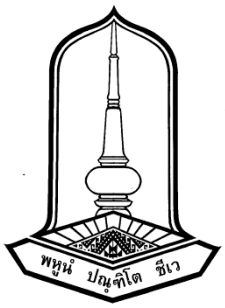

DIVERSITY OF ENTOMOPATHOGENIC FUNGI FROM THE NORTHEASTERN THAILAND AND THEIR BIOLOGICAL CONTROL OF *COLLETOTRICHUM* SPP.

PIYANOOT JAIHAN

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology at Mahasarakham University

January 2018

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The examining committee has unanimously approved this dissertation, submitted by Ms. Piyanoot Jaihan, as a partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology at Mahasarakham University.

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ABSTRACT

The aim of this study was to isolate entomopathogenic fungi from cicada nymphs from the Northeastern Thailand and identify the isolated entomopathogenic fungi using morphological characteristic and genetic studies. The potentially antagonistic isolates were tested against *Colletotrichum* spp., the causal agent of anthracnose disease. The results showed that a total of forty-four entomophathogenic fungi were isolated from cicada nymphs collected from six areas in the Northeastern Thailand, including Maha Sarakham, Roi Et, Nong Bua Lam Phu, Loei, Nakhon Phanom and Sakon Nakhon provinces. Morphological characters, consisted of stroma, perithecium, ascus, ascospore and colony characteristics on PDA, were investigated. The variation in the morphological characters among the species was recorded. A phylogenetic tree obtained from non-coding (*ITS, nrSSU, nrLSU*) and coding gene (*EF-1α* and *rpb1*) sequencing and their related species was constructed. The phylogenetic tree divided the fungal isolates into four clades: clade 1 consisted of 31 isolates of *Polycephalomyces nipponicus*; clade 2 contained four isolates of *Ophiocordyceps longissima* and one isolate of *O. sobolifera*; clade 3 contained three isolates of *Metacordyceps chlamydosporia;* and clade 4 contained five isolates of *Simplicillium obclavatum*. All of the 44 isolates of entomopathogenic fungi were primarily screened for antagonistic activity to inhibit the mycelial growth of two isolates of *Colletotrichum* spp. by the dual culture method. The screening results revealed that eight isolates of entomopathogenic fungi could inhibit the fungal mycelial growth of one isolate of *Colletotrichum capsici* and one isolate of *Colletotrichum* spp. in the range of 16.67 - 54.55%. These potential isolates were chosen for further

confirmation of their antagonistic effects against five isolates of *C. capsici* and five isolates of *Colletotrichum* spp. by the dual culture method. The results revealed that *O. sobolifera* isolate Cod-NB1302 had the best inhibitory effect on all the isolates of *Colletotrichum* spp. with 25.02- 43.55%. Moreover, the mycelial extract and culture filtrate of *O. sobolifera* isolate Cod-NB1302 were then used to determine the inhibitory effects on the mycelial growth and conidial germination of all isolates of plant pathogenic *Colletotrichum* spp. under *in vitro* conditions. The results indicated that the mycelial extract and culture filtrate of *O. sobolifera* isolate Cod-NB1302 could inhibit the mycelial growth of all 10 isolates of *Colletotrichum* spp. and also inhibited the conidial germination. In addition, abnormal spore shapes and short germ tubes were observed when compared with the control treatment. Moreover, the mycelial extract and culture filtrate had effectively reduced the size of the disease lesions and disease severity on chili fruits after inoculation with the plant pathogenic fungi. Therefore, these results suggest that *O. sobolifera* isolate Cod-NB1302 is a potential candidate, with antagonistic activity, for use as a source of antifungal agents to control anthracnose disease caused by plant pathogenic *Colletotrichum* spp.

Keywords: Antagonistic activity, Anthracnose disease, Entomopathogenic fungi

ชื่อเรื่อง ความหลากหลายของเชื้อราแมลงในภาคตะวันออกเฉียงเหนือของประเทศไทย และการควบคุมเชื้อรา *Colletotrichum* spp.
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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อแยกเชื้อราแมลงจากตัวอ่อนจักจั่นที่พบในภาคตะวันออกเฉียงเหนือของประเทศไทย และระบุชนิดของเชื้อแมลงที่แยกได้โดยใช้ลักษณะสัณฐานวิทยาและอณูพันธุศาสตร์ จากนั้นนำเชื้อราแมลงมาทดสอบการเป็นปฏิปักษ์ต่อเชื้อรา *Colletotrichum* spp. สาเหตุโรคแอนแทรกโนสในพริก จากการศึกษาพบว่า แยกเชื้อราแมลงจากตัวอ่อนจักจั่นได้ทั้งหมด จำนวน 44 ไอโซเลต ใน 6 จังหวัดภาคตะวันออกเฉียงเหนือของประเทศไทย ได้แก่ จังหวัดมหาสารคาม ร้อยเอ็ด หนองบัวลำภู เลย นครพนม และสกลนคร จากนั้นศึกษาลักษณะสัณฐานวิทยาประกอบด้วย สโตรมา เพอริทีเซีย แอสคัส แอสโคสปอร์ และลักษณะโคโลนีที่เจริญบนอาหาร PDA โดยพบว่าลักษณะสัณฐานวิทยามีความแปรผันขึ้นอยู่กับสปีชีส์ของเชื้อราแมลง จากนั้นสร้างสายสัมพันธ์ทางวิวัฒนาการจากลำดับนิวคลีโอไทด์ในส่วนของยีน non-coding (*ITS*, *nrSSU, nrLSU*) และ coding (*EF-1α* และ *rpb1*) และสร้างสายสัมพันธ์กับเชื้อราแมลงชนิดอื่นๆ จากการศึกษาพบว่าเชื้อราแมลงแบ่งออกเป็น 4 กลุ่ม ตามสายวิวัฒนาการ ได้แก่ กลุ่มที่ 1 *Polycephalomyces nipponicus* จำนวน 31 ไอโซเลต กลุ่มที่ 2 ประกอบด้วย *Ophiocordyceps longissima* จำนวน 4 ไอโซเลต และ *O. sobolifera* จำนวน 1 ไอโซเลต กลุ่มที่ 3 *Metacordyceps chlamydosporia* จำนวน 3 ไอโซเลต และกลุ่มที่ 4 *Simplicillium obclavatum* จำนวน 5 ไอโซเลต ตามลำดับ จากนั้นนำเชื้อราแมลงที่แยกได้ทั้งหมด 44 ไอโซเลต มาคัดเลือกเบื้องต้นในการยับยั้งการเจริญของเส้นใยเชื้อรา *Colletotrichum* spp. จำนวน 2 ไอโซเลต ด้วยวิธีเลี้ยงเชื้อร่วมกัน (dual culture method) พบว่า มีเชื้อราแมลงทั้งหมด 8 ไอโซเลต ที่สามารถยับยั้งการเจริญของเส้นใยของเชื้อรา *Colletotrichum capsici* จำนวน 1 ไอโซเลต และ *Colletotrichum* spp. จำนวน 1 ไอโซเลต อยู่ในช่วง 16.67 - 54.55 เปอร์เซ็นต์ จากนั้นทำการยืนยันผลของเชื้อราแมลงไอโซเลตที่มีศักยภาพในการยับยั้งการเจริญของเส้นใยเชื้อรา *C. capsici* จำนวน 5 ไอโซเลต และ *Colletotrichum* spp. จำนวน 5 ไอโซเลต ด้วยวิธีเลี้ยงเชื้อร่วมกัน ผลการศึกษาพบว่า เชื้อรา *O. sobolifera* ไอโซเลต Cod-NB1302 มีประสิทธิภาพในการยับยั้งการเจริญของเส้นใยของเชื้อรา *Colletotrichum* spp. ทุกไอโซเลตได้ดีที่สุด โดยมีเปอร์เซ็นต์การยับยั้งอยู่ในช่วง 25.02- 43.55 จากนั้นนำสารสกัดจากเส้นใย และน้ำเลี้ยงของเชื้อรา *O. sobolifera* ไอโซเลต Cod-NB1302 มาทดสอบการยับยั้งการเจริญของเส้นใย และการยับยั้งการงอกสปอร์ของเชื้อรา *Colletotrichum* spp. ทุกไอโซเลตในสภาวะหลอดทดลอง (*in vitro*) ผลการศึกษาพบว่า สารสกัดจากเส้นใยและน้ำเลี้ยงของเชื้อรา *O. sobolifera* ไอโซเลต Cod-NB1302 สามารถยับยั้งการเจริญของเส้นใยและยับยั้งการงอกของสปอร์ราของเชื้อรา *Colletotrichum* spp. ได้

ทั้ง 10 ไอโซเลต โดยพบความผิดปกติของเส้นใยที่งอกจากสปอร์ และ germ tube สั้นเมื่อเทียบกับชุดควบคุม นอกจากนี้สารสกัดจากเส้นใย และน้ำเลี้ยงยังมีประสิทธิภาพในการลดขนาดแผล และลดระดับความรุนแรงในการเกิดอาการของโรคแอนแทรกโนสบนผลพริกได้ ดังนั้นจากผลการทดลองแสดงให้เห็นว่าเชื้อรา *O. sobolifera* ไอโซเลต Cod-NB1302 มีศักยภาพสามารถนำไปใช้เป็นเชื้อปฏิปักษ์ และใช้เป็นแหล่งของสารต้านเชื้อราในการควบคุมเชื้อรา *Colletotrichum* spp. สาเหตุโรคแอนแทรกโนสในพริกได้

คำสำคัญ: เชื้อปฏิปักษ์ โรคแอนแทรกโนส เชื้อราแมลง

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List of Abbreviations

bp	base pairs
°C	degree Celsius
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EF-1α	elongation factor 1α
et al.	et alibi
ITS	Internal transcribed spacer
m	meter
mg	miligram
mL	milliliter
min	minute
mM	millimolar
mm	millimeter
nrSSU	nuclear ribosomal small subunits
nrLSU	nuclear ribosomal large subunits
PCR	polymerase chain reaction
PDB	potato dextrose broth
PDA	potato dextrose agar
rpb1	largest subunit of RNA polymerase II
rpb2	second largest subunit of RNA polymerase II
TBE	Tris base, boric acid and EDTA
UV	Ultraviolet
µm	micrometer/ micron
µg	microgram
µl	micro liter

CHAPTER 1

INTRODUCTION

1.1 Background

Cordyceps spp. are insect pathogenic fungi in the family Clavicipitaceae, order Hypocreales that specifically infect the immature stages of insects (Spatafora and Blackwell, 1993). *Cordyceps* species are the most diverse in the family Clavicipitaceae, and more than 400 species have been reported (Sung *et al.,* 2007a). The fungal *Cordyceps* have been found at high altitudes, hot and humid climates, such as *Cordyceps sinensis* found in the cold, grassy, alpine meadows on the Himalayan Mountains, 3,800 meters above sea level, in Nepal and China (Holliday and Cleaver, 2008). *C. cardinalis* has been found in the southern Appalachian Mountains of the eastern United States and southeastern Japan (Sung and Spatafora, 2004). Shrestha and Sung (2005) reported that eight *Cordyceps* species, including *C. gracilis, C. ishikariensis, C. liangshanensis, C. martialis, C. militaris, C. pruinosa, C. sphecocephala* and *C. tricentri,* were found and collected from the Central Region of Nepal. Moreover, a new species of *C. cuncunae* has been found on large caterpillars of an unidentified ghost moth species in temperate rainforest in the Valdivian Lake Region in southern Chile (Palfner *et al*., 2011). In Thailand, fungal *Cordyceps* are most common and widely distributed in natural forests of the north, northeast and southern regions (Kobayasi, 1941; Kobayasi, 1982; Hywel-Jones, 2001; Aung *et al*., 2008; Luangsa-ard *et al*., 2011; Srivilai *et al*., 2013).

The fungi in the genus *Cordyceps* have been known as important ingredients in Chinese medicine for thousands of years (Zhu *et al*., 1998a). They can produce biological components with excellent value in medical treatment, such as for kidney, lung and heart disease, hyperglycemia, respiratory disease, male and female sexual dysfunction, to relieve pain and restore general health to promote longevity (Holliday and Cleaver, 2008). Moreover, the biological compounds were also of interest in the pharmacological area, immune regulation, immune inhibition in organ transplant, anti-tumor, antibiotic, antibacterial and antioxidant activities (Kuo *et al.,* 2005). Several

potent bioactive compounds and new chemicals have been isolated from *Cordyceps* species. New bioactive compounds, such as cordypyridones A-D that have antimalarial activity, were isolated from *C. nipponica*. Cordyanhydride that has antimalarial and antituberculosis activities was found from *C. psuedomilitaris* (Isaka *et al*., 2000; Isaka and Tanticharoen, 2001; Isaka *et al*., 2005).

Recently, some *Cordyceps* species with inhibitory effects on the growth of microorganisms, including bacteria, fungi and viruses, have been used as biocontrol agents. Imtiaj and Lee (2007) reported that *C. sobolifera* produced bioactive compounds to inhibit the growth of human pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) under *in vitro* conditions. Moreover, this insect fungus also inhibits the growth of some plant pathogenic fungi (*Botrytis cinerea*, *Colletotrichum gloeosporioides* and *C. miyabeanus*). Wong *et al*. (2011) reported that the antimicrobial peptide 'cordymin' isolated from *C. militaris* could inhibit the mycelial growth of *Bipolaris maydis*, *Mycosphaerella arachidicola*, *Rhizoctonia solani* and *Candida albicans*. Moreover, the active compound cordymin also displayed anti-proliferative activity toward breast cancer cells (MCF-7) under *in vitro* condition. Varughese *et al*. (2012) successfully used the *C. dipterigena* to inhibit the mycelial growth of the plant pathogenic fungus *Gibberella fujikuroi*. Kumar and Aparna (2014) reported that *Cordyceps* species were found to be a naturally occurring potential biocontrol agent of coconut root grub (*Leucopholis coneophora*). In addition, Mar and Lumyong (2012) reported that entomopathogenic fungi were used against fruit fly pupa, *Bactrocera* spp. Based on the literature, it indicates that the insect pathogenic fungi *Cordyceps* are a potential candidate with broad antagonistic activity that could be used as a biocontrol agent.

Given the novel potential strains of *Cordyceps* have been isolated, it is important that researchers reliably identify the species they are analyzing. Molecular techniques are some of the tools that have been used for characterization and identification of many organisms (Ban *et al*., 2009; Tian *et al*., 2010). The DNA sequencing of the internal transcribed spacer (*ITS*), the nuclear ribosomal small subunits (*nrSSU*), the nuclear ribosomal large subunits (*nrLSU*), the elongation factor 1α (*EF-1α*) and the largest subunit of RNA polymerase II (*rpb1*) genes have successfully been used to analyze the phylogenetic relationships of many fungi, including *Cordyceps*. Nikoh and Fukatsu

(2000) has successfully used the nuclear and mitochondrial rDNA sequences, including nuclear *SSU* rDNA, nuclear *LSU* rDNA and mitochondrial *SSU* rDNA, for investigation of the phylogenetic relationships among insect pathogenic fungi in the genus *Cordyceps*. Sung *et al*. (2007b) used five to seven gene loci, including *nrSSU*, *nrLSU*, *EF-1α* , *rpb1* and second largest subunit of RNA polymerase II (*rpb2*), to generate the phylogenetic relationships of *Cordyceps* and Claviciptaceous fungi. Ban *et al*. (2009) reported that two regions of rDNA involving the *ITS* region and large subunit (*LSU*) D1/D2 region of rDNA could be used to analyze the phylogenetic relationships of *C. cuboidea*. Chan *et al*. (2011) reported that the DNA sequences from three genes, including *nrLSU*, *EF-1α* and *rpb*1, could be used to classify *C. gunnii*. Moreover, the nucleotide sequences of four genes (*nrSSU*, *nrLSU*, *EF-1α* and *rpb1)* have also been used to classify insect pathogenic fungi as *Ophiocordyceps longissima* isolate Cod-MK1 (Sangdee and Sangdee, 2013).

Therefore, this research aims to isolate insect pathogenic fungi from cicada nymphs from the Northeast of Thailand and to investigate their potential to inhibit the growth of plant pathogenic fungi, *Colletotrichum* spp., the causal agent of chili anthracnose disease, under *in vitro* conditions. The potential isolate was identified by non-coding and coding gene sequences and its phylogenetic relationship was also investigated.

1.2 Objectives of the research

The objectives of the present study are:

1) To study the insect pathogenic fungi from cicada nymphs in the Northeastern Thailand.

2) To identify the isolated insect pathogenic fungi using the DNA sequences of non-coding and coding genes.

3) To investigate the phylogenetic relationship of the isolated insect pathogenic fungi and their related species.

4) To investigate the potential antagonistic activity of the isolated insect pathogenic fungi against *Colletotrichum* spp.

1.3 Scope of the research

In this study, the entomopathogenic fungi should be isolated from the cicada nymph that were collected from mixed deciduous forest in Maha Sarakham Province, Roi Et Province, Nong Bua Lam Phu Province, Nakhon Phanom Province, Sakon Nakhon Province and Loei Province. The entomopathogenic fungi should be initially identified based on morphological characteristics. After that, all isolates of the entomopathogenic fungi should be identified using internal transcribed spacer (*ITS*) gene, small subunit (*nrSSU*) rDNA gene, large subunit (*nrLSU*) rDNA gene, the elongation factor 1α (*EF-1α*) gene and the largest subunit of RNA polymerase II (*rpb1*) gene. The phylogenetic relationship of the entomopathogenic fungi isolates and their related species should be analyzed using molecular genetic tool programs. Next, all of the isolates of the entomopathogenic fungi should be primarily screened for antagonistic activity to inhibit the mycelial growth of *Colletotrichum* spp. by the dual culture technique. Then potential isolates were chosen for further confirmation of their antagonistic effects against 10 isolates of *Colletotrichum* spp. by the dual culture method. Then, the mycelium extract and culture filtrate of potential isolates should be tested on the mycelial growth and conidial germination of all isolates of the plant pathogenic *Colletotrichum* spp. under *in vitro* conditions. Finally, the potential isolates should be tested against the plant pathogenic *Colletotrichum* spp. using the detached fruit bioassay method.

CHAPTER 2

LITERATURE REVIEW

2.1 Classification of the genus *Cordyceps* spp.

Cordyceps spp. are insect pathogenic fungi in the family Cordycipitaceae of the order Hypocreales (Spatafora and Blackwell, 1993). The morphological characters, including the perithecia and asci, ascospore and part-spore, have been used for classification of the entomopathogenic fungi in this family (Mains, 1958; Kobayasi, 1941; 1982). In addition, the insect host has also been used for fungal classification (Massee, 1895; Kobayasi, 1982). For example, Sung *et al*. (2007a) used the morphological characters, such as perithecia, ascospore fragmentation, etc., for classification of *Cordyceps.* Recently, molecular tools have successfully been used to classify the entomopathogenic fungi into three families: Clavicipitaceae, Ophiocordycipitaceae and Cordycipitaceae. The taxonomic levels were described as below (Sung *et al*., 2007a; Spatafora *et al*., 2007):

Kingdom Fungi
Phylum Ascomycota
Class Ascomycetes
Order Hypocreales
Family Clavicipitaceae
Genus Metacordyceps
Family Ophiocordycipitaceae
Genus Ophiocordyceps,
Elaphocordyceps
Family Cordycipitaceae
Genus Cordyceps
Species more than 400 species

(Sung *et al*., 2007a)

2.2 General characteristics of *Cordyceps* spp.

Cordyceps spp. is a group of insect pathogenic fungi. These fungi could attack different hosts from 12 orders: Arthropoda (Aranae, Acari, Blattaria, Coleoptera), Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Odonota, Orthoptera and Phasmida (Spatafora *et al*., 2007). The name "*Cordyceps*" comes from the Latin words *cord* and *ceps*, meaning "club" and "head", respectively. The Latin conjugation accurately describes the appearance of the club fungus (Holliday and Cleaver, 2008). The fungi in the genus *Cordyceps* have teleomorph (sexual) and anamorph (asexual) reproduction stages. For example, life cycle of *Cordyceps militaris* (Cordycipitaceae) and its anamorph (*Lecanicillium*) (Fernando *et al*., 2012) are shown in Figure 2.1. In the sexual reproduction stage, they can produce sexual spores called ascospores in the ascus within the perithecia (fruiting body). In addition, the ascospores can discharge from the asci and then contact and infect to new insect host. Whereas, in the asexual stage, the asexual spores or conidia are produced on the conidiophore without a fruiting body (Dennis, 1978; Arora, 1986 and Hanlin, 1990). The general characteristics of the fungal part were described as below:

2.2.1 Stromata are several shapes, i.e., cylindric or capitate. Generally a cylindrical stem is straight or curved, sometimes with branching. The color are different from white to yellow, orange, red to brown or black (Noramly and Homathevi, 2010). The size of the stroma is different based on species and host.

2.2.2 Perithecia are arranged embedded in the stroma or semi-immersed, the perithecia are ovoid, ellipsoid and oval shaped. The size of the perithecia are different based on the host.

2.2.3 Ascus is typically sac-like cell and similar to a rod or barrel length, colorless wall with single layer. One ascus contains eight ascospores. The tip of the ascus has an apical pore for releasing the mature ascospores.

2.2.4 Ascospores are smooth skinned and often with multi-septate, the length of the ascospore is close to the length of the asci. Most spores are broken into short pieces on a single cell called a part-spore. The part-spores have different shapes such as cylinder short, cylindric, barrel-shaped or fusoid (Hywel-Jones, 1995c; 1996).

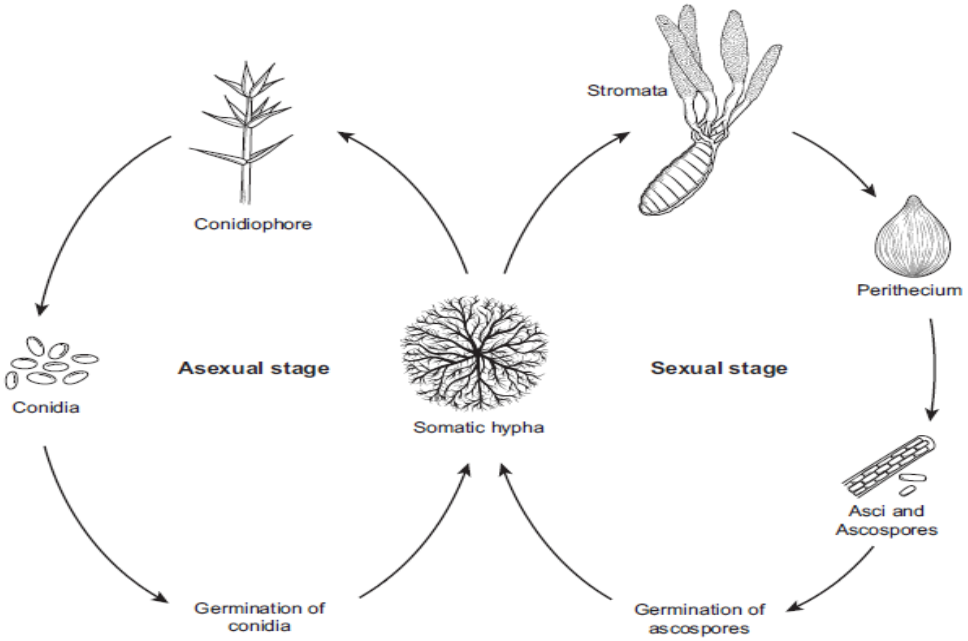

Figure 2.1 Life cycle of *Cordyceps militaris* that contains two reproductive states, anamorph (asexual state) and teleomorph (sexual state) (Fernando *et al*., 2012)

2.3 The importance of *Cordyceps* spp.

2.3.1 Medical and pharmaceutical area

Cordyceps is an important genus of insect pathogenic fungi that have been used in traditional Chinese medicine for more than one thousand years, such as *Cordyceps sinensis*. These fungi can produce several elements that have biological activity. The substances from the fungus *Cordyceps* have the biological activities such as anticancer and antioxidants (Zhang *et al*., 2005; Lin and Chiang, 2008; Zhang *et al*., 2008; Jiang and Gao, 1995). Moreover, the promotion of kidney, lung and immune systems have also been reported (Shin *et al*., 2010; Sheng *et al*., 2011; Lee and Hong, 2011). The importance *Cordyceps* species that can produce bioactive compounds is described below and in Table 2.1.

Cordyceps cicadae can produce two biological active compounds, galactomannans CI-P and CI-A, that have antitumor activity against sarcoma 180 in mouse (Kiho *et al*., 1990) and ergosterol peroxide that has been recognized in various biological activities, such as immunosuppressive, anti-viral, anti-inflammatory, anti-tumor activities and treating kidney disease (Fujimoto *et al*., 1994; Kreisel *et al*., 1990;

Yasukawa *et al*., 1996; Bok *et al*., 1999; Zhu *et al*., 2014). Wang *et al*. (2014) reported several bioactive compounds from *C. cicadae* including cyclodepsipeptide, cordycecin A, beauvericin E, beauvericin J, beauvericin and beauvericin A. Among which the compounds beauvericin E and beauvericin A exhibited a significant inhibitory effect on HepG2 and HepG2/ADM cells; whereas, beauvericin J showed cytotoxic activity against a multiple drug resistant HepG2 cell line (HepG2/ADM).

Cordyceps militaris can produce many bioactive compounds, such as cordycepin (3'-deoxyadenosine), polysaccharides, amino acids, ergosterol and mannitol and acts with anti-tumor, anti-fungal, antivirus, immune-modulatory, anti-tumor, anti-inflammatory, anti- oxidation, anti-proliferative, anti-metastatic, insecticidal and anti-bacterial activity (Liu *et al*.,1997; Mizuno, 1999; Song *et al*., 1998; Rukachaisirikul *et al*, 2004; Yoo *et al*, 2004; Won and Park, 2005; Nag and Wang, 2005; Chen *et al*, 2013; Fan and Lin, 2013). Moreover, Kim *et al*. (2014) reported that two new compounds consist of cordyrroles A and B can significantly inhibit adipocyte differentiation and pancreatic lipase activity.

Cordyceps ophioglossoides can produce a bioactive compound, alkali-soluble polysaccharide, that has an inhibitory effect on the growth of Sarcoma 180 solid-type tumor, solid Ehrlich carcinoma and solid tumor (MM46 mammary carcinoma) (Yamada *et al*., 1984; Ohmort *et al*., 1989). Kneifel *et al*. (1977) reported that *C. ophioglossoides* strain TU 276 can produce ophiocordin ($C_{21}H_{22}N_2O_8$), which is an antibiotic and antifungal agent.

Cordyceps pseudomilitaris can produce a bioxanthracenes compound group that has anti-malarial activity and cytotoxicity (Isaka *et al*., 2001; Jaturapat *et al*., 2001). Moreover, the chemical substances called "anhydride groups" consist of cordyanhydride A and B have been isolated, however the bioactivity of these compounds has not yet been studied (Isaka *et al*., 2000).

Cordyceps sp. can produce bioactive compounds, such as cordytropolone, bioxanthracenes, cordyol A-C and cordyheptapeptide A and B that act as antimararial agents and is found to exhibit cytotoxicity against two cell- lines (KB,BC-1) and Vero cells, anti- HSV-1 activity, antimycobacterial and cytotoxic activity against human breast cancer (BC) and human small cell lung cancer (NCI-H187) cancer cell lines (Seephonkai *et al*., 2001; Isaka *et al*., 2005; Rukachaisirikul *et al*., 2006; Isaka *et al*.,

2007; Bunyapaiboonsri *et al*., 2007). Isaka *et al*. (2013) reported that *Cordyceps* sp. BCC 12671 can produce "cordylactum" from a spider pathogenic fungus. Moreover, Grudniewska *et al*. (2014) reported "opaliferin" that can exhibit weak cytotoxicity against three tumor cell lines: HSC-2, HeLa, and RERF-LC-KJ.

Cordyceps unilateralis has been reported to produce six bioactive naphthoquinones consisting of erythrostominone, deoxyerythrostominone, 4-O-methyl erythrostominone, epierythrostominol, deoxyerythrostominol and 3,5,8-trihydroxy-6-methoxy-2-(5-oxohexa-1,3-dienyl)-1,4-naphthoquinone that act as antibacterial, antimalarial and cytotoxicity against human breast cancer (BC), human epidermoid carcinoma in the mouth (KB) and Vero cell lines (Kittakoop *et al*., 1999; Unagul *et al*., 2005; Wongsa *et al*., 2005).

Ophiocordyceps sinensis (=*Cordyceps sinensis*) can produce many biological components, such as cordycepin, cordycepic acid, adenosine, polysaccharides, ergosterol, nucleosides and peptides, which have anti-inflammatory, antioxidant, anti-tumor, anti-metastatic, immunomodulatory, antimicrobial, insecticidal, hypolipidaemic, hypoglycemic, antiageing, lipolytic, neuroprotective and renoprotective effects (Yoshida *et al*., 1989; Shin *et al*., 2003; Li *et al*., 2006a; Yang *et al*., 2006; Wu *et al*., 2007; Wang *et al*., 2009b; Shrestha *et al*., 2014). Holliday and Cleaver (2008) reported several pharmacological activities, such as improvement of physical performance, circulatory functions, hepatoprotection, atherosclerosis, hyperglycemia, respiratory disease, male and female sexual dysfunction, anti-tumor and anti-metastatic activities. In addition, Jia *et al*. (2009) reported that two new aurantiamides, named as cordyceamides A and B, were isolated from the liquid culture of *C. sinensis* that have anti-cancer activities.

Ophiocordyceps sobolifera (=*Cordyceps sobolifera*) can produce cordysobin that can exhibit significant HIV-1 reverse transcriptase inhibitory activity (Wang *et al*., 2012). Chiu *et al*. (2014) reported that a polysaccharide from *C. sobolifera* (CS-P) could protect against LPS-triggered inflammatory responses and renal injury in rats. In addition, Yang and Zhang (2016) observed that *C. sobolifera* can produce extracellular polysaccharide (Se-CEPS), which improved the anti-tumor activity in a mice model.

Polyephalomyces nipponicus (=*Cordyceps nipponica*) can produced Cordytropolones A-D, which possess antimalarial activity (*Plasmodium falciparum* K1)

(Isaka *et al*., 2001). Moreover, Sangdee *et al*. (2015) reported bioactive compounds from this entomopathogenic fungus, such as adenosine, flavonoid and phenolic, which exhibited antibacterial activity and anti-cancer activities (Sangdee *et al*., 2016).

Table 2.1 The lists bioactive compounds of *Cordyceps* species

Species	Bioactive compounds	Biological activity	References
C. cicadae	Galactomannans	Anti-tumor	Kiho *et al*., 1990
	Cyclopentenone	-	Zhang and Xuan, 2008
	Ergosterol peroxide	Immunosuppressive, anti-viral, anti-inflammatory, anti-tumor activities and treat kidney disease	Fujimoto *et al*., 1994; Kreisel *et al*., 1990; Yasukawa *et al*., 1996; Bok *et al*., 1999; Zhu *et al*., 2014
	Cyclodepsipeptide cordycecin A, Beauvericin A, E, and J	Cytotoxic activity	Wang *et al*., 2014
C. ophioglossoides	Alkali-soluble Polysaccharide	Anti-tumor (MM46 mammary carcinoma),	Yamada *et al*., 1984; Ohmort *et al*., 1989
	Ophiocordin	antibiotic and antifungal	Kneifel *et al*., 1977
C. pseudomilitaris	Anhydride groups,	-	Isaka *et al*., 2000
	Bioxanthracenes	Anti-malarial and cytotoxicity	Isaka *et al*., 2001; Jaturapat *et al*., 2001

Table 2.1 (Cont.)

Species	Bioactive compounds	Biological activity	References
C. militaris	Cordycepin, Polysaccharides, Ergosterol, Mannitol, etc.	Anti-tumour, anti-cancer, anti-leukemic	Liu *et al*., 1997; John and Adamson, 1976; Muller *et al*., 1977; Kodama *et al*., 2000; Shih *et al*., 2007 Chen et al, 2013; Fan and Lin, 2013;
		Anti-proliferative	
		Anti-metastatic	Sone *et al*., 1985; Mao and Zhong, 2006; Shih *et al*., 2007; Lin and Chiang, 2008
		Immunomodulatory	
		Anti-microbial	Park ,1996
		Anti-bacterial	Ahn *et al*., 2000
		Anti-viral	Ortiz *et al*.,1999; Mao and Zhong, 2006; Lin and Chiang, 2008
		Anti-fungal	
	Cordyrroles A and B	Adipocyte differentiation and pancreatic lipase activity	Mao and Zhong, 2006; Shih *et al*., 2007 Kim *et al*., 2014
C. unilateralis	Naphthoquinones	Anti-malaria antibacterial, antimalarial activity and cytotoxicity	Kittakoop *et al*., 1999; Unagul *et al*., 2005; Wongsa *et al*., 2005

Table 2.1 (Cont.)

Species	Bioactive compounds	Biological activity	References
***Cordyceps* sp.**	Cordytropolone,	Anti-malarial, anti-cancer, anti-tuberculous	Seephonkai *et al*., 2001; Isaka *et al*., 2005
	Bioxanthracenes, Cordyheptapeptide A and B,	Cytotoxic activity	Rukachaisirikul *et al*., 2006; Isaka *et al*., 2007
	Cordyol A-C,	Antimycobacterial, anti-HSV-1	Bunyapaiboonsri *et al*., 2007
	Cordylactum ,	-	Isaka *et al*., 2013
	Opaliferin	Cytotoxic activity	Grudniewska *et al*., 2014
C. sinensis	Adenosine	Antioxidant and anti-asthemia	Yue *et al*., 2008; Shrestha *et al*, 2014
	Cordycepin,		Yoshida *et al*., 1989; Shin *et al*., 2003; Yang *et al*., 2006; Wu *et al*., 2007
	Cordycepic acid,	Anti-tumor	
	Polysaccharides,	Anti-cancer	Chen *et al*., 1997
	Mannitol,	Anti-leukemia	Zhang *et al*., 2005; Yoon *et al*., 2008; Jordan *et al*., 2009
	Nucleosides,	Immunostimulating	
	Ergosterol,	Antioxidant	Wang *et al*., 2005; Zhou *et al*., 2009
	Aminophenol,	-	
	Cordyceamides A and B	Anti-cancer	Jia *et al*., 2009

Table 2.1 (Cont.)

Species	Bioactive compounds	Biological activity	References
C. sobolifera	Cordysobin,	Inhibitors phenoloxidase-activating systems of insects Anti- HIV-1	Leger *et al*., 1978; Watanabe *et al*., 2006; Wang *et al*., 2012
	Polysaccharide	Improvement of renal function Anti-tumor	Chiu *et al*., 2014
	Extracellular polysaccharide		Yang and Zhang., 2016
C. nipponica	Cordypyridones A-D	Anti-malaria	Isaka *et al*., 2001
	Adenosine, Flavonoid , Phenolic	Antibacterial	Sangdee *et al*., 2015

2.3.2 Agricultural area

The entomopathogenic fungi in the genus *Cordyceps* play an important role in ecological associations and are widely used in the agricultural field. In entomology, it has been used to inhibit the growth of insect pest such as entomopathogenic nematodes or microbial pathogens to suppress populations of different pest insects (Pal and Gardener, 2006). Moreover, the entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae*, have been used to suppress many insect pests (Kirkland *et al*., 2004; Meyling and Eilenberg, 2007; Amóra *et al*., 2009). In plant pathology, the entomopathogenic fungi have also been used to suppress many plant pathogens, for example, the extracts from *Cordyceps* species could inhibit mycelial growth of the plant pathogens in the genera *Rosellinia* (92%), *Phytophthora* (71%), *Fusarium* (69%), *Colletotrichum* (20%) and *Penicillium* (21%) (Miazzi *et al*., 2012). Based on the data described above, entomopathogenic fungi have the potential to be used as biological control agents.

