordycepin concentrations of there strains were 208.27 ± 46.02 mg/l, 187.11 ± 107.61 mg/l, 165.92 ± 5.60 mg/l, 161.76 ± 8.39 mg/l, 132.11 ± 17.41 mg/l, 126.56 ± 20.76 mg/l, 109.26 ± 36.86 mg/l, 104.46 ± 45.45 mg/l and 93.81 ± 37.67 mg/l, which correspond to 13.88 ± 3.07 mg/l/d, 12.47 ± 7.17 mg/l/d, 11.06 ± 0.37 mg/l/d, 10.78 ± 0.56 mg/l/d, 8.81 ± 1.16 mg/l/d, 8.44 ± 1.38 mg/l/d, 7.28 ± 2.46 mg/l/d, 6.96 ± 3.03 mg/l/d and 6.25 ± 2.51 mg/l/d respectively. However, these cordycepin productions and productivities were much lower than those of two strains *C. militaris* BCC 2819 and *C. militaris* BCC 2816. In addition, the pre-investigated strain *C. militaris* NBRC 9787 could produce only 104.46 ± 45.45 mg/l (with productivity of 6.96 ± 3.03 mg/l/d), this result of cordycepin production was much lower than that previously investigated report (2.5 g/l, Masuda *et al.*, 2007). Three strains, *C. militaris* BCC 2824, BCC 2838 and BCC 1975 produced very low cordycepin amounts in culture medium with the total cordycepin concentrations reached highest only 46.99 ± 1.38 mg/l, 42.51 ± 21.21 mg/l and 36.31

± 12.89 mg/l at 25°C, which correspond to 3.13 ± 0.09 mg/l/d, 2.83 ± 1.41 mg/l/d and 2.42 ± 0.86 mg/l/d respectively.

In order to investigate the effect of cultivation temperatures on cordycepin production of 15 *C. militaris* strains, an analysis of variance was also estimated according to the data of total cordycepin productions. The ANOVA result shown in Table 18 indicate that, except *C. militaris* BCC 2515 (P=0.060) and *C. militaris* NBRC 5298 (P=0.123), other *C. militaris* strains showed the significant difference of cordycepin production among four fermentation temperature conditions. The strain *C. militaris* BCC 2515 could produce high cordycepin at 25°C, however the significantly different results of two replicated experiments caused the high range of standard deviation. The strain *C. militaris* NBRC 5298, however, gave either negative or very poor results of cordycepin production in all fermentation temperature conditions.

Surface culture of *C. militaris* has been used for cordycepin production in some researches and studies after this compound was first discovered by Cunningham *et al.* (1950), however, the purposes of these studies was not to promote for the commercial use (Frederiksen *et al.*, 1965; Furuya *et al.*, 1983). Later works of Masuda *et al.* (2006, 2007) promoted a more efficient method for commercial production of cordycepin by screening for effective additives to the liquid medium and applying the repeated batch operation under the surface culture using *C. militaris*. NBRC 9787. In those studies, optimization of culture conditions for cultivating *C. militaris* NBRC 9787 such as medium composition and medium depth was investigated. It was found that the preferable nitrogen source for the cordycepin production was the mixture of peptone and yeast extract. In the mixture, the ratio of yeast extract was 75% or greater and the optimal carbon/nitrogen ratio (w/w) was 2/1. When glucose was used as the carbon source and in this optimum condition, the maximum cordycepin concentration in the culture medium reached 640 mg/l, and the maximum cordycepin productivity was 32 mg/l/d (Masuda *et al.*, 2006). After a year, the research in 2007 found that when adding a combination of 1 g/l of adenine and 16 g/l of glycine, the maximum cordycepin production reached 2.5 g/l, which corresponds to 4.1 times. The fermentation of that research used basal medium, and these works proved that most of

cordycepin (about 97 to 98%) produced by this fungus was discharged into culture medium. It was also demonstrated that in the shaking culture of *C. militaris* cordycepin production was suppresses to 1/10 (buffled Erlenmeyer flask) ~1/3 (no-buffled) relative to the surface culture, and they suspected that shear stress might have adverse effect on the fungus.

In a contrast, Mao *et al.* (2005) demonstrated that the maximum cordycepin production was achieved under 110 rpm of agitation. Previous study also developed the submerged culture method of *C. militaris* for cordycepin production in commercial scale using a two-stage dissolved oxygen control. It was found that the reduction of dissolved oxygen about 15 to 30% would enhance cordycepin production and productivity (Mao and Zhong, 2004); it mean that the production of cordycepin increased at low oxygen concentration.

From the results and observations of previous studies, it can be suggested that the surface culture method for cordycepin production can be use for further investigation because the cost for the process would be less and the production would be higher when comparing with submersed culture. However, the disadvantages of surface culture are the working volume of fermentation is not high and it may require more space for the fermentation room. Therefore, more studies should be done in order to improve process for high yield production as well as to save cost for the commercial purpose.

The limitation of previous researches and studies was all of those results on cordycepin production were from only single strain of *C. militaris*. Even though cordycepin production of *C. militaris* NBRC 9787 had been done before, however in this study, we reinvestigated this strain in a comparison with other 14 strains in order to search for excellence strain(s) with high cordycepin production for further commercial purposes. The result in this work showed that cordycepin production and productivity of *C. militaris* NBRC 9787 were much lower than that of previous reports (Masuda *et al.*, 2006, 2007). The possible reason is the culture medium component and fermentation condition of this study were not in an optimized

condition. It can be said that this study was the first step in selective strains for further studies on production; therefore, the optimization of culture medium and fermentation conditions would be carried out in further studies, thus, it was not shown in this work. *C. militaris* NBRC 9787 gave a very high yield of cordycepin production from previous studies. However, in this study, this strain produced much lower cordycepin amount when comparing to other strains. Especially, two strains *C. militaris* BCC 2819 and BCC 2816 could yield up to 5.6 fold higher cordycepin production than *C. militaris* NBRC 9787 could in basal medium and fermentation condition. Other study also reported that *C. militaris* BCC 2816 produces 10-membered macrolides in surface culture using PDB medium together with 6 known compounds including cepharosporolides C, cepharosporolides E, cepharosporolides F, 2-carboxymethyl-4-(3'-hydroxybutyl)furan, pyridine-2,6-dicarboxylic acid and cordycepin (Rukachaisirikul *et al.*, 2004). Result of that study, however, indicated a very low amount of cordycepin that could be purified in culture filtrate. In total 5 litres fermentation, only 36 mg of cordycepin could be harvested as pure compound. These reviews have shown the important effects of culture medium and fermentation condition in order to achieve high yield production of cordycepin by *C. militaris*. From our result, it can be concluded that two *C. militaris* strains (*C. militaris* BCC 2819 and *C. militaris* BCC 2816) are the excellence strains in case of cordycepin production. So, these strains can be recommended for further investigations to achieve highest production by an optimization of fermentation medium and condition as well as for studies on production of other metabolites (e.i. adenosine and exopolysaccharides) to get more valuable information for research and utilization.

Table 16 Effect of different cultivation temperatures on cordycepin production of 15 *C. militaris* strains.

Fungal strains	Cultivation Temperatures			
	15°C	20°C	25°C	30°C
Cordyceps militaris BCC 1974	19.03 ± 0.83a	101.29 ± 21.89b	126.56 ± 20.76b	9.28 ± 0.18a
C. militaris BCC 1975	27.79 ± 4.60ab	16.11 ± 1.39ab	36.31 ± 12.89b	1.45 ± 0.16a
C. militaris BCC 2790	21.00 ± 0.00a	79.39 ± 14.43b	132.11 ± 17.41c	8.42 ± 0.08a
C. militaris BCC 2814	0.00 ± 0.00a	43.80 ± 30.49a	208.27 ± 46.02b	0.25 ± 0.25a
C. militaris BCC 2815	1.40 ± 0.81a	7.16 ± 0.10a	187.11 ± 107.61a	0.34 ± 0.03a
C. militaris BCC 2816	13.66 ± 8.27a	230.40 ± 29.67b	544.82 ± 99.80c	4.59 ± 0.19a
C. militaris BCC 2817	0.00 ± 0.00a	86.54 ± 11.04b	165.92 ± 5.60c	0.40 ± 0.02a
C. militaris BCC 2818	0.18 ± 0.00a	7.79 ± 0.60a	161.76 ± 8.39b	1.26 ± 0.09a
C. militaris BCC 2819	1.55 ± 0.11a	240.13 ± 20.22b	587.68 ± 16.82c	8.65 ± 0.40a
C. militaris BCC 2824	1.60 ± 0.73a	17.23 ± 4.31b	46.99 ± 1.38c	0.66 ± 0.00a
C. militaris BCC 2826	0.00 ± 0.00a	184.37 ± 60.86b	109.26 ± 36.86ab	8.24 ± 0.28a
C. militaris BCC 2838	0.00 ± 0.00a	22.71 ± 1.22a	42.51 ± 21.21a	0.63 ± 0.19a
C. militaris NBRC 5298	0.00 ± 0.00a	0.55 ± 0.02a	0.55 ± 0.03a	0.42 ± 0.39a
C. militaris NBRC 9787	1.15 ± 0.06a	8.52 ± 3.66a	104.46 ± 45.45b	0.69 ± 0.43a
C. militaris NBRC 100741	1.27 ± 0.15a	26.69 ± 7.96ab	93.81 ± 37.67b	1.17 ± 0.06a

Note: Values are means of duplicate experiments ± SD. Values with same letters within a rows are not significantly different at P=0.05
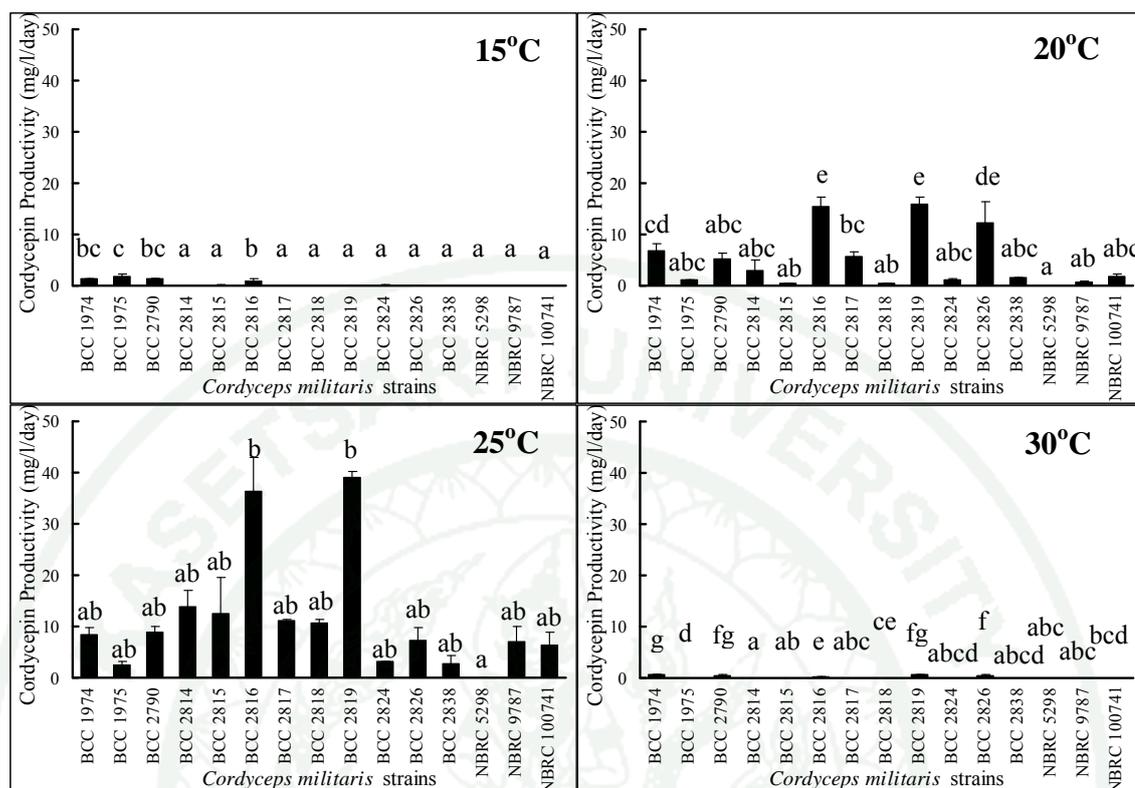
Figure 30 Effect of different cultivation temperatures on cordycepin productivities of
15 *C. militaris* strains.

Note: Error bars show SD of means of duplicate experiments; values with same
letters within a chart are not significantly different at P=0.05 according to
Tukey's test.

Table 17 ANOVA result on effect of different *C. militaris* strains on cordycepin production in four temperature conditions.

Temperatures		Sum of Squares	df	Mean Square	F	*P*
15°C	Between Groups	2496.974	14	178.355	29.241	0.000
	Within Groups	91.494	15	6.100		
	Total	2588.468	29			
20°C	Between Groups	191940.681	14	13710.049	30.107	0.000
	Within Groups	6830.740	15	455.383		
	Total	198771.421	29			
25°C	Between Groups	823730.961	14	58837.926	29.187	0.000
	Within Groups	30238.054	15	2015.870		
	Total	853969.015	29			
30°C	Between Groups	367.770	14	26.269	504.674	0.000
	Within Groups	0.781	15	0.052		
	Total	368.551	29			

Table 18 ANOVA result on the effect of different cultivation temperatures on cordycepin production of 15 *C. militaris* strains.

Fungal strains		Sum of Squares	df	Mean Square	F	P
Cordyceps militaris BCC 1974	Between Groups	20642.297	3	6880.766	30.217	0.003
	Within Groups	910.842	4	227.711		
	Total	21553.139	7			
C. militaris BCC 1975	Between Groups	1370.674	3	456.891	9.655	0.026
	Within Groups	189.288	4	47.322		
	Total	1559.962	7			
C. militaris BCC 2790	Between Groups	19513.868	3	6504.623	50.876	0.001
	Within Groups	511.406	4	127.852		
	Total	20025.274	7			
C. militaris BCC 2814	Between Groups	58755.088	3	19585.029	25.704	0.004
	Within Groups	3047.837	4	761.959		
	Total	61802.925	7			
C. militaris BCC 2815	Between Groups	50918.793	3	16972.931	5.862	0.060
	Within Groups	11581.162	4	2895.291		
	Total	62499.955	7			

Table 18 (Continued).

Fungal strains		Sum of Squares	df	Mean Square	F	P
C. militaris BCC 2816	Between Groups	385441.091	3	128480.364	47.114	0.001
	Within Groups	10907.988	4	2726.997		
	Total	396349.079	7			
C. militaris BCC 2817	Between Groups	38070.604	3	12690.201	331.286	0.000
	Within Groups	153.224	4	38.306		
	Total	38223.827	7			
C. militaris BCC 2818	Between Groups	37838.265	3	12612.755	712.578	0.000
	Within Groups	70.801	4	17.700		
	Total	37909.066	7			
C. militaris BCC 2819	Between Groups	455082.778	3	151694.259	876.920	0.000
	Within Groups	691.941	4	172.985		
	Total	455774.719	7			
C. militaris BCC 2824	Between Groups	2806.919	3	935.640	177.897	0.000
	Within Groups	21.038	4	5.259		
	Total	2827.957	7			

Table 18 (Continued).

Fungal strains		Sum of Squares	df	Mean Square	F	P
C. militaris BCC 2826	Between Groups	46433.817	3	15477.939	12.231	0.018
	Within Groups	5061.857	4	1265.464		
	Total	51495.674	7			
C. militaris BCC 2838	Between Groups	2478.304	3	826.101	7.318	0.042
	Within Groups	451.541	4	112.885		
	Total	2929.845	7			
C. militaris NBRC 5298	Between Groups	0.409	3	0.136	3.623	0.123
	Within Groups	0.150	4	0.038		
	Total	0.559	7			
C. militaris NBRC 9787	Between Groups	15381.410	3	5127.137	9.864	0.026
	Within Groups	2079.210	4	519.803		
	Total	17460.620	7			
C. militaris NBRC 100741	Between Groups	11475.345	3	3825.115	10.319	0.024
	Within Groups	1482.783	4	370.696		
	Total	12958.128	7			

As mentioned before, fermentation is a useful tool for producing biological materials with health promoting properties. Culture medium is very important to the yield of bioactive products because these nutrients are directly related to cell proliferation and metabolite biosynthesis of *C. militaris*. When carbon and nitrogen sources in the medium are not enough, it might stimulate the microorganisms to go to a different pathway and produce different biosynthesis metabolites. In case of cordycepin production, several factors of fermentation condition have been investigated in some previous studies, such as medium composition, medium pH, fermentation time, dissolved oxygen, etc. However it was lack of information about the effect of fermentation temperature on cordycepin production. It might be because the setting up of a temperature experiment requires several available incubators to work at a time and this requirement might not be easily to meet in many laboratories. In this study, the effect of four fermentation temperature conditions was successfully investigated. Both mycelium growth and cordycepin production were affected by cultivation temperatures in surface culture of *C. militaris*. It was, however, found that mycelium growth and cordycepin production of *C. militaris* were not correlated together among the gradient of temperatures. Most *C. militaris* strains showed the better growth at temperature of 15°C and 20°C, a decreased growth when temperature increased to 25°C, and inhibited cell growth at the temperature of 30°C. In case of cordycepin production, fermentation temperature at 25°C was most suitable for almost strains. A reduction of cordycepin production was observed when fermentation temperature was at 20°C in spite of the mycelial biomass induction in this temperature condition. Finally, cordycepin productions of these *C. militaris* strains were very low or completely inhibited when fermentation temperatures were at 15°C or 30°C. It can be discussed that cordycepin production of *C. militaris* was very strictly dependent on temperature. As the results shown from previous experiment, cordycepin was found to be presented from only temperate distributed entomopathogenic fungal species. The Chinese term "Dong Chong Xia Cao", which was temporary translated to "Winter Worm, Summer Herb", could remind us about the life cycle of this fungus. In winter season, when temperature reduces to under zero, the larvae (or pupae) of Lepidoptera living under ground had been infected by fungal spore and mycelium might be developing in insect body from this stage through spring. Until summer season comes,

when the weather become warmer, it is the suitable condition for the development of fruiting body. In addition, in some researches, various metabolites have been found from fruiting body of this fungus.

