สำนักหอสมุดกลาง พระจอมเกล้าลาดกระบัง

ENDOPHYTIC FUNGI ASSOCIATED WITH PALM TREES AND THEIR BIOLOGICAL EFFICACY AGAINST PLANT PATHOGENS

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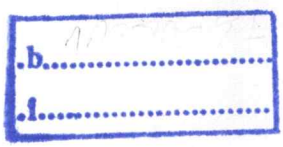

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Thesis	Endophytic Fungi Associated with Palm Trees and Their Biological Efficacy against Plant pathogens
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ABSTRACT

105 isolates were isolated from 10 species healthy palms at King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Among them, 45 unidentified isolates were group to mycelia sterilia fungi. Other 60 isolates were identified as 15 species belong to *Fusarium* spp., *Xylaria* spp., *Cladosporium* spp., *Phialophora* spp., *Pestalotiopsis* spp., *Rhizoctonia* spp., *Colletotrichum spp.*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella* spp., *Emericella* spp., *Aspergillus* spp., *Curvalaria lunata*, *Phoma* spp. and *Nigrospora* spp. by morphological characters.

According to identification, which based on morphology and molecular phylogeny on the basis of ITS1-5.8S-ITS4 ribosomal gene sequence acquisition and analyses, endophytic isolates 17-6 (*Nigrospora* spp.); 5r-1 (*Fusarium* spp.); 4r-3 (*Pestalotiopsis* spp.); 22s-2 (*Giberrella* spp.); 7s-1 (*Chaetomium globosum*) were identified and confirmed as *Nigrospora sphaerica*, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Giberrella moniliformis* and *Chaetomium globosum* when compared to genebank those available in public database.

Pestalotiopsis maculformans, Fusarium falciforme, Phialophora spp., *Nigrospora sphaerica*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp. were selected to test in preliminary screening of bioactivities experiments against plant pathogens *Colletotrichum coffeanum* isolated from coffee leaf anthracnose and *Colletotrichum capsici* isolated from chili fruit anthracnose by bi-culture method. *Fusarium falciforme* showed the best inhibition for the spore production of both *Colletotrichum coffeanum* and *Colletotrichum capsici*. Of which, *Pestalotiopsis maculformans, Nigrospora sphaerica*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* showed high inhibition of colony growth and spore production of this two pathogens. So, this 6 species were selected to extract biological activity substances and used to test their ability to control the growth of plant pathogens (*Colletotrichum coffeanum* and *Colletotrichum capsici*).

Crude methanol extract of *Pestalotiopsis maculformans* gave highest inhibition of *C. coffeanum* colony growth which was 70.25% at the concentration of 1,000 ppm when compared to the control. Methanol crude extract from *Chaetomium globosum* gave significantly highest inhibition of spore production of *C. coffeanum* which was 89.63% at the concentration of 1,000 ppm with ED_{50} at concentration 65.52 ppm. Methanol crude extract from *Chaetomium aureum* gave highest inhibition of *C. capsici* colony growth which was 82.25% at the concentration of 1,000 ppm when compared to the control. Methanol crude extract from *Nigrospora sphaerica* gave significantly highest inhibition of spore production of *C. apsici* which was 87.26% at the concentration of 1,000 ppm with ED_{50} at concentration 41.51 ppm.

บทคัดย่อ

105ไอโซเลทของเชื้อราเอ็นโดไฟต์ ที่ทำการแยกจาก 10 สปีชีส์ ของปาล์มที่ สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง (KMITL), ลาดกระบัง, กรุงเทพ 10520 พบว่า 45 สปีชีส์ของเชื้อราเอ็นโดไฟต์สามารถจัดจำแนกจึงจัดอยู่ในกลุ่มของเชื้อรา sterilia และ เชื้อราเอ็นโดไฟต์ 60ไอโซเลทจัดจำแนกโดยใช้ลักษณะทางสัณฐานวิทยาเป็น15สปีชีส์ดังนี้ *Fusarium* spp., *Xylaria* spp., *Cladosporium* spp., *Phialophora* spp., *Pestalotiopsis* spp., *Rhizoctonia* spp., *Colletotrichum spp.*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella* spp., *Emericella* spp., *Aspergillas* spp., *Curvalaria lunata*, *Phoma* spp. และ *Nigrospora* spp.