Currently, the entomopathogenic fungi in the genera *Ophiocordyceps* and *Cordyceps* have been reported to have *in vitro* activity against some plant pathogenic fungi. For example, *Cordyceps sobolifera* has been reported to be used as a biocontrol agent against plant pathogenic fungi *Botrytis cinerea*, *C. gloeosporioides* and *C. miyabeanus* and could inhibit the growth of the human pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Moreover, Evans *et al*. (1999) has reported that *Cordyceps barnesii* has the activity to control sugar-cane white grubs in East Africa. Varughese *et al*. (2012) observed that *C. dipterigena* could inhibit the mycelial growth of the plant pathogenic fungus *Gibberella fujikuroi*, causal agent of bakanae disease in rice seedlings. Kumar and Aparna (2014) have reported that *Cordyceps* species could be used as a potential biocontrol agent to control coconut root cub (*Leucopholis coneophora*).

2.3.3 Nutritional value of *Cordyceps*

Cordyceps has a wide range of nutritionally benefits, such as amino acids and essential vitamins including vitamins E, K, B1, B2 and B12. The carbohydrates, including monosaccharides, disacchaides, oligosaccharides and polysaccharides, have also been reported in many *Cordyceps* species. Moreover, the protein components, cholesterol, nucleotides and essential elements, such as K, P, Na, Ca, Mg, Fe, Cu, Mn,

Zn, Pi, Se, Al, Si, Ni, Sr, Ti, Cr, Ga, V and Zr, have been reported from *Cordyceps* species too (Holliday *et al*., 2005, Zhou *et al*., 2009).

2.4 Biology of the *Cordyceps*

2.4.1 Life cycle and pathogenesis

These fungi can attack hosts from many orders, especially arthropod groups. Insect hosts are infected by entomopathogenic fungi at different stages of their development, which ranges from the larvae to adult. In the rainy season, infection begins with the dispersion of ascospores of the fungi on insect surface germinates and directly penetrates the insect's cuticle. After that, the fungus grows and forms hyphae in the insect's body. After the death of the host, the fungus produces hyphae inside the body of the insect and then grows out from the head. The stroma is formed, which grows straight up through the soil surface and forms a stalked fruiting body, which a sexual stage. Ascospores are released from perithecia and can infect new insect larva under suitable environmental conditions. Moreover, the asexual stage can produce conidia by conidiogeous cells without the aid of the fruiting body (Dworecka-Kaszak, 2014).

2.4.2 Distribution of the *Cordyceps*

The entomopathogenic fungi are worldwide in their distribution in both temperate and tropical climates. The fungi *Cordyceps sinensis* has been found on the Himalayan Mountains, Tibetan Plateau in Nepal, China and southwestern China (Holliday and Cleaver, 2008; Liang *et al*., 2008; Kinjo and Zang, 2001). *Cordyceps cardinalis* has been found in the southern Appalachian Mountains of the eastern United States and southeastern Japan (Sung and Spatafora 2004). *Cordyceps militaris* has been reported as being widely distributed in North America, South America, Europe and Asia from sub-tropical to temperate regions around the world (Mains, 1958). Moreover, Shrestha and Sung (2005) reported that eight *Cordyceps* species including *C. gracilis*, *C. ishikariensis*, *C. liangshanensis*, *C. martialis*, *C. militaris*, *C. pruinosa*, *C. sphecocephala* and *C. tricentri* were found around Kathmandu Valley and a few high altitude locations of Nepal. The diversity of entomopathogenic fungi is found in subtropical and tropical regions like Asia with a hot and humid climate as in Thailand.

Cordyceps species are most common and widely distributed in the natural forests of the north, northeast and south regions of Thailand (Kobayasi, 1941; 1982; Hywel-Jones, 2001; Aung *et al*., 2008; Luangsa-ard *et al*., 2011; Srivilai *et al*., 2013; Sangdee and Sangdee 2013). In Thailand, *Cordyceps* sp. can be found in the forest during the rainy season, such as *Cordyceps* sp. infecting cicada nymphs that are found in mixed deciduous forest at the start of the rainy season, while those on scale insects can be found over most of the year. The majority of entomopathogenic fungi that are reported in Thailand are members of the families Clavicipitaceae, Cordycipitaceae and Ophiocodycipitaceae (Order Hypocreales), as described in Table 2.2 (Mongkolsamrit *et al*., 2010; Sung *et al*., 2007a).

Table 2.2 Common genera of the three families with species number of invertebrate-pathogenenic fungi found in Thailand

Family	Genus	Identified number of species
Cordycipitaceae	*Akanthomyces*	10
	Beauveria	3
	Cordyceps	16
	Gibellula	8
	Hyperdermium	2
	Isaria	12
	Torrubiella	5
	Verticillium	3
Total		59
Clavicipitaceae	*Aschersonia*	13
	Conoideocrella	2
	Hypocrella	5
	Metacordyceps	3
	Metarhizium	3
	Moelleriella	4
	Orbiocrella	1
	Paecilomyces	4
	Shimizuomyces	1
	Stilbella	1
Total		38
Ophiocordycipitaceae	*Elaphocordyceps*	2
	Hirsutella	12
	Hymenostilbe	11
	Nomuraea	3
	Ophiocordyceps	24
	Paecilomyces	2
Total		54

2.4.3 Host affiliation

Entomopathogenic fungi have a broad insect host range and are dominantly distributed in tropical regions and humid tropical forests (Evans, 1982). *Cordyceps* is a big genus placed in the family Clavicipitaceae of order Hypocreales, comprising more than 400 species that parasitize a wide range of insect hosts, as shown in Table 2.3. The hosts of *Cordyceps* are a very broad range and includes 12 orders: Homoptera, Lepidoptera, Coleoptera, Diptera, Hymenoptera, Isoptera, Odonota, Orthoptera, Aranae, Acari, Blattaria and Phasmida (Nikoh and Fukatsu, 2000; Spatafora *et al*., 2007). Previously, the published literature reported that nearly 60% of the species of *Cordyceps* were recorded in two orders of Coleoptera and Lepidoptera. More than 95% of the hosts are infected in the larvae stage on Lepidoptera (moths, butterflies) and Coleoptera (beetle). Whereas, the majority of hosts in other orders, such as Araneae (spiders), Diptera (fly), Hymenoptera (ant, wasp and bee), Orthoptera (cricket, grasshoppander and locust), Hemiptera (cicada, bug, scale-insect and coccid), Odonata (dragonfly), Blattaria (cockroach and termite) and Phasmatodea (stick-insect) were infected in the adult stage (Shrestha *et al*., 2016). The major insect hosts infected by these fungi are as follows:

Coleoptera: the Coleoptera or beetles is the largest single order of insects with over 360,000 species and many beetles are the main host of *Cordyceps* fungi, as shown in Figure 2.2 (Mongkolsamrit *et al*., 2010).

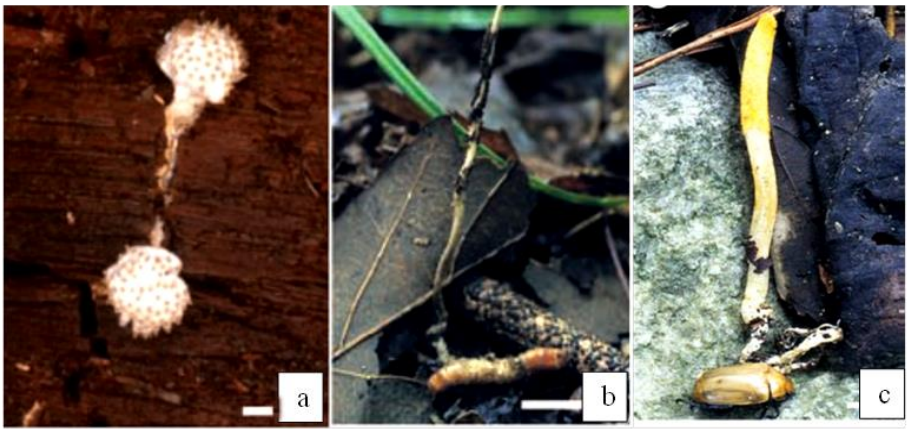

Figure 2.2 a *C. subsessilis* OSC 128581on scarabaeid beetle in decaying wood (Coleoptera); **b** *C. agriotidis* EFCC 5274 on coleopteran larva; **c** *C. scarabaeicola* on scarabaeid beetle (Coleoptera). Scale bars = 1 mm (a), 10 mm (b, c) (Sung *et al*., 2007a)

Hemiptera and Homoptera: comprising 80,000 species, Hemiptera is a considerably large group with a capability to change its life cycle when compared to other insects (involving pupa and nymphs), such as cicadas, true bugs, aphids and scale insects. They are also the main host of insect pathogenic fungi as shown in Figure 2.3 (Mongkolsamrit *et al*., 2010).

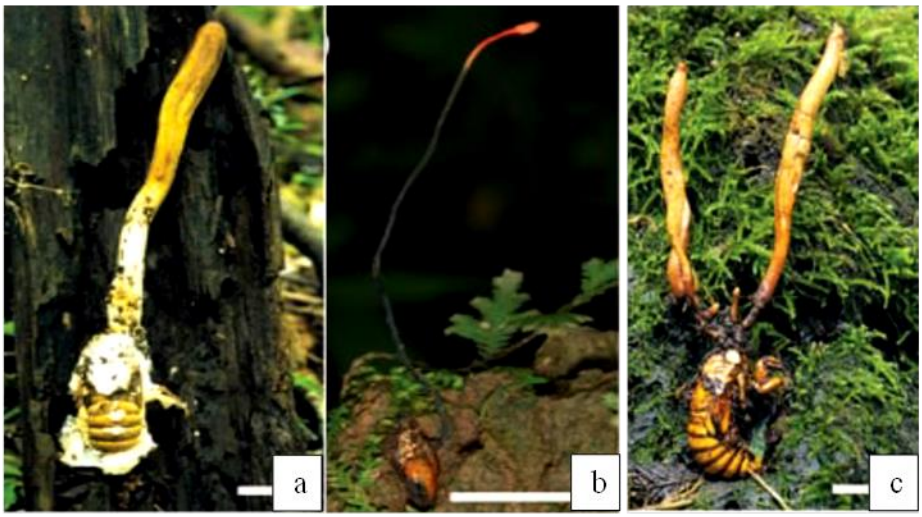

Figure 2.3 a *C. sobolifera* EFCC 7768 on cicada nymph (Hemiptera); **b** *C. nutans* on stink bug (Hemiptera); **c** *C. longissima* EFCC 8576 on cicada nymph (Hemiptera). Scale bars = 10 mm (a-c) (Sung *et al*., 2007a)

Hymenoptera: comprising of 150,000 species, Hymenoptera is a large order of insects including bees, ant and wasps. They are also the host of insect pathogenic fungi as shown in Figure 2.4 (Mongkolsamrit *et al*., 2010).

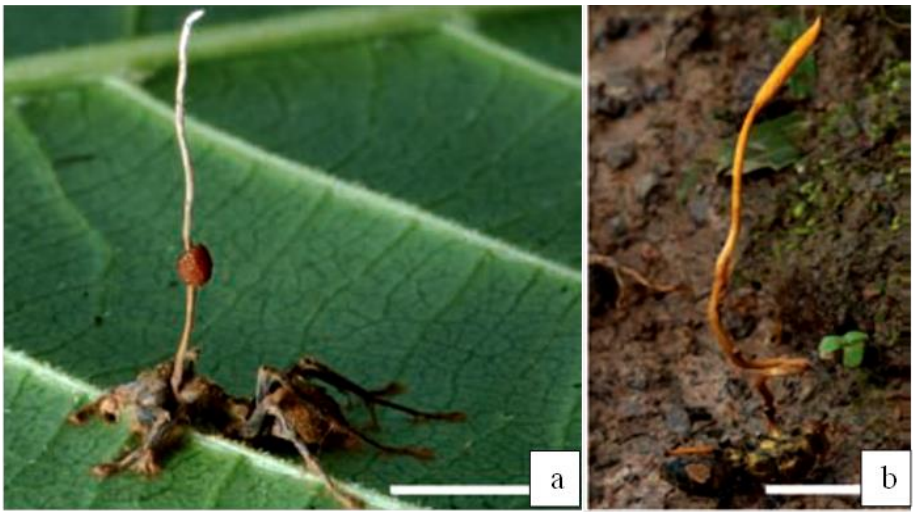

Figure 2.4 a *C. unilateralis* on ant (Hymenoptera); **b** *C. sphecocephala* on wasp (Hymenoptera). Scale bars = 5 mm (a), 10 mm (b) (Sung *et al*., 2007a)

Lepidoptera: comprising around 300,000 species and known as moths, Lepidoptera or butterflies and moths while being among the largest orders of insects. They are hosts of insect pathogenic fungi as shown in Figure 2.5 (Mongkolsamrit *et al*., 2010).

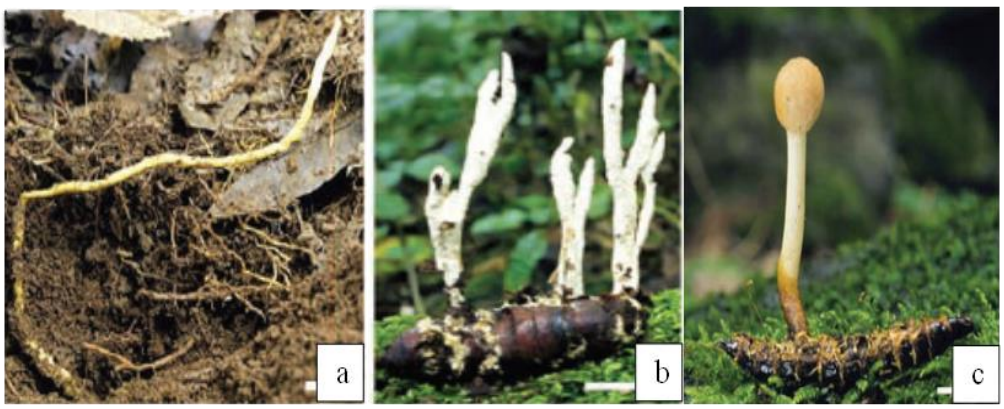

Figure 2.5 a *C. liangshanensis* EFCC 1452 on lepidopteran larva; **b** *Cordyceps* sp. EFCC 12285 on lepidopteran pupa; **c** *C. gracilis* EFCC 10121 on lepidopteran larva. Scale bars = 10 mm (a-c) (Sung *et al*., 2007a)

Orthoptera: comprising of over 20,000 known species, Orthoptera is a large order of insects including grasshoppers, crickets and locusts. Many species of insect pathogenic fungi can infect the orthoptera insects as shown in Figure 2.6 (Mongkolsamrit *et al*., 2010).

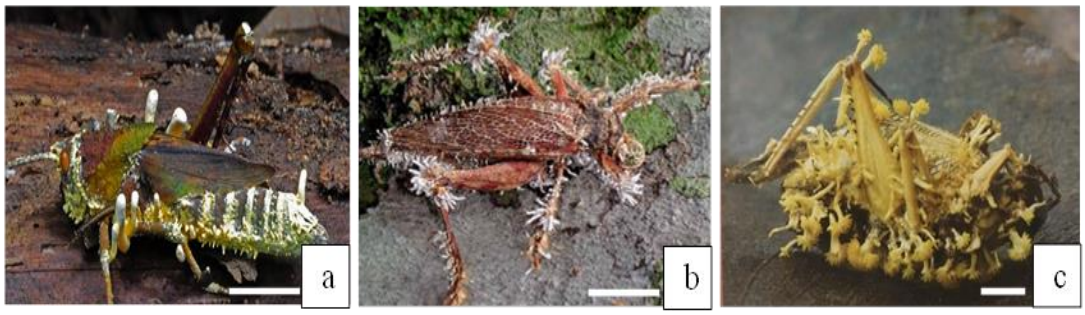

Figure 2.6 a *C. acridophila* on *Agriacris plagiata* (Orthoptera: Romaleidae); **b** *Isaria amorpha* growing on a Orthoptera (Petch); **c** *C. loeiensis* on leaf-rolling cricket. Scale bars = 10 mm (a-c). Available from: http://mushroaming.com/blog?page=2 [Cited 25 June 2014], (Mongkolsamrit *et al*., 2010)

Table 2.3 *Cordyceps* species infected on insect host

Species	Host	Order/ References
C. acicularis	Coleopteran larva	Coleoptera (Sung *et al*., 2007a)
C. alboperitheciata	Beetle	Coleoptera (Ban *et al*., 2009)
C. australis	Ant	Hymenoptera (Evan *et al*., 1999)
C. barnesii	Beetle larva	Coleoptera (Evan *et al*., 1999)
C. bassiana	Moths larva	Lepidoptera (Sung *et al*.,2006; Lee *et al*., 2007)
C. brunneapunctata	Beetle larvae	Coleoptera (Hywel-Jones,1995a)
C. cardinalis	Lepidopteran larva	Lepidoptera (Sung and Spatafora, 2004)
C. chiangdaoensis	Coleopteran larva	Coleoptera (Tasanathai *et al*., 2016)
C. cicadae	Cicada nymph	Hemiptera (Kiho *et al*., 1990; Zhang and Xuan, 2008; Wang *et al*., 2014)
C. coccidiicola	Scale Insect	Hemiptera (Sung *et al*., 2007a)
C. coccinea	Grubs	Coleoptera (Shrestha, 2011)
C. cuboidea	Beetle	Coleoptera (Ban *et al*., 2009)
C. cuncunae	Ghost moth	Lepidoptera (Palfner *et al*., 2011)
C. curculionum	Beetle	Coleoptera (Samson *et al*., 1988)
C. cylindrica	Spider	Araneae (Hywel-Jones and Sivichai, 1995; Luagsa-ard *et al*., 2012)
C. dipterigena	Flies	Diptera (Sung *et al*., 2007a; Varughese *et al*., 2012)
C. forquignoni	Flies	Diptera (Arora,1986; Dennis,1978)
C. gryllotalpidicola	Crickets	Orthoptera (Luagsa-ard *et al*., 2012)
C. heteropoda	Cicada nymph	Hemiptera (Nikoh and Fukatsu, 2000; Sung *et al*., 2007a)

Table 2.3 (Cont.)

Species	Host	Order/ References
C. inegoensis	Cicada	Hemiptera (Nikoh and Fukatsu, 2000)
C. irangiensis	Formicine ant	Hymenoptera (Hywel-Jones,1996)
C. ishikariensis	Cicada nymph	Hemiptera (Shrestha and Sung, 2005; Shrestha, 2011)
C. japonensis	Wasp	Hymenoptera (Imazeki *et al*., 1988)
C. kanzashiana	Cicada nymph	Hemiptera (Nikoh and Fukatsu, 2000;Kepler *et al*., 2013)
C. khaoyaiensis	Lepidopteran larva	Lepidoptera (Hywel-Jones,1994)
C. konnoana	Beetle	Coleoptera(Nikoh and Fukatsu, 2000)
C. kyushuensis	Moth	Lepidoptera (Sung *et al*., 1995; Zhang *et al*., 2015)
C. locustiphila	Grasshopper	Orthoptera (Arora,1986)
C. loeiensis	Leaf-rolling cricket	Orthoptera (Luagsa-ard *et al*., 2012)
C. longissima	Cicada nymph	Hemiptera (Sung *et al*., 2007a)
C. martialis	Lepidopteran pupa	Lepidoptera (Shrestha and Sung, 2005; Shrestha, 2011)
C. militaris	Lepidopteran pupa	Lepidoptera (Mains,1958; Sung *et al*., 2007a; Shrestha, 2011)
C. myrmecophila	Formicine ant	Hymenoptera (Hywel-Jones,1996)
C. nelumbodides	Spider	Araneae (Luagsa-ard *et al*., 2012)
C. nigrella	Coleopteran larva	Coleoptera (Sung *et al*., 2007a)

Table 2.3 (Cont.)

Species	Host	Order/ References
C. ninchukispora	Lepidopteran pupae	Lepidoptera (Luagsa-ard *et al*., 2008)
C. nipponica	Ant lion, Cicada nymph	Hemiptera (Isaka *et al*., 2005; Sangdee *et al*., 2015)
C. nutans	Stink buds	Hemiptera (Hywel-Jones, 1995c; Shrestha and Sung, 2005)
C. oxycephala	Wasp	Hymenoptera (Imazeki *et al*., 1988)
C. paradoxa	Cicada	Hemiptera (Nikoh and Fukatsu, 2000)
C. prolifica	Cicada nymph	Hemiptera (Ban *et al*., 2009)
C. pruinosa	Lepidopteran pupae	Lepidoptera (Shrestha, 2011)
C. pseudolloydii	Ant	Hymenoptera (Evans and Samson, 1984)
C. pseudomilitaris	Lepidopteran larva	Lepidoptera (Hywel-Jones,1994; Isaka *et al*., 2001; Isaka *et al*., 2005)
C. ramosopulvinata	Cicada larva	Hemiptera (Imazeki *et al*., 1988)
C. rhizoidea	Termite	Isoptera (Sung *et al*., 2007a)
C. roseostromata	Beetle larva	Coleoptera (Imazeki *et al*., 1988)
C. scarabaeicola	Scarabaeid	Coleoptera (Sung *et al*., 2007a)
C. sisnensis	Lepidopteran larva	Lepidoptera (Isaka *et al*., 2005; Sung *et al*., 2007a)
C. sobolifera	Cicada nymph	Hemiptera (Nikoh and Fukatsu, 2000; Sung *et al*., 2007a)

Table 2.3 (Cont.)

Species	Host	Order/ References
C. sphecocephala	Bee and wasp	Hymenoptera (Hywel-Jones,1995b; Shrestha and Sung, 2005; Sung *et al*., 2007a)
C. stylophora	Elaterid larva	Coleoptera (Sung *et al*., 2007a)
C. tricentri	Spittlebug	Coleoptera (Nikoh and Fukatsu, 2000)
C. tuberculata	Moth	Lepidoptera (Luagsa-ard *et al*., 2010)
C. unilateralis	Ant	Hymenoptera (Kobmoo *et al*.,2015; Isaka *et al*., 2005; Sung *et al*., 2007a)
C.bifusispora	Lepidopteran pupa	Lepidoptera (Sung *et al*., 2007a; Lu *et al*., 2013)
Cordyceps sp.	Elaterid larva	Coleoptera (Isaka *et al*., 2005)
Cordyceps sp.	Termite	Isoptera (Sung *et al*., 2007a)
Cordyceps sp.	Coleoptera	Coleoptera (Sung *et al*., 2007a)
Cordyceps sp.	Ant	Hymenoptera (Sung *et al*., 2007a)
Cordyceps sp.	Lepidopteran pupa	Lepidoptera (Sung *et al*., 2007a)

2.5 Identification of *Cordyceps*

The identification method of the entomopathogenic fungi can be separated into two levels: morphological and molecular. These have different efficiencies and limitations.

2.5.1 Morphological identification

The entomopathogenic fungi have been primarily identified based on similarities and differences in morphological characteristics. Morphological characters were identified based on host, stroma, perithecia, asci and ascospores (Luangsa-Ard *et al*., 2011), which are shown in Figure 2.7. The shape, length and number of stroma were used for fungal identification. The sizes and shape of perithecia, asci and ascospores were also used as a tool for identification (Sung and Spatafora, 2004). For example,

C. cardinalis is characterized by gregarious, 1~26 stromata per host, orange reddish to reddish, 10~40 long and 0.5~1.5 mm wide stromata growing on lepidopteran larva. The head is 2~9 × 1~4 mm in size. Perithecia are ovoid and semi-immersed. Asci have a distinct cap. The ascospores are irregularly septate but do not disarticulate into partspores, are shown in Figure 2.8 (Sung *et al*., 2010).

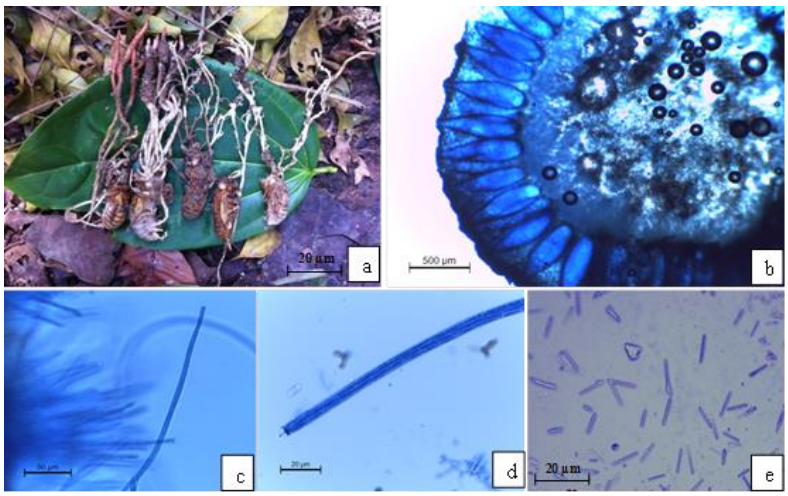

Figure 2.7 Morphological characteristics of the entomopathogenic fungi were classified based on **a** host and stroma; **b** perithecia; **c** ascus; **d** ascus with ascus tip; **e** ascospores or part-spore. Scale bars = 20 mm (a), 500 µm (b), 50 µm (c), 20 µm (d), 50 µm (e)

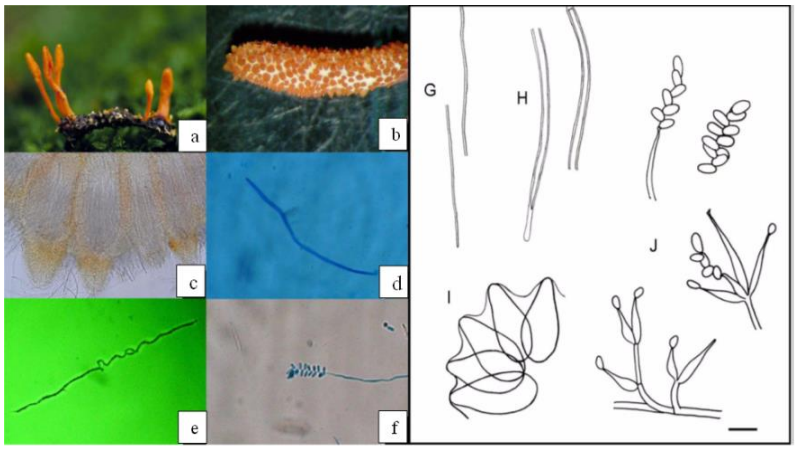

Figure 2.8 Morphological characteristics of the *C. cardinalis* CRI C-10376 (Source: Sung *et al*., 2010) **a** natural specimen; **b** enlarged stroma; **c, i** perithecia; **d, h** Ascus; **e, g** Ascospores; **f, j** Conidial stage. Scale bars = 20 µm (g, h), 200 µm (i) 10 µm (j)

2.5.2 Molecular identification

The entomopathogenic fungi in the genus *Cordyceps* have been reported to include over 400 species worldwide. Sometimes, the morphological characteristics cannot be used to identify these fungi due to the morphological characters proving to have high variation and depending on the insect host and environment.

In recent years, the molecular technique is one of the tools that has been used for characterization and identification of many organisms (Ban *et al*., 2009; Tian *et al*., 2010). Moreover, the advantage of using molecular identification is that it is fast and suitable for identification of invertebrate pathogenic fungi. The molecular technique with a specific primer to a particularly genome has also been used to identify many fungi, including *Cordyceps*. For example, the Polymerase Chain Reaction (PCR) technique based on the intergenic transcribed spacer (*ITS*) has evolved rapidly and can be used for discrimination of closely related species and subspecies (Chen *et al*., 1992). Currently, DNA sequence data sets of *ITS*, nuclear ribosomal small subunits (*nrSSU*), nuclear ribosomal large subunits (*nrLSU*), elongation factor 1α (*EF-1α*), largest and second largest subunits of RNA polymerase II (*rpb1* and *rpb2*), β-tubulin (*tub*) and mitochondrial ATP6 (*atp6*) sequences have been used for identification of the entomopathogenic fungi (Sung *et al*., 2007b). Chan *et al*. (2011) has successfully used the DNA sequence from three genes, including *nrLSU*, *EF-1α* and *rpb1* to identify *Cordyceps gunnii*. Luangsa-ard e*t al*. (2011) has used the sequences of two regions of ITS1-5.8S-ITS2 rDNA and *EF-1α* to analyze the phylogenetic relationships of *Ophiocordyceps halabalaensis*, a new species of genus *Ophiocordyceps*. Kepler *et al*. (2012) used five of nuclear loci: fragments of small and large subunit of ribosomal DNA, elongation factor 1α and the largest and second largest subunits of RNA polymerase II, for investigation of the phylogenetic relationships among the entomopathogenic genus *Metacordyceps*. Ciancio *et al*. (2013) successfully used the sequence of *ITS* region and β-tubulin gene region to identify *Hirsutella tunica* sp. nov. (Ophiocordyceptaceae). Wen *et al*. (2013) successfully used the combined sequence data of *ITS* rDNA, *nrSSU*, *EF1α* and *rpb1* genes to identify a new species, *O. xuefengensis* sp. nov. in Hunan province, southern China. Sangdee and Sangdee (2013) used the nucleotide sequences of *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* to identify the insect pathogenic fungi that infected cicada nymphs as *O. longissima* isolate Cod-MK1.

Ban *et al*. (2015) demonstrated that three new species, *O. coenomyia*, *O. arborescens* and *O. macroacicularis,* could be identified using a combined data set consisting of *ITS*, *nrSSU*, *nrLSU*, *EF1α* and *rpb2*. Kepler *et al*. (2013) used the combined data of *nrSSU*, *nrLSU*, *EF-1α*, *rpb1* and *rpb2* to identify insect pathogens in the genus *Polycephalomyces*. Moreover, the combined data set of *ITS*, *nrSSU*, *nrLSU*, *EF-1α, rpb1, β-tubulin* and *atp6* genes have successfully been used to identify *P. nipponicus* isolate Cod-MK1201 (Sangdee *et al*., 2015, 2016). Therefore, the combined data set technique can be used to identify the entomopathogenic fungi, which is more accurate and reliable than other methods.

CHAPTER 3

METHODOLOGY

3.1 Research designs

In this research, the entomopathogenic fungi were initially identified based on morphological characteristics including stromata, perithecia, asci, and ascospore. Then, all isolates of entomopathogenic fungi were identified using non-coding (*ITS*, *nrSSU*, *nrLSU*) and coding gene (*EF-1α* and *rpb1*). After that, the phylogenetic relationship of entomopathogenic fungi isolates and their related species were analyzed using molecular genetic tool programs. Next, the biological activity of entomopathogenic fungi was investigated against *Colletotrichum* spp. causing agent of chili anthracnose by dual culture technique. The potential isolates were chosen for further confirmation of their antagonistic effects against ten isolates of *Colletotrichum* spp. by the dual culture method. In addition, the biological activities of the potential isolates were tested on the mycelial growth, conidial germination under in *vitro* conditions. Finally, the potential isolates should be tested against plant pathogenic *Colletotrichum* spp. using detached fruit bioassay. The experiments was designed as in Figure 3.1

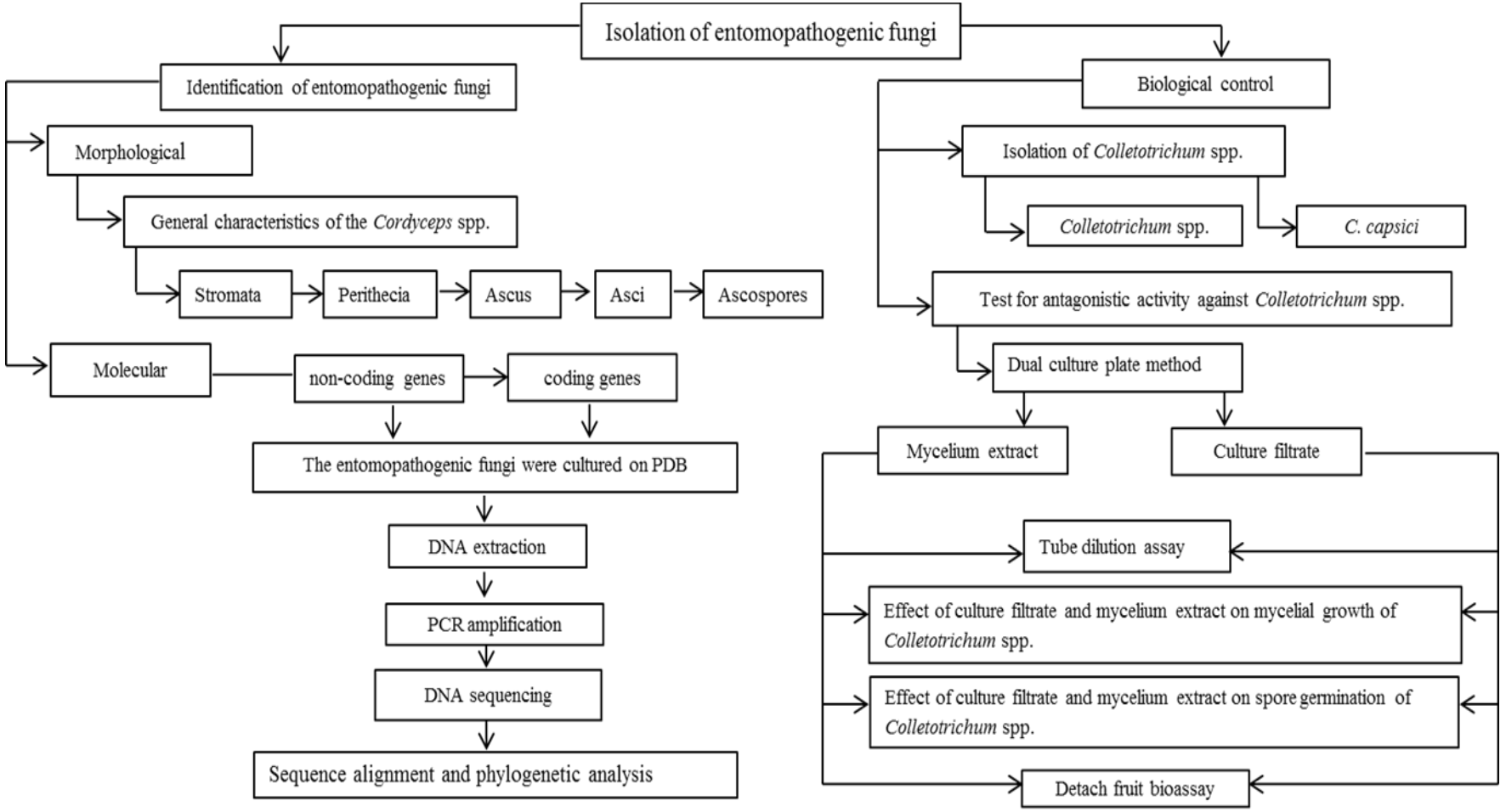

Figure 3.1 The schematic of the experiments in this study

3.2 Sample collections

Cicada nymphs infected with entomopathogenic fungi were collected from six Provinces (Maha Sarakham, Roi Et, Nong Bua Lam Phu, Nakhon Phanom, Sakon Nakhon and Loei) in mixed deciduous forest from the Northeastern Thailand (Figure 3.2). Details (latitude, longitude, and altitude) of collection sites are shown in Table 3.1.