In recent research, Lin and Chiang (2008) investigated in using anti-tumour activity as marker in four different fermentation temperatures (15, 20, 25 and 30°C) on four tumour cell lines (AGS, MCF-7, Hep G2, and CT26), *C. militaris* grew successfully at 15°C, 20°C and 25°C and did not grow well when culture temperature reached 30°C. Anti-tumour activity of *C. militaris* was highest at 25°C, with the IC_{50} of fermentation broth obtained at 25°C for AGS, MCF-7, Hep G2, CT26 cell lines were lowest compared to other three fermentation temperatures. Further more, the anti-tumour activity of fermentation broth without agitation increased with the cultivation time, and there was a sharp increase in anti-tumour activity from three to four weeks of cultivation, however, the fermentation at 100 rpm of agitation, practically no anti-tumour activity was found in the broth after two weeks of cultivation. These results clearly showed that the static culture of *C. militaris* had better anti-tumour activity than the agitated culture. Other research examined the growth of mycelia of three *Ophiocordyceps nutans* strains in a range of temperatures at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C. Mycelium growth could be observed at the temperatures ranged from 10°C to 25°C and was greater at 20°C and 25°C. At 30°C and 35°C, mycelium growth was not observed in any of the strains (Sasaki *et al.*, 2005). Unagul *et al.* (2005) concluded that the temperature has effect on the growth of the fungus *Ophiocordyceps unilateralis*, the maximum growth was at 22°C and the growth was completely inhibited at 30°C. Also in her study, the production of secondary metabolites by this fungus was affected by temperature, the highest production of naphthoquinone were detected at 28°C, which was not suitable for mycelium growth.

3.3 Specific cordycepin production of *C. militaris* strains

In order to investigate the specific cordycepin production of different *C. militaris* strains, the cordycepin yields on biomass (mg/g DW) were calculated. The

hypothesis is that in a fixed culture medium and fermentation condition, the amount of cordycepin produced by *C. militaris* will be affected by two factors: the quantity of initial biomass from the stock inoculum and the capacity (characteristic) of strain in cordycepin production. The initial biomass is an important factor; maximum growth and cordycepin production times will be varied depending on the amount of initial cell. If quantity of biomass is high, it will take shorter time to reach highest cell growth and cordycepin production. Otherwise, if the amount of initial cell is less, the fermentation may take longer time. For better cordycepin production and productivity, the initial biomass can be controlled in the inoculum preparation. However, this study did not concern on this issue. In the other case, if cordycepin production depends on the capacity of fungal strain, some strains may produce low biomass but high cordycepin.

Cordycepin yields on biomass of 15 *C. militaris* strains were successfully calculated. The ANOVA result shown in Table 20 indicated that there were significant differences among strains in cordycepin yields on biomass (P=0.05). Significant differences could be observed in all cultivation conditions (15°C, 20°C, 25°C and 30°C). The result of cordycepin yield on biomass of 15 *C. militaris* strains in four fermentation temperature conditions is shown in Table 19 and the specific cordycepin formation rate is shown in Figure 31. The highest cordycepin yield on biomass were also found from two entomopathogenic fungal strains *C. militaris* BCC 2819 and *C. militaris* BCC 2816 (57.65 ± 0.76 mg/g and 64.64 ± 4.10 mg/g) at cultivation temperature of 25°C, which correspond to 3.84 ± 0.05 and 4.31 ± 0.27 mg/g/d respectively. At the same cultivation temperature, other *C. militaris* strains (BCC 2815, BCC 2814, BCC 2818) also indicated high rates of cordycepin production based on mycelial mass with cordycepin yields reached 41.24 ± 31.00 mg/g, 32.06 ± 2.08 mg/g and 25.11 ± 2.81 mg/g, which correspond to 2.75 ± 2.07 mg/g/d, 2.14 ± 0.14 mg/g/d and 1.67 ± 0.19 mg/g/d respectively. Other *C. militaris* strains presented low or very low cellular abilities on cordycepin production.

Comparing cordycepin yields on biomass among different cultivation temperatures, the ANOVA result shown in Table 21 indicated that significant

differences could be observed from most strains, except *C. militaris* NBRC 5298 and *C. militaris* BCC 2815. *C. militaris* NBRC 5298 indicated very low cordycepin yields in all temperature conditions due to it poor capacity of cordycepin production even mycelial biomass developed well in culture medium. Other 14 strains indicated the significantly differences of cordycepin yields on biomass. Cordycepin yields on biomass from these strains reached highest at the cultivation temperature of 25$^{\mathrm{o}}$C. Whereas, the yields of cordycepin on biomass were lower to significantly lower at the cultivation temperature of 20$^{\mathrm{o}}$C and most strains could not produce or produced very low amounts at 15$^{\mathrm{o}}$C and 30$^{\mathrm{o}}$C.

Cordycepin production by *C. militaris* was investigated in several studies. In these study, there were two method of cultivation have been investigated. Submersed culture of *C. militaris* mycelia have been presented in some studies (Mao *et al.*, 2005; Mao and Zhong, 2004; Kim *et al.*, 2003 a,b; Park *et al.*, 2001), while other studies concentrated on surface culture (Masuda *et al.*, 2006; 2007). Results from these studies indicated that the surface culture method shows more advantages in higher cordycepin production and productivity, and lower cost by less energy consumption.

Study of Mao and co-workers (2005) obtained cordycepin yield on biomass only about 20.17 mg/g in an optimized medium and culture condition in bioreactor, this result was about 3.2 and 2.9 times lower than our result of *C. militaris* BCC 2816 and BCC 2819. However, the result of Mao showed the higher of biomass production (17.12 ± 0.15 g/l), caused by high oxygen supply in bioreactor. As other filamentous fungi, *C. militaris* mycelium develops very well in aerobic condition with high oxygen concentration. However, in some cases, higher development of mycelial biomass may cause the lower metabolite production, especially for secondary metabolites. In case of cordycepin, this literature had been proved in the research of Mao and Zhong (2004).

Table 19 Effect cultivation temperatures on cordycepin yield on biomass (mg/g) of 15 *C. militaris* strains.

Fungal strains	Cultivation Temperatures			
	15	20	25	30
Cordyceps militaris BCC 1974	2.27 ± 0.65a	10.59 ± 1.78b	16.13 ± 0.88c	2.45 ± 0.11a
C. militaris BCC 1975	2.41 ± 0.03ab	1.44 ± 0.06ab	3.71 ± 1.09b	0.39 ± 0.01a
C. militaris BCC 2790	1.27 ± 0.02a	11.25 ± 1.93b	19.82 ± 2.23c	1.68 ± 0.20a
C. militaris BCC 2814	0.00 ± 0.00a	4.33 ± 1.80a	32.06 ± 2.08b	0.07 ± 0.06a
C. militaris BCC 2815	0.26 ± 0.14a	1.14 ± 0.12a	41.24 ± 31.00a	0.09 ± 0.02a
C. militaris BCC 2816	4.39 ± 4.04a	20.51 ± 0.83b	64.64 ± 4.10c	1.04 ± 0.14a
C. militaris BCC 2817	0.00 ± 0.00a	9.39 ± 0.64b	21.26 ± 0.16c	0.10 ± 0.02a
C. militaris BCC 2818	0.05 ± 0.01a	1.01 ± 0.01a	25.11 ± 2.81b	0.27 ± 0.01a
C. militaris BCC 2819	0.34 ± 0.14a	23.15 ± 1.56b	57.65 ± 0.76c	2.48 ± 0.18a
C. militaris BCC 2824	0.29 ± 0.08a	2.61 ± 0.64b	7.01 ± 0.48c	0.14 ± 0.01a
C. militaris BCC 2826	0.00 ± 0.00a	17.56 ± 3.52b	16.85 ± 5.53b	2.06 ± 0.19a
C. militaris BCC 2838	0.00 ± 0.00a	4.27 ± 0.75ab	8.62 ± 3.94b	0.20 ± 0.02a
C. militaris NBRC 5298	0.00 ± 0.00a	0.07 ± 0.01a	0.12 ± 0.00a	0.07 ± 0.06a
C. militaris NBRC 9787	0.09 ± 0.02a	1.20 ± 0.51a	15.39 ± 2.74b	0.15 ± 0.09a
C. militaris NBRC 100741	0.17 ± 0.00a	3.98 ± 1.04a	17.35 ± 5.05b	0.30 ± 0.08a

Note: Values are means of duplicate experiments ± SD. Values with same letters within a rows are not significantly different (P=0.05).
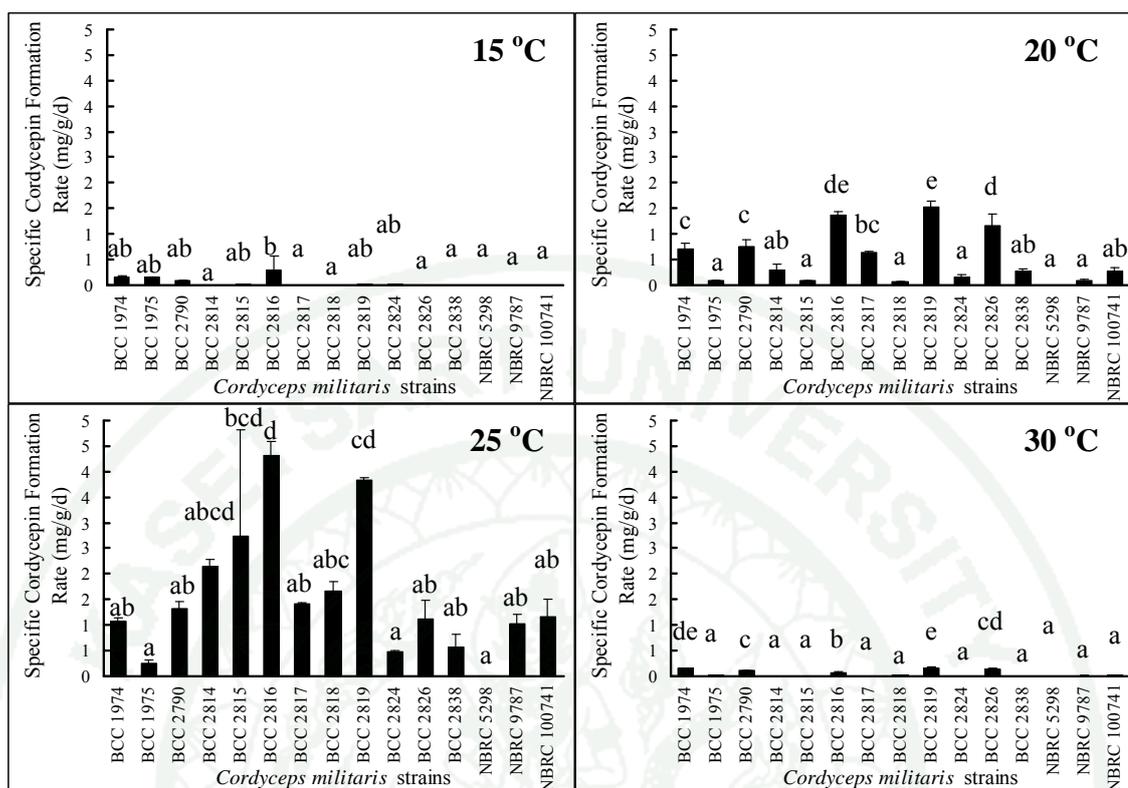
Figure 31 Effect of different cultivation temperatures on the specific cordycepin
formation rates of 15 *C. militaris* strains.

Note: Error bars show SD of means of duplicate experiments; values with same
letters within a chart are not significantly different at P=0.05 according to
Tukey's test.

Table 20 ANOVA result on effect of different *C. militaris* strains on cordycepin yield on biomass in four temperature conditions.

Temperatures		Sum of Squares	df	Mean Square	F	P
15°C	Between Groups	46.568	14	3.326	2.975	0.022
	Within Groups	16.773	15	1.118		
	Total	63.341	29			
20°C	Between Groups	1626.767	14	116.198	61.491	0.000
	Within Groups	28.345	15	1.890		
	Total	1655.112	29			
25°C	Between Groups	9799.130	14	699.938	9.748	0.000
	Within Groups	1077.036	15	71.802		
	Total	10876.167	29			
30°C	Between Groups	23.847	14	1.703	155.817	0.000
	Within Groups	0.164	15	0.011		
	Total	24.011	29			

Table 21 ANOVA result on the effect of different cultivation temperatures on cordycepin yield on biomass of 15 *C. militaris* strains.

Fungal strains		Sum of Squares	df	Mean Square	F	P
Cordyceps militaris BCC 1974	Between Groups	272.971	3	90.990	83.193	0.000
	Within Groups	4.375	4	1.094		
	Total	277.346	7			
C. militaris BCC 1975	Between Groups	12.022	3	4.007	13.506	0.015
	Within Groups	1.187	4	0.297		
	Total	13.209	7			
C. militaris BCC 2790	Between Groups	469.234	3	156.411	71.680	0.001
	Within Groups	8.728	4	2.182		
	Total	477.962	7			
C. militaris BCC 2814	Between Groups	1428.890	3	476.297	251.733	0.000
	Within Groups	7.568	4	1.892		
	Total	1436.458	7			
C. militaris BCC 2815	Between Groups	2490.608	3	830.203	3.455	0.131
	Within Groups	961.160	4	240.290		
	Total	3451.768	7			

Table 21 (Continued).

Fungal strains		Sum of Squares	df	Mean Square	F	P
C. militaris BCC 2816	Between Groups	5136.441	3	1712.147	202.335	0.000
	Within Groups	33.848	4	8.462		
	Total	5170.289	7			
C. militaris BCC 2817	Between Groups	607.354	3	202.451	1882.337	0.000
	Within Groups	0.430	4	0.108		
	Total	607.784	7			
C. militaris BCC 2818	Between Groups	914.016	3	304.672	154.288	0.000
	Within Groups	7.899	4	1.975		
	Total	921.915	7			
C. militaris BCC 2819	Between Groups	4235.073	3	1411.691	1831.948	0.000
	Within Groups	3.082	4	0.771		
	Total	4238.155	7			
C. militaris BCC 2824	Between Groups	61.629	3	20.543	127.337	0.000
	Within Groups	0.645	4	0.161		
	Total	62.274	7			

Table 21 (Continued).

Fungal strains		Sum of Squares	df	Mean Square	F	P
C. militaris BCC 2826	Between Groups	528.023	3	176.008	16.396	0.010
	Within Groups	42.939	4	10.735		
	Total	570.962	7			
C. militaris BCC 2838	Between Groups	99.446	3	33.149	8.255	0.035
	Within Groups	16.063	4	4.016		
	Total	115.509	7			
C. militaris NBRC 5298	Between Groups	0.014	3	0.005	4.525	0.089
	Within Groups	0.004	4	0.001		
	Total	0.018	7			
C. militaris NBRC 9787	Between Groups	335.040	3	111.680	57.631	0.001
	Within Groups	7.751	4	1.938		
	Total	342.791	7			
C. militaris NBRC 100741	Between Groups	396.360	3	132.120	19.840	0.007
	Within Groups	26.637	4	6.659		
	Total	422.998	7			

3.4 Estimation the effects of fungal sources and fermentation temperatures

In order to estimate the effect of different sources of strains and cultivation temperatures on growth and cordycepin production and their correlation, The General Linear Model (GLM) multivariate procedure was performed to provide regression analysis and analysis of variance (ANOVA) for two dependent variables.

In the regression of different sources of *C. militaris* strains, five strain samples collected from Appi Highlands and four strain samples collected from Nyutu Spa were successfully estimated for growth and cordycepin production in four fermentation temperature conditions.

For the regression of different temperature effects on growth and cordycepin production and the correlation between growth and cordycepin production in the dependence on fermentation temperatures, the results of DWs and cordycepin productions of 15 *C. militaris* strains in four temperature conditions were used to estimate. The following profile plots (interaction plots) were conducted in the model for comparing marginal means of DWs and cordycepin concentrations of multiple samples.

3.4.1 Effect of different sources of strains

The analysis of variance in Table 22 showed that, in case of comparison of DWs from two different sources of strains the regression was not statistically significant at 95% of confidence interval (P=0.420>0.05). However, cordycepin production was significantly different between strains from Appi Highlands and strains from Nyutu Spa (P=0.002<0.05). The profile plot of estimated marginal means of total DW is shown in Figure 32. In average, DW produced by strains obtained from Nyutu Spa's was higher than those from Appi Highlands at 15°C, however it was not significantly different at 95% of confidence interval of regression and the regression coefficient was low (R^2=0.470). For cordycepin production, the profile plot of estimated marginal means of cordycepin production is

shown in Figure 33. It indicated that the temperature at 25°C was the suitable temperature for cordycepin production of both strain sources. There was a significant difference in cordycepin production by *C. militaris* strains from Appi Highlands and Nyutu Spa. Cordycepin production of *C. militaris* strains collected from Appi Highlands was significantly higher than that from Nyutu Spa with the regression coefficient was R^2=0.606.