จากการจัดจำแนกทางสัณฐานวิทยาและทางชีววิทยาระดับโมเลกุลโดยใช้ ITS1-5.8S-ITS4 ribosomal gene เพื่อจัดลำดับ DNA ของเชื้อราไอโซเลท 17-6 (*Nigrospora* spp.); 5r-1 (*Fusarium* spp.); 4r-3 (*Pestalotiopsis* spp.); 22s-2 (*Giberrella* spp.); 7s-1 (*Chaetomium globosum*) พบว่าเชื้อราทั้ง5ไอโซเลทยืนยันว่าเป็นเชื้อรา *Nigrospora sphaerica*, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Giberrella moniliformis* และ *Chaetomium globosum* เมื่อเปรียบเทียบกับ genebank

Pestalotiopsis maculformans, Fusarium falciforme, Phialophora spp., *Nigrospora sphaerica*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp.ถูกนำมาใช้ในการทดสอบสารออกฤทธิ์ทางชีวภาพโดยวิธี bi-culture ในการควบคุม *Colletotrichum coffeanum*ซึ่งเป็นไอโซเลทที่รุนแรงต่อการเกิดโรคแอนแทรคโนสของกาแฟและ *Colletotrichum capsici* เป็นไอโซเลทที่รุนแรงต่อการเกิดโรคแอนแทรคโนส ของพริก นอกจากนี้ *Fusarium falciforme* เป็นเชื้อราเอ็นโดไฟต์ที่สามารถยับยั้งสปอร์ของ *Colletotrichum coffeanum* และ *Colletotrichum capsici* ได้ดีที่สุด *Pestalotiopsis maculformans, Nigrospora sphaerica*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* เป็นเชื้อราเอ็นโดไฟต์ สามารถยับยั้งขนาดโคโลนีและควบคุมการสร้างสปอร์ของเชื้อก่อโรคได้ดี ดังนั้นเชื้อราเอ็นโดไฟต์ ทั้ง**6**สปีชีส์จึงถูกนำมาใช้ในการสกัดสารออกฤทธิ์ทางชีวภาพเพื่อทดสอบความสามารถในการควบคุมเชื้อราสาเหตุโรค *Colletotrichum coffeanum* และ *Colletotrichum capsici*

crude เมทานอลที่สกัดจาก *Pestalotiopsis maculformans*สามารถยับยั้งโคโลนีของ *C. coffeanum* สูงที่สุดที่ 70.25% ที่ความเข้มข้น1,000 ppm เมื่อเปรียบเทียบกับทรีทเมนต์ควบคุม crude เมทานอลที่สกัดจาก *Chaetomium globosum* ยับยั้งโคโลนีและยับยั้งการสร้างสปอร์ของ *C. coffeanum* ที่ 89.63% ที่ความเข้มข้น1,000 ppm. และมีค่า ED_{50}ที่ความเข้มข้น65.52 ppm. crude เมทานอลจาก *Chaetomium aureum*ให้ค่าสูงสุดในการควบคุมขนาดโคโลนีของ *C. capsici* เท่ากับ 82.25% ที่ความเข้มข้น1,000 ppm เมื่อเปรียบเทียบกับทรีทเมนต์ควบคุม crude เมทานอลที่ได้จาก *Nigrospora sphaerica* ยับยั้งโคโลนีและการยับยั้งการสร้างสปอร์ของ *C. capsici* เท่ากับ87.26%ที่ความเข้มข้น1,000 ppm. และมีค่า ED_{50}ที่ความเข้มข้น41.51 ppm.