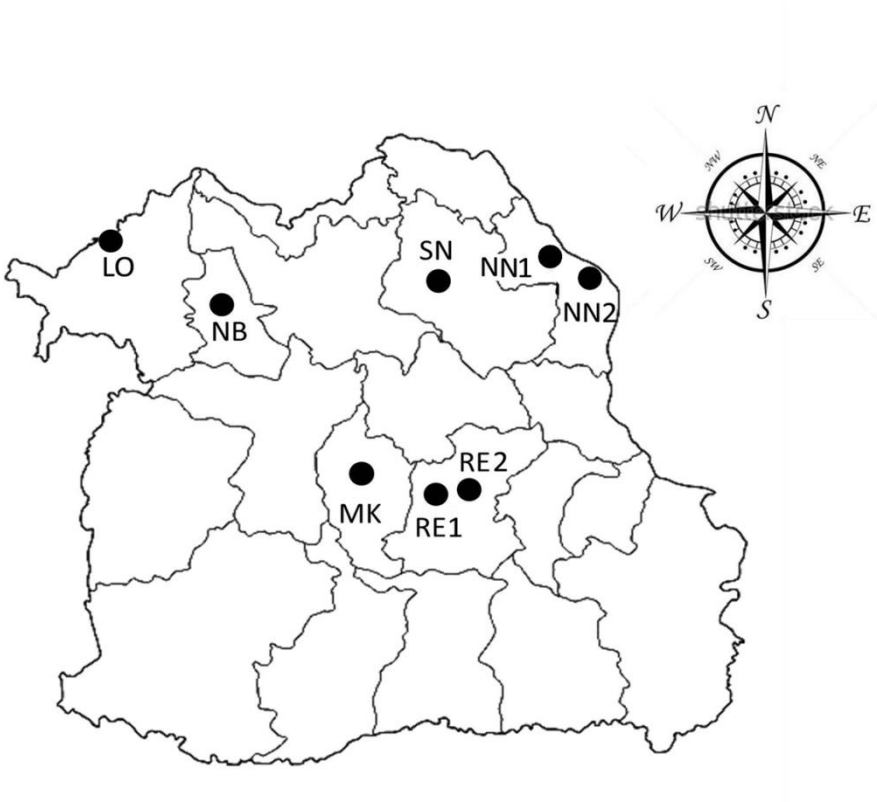

Figure 3.2 Sample collection sites of the entomopathogenic fungi from Northeastern Thailand; MK – Maha Sarakham, RE – Roi Et (RE1, RE2), NB–Nong Bua Lam Phu, NN – Nakhon Phanom (NN1, NN2), SN– Sakon Nakhon, LO– Loei

Table 3.1 Sample collection sites of entomopathogenic fungi this study

Location	Code*	Name of isolate	Latitude/longitude	Altitude (m)	Collection date
Ban na pang, Muang Maha Sarakham	MK	Cod-MK1201	16°10′44″N 103°28′36″E	135	11/06/2012
		Cod-MK1202	16°10′44″N 103°28′36″E	135	
		Cod-MK1203	16°10′44″N 103°28′36″E	135	
		Cod-MK1204	16°10′44″N 103°28′36″E	135	
		Cod-MK1205	16°10′44″N 103°28′36″E	135	
		Cod-MK1206	16°10′44″N 103°28′36″E	135	
		Cod-MK1207	16°10′44″N 103°28′36″E	135	
		Cod-MK1208	16°10′44″N 103°28′36″E	135	
		Cod-MK1209	16°10′44″N 103°28′36″E	135	
		Cod-MK1210	16°10′44″N 103°28′36″E	135	
Ban na pang, Muang Maha Sarakham	MK	Cod-MK1301	16°10′44″N 103°28′36″E	135	28/05/2013
		Cod-MK1302	16°10′44″N 103°28′36″E	135	
		Cod-MK1303	16°10′44″N 103°28′36″E	135	
		Cod-MK1304	16°10′44″N 103°28′36″E	135	
		Cod-MK1305	16°10′44″N 103°28′36″E	135	
		Cod-MK1309	16°10′44″N 103°28′36″E	135	
		Cod-MK1311	16°10′44″N 103°28′36″E	135	
		Cod-MK1319	16°10′44″N 103°28′36″E	135	
		Cod-MK1321	16°10′44″N 103°28′36″E	135	
		Cod-MK1324	16°10′44″N 103°28′36″E	135	
		Cod-MK1325	16°10′44″N 103°28′36″E	135	
		Cod-MK1329	16°10′44″N 103°28′36″E	135	

Table 3.1 (Cont.)

Location	Code*	Name of isolate	Latitude/longitude	Altitude (m)	Collection date
Ban Ngu Luam, Suwan Phum, Roi Et	RE1	Cod-RE1201 Cod-RE1202 Cod-RE1203	15°40'52.6"N 103°44'58.0"E 15°40'52.6"N 103°44'58.0"E 15°40'52.6"N 103°44'58.0"E	145 145 145	16/07/2012
Ban Mek, Suwan Phum, Roi Et	RE2	Cod-RE1301	15°41'00.8"N 103°46'25.5"E	148	25/09/2013
Tham Erawan, Na Wang, Nong Bua Lam Phu	NB	Cod-NB1301 Cod-NB1302 Cod-NB1303 Cod-NB1304 Cod-NB1305 Cod-NB1306 Cod-NB1307 Cod-NB1308	17°20'20.4"N 102°01'16.8"E 17°20'20.4"N 102°01'16.8"E 17°20'20.4"N 102°01'16.8"E 17°20'20.4"N 102°01'16.8"E 17°20'20.4"N 102°01'16.8"E 17°20'20.4"N 102°01'16.8"E 17°20'20.4"N 102°01'16.8"E 17°20'20.4"N 102°01'16.8"E 17°52'49.9"N 101°39'36.7"E	337 337 337 337 337 337 337 337	24/05/2013
Chiang Khan, Loei	LO	Cod-Loei1301		214	23/08/2013

Table 3.1 (Cont.)

Location	Code*	Name of isolate	Latitude/longitude	Altitude (m)	Collection date
Si Songkhram, Nakhon Phanom	NN1	NN1301	17°39'03.5"N 104°12'43.9"E	146	7/06/2013
		NN1302	17°39'03.5"N 104°12'43.9"E	146	
		NN1303	17°39'03.5"N 104°12'43.9"E	146	
		NN1304	17°39'03.5"N 104°12'43.9"E	146	
		NN1305	17°39'03.5"N 104°12'43.9"E	146	
Tha Uthen, Nakhon Phanom	NN2	NN1306	17°33′25″N 104°36′45″E	156	24/06/2013
		NN1307	17°33′25″N 104°36′45″E	156	
Wanon Niwat, Sakon Nakhon	SN	SN1401	17°37′56″N 103°45′7″E	167	20/06/2014
		SN1402	17°37′56″N 103°45′7″E	167	

* MK – Maha Sarakham, RE – Roi Et, NB–Nong Bua Lam Phu, NN – Nakhon Phanom, SN–Sakon Nakhon, LO– L

3.3 Isolation of entomopathogenic fungi

The 134 cicada nymphs that were collected from different areas of mixed deciduous forest from Northeastern Thailand then had entomopathogenic fungi isolated using the tissue transplanting technique. The cicada samples were washed with sterile distilled water and sectioned into two pieces. The inner tissue of the cicada nymph was cut (5 x 5 mm^2) and surface sterilized by dipping in 10% sodium hypochloride for 2 min, after that the sample was rinsed several times with sterile distilled water before being transferred onto the surface of a potato dextrose agar (PDA) plate. The plate was incubated at 25 °C for seven days. The mycelium growing out of the cicada nymph tissue was sub-cultured on PDA and incubated at 25-28 °C for further study.

3.4 Identification of entomopathogenic fungi

3.4.1 Morphological characteristics

The morphological characters of the entomopathogenic fungi, such as stroma, perithecia, asci, ascospore and part-spore were examined under a light microscope (Luangsa-Ard *et al*., 2011) and the anamorphic morphology was also examined. The presence (1) or absence (0) of morphology features was scored as a binary matrix. Similarity, coefficients for all pairwise combinations were determined using Dice's coefficients in the SIMQUAL program and clustered by the unweighted paired-group method with arithmetic mean (UPGMA) by means in the SAHN program of the NTSYS-pc package (Rohlf, 2000).

3.4.2 DNA extraction

All isolates of the entomopathogenic fungi were cultured on PDB at 28°C for 20 days. Mycelia were harvested from the PDB medium before being homogenized with liquid nitrogen using a mortar and pestle. The mycelium powder was transferred to a microcentrifuge tube and genomic DNA was extracted using a DNA extraction kit (Vivantis, Malaysia). The DNA samples were analyzed by 1% agarose gel electrophoresis and stored at -20 °C.

3.4.3 PCR Amplification

Three non-coding genes, consisting of internal transcribed spacers of nuclear ribosomal DNA repeats (*ITS*), small subunit of (*nrSSU*) rDNA and large subunit of (*nrLSU*) rDNA and two coding genes consisting of the elongation factor 1α (*EF-1α*) gene and the largest subunit of RNA polymerase II (*rpb1*) gene were amplified by PCR with specific primers (Table 3.3). PCR amplification of the *ITS*, *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* genes was performed in a 50 µl reaction mixture that contained 100 ng of total genomic DNA, 2 µl of each primer (10 uM), 2 µl of 25 mM $MgCl_2$, 5 µl of 10X PCR buffer, 4 µl of 2.5 uM dNTPs, 0.5 µl of *Taq* DNA polymerase (5 U/ µl) (Vivantis, Malaysia) and sterile deionized water to adjust the final volume. The amplification was carried out in a Thermal cycler (Veriti 96-Well Thermal Cycler, Applied Biosystems, USA). The PCR products were separated on a 1% agarose gel using TBE and stained with ethidium bromide. The DNA fragments were purified with a GF1 PCR CLEAN-UP Kit (Vivantis, Malaysia) before being sequenced.

Table 3.2 Forward (F) and reverse (R) PCR primers and the temperature profile of the Polymerase Chain Reaction in this study

Name	Sequences (5′-3′)	Temperature profile of PCR	References
ITS			
ITS1 (F)	TCCGTAGGTGAACCTGCGG	94 °C, 4 min	
ITS4 (R)	TCCTCCGCTTATTGATATGC	94 °C, 1 min 55 °C, 1 min 72 °C, 1.5 min } 35 cycles 72 °C, 10 min	White *et al*. (1990)
nrSSU			
NS1 (F)	GTAGTCATATGCTTGTCTC	94 °C, 4 min	
NS2 (R)	GGCTGCTGGCACCAGACTTGC	94 °C, 1 min 55 °C, 1 min 72 °C, 1.5 min } 35 cycles 72 °C, 10 min	White *et al*. (1990)
nrLSU			
LROR (F)	ACCCGCTGAACTTAAGC	94°C, 4 min	
LR7 (R)	TACTACCACCAAGATCT	94°C, 1 min 47°C, 45 s 72°C, 2 min } 38 cycles 72°C, 10 min	Vilgalys and Hester (1990)
EF-1α			
EF-983F (F)	GCYCCYGGHCAYGGTGAYTTYAT	95°C, 5 min	
EF-2218R (R)	GACTTGACTTCRGTVGTGAC	95°C, 1 min 55°C, 1 min 72°C, 1 min } 35 cycles 72°C, 10 min	Currie *et al*. (2003)
rpb1			
CRPB1 (F)	CCWGGYTTYATCAAGAARGT	95°C, 5 min	
RPB1Cr (R)	CCNGCDATNTCRTTRTCCATRTA	95°C, 1 min 55°C, 1 min 72°C, 1 min } 35 cycles 72°C, 10 min	Castlebury *et al*. (2004)

3.4.4 DNA sequencing and phylogenetic analysis of entomopathogenic fungi

The sequencing was performed using Macrogen Advancing through Genomics (Macrogen Inc., Korea). The sequence data of the partial *ITS*, *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* were compared with sequences in the National Center for Biotechnology Information data bank using the BLAST program (www.ncbi.nih.gov/blast). The novel partial sequences were deposited in the GenBank nucleotide sequence database. The phylogenetic analyses of the *ITS* and the combined data sets of *ITS*, *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* were generated using MEGA 6 (Tamura *et al*., 2013), PhyML 3.0 (Guindon *et al*., 2010) and MRBAYES 3.04b (Huelsenbeck and Ronquist, 2001). The Neighbor Joining (NJ) tree was analyzed using the Kimura 2 parameter method for pairwise deletion at uniform rates by MEGA 6

(Tamura *et al*., 2013). Bootstrap support was estimated for 1000 replicates. The Maximum Likelihood (ML) was implemented in PhyML 3.0 (Guindon *et al*., 2010). Branch support was calculated using approximate likelihood ratio tests (Anisimova and Gascuel, 2006). The phylogenetic relationship based on Bayesian methods was performed in MRBAYES 3.04b with a general time reversible plus proportion invariant plus gamma (GTR+I+G) model of nucleotide substitution as the best fit and a gamma distribution rate variation across sites for both combined data sets (Huelsenbeck and Ronquist 2001). The best-fit model for Bayesian analysis was selected by hierarchical likelihood ratio tests and implemented in jModeltest v2.1.4 (Darriba *et al*., 2015). Bayesian analysis was run for 10,000,000 generations, with a sampling frequency of 100 generations. The fungus *Bionectria ochroleuca* was used as the outgroup.

3.5 Isolation of *Colletotrichum* spp. causal agent of chili anthracnose

The plant pathogenic fungi *Colletotrichum* spp. were isolated from infected chili fruits with typical disease symptom using the tissue transplanting technique as described in 3.3. The fungal was identified based on conidia morphological characteristics and then confirmed by carrying out pathogenicity tests in strict conformity with Koch's postulates.

3.6 *In vitro* primary screening of antagonistic entomopathogenic fungi

Forty four isolates of entomopathogenic fungi were tested for their antagonistic activity against two isolates of *Colletotrichum* spp. by the dual culture plate method. Fungal hypha tips of all isolates of the entomopathogenic fungi were cut with a 7 mm diameter cork borer and placed on PDA 10 mm from the plate periphery, while a mycelium plug of the pathogen was placed on the opposite side at 70 mm length. The dual culture plate was incubated at 27-30 °C for 14 days. The control plates consisted of individual cultures of the pathogen. After 14 days, the dual culture plates were evaluated for antagonistic activity to reduce pathogen colony expansion. The percentage of mycelial growth reduction (PGI-1) was calculated using the formula:

PGI-1(%) = KR-R1/KRx100,

where KR represents the fungal growth radius (mm) of the control culture and R1 represents the fungal growth radius distance (mm) in the direction of the entomopathogenic fungi (Korsten *et al*., 1995). The PGI-1 was categorized from 0 to 4, where 0 = no growth inhibition, 1 = 1-25% growth inhibition, 2 =26-50% growth inhibition, 3 = 51-75% growth inhibition and 4 = 76-100% growth inhibition. The data from each experiment was analyzed with an analysis of variance, and means were compared by Duncan's Multiple Range Test (DMRT) (at P = 0.05). The isolates that inhibited the mycelial growth of the plant pathogenic fungi were selected as candidate antagonistic biocontrol agents for further study.

3.7 Confirmation of antagonistic activity against the plant pathogenic *Colletotrichum* spp.

The potential selected isolates of antagonistic entomopathogenic fungi were tested for their antagonistic activity against five isolates of *C. capsici* (CcC) and five isolates of *Colletotrichum* spp. (CgC) using the dual culture method as described in the primary screening test. After 14 days, the dual culture plates were evaluated for antagonistic activity that reduced the pathogen colony expansion, as described in 3.6.

3.8 Effect of mycelial extract and culture filtrate on mycelial growth of *Colletotrichum* spp.

3.8.1 Preparation of culture filtrate

The 20-day-old mycelial discs of potential selected antagonistic isolates were cut with a sterilized cork borer to a diameter of 7 mm under aseptic conditions and placed into 25 mL of induced medium (Huang *et al*., 2009). The seed culture was incubated without shaking at 28°C for 20 days. The induced medium was prepared by dissolving 35 g of sucrose, 5 g of peptone, 2.5 g of yeast extract, 0.5 g of $MgSO_4$, 1 g of KH_2PO_4 and 0.05 g of thiamine in distilled water, which was adjusted to a final volume of 1000 mL. The mycelium on the surface of the induced culture was collected and

dried at 50°C for 2-3 days, while the culture filtrate was also collected and then filtered through a 0.2 μm filter before being used.

3.8.2 Preparation of mycelial extract

The dried mycelium from 3.8.1 was powdered using a mortar and pestle. A known weight of the dried mycelium was placed in a conical screw cap tube. A known volume of 50% ethanol was used as a solvent and added to the dried mycelium. The mycelium suspension (100 mg/mL) was sonicated with a High Intensity Ultrasonic Processor (Model VCX 750, USA). This step was performed on ice for a total of 5 min in 10 s bursts and 2 s gaps for cooling. The sonicated solutions were centrifuged (Tomy MX-301, Japan) at 9,100 g for 5 min and filtered through a 0.2 μm filter before being used further (Sangdee *et al.*, 2015).

3.8.3 *In vitro* antagonistic activity test

3.8.3.1 Tube dilution assay

The culture filtrate and mycelium extracts from 3.8.1 and 3.8.2, respectively, were diluted with twofold dilutions in PDB medium for a 5 mL total volume. The diluted mycelial extracts and culture filtrates were incubated with a mycelial disk of the plant pathogenic fungi *Colletotrichum* spp. for 14 days at 28ºC. The control tube contained 5 mL PDB that was incubated with the individual plant pathogenic fungi. The mycelial growth was categorized on a scale of 0 to 2, where 0 = no mycelial growth, 1 = growth limited around mycelial disk and 2 = mycelia overgrown into liquid medium (Alvindia and Natsuaki, 2008). The experiment was done with three replications.

3.8.3.2 Pore plate technique

For this, 5 ml of sterile culture filtrates and mycelial extracts from 3.8.1 and 3.8.2, respectively, were mixed in 100 ml PDA medium before being plated into 90 mm Petri dishes. Seven-day-old mycelial discs of each pathogen were cut with a sterilized cork borer to a diameter of 7 mm under aseptic conditions and placed into the 25 mL PDA plates containing sterile culture filtrate and mycelial extracts. The plates

were incubated at 28°C. The mycelial growth was determined daily for up to 14 days. The percentage of mycelial growth reduction (PGI-2) was calculated using the formula:

PGI-2(%) = KR-R1/KRx100,

where KR represents the fungal growth radius (mm) of the control culture and R1 represents the fungal growth radius distance (mm) in the direction of the entomopathogenic fungi (Korsten *et al*., 1995). The PGI was categorized from 0 to 4, where 0 = no growth inhibition, 1 = 1-25% growth inhibition, 2 =26-50% growth inhibition, 3 = 51-75% growth inhibition and 4 = 76-100% growth inhibition. The data from each experiment was analyzed with an analysis of variance, and means were compared by Duncan's Multiple Range Test (DMRT) (at $P = 0.05$).

3.9 Effect of mycelium extract on spore germination of *Colletotrichum* spp.

The inoculums of *Colletotrichum* spp. were prepared by culturing on PDA medium until sporulation. Conidia were harvested by flooding the cultures with distilled water. The concentrations of propagules in suspension were standardized with the aid of a hemacytometer to 1 x 10^4 conidia ml^{-1} for each fungus. The conidial germination test was determined on PDA plates containing 10% sterile mycelial extract, 10% culture filtrate and 10% of 50% ethanol. The plates were incubated at 28 °C. Then 100 spores of all treatments were determined within 24 hours under a light microscope. The inhibition rate of conidial germination (ICG) was calculated using the formula:

ICG (%) = (1-T/C) x 100,

where T represents the germination rate of the treatment and C represents the germination rate of the control (Kwak *et al*., 2012). The experiment was done with five replicates. Geminated germ tube = the length of the germ tube more than the diameter of the spore, not germinated germ tube = the length of the germ tube less than or equal to spore diameter.

3.10 Control of anthracnose on chili fruit by detached fruit bioassay

Mature chili fruits were surface sterilized in 70% alcohol for five minutes, washed with sterile distilled water and blotted dry on sterilized filter paper before being used in this study.

Inoculum of *Colletotrichum* spp. was cultured on PDA medium until sporulation and prepared as described in 3.9. The mycelial extract and culture filtrate of the potential selected antagonistic isolates were applied to a pin point wound of mature chili fruits one day before the conidia suspensions of the pathogen were applied. The disease severity was observed daily for seven days. The severity index (SI) of the anthracnose symptoms was categorized based on the lesion diameter, SL = no lesion or symptomless, HR = highly resistant (1.0-4.9 mm), MR = moderately resistant (5.0-9.9 mm), MS = moderately susceptible (10.0-19.9) and HS = highly susceptible (>20.0 mm) (Hartman and Wang, 1992).

3.11 Data analysis

The experiments from 3.6, 3.7 and 3.8 (3.8.3.2) were performed in triplicate and the results were expressed as mean ± S.D. (standard deviation). Analysis of variance (ANOVA) was carried out to determine any significant differences in measurements using SPSS statistical software (SPSS 14 for Windows; SPSS Inc., Chicago, IL, USA). The significance of the differences between the means was determined using Duncan's Multiple Range Test (DMRT) and the differences were considered to be significant at $p = 0.05$.

CHAPTER 4

RESULTS

4.1. Sample collection and isolation of entomopathogenic fungus from Northeastern Thailand

A total of 134 samples of cicada nymphs infected with entomopathogenic fungi were collected from six areas of mixed deciduous forest from Northeastern Thailand during 2012-2014. Only 44 isolates of entomopathogenic fungi were isolated as described in Table 4.1 and they were cultured on PDA medium before being used for further study.

Table 4.1 Location, code and number of isolate of entomopathogenic fungi from Northeastern Thailand

Provinces	Year	Number of isolate	Code
Maha Sarakham (MK)	2012	10	Cod-MK1201, Cod-MK1202,Cod-MK1203,Cod-MK1204, Cod-MK1205,Cod-MK1206,Cod-MK1207,Cod-MK1208, Cod-MK1209,Cod-MK1210
Maha Sarakham (MK)	2013	12	Cod-MK1301,Cod-MK1302,Cod-MK1303,Cod-MK1304, Cod-MK1305,Cod-MK1309,Cod-MK1311,Cod-MK1319, Cod-MK1321, Cod-MK1324, Cod-MK1325,Cod-MK1329
Roi Et (RE)	2012	3	Cod-RE1201,Cod-RE1202,Cod-RE1203
Roi Et (RE)	2013	1	Cod-RE1301
Nong Bua Lam Phu (NB)	2013	8	Cod-NB1301,Cod-NB1302,Cod-NB1303,Cod-NB1304, Cod-NB1305, Cod-NB1306,Cod-NB1307, Cod-NB1308
Loei (LO)	2013	1	Cod-Loei1301
Nakhon Phanom (NN)	2013	7	Cod-NN1301,Cod-NN1302,Cod-NN1303,Cod-NN1304, Cod-NN1305,Cod-NN1306,Cod-NN1307
Sakon Nakhon (SN)	2014	2	Cod-SN1401,Cod-SN1402

4.2 Colony morphology of the entomopathogenic fungi

The fungal colony tips of all isolates were subcultured and grown on PDA at 28°C for 25-30 days. After the incubation period, the fungal isolates were grouped. Based on colony characteristics, the fungal isolates were divided into five groups. The first group (group A) comprised of the 31 isolates of the entomopathogenic fungi from 5 provinces (MK, RE, NB, NN and SN) that produced whitish cream colonies with slimy cream-yellow conidia at the center of colony. Colony diameter was ranged from 75-80 mm. Moreover, they had differences in their stroma-like stalks on PDA, especially isolate Cod-MK1305, Cod-NB1305, Cod-NB1306, Cod-SN1401, and some isolates (Cod-MK1203, Cod-RE1201) could not determine the stroma-like stalks (Figure 4.1). The second group (group B) consisted of four isolates from 4 provinces (MK, RE, LO and SN). The colony morphology of the isolates on PDA was categorized in to 2 groups. Group B-1 comprised of three isolates that produced white colonies with a slow growth rate (50-60 mm) and they did not produce conidia on PDA (Cod-RE1301, Cod-MK1202, Cod-SN1402). The remaining isolate had produced white colony with a fast growth rate (75 mm) without conidia production were assigned to group B-2 (Cod-Loei1301) (Figure 4.2). The third group (group C) consisted of five isolates from MK provinces that also produced white colonies but with a fast growth rate (75-80 mm). Moreover, they could produce conidia on the colonies. The forth group (group D) consisted of three isolates from 2 provinces (NN and RE) that produced white-yellow colonies on PDA with a fast growth rate (75-80) mm. The remaining isolate (Cod-NB1302) was categorized in group 5 (group E) and produce a pink colony with extremely slow growth rate (35-45 mm) and could produce mycelium like stalks and pink conidia on the colony (Figure 4.3). However, the colony morphology variations in each group were observed and these depended on isolates of the fungus. Moreover, the dendrogram that constructed form these morphological characters using the UPGMA cluster analysis was supported this study (Figure 4.4).

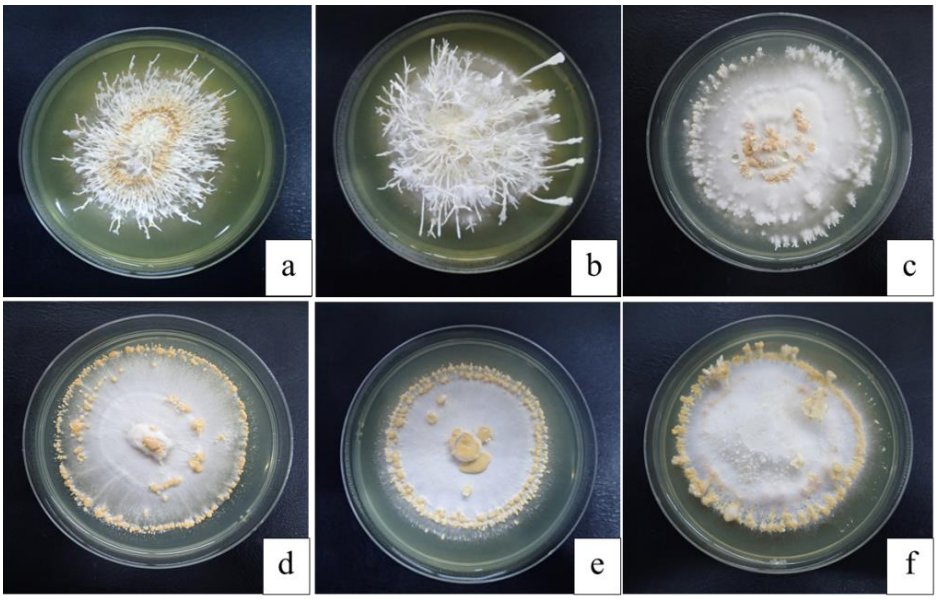

Figure 4.1 The colony morphology of entomopathogenic fungi on potato dextrose agar (PDA) that was categorized in group 1 after 25-30 days. **a** Cod-MK1305; **b** Cod-NB1305; **c** Cod-NB1306; **d** Cod-MK1203; **e** Cod-RE1201; **f** Cod-SN1401

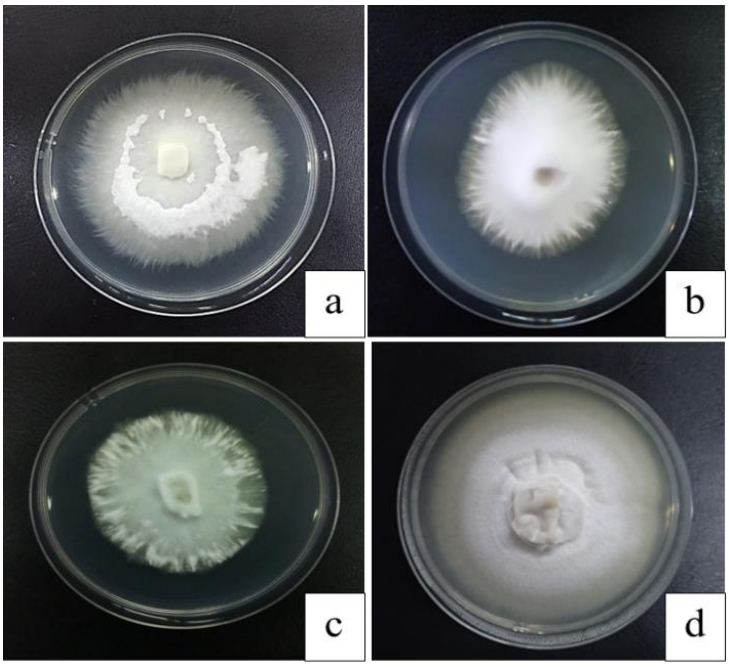

Figure 4.2 The colony morphology of entomopathogenic fungi on potato dextrose agar (PDA) that was categorized in group 2 after 25-30 days. **a** Cod-RE1301 (group B-1); **b** Cod-MK1202 (group B-1); **c** Cod-SN1402 (group B-1); **d** Cod-Loei1301 (group B-2)

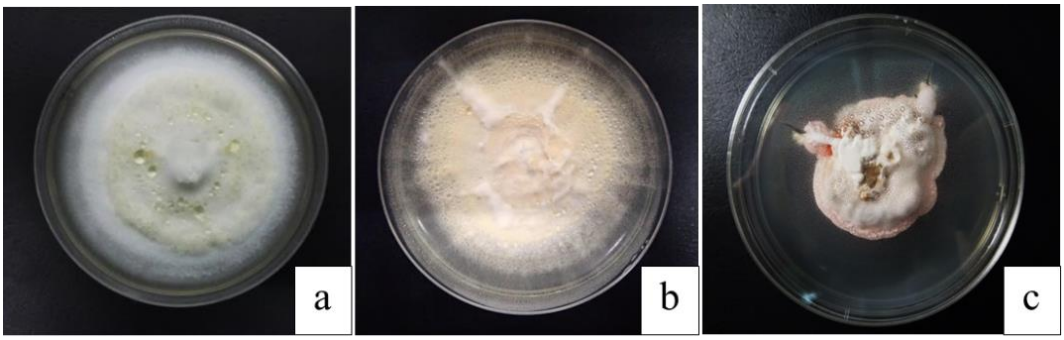

Figure 4.3 The colony morphology of entomopathogenic fungi on potato dextrose agar (PDA) after 25-30 days. **a** Cod-MK1311 (group C); **b** Cod-NN1302 (group D); **c** Cod-NB1302 (group E)

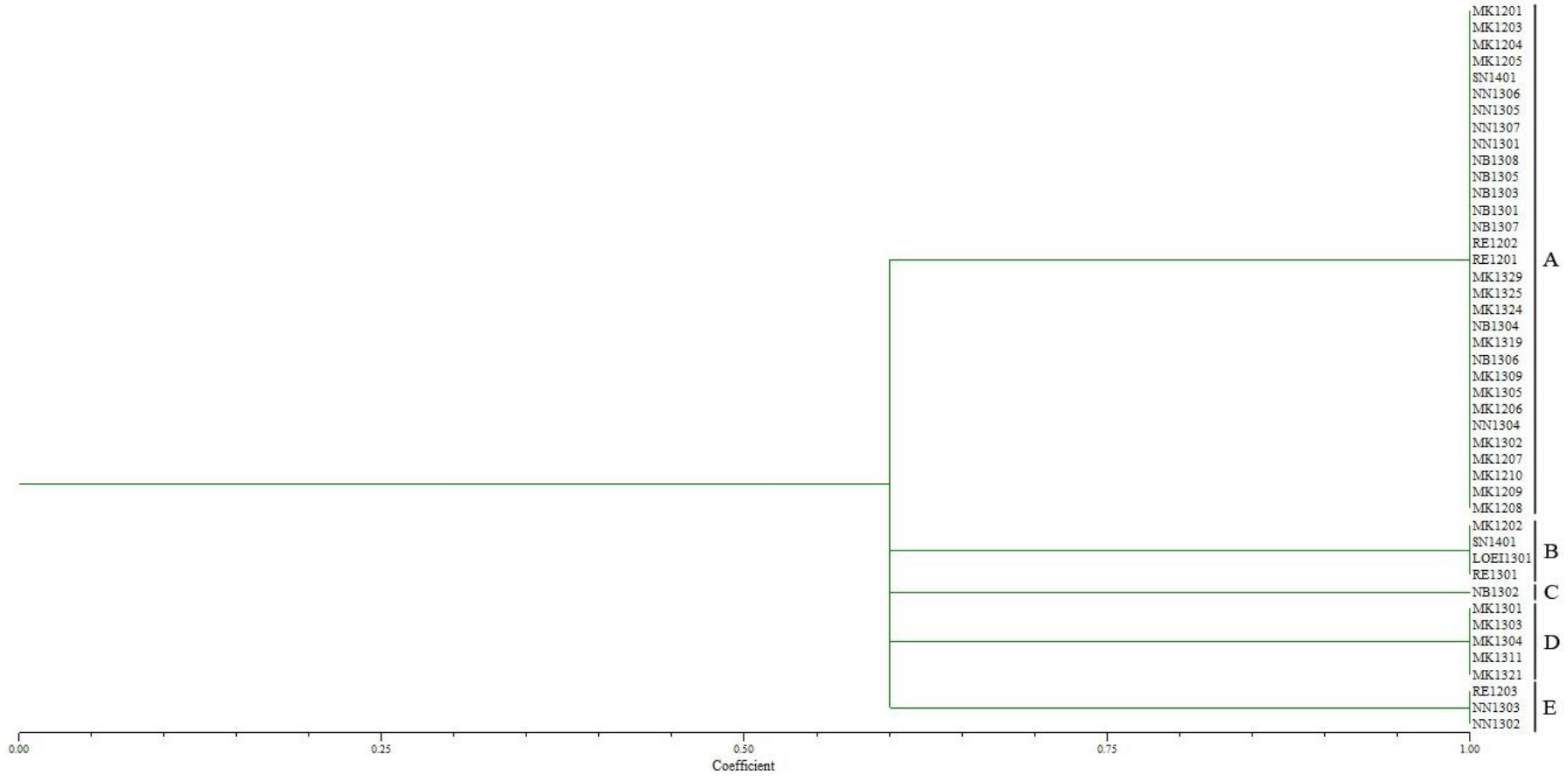

Figure 4.4 A dendrogram showing the relationships between 44 isolates of the entomopathogenic fungi were derived from cluster analysis of colony characteristics

4.3 Microscopic characteristics

The stromata, perithecia, ascus and ascospores of all the isolates of the entomopathogenic fungi were observed under a light microscope. The results revealed that the 44 isolates of the entomopathogenic fungi showed some variation in stroma structure, size and color of stromata size of perithecia and the stipe. Based on the stromata structures and perithecium shapes characteristics, the fungal isolated were divided into five groups. Group 1 comprised of six isolates that have the following characteristics: stroma solitary emerging from the head regions of the host, stalk cylindrical long, light brown to dark brown. Perithecia were completely immersed with long ovoid in shape measuring, 100–180 x 342-616 µm in size are shown in Figure 4.5. Group 2 consisted of 5 isolates that have the following characteristics: stroma arising from head per host, cylindrical reddish brown stromata erect, mostly branched, stalk cylindrical, fertile part are located at the terminal end with many at the sub-terminal part of the stroma, orange brown perithecial plates. Perithecia were semi-immersed narrowly ovoid to conoid in shape measuring, 184-284 x 606-866 µm in size are shown in Figure 4.6. Group 3 consisted of 11 isolates that have the following characteristics: stromata solitary, gregarious and branched emerging from head per host, stalk cylindrical, acuminate apex with branched, fertile part are clavate and acuminate apex, orange brown, pink-brownish, dark brown and brown to black. Perithecia were semi-immersed and completely immersed, narrowly ovoid to conoid and 100-240 x 499-837 µm in size are shown in Figure 4.7.Group 4 consisted of 17 isolates that have the following characteristics: stroma arising from the head and abdomen regions of the host, stalk cylindrical with mostly branched, acuminate apex, light brown to brown and perithecim could not determine are shown in Figure 4.8. Group 5 comprised five isolates have the following characteristics: stroma and perithecim on cicada nymph samples could not determine are shown in Figure 4.9. The microscopic characteristics of all isolates are shown in Table 4.2.