The profile plots (interaction plots) were used for comparing marginal means in these models. Each line in a plot showed the variable (DW or cordycepin production) of each fungal source (Appi highlands or Nyutu Spa). Two lines of the plot made a comparison the difference of variables which depend on cultivation temperatures. Our belief is that *C. militaris* strains from Appi Highlands and Nyutu Spa may provide different abilities of growth and cordycepin production. The results from the regression indicated that only cordycepin production was significantly different between two sources. However, the result of regression coefficient was not high (0.606) because the numbers of samples were less (only five strains from Appi Highlands and four strains from Nyutu Spa were applied in the model). Two strains, *C. militaris* BCC 2816 and *C. militaris* BCC 2819, which indicated highly cordycepin productions and productivities, were collected from Appi Highlands. It can be discussed that there were two reasons would cause the significant increase of cordycepin production of Appi Highlands' strains compared with strains from Nyutu Spa's one. The first, some *C. militaris* strains that collected from Appi Highlands might be derived from an initial fungal population which has closely genetic relationship between individuals, and strains that collected from Nyutu Spa might come from an other fungal population. Because the distribution of specimens were not far from each other in an area, ascospores infected Lepidoptera larvae under ground might be discharged from one mother fruiting body or some closely related individuals. Therefore, they got a similar characteristic to each other in their cordycepin production abilities. The second reason is the difference of geographical positions of Appi Highlands and Nyutu Spa resulted different conditions such as weather and other biotic or abiotic factors that caused the different characteristics of cordycepin production of *C. militaris* strains collected from these locations. From the

result, it can be suggested that more samples in these areas should be examined in order to prove this hypothesis in future studies.
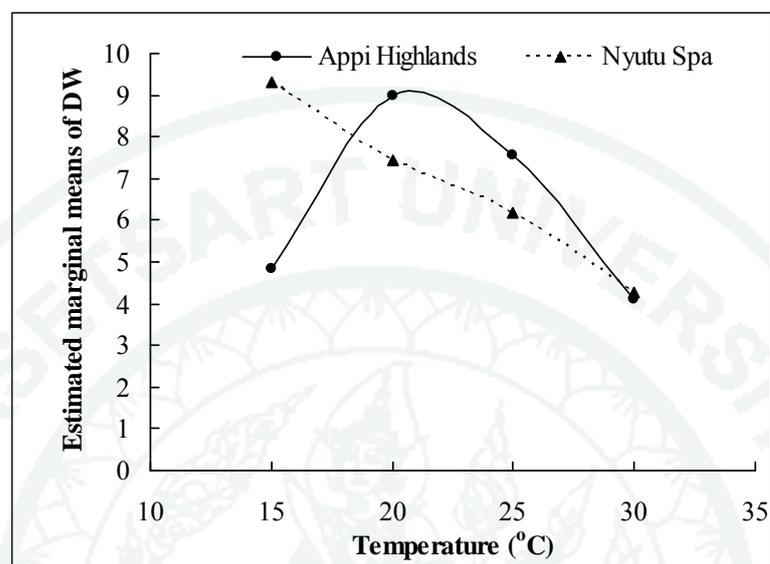

Figure 32 Profile plot showing estimated marginal means of mycelial dry weights of *C. militaris* strains from two sources.
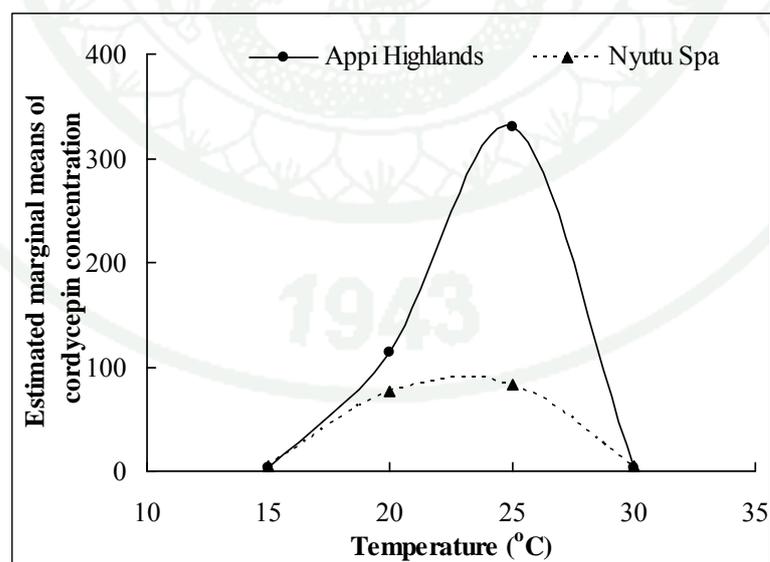

Figure 33 Profile plot showing estimated marginal means of cordycepin productions of *C. militaris* strains from two sources.

Table 22 ANOVA regression for the effect of strain sources on mycelium growth
and cordycepin production of *C. militaris*.

Source	Dependent Variable	Sum of Squares (type III)	R^2	df	F	P
Location	DW	3.097	0.470	1	0.659	0.420
	Cordycepin concentration	88036.075	0.606	1	10.044	0.002

3.4.2 Effect of different temperature conditions

In order to investigate the correlation between mycelium growth
and cordycepin production in the dependence of temperatures, the GLM Multivariate
procedure was performed to provide regression analysis and analysis of variance
(ANOVA) for two dependent variables (total DW and cordycepin concentration) by
one-factor variables (temperature). Total fifteen samples (15 *C. militaris* strains) were
applied in the model, and the temperature variables were $15^{o}C$, $20^{o}C$, $25^{o}C$ and $30^{o}C$.
The observed means of mycelial DW and cordycepin production of 15 *C. militaris*
strains from each temperature variable were then subjected to the Profile Plots to
estimate the marginal means. The ANOVA result of the model is shown in Table 23.
It indicated that the regression was statistically significant at 95% of confidence
interval in both growth (DW) and cordycepin production. It also indicated that
different cultivation temperatures gave significant affects on the growth and
cordycepin production (P=0.05). The regression coefficient were high for both growth
and cordycepin production, R^2=0.919 and 0.977 respectively.

Figure 34 provides the estimation of growth and Figure 35
shows the estimation of cordycepin production on the dependence of temperatures.
Both line plots, which showed estimated marginal means of growth and cordycepin
production, were not produced in parallel. It proved that mycelium growth and
cordycepin production of *C. militaris* are depended on cultivation temperature but
they do not correlate together. In detail, the profile plot shown in Figure 34 indicated

that *C. militaris* grew significantly better at the cultivation temperatures from 15°C to 20°C. Higher temperature at 25°C caused a reduction of growth, and the growth of *C. militaris* was inhibited at 30°C. In other way, the estimated result of cordycepin production was shown to be different. This species produced highest cordycepin at the cultivation temperature at 25°C, lower temperature at 20°C caused the lower cordycepin production and the cordycepin productions were very low or completely inhibited at 15°C and 30°C.

Table 23 ANOVA of regression the effect of fermentation temperatures on mycelium growth and cordycepin production of *C. militaris*.

Source	Dependent Variable	Sum of Squares (type III)	R^2	df	F	P
Temperatures	DW	327.081	0.916	3	79.989	0.000
	Cordycepin concentration	550263.626	0.977	3	296.151	0.000

Several studies have been successfully in production of cordycepin by submersed culture of *C. militaris*. The medium components have also been optimized to achieve high yield production and productivity. However, the temperature for fermentation of these studies was fixed at 25°C. So far, the relationship between growth and cordycepin production under the effect of cultivation temperature has not yet been confirmed. This study has proved the different effect of temperature on mycelium growth and cordycepin production, lower temperatures at 15°C and 20°C promoted biomass production but were not favourable for cordycepin production meanwhile higher temperature at 25°C was suitable for cordycepin production irrespective its growth reduction.
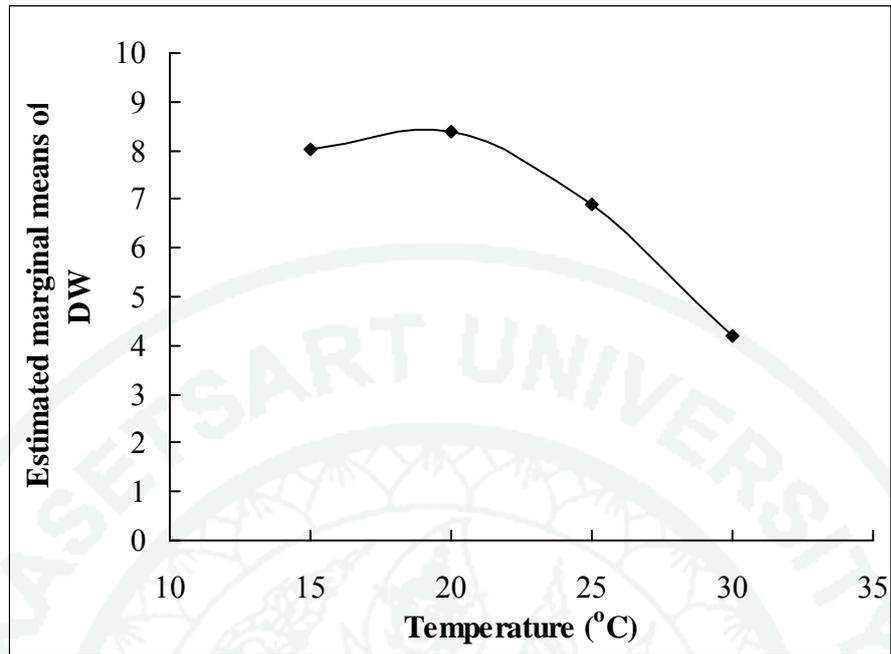
Figure 34 Profile plot showing estimated marginal means of mycelium growths in the dependence of temperatures.
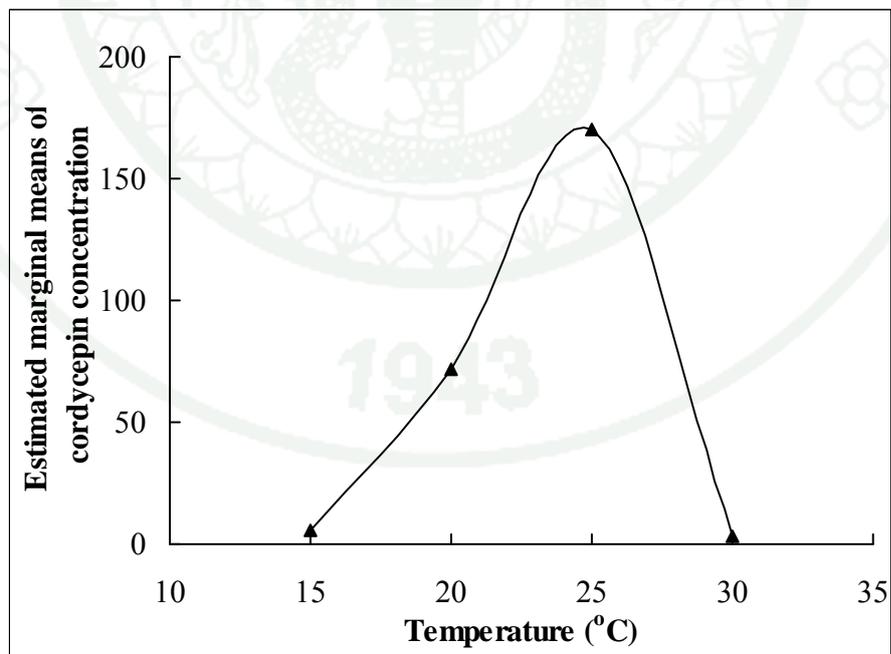

Figure 35 Profile plot showing estimated marginal means of cordycepin productions in the dependence of temperatures.

Different from other *Cordyceps* species, at the cultivation temperature at 25°C, growth and exobiopolymer production in shake flask culture of *Ophiocordyceps sphecocephala* J-201 (Oh *et al.*, 2007) and the production of mycelia and polysaccharide in submerged cultivation of *C. jiangxiensis* (Xiao *et al.*, 2004; 2006) were increased together. Kim and Yun (2005) investigated that, in case of *C. militaris*, maximum exopolysaccharide concentrations were obtained at 30°C, whereas maximum mycelial biomasses were found at 20°C. Both EPS yield and mycelium growth were found to be noticeably decreased when incubated in temperatures above 30°C. In case of *Ophiocordyceps sinensis*, maximum productions of exopolysaccharide and mycelial biomass were both achieved at 20°C.

To date, nucleosides are believed to be the active components in *Cordyceps* and more than ten nucleosides and their related compounds such as adenine, adenosine, uracil, uridine, guanine, guanosine, hypoxanthine, inosine, thymine, thymidine and deoxyuridine have been isolated (Li *et al.*, 2006a,b). Kim *et al.* (2002) discussed that the nutritional requirement for exopolysaccharide production in basidiomycetes and ascomycetes differed in strains and culture conditions. It was known that several complex nitrogen sources such as polypeptone and corn steep powder or amino acids were desirable while inorganic nitrogen sources were inefficient for the production of exopolysaccharide in submerged cultures of mushrooms (Bae *et al.* 2001; Kim *et al.* 2002; Chi *et al.* 1996). Unagul *et al.* (2005) concluded that the temperature had effect on growth of the fungus *Ophiocordyceps unilateralis*, the maximum growth was at 22°C and its growth was completely inhibited at 30°C. In other case, production of secondary metabolites by this fungus was also affected by temperature; the highest production of naphthoquinone was detected at 28°C, which was not suitable for mycelium growth (Unagul *et al.*, 2005).

In addition, cordycepin is a nucleoside secondary metabolite, its biosynthesis is related to metabolism of adenosine and adenylate, and ADP may be a direct precursor of cordycepin (Lennon and Suhadolnik, 1976). Indeed, it was reported that acetate and isovalerate were not precursors for cordycepose. In addition, glucose-l-C^{14} and glucose-6-C^{14} were incorporated into cordycepose, whereas ribose-

l-C^{14} was essentially not utilized; adenine-8-C^{14} was incorporated into the purine moiety of cordycepin and glucose was the sole carbon source (Kredich and Guarino, 1961). It was also discussed that the biosynthesis of cordycepin by *C. militaris* may be thought of to involve three individual problems: the synthesis of adenine, the synthesis of cordycepose, and the synthesis of the N-glycosidic bond (Kredich and Guarino, 1961). Later study of Grant (1964) investigated that Adenosine-U-C^{14} and formate-C^{14} were both incorporated into cordycepin and it was proved that the biosynthesis of cordycepin from adenosine occurs in a direct conversion of adenosine. These findings also ruled out the hydrolysis of adenosine prior to the formation of cordycepin, these studies provided strong evidence that adenosine converted to 3′-deoxyadenosine without hydrolysis of the N-riboside bond (Lennon and Suhadolnik, 1976).

In conclusion, our results of mycelium growth and cordycepin production are agreed with the reports in literatures that the optimal temperatures for the growth of *Cordyceps* mycelium and exopolysaccharide production ranged from 20°C to 28°C (Xiao *et al.*, 2004; Park *et al.*, 2001 and Kim *et al.*, 2003a). In addition, it is accepted that the production of cordycepin as corresponds with secondary metabolite production in other microorganisms, in that, the optimum temperature is generally higher than that for growth (Bhosale and Gadre, 2002; Kuznetsov *et al.*, 1984).

CONCLUSION AND RECOMMENDATIONS

Conclusion

Cat Tien National Park of Vietnam has diverse numbers of entomopathogenic fungi with at least 41 species belonging to 17 genera have been recorded within three-day collecting trip in rainy season. The distribution of entomopathogenic fungal specimens in Cat Tien National Park was higher on leaves of forest plants while very few specimens could be found from other habitats.

Among 259 specimens collected, the number of anamorphs were much higher than the number of teleomorphs. Species of the entomopathogenic fungus *Aschersonia* and their teleomorphs (*Hypocrella* and *Moelleriella*) were frequently found in the collection, all of these species infected scale insect living on leaves of forest plants. *Cordyceps* and related species with brightly and freshly stromata were very rare.

In the association between fungi and their insect and other arthropod hosts, eight arthropod Orders were the hosts of entomopathogenic fungi in Cat Tien National Park in which the highest number of specimens was found on Homoptera.

One hundred and forty night cultures were successfully isolated and preserved in the culture collection. These cultures will be the potential materials for future research and application purposes.

Cordycepin (3'-deoxyadenosine) is a nucleoside analogue, which was found from *Cordyceps militaris*, *C. kyushuensis* and *Ophiocordyceps sinensis*. In the current study, 35 cultures belonging to 32 fungal species were successfully investigated for cordycepin production. Only well-known *C. militaris* species, which originally collected from Japan, showed the ability of cordycepin production in both PDB and

PG media while other species from Thailand and Vietnam could not produce cordycepin.

The cordycepin production ability of entomopathogenic fungi was not correlated with their phylogenetic classification. For cordycepin production, glucose and peptone were the favourable carbon and nitrogen sources in fermentation medium.

The capacities for cordycepin production of *C. militaris* were different among different strains. Two strains *C. militaris* BCC 2819 and *C. militaris* BCC 2816 gave excellence results of cordycepin productions and productivities. The effect of fermentation temperatures on growth and cordycepin production was successfully estimated using General Linear Model. Mycelium growth and cordycepin production were not correlated together in the dependence of fermentation temperature. Mycelium grew best at temperature from $15^{o}C$ to $20^{o}C$ but cordycepin production was low in these temperatures. The most suitable temperature for cordycepin production was $25^{o}C$. The temperature at $30^{o}C$ inhibited both mycelial growth and cordycepin production of *C. militaris*.

Recommendations

In order to achieve better information about biodiversity and distribution of entomopathogenic fungi in Cat Tien National Park, More surveys should be conducted regularly from other locations in the park and both in rainy and dry seasons. Collaborative research with other institutions nationwide should be established to build the data of entomopathogenic in Vietnam.

Two strains, *C. militaris* BCC 2816 and *C. militaris* BCC 2819, were the excellence strains for use in cordycepin production. These strains are highly recommended for use in further research on cordycepin as well as other metabolite production. For further research and utilization on cordycepin production, the medium component should be optimized basing on the compositions of PG medium.

LITERATURE CITED

Ahn, Y.J., S.J. Park, S.G. Lee, S.C., Shin and D.H. Choi. 2000. Cordycepin: Selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp. **J. Agric. Food Chem.** 48: 2744-2748.

Aman, S., D.J. Anderson, T.J. Connolly, A.J. Crittall, and G.J. Ji. 2000. From adenosine to 3′-deoxyadenosine: development and scale up. **Org. Process Res. Dev.** 4: 601-605.

Artjariyasripong, S., J.L. Mitchell, N.L. Hywel-Jones, E.B.G. Jones. 2001. Relationships of the genus *Cordyceps* and related genera, based on parsimony and spectral analysis of partial 18S and 28S ribosomal gene sequences. **Mycoscience** 42: 503–517.

Bae, T., J.P. Park, C.H. Song, C.B. Yu, M.K. Park, and J.W. Yun. 2001. Effect of carbon source on the mycelial growth and exo-biopolymer production by submerged culture of *Paecilomyces japonica*. **J. Biosci. Bioeng.** 91: 522–524.

Bazin, H. and J. Chattopadhyaya. 1985. A convenient preparation of 3'-deoxyadenosine (cordycepin) and 9-[3'(R)-deuterio-b-D-2'(R)-pentofuranosyl]-adenine. **Synthesis** 12: 1108-1111.

Bhosale, P. and R.V. Gadre. 2002. Manipulation of temperature and illumination conditions for enhanced beta-carotene production by mutant 32 of *Rhodotorula glutinis*. **Lett. Appl. Microbiol**. 34: 349-353.

Borel, J.F.. 2002. History of the discovery of cyclosporin and of its early pharmacological development. **Wien. Klin. Wochenschr**. 114(12): 433-437.