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Jiaojiao Song

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CHAPTER 1

INTRODUCTION

1.1 General Introduction to Endophytes

1.1.1 Introduction of Endophytes

Endophytes are microorganisms that internally infect living plant tissues without causing any visible manifestation of disease, and live in mutualistic association with plants for at least a part of their life cycle (Bacon and white, 2000). A wide range of plants have now been examined for endophytes, and endophytes have been found in nearly all of them, including trees, grass, algae and herbaceous plants. Evidence of plant-associated microorganisms found in the fossilized tissues of stems and leaves has revealed that endophyte-plant associations may have evolved from the time higher plants first appeared on the earth (Redecker *et al.*, 2000). Hawksworth and Rossman estimated that nearly one million species of endophytes may exist in the unexplored plants (Strobel and Daisy, 2003; Arnold, 2005). Endophytes microorganisms were discovered including fungi, bacteria, and actinomycetes. And fungal endophytes are the most frequently encountered endophytes (Staniek *et al.*, 2008). It have great promise with diverse potential for exploitation (Li *et al.*, 2012; Staniek *et al.*, 2008). The existence of fungi inside the tissues of asymptomatic plants has been known since the end of the nineteenth century (Guerin, 1898). Ever since the discovery of the rich diversity of the endophytic fungi, their population dynamics, their role in improving plant growth, plant health (Hallmann *et al.*, 2007), their distribution in the plant, the metabolites they secrete and their potency to produce novel compounds within the plants (Tan and Zou, 2001), have formed an important aspect of present day research. Since endophytes were first described in the Darnel (Freeman, 1904), various investigators have isolated endophytes from different plant species. These discoveries led to a worldwide search for novel endophytes for the better understanding and applicability of such a promising group of microorganisms. On the one hand, the ecological aspects of endophytic fungi such as host range, evolutionary relatedness, infection, colonization, transmission patterns, tissue specificity, and mutualistic fitness benefits have been investigated relating to a plethora of plants (Arnold *et al.*, 2003, 2007; Arnold, 2005, 2007; Stone *et al.*, 2004; Schulz and Boyle, 2005; Rodriguez *et al.*, 2009). On the other hand, many discoveries have been made in isolating

endophytic fungi, which have been shown to have the potential for *de novo* synthesis of various bioactive metabolites that may directly or indirectly be used as therapeutic agents against numerous ailments (Strobel and Daisy, 2003; Strobel *et al.*, 2004; Zhang *et al.*, 2006; Gunatilaka, 2006; Staniek *et al.*, 2008; Suryanarayanana *et al.*, 2009; Aly *et al.*, 2010; Kharwar *et al.*, 2011; Kusari and Spiteller, 2010, 2011).

1.1.2 Introduction to Bioactivity Compound of Fungal Endophytes

Endophytic fungi have attracted great attention in the past few decades due to its ability to produce novel secondary metabolites for medical, agricultural and industrial use. And they are also considered as an outstanding source of bioactive compounds due to its ability to occupy any plants at any environments (Strobel and Daisy, 2003).

Several studies on the use of bioactive compounds from endophytic fungi have been reported. Endophytic fungi are able to produce antimicrobial, anticancer such as Taxol (Walker and Croteau, 2001) and antimalarial activities (Wiyakrutta *et al.*, 2004). Study done by Woropong *et al.* (2001) showed that isolated endophytic fungi are able to produce mixture of volatile organic compounds that are lethal to human and plant pathogenic fungi and bacteria. The secondary metabolites of some endophytic fungal which isolated from 29 traditional chinese medicinal plants in Hong Kong exhibited strong anti-oxidant capacity, and anti-microbial activity. (Kumar and Hyde, 2004; Kumar *et al.*, 2005; Huang *et al.*, 2007 a,b) Antifungal products are vastly produced by majority of the endophytes. Griseofulvin-producing endophyte was first reported in fungus from *Abies holophylla* and was evaluated *in vivo* antifungal activity against plant pathogenic fungi (Park *et al.*, 2004).

Most studies have been concerned with graminaceous endophytes (Dahlman *et al.*, 1991), and have been undertaken in temperate countries. Recently, there has been interest in endophytes of tropical hosts (Dreyfuss and Petrini, 1984; Lodge *et al.*, 1996; Rodrigues and Petrini, 1996) because endophytes are believed to be both diverse and to provide an excellent potential source of biologically active novel compounds (Dreyfuss and Petrini, 1984; Hyde,2001)., Studies in the tropics have included endophytes in bamboo (Umali, Quimio & Hyde, 1999; Lumyong *et al.*, 2000) and palms, including Amazon palm *Euterpe oleracea* (Rodrigues, 1994); *Trachycarpus fortunei* (Taylor, Hyde and Jones, 1999), and so on.

1.2 Introduction to Palm

Palms are the quintessential tropical plants. In the popular imagination they symbolize the tropical landscape. Palms are confined almost exclusively to the tropics, where they have diversified into dozens of genera and hundreds of species and have occupied almost every habitate. Indeed, palms are among the world's largest plant families, both in terms of number of species and in abundance (Henderson *et al.*, 1995).

Palm trees are a botanical family of perennial lianas, shrubs, and trees. They are in the family Arecaceae (Due to historical usage, the family is alternatively called Palmae or Palmaceae). They are flowering plants, the only family in the monocot order Arecales. Roughly 202 genera with around 2600 species are currently known, most of them restricted totropical, subtropical, and warm temperate climates. Most palms are distinguished by their large, compound, evergreen leaves arranged at the top of an unbranched stem. Palms are one of the best known and most widely planted tree families. They have held an important role for humans throughout much of history. Many common products and foods come from palms. They are often used in parks and gardens that are in areas that do not have heavy frosts. Endophytic fungi associated with palms had reported, including temperature palms and tropic palms (Rodrigues and Samuels, 1990; Fröhlich and Hyde 2000; Hyde *et al.*, 2000).