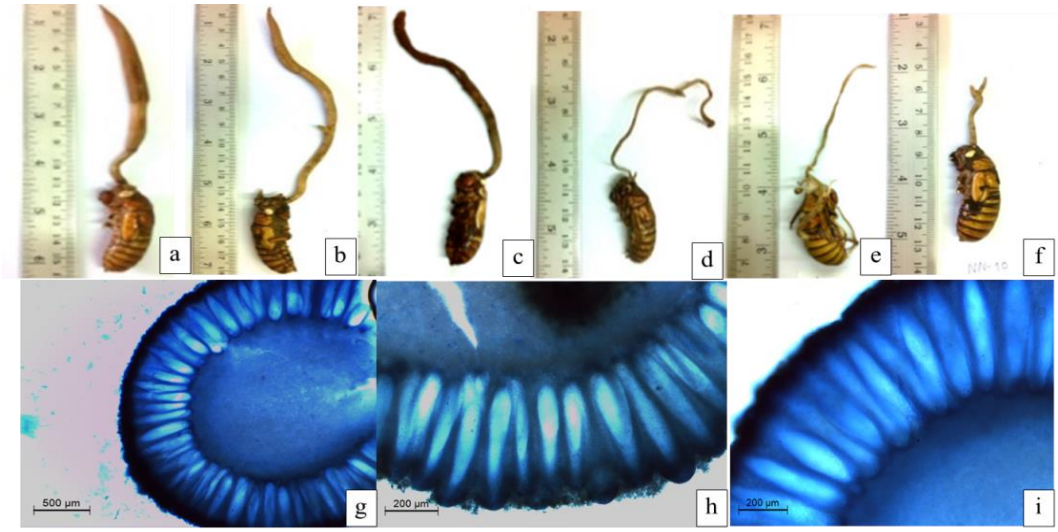

Figure 4.5 The stroma structure and perithecia of the entomopathogenic fungi group 1. **a-f** variation of the stroma structure on cicada nymph samples; **g-i** the cross section of perithecia structure examined under a light microscope. Scale bars = 500 µm (g), 200 µm (h-i)

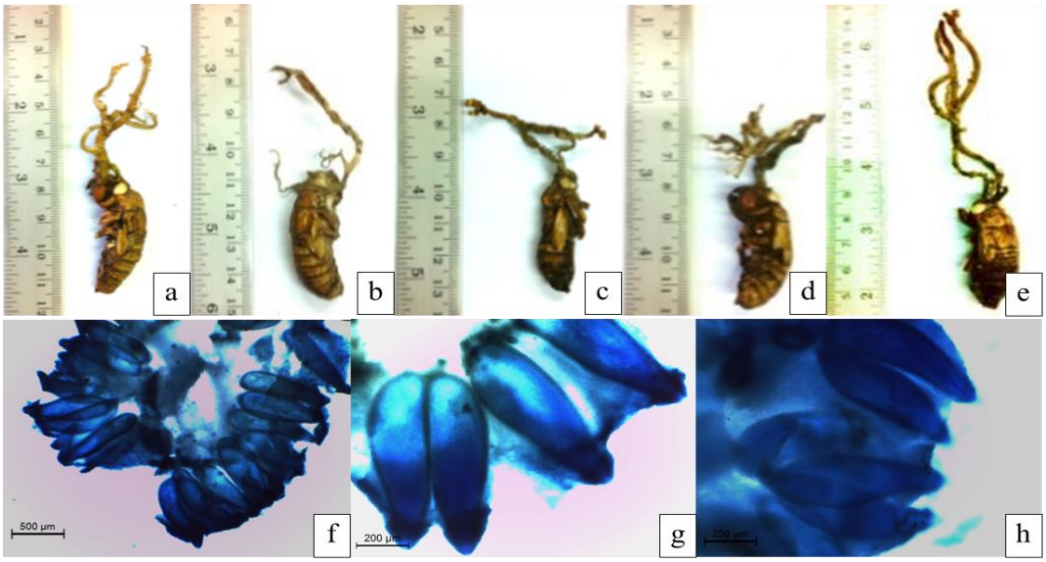

Figure 4.6 The stroma structure and perithecia of the entomopathogenic fungi group 2. **a-e** variation in the stroma structure on cicada nymph samples; **f-h** the cross section of perithecia structure examined under a light microscope. Scale bars = 500 µm (f), 200 µm (g-h)

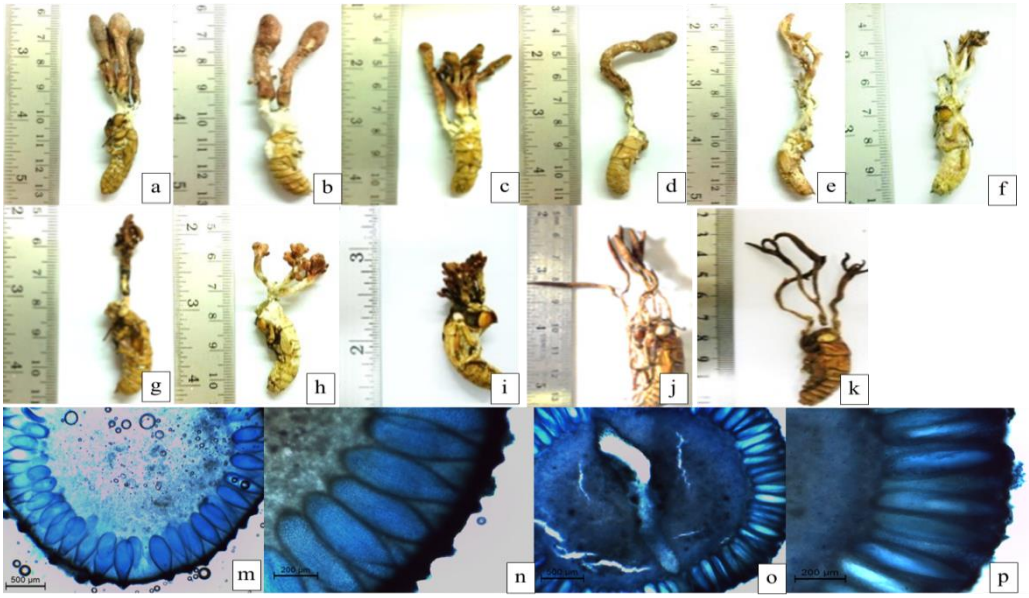

Figure 4.7 The stroma structure and perithecia of the entomopathogenic fungi group 3. **a-l** variation in stroma structure on cicada nymph samples; **m-p** the cross section of perithecia structure examined under a light microscope. Scale bars = 500 µm (m, o), 200 µm (n-p)

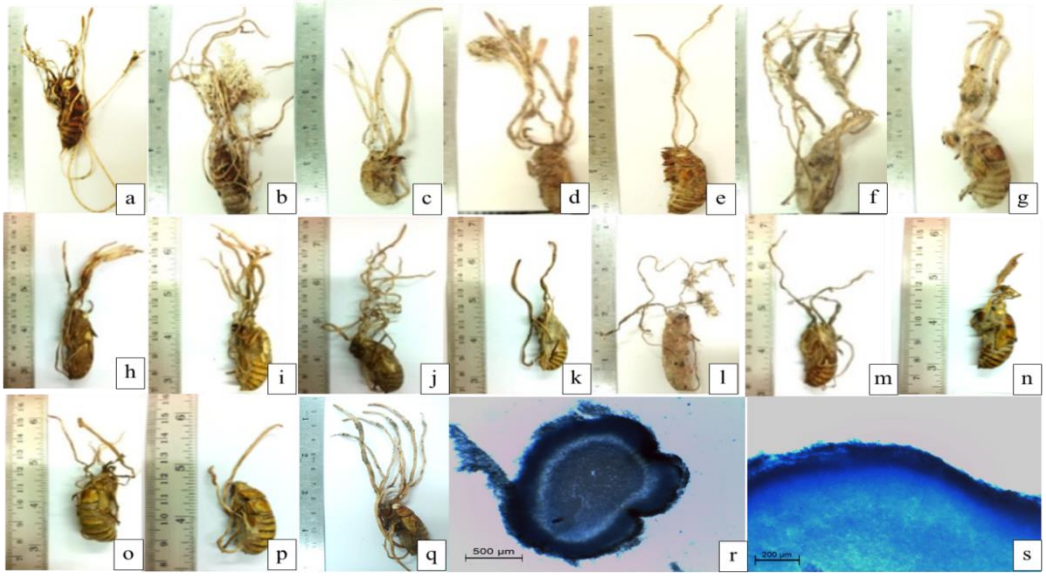

Figure 4.8 The stroma structure and perithecia of the entomopathogenic fungi group 4. **a-q** variation in stroma structure on cicada nymph samples; **r-s** the cross section of perithecia structure examined under a light microscope. Scale bars = 500 µm (r), 200 µm (s)

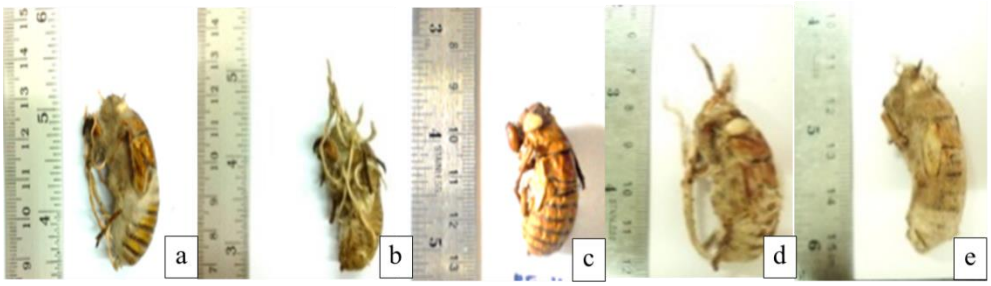

Figure 4.9 The stroma structure and perithecia of the entomopathogenic fungi group 5. **a-e** cicada nymph samples

4.4 Morphological grouping of the entomopathogenic fungi based on colony and microscopic characteristics

After the colony morphology and microscopic characteristic were categorized and assayed, these characters were scored as binary data based on the presence (1) or absence (0) of their morphological characters. Next, the mophological characteristic relationships were determined and a dendrogram was constructed using the UPGMA cluster analysis. Two main groups were categorized (group A and B) with similarity coefficients of 0.27 (Figure 4.10). The first group (group A) was subdivided into two distinct groups, A1 and A2, at a similarity coefficient of 0.34. Group A1 included 22 isolates and group A2 consisted of 12 isolates. The entomophathogenic fungi in group A had the following characteristics: 1-15 stromata arising from the head and abdomen regions of the host, stalk cylindrical with mostly branched, acuminate apex, clavate, light brown to brown, reddish brown and dark brown. However, some isolates could produce cottony colonies on PDA with a color of white-cream and could produce slimy cream-yellow conidia. Some isolates could produce white color colonies and that did not produce conidia. Whereas group B comprised of 10 isolates that had 1-8 stromata arising from the head and abdomen per host, reddish brown, stalk cylindrical, acuminate apex with branched, fertile part: clavate , orange brown, pink-brownish and dark brown. In addition, the colonies of group B on PDA were white in color, while one isolate (Cod-NB1302) had a pink color and slow growth rate. These were indicated that the entomopathogenic fungi have a highly variations on external morphology, colony morphology and microscopic characteristics.

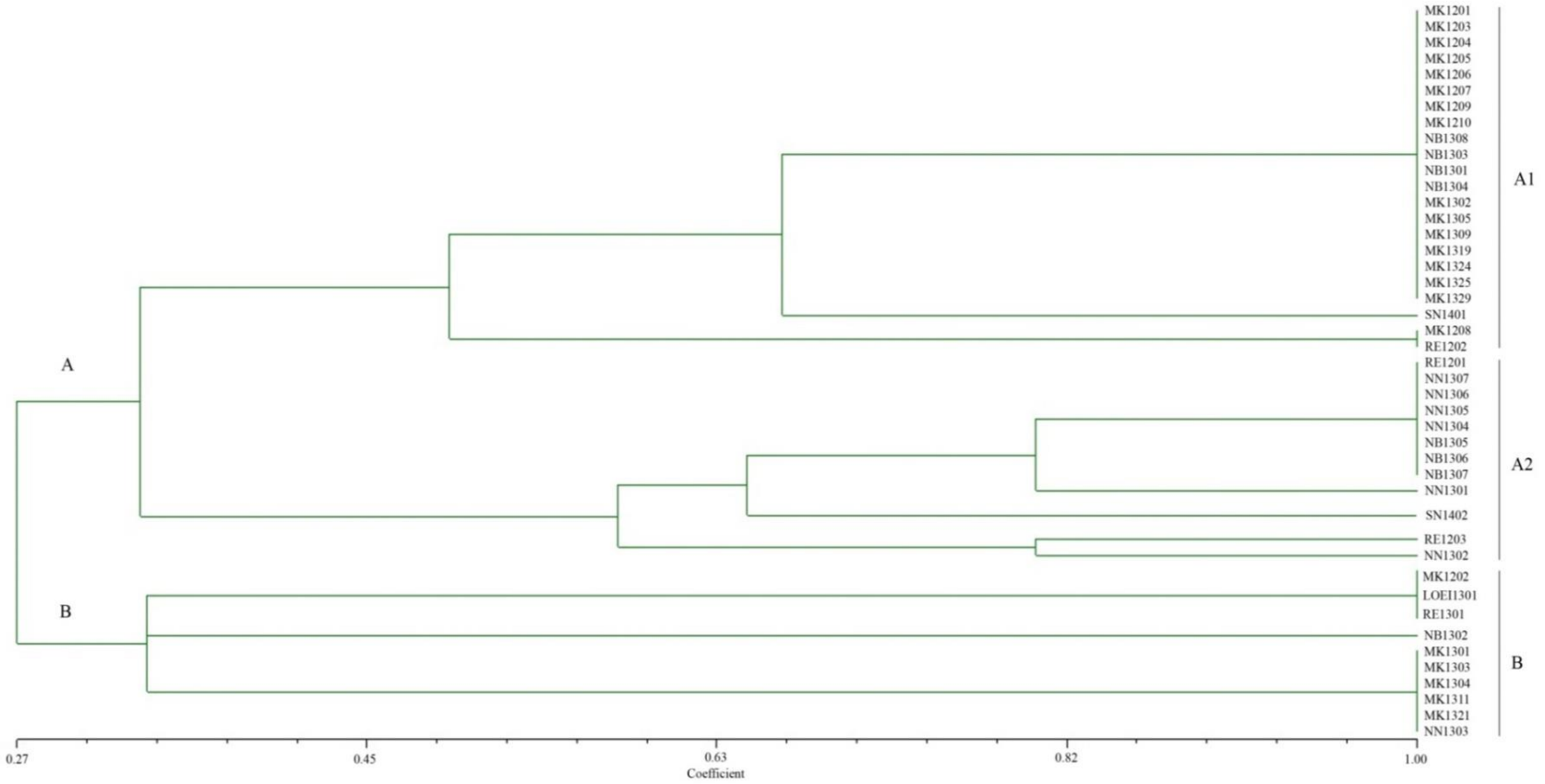

Figure 4.10 A dendrogram showing the relationships between 44 isolates of the entomopathogenic fungi were derived from cluster analysis of morphological character

Table 4.2 Morphological characteristics of the entomopathogenic fungi collected from 6 provinces in Northeastern Thailand

Species	Location	Morphology features						
		Stromata (mm)	Perithecium length (µm)	Asci length (µm)	Ascospores or part-spore length (µm)	Colony group	Microscopic group	Morphological group
P. nipponicus Cod-MK1201	MK	10 stromata arising from head and abdomen regions of the host, cylindrical, acuminate apex, 1x40-70 mm, brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1203	MK	8-10 stromata arising from head and abdomen regions of the host, cylindrical, acuminate apex,1x50-60 mm, brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1204	MK	5 stromata arising from head and abdomen regions of the host, cylindrical, acuminate apex,1x50-60 mm, light brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1205	MK	4 stromata arising from head regions of the host,cylindrical, acuminate apex,1-2x50-55 mm, light brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1206	MK	4 stromata arising from head and abdomen regions of the host, cylindrical, acuminate apex,1x40-60 mm, brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1207	MK	6 stromata arising from head regions of the host,cylindrical, acuminate apex,1-2x80-90 mm, brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1208	MK	-	-	-	-	A	5	A1

Table 4.2 (Cont.)

Species	Location	Morphology features						
		Stromata (mm)	Perithecium length (µm)	Asci length (µm)	Ascospores or part-spore length (µm)	Colony group	Microscopic group	Morphological group
P. nipponicus Cod-MK1209	MK	2 stromata arising from head regions of the host,cylindrical, acuminate apex,1-2x75-120 mm, brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1210	MK	3 stromata arising from head and abdomen regions of the host ,cylindrical, acuminate apex,1x20-50 mm, light brown	-	-	-	A	5	A1
P. nipponicus Cod-MK1302	MK	7 stromata arising from head and abdomen regions of the host ,cylindrical, acuminate apex,1x17-35 mm, brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1305	MK	3 stromata arising from head and abdomen regions of the host ,cylindrical, acuminate apex,1-2x30-60 mm, brown	-	-	-	A	1	A1
P. nipponicus Cod-MK1309	MK	3 stromata arising from abdomen regions of the host ,cylindrical, acuminate apex,1x25-50 mm, brown	-	-	-	A	4	A1
P. nipponicus Cod-MK1319	MK	1 stromata arising from abdomen regions of the host , cylindrical, acuminate apex,1x30 mm, brown	-	-	-	A	5	A1
P. nipponicus Cod-MK1324	MK	3 stromata arising from head and abdomen regions of the host ,cylindrical, acuminate apex,2x50-70 mm, brown	-	-	-	A	4	A1

Table 4.2 (Cont.)

Species	Location	Morphology features						
		Stromata (mm)	**Perithecium length (µm)**	**Asci length (µm)**	**Ascospores or part-spore length (µm)**	**Colony group**	**Microscopic group**	**Morphological group**
P. nipponicus Cod-MK1325	MK	6 stromata arising from head and abdomen regions of the host, cylindrical, acuminate apex,1x15-20 mm, brown	-	-	-	A	5	A1
P. nipponicus Cod-MK1329	MK	3 stromata arising from head and abdomen regions of the host, cylindrical, acuminate apex,1x30-70 mm, brown	-	-	-	A	4	A1
P. nipponicus Cod-RE1201	RE	6 stromata arising from head of the host ,cylindrical, 1x15 mm; fertile part1-3x20-25 mm, acuminate apex, dark brown	Completely immersed, narrowly ovoid to conoid,100-120x519-557 µm	Cylindrical,3-4.7x 150-166 µm	Filiform, 0.1-83.5 µm	A	3	A2
P. nipponicus Cod-RE1202	RE	-	-	-	-	A	5	A1
P. nipponicus Cod-NB1301	NB	1-15 stromata arising from head per host, gregarious, clavate, pink-brownish, stalk cylindrical with mostly branched; 1x10 mm	-	-	-	A	3	A1
P. nipponicus Cod-NB1303	NB	1-10 stromata arising from head per host, gregarious, dark brown, stalkcylindrical;5x20mm	-	-	-	A	3	A1
P. nipponicus Cod-NB1304	NB	3 stromata arising from head per host, gregarious, pink-brownish, stalk cylindrical with many branched; 1.5-2x15 mm	-	-	-	A	3	A1

Table 4.2 (Cont.)

Species	Location	Morphology features						
		Stromata (mm)	**Perithecium length (µm)**	**Asci length (µm)**	**Ascospores or part-spore length (µm)**	**Colony group**	**Microscopic group**	**Morphological group**
P. nipponicus Cod-NB1305	NB	2 stromata arising from head per host, reddish brown, stalk cylindrical; 4x40-45 mm; fertile part;4-5x7-10 mm, clavate , dark brown	Semi- immersed narrowly ovoid to conoid,200-240x 623-684 µm	Cylindrical; 4.9-5.1x270-331 µm, ascus cap; 2x4.9 µm	Filiform, part-spore; 0.6-0.7x8.7-9.2 µm	A	3	A2
P. nipponicus Cod-NB1306	NB	3 stromata arising from head per host, reddish brown, stalk cylindrical; 5x35-40 mm; fertile part;2-6x7-10 mm, clavate, dark brown	Semi- immersed narrowly ovoid to conoid,200-220x 622-700 µm	Cylindrical; 4-6 x252-264 µm, ascus cap; 2.7-2.8x6.0-6.2 µm	Filiform, part-spore; 0.4-0.7x9.4-10.5 µm	A	3	A2
P. nipponicus Cod-NB1307	NB	1 stromata arising from head per host, reddish brown, stalk cylindrical; 4x45 mm; fertile part;5x10 mm, clavate, dark brown	Semi- immersed narrowly ovoid to conoid,200-220x 773-837 µm	Cylindrical; 5.4-5.9x250-289 µm, ascus cap; 1.5-2.7 x4.0-4.7 µm	Filiform, part-spore; 0.4-1.0x8.9-11.7 µm	A	3	A2
P. nipponicus Cod-NB1308	NB	2 stromata arising from head per host, clavate, brown, stalk cylindrical with mostly branched; 3x40-45 mm	-	-	-	A	3	A1
P. nipponicus Cod-NN1301	NN	1 stromata arising from head per host, cylindric with acuminate apex, dark brown, stalk cylindrical; 3x75 mm, fertile part;4x40 mm,	Completely immersed narrowly ovoid,111-120 x556-592 µm	Cylindrical; 1.9-3.8x168-262 µm,	not determined	A	1	A2

Table 4.2 (Cont.)

Species	Location	Morphology features						
		Stromata (mm)	**Perithecium length (µm)**	**Asci length (µm)**	**Ascospores or part-spore length (µm)**	**Colony group**	**Microscopic group**	**Morphological group**
P. nipponicus Cod-NN1304	NN	1 stromata arising from head per host, cylindric reddish brown stromata, erect, mostly branched , stalk cylindrical; 3-4x25-40 mm, fertile part are located at the terminal end with many at the sub-terminal part of the stroma,orange brown perithecial plates	Semi-immersed narrowly ovoid to conoid,215-284x716-842 µm	Cylindrical; 0.5-1.0 x157-252 µm	Filiform, part-spore; 0.2-0.4x5.1-6.0 µm	A	2	A2
P. nipponicus Cod-NN1305	NN	1 stromata arising from head per host, cylindric brown-black stromata, erect, mostly branched , stalk cylindrical; 1-3x25-30 mm, fertile part are located at the terminal end with many at the sub-terminal part of the stroma	Semi-immersed narrowly ovoid to conoid,184-250x720-866 µm	Cylindrical; 0.5-0.8 x180-189 µm	Filiform, part-spore; 0.2-0.4x5.2-5.8 µm	A	2	A2
P. nipponicus Cod-NN1306	NN	1 stromata arising from head per host, cylindric dark brown stromata, erect, branched , stalk cylindrical; 1-3x45 mm, fertile part are located at the terminal end with many at the sub-terminal part of the stroma	Semi-immersed narrowly ovoid to conoid,190-214x606-678 µm	Cylindrical; 0.5-0.6 x156-180 µm	Filiform, part-spore; 0.2-0.5x4.2-5.5 µm	A	2	A2

Table 4.2 (Cont.)

Species	Location	Morphology features						
		Stromata (mm)	**Perithecium length (µm)**	**Asci length (µm)**	**Ascospores or part-spore length (µm)**	**Colony group**	**Microscopic group**	**Morphological group**
P. nipponicus Cod-NN1307	NN	1 stromata arising from head per host, cylindric reddish brown stromata, erect, mostly branched , stalk cylindrical; 1-2x35 mm, fertile part are located at the terminal end with many at the sub-terminal part of the stroma,orange brown perithecial plates	Semi- immersed narrowly ovoid to conoid,180-200x610-755 µm	Cylindrical; 0.5-1.2 x198-250 µm,	Filiform, part-spore; 0.2-0.5 x 5.0-5.2 µm	A	2	A2
P. nipponicus Cod-SN1401	SN	2 stromata arising from head per host, cylindric reddish brown stromata, erect, branched , stalk cylindrical; 1-3x75-80 mm, fertile part are located at the terminal end with many at the sub-terminal part of the stroma	Semi- immersed narrowly ovoid to conoid,200-284x747-808 µm	-	-	A	2	A1
O. longissima Cod-MK1202	MK	8 stromata arising from head and thorax regions of the host ,long stalk cylindrical, acuminate apex,1x70-80 mm, light brown	-	-	-	B	4	B
O. longissima Cod-RE1301	RE	1 stromata arising from head regions of the host ,long stalk cylindrical, acuminate apex,2x75 mm, fertile part;1x10 mm, dark brown	Completely immersed long ovoid,100-120 x499-571 µm	Cylindrical;1 .6-2.0 x116-140 µm,	Filiform, part-spore; 0.2-0.3x 8.0-10.2 µm	B	1	B
O. longissima Cod-SN1402	SN	1 stromata arising from head of the host ,long stalk cylindrical, acuminate apex,5x80 mm, fertile part;5x20 mm, dark brown	Completely immersed long ovoid,150-180 x590-616 µm	Cylindrical;2 .0-3.2 x150-170 µm	-	B	1	A2

Table 4.2 (Cont.)

Species	Location	Morphology features						
		Stromata (mm)	Perithecium length (µm)	Asci length (µm)	Ascospores or part-spore length (µm)	Colony group	Microscopic group	Morphological group
O. longissima Cod-Loei1301	LO	1 stromata arising from head per host, gregarious, clavate, dark brown, stalk cylindrical with mostly branched; 1-2x10-20 mm	-	-	-	B	3	B
Simplicillium spp. Cod-MK1301	MK	8 arising from head and abdomen regions of the host , cylindrical, acuminate apex,1x50-80 mm, light brown	-	-	-	D	4	B
Simplicillium spp. Cod-MK1303	MK	2 arising from head and abdomen regions of the host, cylindrical, acuminate apex,1x35 mm, light brown	-	-	-	D	4	B
Simplicillium spp. Cod-MK1304	MK	4 arising from head and abdomen regions of the host, cylindrical, acuminate apex,1x40 mm, light brown	-	-	-	D	4	B
Simplicillium spp. Cod-MK1311	MK	10 arising from head and abdomen regions of the host, cylindrical, acuminate apex,1x40-80 mm, light brown	-	-	-	D	4	B
Simplicillium spp. Cod-MK1321	MK	7 arising from head and abdomen regions of the host, cylindrical, acuminate apex,2x40-70 mm, light brown	-	-	-	D	4	B

Table 4.2 (Cont.)

Species	Location	Morphology features						
		Stromata (mm)	**Perithecium length (µm)**	**Asci length (µm)**	**Ascospores or part-spore length (µm)**	**Colony group**	**Microscopic group**	**Morphological group**
M. chlamydosporia Cod-RE1203	RE	4 stromata arising from head regions of the host, cylindrical, acuminate apex with branched;1-2x30-45 mm, fertile part;2-3x20-30 mm, brown to black	Completely immersed long ovoid,104-130 x459-520 µm	Cylindrical; 1.5-2.0x120-170 µm	Filiform, 1x102 µm part-spore; Rod-shape; 0.4-0.9x8.7-11.5 µm	E	3	A2
M. chlamydosporia Cod-NN1302	NN	1 stromata arising from head per host, cylindric with acuminate apex, dark brown, stipe cylindrical; 3x85 mm, fertile part;5x40 mm,	Completely immersed long ovoid,65-69 x342-403 µm	Cylindrical; 3.0-3.2x148-170 µm	-	E	1	A2
M. chlamydosporia Cod-NN1303	NN	1 stromata arising from head per host, acuminate apex with branched , brown, stipe cylindrical; 2x30 mm	-	-	-	E	1	B
O. sobolifera Cod-NB1302	NB	3-6 stromata arising from head per host, clavate, pink-brownish, stalk cylindrical; 2-3x25-30 mm	-	-	-	C	3	B

***** Abbreviation of location; MK, Maha Sarakham; RE, Roi Et; NB, Nong Bua Lam Phu; LO, Loei; NN, Nakhon Phanom; SN, Sakon Nakhon; -, Not determined

4.5 Identification of entomopathogenic fungi by molecular method

The novel 44 ITS sequences from the isolated entomopathogenic fungi were used to compare and initially identify the fungal species by comparison with the sequence data from GenBank using A BLAST search program (www.ncbi.nih.gov/blast). The results showed that the 44-taxa of entomopathogenic fungi consisted of 31 isolates, four isolates, five isolates, three isolates and one isolate that were most similar to the sequences of *P. nipponicus* (=*C. nipponica*) with 95-99% homology, *O. longissima* (=*C. longissima*) with 96-99% homology, whereas one isolate had 86% homology (Cod-MK1202), *S. obclavatum* with 99% homology, *M. chlamydosporia* with 99% homology and *O. sobolifera* (=*C. sobolifera*) with 91% homology, respectively. Next, a phylogenetic tree of the ITS region was generated with three different methods, neighbor joining (NJ), maximum likelihood (ML) and Bayesian analysis. The results showed that all of the phylogenetic trees gave similar tree topologies with four main clades. Thirty-one isolates of the entomopathogenic fungi were located in the same clade as *P. nipponicus* with a 0-1% genetic distance, four isolates were located in the same clade as *O. longissima* with genetic distance ranging from 0.5-5%, five isolates were located in the same clade as *S. obclavatum* with a 0% genetic distance, three isolates were located in the same clade as *M. chlamydosporia* with a 0% genetic distance and one isolate was located in the same clade as *O. sobolifera* with a 3-6% genetic distance (Figure 4.11). Similar findings were obtained from the combined data set results. Thirty-one isolates were also located in the same clade with *P. nipponicus* with a 0.9-2.8% genetic distance, four isolates were located in the same clade *O. longissima* with 1.4-3.7% genetic distance, five isolates were located in the same clade as *S. obclavatum* with a 0.1% genetic distance, three isolates were located in the same clade *M. chlamydosporia* with a 0.2% genetic distance and one isolate was also placed within the same clade as *O. sobolifera* with 3-8% genetic distance Based on the data from ITS and combined data set, therefore, the member isolates in Clade I were identified as *P. nipponicus*, clade II were identified as *O. longissima* and *O. sobolifera*, clade III were identified as *M. chlamydosporia* and clade IV were identified as *S. obclavatum* (Figure 4.12).

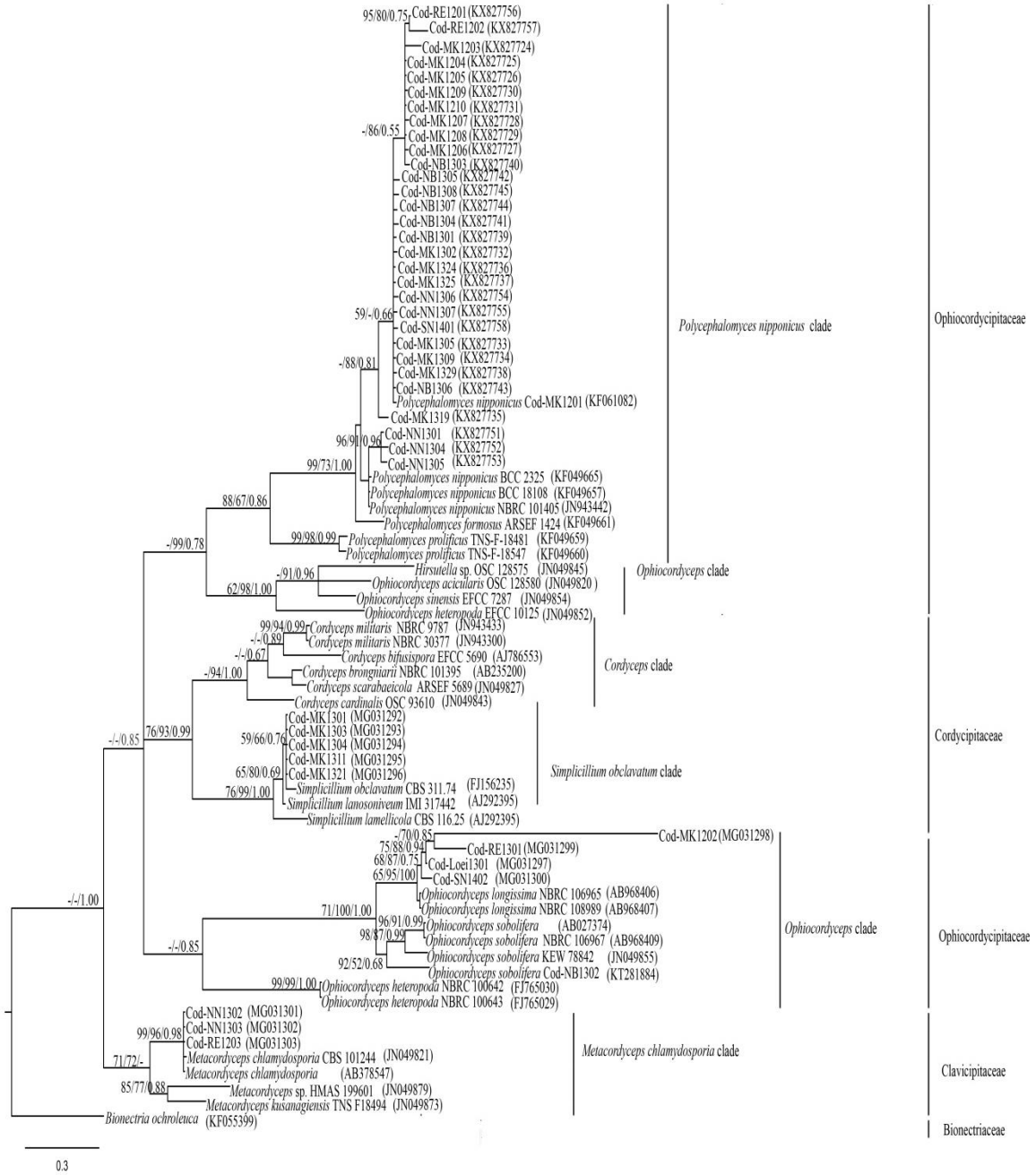

Figure 4.11 Bayesian tree for the partial *ITS* sequences of the entomopathogenic fungal 44 taxa, the 31 related species and accession number of *ITS* sequence. Bootstrap support for neighbor-joining, likelihood-ratio test for maximum likelihood and posterior probabilities for Bayesian analysis are shown above or near the branch. -, denote bootstrap support less than 50%. Scale bar represents 0.3 substitutions per nucleotide position.

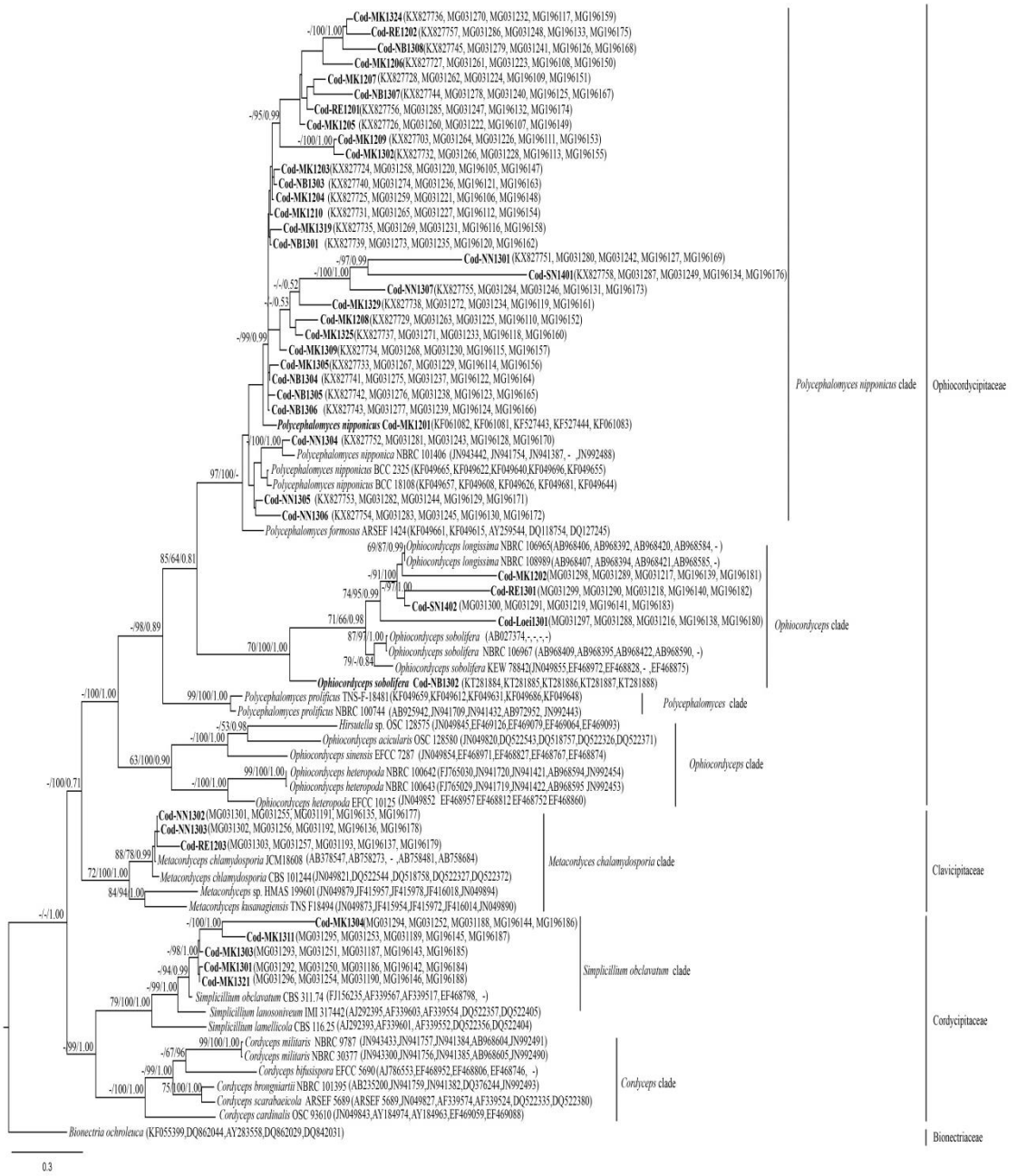

Figure 4.12 Bayesian tree of the entomopathogenic fungal 44 taxa, the 31 related species based on partial *ITS*, *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* sequences with accession number of *ITS*, *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* respectively. Bootstrap support for neighbor-joining, likelihood-ratio test for maximum likelihood and posterior probabilities for Bayesian analysis are shown above or near the branch. -, denote bootstrap support less than 50%. Scale bar represents 0.3 substitutions per nucleotide position

4.6 Description of entomopathogenic fungi from cicada nymph

4.6.1 Scientific name: *Cordyceps caloceroides* Berk. & M.A. Curtis, Journal of the Linnean Society. Botany 10: 375 (1869) [MB#184173]

Anamorph. *Simplicillium obclavatum* (W. Gams) Zare & W. Gams, Nova Hedwigia 73 (1-2): 41 (2001) [MB#484549]

Host. Nymph of Cicadidae (Hemiptera)

Description. Stromata are solitary or branched per host and arose from head and abdomen regions of host insect. Stipe is irregular on surface, brownish, cylindrical and acuminate apex. Stipe ranges from 35-80 mm in length and 1-2 mm in breadth. Perithecia, asci and ascospores are not determined.