Bowman, S.M. and S.J. Free. 2006. The structure and synthesis of the fungal cell wall. **BioEssays** 28: 799–808.

Bruns, T.. 2006. Evolutionary biology: a kingdom revised. **Nature** 443: 758–761.

Carruthers, R.I., T.S. Larkin, and H. Firstencel. 1992. Influence of thermal ecology on the mycosis of a rangeland grasshopper. **Ecology** 73: 190-204.

Charnley, A.K. and R.J. St. Leger. 1991. The role of cuticle-degrading enzymes in fungal pathogenesis in insects. *In:* G. T. Cole and H. C. Hoch (eds.), **The Fungal Spore and Disease Initiation in Plants and Animals,** Plenum, New York, pp. 267-286.

Charnley, A.K.. 1989. Mechanisms of fungal pathogenesis in insects. *In*: J. M. Whipps and R. D. Lumsden (eds.), **The Biotechnology of Fungi for Improving Plant Growth,** Cambridge Univ. Press, London, pp. 85–125.

Chaverri, P., M. Liu and K.T. Hodge. 2008. A monograph of the entomopathogenic genera *Hypocrella*, *Moelleriella*, and *Samuelsia* gen. nov. (*Ascomycota*, *Hypocreales*, *Clavicipitaceae*), and their aschersonia-like anamorphs in the Neotropics. **Stud. Mycol.** 60: 1–66.

Chen, Y.Q., B. Hu, F. Xu, W. Zhang, H. Zhou, and L.H. Qu. 2004. Genetic variation of *Cordyceps sinensis*, a fruit-body-producing entomopathogenic species from different geographical regions in China. **FEMS Microbiol. Lett.** 230: 153-158.

Chen, Y.C., Y.L. Huang, and B. Huang. 2005. *Cordyceps sinensis* mycelium activates PKA and PKC signal pathways to stimulate steroidogenesis in MA-10 mouse Leydig tumor cells**. Int. J. Biochem. Cell B.** 37: 214–223.

Cheng, M. and Y. Zhang. 1995. **Chin. Trad. Pat. Med.** 17: 40-41.

Chi, J.H., T.M. Ha, Y.H. Kim, and Y.D. Rho. 1996. Studies on the main factors affecting the mycelial growth of *Phellinus linteus*. **Kor. J. Mycol**. 24: 214–222.

Cho, H.J., J.Y. Cho, M.H. Rhee, and H.J. Park. 2007. Cordycepin (3′-deoxyadenosine) inhibits human platelet aggregation in a cyclic AMP-and cyclic GMP-dependent manner. **Eur. J. Pharmacol.** 558, 43–51.

Clark, T.B., W.R. Kellen, R. Fukuda, and J.E. Lindegren. 1968. Field and Laboratory studies on the pathogenicity of the fungus *Beauveria bassiana* to three genera of mosquitoes. **J. Invertebrate Path.** 11. 2-8.

CTNP. 2003. Conservation management and operational plan for Cat Tien National Park, Dong Nai, Lam Dong and Binh Phuoc provinces, Vietnam: 2003-2008. Cat Tien: Cat Tien National Park Management Board.

Cui, X.M.. 1999. Artificial culture of *Cordyceps sinensis*. *Asia-Pacific Biotech News* 3: 333–337.

Cunningham, K.G., S.A. Hutchinson, W. Manson, and F.S. Spring. 1951. Cordycepin. A metabolic product from cultures *Cordyceps militaris* (Linn.) Link. Part I. Isolation and characterization. **J. Chem. Soc.** 2299–3000.

Cunningham, K.G., W. Manson, F.S. Spring, and S.A. Hutchison. 1950. Cordycepin, a metabolic product isolated from cultures of *Cordyceps militaris* (Linn.) Link. **Nature** 166, 949-952.

Dai, G., T. Bao, C. Xu, R. Cooper, and J.S. Zhu. 2001. CordyMax Cs-4 improves steady-state bioenergy status in mouse liver. **J. Alternat. Complement. Med.** 7: 231–240.

de Julian-Ortiz, J.V., J. Gálvez, C. Muñoz-Collado, R. Garcia-Domenech, and C. Jimeno-Cardona. 1999. Virtual combinatorial syntheses and computational screening of new potential anti-herpes compounds. **J. Med. Chem.** 42: 3308-3314.

Dillon, R.J. and A.K. Charnley. 1989. Initiation on germination in conidia of the entomopathogenic fungus, *Metarhizium anisopliae*. *In:* A.K. Charnley (ed.): **Mycoinsecticides: Present use and Future prospects. Progress and Prospects in Insect control**. Monograph No.43, British Crop Protect. Council. pp.165-181

Evans, H.C. and R.A. Samson. 1984. *Cordyceps* species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems. 2. The Camponotus (Formicinae) complex. **Trans. Br. Mycol. Soc.** 82: 127-150.

Evans, H.C.. 1988. Coevolution of entomogenous fungi and their insect hosts. *In*: K.A. Pirozynski and D.L. Hawksworth (eds.): **Coevolution of Fungi with Plants and Animals**, Academic Press, London, pp. 149–171.

Fargues, J., O. Reisinger, P.H. Robert, and C. Aubart. 1983. Biodegradation of entomopathogenic hyphomycetes: influence of clay coating on *Beauveria bassiana* blastospore survival in soil**. J. Invert. Pathol.** 41: 131–142.

Feng, M.G., Q.G. Zhou, and G.H. Feng. 1987. Vasodilating effect of cultured *Cordyceps sinensis* (Berk) Sacc. mycelia in anesthetized dogs. **Zhong Yao Tong Bao** 12, 41–45.

Ferron, P.. 1981. Pests control by the fungi *Beauveria* and *Metarhizium. In*: H.D. Burges (ed.). **Microbial control of pests and plant diseases 1970-1980**, Academic Press. London. pp. 465-482.

FIPI. 1993. **Feasibility study for Cat Tien National Park**. Hanoi: Forest Inventory and Planning Institute.

Frederiksen, S., H. Malling, and H. Klenow. 1965. Isolation of 3'-deoxyadenosine (cordycepin) from the liquid medium of *Cordyceps militaris* (L. EX FR.) Link. **Biochim. Biophys. Acta** 95: 189–193.

Fukatzu, T., H. Sato, and H. Kuriyama. 1997. Isolation, inoculation to insect host, and molecular phylogeny of an entomogenous fungus *Paecilomyces tenuipes*. **J. Inver-tebr. Pathol.** 70: 203–208.

Furuya, T., M. Masao, and M. Masuzawa. 1983. N^6-(2-hydroxyethel)adenosine, a biologically active compound from cultured mycelia of *Cordyceps* and *Isaria* species. **Phytochemistry** 22: 2509-2512.

Gao, Q., G. Wu, and D. He. 2000. Effect of *Cordyceps sinensis* on the Th1/Th2 cytokines in patients with Condyloma Acuminatum. **Zhong Yao Cai** 23(7): 402-404.

Georg, L.K., W.M. Williamson, E.B. Tilden, and R.E. Getty. 1962. Mycotic pulmonary disease of captive giant tortoise due to *Beauvaria bassiana* and *Paecilomyces* fumoso-roseus. **Sabouraudia** 2, 80-86.

Goettel, M.S., R.J. St. Leger, N.W. Rizzo, R.C. Staples, and D.W. Roberts. 1989. Ultrastructural localization of a cuticle degrading protease produced by the entomopathogenic fungus, *Metarhizium anisopliae*. **J. Gen. Microbiol**. 135: 2223-2239.

Gong, H.Y., K.Q. Wang, and S.G. Tang. 2000. Effects of *Cordyceps sinensis* on T lymphocyte subsets and hepatofibrosis in patients with chronic hepatitis B. **Hunan Yi Ke Da Xue Xue Bao** 25(3): 248-250.

Griesch, J. and A. Vilcinskas. 1998. Proteases released by entomopathogenic fungi impair phagocytic activity, attachment and spreading of plasmatocytes isolated from haemolymph of the greater wax moth, *Galleria mellonella*. **Biocontrol Sci. Technol**. 8: 517-531.

Griffin, D.M.. 1963. Soil moisture and the ecology of soil fungi. **Biol. Rev. Cambridge Phil. Soc.** 38: 141–166.

Guo, C., J. Zhu, C. Zhang, and L.J. Zhang. 1998. Determination of adenosine and 3'-deoxyadenosine in *Cordyceps militaris* (L.) Link. by HPLC. **China J. Chinese Mater. Med.** 23: 236-237.

Hajek, A.E. and R.J. St. Leger. 1994. Interactions between fungal pathogens and insect hosts. **Annu. Rev. Entomol**. 39. 293-322.

Hall, F.R. and J.J. Menn (eds.), 1999. **Biopesticides: Use and Delivery**. Humana Press, Totowa. 640pp.

Hamburger, M.. 2007. Comment on comparison of protective effects between cultured *Cordyceps militaris* and natural *Cordyceps sinensis* against oxidative damage. **J. Agric. Food Chem**. 55: 7213–7214.

Hansske, F. and M.J. Robins. 1985. Nucleic-acid related-compounds .49. regiospecific and stereoselective conversion of ribonucleosides to 3'-deoxynucleosides-a high-yield 3-stage synthesis of cordycepin from adenosine. **Tetrahedron Lett.** 26(36): 4295-4298.

Hashimoto, K. and B. Simizu. 1976. Effect of cordyeepin on the replieation of western equine encephalitis virus. Brief Report. **Arch. Virol.** 52: 341-345.

Hassan, A.E.M., R.M. Dillon, and A.K. Charnley. 1989. Influence of accelerated germination of conidia on the pathogenicity of *Metarhizium anisopliae* for *Manduca sexta*. **J. Invert. Pathol.** 54: 277–279.

Hawksworth, D.L., P.M. Kirk, B.C. Sutton, and D.N. Pegler. 1995. **Ainsworth and Bisby's Dictionary of the Fungi** (8th Ed.). CAB International, Wallingford, United Kingdom. 616pp.

Herdewijn, P.. 1989. Reaction of adenine nucleosides, tosylated in the carbohydrate moiety, with lithium triethylborohydride. **Tetrahedron** 45: 6563-6580.

Hodge, K.T.. 2003. Clavicipitaceous anamophs. *In*: White Jr.J.F., Bacon C.W., Hywel-Jones N.L. and Spatafora J.W. (eds.), **Clavicipitalean Fungi**, Marcel Dekker, New York, pp. 75–123.

Hoffmann, M.P. and A.C. Frodsham. 1993. **Natural Enemies of Vegetable Insect Pests**. Cooperative Extension, Cornell University, Ithaca, NY. 63pp.

Hsu, C.C., Y.L. Huang, S.J. Tsai, C.C. Sheu, and B.M. Huang. 2003. *In vivo* and *in vitro* stimulatory effects of *Cordyceps sinensis* on testosterone production in mouse Leydig cells. **Life Sci.** 73(16): 2127-2136.

Hsu, T.H. and H.C. Lo. 2002. Biological activity of *Cordyceps* (Fr.) Link species (Ascomycetes) derived from a natural source and from fermented mycelia on diabetes in STZ-induced rats. **Int. J. Med. Mushroom** 4: 111-125.

Huang, L.F., Y.Z. Liang, F.Q. Guo, Z.F. Zhou, and B.M. Cheng. 2003. Simultaneous separation and determination of active components in *Cordyceps sinensis* and *Cordyceps militarris* by LC/ESI-MS. **J. Pharm. Biomed. Anal.** 33: 1155-1162.

Huang, Y.L., S.F. Leu , B.C. Liu , C.C., Sheu and B.M. Huang . 2004. In vivo stimulatory effect of *Cordyceps sinensis* mycelium and its fractions on reproductive functions in male mouse. **Life Sci.** 75(9): 1051-1062.

Huxham, I.M., A.M. Lackie, and N.J. Mccorkindale. 1989. Inhibitory effects of cyclodepsipeptides, destruxins, from the fungus *Metarhizium anisopliae*, on cellular immunity in insects. **J. Insect Physiol.** 35: 97-107.

Hywel-Jones, N.L., R.D. Goos, and E.B.G. Jones. 1998. **Hirsutella petchabunensis** sp. nov. from Thailand, with a **Helicoma** synanamorph. **Mycol. Res.** 102: 577-581.

Hywel-Jones, N.L.. 1994. *Cordyceps khaoyaiensis* and *C. pseudomilitaris*, two new pathogens of Lepidopteran larvae from Thailand. **Mycol. Res.** 98: 939-942.

Hywel-Jones, N.L.. 1995a. *Cordyceps nutans* and its anamorph a pathogen of Hemipteran bugs in Thailand**. Mycol. Res**. 99: 724-726.

Hywel-Jones, N.L.. 1995b. *Cordyceps sphecocephala* and a *Hymenostilbe* sp. infecting wasps and bees in Thailand. **Mycol. Res.** 99: 154-158.

Hywel-Jones, N.L.. 1996. *Cordyceps myrmecophila*-like fungi infecting ants in the leaf litter of tropical forest in Thailand. **Mycol. Res.** 100: 613-619.

Hywel-Jones, N.L.. 2002. The importance of invertebrate-pathogenic fungi from the tropics. **Trop. Mycol**. 2: 133-144.

Hywel-Jones, N.L.. 2004. The relationship between the entomopathogenic fungal genera *Cordyceps* and *Beauveria*. Laimburg J., 1: 299−304.

Inglis, G.D., M.S. Goettel, T.M. Butt, and H. Strasser. 2001. Used of hyphomycetous fungi for managing insect pests. *In*: Butt T.M., Jackson C., and Magan N.

(eds.), **Fungi as Biocontrol Agents**, CAB International, Wallingford. pp. 23-69.

Isaka, M., P. Kongsaeree, and Y. Thebtaranonth. 2001. Bioxanthracenes from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620, II. Structure elucidation**. J. Antibiot.** 54(1): 36-43.

Ito, Y., T. Shibata, M. Arita, H. Sawai, and M.J. Ohno. 1981. Chirally selective synthesis of sugar moiety of nucleosides by chemicoenzymatic approach: L- and D-riboses, showdomycin, and cordycepin. **J. Am. Chem. Soc.** 103(22): 6739-6741.

Jain, T.C., I.D. Jenkins, A.F. Russell, J.P.H. Verheyden, and J.G.J. Moffatt. 1974. Reactions of 2-Acyloxyisobutyryl Halides with Nucleosides. IV. A Facile Synthesis of 2',3'-Unsaturated Nucleosides Using Chromous Acetate. **Org. Chem.** 39: 30-38.

James, H.C.. 1946. The bionomics and control of *Tomaspis fluvilatera* Ur., The Demerara sugar cane froghopper. **Proc. Brit. W. Ind. Sug. Tech.** 34-79.

Johnson, D., G.H. Sung, N.L. Hywel-Jones, J. J. Luangsa-ard, J.F. Bischoff, R.M. Kepler, and J.W. Spatafora. 2009. Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota). **Mycol. Res.** 113: 279–289

Kendrick, B.. 2000. **The Fifth Kingdom** (3rd Ed.), Focus Publishing, Newburyport, Massachusetts.

Kiho, T., A. Yamane, J. Hui, S. Usui, and S. Ukai. 1996. Polysaccharides in fungi. XXXVI. Hypoglycemic activity of polysaccharide (CS-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver. **Biol. Pharm. Bull.** 19: 294-296.

Kim, H.O. and J.W. Yun. 2005. A comparative study on the production of exopolysaccharides between two entomopathogenic fungi *Cordyceps militaris* and *Cordyceps sinensis* in submerged mycelial cultures. **J. Appl. Microbiol.** 99: 728–738.

Kim, H.W., Y.H. Kim, C.X. Fu, K.S. Nam, S.J. Lee, H.S. An, E.H. Jeoung, S.H. Yun, S.K. Sung, S.J. Lee, and J.W. Hyun. 2001. *In vitro* antitumor activity of ergosterol peroxide isolated from *Cordyceps militaris* on cancer cell line from Korea patients. **Korean J. Mycol.** 29: 61-66.

Kim, J.R., S.H. Yeon, H.S. Kim, and Y.J. Ahn. 2002. Larvicidal activity against *Plutella xylostella* of cordycepin from the fruiting body of *Cordyceps militaris*. **Pest Manage. Sci.** 58: 713-717.

Kim, S.W., C.P. Xu, H.J., Hwang J.W. Choi, C.W. Kim, and J.W. Yun. 2003b. Production and characterization of exopolysaccharides from an enthomopathogenic fungus *Cordyceps militaris* NG3. **Biotechnol. Prog.** 19: 428-435.

Kim, S.W., H.J. Hwang, C.P. Xu, J.M. Sung, J.W. Choi, and J.W. Yun. 2003a. Optimization of submerged culture process for the production of mycelial biomass and exo-polysaccharides by *Cordyceps militaris* C738. **J. Appl. Microbiol.** 94: 120-126.

Kirk, P.M., P.F. Cannon, J.C. David, and J.A Stalpers. 2001. **Ainsworth and Bisby's Dictionary of the Fungi**, 9th Edition. CABI Publishing. 666pp.

Kittakoop, P., J. Punya, P. Kongsaeree, Y. Lertwerawat, A. Jintasirikul, M. Tanticharoen, and Y. Thebtaranonth. 1999. Bioactive naphthoquinones from *Cordyceps unilateralis*. **Phytochemistry**. 52(3): 453-457.

Kocharin, K. and P. Wongsa. 2006. Semi-defined medium for in vitro cultivation of the fastidious insect pathogenic fungus *Cordyceps unilateralis*. **Mycopathologia** 161(4): 255-260.

Koh, J.H., K.M. Kim, J.M. Kim, J.C. Song, and H.J. Suh. 2003. Antifatigue and antistress effect of the hot-water fraction from mycelia of *Cordyceps sinensis*. **Biol. Pharm. Bull.** 26(5): 691-694.

Koh, J.H., K.W Yu, H.J. Suh, Y.M. Choi, and T.S. Ahn. 2002. Activation of macrophages and the intestinal immune system by an orally administered decoction from culture mycelia of *Cordyceps sinensis*. **Biosci. Biotechnol. Biochem.** 66(2): 407-411.

Koh, J.H., K.W. Yu, H.J. Suh, and T.S. Ahn. 2001. Pharmacological activities of the mycelial extract of cultured *Cordyceps sinensis*. **Agri. Chem. Biotechnol.** 44(2): 77-83.