1.3 Anthracnose of Chili

Chili (*Capsicum* spp.) is the fourth important vegetable in the world and the first in Asia, with world production in 2006 at approximately 25.9 million t for fresh chili and 2.8 million t for dry chili (FAOSTAT, 2008). But, anthracnose caused by *Colletotrichum* spp., is a serious problem for chili production in the tropics and subtropics worldwide. *Colletotrichum* isolates typically have a wide host range and may also infect other solanaceous crops such as tomato, potato and eggplant and members of Cucurbitaceae, Leguminosae and Malvaceae. Anthracnose incidence on fruit in pepper ranged from 5 to 75% (Hadden 1989). Anthracnose is more likely to develop on mature fruit that is present for longer periods of time on the plant, but immature fruit are also susceptible. Aspersoria form germinated conidia and attach to the fruit epidermis. Infection hyphae directly penetrate the host surface. Although all fruit is susceptible to infection, more mature, red pepper fruit are infected more frequently than green, immature fruit, which may be due to physical and chemical differences in the cuticle and exocarp (Kim *et al.*, 1999,

Manandhar *et al.*, 1995). Immature fruit that are infected may not express symptoms until the fruit matures.

1.4 Anthracnose of Coffee

Coffee belongs to Rubiaceae, is a perennial evergreen shrub and it is a perennial horticultural crop. Leaves which are opposite elongated oval, glossy, at the end of a long branches, small branches, and flowers are white, open branches in the base of the petiole link. Coffee is a major source of income for Thailand, being the third largest producer in Southeast Asia - after Vietnam and Indonesia. While there are many species of coffee in the world, only Coffee arabica (Arabica coffee) and *C. robusta* (robusta coffee) have major economic significance. Arabica coffee is grown in the cooler highland areas of the northern part of Thailand, while robusta coffee is grown in southern part of Thailand (Angkasith, 2001). The main important factors for poor quality of coffee are disease and insect pests, especially coffee bean anthracnose caused by *Colletotrichum coffeanum* Noack. The coffee growers are usually applied chemical fungicides but later the pathogen become resistant to those fungicides (Soytong *et al.*, 2001) leading to low quality of coffee beans. Biological control is increasingly interested by many researchers to investigate the new antagonists against plant pathogens. As reports in Ascomycetous fungi like *Chaetomium* spp. are reported to be antagonizing many plant pathogens (Soytong and Quimio, 1992).

1.5 Research Objective

Use of chemical for control plant disease is one of the most commonly used strategy usually what farmers followed, but nowadays people are more aware of various side effects caused by pesticide residues present in food and water, and also may lead to environmental pollution. There is needed to research biological product for control plant disease. In the past two decades, a great deal of information on the role of endophytic microorganisms in nature has been collected. The capability of colonizing internal host tissues has made endophytes valuable for agriculture as a tool to improve crop performance. Endophytes are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural and industrial arenas (Frohlich and Hyde, 2000).

The aim of this research focused on preliminary test for bioactivity substances from

endophytic fungi associated with palm trees to inhibit *Colletotrichum capsici* causing anthracnose of chili and *Colletotrichum coffeanum* causing anthracnose of coffee.

The objectives of this study were as follows:

1) To isolate endophytic fungi from leaves, petioles and roots of 10 species palm trees ;

2) To identify morphological and molecular phylogeny to confirm species.

3) To test for biological properties of some species endophytic fungi against plant pathogen *Colletotrichum capsici* causing anthracnose of chili and *Colletotrichum coffeanum* causing anthracnose of coffee.