Colony. Colonies on potato dextrose agar are fast growing and attain a diameter of 75-80 mm in 30 days at 28 °C. Colonies are convex with white floccose aerial mycelium. Phialides always solitary, long and slender, 0.5-1.0 x20-65µm. Conidia are obliquely, forming short imbricate chains, obclavate to ellipsoidal, 0.1-0.3x1.0-2.0 µm (Figure 4.13).

Distribution and Habitat. The specimen found mostly buried in forest soil from a mixed deciduous forest.

Additional specimen examined. THAILAND, Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N; 103° 28′ 36″ E, 135 m, mixed deciduous forest. 28 May 2013, Jaihan 06 (MSUT_7216), Jaihan 07 (MSUT_7217), Jaihan 08 (MSUT_7218), Jaihan 09 (MSUT_7219), Jaihan 10 (MSUT_7220)

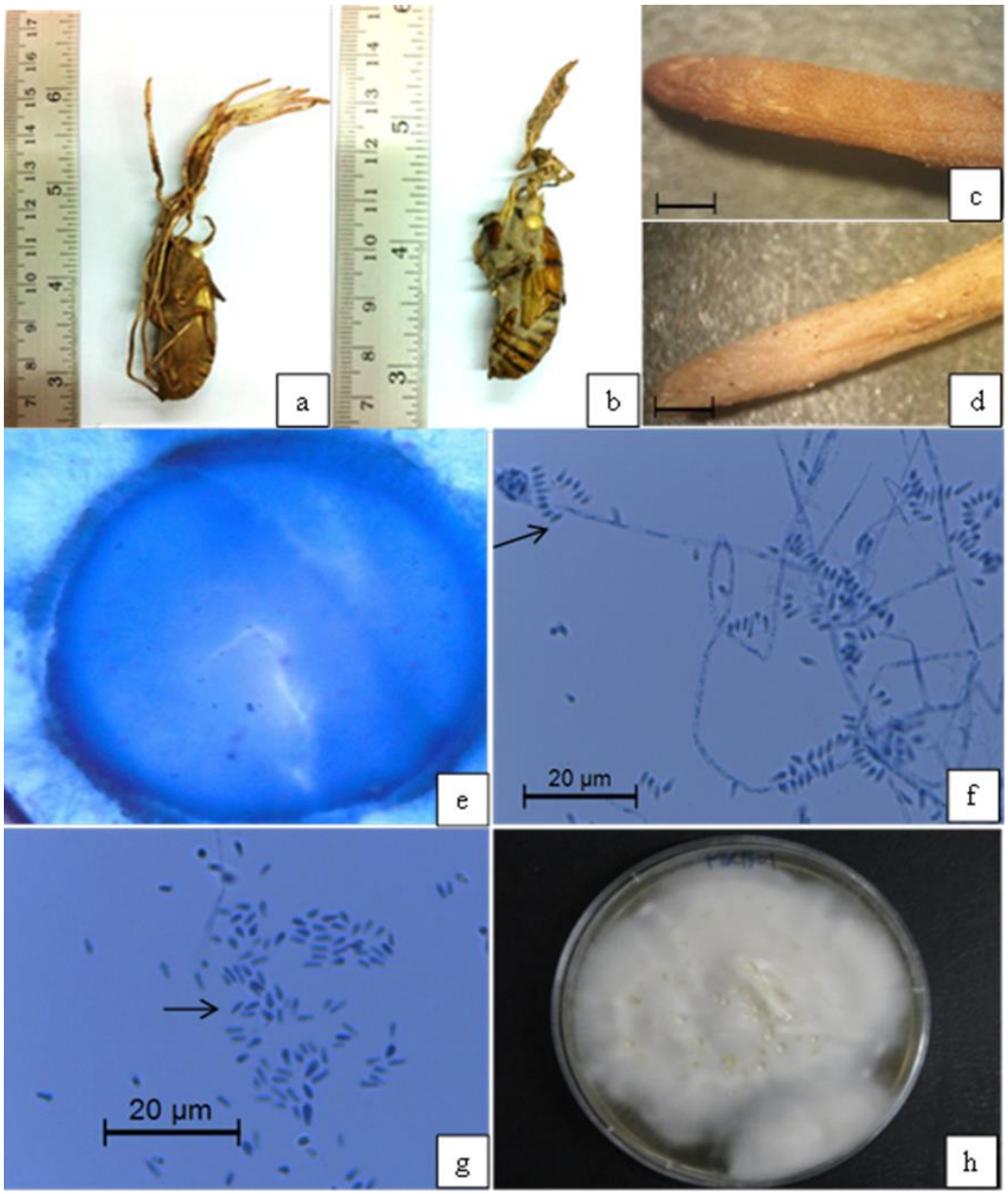

Figure 4.13 Morphological characters of entomopathogenic fungus *Simplicillium obclavatum.* **a-b** stroma arising from a cicada nymph; **c-d** part of stroma; **e** cross section of the stroma; **f** conidiophores; **g** conidia; **h** colony on PDA. Scale bars = 10 mm (c, d), 500 µm (e), 200 µm (f), 20 µm (f, g)

4.6.2 Scientific name: *Metacordyceps chlamydosporia* (H.C. Evans) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, in Sung *et al*. Studies in Mycology 57:5-59 (2007) [MB#504186]

Bas. *Cordyceps chlamydosporia* H.C. Evans in Zare *et al*. Nova Hedwigia 73(1-2):51-86 (2001) [MB#484555]

Anamorph. *Pochonia chlamydosporia*

Host. Nymph of Cicadidae (Hemiptera)

Description. Stromata are solitary or sometimes branched arose from the head regions per host insect and consisted of brownish, brown to black stipe. The stipe is 20–85 mm in length and 1–3 mm in breadth. Fertile part is cylindrical, acuminate apex with branched, 1-5x20-40 mm in size. Perithecia are completely immersed with long ovoid shape measuring, 65–130x 342-520 µm in size. Asci cylindrical, size of ascus is 1.5-3.2x120-170 µm. Ascospores cylindrical, multiseptate and disarticulating into part-spores are measured as, 1x102 µm. Size of part-spore is 0.1-0.9x8.7-11.5 µm.

Colony. Colonies are fast growing, floccose, white-yellow mycelium, with age produced dictyochlamydospores and attain a diameter of 75-80 mm in 30 days at 28 °C. Dictyochlamydospores are produced on the surface of colony or aerial mycelium. Conidia are ellipsoidal in shape and measure approximately 0.5-1 .0 x 1.8-2.0 µm (Figure 4.14).

Distribution and Habitat. The specimen found mostly buried in forest soil from a mixed deciduous forest.

Additional specimen examined. THAILAND, Roi Et Province, Ban Ngu Luam, Suwan Phum District, 15° 40' 52.6" N; 103° 44' 58.0" E, 145 m, mixed deciduous forest. 16 July 2012, Jaihan 11 (MSUT_7221)

Nakhon Phanom Province, Si Songkhram District, 17° 39' 03.5" N; 104° 12' 43.9" E, 146 m, mixed deciduous forest. 7 June 2013, Jaihan 12 (MSUT_7222), Jaihan 13 (MSUT_7223)

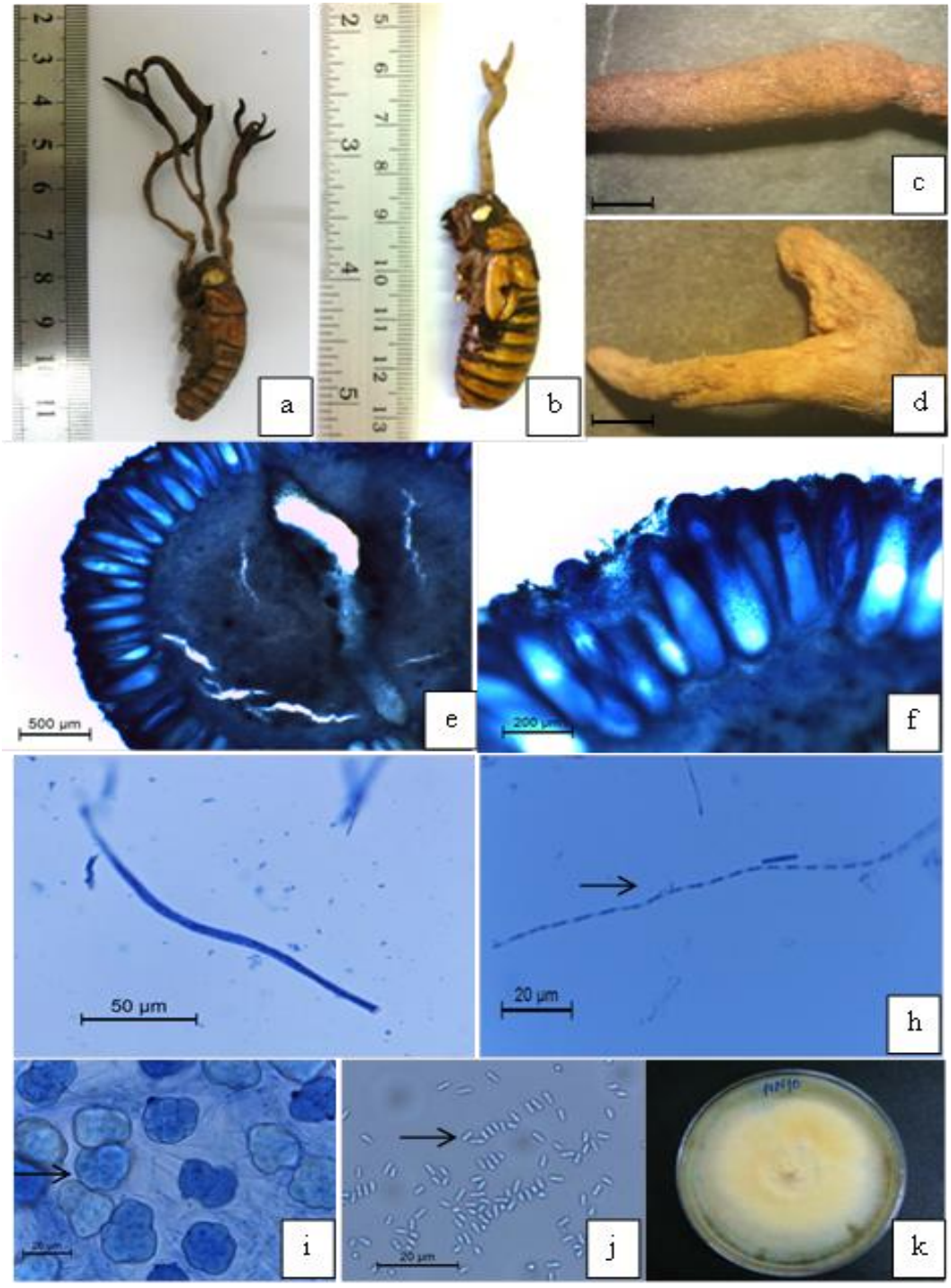

Figure 4.14 Morphological characters of entomopathogenic fungus *Metacordyceps chlamydosporia*. **a-b** stroma emerging from a cicada nymph; **c-d** part of stroma showing perithecial ostioles; **e-f** cross section of the stroma showing perithecia; **g** ascus; **h** ascospore; **i** dictyochlamdospore; **j** conidia; **k** colony on PDA. Scale bars = 10 mm (c, d), 500 µm (e), 200 µm (f), 50 µm (g), 20 µm (h-k)

4.6.3 Scientific name: *Ophiocordyceps longissima* (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora in Sung *et al*. Studies in Mycology 57: 5–59 (2007) [MB#504298]

Bas. *Cordyceps longisima* in Kobayasi Y. & Shimizu D., 1963 Bulletin of the National Science Museum Tokyo 6:286-314 (1963) [MB#328905]

Anamorph. *Hirsutella/Hymenostibe-like*

Host. Nymph of Cicadidae (Hemiptera)

Description. The stromata solitary or branched, light brown to dark brown, emerging from the head and dorsal region of the host insect. The stipe is long and smooth in surface, 1-5x10-80 mm. The fertile part is cylindrical to narrowly ovoid, with orange, brown to dark brown colored head, 1-5x10-20 mm. Perithecia are completely immersed with long ovoid shape and 100-180x482-616 µm in size. Asci are cylindrical and size of ascus is 1.2-3.2x116-200 µm. Ascospores filiform readily dissociating into part-spores while still in the ascus, 0.2-0.3x8.0-10.2 µm.

Colony. Colonies on PDA are extremely slow-growing, attaining a diameter of 50-60 mm in 30 days at 28 °C. Colonies are white and floccose (Figure4.15).

Distribution and Habitat. The specimen found mostly buried in forest soil from a mixed deciduous forest.

Additional specimen examined. THAILAND, Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N; 103° 28′ 36″ E, 135 m, mixed deciduous forest. 11 June 2012, Jaihan 01 (MSUT_7211)

Roi Et Province, Ban Mek, Suwan Phum District, 15° 41' 00.8" N; 103° 46' 25.5" E, 148 m, mixed deciduous forest. 25 September 2013, Jaihan 02 (MSUT_7212)

Loei Province, Chiang Khan District, 17° 52' 49.9" N; 101° 39' 36.7" E, 214 m, mixed deciduous forest. 23 August 2013, Jaihan 03 (MSUT_7213)

Sakon Nakhon Province, Wanon Niwat District, 17° 37′ 56″ N; 103° 45′ 7″ E, 167 m, mixed deciduous forest. 20 June 2014, Jaihan 04 (MSUT_7214)

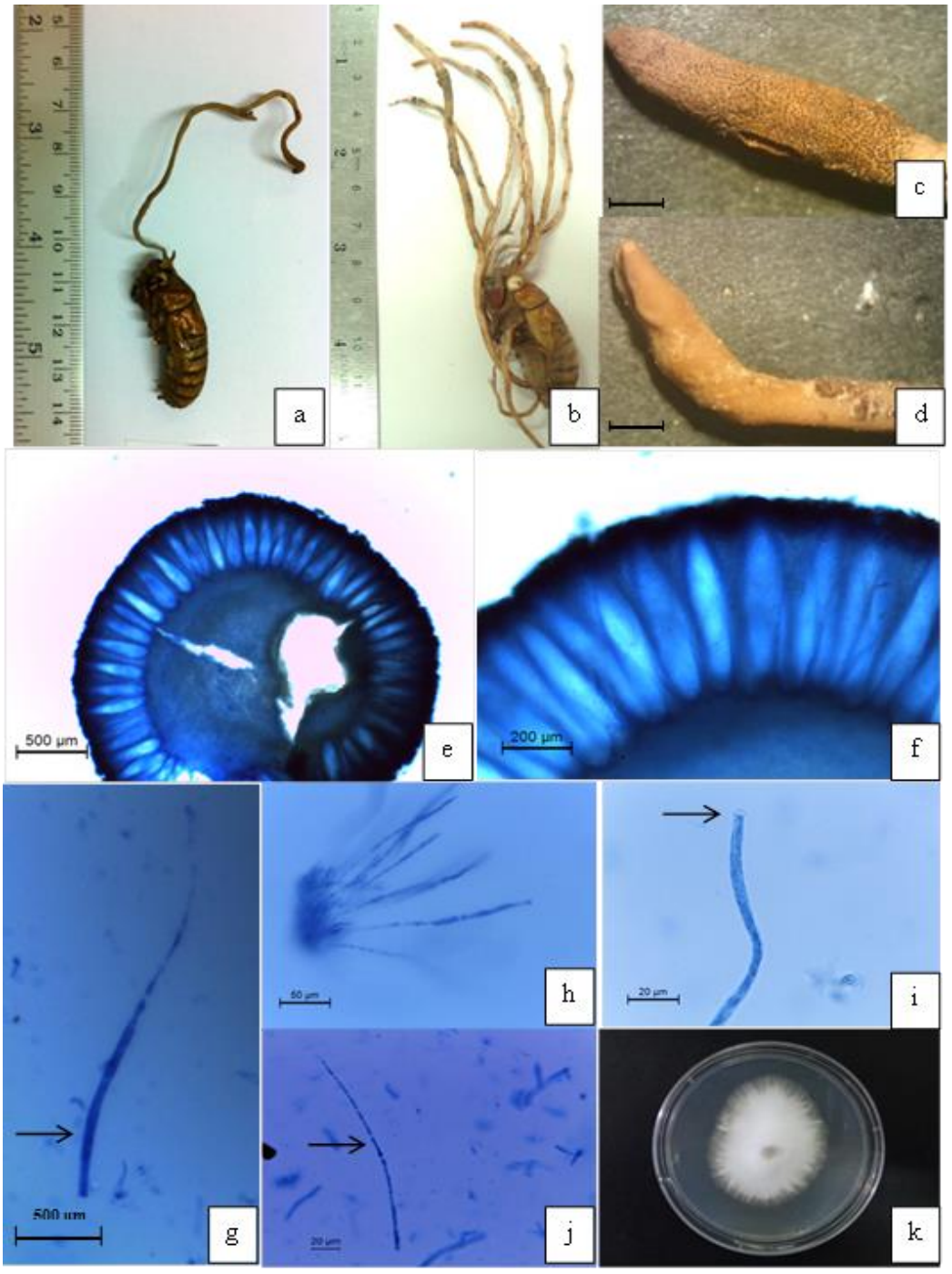

Figure 4.15 Morphological characters of entomopathogenic fungus *Ophiocordyceps longissima*.**a-b** stroma arising from infected cicada nymph; **c-d** part of stroma showing perithecial ostioles; **e-f** cross section of the stroma showing perithecia; **g-h** part of ascus; **i** tip of ascus; **j** part-spore; **k** colonies on PDA. Scale bars = 10 mm (c, d), 500 µm (e), 200 µm (f), 50 µm (g, h), 20 µm (i, j)

4.6.4 Scientific name: *Ophiocordyceps sobolifera* (Hill ex Watson) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, in Sung *et al*. Studies in Mycology 57:5-59 (2007) [MB#504342]

Bas. *Cordyceps sobolifera* (Hill ex Watson) in Berk & Broome, Botanical Journal of the Linnean Society 14: 110 (1875) [MB#215259]

Anamorph. *Beauveria sobolifera*

Host. Nymph of Cicadidae (Hemiptera)

Description. Stromata are gregarious, pink-brownish, arising from head regions of host insect. The stipe is smooth in surface and clavate in shape ranges from 2-3x25-30 mm**.** Perithecia, asci and ascospores not determined.

Colony. Colony on PDA is extremely slow-growing, attaining a diameter of 35-45 mm in 30 days at 28 °C. Colony is floccose, white to pink mycelium at beginning and becoming brown-black with pink conidia. Conidiophores hyaline, 1.5 -2.5x 4.6-5.8 µm, solitary, growing densely on swollen cells and mostly ellipsoidal. Conidia are produced on conidiogenous cells of sympodial elongation, long ellipsoidal; 1.8-2.7x7.2-7.6 µm (Figure 4.16).

Distribution and Habitat. The specimen found mostly buried in forest soil from a mixed deciduous forest.

Additional specimen examined. THAILAND, Nong Bua Lam Phu Province, Tham Erawan, 17° 20' 20.4" N; 102° 01' 16.8" E, 337 m, mixed deciduous forest. 24 May 2013, Jaihan 05 (MSUT_7215)

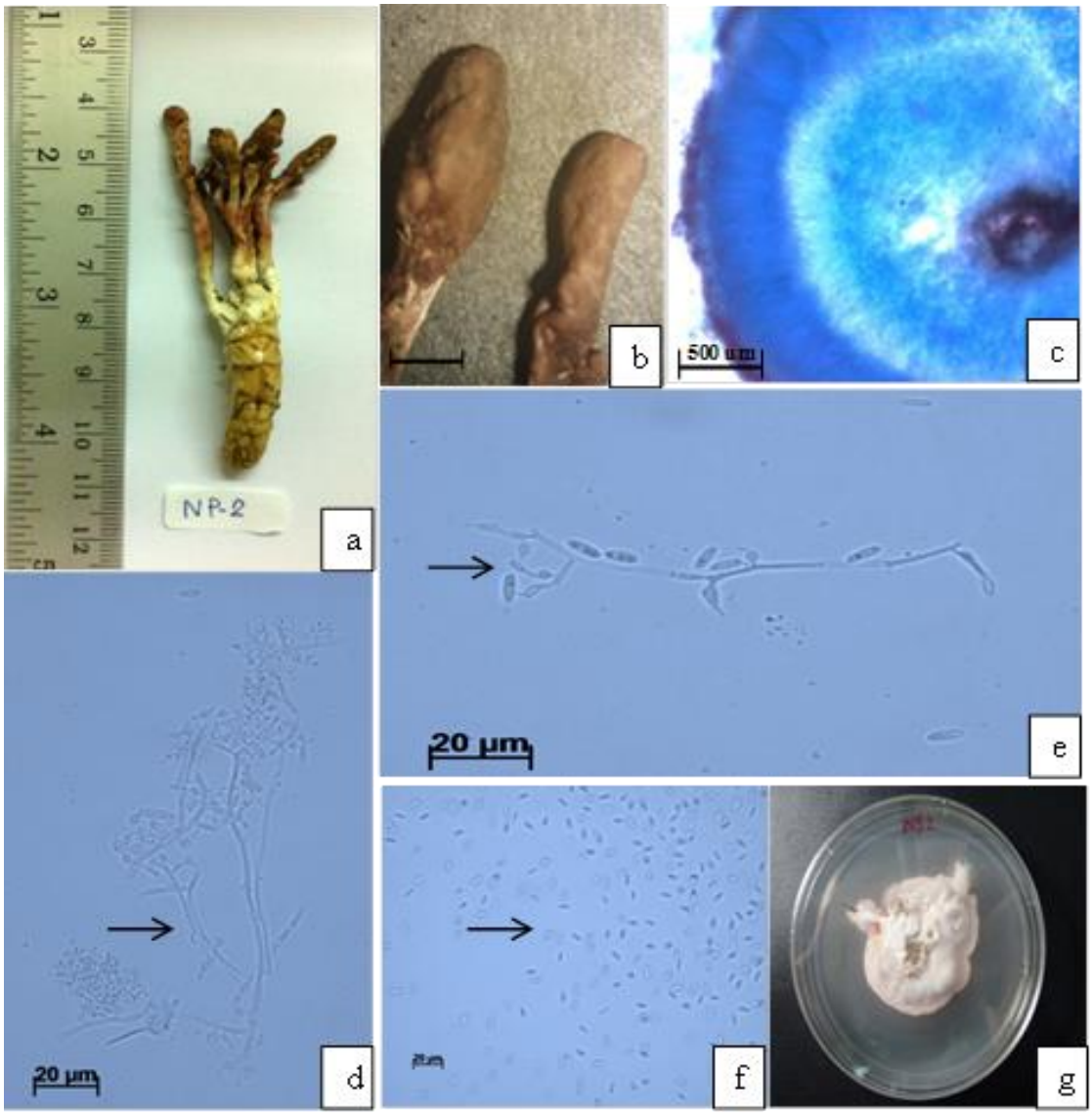

Figure 4.16 Morphological characters of entomopathogenic fungus *Ophiocordyceps sobolifera*. **a** stroma of fungus emerging from a cicada nymph; **b** part of stroma; **c** cross section of the stroma; **d-e** relationship between conidia, conidiogenous cell and conidiophores; **g** conidia; **h** colony on PDA. Scale bars =10 mm (b), 20 µm(c-f)

4.6.5 Scientific name: *Polycephalomyces nipponicus* (Kobayasi) Kepler & Spatafora in Kepler *et al*. Fungal Biology 117: 611- 622 (2013) [MB#804389]

Bas. *Cordyceps nipponica* Kobayasi in Kobayasi Y., 1939 Bulletin of the Biogeographical Society of Japan 9: 145-176 (1939) [MB#253573]

Anamorph. unknown

Host. Nymph of Cicadidae (Hemiptera)

Description. Stromata are solitary or branched, light brown to dark brown, arose from the head, abdomen and thorax regions of the host insect. The stromata were erect mostly branched and measured at 1–3 mm in width and 15–80 mm in length. Fertile parts were located at the terminal end or at the sub-terminal part of the stroma. Some isolates had a dark brown clavate fertile part, 1-6 mm in width and 10–40 mm in length. Perithecia were semi-immersed and completely immersed, narrowly ovoid to conoid and 100-284x606-866 µm in size. Asci cylindrical, 0.5-6x150-331 µm. Size of ascus cap was 1.5-2.8x4.0-6.2 µm. Ascospores filiform, size of part-spore was 0.2-1.0 x 4.2-11.7 µm.

Colony. Colonies on PDA are first floccose with white mycelium and with age producing patches of slimy cream-yellow conidia, attaining a diameter of 75-80 mm in 30 days at 28 °C (Figure4.17).

Distribution and Habitat. The specimen found mostly buried in forest soil from a mixed deciduous forest.

Additional specimen examined. THAILAND, Maha Sarakham Province, Ban Na Pang, Muang District, 16°10′44″N 103°28′36″E, 135 m, mixed deciduous forest. 11 June 2012, Sangdee 01 (MSUT_7171), Sangdee 02 (MSUT_7172), Sangdee 03 (MSUT_7173), Sangdee 04 (MSUT_7174), Sangdee 05 (MSUT_7175), Sangdee 06 (MSUT_7176), Sangdee 07 (MSUT_7177), Sangdee 08 (MSUT_7178), Sangdee 09 (MSUT_7179)

Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N; 103° 28′ 36″ E, 135 m, mixed deciduous forest. 28 May 2013, Sangdee 10 (MSUT_7180), Sangdee 11 (MSUT_7181), Sangdee 12 (MSUT_7182), Sangdee 13 (MSUT_7183), Sangdee 14 (MSUT_7184), Sangdee 15 (MSUT_7185), Sangdee 16 (MSUT_7186)

Roi Et Province, Ban Ngu Luam, Suwan Phum District, 15° 40' 52.6" N; 103° 44' 58.0 " E, 145 m, mixed deciduous forest. 16 July 2012, Sangdee 17 (MSUT_7187), Sangdee 18 (MSUT_7188)

Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20' 20.4" N; 102° 01' 16.8" E, 337 m, mixed deciduous forest. 24 May 2013, Sangdee 19 (MSUT_7189), Sangdee 20 (MSUT_7190), Sangdee 21 (MSUT_7191), Sangdee 22 (MSUT_7192), Sangdee 23 (MSUT_7193), Sangdee 24 (MSUT_7194), Sangdee 25 (MSUT_7195)

Nakhon Phanom Province, Si Songkhram District, 17° 39' 03.5" N; 104° 12' 43.9" E, 146 m, mixed deciduous forest. 7 June 2013, Sangdee 26 (MSUT_7196), Sangdee 27 (MSUT_7197), Sangdee 28 (MSUT_7198)

Nakhon Phanom Province, Tha Uthen District, 17° 33′ 25″ N; 104° 36′ 45″ E, 156 m, mixed deciduous forest. 24 June 2013, Sangdee 29 (MSUT_7199), Sangdee 30 (MSUT_7200)

Sakon Nakhon Province, Wanon Niwat District, 17° 37′ 56″ N; 103° 45′ 7″ E, 167 m, mixed deciduous forest. 20 June 2014, Sangdee 31(MSUT_7201)

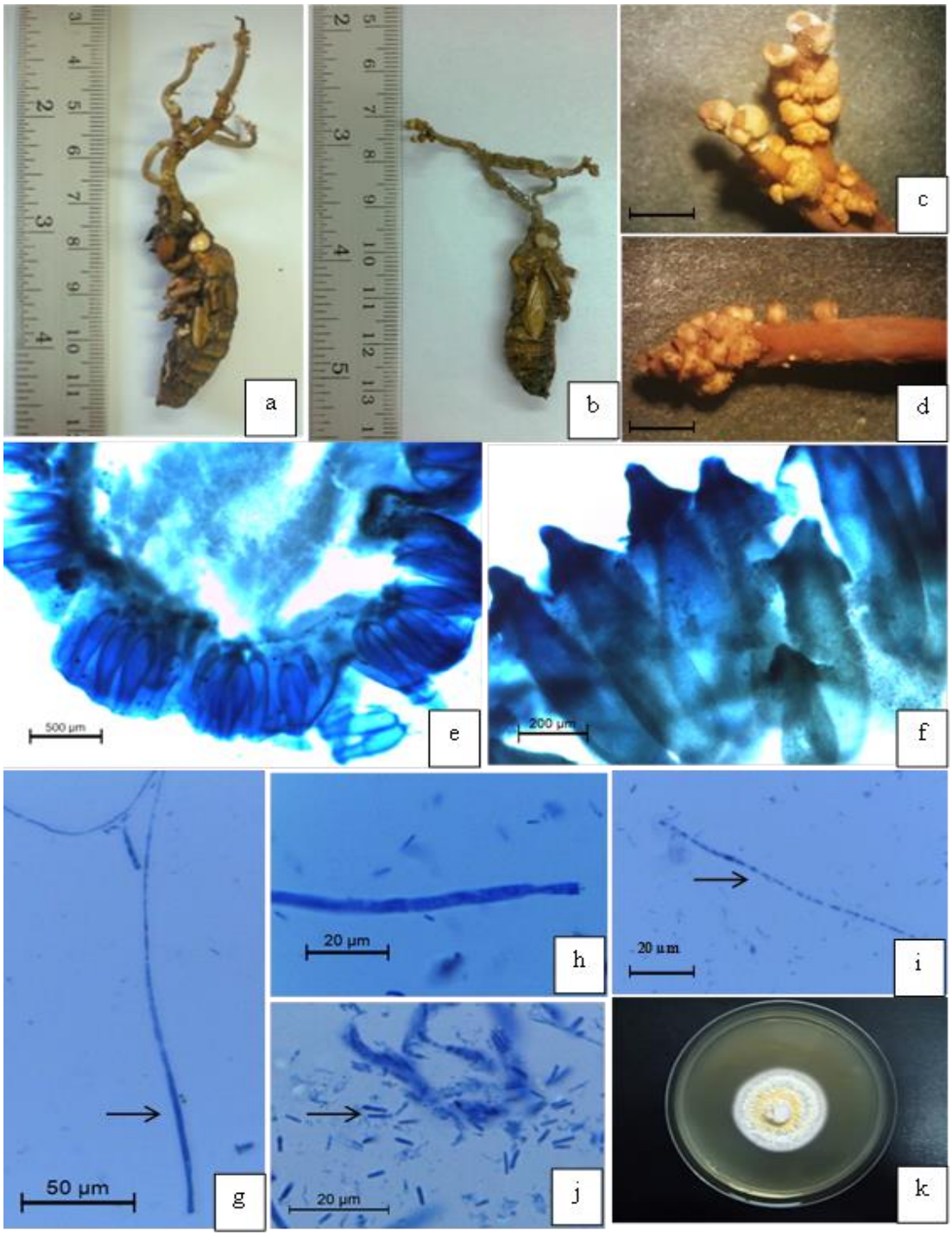

Figure 4.17 Morphological characters of entomopathogenic fungus *Polycephalomyces nipponicus*. **a-b** stroma of fungus emerging from a cicada nymph; **c-d** part of stroma showing perithecial ostioles; **e-f** cross section of the stroma showing perithecia; **g** part of ascus; **h** part of ascus with ascus tip; **i** discharged ascospore; **j** colony on PDA. Scale bars = 10 mm (c, d), 500 µm (e), 200 µm (f), 50 µm (g), 20 µm (h-j)

4.7 Application in agricultural area (biological control)

4.7.1 Isolation of *Colletotrichum* spp. causal agent of chilli anthracnose

Ten isolates of *Colletotrichum* spp. were isolated from infected chili fruits and were then identified as *C. capsici* and *Colletotrichum* spp. based on size and shape of conidia and their fruiting body structure. *C. capsici* consisting of five isolates in which the colonies were mainly black and produced acervulus fruiting bodies with setae, and it could produce pale orange and falcate conidia. Whereas, the *Colletotrichum* spp. also consisted of five isolates that produced cottony colonies on PDA with a color of grayish-white to dark grey on the ventral surface and it could produce cylindrical conidia.

4.7.2 Primary screening of antagonistic entomopathogenic fungi

Forty-four isolates of the entomopathogenic fungi were evaluated for their antagonistic activity against the two isolates of plant pathogenic fungi *Colletotrichum* spp. (*C. capsici* and *Colletotrichum* spp.) The results revealed that the interaction between the mycelial growth of the entomopathogenic fungi with the test plant pathogenic *Colletotrichum* spp. were categorized into two groups. In the first group, the mycelium of the entomopathogenic fungi and plant pathogenic fungi grow well and inhibited each other without an inhibition zone. In the second group, the mycelial growth of plant pathogenic *Colletotrichum* spp. was inhibited by the mycelium of the entomopathogenic fungi with an inhibition zone. The percentage of mycelial growth reduction in all isolates of entomopathogenic fungi against plant pathogenic *Colletotrichum* spp. ranged from 25.00- 47.06% and 14.76- 54.55% for *C. capsici* and *Colletotrichum* spp., respectively (Table 4.3). The high percentage of mycelial growth reduction and large inhibition zone compared with the control were used as criteria for selection of the antagonistic strain (Figure 4.18 and 4.19). Therefore, eight isolates of antagonistic entomopathogenic fungi consisting of Cod-NB1301, Cod-NB1302, Cod-NB1305, Cod-NN1307, Cod-MK1208, Cod-MK1305, Cod-MK1319 and Cod-Loei1301, which showed a high percentage of mycelium reduction in the range from 38.46- 47.06% for *C. capsici* and 26.67 - 54.55% for *Colletotrichum* spp, were selected to investigate their antifungal activity further.