Krasnoff, S.B., D.W. Watson, D.M. Gibson, and E.C. Kwan. 1995. Behavioral effects of the entomopathogenic fungus, *Entomophthora muscae* on its host *Musca domestica*: postural changes in dying hosts and gated pattern of mortality. **J. Insect Physiol.** 41: 895-903.

Krassilstchik, I.M., 1888. La production industrial des parasites végétaux pour la destruction des insects nuisibles. **Bull. Scient. Fr. Belg.** 19: 461-472.

Kredich, N.M. and A.J. Guarino. 1961. Studies on the biosynthesis of cordycepin. **Biochim. Biophys. Acta**. 47: 529-534.

Kuo, Y.C., C.Y. Lin, W.J. Tsai, C.L. Wu, C.F. Chen, and M.S. Shiao. 1994. Growth inhibitors against tumor cells in *Cordyceps sinensis* other than Cordycepin and polysaccharides. **Cancer Invest.** 12(6): 611-615.

Kuznetsov, V.D., S.N. Filippova, T.I. Orlova, and A.M. Rybakova. 1984. Regulation of the biosynthesis of secondary metabolites in *Streptomyces galbus*. **Mikrobiologia** 53: 357-363.

Kwong, T.F.N.. 2003. **A study of the distribution and molecular phylogeny of arthropod-pathogenic fungi**. Ph.D Thesis. City University of Hong Kong. 372pp.

Lallas, G.C., N. Courtis, and M. Havredaki. 2004. K562 cell sensitization to 5-Xuorouracil-or interferon-alpha-induced apoptosis via cordycepin (3'-deoxyadenosine): Wne control of cell apoptosis via poly (A) polymerase upregulation. *Int. J. Biol. Markers.* 19: 58-66.

Latgé, J.P., G. Remaudière, and M. Diaquin. 1978. Un nouveau milieu pour la croissance et la sporulation d'Entomophthorales pathogènes d'aphides. **Ann. Microbiol. (Inst. Pasteur)** 129B: 463-476.

Lee, C. and R.J. Suhadolnik. 1983. Inhibition of protein synthesis by the cordycepin analog of (2'-5')ppp (Ap)nA, (2'-5')ppp (3'dAp)n3'dA, in intact mammalian cells. **FEBS lett.** 157(1): 205-209.

Lennon, M.B. and R.J. Suhadolnik. 1976. Biosynthesis of 3'-deoxyadenosine by *Cordyceps militaris*: mechanism of reduction. **Biochim. Biophys. Acta** 425: 532–536.

Li, S.P., F.Q. Yang, and K.W.K. Tsim. 2006a. Quality control of *Cordyceps sinensis*, a valued traditional Chinese medicine. **J. Pharm. Biomed. Anal.** 41: 1571-1584.

Li, S.P., G.H. Zhang, Q. Zeng, Z.G. Huang, Y.T. Wang, T.T.X. Dong, and K.W.K. Tsim. 2006b. Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured *Cordyceps* mycelia. **Phytomedicine** 13: 428-433.

Li, S.P., P. Li, T.T.X. Dong, and K.W.K. Tsim. 2001. Anti-oxidation activity of different types of natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia, **Phytomedicine** 8: 207-212.

Lichtiger, S., D.H. Present, A. Kornbluth, I. Gelernt, J. Bauer, G. Galler, F. Michelassi, and S. Hanauer. 1994. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. **N. Engl. J. Med.** 330(26): 1841-1845.

Lin, Y.W. and B.H. Chiang. 2008. Anti-tumor activity of the fermentation broth of *Cordeceps militaris* cultured in the medium of *Radix astragali*. **Process Biochem.** 43: 244-250.

Ling, J.Y., Y.J. Sun, H. Zhang, P. Lv, and C.K. Zhang. 2002. Measurement of cordycepin and adenosine in stroma of *Cordyceps* sp. by capillary zone electrophoresis (CZE). **J. Biosci. Bioeng.** 94: 371-374.

Liu, Y.K. and W. Shen. 2003. Inhibitive effect of *Cordyceps sinensis* on experimental hepatic fibrosis and its possible mechanism. **World J. Gastroenterol.** 9: 529-533.

Lomako, J., W.M. Lomako, and W.J. Whelan. 2004. Glycogenin: the primer for mammalian and yeast glycogen synthesis. **Biochem. Biophys. Acta.** 1673: 45–55.

Luangsa-ard, J.J., N.L. Hywel-Jones, L. Manoch and R.A. Samson. 2005. On the relationships of *Paecilomyces* sect. *Isarioidea* species. **Mycol. Res.** 109 (5):581-589.

Mahr, S.. 1997. **Know Your Friends: *Beauveria bassiana***. Midwest Biological Control News Online 4(10). Available Source: http://www.entomology.wisc.edu/mbcn/kyf410.html. October 1997.

Mains, E.B.. 1958. North American Entomogenous Species of *Cordyceps*. **Mycologia** 50: 169-222.

Mao, X.B. and J.J. Zhong. 2004. Hyperproduction of cordycepin by two-stage dissolved oxygen control in submerged cultivation of medicinal mushroom *Cordyceps militaris* in bioreactors. **Biotechnol. Prog.** 20: 1408-1413.

Mao, X.B. and J.J. Zhong. 2006. Significant effect of NH4$^+$ on cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. **Enzyme Microb. Technol**.38: 343-350.

Mao, X.B., T. Eksriwong, S. Chauvatcharin, J.J. Zhong. 2005. Optimization of carbon source and carbon/nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. **Process Biochem.** 40: 1667-1672.

Marikovsky, P.I.. 1962. On some features of behavior of the ants *Formica rufa* L. infected with fungus disease. **Insect Soc.** 9: 173-79.

Masuda, M., E. Urabe, H. Honda, A. Sakurai, and M. Sakakibara. 2007. Enhanced production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. **Enzyme Microb. Tech.** 40: 1199-1205.

Masuda, M., E. Urabe, H. Honda, A. Sakurai, and M. Sakakibara. 2006. Production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris.* **Enzyme Microb. Tech.** 39: 641-646.

McCoy, C.W., R.A. Samson, and D.G. Boucias. 1988. Entomogenous fungi. *In* C.M. Ignoffo, and N.B. Mandava (eds.), **Handbook of Natural Pesticides**, Vol. V. Microbial Pesticides. Part A. Entomogenous Protozoa and Fungi. CRC Press, Inc., Boca Raton, FL. pp. 151-236.

McCoy, C.W.. 1981. Pest Control by the fungus *Hirsutella thompsonii*. *In*: H.D. Burges (ed.), **Microbial Control of Pests and Plant Diseases**, Academic Press, London, pp. 499-512.

McCoy, C.W.. 1990. Entomogenous fungi as microbial pesticides, pp. 139-159. *In* R.R. Baker, and P.E. Dunn (eds.), **New Directions in Biological Control**. Alan R. Liss, New York. pp. 139-159.

McDonald, F.E. and M.M. Gleason. 1995. Asymmetric syntheses of stavudine (d4t) and cordycepin by cycloisomerization of alkynyl alcohols to endocyclic enol ethers**. Angew. Chem., Int. Ed. Engl.** 34(3): 350-352.

McDonald, F.E., M.M. Gleason. 1996. Asymmetric synthesis of nucleosides via molybdenum-catalyzed alkynol cycloisomerization coupled with stereoselective glycosylations of deoxyfuranose glycals and 3-amidofuranose glycals. **J. Am. Chem. Soc.** 118(28): 6648-6659.

Meekes, E.T.M.. 2001. **Entomopathogenic fungi against whiteflies: tritrophic interactions between *Aschersonia* species, *Trialeurodes vaporariorum* and *Bemisia argentifolii* and glasshouse crops.** PhD thesis, Wageningen University.

Metschnikoff, E., 1879. Zur Naturgeschichte der Orthonectiden**. Zoologischer Anzeiger** 2: 547-549.

Misra, J.K. and B.W. Horn (eds.), 2001. **Trichomycetes and other fungal groups**. Science Publishers, Inc., Enfield, New Hampshire, USA. 396pp.

Mode, C.J.. 1958. A mathematical mode for the co-evolution of obligates parasites and their hosts. **Evolution**. 12: 158-165.

Mongkolsamrit, S., J.J. Luangsa-ard, J.W. Spatafora, G.H. Sung, and N.L. Hywel-Jones. 2009. A combined ITS rDNA and β-tubulin phylogeny of Thai species of *Hypocrella* with non-fragmenting ascospores. **Mycol. Res.** 113: 684-699.

Mueller, W.E.G., B.E. Weiler, R. Charubala, W. Pfleiderer, L. Leserman, R.W. Sobol, R.J. Suhadohtih, and H.C. Schroeder. 1991. Cordycepin analogues of 2'5'-oligoadenylate inhibit human immunodeficiency virus infection via inhibition of reverse transcriptase. **Biochem. Wash.** 30: 2027-2033.

Nakamura, K., N. Yoshikawa, Y. Yamaguchi, S. Kagota, K Shinozuka., and M. Kunitomo. 2006. Antitumor effect of cordycepin (3'-deoxyadenosine) on mouse melanoma and lung carcinoma cells involves adenosine A3 receptor stimulation. **Anticancer Res.** 26(1A): 43–47.

Nikoh, N. and T. Fukatsu. 2001. Evolutionary dynamics of multiple group I introns in nuclear ribosomal RNA genes of entomoparasitic fungi of the genus *Cordyceps*. **Mol. Biol. Evol.** 18: 1631-1642.

Oh, J.Y., E.J. Cho, S.H. Nam, J.W. Choi, and J.W. Yun. 2007. Production of polysaccharide-peptide complexes by submerged mycelial culture of an entomopathogenic fungus *Cordyceps sphecocephala.* **Process Biochem.** 42(3): 352-362.

Park, J.P., S.W. Kim, H.J. Hwang, and J.W. Yun. 2001. Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production by *Cordyceps militaris*. **Lett. Appl. Microbiol.** 33: 76-81.

Pendland, J.C.. 1982. Resistant structures in the entomogenous hypomycete, *Nomuraea rileyi*: an ultrastructural study. **Can. J. Bot.** 60: 1569-1576.

Penman, S. and M. Rosbach. 1970. Messenger and heterogeneous nuclear RNA in HeLa cells: differential inhibition by cordycepin. **Proc. Natl. Acad. Sci. USA** 67: 1878-l 885.

Pickles, A.. 1945. Entomology. **Adm. Rep. Dir. Agric. Trin. Tob.** pp. 17-18.

Polet, G. and S. Ling. 2004. Protecting mammal diversity: opportunities and constraints for pragmatic conservation management in Cat Tien National Park and surrounding forests in Vietnam. **Oryx** 38(2): 186-196. Cambridge University Press.

Poulin, R.. 1995. "Adaptive" changes in the behavior of parasitized animals: a critical review. **J. Parasitol.** 25: 1371-1383.

Pu, Z.L. and Z.Z. Li. 1996. **Insect Mycolgy**. Hefei: Anhui Science and Technology Press. 674pp.

Rao, Y.K., C.H. Chou, and Y.M. Tzeng. 2006. A simple and rapid method for identification and determination of cordycepin in *Cordyceps militaris* by capillary electrophoresis. **Anal. Chim. Acta** 566(2): 253-258.

Roberts, D.V.. 1973. Means for insect regulation-Fungi. **Ann. N.Y. Acad. Sci.** 217: 76-84.

Rogerson, C.T.. 1970. The hypocrealean fungi (Ascomycetes, Hypocreales). **Mycologia** 62: 865-910.

Rombach, M.C., B.M. Shepard, and F.R.S. Nelson. 1987. Compatibility of *M. anisopliae* var. *anisopliae* with chemical pesticides. **Mycopathologia** 99: 99-105.

Roy, H.E., D.C. Steinkraus, J. Eilenberg, A.E. Hajek, and J. K. Pell. 2006. Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts. **Annu. Rev. Entomol.** 51: 331-357.

Rukachaisirikul, V., S. Pramjit, C. Pakawatchai, M. Isaka, and S. Supothina. 2004. 10-membered macrolides from the insect pathogenic fungus *Cordyceps militaris* BCC 2816. **J. Nat. Prod.** 67(11): 1953-1955.

Russell, A.F., S. Greenberg, and J.G. Moffatt. 1973. Reactions of 2-acyloxyisobutyryl halides with nucleosides. II. Reactions of adenosine. **J. Am. Chem. Soc.** 13: 95(12): 4025-4030.

Russell, R. and M. Paterson. 2008. *Cordyceps* - A traditional Chinese medicine and another fungal therapeutic biofactory?. **Phytochemistry** 69: 1469-1495.

Samson, R.A., E.S. van Reenen-Hoekstra, and H.C. Evans. 1989. New species of *Torrubiella* (Ascomycotina: Clavicipitales) on insects from Ghana. **Stud. Mycol.** 31: 123-132.

Samson, R.A., H.C. Evans, and J.P. Latgé. 1988. **Atlas of Entomopathogenic Fungi**. Springer-Verlag, Berlin. Heidelberg, New York. 187pp.

Samuels, R.I., S.E. Reynolds, and A.K. Charnley. 1988. Calcium channel activation of insect muscle by destruxins, insecticidal compounds produced by the entomopathogenic fungus *Metarhizium anisopliae*. **Comp. Biochem. Physiol**. 90C: 403-412.

Sasaki, F., T. Miyamoto, Y. Tamai, and T. Yajima. 2005. Optimum temperature and pH for mycelial growth of *Cordyceps nutans* Pat. (Ascomycetes). **Int. J. Med. Mushr.** 7: 301–304.

Shah, P.A. and J.K. Pell. 2003. Entomopathogenic fungi as biological control agents. **Appl. Microbiol. Biotech.** 61: 413-423.

Shih, I.L., K.L. Tsai and C.Y. Hsieh. 2007. Effects of culture conditions on the mycelial growth and bioactive metabolite production in submerged culture of *Cordyceps militaris*. **Biochem. Eng. J.** 33: 193-201.

Shimazu, M., W. Mitsuhashi, and H. Hashimoto. 1998. *Cordyceps brongniartii* sp. nov., the teleomorph of *Beauveria brongniartii*. **Trans. Mycol. Soc. Jpn.** 29: 323-330.

Shoji, J.Y., M. Arioka, and K. Kitamoto. 2006. Possible involvement of pleiomorphic vacuolar networks in nutrient recycling in filamentous fungi. **Autophagy** 2: 226-227.

Sivichai, S., N.L. Hywel-Jones, and S. Somrithipol. 2000. Lignicolous freshwater *Ascomycota* from Thailand: *Melanochaeta* and *Sporoschisma* anamorphs. **Mycol. Res.** 104(4): 478-485.

Spatafora, J.W., G.H. Sung, J.M. Sung, N.L. Hywel-Jones, and J.F.Jr. White. 2007. Phylogenetic evidence for an animal pathogen origin for ergot and the grass endophytes. **Mol. Ecol.** 16: 1701-1711.

St. Leger, R.J., R.M. Cooper, and A.K. Charnley. 1986a. Cuticle-degrading enzymes of entomopathogenic fungi: Cuticle degradation *in vitro* by enzymes from entomopathogens**. J. Invertebr. Pathol.** 47: 167-177.

St. Leger, R.J., R.M. Cooper, and A.K. Charnley. 1986b. Cuticle degrading enzymes of entomopathogenic fungi: Synthesis in culture on cuticle. **J. Invertebr. Pathol.** 48: 85-95.

St. Leger, R.J., R.M. Cooper, and A.K. Charnley. 1986c. Cuticle degrading enzymes of entomopathogenic fungi: Regulation of production of chitinolytic enzymes. **J. Gen. Microbiol.** 132: 1509-1517.

St. Leger, R.J.. 1995. The role of cuticle-degrading proteases in fungal pathogenesis of insects. **Can. J. Bot.** 73(S1): 1119-1125.

Starzl, T.E., G.B. Klintmalm, K.A. Porter, S. Iwatsuki, and G.P. Schröter. 1981. Liver transplantation with use of cyclosporin a and prednisone. **N. Engl. J. Med.** 305(5): 266-269.

Steinhaus, E.A.. 1956. Microbial control, the emergence of an idea. **Hilgardia** 26: 107-157.

Steinhaus, E.A.. 1975. **Disease in a minor chord** (1st edit.). Ohio State University Press, Columbus, 488pp.

Steinkraus, D.C.. 1994. Whitfield, J. B. Chinese Caterpillar Fungus and World Record Runners. **Am. Entomol.** 40: 235-239.

Stensrud, Ø., N.L. Hywel-Jones, and T. Schumacher. 2005. Towards a phylogenetic classification of *Cordyceps*: ITS nrDNA sequencedata confirm divergent lineages and paraphyly. **Mycol. Res.** 109: 41-56.

Sugar, A.M. and R.P. McCaffrey. 1998. Antifungal activity of 3'-deoxyadenosine (cordycepin). **Antimicrob. Agents Chemother.** 42: 1424-1427.

Suhadolnik, R.J., G. Weinbaum, and H.P. Meloche. 1964. The biosynthesis of cordycepin. **J. Am. Chem. Soc.** 86(5): 948-949.

Sullivan, P.G., M. Thompson, and S.W. Scheff. 2000. Continuous infusion of
cyclosporin A postinjury significantly ameliorates cortical damage following
traumatic brain injury. **Exp. Neurol.** 161(2): 631-637.

Sung, G.H., J.M. Sung, N.L. Hywel-Jones, and J.W. Spatafora. 2007a. A multi-gene
phylogeny of *Clavicipitaceae (Ascomycota,* Fungi): Identification of localized
incongruence using a combinational bootstrap approach. **Mol. Phylogenet.
Evol.** 44(3): 1204-1223.

Sung, G.H., J.W. Spatafora, R. Zare, K.T. Hodge, and W. Gams. 2001. A revision of
Verticillium sect. *Prostrata.* II. Phylogenetic analyses of SSU and LSU
nuclear rDNA sequences from anamorphs and teleomorphs of the
Clavicipitaceae. **Nova Hedw.** 71: 311–328.

Sung, G.H., N.L. Hywel-Jones, J.M. Sung, J.J. Luangsa-ard, B. Shrestha, and J.W.
Spatafora. 2007b. Phylogenetic classification of *Cordyceps* and the
clavicipitaceous fungi. **Stud. Mycol.** 57: 5–59.