CHAPTER 2

REVIEW LITERATURE

2.1 General of Endophytic Fungi

2.1.1 History and Definition of Endophytic Fungi

Many mycologists have provided definitions for the term endophyte. The term 'endophyte' is an all-encompassing topographical term which includes all organisms that, during a variable period of their life, symptomlessly colonise the living internal tissues of their hosts (Stone *et al.*, 2000). This definition is broad enough to include virtually any organism residing inside a plant host. Evidence of plant-associated microorganisms found in the fossilized tissues of stems and leaves has revealed that endophyte–host associationsmay have evolved fromthe time that higher plants first appeared on the Earth (Strobel, 2003; Andrzej, 2002). Endophytic microbes fall into several identifiable classes often in relation to their plant organ source, with the major groups as follows: 1) endophytic Clavicipitaceae; 2) fungal endophytes of dicots; 3) other systemic fungal endophytes; 4) fungal endophytes of lichens; 5) endophytic fungi of bryophytes and ferns; 6) endophytic fungi of tree bark; 7) fungal endophytes of xylem; 8) fungal endophytes of root; 9) fungal endophytes of galls and cysts; 10) prokaryotic endophytes of plants (includes endophytic bacteria and actinomycetes) (Stone *et al.*, 2000; Bills *et al.*, 2003).

The symbiosis of fungi with plants most probably dates back to the emergence of vascular plants (Rodriguez and Redman, 1997). An enormous number of different fungi can be isolated from plants growing in their native habitat. Most of the fungi are uncommon and narrowly distributed, taxonomically and geographically. However a few fungi are widely distributed with the host, suggesting a long standing, close and mutually beneficial interaction. Fungal endophytes have evolved two transmission modes. These are vertical and horizontal transmission, of which the former transmits the systemic fungus from plant to offspring *via* host seeds, and the latter operates by sexual or asexual spore transfer. (Saikkonen *et al.*, 2004) Endophytic fungi are found in all divisions of fungi so have presumably evolved the association independently on many occasions. And, some are closely related to fungi known to cause disease in plant or animal (especially insect). Phylogenetic evidence is used to suggest that some endophytes have evolved from pathogens and for others, vice versa. George Carroll (1988) has

suggested that some phytopathogens in the environment are related to endophytes and have an endophytic origin

The most commonly used definition of fungal endophyte is that of Petrini (1991). He defined endophytic fungi "as those fungi that live within healthy plant tissues without causing any symptoms or apparent injury to the host" (Fisher and Petrini, 1990; Petrini, 1991). These symbioses are diverse in nature and can be mutualistic, neutral or antagonistic. The colonization of plant tissues by endophytes occurs in a similar way to that of plant pathogens and mycorrhizae (Lumyong *et al.*, 2004). Colonization comprises a sequence of steps involving host recognition by the fungus, spore germination, penetration of the epidermis and tissue colonization (Petrini, 1991).

2.1.2 Taxonomy of Endophytic Fungi

Based on phylogeny and life history traits, endophytic fungi were originally organized into two broad groups – clavicipitaceous endophytes (CEs) colonize shoots and rhizomes of a narrow host range of cool- and warm-season grasses (*Poaceae*) and non-clavicipitaceous endophyts (NCEs) have been isolated from shoots and/or roots of almost all sampled plants and are plentifully diverse. More recently, these groups have been divided into four distinct functional classes based on a number of different criterion related to life history. The classification into one of the four classes based on: host range (single species to highly ubiquitous), tissue colonization (localized to general), extent in plantcolonization (limited to extensive) and biodiversity (low to high), mode of transmission (vertical to horizontal) and host, most of them belonging to Ascomycota.(Rodriguez *et al.*, 2009). Within the Ascomycetes they are very diverse, including Loculoascomycetes, Discomycetes and Pyrenomycetes (Petrini, 1986).

The CEs (class 1 endophytes) belong to the fungal teleomorphic genera *Epichloë* and *Balansia* correspond to the anamorphs *Neotyphodium* and *Ephelis*, respectively. They form systemic intercellular infections with a hyphal gradient along the plant axis and primarily transmitted vertically by seeds (Schulz and Boyle, 2005; Kuldau and Bacon, 2008). The NCEs have been divided into three functional groups, class 2 class 3 and class 4. Class 2 endophytes broadly colonize both above and below ground tissues, but with limited biodiversity in individual plant hosts. They are both transmitted vertically and horizontally, sometime colonize non-habitat or habitat adapted benefits to the plant host. Endophytic fungi in this class belong to a few