Table 4.3 Percentages of mycelial growth reduction of 44 isolates of the entomopathogenic fungi on mycelial growth of plant pathogenic *Colletotrichum* spp. by dual culture method after 14 days

Isolate	Location	Percentages of mycelial growth reduction (%)	
		C. capsici	***Colletotrichum* spp.**
Cod-MK1201	MK	34.82±3.19^{de}	31.81±2.14^{fg}
Cod-MK1202	MK	36.81±.000^{c}	31.27±2.85^{f}
Cod-MK1203	MK	34.82±3.19^{de}	32.09±1.28^{efg}
Cod-MK1204	MK	34.12±4.15^{defg}	32.31±0.00^{e}
Cod-MK1205	MK	38.24±0.00^{bc}	30.91±3.14^{fg}
Cod-MK1206	MK	31.18±0.00^{efgh}	38.08±3.85^{bc}
Cod-MK1207	MK	31.18±0.00^{efgh}	34.54±2.56^{def}
Cod-MK1208	MK	41.18±0.00^{bcd}	38.54±1.27^{b}
Cod-MK1209	MK	31.18±0.00^{efgh}	20.00±0.00^{ijk}
Cod-MK1210	MK	31.18±0.00^{efgh}	32.73±0.00^{e}
Cod-MK1301	MK	30.77±0.00^{f}	34.61±2.72^{de}
Cod-MK1302	MK	35.57±3.08^{cde}	23.08±0.00^{h}
Cod-MK1303	MK	35.46±0.00^{cde}	32.69±0.00^{ef}
Cod-MK1304	MK	35.46±0.00^{cde}	34.62±0.00^{de}
Cod-MK1305	MK	39.46±0.00^{b}	37.88±2.79^{bc}
Cod-MK1309	MK	38.00±2.17^{bc}	29.80±3.08^{fgh}
Cod-MK1311	MK	33.33±0.00^{e}	11.90±0.00^{m}
Cod-MK1319	MK	41.66±2.35^{ab}	38.00±3.13^{bc}
Cod-MK1321	MK	33.33±0.00^{e}	21.00±1.41^{i}
Cod-MK1324	MK	28.33±2.35^{gh}	22.22±0.00^{hijk}
Cod-MK1325	MK	27.50±3.53^{hij}	19.89±2.98^{j}
Cod-MK1329	MK	33.33±0.00^{e}	17.16±3.39^{kl}
Cod-RE1201	RE	31.18±0.00^{efgh}	37.22±1.28^{bcd}
Cod-RE1202	RE	30.14±3.19^{fg}	37.27±1.24^{bcd}
Cod-RE1301	RE	31.66±0.00^{ef}	14.76±0.00^{l}
Cod-NN1301	NN	25.00±0.00^{i}	17.14±0.00^{kl}
Cod-NN1302	NN	36.67±0.00^{cd}	14.28±3.36^{lm}
Cod-NN1303	NN	34.40±2.36^{d}	11.90±0.00^{m}
Cod-NN1304	NN	27.50±3.53^{hij}	14.76±3.36^{l}
Cod-NN1305	NN	31.33±0.00^{efg}	16.67±0.00^{klm}
Cod-NN1306	NN	30.00±0.00^{fgh}	14.76±0.00^{ij}
Cod-NN1307	NN	39.00±0.00^{b}	37.90±0.00^{bc}
Cod-SN1401	SN	28.67±3.11^{g}	22.72±3.42^{hij}
Cod-SN1402	SN	26.47±0.00^{ijk}	22.27±0.00^{hijk}
Cod-NB1301	NB	38.46±3.35^{b}	35.80±3.65^{cd}
Cod-NB1302	NB	47.06±2.07^{a}	54.55±0.00^{a}
Cod-NB1303	NB	34.46±0.00^{def}	23.00±1.31^{h}

Table 4.3 (Cont.)

Isolate	Location	Percentages of mycelial growth reduction (%)	
		C. capsici	***Colletotrichum* spp.**
Cod-NB1304	NB	30.77±0.00^{f}	26.00±4.24^{ghi}
Cod-NB1305	NB	40.00±2.17^{ab}	35.75±1.07^{cd}
Cod-NB1306	NB	30.00±2.17^{gh}	20.00±0.00^{ijk}
Cod-NB1307	NB	31.54±0.00^{efg}	22.00±2.82^{hijk}
Cod-NB1308	NB	36.46±0.00^{cd}	26.00±3.24^{ghi}
Cod-Loei1301	LO	34.33±0.00^{def}	26.67±0.00^{g}

* Abbreviations of location: MK, Maha Sarakham; RE, Roi Et; NB, Nong Bua Lam Phu; LO, Loei; NN, Nakhon Phanom; SN, Sakon Nakhon

*Averaged from three replications. Values are the means (±SD), inhibition percentages against *Colletotrichum* spp. Values in the same column followed by the same letter are not significantly different according to analysis of variance and Duncan's Multiple Range Test (DMRT) (at P = 0.05).

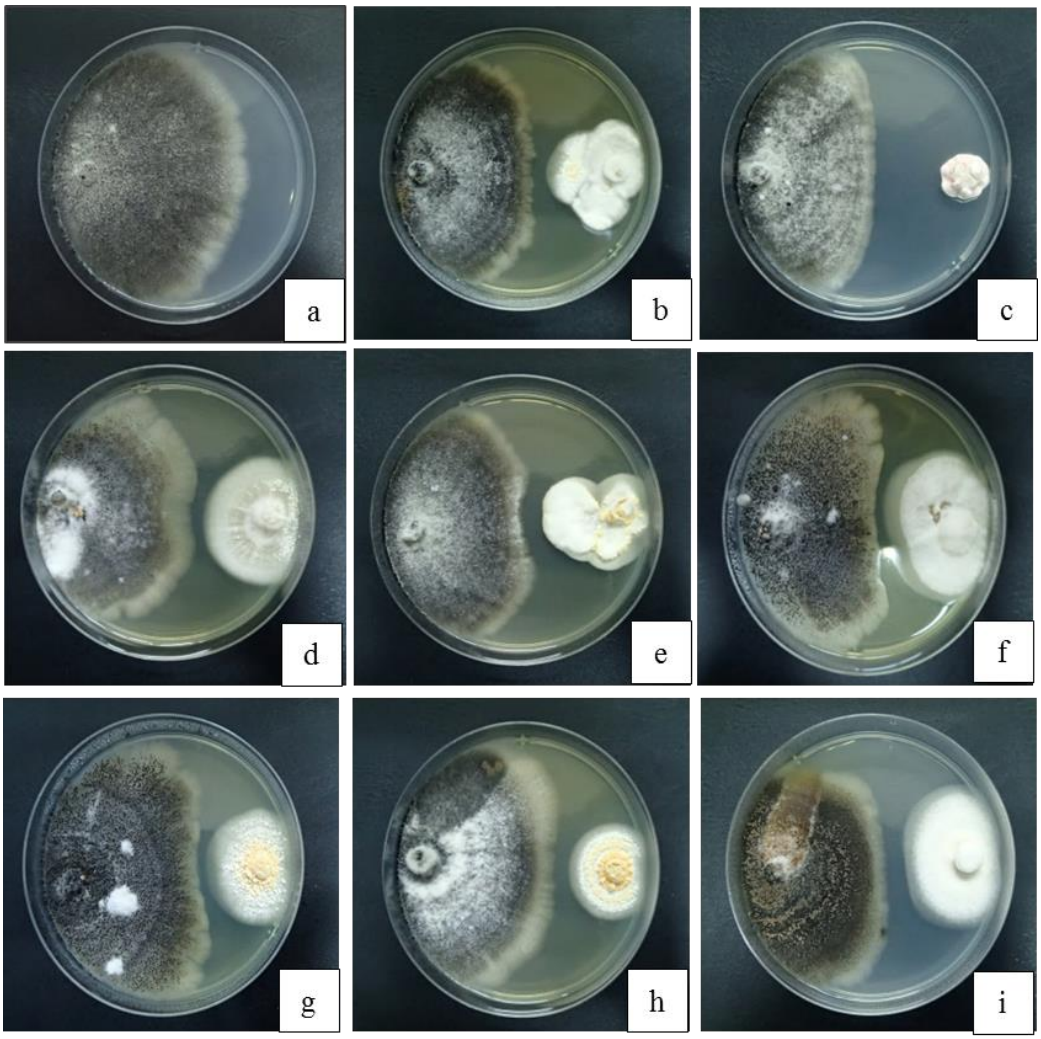

Figure 4.18 Interaction between mycelial growth of the entomopathogenic fungi and *C. capsici* by dual culture method on PDA after 14 days. **a** Control *C. capsici*; **b** Cod-NB1301; **c** Cod-NB1302; **d** Cod-NB1305; **e** Cod-NN1307; **f** Cod-MK1208; **g** Cod-MK1305; **h** Cod-MK1319; **i** Cod-Loei1301

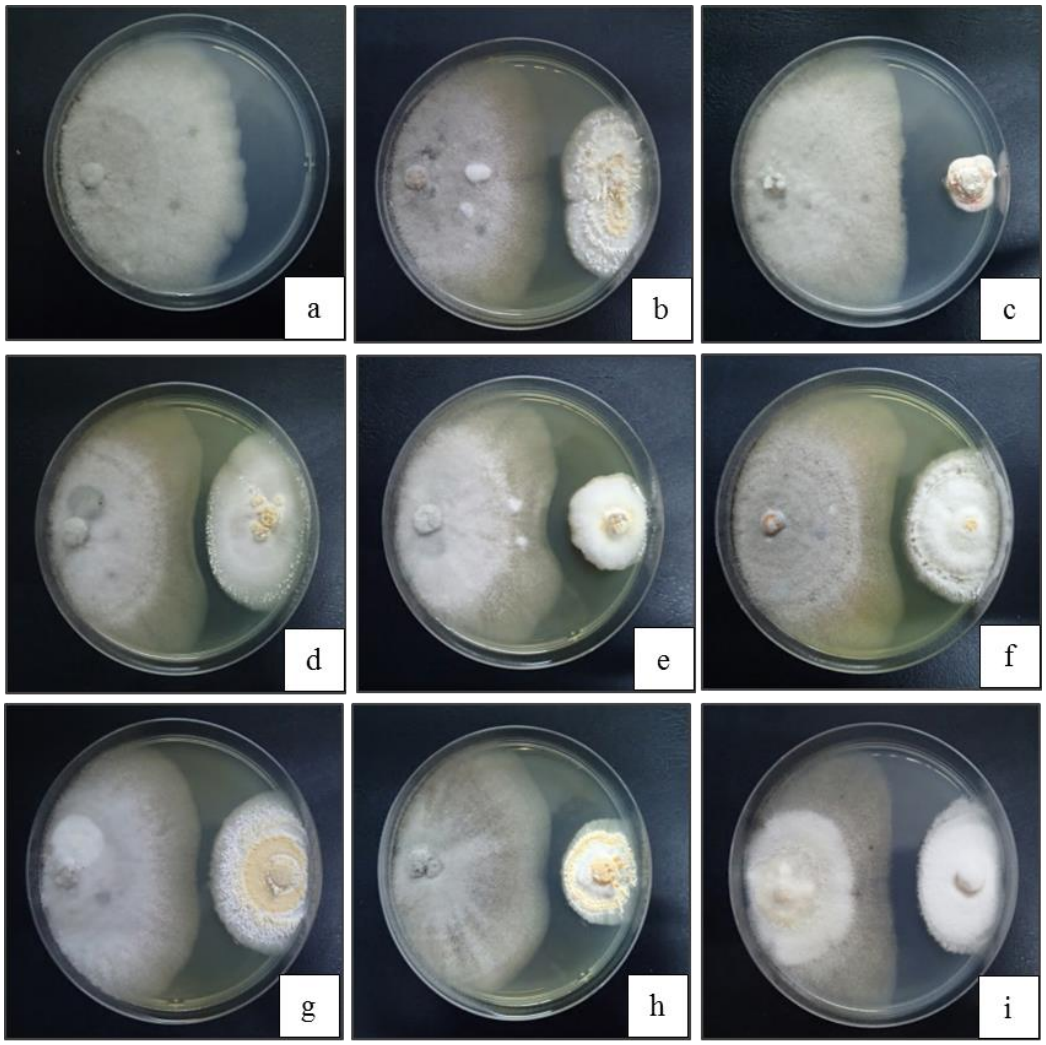

Figure 4.19 Interaction between mycelial growth of the entomopathogenic fungi and *Colletotrichum* spp. by dual culture method on PDA after 14 days. **a** Control *Colletotrichum spp.*; **b** Cod-NB1301; **c** Cod-NB1302; **d** Cod-NB1305; **e** Cod-NN1307; **f** Cod-MK1208; **g** Cod-MK1305; **h** Cod-MK1319; **i** Cod-Loei1301

4.7.3 Confirmation of antagonistic activity against plant pathogenic *Colletotrichum* spp.

The confirmation of antagonistic activity of the eight selected antagonistic entomopathogenic fungi were investigated against five isolates of *C. capsici* and five isolates *Colletotrichum* spp. The results showed that all the selected isolates inhibited the growth of *C. capsici* and *Colletotrichum* spp. in the ranges from 25.00- 43.55% and 11.33-34.45%, respectively (Table 4.4). The variation in percentage of fungal mycelial growth reduction depended on the isolate of entomopathogenic fungi and plant pathogenic *Colletotrichum* spp. Among the selected antagonistic isolates, the entomopathogenic fungi isolate Cod-NB1302 showed the highest percentage of mycelial growth reduction in both *C. capsici* and *Colletotrichum* spp., which was significantly greater than the other isolates (Figure 4.20). Therefore, isolate Cod-NB1302 was chosen for further evaluation of the antagonistic activity.

Table 4.4 Inhibitory effect of eight isolates of entomopathogenic fungi on mycelial growth of ten isolates of plant pathogenic *Colletotrichum* spp. by dual culture method after 14 days

Isolate	Mycelial growth reduction (%)*									
	C. capsici					*Colletotrichum* spp.				
	CcC1	CcC2	CcC4	CcC5	CcC6	CgC6	CgC7	CgC10	CgC11	CgC12
Cod-MK1208	36.11±3.47$^{b, B}$	38.33±2.88$^{b, A}$	36.92±2.66$^{ab, B}$	38.17±2.46$^{b, A}$	36.01±0.92$^{a, B}$	30.43±0.15$^{ab, A}$	30.91±0.00$^{bc, A}$	23.08±0.00$^{ab, B}$	28.57±0.00$^{b, A}$	24.66±4.16$^{a, B}$
Cod-MK1305	25.00±0.00$^{d, C}$	25.00±0.00$^{e, C}$	34.35±3.87$^{b, A}$	31.71±4.05$^{cd, B}$	30.10±2.45$^{b, B}$	28.48±2.10$^{bcd, B}$	28.48±2.10^{bcd,B}	19.87±5.55$^{ab, B}$	22.61±1.02$^{de, B}$	16.66±5.03$^{de, B}$
Cod-MK1319	26.11±1.92$^{d, C}$	25.00±0.00$^{e, C}$	34.35±3.87$^{b, A}$	30.64±4.29$^{d, B}$	30.64±2.78$^{b, AB}$	19.39±2.10$^{e, A}$	21.21±1.05$^{e, A}$	17.30±3.33$^{b, A}$	20.82±1.02$^{de, A}$	18.66±2.30$^{bc, A}$
Cod-NB1301	34.44±0.96$^{bc, B}$	30.55±2.54$^{d, C}$	34.87±0.88$^{b, B}$	38.17±2.46$^{b, A}$	35.48±0.00$^{a, AB}$	24.24±3.78$^{d, AB}$	27.87±1.05$^{cd, A}$	18.58±4.00$^{ab, C}$	25.59±5.15$^{bc, A}$	11.33±2.30$^{e, A}$
Cod-NB1302	40.55±1.92$^{a, A}$	42.77±1.92$^{a, A}$	41.35±1.81$^{a, B}$	43.55±0.00$^{a, A}$	37.63±1.86$^{a, B}$	32.91±0.31$^{a, A}$	34.45±1.25$^{a, A}$	25.02±1.92$^{a, B}$	30.83±2.77$^{a, B}$	25.52±1.84$^{a, B}$
Cod-NB1305	33.33±0.00$^{bc, B}$	31.11±1.92$^{d, C}$	36.40±1.77$^{ab, A}$	36.01±0.92$^{bc, A}$	35.48±0.00$^{a, AB}$	27.27±3.63$^{bcd, A}$	30.30±2.10$^{bc, A}$	23.08±0.00$^{ab, B}$	23.81±2.06$^{cd, C}$	22.66±2.30$^{ab, C}$
Cod-NN1307	33.33±0.00$^{bc, A}$	33.33±0.00$^{cd, A}$	38.46±0.00$^{b, A}$	37.63±2.46$^{bc, A}$	37.29±1.93$^{a, A}$	26.66±1.05$^{cd, A}$	26.66±1.05$^{d, A}$	21.15±0.00$^{ab, B}$	19.64±2.06$^{e, B}$	18.00±2.30$^{cd, B}$
Cod-Loei1301	32.22±1.92$^{c, B}$	36.11±2.54$^{bc, A}$	38.46±0.00$^{ab, A}$	36.55±1.86$^{bc, A}$	35.48±0.00$^{a, A}$	30.36±0.47$^{ab, A}$	32.91±0.66$^{ab, A}$	23.08±0.00$^{ab, B}$	32.19±1.62$^{a, A}$	24.00±4.00$^{ab, B}$

*Averaged from three replications. Values are the means (±SD), inhibition percentages against *C. capsici* and *Colletotrichum* spp. The values in the same column or row followed by the same letter are not significantly different according to analysis of variance and Duncan's Multiple Range Test (DMRT) (at P = 0.05).

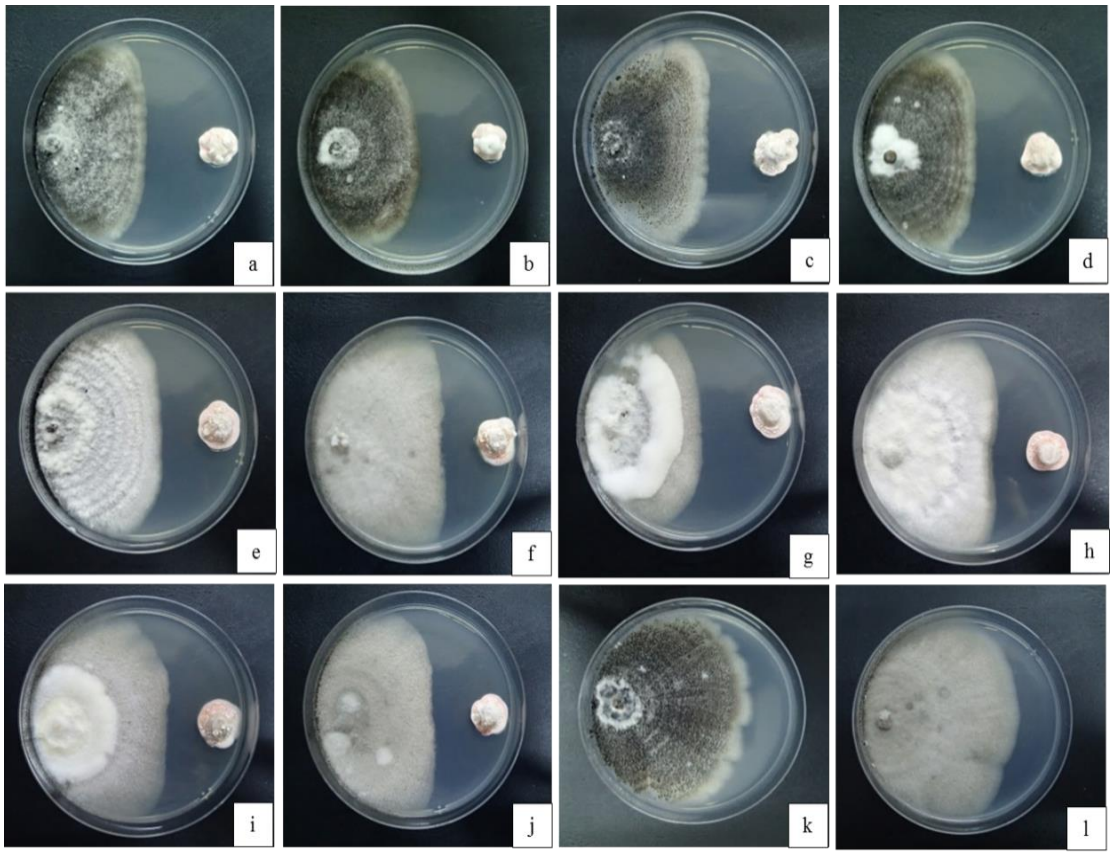

Figure 4.20 Antifungal activities of entomopathogenic fungus isolate Cod-NB1302 against *C. capsici* and *Colletotrichum* spp. by dual culture method on PDA incubated at 28 °C after 14 days. **a** Cod-NB1302+*C. capsici* CcC1; **b** Cod-NB1302+ *C. capsici* CcC2; **c** Cod-NB1302+*C. capsici* CcC4; **d** Cod-NB1302+*C. capsici* CcC5; **e** Cod-NB1302+*C. capsici* CcC6; **f** Cod-NB1302+*Colletotrichum* spp.CgC6**; g** Cod-NB1302+ *Colletotrichum* spp. CgC7; **h** Cod-NB1302+*Colletotrichum* spp. CgC10; **i** Cod-NB1302+ *Colletotrichum* spp. CgC11; **j** Cod-NB1302+*Colletotrichum* spp. CgC12; **k** control *C. capsici* **l** control *Colletotrichum* spp.

4.7.4 Effect of mycelial extract and culture filtrate on mycelial growth of *Colletotrichum* spp.

4.7.4.1 Tube dilution assay

The antifungal activity of the mycelial extract and culture filtrate of the isolate Cod-NB1302 was investigated using a tube dilution assay. The results showed that the mycelial extract exhibited greater antifungal activity against all 10 isolates of the tested plant pathogenic fungi than the culture filtrate and 50% ethanol (Table 4.5). The mycelial extract completely controlled the mycelial growth of five isolates of *C. capsici* and five isolates of *Colletotrichum* spp. after 14 days at dilutions of 1:1 up to 1:8. The culture filtrate had no inhibitory affected on the mycelial growth all 10 isolates of the tested plant pathogenic fungi. The 50% ethanol, which was used as an organic solvent, also completely inhibited the fungal mycelial growth for five isolates of *C. capsici* and five isolates of *Colletotrichum* spp. at dilutions of 1:1 and 1:2, while at the dilution of 1:4 it could inhibit the mycelial growth of some isolates (Figure 4.21).

Table 4.5 Antifungal activity of dilutions of mycelial extract and culture filtrate from entomopathogenic fungal isolate Cod-NB1302 against plant pathogenic *Colletotrichum* spp. compared with 50%ethanol for 14 days

***Colletotrichum* spp.**	**Mycelial extract**					**Culture filtrate**					**50% ethanol**				
	PDB	**1:1**	**1:2**	**1:4**	**1:8**	**PDB**	**1:1**	**1:2**	**1:4**	**1:8**	**PDB**	**1:1**	**1:2**	**1:4**	**1:8**
C. capsici CcC1	2	0	0	0	0	2	2	2	2	2	2	0	0	0	2
C. capsici CcC2	2	0	0	0	0	2	1	1	1	1	2	0	0	0	1
C. capsici CcC4	2	0	0	0	0	2	1	1	1	1	2	0	0	0	1
C. capsici CcC5	2	0	0	0	0	2	2	2	2	2	2	0	0	0	1
C. capsici CcC6	2	0	0	0	0	2	1	1	1	1	2	0	0	0	2
Colletotrichum spp. CgC6	2	0	0	0	0	2	1	1	1	2	2	0	0	1	1
Colletotrichum spp. CgC7	2	0	0	0	0	2	1	2	2	2	2	0	0	0	2
Colletotrichum spp. CgC10	2	0	0	0	0	2	1	2	2	2	2	0	0	0	2
Colletotrichum spp. CgC11	2	0	0	0	0	2	1	2	2	2	2	0	0	0	2
Colletotrichum spp. CgC12	2	0	0	0	0	2	1	2	2	2	2	0	0	1	1

*Mycelial growth was categorized on a scale of 0 to 2; 0 = no mycelial growth, 1 = growth limited around mycelial disk and 2 = mycelia overgrows into liquid medium

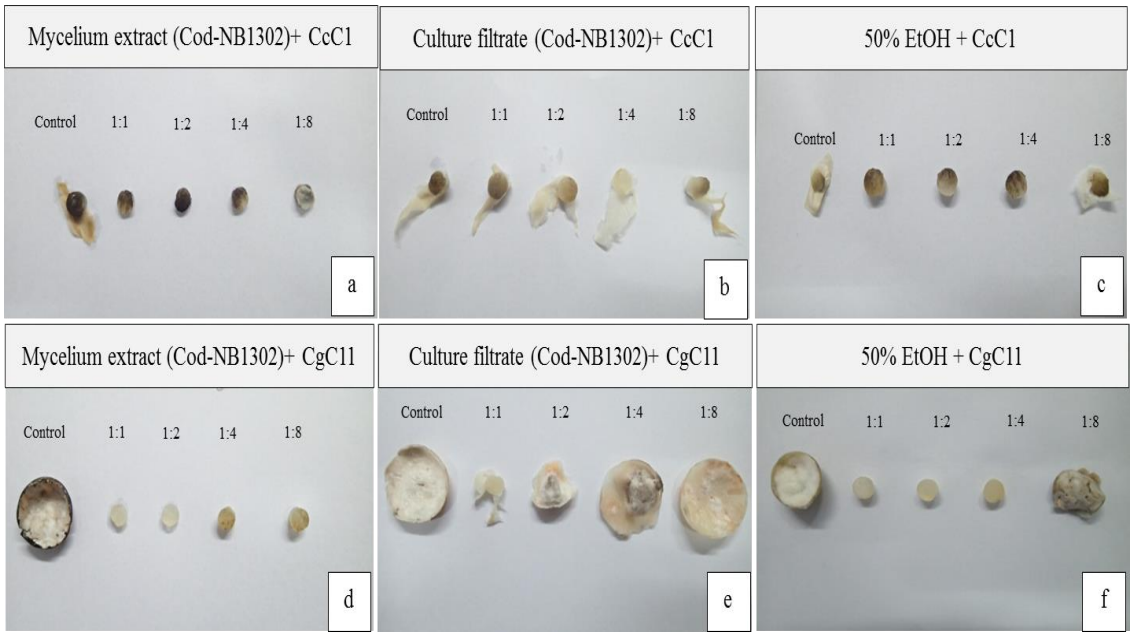

Figure 4.21 Effect of the mycelial extract of Cod-NB1302 of inhibit mycelial growth of *C.capsici* and *Colletotrichum* spp. after 14 days. **a** mycelial extract + *C. capsici*; **b** culture filtrate + *C. capsici*; **c** 50% ethanol + *C. capsici*; **d** mycelial extract + *Colletotrichum* spp.; **e** culture filtrate + *Colletotrichum* spp.; **f** 50% ethanol + *Colletotrichum* spp.

4.7.4.2 Pour plate technique

The antifungal activity of the mycelial extract and culture filtrate of isolate Cod-NB1302 was further evaluated by the pour plate technique. The results showed that the mycelial extract exhibited greater antifungal activity against mycelial growth of all 10 isolates of *C. capsici* and *Colletotrichum* spp. than the culture filtrate and 50% ethanol. The percentage mycelial growth reductions for the mycelial extract ranged from 50.47-71.09% for all 10 isolates of the tested fungi after 14 days. Whereas, the culture filtrate had no effect on the mycelial growth for all 10 isolates of the tested fungi after 14 days. The 50% ethanol showed percent mycelial growth reductions ranging from 34.89-57.35% after 14 days for all 10 isolates of the tested fungi. Interestingly, the mycelial extract exhibited greater antifungal activity than the 50% ethanol in the range from 1.0-2.0 folds, as shown in the Table 4.6 and Figures 4.22 and 4.23.

Table 4.6 Inhibition effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 on the mycelial growth of *Colletotrichum* spp.

***Colletotrichum* spp.**	**Days**	**Percentage of mycelial growth reduction (PGI)**					
		Mycelial extract	**PGI-2 category**	**Culture filtrate**	**PGI-2 category**	**50% EtOH**	**PGI-2 category**
C. capsici CcC1	14	71.09±3.48$^{a, A}$	3	0±0.00$^{a, C}$	0	47.25±1.93$^{bc, B}$	2
C. capsici CcC2	14	57.95±3.35$^{bc, A}$	3	0±0.00$^{a, C}$	0	38.56±2.99$^{de, B}$	2
C. capsici CcC4	14	51.33±2.00$^{d, A}$	3	0±0.00$^{a, C}$	0	45.77±4.01$^{bcd, B}$	2
C. capsici CcC5	14	69.26±3.79$^{a, A}$	3	0±0.00$^{a, C}$	0	39.82±1.35$^{cde, B}$	2
C. capsici CcC6	14	70.51±3.39$^{a, A}$	3	0±0.00$^{a, C}$	0	40.17±6.42$^{cde, B}$	2
Colletotrichum spp. CgC6	14	70.47±0.81$^{a, A}$	3	0±0.00$^{a, C}$	0	40.71±9.66$^{cde, B}$	2
Colletotrichum spp. CgC7	14	50.47±2.06$^{d, A}$	3	0±0.00$^{a, C}$	0	48.81±1.09$^{b, A}$	2
Colletotrichum spp. CgC10	14	69.77±3.35$^{a, A}$	3	0±0.00$^{a, C}$	0	34.89±1.68$^{e, B}$	2
Colletotrichum spp. CgC11	14	61.54±6.41$^{b, A}$	3	0±0.00$^{a, C}$	0	42.73±0.73$^{bcde, B}$	2
Colletotrichum spp. CgC12	14	54.33±0.38$^{cd, B}$	3	0±0.00$^{a, C}$	0	57.35±0.75$^{a, A}$	3

*Percentage inhibition of mycelial growth was measured after 14 days of incubation at 28 °C

*Averaged from three replications and values is the means (±SD), inhibition percentages against *Colletotrichum* spp. The values in the same column or row followed by the same letter are not significantly different according to analysis of variance and Duncan's Multiple Range Test (DMRT) (at P = 0.05).

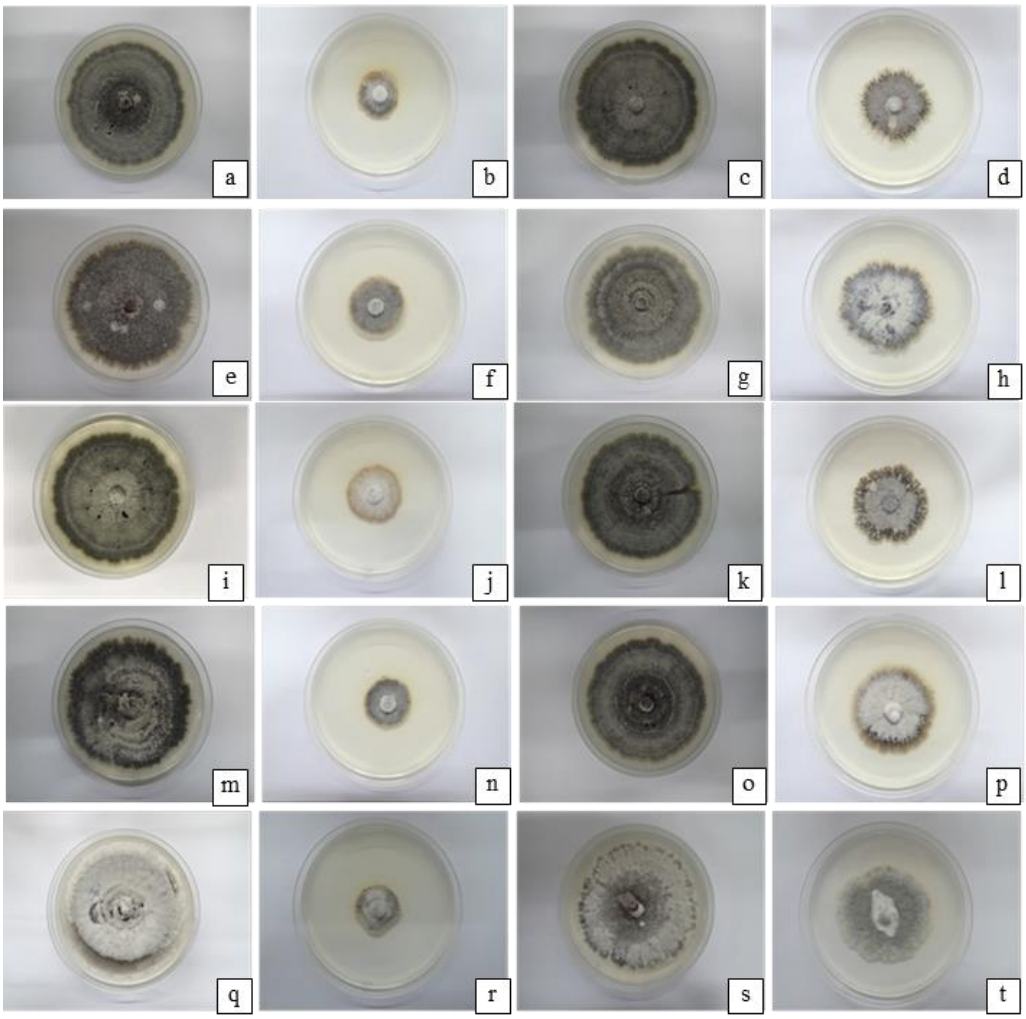

Figure 4.22 Effect of the mycelial extract of the entomopathogenic fungal isolate Cod-NB1302 on PDA to inhibit mycelial growth of *C. capsici* after 14 days. **a** control *C. capsici* CcC1; **b** mycelial extract + *C. capsici* CcC1; **c** culture filtrate + *C. capsici* CcC1; **d** 50% ethanol + *C. capsici* CcC1; **e** control *C. capsici* CcC2; **f** mycelial extract + *C. capsici* CcC2; **g** culture filtrate + *C. capsici* CcC2; **h** 50% ethanol + *C. capsici* CcC2; **i** control *C. capsici* CcC4; **j** mycelial extract + *C. capsici* CcC4; **k** culture filtrate + *C. capsici* CcC4; **l** 50% ethanol + *C. capsici* CcC4; **m** control *C. capsici* CcC5; **n** mycelial extract + *C. capsici* CcC5; **o** culture filtrate + *C. capsici* CcC5; **p** 50% ethanol + *C. capsici* CcC5; **q** control *C. capsici* CcC6; **r** mycelial extract + *C. capsici* CcC6; **s** culture filtrate + *C. capsici* CcC6; **t** 50% ethanol + *C. capsici* CcC6

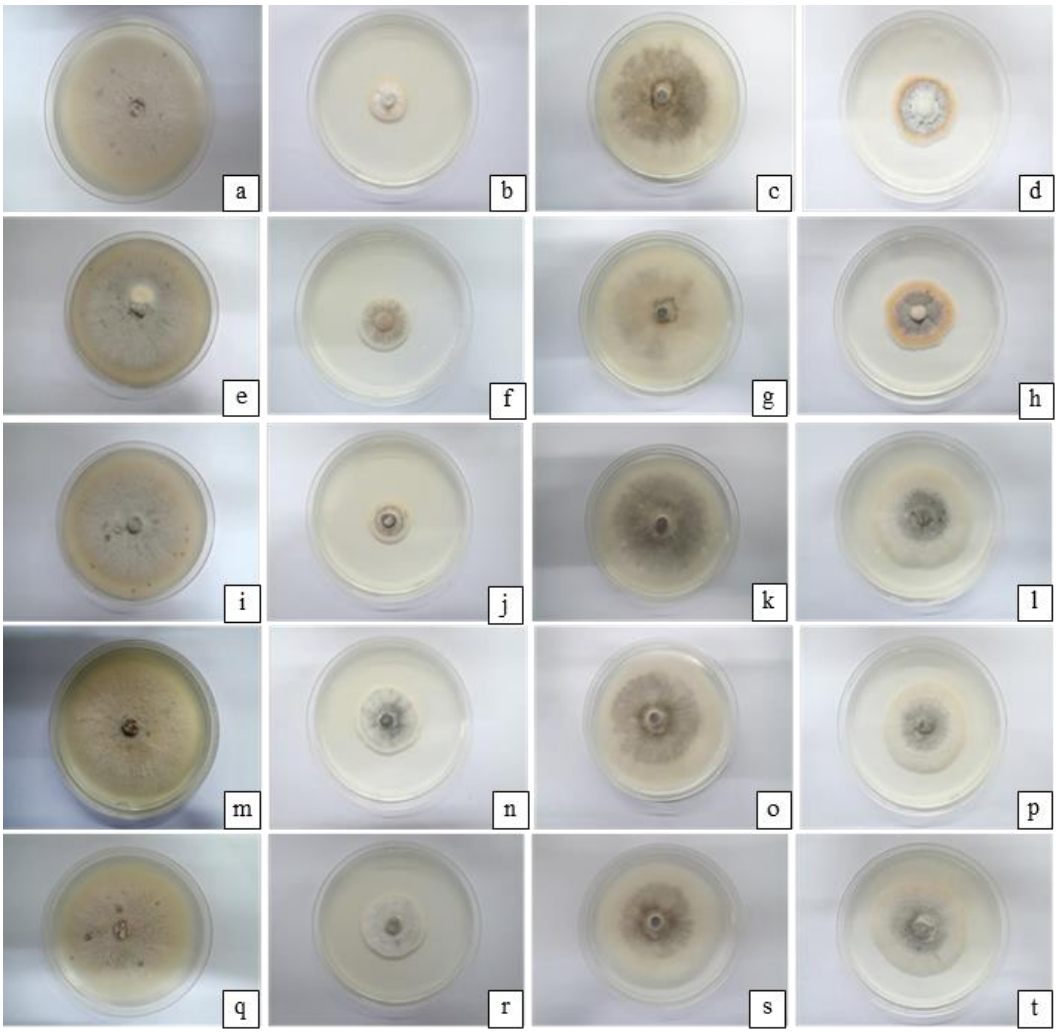

Figure 4.23 Effect of the mycelial extract of the entomopathogenic fungal isolate Cod-NB1302 on PDA to inhibit mycelial growth of *Colletotrichum* spp. after 14 days. **a** control *Colletotrichum* spp. CgC6; **b** mycelial extract + *Colletotrichum* spp. CgC6; **c** culture filtrate + *Colletotrichum* spp. CgC6; **d** 50% ethanol + *Colletotrichum* spp. CgC6; **e** control *Colletotrichum* spp. CgC7; **f** mycelial extract + *Colletotrichum* spp. CgC7; **g** culture filtrate + *Colletotrichum* spp. CgC7; **h** 50% ethanol + *Colletotrichum* spp. CgC7; **i** control *Colletotrichum* spp. CgC10; **j** mycelial extract + *Colletotrichum* spp. CgC10; **k** culture filtrate + *Colletotrichum* spp. CgC10; **l** 50% ethanol + *Colletotrichum* spp. CgC10; **m** control *Colletotrichum* spp. CgC11; **n** mycelial extract + *Colletotrichum* spp. CgC11; **o** culture filtrate + *Colletotrichum* spp. CgC11; **p** 50% ethanol + *Colletotrichum* spp. CgC11; **q** control *Colletotrichum* spp. CgC12; **r** mycelial extract + *Colletotrichum* spp. CgC12; **s** culture filtrate + *Colletotrichum* spp. CgC12; **t** 50% ethanol + CgC12

4.7.5 Effect of mycelial extract and culture filtrate on spore germination of *Colletotrichum* spp.

The effects of the mycelial extract and culture filtrate of the selected entomopathogenic fungal isolate Cod-NB1302 on spore germination of *Colletotrichum* spp. was investigated. After 24 h, spore germination was observed by light microscopy. The results showed that the mycelial extract of isolates Cod-NB1302 and 50% ethanol could inhibit the spore germination of all 10 isolates of the tested pathogenic fungi. The spore germination inhibitions ranged from 38.63-48.25% and 33.98-43.94%, respectively. While, the culture filtrate could suppress spore germination ranging from 20.16-32.33% (Table 4.7). Moreover, the length of the germ tube on PDA plus mycelial extract ranged from 90 to 200 μm for *C. capsici* and *Colletotrichum* spp. The lengths of germ tubes of *C. capsici* and *Colletotrichum* spp. in PDA plus culture filtrate ranged from 220 to 400 μm and 170 to 380 μm, respectively. For the 50% ethanol treatment, the lengths of the germ tubes ranged from 100 to 210 μm and 100 to 230 μm for *C. capsici* and *Colletotrichum* spp., respectively. In the control group, the lengths of the germ tubes were 390 to 520 μm and 300 to 620 μm for *C. capsici* and *Colletotrichum* spp., respectively (Figure4.24- 4.25).