Sutton B.C. 1980. **The Coelomycetes, fungi imperfecti with pycnidia, acervuli and
stromata**. Commonwealth Mycological Institute, Kew, Surrey,. England.
696pp.

Taborsky, V.. 1992. Small-scale processing of microbial pestcides. **FAO Agric.
Service Bull.** 96. Food and Agriculture Organization of the United Nations
Rome.

Tanada, Y., and H.K. Kaya. 1993. **Insect Pathology**. Academic Press, Inc., San
Diego. 666pp.

Tefera, T. and K.L. Pringle. 2003. Food consumption by *Chilo partellus* (Lepidoptera:
Pyralidae) larvae infected with *Beauveria bassiana* and *Metarhizium*

anisopliae and effects of feeding natural versus artificial diets on mortality and mycosis. **J. Invertebr. Pathol.** 84: 220-225.

Thomadaki, H., A. Scorilas, C.M. Tsiapalis, and M. Havredaki. 2008b. The role of cordycepin in cancer treatment via induction or inhibition of apoptosis: implication of polyadenylation in a cell type specific manner. *Cancer Chemother. Pharmacol.* 61: 251-165.

Thomadaki, H., C.M. Tsiapalis, A. Scorilas. 2008a. The effect of the polyadenylation inhibitor cordycepin on human Molt-4 and Daudi leukaemia and lymphoma cell lines. *Cancer Chemother. Pharmacol.* 61: 703-711.

Thomadaki, H., C.M. Tsiapalis, and A. Scorilas. 2005. Polyadenylate polymerase modulations in human epithelioid cervix and breast cancer cell lines, treated with etoposide or cordycepin, follow cell cycle rather than apoptosis induction. **Biol. Chem.** 386: 471-480.

Toumanoff, C.. 1931. Action des champignons entomophytes sur les abeilles. **Annls Parasit. Hum.Comp.** 9: 464-482.

Trigg, P., W.E. Gutteridge, and J. Williamson. 1971. The effect of cordycepin on malaria parasites. **Trans. R. Sot. Trop. Med. Hyg.** 65: 514-520.

Tzean, S.S., L.S. Hsieh, and W.J. Wu. 1997. **Atlas of Entomopathogenic Fungi from Taiwan**. Council of Agriculture, Executive Yuan, Taiwan, R.O.C. 214pp.

Unagul, P., P. Wongsa, P. Kittakoop, S. Intamas, P. Srikitikulchai, and M. Tanticharoen. 2005. Production of red pigments by the insect pathogenic fungus *Cordyceps unilateralis* BCC 1869. **J. Ind. Microbiol. Biotechnol.** 32: 135-140.

Vimala Devi, P.S., Y.G. Prasad, B. Rajeswari, and B.L. Vijay. 1996. Epizootic of the entomo-fungal pathogen, *Nomuraea rileyi*, on lepidopterous pests of oilseeds. **J. Oilseeds Res.** 13: 144–148.

Watson, D.W., B.A. Mullens, and J.J. Petersen. 1993. Behavioral fever response of *Musca domestica* (Diptera: Muscidae) to infection by *Entomophthora muscae* (Zygomycetes: Entomophthorales). **J. Invertebr. Pathol.** 61: 10-16.

Weiguo, F., L. Bo, X. Yuehua, J. Kai, M. Jincheng, F. Yanhua, F. Jing, Y. Xingyong, Z. Yongjun, and P. Yan. 2005. Cloning of *Beauveria bassiana* chitinase gene *Bbchit1* and its application to improve fungal strain virulence. **Appl. Environ. Microbiol.** 71: 363-370.

Wongsa, P., K. Tasanatai, P. Watts, and N.L. Hywel-Jones. 2005. Isolation and in *vitro* cultivation of the insect pathogenic fungus *Cordyceps unilateralis*. **Micol. Res.** 109(8): 936-940.

Wu, S., M. Schalk, A. Clark, R.B. Miles, R. Coates, and J. Chappell. 2007. Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. **Nat. Biotechnol.** 24: 1441-1447.

Wu, Z.L., X.X. Wang, and W.Y. Chen. 2000. Inhibitory effect of *Cordyceps sinensis* and *Cordyceps militaris* on human glomerular mesangial cell proliferation induced by native LDL. **Cell Biochem. Function** 18: 93-97.

Xiao, J.H., D.X. Chen, J.W. Liu, Z.L. Liu, W.H. Wan, N. Fang, Y. Xiao, Y. Qi, and Z.Q. Liang. 2004. Optimization of submerged culture requirements for the production of mycelial growth and exopolysaccharide by *Cordyceps jiangxiensis* JXPJ 0109. **J. Appl. Microbiol**. 96: 1105-1116.

Xiao, J.H., D.X. Chen, W.H. Wan, X.J. Hu, Y. Qi, and Z.Q. Liang. 2006. Enhanced simultaneous production of mycelia and intracellular polysaccharide in

submerged cultivation of *Cordyceps jiangxiensis* using desirability functions. **Process Biochem.** 41: 1887-1893.

Yang, H.Y., S.F. Leu, Y.K. Wang, C.S. Wu, and B.M. Huang. 2006. *Cordyceps sinensis* mycelium induces MA-10 mouse leydig tumor cell apoptosis by activating the caspase-8 pathway and suppressing the NF-kB pathway. **Arch. Androl.** 52: 103-110.

Yoshikawa, N., K. Nakamura, Y. Yamaguchi, S. Kagota, K. Shinozuka, and M. Kunitomo. 2004. Antitumour activity of cordycepin in mice. **Clin. Exp. Pharmacol. Physiol.** 31(S2): 51-53.

Zhou, L., W. Yang, Y. Xu, Q. Zhu, Z. Ma, T. Zhu, X. Ge, and J. Gao. 1990. Short-term curative effect of cultured *Cordyceps sinensis* (Berk.) Sacc. mycelia in chronic hepatitis B. **China J. Chin. Mater. Med.** 15: 53-55.

Zhou, X.X., C.U. Meyer, P. Schmidtke, and F. Zepp. 2002. Effect of cordycepin on interleukin-10 production of human peripheral blood mononuclear cells. **Eur. J. Pharmacol.** 453: 309-317.

Zhu, J.L. and C. Liu. 1992. Modulating effects of extractum semen Persicae and cultivated *Cordyceps* hyphae on immuno-dysfunction of inpatients with posthepatitic cirrhosis. **Zhongguo Zhong Xi Yi Jie He Za Zhi** 12: 207-209.

Zhu, J.S., G.M. Halpern, and K. Jones. 1998a. The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*. Part I. **J. Altern. Complement. Med.** 4(3): 289-303.

Zhu, J.S., G.M. Halpern, and K. Jones. 1998b. The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*. Part II. **J. Altern. Complement. Med.** 4(4): 429-457.

Zimmermann, G., P. Bernard, and G. Travis. 1995. Elias Metschnikoff, Elie Metchnikoff or Ilya Ilich Mechnikov (1845-1916): A pioneer in insect pathology, the first describer of the entomopathogenic fungus *Metarhizium anisopliae* and how to translate a Russian name. **Biocon. Sci. Tech.** 5(4): 527-530.
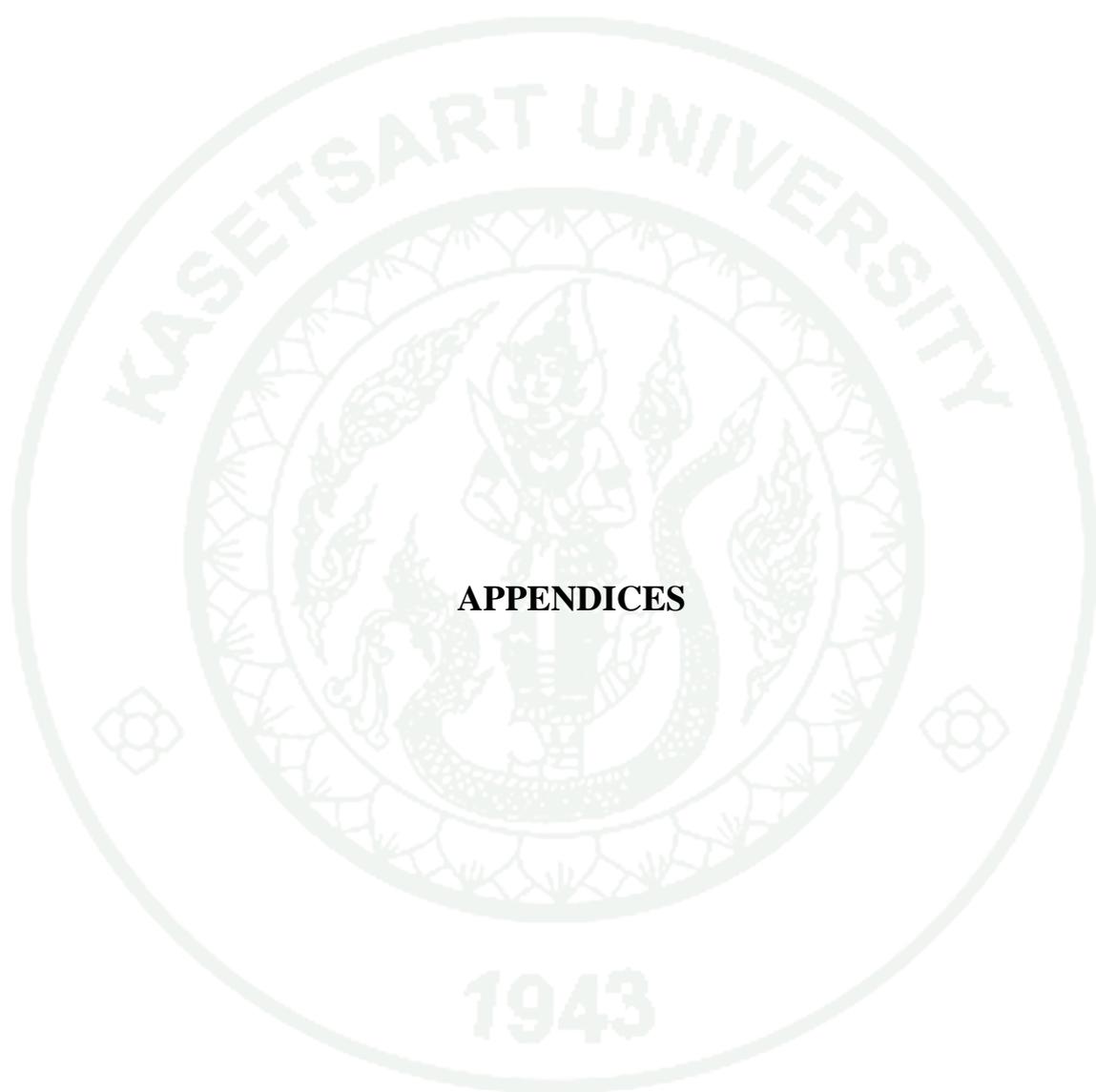
APPENDICES
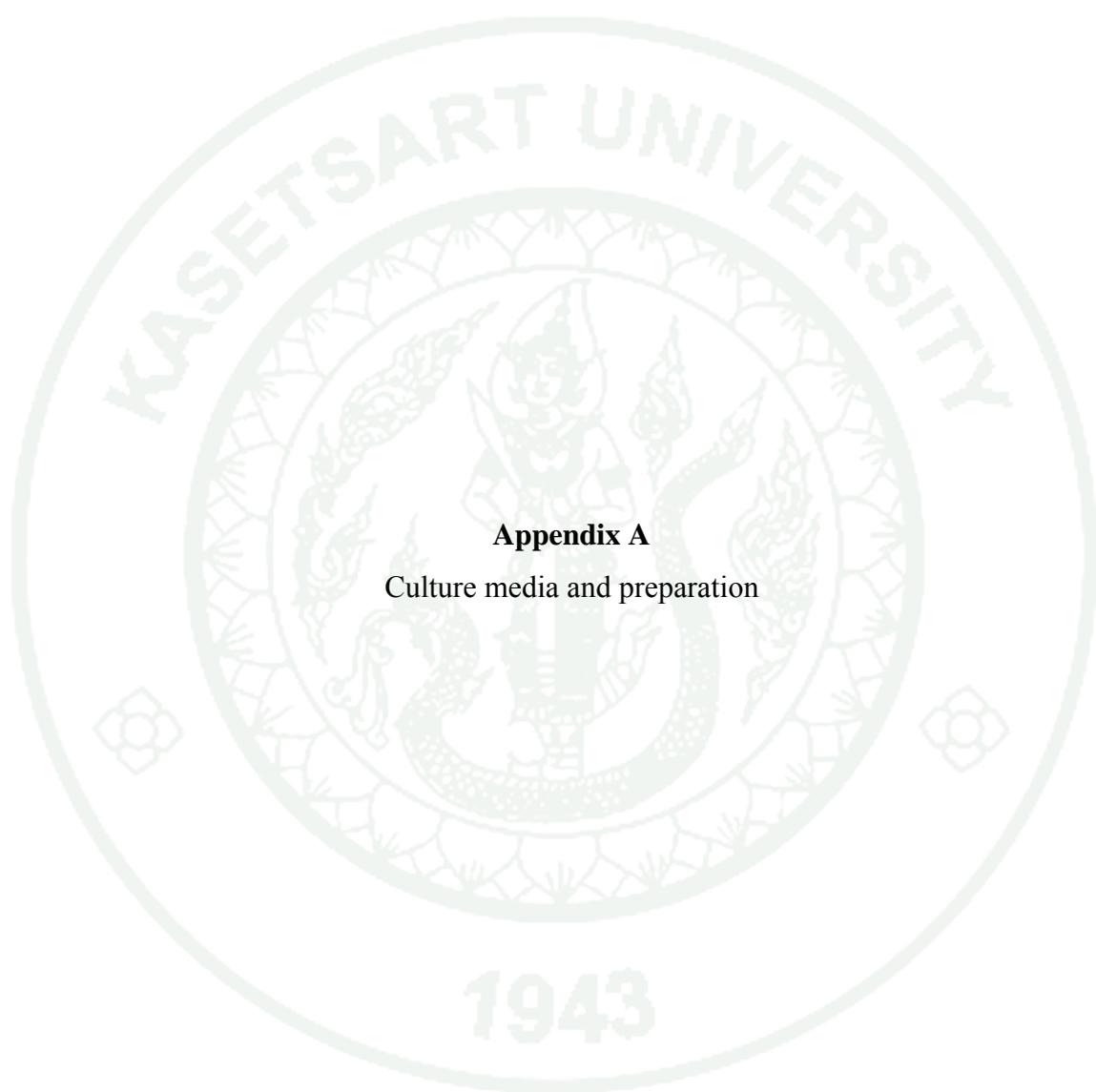
Appendix A
Culture media and preparation

1. Manual Potato Dextrose Agar (PDA)

    1.1 Composition per litre

Sliced potato	300g
Dextrose (glucose)	20g
Agar	15g

    1.2 Preparation of medium

Dice potatoes and place in 500 ml of boiling water for 30 minutes. Strain through cheesecloth. Adjust the volume to 1.0 litre with distilled water mix thoroughly. Add agar. Gently heat and bring to boiling. Add 20g glucose and mix thoroughly. Distribute to bottles and tubes. Autoclave for 15 mins at 121 $^{\circ}$C. Pour in to sterilized Petri dishes or leave in tubes.

    1.3 Use

For cultivation and maintenance of entomopathogenic fungi.

2. Potato Dextrose Agar with Antibiotics

    2.1 Composition per litre

Sliced potato	300g
Dextrose (glucose)	20g
Agar	15g
Antibiotic solution	20ml

    2.2 Antibiotic Solution: Composition per 100ml

Chlortetracycline-HCL	0.5g

Chloramphenicol   0.5g

2.3 Preparation of antibiotic solution

Add component to distilled water and bring volume to 100ml. mix thoroughly. Filter sterilize.

2.4 Preparation of medium

Dice potatoes and place in 500 ml of boiling water for 30 minutes. Strain through cheesecloth. Adjust the volume to 980 ml with distilled water mix thoroughly. Add agar. Gently heat and bring to boiling. Add 20g glucose and mix thoroughly. Distribute to bottles and tubes. Autoclave for 15 mins at 121 $^{\circ}$C. Cool to 45-50 $^{\circ}$C. Aseptically add 20ml of sterile antibiotic solution. Pour in to sterilized Petri dishes.

2.5 Use

For isolation of entomopathogenic fungi.

3. Potato Dextrose Broth (PDB)

3.1 Composition per litre

Potato starch   4g
Dextrose   20g

3.2 Preparation of medium

Suspend 24 g powder in 1.0 litre of distilled water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Distribute to flasks. Autoclave at 121°C for 15 minutes.

3.3 Use

For fermentation for cordycepin production from selected entomopathogenic fungi.

4. Peptone Glucose Medium (PG)

4.1 Composition per litre:

Glucose	40g
Peptone	15g
KH_2PO_4	0.5g
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.5g

4.2 Preparation of medium

Suspend components in 1.0 litre of distilled water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve. Distribute to flasks. Autoclave at 110°C for 15 minutes.

4.3 Use

For fermentation for cordycepin production of entomopathogenic fungi.
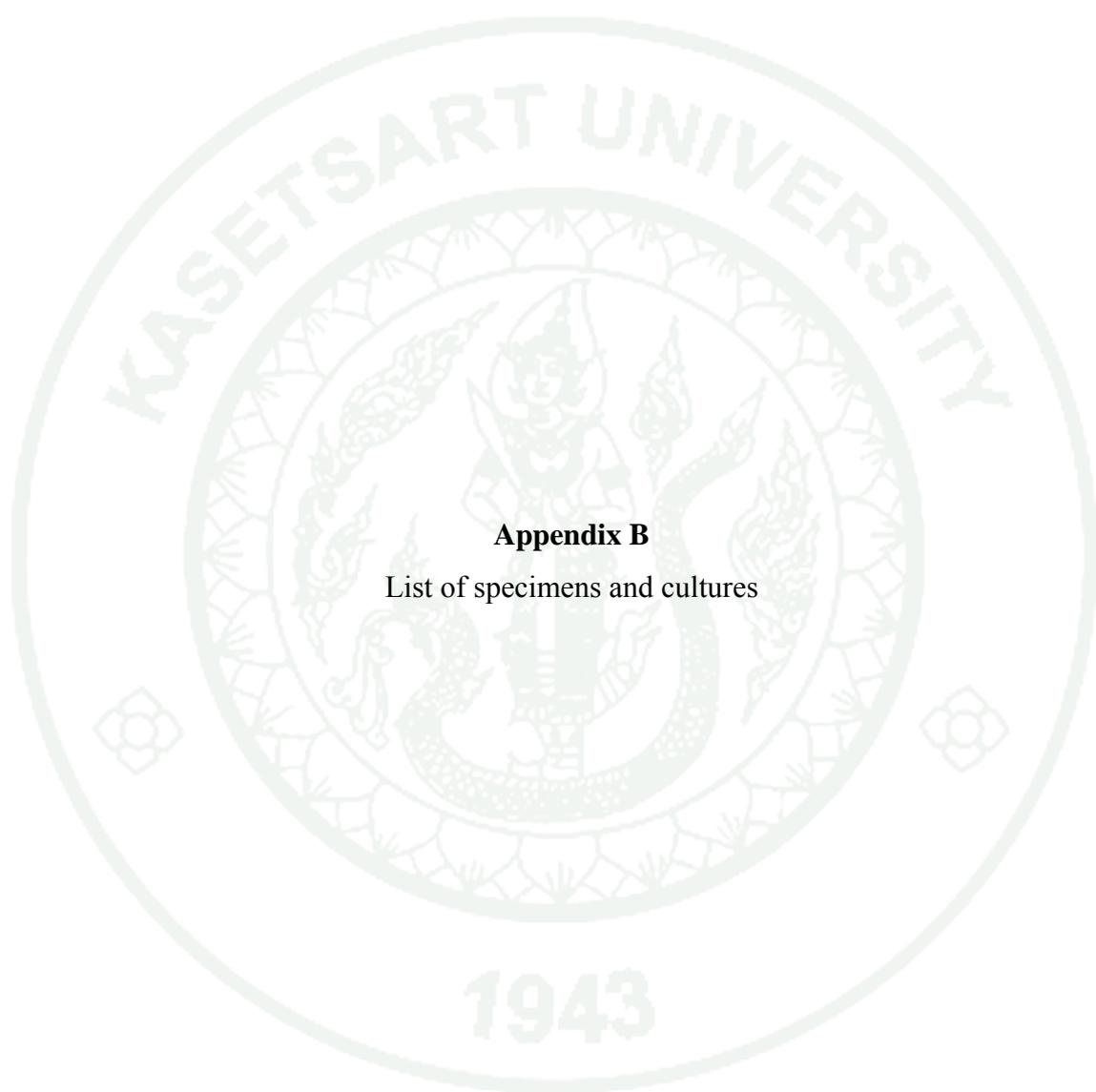
Appendix B

List of specimens and cultures

Appendix Table B1 List and details of specimens and its arthropod hosts, colleting locations, and microhabitats.

No.	Code	Genus	Species	Host	Habitat	Location
1	ITB 305	*Isaria*	*tenuipes*	Lepidoptera-pupa	leaf litter	Track behind the Headquarter
2	ITB 306	*Isaria*	*tenuipes*	Lepidoptera-pupa	leaf litter	Track behind the Headquarter
3	ITB 307	*Isaria*	*tenuipes*	Lepidoptera-pupa	leaf litter	Track behind the Headquarter
4	ITB 308	*Isaria*	*javanica*	Araneida-spider	leaf litter	Track behind the Headquarter
5	ITB 309	*Hirsutella*	*formicarum*	Hymenoptera-ant	Underside of leaf	Track behind the Headquarter
6	ITB 310	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Track behind the Headquarter
7	ITB 311	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Track behind the Headquarter
8	ITB 312	*Hirsutella*	*formicarum*	Hymenoptera-ant	Underside of leaf	Track behind the Headquarter
9	ITB 313	*Hirsutella*	*formicarum*	Hymenoptera-ant	Underside of leaf	Track behind the Headquarter
10	ITB 314	*Isaria*	*javanica*	Araneida-spider	Underside of leaf	Track behind the Headquarter
11	ITB 315	*Isaria*	sp.	Lepidoptera larva	leaf litter	Track behind the Headquarter
12	ITB 316	*Hymenostilbe*	*aurantiaca*	Hymenoptera-ant	leaf litter	Track behind the Headquarter
13	ITB 317	*Ophiocordyceps*	*irangiensis/ myrmecophila*	Hymenoptera-ant	leaf litter	Track behind the Headquarter
14	ITB 318	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Track behind the Headquarter
15	ITB 319	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Track behind the Headquarter

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
16	ITB 320	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Track behind the Headquarter
17	ITB 321	*Torrubiella*	sp.1	Araneida-spider	Underside of leaf	Track behind the Headquarter
18	ITB 322	*Verticillium*	sp.	Hemiptera-hopper	Underside of leaf	Track behind the Headquarter
19	ITB 323	*Nomuraea*	*atypicola*	Araneida-spider	leaf litter	Track behind the Headquarter
20	ITB 324	*Nomuraea*	*atypicola*	Araneida-spider	leaf litter	Track behind the Headquarter
21	ITB 325	*Cordyceps*	sp.	Mantodea-mantis egg	leaf litter	Track behind the Headquarter
22	ITB 326	*Paecilomyces*	*lilacinus*	Hemiptera-cydnidae bug	leaf litter	Track behind the Headquarter
23	ITB 327.01	*Ophiocordyceps*	cf. *brunneipunctata*	Coleoptera-elaterid larva	leaf litter	Track behind the Headquarter
24	ITB 327.02	*Hirsutella*	cf. *brunneipunctata*	Coleoptera-elaterid larva	leaf litter	Track behind the Headquarter
25	ITB 328	*Ophiocordyceps*	cf. *brunneipunctata*	Coleoptera-elaterid larva	leaf litter	Track behind the Headquarter
26	ITB 329	*Paecilomyces*	*cinnamomeus*	Homoptera-scale insect	leaf litter	Track behind the Headquarter
27	ITB 330.01	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
28	ITB 330.02	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
29	ITB 330.03	*Aschersonia*	*confluens*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
30	ITB 330.04	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
31	ITB 331.01	*Aschersonia*	*placenta*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
32	ITB 331.02	*Aschersonia*	*placenta*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
33	ITB 332.01	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
34	ITB 332.02	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
35	ITB 332.03	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
36	ITB 333.01	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
37	ITB 333.02	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
38	ITB 334.01	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
39	ITB 334.02	*Aschersonia*	*placenta*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
40	ITB 334.03	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
41	ITB 335.01	*Aschersonia*	*placenta*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
42	ITB 335.02	*Aschersonia*	*placenta*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
43	ITB 336.01	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
44	ITB 336.02	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
45	ITB 336.03	*Aschersonia*	*samoensis*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
46	ITB 336.04	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
47	ITB 337.01	*Aschersonia*	*marginata*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
48	ITB 337.02	*Aschersonia*	*marginata*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
49	ITB 338.01	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
50	ITB 338.02	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
51	ITB 338.03	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
52	ITB 339.01	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
53	ITB 339.02	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
54	ITB 340.01	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
55	ITB 340.02	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
56	ITB 341.01	*Hypocrella*	*siamensis*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
57	ITB 341.02	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
58	ITB 342.01	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
59	ITB 342.02	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
60	ITB 343	*Aschersonia*	*confluens*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
61	ITB 344	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
62	ITB 345.01	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
63	ITB 345.02	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
64	ITB 346.01	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
65	ITB 346.02	*Aschersonia*	*samoensis*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
66	ITB 347.01	*Hypocrella*	*calendulina*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
67	ITB 347.02	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
68	ITB 348	Unidentified		Hemiptera-hopper	Underside of leaf	Track behind the Headquarter
69	ITB 349	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
70	ITB 350	*Metarhizium*	sp.3	Homoptera-scale insect	Underside of leaf	Track behind the Headquarter
71	ITB 351	*Paecilomyces*	*lilacinus*	Hemiptera-Cydnidae bug	Leaf litter	Left area along the main road
72	ITB 352	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Left area along the main road
73	ITB 353	*Gibellula*	*pulchra*	Araneida-spider	Underside of leaf	Left area along the main road
74	ITB 354.01	*Hypocrella*	*calendulina*	Homoptera-scale insect	Underside of leaf	Left area along the main road
75	ITB 354.02	*Aschersonia*	*samoensis*	Homoptera-scale insect	Underside of leaf	Left area along the main road

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
76	ITB 354.03	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Left area along the main road
77	ITB 354.04	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Left area along the main road
78	ITB 355.01	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Left area along the main road
79	ITB 355.02	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Left area along the main road
80	ITB 356	*Torrubiella*	sp.1	Araneida-spider	Underside of leaf	Cay Go Bac Dong trail Trail
81	ITB 357	*Ophiocordyceps*	*irangiensis/myrmecophila*	Hymenoptera-ant	Underside of leaf	Cay Go Bac Dong trail Trail
82	ITB 358	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Left area along the main road
83	ITB 359	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Left area along the main road
84	ITB 360	*Akanthomyces*	sp.	Araneida-spider	Underside of leaf	Left area along the main road
85	ITB 361	*Gibellula*	*pulchra*	Araneida-spider	Underside of leaf	Left area along the main road
86	ITB 362	*Gibellula*	*pulchra*	Araneida-spider	Underside of leaf	Left area along the main road
87	ITB 363	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Cay Go Bac Dong trail
88	ITB 364	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Cay Go Bac Dong trail
89	ITB 365	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Cay Go Bac Dong trail
90	ITB 366	*Isaria*	sp.	Lepidoptera-larva	Underside of leaf	Cay Go Bac Dong trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
91	ITB 367	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Cay Go Bac Dong trail
92	ITB 368	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Cay Go Bac Dong trail
93	ITB 369	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Cay Go Bac Dong trail
94	ITB 370	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Cay Go Bac Dong trail
95	ITB 371	*Gibellula*	sp.2	Araneida-spider	Underside of leaf	Cay Go Bac Dong trail
96	ITB 372	*Isaria*	sp.	Unknown host	Tree twig	Cay Go Bac Dong trail
97	ITB 373	*Isaria*	*javanica*	Araneida-spider	Underside of leaf	Cay Go Bac Dong trail
98	ITB 374	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Cay Go Bac Dong trail
99	ITB 375	*Hirsutella*	*formicarum*	Hymenoptera-ant	Underside of leaf	Cay Go Bac Dong trail
100	ITB 376	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Cay Go Bac Dong trail
101	ITB 377.01	*Aschersonia*	*samoensis*	Homoptera-scale insect	Underside of leaf	Cay Go Bac Dong trail
102	ITB 377.02	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Cay Go Bac Dong trail
103	ITB 378	*Akanthomyces*	*pistellaliformis*	Lepidoptera-adult moth	Leaf litter	Bang Lang Trail
104	ITB 379.01	*Torrubiella*	sp.2	Hemiptera-hopper	Underside of leaf	Bang Lang Trail
105	ITB 379.02	*Torrubiella*	sp.2 (anamorph)	Hemiptera-hopper	Underside of leaf	Bang Lang Trail

178

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
106	ITB 380	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Bang Lang Trail
107	ITB 381	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Bang Lang Trail
108	ITB 382	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Bang Lang Trail
109	ITB 383	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Bang Lang Trail
110	ITB 384	*Isaria*	sp.	Lepidoptera-larva	Underside of leaf	Bang Lang Trail
111	ITB 385	*Isaria*	sp.	Unknown host	Underside of leaf	Bang Lang Trail
112	ITB 386	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
113	ITB 387	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
114	ITB 388	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
115	ITB 389	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
116	ITB 390	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
117	ITB 391	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
118	ITB 392	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
119	ITB 393	*Hirsutella*	*formicarum*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
120	ITB 394	*Hirsutella*	*formicarum*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
121	ITB 395	Hyperparasite		on *H. formicarum*	Underside of leaf	Bang Lang Trail
122	ITB 396	Hyperparasite		on *H. formicarum*	Leaf litter	Bang Lang Trail
123	ITB 397	Hyperparasite		on *H. formicarum*	Underside of leaf	Bang Lang Trail
124	ITB 398	Hyperparasite		on *H. formicarum*	Underside of leaf	Bang Lang Trail
125	ITB 399	*Ophiocordyceps*	*irangiensis/myrmecophila*	Hymenoptera-ant	Leaf litter	Bang Lang Trail
126	ITB 4	*Hymenostilbe*	*aurantiaca*	Hymenoptera-ant	Leaf litter	Bang Lang Trail
127	ITB 401	*Gibellula*	sp.2	Araneida-spider	Underside of leaf	Bang Lang Trail
128	ITB 402	*Metarhizium*	sp.1	Blattaria-cockroach	Underside of leaf	Bang Lang Trail
129	ITB 403	*Gibellula*	sp.2	Araneida-spider	Underside of leaf	Bang Lang Trail
130	ITB 404	*Gibellula*	*pulchra*	Araneida-spider	Underside of leaf	Bang Lang Trail
131	ITB 405	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
132	ITB 406	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
133	ITB 407	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
134	ITB 408	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
135	ITB 409	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
136	ITB 410	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
137	ITB 411	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
138	ITB 412	*Gibellula*	sp.2	Araneida-spider	Underside of leaf	Bang Lang Trail
139	ITB 413	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
140	ITB 414.01	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
141	ITB 414.02	*Aschersonia*	*placenta*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
142	ITB 414.03	*Aschersonia*	*placenta*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
143	ITB 415.01	*Aschersonia*	*samoensis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
144	ITB 415.02	*Aschersonia*	*samoensis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
145	ITB 416.01	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
146	ITB 416.02	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
147	ITB 416.03	*Aschersonia*	*placenta*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
148	ITB 417.01	*Hypocrella*	*calendulina*	Homoptera-scale insect	Underside of bamboo leaf	Bang Lang Trail
149	ITB 417.02	*Hypocrella*	*calendulina*	Homoptera-scale insect	Underside of bamboo leaf	Bang Lang Trail
150	ITB 417.03	*Hypocrella*	*calendulina*	Homoptera-scale insect	Underside of bamboo leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
151	ITB 417.04	*Aschersonia*	*samoensis*	Homoptera-scale insect	Underside of bamboo leaf	Bang Lang Trail
152	ITB 417.05	*Aschersonia*	*samoensis*	Homoptera-scale insect	Underside of bamboo leaf	Bang Lang Trail
153	ITB 418	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
154	ITB 419	*Aschersonia*	*confluens*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
155	ITB 420	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
156	ITB 421	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
157	ITB 422.01	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
158	ITB 422.02	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
159	ITB 423.01	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
160	ITB 423.02	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
161	ITB 423.03	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
162	ITB 424.01	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
163	ITB 424.02	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
164	ITB 425.01	*Hypocrella*	*calendulina*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
165	ITB 425.02	*Hypocrella*	*calendulina*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
166	ITB 426	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
167	ITB 427	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
168	ITB 428.01	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
169	ITB 428.02	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
170	ITB 429	*Aschersonia*	*confluens*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
171	ITB 430	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
172	ITB 431	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
173	ITB 432.01	*Aschersonia*	*marginata*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
174	ITB 432.02	*Aschersonia*	*marginata*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
175	ITB 432.03	*Aschersonia*	*marginata*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
176	ITB 432.04	*Aschersonia*	*marginata*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
177	ITB 432.05	*Aschersonia*	*marginata*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
178	ITB 433	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
179	ITB 434	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
180	ITB 435	*Isaria*	sp.	Lepideptera-pupa	Underside of leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
181	ITB 436	*Isaria*	sp.	Lepideptera-pupa	Underside of leaf	Bang Lang Trail
182	ITB 437	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
183	ITB 438	*Hirsutella*	*formicarum*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
184	ITB 439	Hyperparasite		on *H. formicarum*	Underside of leaf	Bang Lang Trail
185	ITB 440	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
186	ITB 441	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
187	ITB 442	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
188	ITB 443	*Hirsutella*	*saussurei*	Hymenoptera-wasp	Underside of leaf	Bang Lang Trail
189	ITB 444	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
190	ITB 445	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
191	ITB 446	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Bang Lang Trail
192	ITB 447	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
193	ITB 448	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
194	ITB 449	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
195	ITB 450	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
196	ITB 451	*Hypocrella*	*siamensis*	Homoptera-scale insect	Under and upper sides of leaf	Bang Lang Trail
197	ITB 452	*Paecilomyces*	*lilacinus*	Hemiptera-Cydnidae bug	Leaf litter	Bang Lang Trail
198	ITB 453	*Paecilomyces*	*lilacinus*	Hemiptera-Cydnidae bug	Leaf litter	Bang Lang Trail
199	ITB 454	*Paecilomyces*	*lilacinus*	Hemiptera-Cydnidae bug	Leaf litter	Bang Lang Trail
200	ITB 455	*Isaria*	*tenuipes*	Lepideptera-pupa	Leaf litter	Bang Lang Trail
201	ITB 456	*Isaria*	*tenuipes*	Lepideptera-pupa	Underside of leaf	Bang Lang Trail
202	ITB 457	*Isaria*	*tenuipes*	Lepideptera-pupa	Leaf litter	Bang Lang Trail
203	ITB 458	*Isaria*	*tenuipes*	Lepideptera-pupa	Underside of leaf	Bang Lang Trail
204	ITB 459	*Isaria*	*tenuipes*	Lepideptera-pupa	Underside of leaf	Bang Lang Trail
205	ITB 460	*Isaria*	*javanica*	Araneida-spider	Underside of leaf	Bang Lang Trail
206	ITB 461	*Isaria*	sp.	Lepideptera-larva	Underside of leaf	Bang Lang Trail
207	ITB 462	*Isaria*	sp.	Lepideptera-larva	Underside of leaf	Bang Lang Trail
208	ITB 463	*Beauveria*	sp.	Hymenoptera-ant	Leaf litter	Bang Lang Trail
209	ITB 464	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail
210	ITB 465	*Gibellula*	sp.1	Araneida-spider	Underside of leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
211	ITB 466	*Gibellula*	*pulchra*	Araneida-spider	Underside of leaf	Bang Lang Trail
212	ITB 467	*Hirsutella*	*formicarum*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
213	ITB 468	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
214	ITB 469	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
215	ITB 470	*Ophiocordyceps*	*unilateralis*	Hymenoptera-ant	Underside of leaf	Bang Lang Trail
216	ITB 471	*Verticillium*	sp.	Hemiptera-hopper	Underside of leaf	Bang Lang Trail
217	ITB 472	*Hirsutella*	sp.	Unknown host	Leaf litter	Bang Lang Trail
218	ITB 473	*Akanthomyces*	sp.	Araneida-spider	Underside of leaf	Bang Lang Trail
219	ITB 474	*Conoideocrella*	*tenuis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
220	ITB 475	*Paecilomyces*	*cinnamomeus*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
221	ITB 476	*Ophiocordyceps*	cf. *brunneipunctata*	Coleoptera-elaterid larva	Leaf litter	Bang Lang Trail
222	ITB 477	*Ophiocordyceps*	cf. *brunneipunctata*	Coleoptera-elaterid larva	Leaf litter	Bang Lang Trail
223	ITB 478	*Isaria*	sp.	Lepideptera-larva	Underside of leaf	Bang Lang Trail
224	ITB 479	*Hirsutella*	*citriformis*	Hemiptera-hopper	Underside of leaf	Behind Bangalow
225	ITB 480	*Metarhizium*	sp.2	Hemiptera-hopper	Underside of leaf	Behind Bangalow

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
226	ITB 481.01	*Hypocrella*	*siamensis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
227	ITB 481.02	*Hypocrella*	*siamensis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
228	ITB 481.03	*Hypocrella*	*siamensis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
229	ITB 481.04	*Hypocrella*	*siamensis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
230	ITB 481.05	*Hypocrella*	*siamensis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
231	ITB 481.06	*Hypocrella*	*siamensis*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
232	ITB 482	*Hypocrella*	*calendulina*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
233	ITB 483.01	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
234	ITB 483.02	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
235	ITB 483.03	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
236	ITB 484.01	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
237	ITB 484.02	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
238	ITB 484.03	*Hypocrella*	*luteola*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
239	ITB 484.04	*Aschersonia*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
240	ITB 485.01	*Aschersonia*	*confluens*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
241	ITB 485.02	*Aschersonia*	*confluens*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
242	ITB 486.01	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
243	ITB 486.02	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
244	ITB 487.01	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
245	ITB 487.02	*Aschersonia*	*badia*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
246	ITB 488	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
247	ITB 489	*Aschersonia*	*oxystoma*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
248	ITB 490.01	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
249	ITB 490.02	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
250	ITB 490.03	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
251	ITB 490.04	*Moelleriella*	*raciborskii*	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
252	ITB 490.05	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
253	ITB 490.06	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
254	ITB 490.07	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
255	ITB 491.01	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail

Appendix Table B1 (Continued).