Table 4.7 Percentage inhibition of entomophathogenic fungal isolate Cod-NB1302 against plant pathogenic *Colletotrichum* spp.

***Colletotrichum* spp.**	**Inhibition rate of conidial germination (%)***				**length of the germ tube (µm)****			
	Mycelial extract	**Culture filtrate**	**50% ethanol**	**Control**	**Mycelial extract**	**Culture filtrate**	**50% ethanol**	**Control**
C. capsici CcC1	$43.04^{cd, B}$	$28.84^{ab, C}$	$41.61^{ab, B}$	$100^{a, A}$	$120^{a, A}$	$312^{de, C}$	$161^{a, B}$	$450^{a, D}$
C. capsici CcC2	$45.15^{ab, B}$	$26.03^{bc, C}$	$43.94^{a, B}$	$100^{a, A}$	$137^{b, A}$	$325^{de, B}$	$167^{ab, A}$	$463^{a, C}$
C. capsici CcC4	$40.72^{cd, B}$	$31.42^{a, C}$	$38.11^{bc, C}$	$100^{a, A}$	$136^{b, A}$	$327^{e, B}$	$165^{ab, A}$	$455^{a, C}$
C. capsici CcC5	$46.11^{ab, B}$	$26.18^{bc, C}$	$41.65^{ab, B}$	$100^{a, A}$	$144^{b, A}$	$307^{de, B}$	$159^{a, A}$	$464^{a, C}$
C. capsici CcC6	$48.25^{a, B}$	$32.33^{a, C}$	$40.91^{ab, B}$	$100^{a, A}$	$158^{c, A}$	$301^{d, B}$	$178^{b, A}$	$453^{a, C}$
Colletotrichum spp. CgC6	$38.63^{d, B}$	$28.05^{ab, C}$	$33.98^{c, B}$	$100^{a, A}$	$118^{a, A}$	$260^{c, B}$	$166^{ab, A}$	$422^{a, C}$
Colletotrichum spp. CgC7	$39.99^{cd, B}$	$22.09^{cd, C}$	$39.58^{b, B}$	$100^{a, A}$	$112^{a, A}$	$209^{a, C}$	$171^{ab, B}$	$519^{b, D}$
Colletotrichum spp. CgC10	$40.06^{cd, B}$	$23.25^{cd, C}$	$38.76^{bc, B}$	$100^{a, A}$	$109^{a, A}$	$236^{b, C}$	$179^{b, B}$	$515^{b, D}$
Colletotrichum spp. CgC11	$39.25^{cd, B}$	$22.66^{cd, C}$	$37.81^{bc, B}$	$100^{a, A}$	$109^{a, A}$	$225^{ab, C}$	$180^{b, B}$	$528^{b, D}$
Colletotrichum spp. CgC12	$44.79^{ab, B}$	$20.16^{d, D}$	$39.49^{b, C}$	$100^{a, A}$	$112^{a, A}$	$246^{bc, C}$	$177^{b, B}$	$517^{b, D}$

*Mean of spore germination calculated from three replications (100 spores per replication). Percentage inhibition of spore germination in a column or row followed by the same letter(s) are not significantly different according to DMRT (p = 0.05).

**The length of the germ tube in a column or row followed by the same letter(s) are not significantly different according to DMRT (p = 0.05).

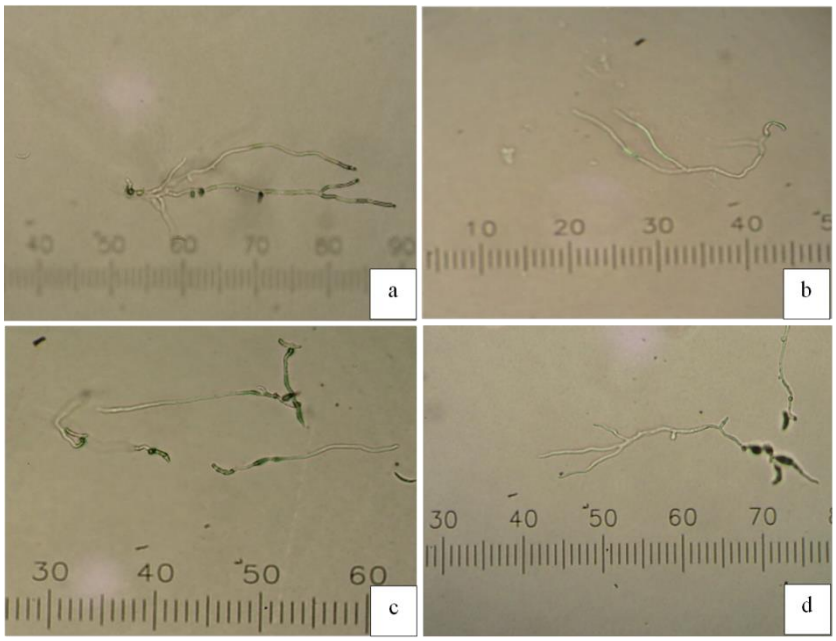

Figure 4.24 Effect of the entomopathogenic fungus isolate Cod-NB1302 on conidial germination of *C. capsici* after 24 hours. **a** Control *C. capsici*; **b** 50% ethanol+ *C. capsici*; **c** mycelial extract+ *C. capsici*; **d** culture filtrate+ *C. capsici*

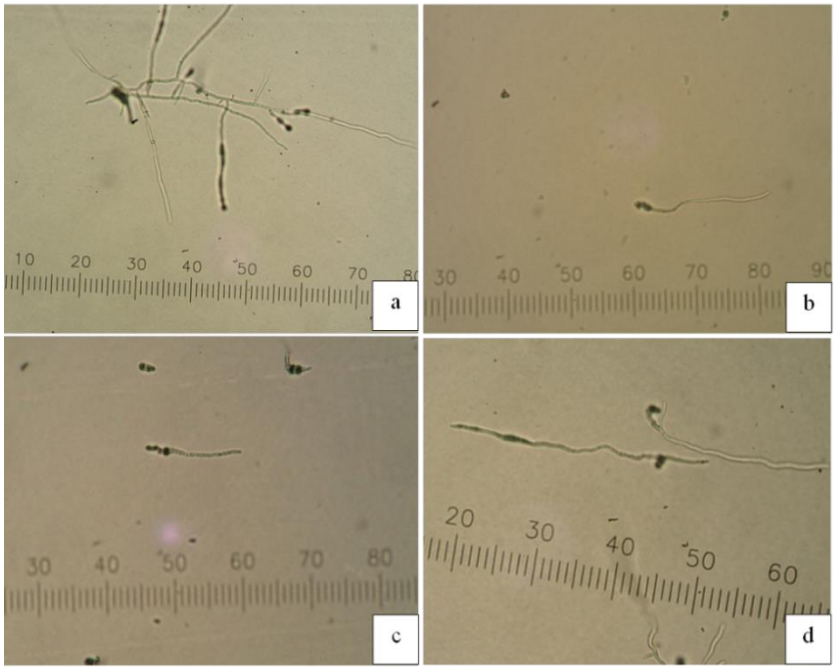

Figure 4.25 Effect of the entomopathogenic fungus isolate Cod-NB1302 on conidial germination of *Colletotrichum* spp. after 24 hours. **a** Control *Colletotrichum* spp.; **b** 50% ethanol+ *Colletotrichum* spp.; **c** mycelial extract+ *Colletotrichum* spp.; **d** culture filtrate+ *Colletotrichum* spp.

4.7.6 Control of anthracnose on chili fruit by detached fruit bioassay

The potential of the selected entomopathogenic fungal isolate Cod-NB1302 to inhibit 10 isolates of the tested plant pathogenic *Colletotrichum* spp. was evaluated by the detached fruit technique. The chili fruits were inoculated with the mycelial extract and culture filtrate of isolate Cod-NB1302 by application to a pin point wound of mature chili fruits one day before the conidial suspensions of 10 isolates of *Colletotrichum* spp. The results indicated that chili fruits inoculated with mycelial extract showed lower disease severity after inoculation compared with those with the culture filtrate, 50% ethanol and positive control. The mycelial extract treatments showed the smallest brown lesions ranging from 3.0 ± 1.2 to 5.8 ± 1.6 mm and 6.8 ± 2.7 to 11.2 ± 4.9 mm for *C. capsici* and *Colletotrichum* spp., respectively. While, the culture filtrate treatments showed larger lesion sizes than the mycelial extract. The disease lesion sizes for the culture filtrate ranged from 3.6 ± 0.9 to 8.0 ± 2.1 mm and 12.4 ± 2.5 to 15.6 ± 2.8 mm for *C. capsici* and *Colletotrichum* spp., respectively. The 50% ethanol treatments also showed large dark sunken lesions. The disease lesion sizes ranged from 14.6 ± 1.1 to 16.4 ± 2.2 mm and 13.8 ± 2.8 to 16.0 ± 4.2 mm for *C. capsici Colletotrichum* spp., respectively, and were obtained in 50% of treatments. Whereas, the positive control showed the highest lesion development, with sunken black lesions, tissue collapse and the black acervuli on the surface of inoculated chili fruits observed. The disease lesion sizes of the positive control treatments ranged from 13.8 ± 3.9 to 18.8 ± 2.3 mm and 15.6 ± 3.3 to 17.6 ± 3.7 mm for *C. capsici* and and *Colletotrichum* spp., respectively. Moreover, the severity index was also observed on chili fruits at seven days after inoculation. The results indicated that the mycelial extract and culture filtrate could protect and reduce disease severity of the tested fungal pathogens with the highly resistant to moderately susceptible *C. capsici* and *Colletotrichum* spp. compared with the 50% ethanol and positive control treatments (Table 4.8 and Figures 4.26- 4.35).

Table 4.8 Effect of mycelial extract and culture filtrate of entomopathogenic fungal isolate Cod-NB1302 on size of anthranose disease lesion and severity index after inoculation with plant pathogenic *Colletotrichum* spp. compared with 50% ethanol.

***Colletotrichum* spp.**	**Mycelial extract**		**Culture filtrate**		**50% ethanol**		**Positive control**	
	Size of lesion (mm)	**Severity index**	**Size of lesion (mm)**	**Severity index**	**Size of lesion (mm)**	**Severity index**	**Size of lesion (mm)**	**Severity index**
C. capsici CcC1	3.0±1.2$^{a, A}$	HR	3.6±0.9$^{a, A}$	HR	16.4±2.2$^{a, B}$	MS	18.8±2.3$^{a, C}$	MS
C. capsici CcC2	3.6±1.5$^{a, A}$	HR	4.0±1.0$^{a, A}$	HR	16.0±1.4$^{a, B}$	MS	15.4±3.8$^{a, C}$	MS
C. capsici CcC4	3.0±1.2$^{a, A}$	HR	4.8±0.4$^{ab, A}$	HR	15.6±2.3$^{a, C}$	MS	15.6±3.3$^{a, C}$	MS
C. capsici CcC5	3.2±1.1$^{a, A}$	HR	7.4±1.8$^{b, B}$	MR	16.4±3.0$^{a, C}$	MS	15.2±2.8$^{a, C}$	MS
C. capsici CcC6	5.8±1.6$^{ab, A}$	MR	8.0±2.1$^{b, A}$	MR	14.6±1.1$^{a, B}$	MS	13.8±3.9$^{a, B}$	MS
Colletotrichum spp. CgC6	7.0±2.1$^{b, A}$	MR	13.0±4.4$^{c, B}$	MS	13.8±2.8$^{a, B}$	MS	17.6±3.7$^{a, B}$	MS
Colletotrichum spp. CgC7	11.2±4.9$^{c, A}$	MS	15.6±2.8$^{c, A}$	MS	14.8±3.6$^{a, A}$	MS	15.6±3.3$^{a, A}$	MS
Colletotrichum spp.CgC10	8.6±2.1$^{bc, A}$	MR	12.4±2.5$^{c, AB}$	MS	15.0±3.1$^{a, B}$	MS	16.4±4.7$^{a, B}$	MS
Colletotrichum spp.CgC11	8.6±2.1$^{bc, A}$	MR	12.8±3.1$^{c, B}$	MS	15.4±1.1$^{a, BC}$	MS	17.6±2.5$^{a, C}$	MS
Colletotrichum spp.CgC12	6.8±2.7$^{b, A}$	MR	13.4±2.3$^{c, B}$	MS	16.0±4.2$^{a, B}$	MS	16.0±2.2$^{a, B}$	MS

*Average size of lesion (mm) on chilli fruit at seven days after inoculation of *Colletotrichum* spp. (five fruits per treatment); symptomless (SL, no lesion), highly resistant (HR, 1.0-4.9 mm), moderately resistant (MR, 5.0-9.9 mm), moderately susceptible (MS, 10.0-19.9) and highly susceptible (HS, >20.0 mm) (Hartman and Wang, 1992). Values is the means (±SE), the values in the same column or row followed by the same letter are not significantly different according to analysis of variance and Duncan's Multiple Range Test (DMRT) (at P = 0.05).

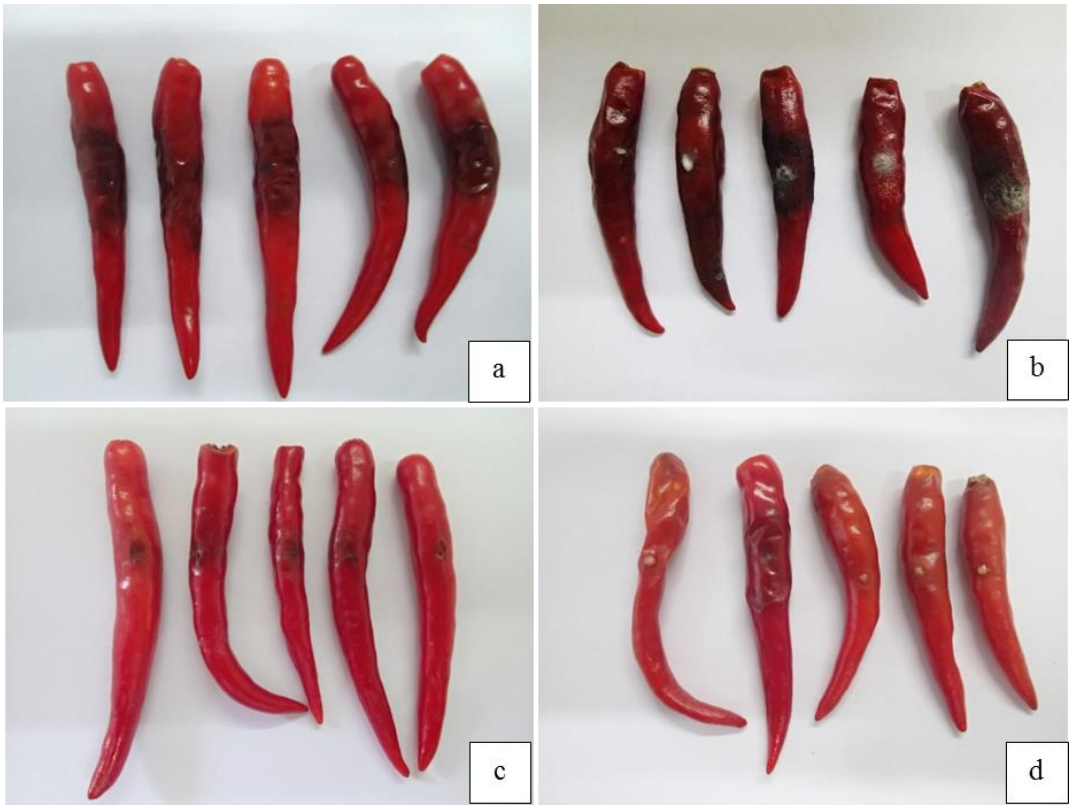

Figure 4.26 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *C. capsici* compared with control group by detach fruit bioassay. **a** control *C. capsici* CcC1; **b** 50% ethanol + *C. capsici* CcC1; **c** mycelial extract + *C. capsici* CcC1; **d** culture filtrate + *C. capsici* CcC1

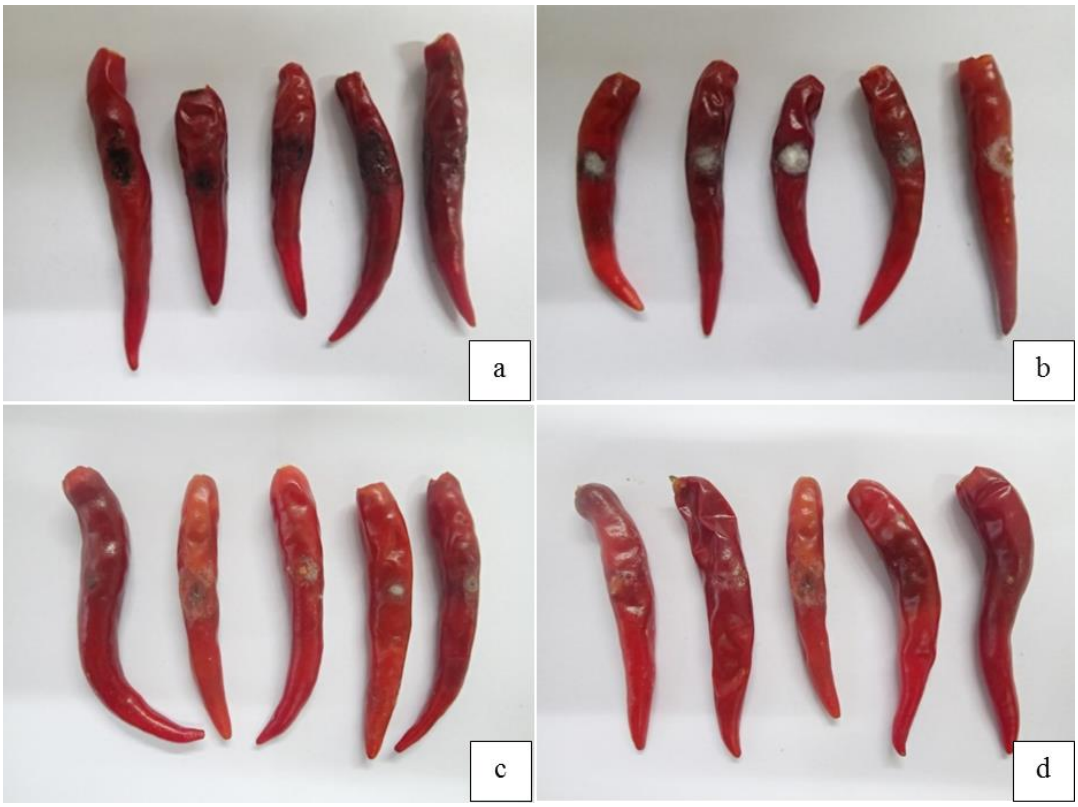

Figure 4.27 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *C. capsici* compared with control group by detach fruit bioassay. **a** control *C. capsici* CcC2; **b** 50% ethanol + *C. capsici* CcC2; **c** mycelial extract + *C. capsici* CcC2; **d** culture filtrate + *C. capsici* CcC2

Figure 4.28 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *C. capsici* compared with control group by detach fruit bioassay. **a** control *C. capsici* CcC4; **b** 50% ethanol + *C. capsici* CcC4; **c** mycelial extract + *C. capsici* CcC4; **d** culture filtrate + *C. capsici* CcC4

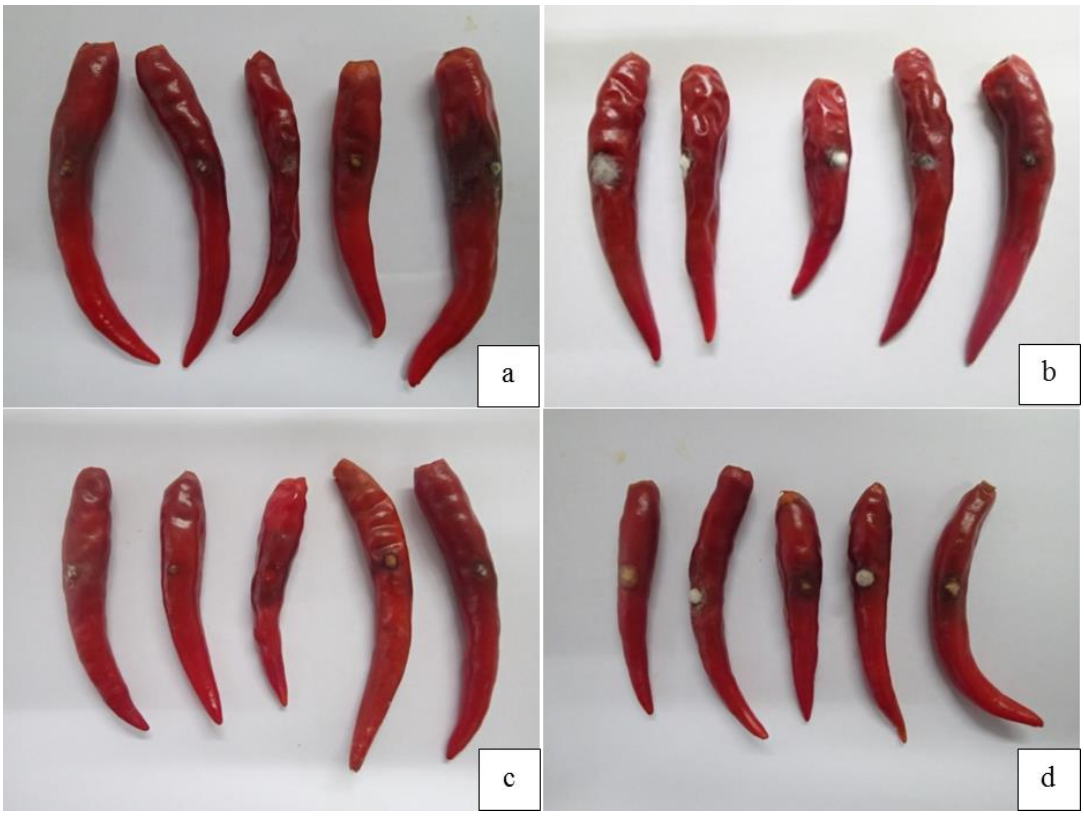

Figure 4.29 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *C. capsici* compared with control group by detach fruit bioassay. **a** control *C. capsici* CcC5; **b** 50% ethanol + *C. capsici* CcC5; **c** mycelial extract + *C. capsici* CcC5; **d** culture filtrate + *C. capsici* CcC5

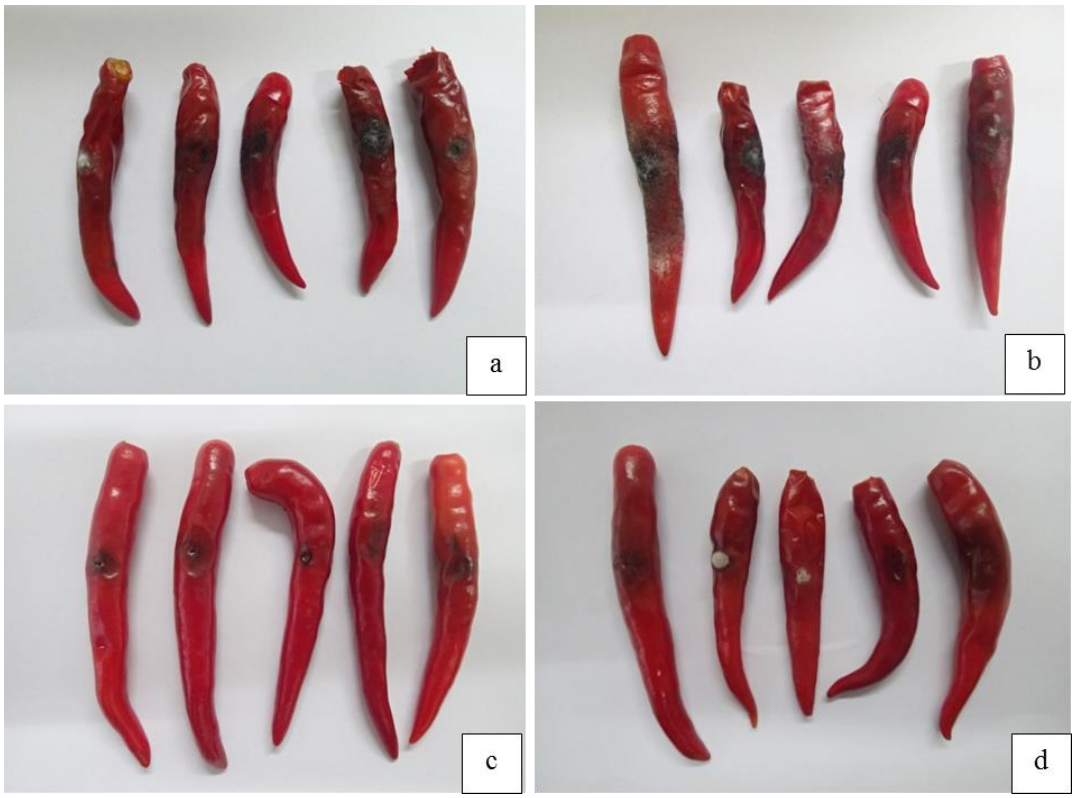

Figure 4.30 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *C. capsici* compared with control group by detach fruit bioassay. **a** control *C. capsici* CcC6; **b** 50% ethanol + *C. capsici* CcC6; **c** mycelial extract + *C. capsici* CcC6; **d** culture filtrate + *C. capsici* CcC6

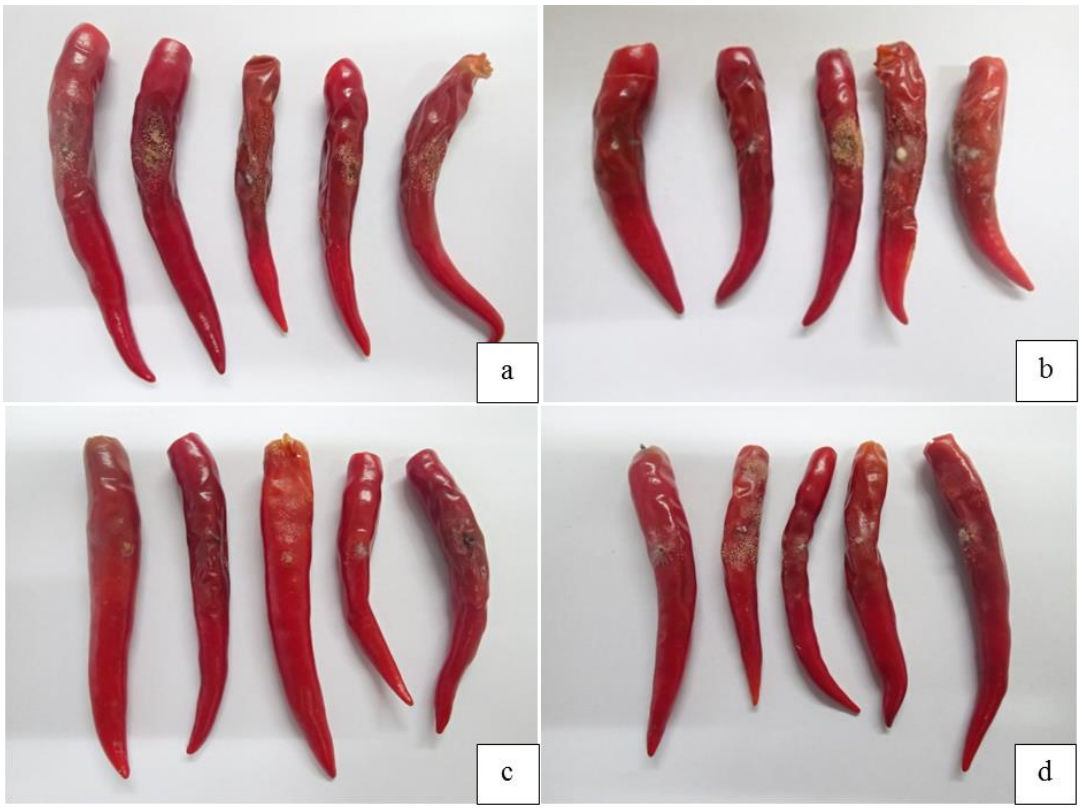

Figure 4.31 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *Colletotrichum* spp. compared with control group by detach fruit bioassay. **a** control *Colletotrichum* spp. CgC6; **b** 50% ethanol + *Colletotrichum* spp. CgC6; **c** mycelial extract + *Colletotrichum* spp. CgC6; **d** culture filtrate + *Colletotrichum* spp. CgC6

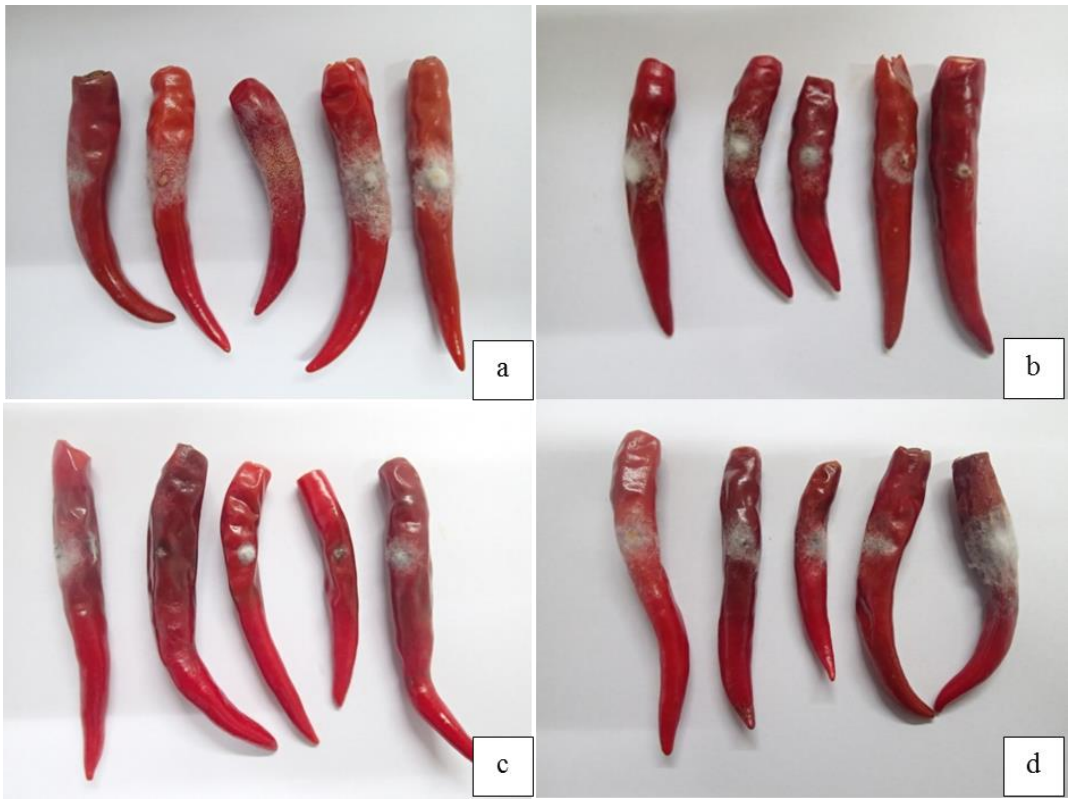

Figure 4.32 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *Colletotrichum* spp. compared with control group by detach fruit bioassay. **a** control *Colletotrichum* spp. CgC7; **b** 50% ethanol + *Colletotrichum* spp. CgC7; **c** mycelial extract + *Colletotrichum* spp. CgC7; **d** culture filtrate + *Colletotrichum* spp. CgC7