No.	Code	Genus	Species	Host	Habitat	Location
256	ITB 491.02	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
257	ITB 491.03	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
258	ITB 491.04	*Moelleriella*	sp.	Homoptera-scale insect	Underside of leaf	Bang Lang Trail
259	ITB 492	*Isaria*	*tenuipes*	Lepidoptera-pupa	Leaf litter	Bang Lang Trail

Appendix Table B2 List of successfully isolated cultures with correlated names, BCC code, original specimen code and arthropod host.

No.	Original code	Culture code	Fungal name	Host
1	ITB 00305	BCC 27368	*Isaria tenuipes*	Lepidoptera-pupa
2	ITB 00306	BCC 27490	*Isaria tenuipes*	Lepidoptera-pupa
3	ITB 00307	BCC 27369	*Isaria tenuipes*	Lepidoptera-pupa
4	ITB 00308	BCC 27370	*Isaria javanica*	Araneida-spider
5	ITB 00314	BCC 27721	*Isaria javanica*	Araneida-spider
6	ITB 00315	BCC 27371	*Isaria* sp.	Lepidoptera larva
7	ITB 00316	BCC 27960	*Hymenostilbe aurantiaca*	Hymenoptera-ant
8	ITB 00318	BCC 27722	*Gibellula* sp.1	Araneida-spider
9	ITB 00319	BCC 27491	*Gibellula* sp.1	Araneida-spider
10	ITB 00320	BCC 27492	*Gibellula* sp.1	Araneida-spider
11	ITB 00321	BCC 27723	*Torrubiella* sp.1	Araneida-spider
12	ITB 00323	BCC 27493	*Nomuraea atypicola*	Araneida-spider
13	ITB 00324	BCC 27518	*Nomuraea atypicola*	Araneida-spider
14	ITB 00326	BCC 27372	*Paecilomyces lilacinus*	Hemiptera-cydnidae bug
15	ITB 00328	BCC 27494	*Ophiocordyceps* cf. *brunneipunctata*	Coleoptera-elaterid larva

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
16	ITB 00330.01	BCC 27519	*Moelleriella raciborskii*	Homoptera-scale insect
17	ITB 00330.02	BCC 27520	*Moelleriella raciborskii*	Homoptera-scale insect
18	ITB 00330.03	BCC 27817	*Aschersonia confluens*	Homoptera-scale insect
19	ITB 00331.01	BCC 27521	*Aschersonia placenta*	Homoptera-scale insect
20	ITB 00331.02	BCC 27522	*Aschersonia placenta*	Homoptera-scale insect
21	ITB 00332.01	BCC 27523	*Moelleriella raciborskii*	Homoptera-scale insect
22	ITB 00332.02	BCC 27524	*Hypocrella luteola*	Homoptera-scale insect
23	ITB 00332.03	BCC 27818	*Moelleriella raciborskii*	Homoptera-scale insect
24	ITB 00333.01	BCC 27525	*Moelleriella raciborskii*	Homoptera-scale insect
25	ITB 00334.01	BCC 27724	*Aschersonia badia*	Homoptera-scale insect
26	ITB 00334.02	BCC 27526	*Aschersonia placenta*	Homoptera-scale insect
27	ITB 00334.03	BCC 27527	*Aschersonia* sp.	Homoptera-scale insect
28	ITB 00335.01	BCC 27528	*Aschersonia placenta*	Homoptera-scale insect
29	ITB 00335.02	BCC 27529	*Aschersonia placenta*	Homoptera-scale insect
30	ITB 00336.01	BCC 27530	*Hypocrella luteola*	Homoptera-scale insect

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
31	ITB 00336.02	BCC 27531	*Hypocrella luteola*	Homoptera-scale insect
32	ITB 00336.03	BCC 27725	*Aschersonia samoensis*	Homoptera-scale insect
33	ITB 00336.04	BCC 27819	*Aschersonia* sp.	Homoptera-scale insect
34	ITB 00337.02	BCC 27820	*Aschersonia marginata*	Homoptera-scale insect
35	ITB 00338.02	BCC 27821	*Aschersonia* sp.	Homoptera-scale insect
36	ITB 00339.01	BCC 27726	*Aschersonia badia*	Homoptera-scale insect
37	ITB 00339.02	BCC 27727	*Aschersonia* sp.	Homoptera-scale insect
38	ITB 00340.01	BCC 27532	*Hypocrella luteola*	Homoptera-scale insect
39	ITB 00342.01	BCC 27822	*Aschersonia* sp.	Homoptera-scale insect
40	ITB 00342.02	BCC 27823	*Aschersonia* sp.	Homoptera-scale insect
41	ITB 00343	BCC 27728	*Aschersonia confluens*	Homoptera-scale insect
42	ITB 00344	BCC 27824	*Aschersonia* sp.	Homoptera-scale insect
43	ITB 00345.01	BCC 27825	*Aschersonia oxystoma*	Homoptera-scale insect
44	ITB 00345.02	BCC 27826	*Aschersonia* sp.	Homoptera-scale insect
45	ITB 00346.01	BCC 27533	*Moelleriella raciborskii*	Homoptera-scale insect

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
46	ITB 00346.02	BCC 27827	*Aschersonia samoensis*	Homoptera-scale insect
47	ITB 00351	BCC 27373	*Paecilomyces lilacinus*	Hemiptera-Cydnidae bug
48	ITB 00352	BCC 27374	*Isaria tenuipes*	Lepidoptera-pupa
49	ITB 00353	BCC 27729	*Gibellula pulchra*	Araneida-spider
50	ITB 00354.02	BCC 27730	*Aschersonia samoensis*	Homoptera-scale insect
51	ITB 00354.03	BCC 27534	*Moelleriella raciborskii*	Homoptera-scale insect
52	ITB 00355.02	BCC 27731	*Aschersonia* sp.	Homoptera-scale insect
53	ITB 00356	BCC 27495	*Torrubiella* sp.1	Araneida-spider
54	ITB 00358	BCC 27375	*Isaria tenuipes*	Lepidoptera-pupa
55	ITB 00359	BCC 27732	*Gibellula* sp.1	Araneida-spider
56	ITB 00360	BCC 27496	*Akanthomyces* sp.	Araneida-spider
57	ITB 00361	BCC 27733	*Gibellula pulchra*	Araneida-spider
58	ITB 00362	BCC 27497	*Gibellula pulchra*	Araneida-spider
59	ITB 00363	BCC 27376	*Isaria tenuipes*	Lepidoptera-pupa
60	ITB 00364	BCC 27377	*Isaria tenuipes*	Lepidoptera-pupa

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
61	ITB 00365	BCC 27378	*Isaria tenuipes*	Lepidoptera-pupa
62	ITB 00366	BCC 27379	*Isaria* sp.	Lepidoptera-larva
63	ITB 00367	BCC 27734	*Gibellula* sp.1	Araneida-spider
64	ITB 00368	BCC 27735	*Gibellula* sp.1	Araneida-spider
65	ITB 00369	BCC 27736	*Gibellula* sp.1	Araneida-spider
66	ITB 00370	BCC 27498	*Gibellula* sp.1	Araneida-spider
67	ITB 00371	BCC 27499	*Gibellula* sp.2	Araneida-spider
68	ITB 00377.01	BCC 27737	*Aschersonia samoensis*	Homoptera-scale insect
69	ITB 00379.01	BCC 27738	*Torrubiella* sp.2	Hemiptera-hopper
70	ITB 00379.02	BCC 27500	*Torrubiella* sp.2 (anamorph)	Hemiptera-hopper
71	ITB 00380	BCC 27380	*Isaria tenuipes*	Lepidoptera-pupa
72	ITB 00381	BCC 27381	*Isaria tenuipes*	Lepidoptera-pupa
73	ITB 00383	BCC 27501	*Isaria tenuipes*	Lepidoptera-pupa
74	ITB 00385	BCC 27382	*Isaria* sp.	Unknown host
75	ITB 00399	BCC 27535	*Ophiocordyceps irangiensis/myrmecophila*	Hymenoptera-ant

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
76	ITB 00401	BCC 27739	*Gibellula* sp.2	Araneida-spider
77	ITB 00402	BCC 27740	*Metarhizium* sp.1	Blattaria-cockroach
78	ITB 00404	BCC 27502	*Gibellula pulchra*	Araneida-spider
79	ITB 00405	BCC 27741	*Gibellula* sp.1	Araneida-spider
80	ITB 00408	BCC 27742	*Gibellula* sp.1	Araneida-spider
81	ITB 00409	BCC 27743	*Gibellula* sp.1	Araneida-spider
82	ITB 00410	BCC 27744	*Gibellula* sp.1	Araneida-spider
83	ITB 00411	BCC 27745	*Gibellula* sp.1	Araneida-spider
84	ITB 00412	BCC 27536	*Gibellula* sp.2	Araneida-spider
85	ITB 00414.03	BCC 27537	*Aschersonia placenta*	Homoptera-scale insect
86	ITB 00415.01	BCC 27746	*Aschersonia samoensis*	Homoptera-scale insect
87	ITB 00415.02	BCC 27961	*Aschersonia samoensis*	Homoptera-scale insect
88	ITB 00416.01	BCC 27538	*Moelleriella raciborskii*	Homoptera-scale insect
89	ITB 00416.02	BCC 27539	*Moelleriella raciborskii*	Homoptera-scale insect
90	ITB 00417.01	BCC 27540	*Hypocrella calendulina*	Homoptera-scale insect

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
91	ITB 00417.02	BCC 27541	*Hypocrella calendulina*	Homoptera-scale insect
92	ITB 00417.03	BCC 27542	*Hypocrella calendulina*	Homoptera-scale insect
93	ITB 00417.04	BCC 27747	*Aschersonia samoensis*	Homoptera-scale insect
94	ITB 00417.05	BCC 27962	*Aschersonia samoensis*	Homoptera-scale insect
95	ITB 00419	BCC 27828	*Aschersonia confluens*	Homoptera-scale insect
96	ITB 00420	BCC 27748	*Aschersonia badia*	Homoptera-scale insect
97	ITB 00421	BCC 27543	*Moelleriella raciborskii*	Homoptera-scale insect
98	ITB 00424.01	BCC 27829	*Aschersonia oxystoma*	Homoptera-scale insect
99	ITB 00425.01	BCC 27544	*Hypocrella calendulina*	Homoptera-scale insect
100	ITB 00428.01	BCC 27830	*Aschersonia badia*	Homoptera-scale insect
101	ITB 00428.02	BCC 27749	*Aschersonia badia*	Homoptera-scale insect
102	ITB 00429	BCC 27831	*Aschersonia confluens*	Homoptera-scale insect
103	ITB 00432.01	BCC 27750	*Aschersonia marginata*	Homoptera-scale insect
104	ITB 00432.02	BCC 27832	*Aschersonia marginata*	Homoptera-scale insect
105	ITB 00432.03	BCC 27751	*Aschersonia marginata*	Homoptera-scale insect

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
106	ITB 00432.04	BCC 27752	*Aschersonia marginata*	Homoptera-scale insect
107	ITB 00432.05	BCC 27753	*Aschersonia marginata*	Homoptera-scale insect
108	ITB 00441	BCC 27963	*Aschersonia* sp.	Homoptera-scale insect
109	ITB 00442	BCC 27754	*Gibellula* sp.1	Araneida-spider
110	ITB 00446	BCC 27503	*Isaria tenuipes*	Lepidoptera-pupa
111	ITB 00452	BCC 27383	*Paecilomyces lilacinus*	Hemiptera-Cydnidae bug
112	ITB 00453	BCC 27384	*Paecilomyces lilacinus*	Hemiptera-Cydnidae bug
113	ITB 00454	BCC 27385	*Paecilomyces lilacinus*	Hemiptera-Cydnidae bug
114	ITB 00455	BCC 27386	*Isaria tenuipes*	Lepideptera-pupa
115	ITB 00456	BCC 27387	*Isaria tenuipes*	Lepideptera-pupa
116	ITB 00457	BCC 27388	*Isaria tenuipes*	Lepideptera-pupa
117	ITB 00458	BCC 27545	*Isaria tenuipes*	Lepideptera-pupa
118	ITB 00459	BCC 27389	*Isaria tenuipes*	Lepideptera-pupa
119	ITB 00460	BCC 27390	*Isaria javanica*	Araneida-spider
120	ITB 00466	BCC 27755	*Gibellula pulchra*	Araneida-spider

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
121	ITB 00477	BCC 27504	*Ophiocordyceps* cf. *brunneipunctata*	Coleoptera-elaterid larva
122	ITB 00478	BCC 27546	*Isaria* sp.	Lepideptera-larva
123	ITB 00480	BCC 27756	*Metarhizium* sp.2	Hemiptera-hopper
124	ITB 00481.02	BCC 27833	*Hypocrella siamensis*	Homoptera-scale insect
125	ITB 00481.03	BCC 27834	*Hypocrella siamensis*	Homoptera-scale insect
126	ITB 00482	BCC 27835	*Hypocrella calendulina*	Homoptera-scale insect
127	ITB 00483.01	BCC 27757	*Aschersonia badia*	Homoptera-scale insect
128	ITB 00483.02	BCC 27836	*Aschersonia badia*	Homoptera-scale insect
129	ITB 00483.03	BCC 27964	*Aschersonia badia*	Homoptera-scale insect
130	ITB 00484.01	BCC 27547	*Hypocrella luteola*	Homoptera-scale insect
131	ITB 00484.02	BCC 27837	*Hypocrella luteola*	Homoptera-scale insect
132	ITB 00484.03	BCC 27548	*Hypocrella luteola*	Homoptera-scale insect
133	ITB 00484.04	BCC 27758	*Aschersonia* sp.	Homoptera-scale insect
134	ITB 00485.01	BCC 27838	*Aschersonia confluens*	Homoptera-scale insect
135	ITB 00485.02	BCC 27839	*Aschersonia confluens*	Homoptera-scale insect

Appendix Table B2 (Continued).

No.	Original code	Culture code	Fungal name	Host
136	ITB 00486.01	BCC 27759	*Aschersonia badia*	Homoptera-scale insect
137	ITB 00486.02	BCC 27760	*Aschersonia badia*	Homoptera-scale insect
138	ITB 00487.01	BCC 27761	*Aschersonia badia*	Homoptera-scale insect
139	ITB 00487.02	BCC 27762	*Aschersonia badia*	Homoptera-scale insect
140	ITB 00489	BCC 27549	*Aschersonia oxystoma*	Homoptera-scale insect
141	ITB 00490.01	BCC 27550	*Moelleriella* sp.	Homoptera-scale insect
142	ITB 00490.02	BCC 27551	*Moelleriella* sp.	Homoptera-scale insect
143	ITB 00490.03	BCC 27552	*Moelleriella* sp.	Homoptera-scale insect
144	ITB 00490.04	BCC 27553	*Moelleriella raciborskii*	Homoptera-scale insect
145	ITB 00490.06	BCC 27840	*Moelleriella* sp.	Homoptera-scale insect
146	ITB 00490.07	BCC 27841	*Moelleriella* sp.	Homoptera-scale insect
147	ITB 00491.03	BCC 27554	*Moelleriella* sp.	Homoptera-scale insect
148	ITB 00491.04	BCC 27763	*Moelleriella* sp.	Homoptera-scale insect
149	ITB 00492	BCC 27505	*Isaria tenuipes*	Lepidoptera-pupa

CURRICULUM VITAE

NAME :   : Mister Le Tan Hung

BIRTH DATE :   : April 27, 1977.

BIRTH PLACE :   : Long An Province, Vietnam

EDUCATION :   YEAR   INSTITUTION   DEGREE

                                   2001   University of Natural Science

                                              in Ho Chi Minh City   B.Sc. (Biology)

FUNDING SOURCE   : Natural Center for Genetic Engineering and Biotechnology

                         (BIOTEC), National Science and Technology Development

                         Agency (NSTDA), Thailand

                         Institute of Tropical Biology (ITB), Vietnamese Academy of

                         Science and Technology, Vietnam.