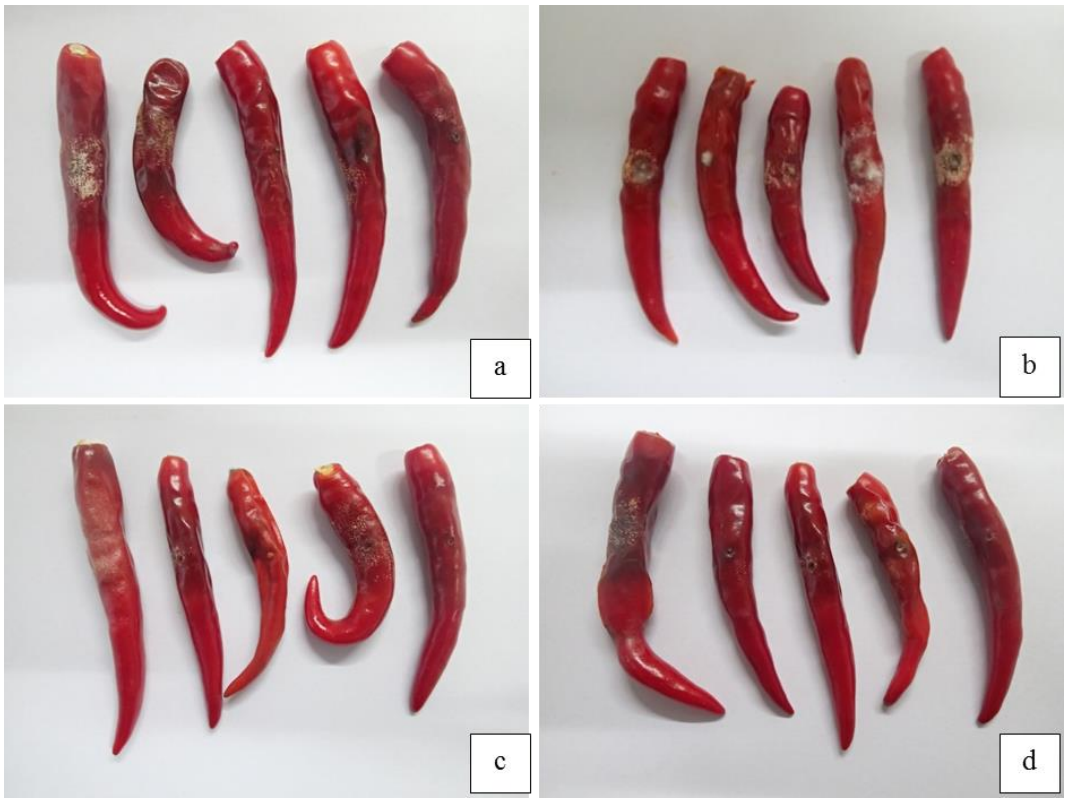

Figure 4.33 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *Colletotrichum* spp. compared with control group by detach fruit bioassay. **a** control *Colletotrichum* spp. CgC10; **b** 50% ethanol + *Colletotrichum* spp. CgC10; **c** mycelial extract + *Colletotrichum* spp. CgC10; **d** culture filtrate + *Colletotrichum* spp. CgC10

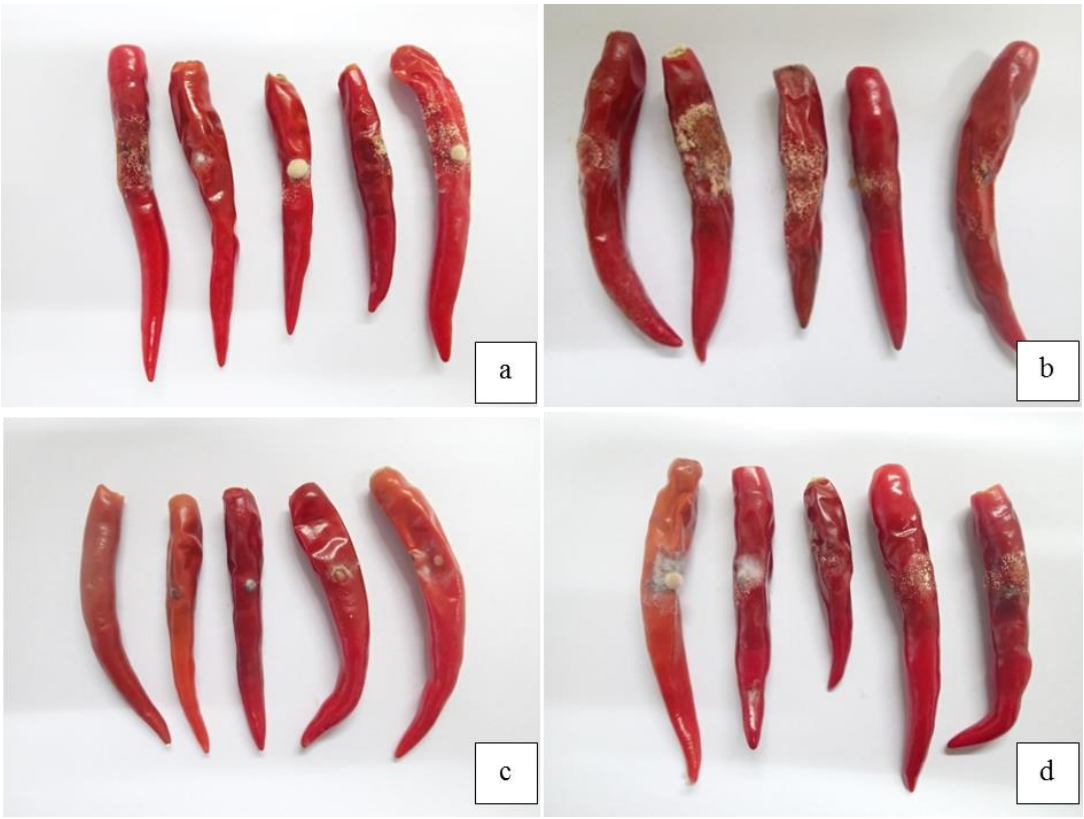

Figure 4.34 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *Colletotrichum* spp. compared with control group by detach fruit bioassay. **a** control *Colletotrichum* spp. CgC11; **b** 50% ethanol + *Colletotrichum* spp. CgC11; **c** mycelial extract + *Colletotrichum* spp. CgC11; **d** culture filtrate + *Colletotrichum* spp. CgC11

Figure 4.35 Effect of mycelial extract and culture filtrate of the entomopathogenic fungal isolate Cod-NB1302 and 50% ethanol on chilli fruits after inoculation with *Colletotrichum* spp. compared with control group by detach fruit bioassay. **a** control *Colletotrichum* spp. CgC12; **b** 50% ethanol + *Colletotrichum* spp. CgC12; **c** mycelial extract + *Colletotrichum* spp. CgC12; **d** culture filtrate + *Colletotrichum* spp. CgC12

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Discussion

In this study, a total of 44 samples of entomopathogenic fungi infected cicada nymphs were isolated and initially identified based on morphological characteristics, such as physical characteristics, cultural morphology, colony color and conidial shape. The result indicated that the isolated entomopathogenic fungi were divided into five groups: the first group consisted of the 31 isolates of the entomopathogenic fungi that produced white cream colonies with slimy cream-yellow conidia at the center of the colony like *Polycephalomyces nipponicus* (=*Cordyceps nipponica*). These findings correlate with those of Luangsa-ard *et al*. (2008) who reported that *P. nipponicus* is an insect pathogen that infected cicada nymphs. The fungus has reddish brown stomata, is erect and mostly branched in appearance. The colonies at first floccose possess white-cream and produce patches of slimy cream-yellow conidia. The second group consisted of four isolates that produced white colonies with a slow growth rate and they did not produce conidia on PDA medium, like *Ophiocordyceps longissima*. These findings correlate with those of Luangsa-ard *et al*. (2010) who found that *O. longissima* is an insect pathogen that infected cicada nymphs. The fungus produces a solitary orange to dark brown stroma emerging from the head or thorax. This fungus produced a white colony color and extremely slow growth rate. The third group consisted of five isolates that also produced white colonies with a fast growth rate. Moreover, they could produce mainly solitary phialides and formed imbricate conidial chains, conidia are obclavate to ellipsoidal like *Simplicillium obclavatum*. Zare and Gams (2001) reported that *Simplicillium* resembled the genus *Lecanicillium* (family Cordycipitaceae). Moreover, a species of *Simplicillium* has been reported to be isolated from soil, nematodes, rust and mushrooms (Nonaka *et al*., 2013). Whereas, in this study, the fungal *S. obclavatum* was isolated from cicada nymphs. The fourth group consisted of three isolates that produced whitish yellow colonies on PDA with a fast growth rate, like *Metacordyceps chlamydosporia*, and this fungus produced white colonies at the beginning that later became cream-colored and appeared powdery with dictyochlamydospores on the

surface of the colony and then pale yellow in appearance. This finding correlates with Li *et al*. (2010) who reported that the fungus *Metacordyceps* is a pathogen on cicada nymphs (Hemiptera: Cicadidae). Kim *et al*. (2016) successfully isolated *M. chlamydosporia* (KNU14-22) from soil in Korea. However, this observation contradicted the opinion of Zare *et al*. (2001) who reported that *M. chlamydosporia* was isolated from mollusc egg sacs and eggs of slugs (Sung *et al*., 2007a). The fifth group consisted of one isolate that produced a pink colony with extremely slow growth rate and could produce mycelium with stalks and pink conidia on the colony, like *O. sobolifera* (=*Cordyceps sobolifera*), and this the fungus produces a solitary yellow to brown stroma arising from the head of the host appearing clavate and roughened. Conidia were produced on conidiogenous cells with sympodial elongation, long ellipsoidal. These finding agreed with the report of Liu *et al*. (2001) who isolated the fungus *C. sobolifera* (=*Beauveria sobolifera*, anamorph state) from cicada nymphs.

In this study, the entomopathogenic fungi were grouped based on colony morphology and microscopic characteristics. The dendrogram derived from morphological features divided the isolates of the entomopathogenic fungi into two main clusters that were categorized in group A and B. For example, 16 isolates from Mahasarakham (MK) and seven isolates from Nong Bua Lam Phu (NB) were categorized in group A. While, six isolates from MK and one isolate from NB were categorized in group B based on the microscopic characteristics (Figure 4.10). Therefore, the colony morphology and microscopic characteristics varied among isolates and strains of the fungus, without influence from the geographic region.

Previous studies reported that molecular techniques based on *ITS* region and combined data sets consisting of *ITS*, *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* regions were effective for identification of *Cordyceps* species (Sung *et al*., 2007b; Chan *et al*., 2011). The DNA sequencing technique has been the most widely used and this method is practical, sensitive and easy to use rapid assay to identify species. In the present study, the phylogenetic relationship between *Cordyceps* and related species based on combined data sets consisting of *ITS*, *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* regions were also investigated. The results proved that the phylogenetic tree based on the *ITS* sequence and the combined data sets of *ITS*, *nrSSU*, *nrLSU*, *EF-1α* and *rpb1* were successfully

used to classify these fungi. The phylogeny showed that 31 isolates of fungi aggregated into one large cluster as a group of *P. nipponicus* clade, four isolates of the fungi were closely related with *O. longissima* in a clade, three isolates of the fungi were located in the same clade with *M. chlamydosporia*, five isolates of the fungi were closely related with *S. obclavatum* and one isolate was closely related with *O. sobolifera* in a clade. These findings were supported by high bootstrap values (99-100%). These results resemble those of Kepler *et al*. (2013) who used the sequence of ribosomal DNA and protein coding DNA to classify the genus *Polycephalomyces.* The *SSU, LSU, EF-1α* and *rpb1* sequences have also been used to identify the entomopathogenic fungi that infected cicada nymphs as *O. longissima* isolate Cod-MK1 (Sangdee *et al*., 2013). Nonaka *et al*. (2013) successfully used the ribosomal RNA gene of the *ITS* region to classify six *Simplicillium* species, including *S. aogashimaense*, *S. cylindrosporum*, *S. obclavatum*, *S. subtropicum* and *S. sympodiophorum*. Kepler *et al*. (2012) determined the phylogenetic relationships among the entomopathogenic genus *Metacordyceps* using small and large subunits of ribosomal DNA, elongation factor 1α and the largest and second largest subunits of RNA polymerase II. Therefore, the identification of the entomopathogenic fungi that infected cicada nymphs can be efficiently carried out using the combined data sets. However, only the *ITS* sequence may be used for a rapid identification.

Information on the bioactivities of *Cordyceps* species has been reported in the medical and pharmaceutical areas, such as for *C. sinensis*, *C. cicadae*, *C. militaris*, *C. sobolifera*, *C. ophioglossoides* and *C. nipponica*. These fungi have potentially salutary effects, such as immunomodulatory, anti-tumor, antioxidant, anti-inflammatory, anti-fungal, anti-viral, antibacterial, anti-metastatic, anti-cancer and anti-malarial activities (Kneifel *et al*., 1977; Kiho *et al*., 1990; Lin and Chiang, 2008; Yue *et al*., 2008; Jia *et al*., 2009; Shrestha *et al*., 2013; Zhu *et al*., 2014). Whereas, the information on the bioactive compounds used in the agricultural area, such as biocontrol agent, is limited. In the present study, the antifungal activity of entomopathogenic fungi isolated from dead cicada nymphs from various locations in the northeastern Thailand against the plant pathogenic fungus *Colletotrichum* spp. was investigated. Eight of 44 isolates from the entomopathogenic fungi showed good inhibitory effect on the mycelial growth of the plant pathogenic fungi. However, the antifungal effects of the

entomopathogenic fungi depended on the strain and species of the entomopathogenic fungi and the strain of plant pathogenic *Colletotrichum*. For example, entomopathogenic fungal isolate Cod-NB1302 showed the highest percentage of mycelial growth reduction of *C. capsici* isolate CcC4 at 43.55%, while the mycelial growth reduction of *Colletotrichum* spp. isolate CgC10 was 25.02%. This study demonstrated that different isolates of entomopathogenic fungi have variations in the percentage of mycelial growth reduction when tested with the same isolates of the plant pathogenic *Colletotrichum* spp.

Among the extracts of the entomopathogenic fungal strains, the mycelial extract of *O. sobolifera* isolate Cod-NB1302 gave the greatest inhibition of the mycelial growth of *Colletotrichum* spp. under *in vitro* conditions. Moreover, the mycelial extract showed high inhibition of spore germination (38.63-48.25%) and germ tube length (90-200 µm) when compared with the culture filtrate (20.16-32.33%, 170-400 µm), 50% ethanol (33.98-43.94%, 100-230 µm) and control treatments. Moreover, abnormal spore shapes and short germ tubes were observed after treatment with the mycelial extract. Based on these results, it indicated that the inhibitory effect of the mycelial extract was not affected by the 50% ethanol that was used as an organic solvent. Therefore, these inhibitory effects may be due to the bioactive compounds that are present in the mycelial extract. The finding agreed with the reported of Imtiaj and Lee (2007) who demonstrated that the culture filtrate of *O. sobolifera* could inhibit the growth of plant pathogen fungi (*Botrytis cinerea, Colletotrichum gloeosporioides* and *C. miyabeanus)*. Chen and Huang (2010) also found that the culture filtrate of the mushroom *Lentinula edodes* could inhibit the mycelial growth and zoospore germination of the plant pathogenic fungus *Phytophtora capsici*. Pandey (2012) reported that the wild mushrooms, including *Cordyceps* sp., could inhibit mycelial growth and conidial germination of *Alternaria brassicae, Fusarium oxysporum, C. capsici* and *Rhynchosporium oryzae*. Ashok *et al*. (2014) reported that crude extracts of a mushroom (*Lycoperdon umbrinum*) could inhibit the mycelial growth of some plant pathogens, such as *C. capsici, C. dematium, C. lindemuthianum, F. oxysporum* and *F. solani,* by the agar well diffusion technique. Miazzi *et al*. (2012) also reported that the extracts from *Cordyceps* species could inhibit mycelial growth of the plant

pathogens *Rosellinia* (92%), *Phytophthora* (71%), *Fusarium* (69%), *Colletotrichum (*20%) and *Penicillium* (21%).

Moreover, the detached chili fruit assay was also used to investigate the antifungal activity of the extract of *O. sobolifera* isolate Cod-NB1302 against the plant pathogenic *Colletotrichum* spp. The results indicated that the mycelial extract and culture filtrate of *O. sobolifera* isolate Cod-NB1302 could reduce the size of the disease lesion and disease severity of all test plant pathogenic *Colletotrichum* spp. Whereas, the 50% ethanol and control could not reduce the disease severity with the appearance of large brownish lesions on chili fruits. The mycelial extract and culture filtrate showed reduced disease severity scores for the test plant pathogens with highly resistant (HR) to moderately susceptible (MS). The 50% ethanol and control treatments showed disease severity scores for all the pathogens with moderately susceptible (MS). These findings resemble the opinion of Chen and Huang (2010) who demonstrated that the culture filtrate of the mushrooms *Clitocybe nuda* (LA82) and *C. aureus* could effectively reduce the disease severity of phytophtora blight of pepper plants caused by *P. capsici*. In addition, Park *et al*. (2009) reported the activity of the entomopathogenic fungus *C. militaris* against the growth of the plant pathogenic fungus *F. oxysporum*. Therefore, isolate Cod-NB1302 could be used as a source of bioactive compounds that will be developed into a new antifungal agent against anthracnose disease. Further studies will focus on green house and field trials for confirmation of the antifungal activity before application. Moreover, isolating the compounds responsible for the observed bioactivities and elucidating their structure should be the focus in further studies.

5.2 Conclusion

The entomopathogenic fungi that infected cicada nymphs in the northeastern Thailand were classified as five species, including *P. nipponicus*, *O. longissima O. sobolifera*, *M. chlamydosporia* and *S. obclavatum*, based on the molecular data from the nucleotide sequence of non-coding and coding gene sequences, host affiliation and morphology characters. However, the fungal genus *S. obclavatum* has not been reported as a parasitic species in cicada nymphs. The entomopathogenic fungus *O. sobolifera* isolate Cod-NB1302 had the best activity against the plant pathogenic fungi

Colletotrichum spp. under *in vitro* conditions. Therefore, isolate Cod-NB1302 might be considered as a new candidate source for biological control agents.

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APPENDICES

Appendix A

Sequences of entomopathogenic fungal and related species and their NCBI accession numbers used in this study

Table 1 Sequences of entomopathogenic fungal and related species and their NCBI accession numbers used in this study.

Species	Strain	NCBI accession number				
		ITS	***nrSSU***	***nrLSU***	***EF-1α***	***rpb1***
Bionectria ochroleuca	-	KF055399	DQ862044	AY283558	DQ862029	DQ842031
Cordyceps bifusispora	EFCC 5690	AJ786553	EF468952	EF468806	EF468746	-
Cordyceps brongniartii	NBRC 101395	AB235200	JN941759	JN941382	DQ376244	JN992493
Cordyceps cardinalis	OSC 93610	JN049843	AY184974	AY184963	EF469059	EF469088
Cordyceps militaris	NBRC 30377	JN943300	JN941756	JN941385	AB968605	JN992490
Cordyceps militaris	NBRC 9787	JN943433	JN941757	JN941384	AB968604	JN992491
Cordyceps scarabaeicola	ARSEF 5689	JN049827	AF339574	AF339524	DQ522335	DQ522380
Hirsutella sp.	OSC 128575	JN049845	EF469126	EF469079	EF469064	EF469093
Metacordyceps chlamydosporia	CBS 101244	JN049821	DQ522544	DQ518758	DQ522327	DQ522372
Metacordyceps chlamydosporia	-	AB378547	AB758273	-	AB758481	AB758684
*Metacordyceps chlamydosporia**	Cod-RE1203	MG031303	MG031257	MG031193	MG196137	MG196179
*Metacordyceps chlamydosporia**	Cod-NN1302	MG031301	MG031255	MG031191	MG196135	MG196177
*Metacordyceps chlamydosporia**	Cod-NN1303	MG031302	MG031256	MG031192	MG196136	MG196178
Metacordyceps kusanagiensis	TNS F18494	JN049873	JF415954	JF415972	JF416014	JN049890
Metacordyceps sp.	HMAS 199601	JN049879	JF415957	JF415978	JF416018	JN049894
Ophiocordyceps acicularis	OSC 128580	JN049820	DQ522543	DQ518757	DQ522326	DQ522371
Ophiocordyceps heteropoda	EFCC 10125	JN049852	EF468957	EF468812	EF468752	EF468860
Ophiocordyceps heteropoda	NBRC 100643	FJ765029	JN941719	JN941422	AB968595	JN992453
Ophiocordyceps heteropoda	NBRC 100642	FJ765030	JN941720	JN941421	AB968594	JN992454
Ophiocordyceps longissima	NBRC 106965	AB968406	AB968392	AB968420	AB968584	-
Ophiocordyceps longissima	NBRC 108989	AB968407	AB968394	AB968421	AB968585	-
*Ophiocordyceps longissima**	Cod-MK1202	MG031298	MG031289	MG031217	MG196139	MG196181
*Ophiocordyceps longissima**	Cod-RE1301	MG031299	MG031290	MG031218	MG196140	MG196182
*Ophiocordyceps longissima**	Cod-SN1402	MG031300	MG031291	MG031219	MG196141	MG196183
*Ophiocordyceps longissima**	Cod-Loei1301	MG031297	MG031288	MG031216	MG196138	MG196180
Ophiocordyceps sinensis	EFCC 7287	JN049854	EF468971	EF468827	EF468767	EF468874
Ophiocordyceps sobolifera	-	AB027374	-	-	-	-
Ophiocordyceps sobolifera	KEW78842	JN049855	EF468972	EF468828	-	EF468875
Ophiocordyceps sobolifera	NBRC 106967	AB968409	AB968395	AB968422	AB968590	-

Table 1 (Cont.)

Species	Strain	NCBI accession number				
		ITS	***nrSSU***	***nrLSU***	***EF-1α***	***rpb1***
Ophiocordyceps sobolifera *	Cod-NB1302	KT281884	KT281885	KT281886	KT281887	KT281888
Polycephalomyces formosus	ARSEF 1424	KF049661	KF049615	AY259544	DQ118754	DQ127245
Polycephalomyces nipponicus	BCC 2325	KF049665	KF049622	KF049640	KF049696	KF049655
Polycephalomyces nipponicus	BCC 18108	KF049657	KF049608	KF049626	KF049681	KF049644
Polycephalomyces nipponicus	NBRC 101405	JN943442	JN941754	JN941387	-	JN992488
Polycephalomyces nipponicus *	Cod-MK1201	KF061082	KF061081	KF527443	KF527444	KF061083
*Polycephalomyces nipponicus**	Cod-MK1203	KX827724	MG031258	MG031220	MG196105	MG196147
*Polycephalomyces nipponicus**	Cod-MK1204	KX827725	MG031259	MG031221	MG196106	MG196148
*Polycephalomyces nipponicus**	Cod-MK1205	KX827726	MG031260	MG031222	MG196107	MG196149
*Polycephalomyces nipponicus**	Cod-MK1206	KX827727	MG031261	MG031223	MG196108	MG196150
*Polycephalomyces nipponicus**	Cod-MK1207	KX827728	MG031262	MG031224	MG196109	MG196151
*Polycephalomyces nipponicus**	Cod-MK1208	KX827729	MG031263	MG031225	MG196110	MG196152
*Polycephalomyces nipponicus**	Cod-MK1209	KX827730	MG031264	MG031226	MG196111	MG196153
*Polycephalomyces nipponicus**	Cod-MK1210	KX827731	MG031265	MG031227	MG196112	MG196154
*Polycephalomyces nipponicus**	Cod-MK1302	KX827732	MG031266	MG031228	MG196113	MG196155
*Polycephalomyces nipponicus**	Cod-MK1305	KX827733	MG031267	MG031229	MG196114	MG196156
*Polycephalomyces nipponicus**	Cod-MK1309	KX827734	MG031268	MG031230	MG196115	MG196157
*Polycephalomyces nipponicus**	Cod-MK1319	KX827735	MG031269	MG031231	MG196116	MG196158
*Polycephalomyces nipponicus**	Cod-MK1324	KX827736	MG031270	MG031232	MG196117	MG196159
*Polycephalomyces nipponicus**	Cod-MK1325	KX827737	MG031271	MG031233	MG196118	MG196160
*Polycephalomyces nipponicus**	Cod-MK1329	KX827738	MG031272	MG031234	MG196119	MG196161
*Polycephalomyces nipponicus**	Cod-RE1201	KX827756	MG031285	MG031247	MG196132	MG196174
*Polycephalomyces nipponicus**	Cod-RE1202	KX827757	MG031286	MG031248	MG196133	MG196175
*Polycephalomyces nipponicus**	Cod-NB1301	KX827739	MG031273	MG031235	MG196120	MG196162
*Polycephalomyces nipponicus**	Cod-NB1303	KX827740	MG031274	MG031236	MG196121	MG196163

Table 1 (Cont.)

Species	Strain	NCBI accession number				
		ITS	***nrSSU***	***nrLSU***	***EF-1α***	***rpb1***
*Polycephalomyces nipponicus**	Cod-NB1304	KX827741	MG031275	MG031237	MG196122	MG196164
*Polycephalomyces nipponicus**	Cod-NB1305	KX827742	MG031276	MG031238	MG196123	MG196165
*Polycephalomyces nipponicus**	Cod-NB1306	KX827743	MG031277	MG031239	MG196124	MG196166
*Polycephalomyces nipponicus**	Cod-NB1307	KX827744	MG031278	MG031240	MG196125	MG196167
*Polycephalomyces nipponicus**	Cod-NB1308	KX827745	MG031279	MG031241	MG196126	MG196168
*Polycephalomyces nipponicus**	Cod-NN1301	KX827751	MG031280	MG031242	MG196127	MG196169
*Polycephalomyces nipponicus**	Cod-NN1304	KX827752	MG031281	MG031243	MG196128	MG196170
*Polycephalomyces nipponicus**	Cod-NN1305	KX827753	MG031282	MG031244	MG196129	MG196171
*Polycephalomyces nipponicus**	Cod-NN1306	KX827754	MG031283	MG031245	MG196130	MG196172
*Polycephalomyces nipponicus**	Cod-NN1307	KX827755	MG031284	MG031246	MG196131	MG196173
*Polycephalomyces nipponicus**	Cod-SN1401	KX827758	MG031287	MG031249	MG196134	MG196176
Polycephalomyces prolificus	TNS-F-18481	KF049659	KF049612	KF049631	KF049686	KF049648
Polycephalomyces prolificus	TNS-F-18547	KF049660	KF049613	KF049632	KF04968	KF049649
Simplicillium lamellicola	CBS 116.25	AJ292393	AF339601	AF339552	DQ522356	DQ522404
Simplicillium lanosoniveum	IMI 317442	AJ292395	AF339603	AF339554	DQ522357	DQ522405
Simplicillium obclavatum	CBS 311.74	FJ156235	AF339567	AF339517	EF468798	-
Simplicillium spp. *	Cod-MK1301	MG031292	MG031250	MG031186	MG196142	MG196184
Simplicillium spp. *	Cod-MK1303	MG031293	MG031251	MG031187	MG196143	MG196185
Simplicillium spp. *	Cod-MK1304	MG031294	MG031252	MG031188	MG196144	MG196186
Simplicillium spp. *	Cod-MK1311	MG031295	MG031253	MG031189	MG196145	MG196187
Simplicillium spp. *	Cod-MK1321	MG031296	MG031254	MG031190	MG196146	MG196188

* In this study

Appendix B

Natural Medicinal Mushroom Museum

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7171
Collector No. *Sangdee*-01
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7172
Collector No. *Sangdee*-02
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7173
Collector No. *Sangdee*-03
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7174
Collector No. *Sangdee*-04
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7175
Collector No. *Sangdee*-05
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7176
Collector No. *Sangdee*-06
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7177
Collector No. *Sangdee*-07
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7178
Collector No. *Sangdee*-08
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7179
Collector No. *Sangdee*-09
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 11 Jun. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7180
Collector No. *Sangdee*-10
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 28 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7181
Collector No. *Sangdee*-11
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 28 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7182
Collector No. *Sangdee*-12
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 28 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7183
Collector No. *Sangdee*-13
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 28 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7184
Collector No. *Sangdee*-14
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 28 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7185
Collector No. *Sangdee*-15
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 28 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7186
Collector No. *Sangdee*-16
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 28 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7187
Collector No. *Sangdee*-17
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Roi Et Province, Ban Ngu Luam, Suwan Phum District, 15° 40′ 52.6″ N, 103° 44′ 58.0″ E
Altitude: 145 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 16 Jul. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7188
Collector No. *Sangdee*-18
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Roi Et Province, Ban Ngu Luam, Suwan Phum District, 15° 40′ 52.6″ N, 103° 44′ 58.0″ E
Altitude: 145 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 16 Jul. 2012
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7189
Collector No. *Sangdee*-19
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20′ 20.4″ N, 102° 01′ 16.8″ E
Altitude: 337 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7190
Collector No. *Sangdee*-20
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20′ 20.4″ N, 102° 01′ 16.8″ E
Altitude: 337 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7191
Collector No. *Sangdee*-21
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20′ 20.4″ N, 102° 01′ 16.8″ E
Altitude: 337 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7192
Collector No. *Sangdee*-22
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20′ 20.4″ N, 102° 01′ 16.8″ E
Altitude: 337 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7193
Collector No. *Sangdee*-23
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20′ 20.4″ N, 102° 01′ 16.8″ E
Altitude: 337 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7194
Collector No. *Sangdee*-24
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20′ 20.4″ N, 102° 01′ 16.8″ E
Altitude: 337 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7195
Collector No. *Sangdee*-25
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20′ 20.4″ N, 102° 01′ 16.8″ E
Altitude: 337 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 May 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7196
Collector No. *Sangdee*-26
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nakhon Phanom Province, Si Songkhram District, 17° 39′ 03.5″ N, 104° 12′ 43.9″ E
Altitude: 146 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 7 Jun. 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7197
Collector No. *Sangdee*-27
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nakhon Phanom Province, Si Songkhram District, 17° 39′ 03.5″ N, 104° 12′ 43.9″ E
Altitude: 146 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 7 Jun. 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7198
Collector No. *Sangdee*-28
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nakhon Phanom Province, Si Songkhram District, 17° 39′ 03.5″ N, 104° 12′ 43.9″ E
Altitude: 146 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 7 Jun. 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7199
Collector No. *Sangdee*-29
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nakhon Phanom Province, Tha Uthen District, 17° 33′ 25″ N, 104° 36′ 45″ E
Altitude: 156 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 Jun. 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7200
Collector No. *Sangdee*-30
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nakhon Phanom Province, Tha Uthen District, 17° 33′ 25″ N, 104° 36′ 45″ E
Altitude: 156 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 24 Jun. 2013
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Polycephalomyces* Herbarium**

MSUT_7201
Collector No. *Sangdee*-31
Sci.name: *Polycephalomyces nipponicus* (=*Cordyceps nipponica*) (Kobayasi) Kepler & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Sakon Nakhon Province, Wanon Niwat District, 17° 37′ 56″ N, 103° 45′ 7″ E **Altitude:** 167 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *A. Sangdee* **Date of coll.** 20 Jun. 2014
Determined by: *A. Sangdee*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Ophiocordyceps* Herbarium**

MSUT_7211
Collector No. *Jaihan*-01
Sci.name: *Ophiocordyceps longissima* (=*Cordyceps longisima*) (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 11 Jun. 2012
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Ophiocordyceps* Herbarium**

MSUT_7212
Collector No. *Jaihan*-02
Sci.name: *Ophiocordyceps longissima* (=*Cordyceps longisima*) (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Roi Et Province, Ban Mek, Suwan Phum District, 15° 41′ 00.8″ N, 103° 46′ 25.5″ E
Altitude: 148 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 25 Sep. 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Ophiocordyceps* Herbarium**

MSUT_7213
Collector No. *Jaihan*-03
Sci.name: *Ophiocordyceps longissima* (=*Cordyceps longisima*) (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Loei Province, Chiang Khan District, 17° 52′ 49.9″ N, 101° 39′ 36.7″ E
Altitude: 214 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 23 Aug. 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Ophiocordyceps* Herbarium**

MSUT_7214
Collector No. *Jaihan*-04
Sci.name: *Ophiocordyceps longissima* (=*Cordyceps longisima*) (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Sakon Nakhon Province, Wanon Niwat District, 17° 37′ 56″ N, 103° 45′ 7″ E
Altitude: 167 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 20 Jun. 2014
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Ophiocordyceps* Herbarium**

MSUT_7215
Collector No. *Jaihan*-05
Sci.name: *Ophiocordyceps sobolifera* (=*Cordyceps sobolifera*) (Hill ex Watson) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
Family: Ophiocordycipitaceae
Collecting locality: THAILAND: Nong Bua Lam Phu Province, Tham Erawan, Na Wang District, 17° 20′ 20.4″ N, 102° 01′ 16.8″ E
Altitude: 337 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 24 May 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Simplicillium* Herbarium**

MSUT_7216
Collector No. *Jaihan*-06

Sci.name: *Simplicillium* spp. Zare R. & Gams W.
Family: Cordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16°10′44″ N, 103°28′36″E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 28 May 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Simplicillium* Herbarium**

MSUT_7217
Collector No. *Jaihan*-07

Sci.name: *Simplicillium* spp. Zare R. & Gams W.
Family: Cordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 28 May 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Simplicillium* Herbarium**

MSUT_7218
Collector No. *Jaihan*-08

Sci.name: *Simplicillium* spp. Zare R. & Gams W.
Family: Cordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 28 May 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Simplicillium* Herbarium**

MSUT_7219
Collector No. *Jaihan*-09

Sci.name: *Simplicillium* spp. Zare R. & Gams W.
Family: Cordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 28 May 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Simplicillium* Herbarium**

MSUT_7220
Collector No. *Jaihan*-10

Sci.name: *Simplicillium* spp. Zare R. & Gams W.
Family: Cordycipitaceae
Collecting locality: THAILAND: Maha Sarakham Province, Ban Na Pang, Muang District, 16° 10′ 44″ N, 103° 28′ 36″ E
Altitude: 135 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 28 May 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Metacordyceps* Herbarium**

MSUT_7221
Collector No. *Jaihan*-11

Sci.name: *Metacordyceps chlamydosporia* (*Cordyceps chlamydosporia*) (H.C. Evans) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
Family: Clavicipitaceae
Collecting locality: THAILAND: Roi Et Province, Ban Ngu Luam, Suwan Phum District, 15° 40′ 52.6″ N, 103° 44′ 58.0″ E
Altitude: 145 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 16 Jul. 2012
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Metacordyceps* Herbarium**

MSUT_7222
Collector No. *Jaihan*-12
Sci.name: *Metacordyceps chlamydosporia* (*Cordyceps chlamydosporia*) (H.C. Evans) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
Family: Clavicipitaceae
Collecting locality: THAILAND: Nakhon Phanom Province, Si Songkhram District, 17° 39′ 03.5″ N, 104° 12′ 43.9″ E
Altitude: 146 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 7 Jun. 2013
Determined by: *P. Jaihan*

Natural Medicinal Mushroom Museum
Faculty of Science, Mahasarakham University
THAILAND
***Metacordyceps* Herbarium**

MSUT_7223
Collector No. *Jaihan*-13
Sci.name: *Metacordyceps chlamydosporia* (*Cordyceps chlamydosporia*) (H.C. Evans) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
Family: Clavicipitaceae
Collecting locality: THAILAND: Nakhon Phanom Province, Si Songkhram District, 17° 39′ 03.5″ N, 104° 12′ 43.9″ E
Altitude: 146 m
Substrate: **Forest type:** mixed deciduous forest
Collector: *P. Jaihan* **Date of coll.** 7 Jun. 2013

BIOGRAPHY

Biography

Name Piyanoot Jaihan

Date of birth 5th November 1989

Place of birth Nong Khai Province, Thailand

Institution attended

2018 Doctor of Philosophy (Biology), Mahasarakham University, Maha Sarakham, Thailand

2012 Bachelor of Science (Biology) (second-class honors), Mahasarakham University, Maha Sarakham, Thailand

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Research grants & awards

- Mahasarakham University (Grant No. 5601010/2556) (Grant year 2013)
- Science Achievement Scholarship of Thailand, SAST

Research output

Jaihan, P., Sangdee, K., and Sangdee, A. (2016) Selection of entomopathogenic fungus for biological control of chili anthracnose disease caused by *Colletotrichum* spp. *European Journal of Plant Pathology*, 146:551-564.

Conferences

Jaihan, P., Sangdee, K., and Sangdee, A., "Identification of entomophathogenic fungi isolated from cicada nymphs in northeastern Thailand using their morphological characteristics and molecular genetic studies", 12th Conference on Science and Technology for Youths Held at the Bangkok international Trade and Exhibition Centre, Bangkok, Thailand, June 3-4, 2017. (Poster presentation)