members of the *Agaricomycotina, Pucciniomycotina Basidiomycota* and also to *Pezizomycotina* (Ascomycota) such as *Phoma* sp., *Arthrobotrys* sp., *Fusarium culmorum, Colletrichum sp.* and *Curvularia protuberata* that can also found in roots, rhizomes, stems and leaves (Rodriguez *et al.*, 2009). NCEs class 3 the colonization has restricted to shoots, but with high biodiversity in individual tissues. They have been isolated broadly from tropical forest to boreal and Arctic plant populations. They are horizontally transmitted by wind, rain and insects (Higgins *et al.*, 2007; Arnold, 2008; Feldman *et al.*, 2008). The Ascomycota that isolated from leaves the most belonging to the *Sordariomycetes, Dothidiomycetes, Pezizomycetes, Leotiomycetes* and *Eurotiomycetes,* while *basidiomycetous* isolates belonging to *Agaricomycotina, Pucciniomycotina* (Arnold *et al.*, 2007; Higgins *et al.*, 2007; Rodriguez *et al.*, 2009, Vega *et al.*, 2010). Class 4 endophytic fungi colonize only roots. They also known as Dark Septate Endophytes (DSEs), are easily distinguished by their highly melanized septate hyphae. These fungi are limited to the roots of their host, coexisting with mycorrhizal fungi but not growing outward into the rhizosphere. They have a broad host range and belong to different phylogenetic groups among Ascomycota and among non-mycorrhizal members of the order *Sebacinales* (Basidiomycota) (Weiss *et al.*, 2004; Addy *et al.*, 2005; Selosse *et al.*, 2009; Schäfer and Kogel, 2009).

2.1.3 Host Range of Endophytic Fungi

Fungal surveys of various hosts during the past 20 years have demonstrated that endophytic colonization of land plants by fungi is ubiquitous (Zhang *et al.*, 2006). Endophytes are known from plants growing in tropical, temperate, and boreal forests; from herbaceous plants from various habitats, including extreme arctic, alpine (Petrini, 1987; Fisher *et al.*, 1995), and xeric environments (Mushin and Booth, 1987; Mushin *et al.*, 1989); and from mesic temperate and tropical forests.

The plant hosts of endophytic fungi including mosses and hepatics (Döbbler, 1979; Pocock and Duckett, 1985a; Ligrone *et al.*, 1993), ferns and fern allies (Fisher *et al.*, 1992; Schmid and Oberwinkler, 1993), numerous angiosperms and gymnosperms, including tropical palms (Rodrigues and Samuels, 1992; Fröhlich and Hyde, 2000; Hyde *et al.*, 2000), broad-leaved trees (Arrhenius and Langenheim, 1986; Lodge *et al.*, 1995), the estuarine plants *Salicornia perennis* (Petrini and Fisher, 1986), *Spartina alterniflora* (Gessner, 1977), and *Suada fruticosa* (Fisher and Petrini, 1987), diverse herbaceous annuals, and many deciduous and evergreen

perennials.

In addition, endophytes are found in a wide variety of plant tissue types, such as seeds and ovules (Siegel and Latch, 1987), fruits (Schena *et al.*, 2003), stems (Gutierrez-Zmora and Martinez-Romero, 2001), roots (Germida *et al.*, 1998), leaves (Smith *et al.*, 1996), tubers (Sturz *et al.*, 1998), buds (Ragazzi *et al.*, 1999), xylem (Hoff *et al.*, 2004), rachis (Rodrigue and Samuls, 1999) and bark (Raviraja, 2005). It is now widely accepted that endophyte-free plants are few, and this is especially true for shrubs and trees (Gennaro *et al.*, 2003). Several studies have shown the presence of fungal endophytes in host species belonging virtually to all plant divisions, frommosses and ferns to monocotyledons (Zhang *et al.*, 2006).

2.1.4 Host and Tissue Specificity

It is possible to isolate hundreds of endophytic species from single plant, and among them, at least one generally shows host specificity (Tan and Zou, 2001). Often a single woody plant will harbour more than 40 fungal endophytes (Petrini *et al.*, 1992; Faeth *et al.*, 1997). Systemic grass endophytes are shown to be significantly more host-specific than fungal and plant phylogenies, leading to host-adapted fungi that are compatiblewith only certain host genotypes (Germaine *et al.*, 2004). In general, endophytic fungal communities demonstrate single host specificity at the plant species level, but this specificity can be influenced by environmental conditions (Susan, 2004). With the exception of *Epichlo¨e typhina*, which has a very broad host range, all other species of the *Epichlo¨e* genus are relatively host-specific (Caruso *et al.*, 2000). Investigations on the endophytic community in *Quercus ilex* have revealed a higher degree of single host specificity within the plant's native geographic range (Fisher *et al.*, 1994). Endophytes are also able to colonise multiple host species belonging to different families within a given geographic site. For instance, dark septate root endophytes (DSE) are conidial or sterile fungi that colonise plant roots. They have been reported for nearly 600 plant species, representing about 320 genera and 100 families (Jumpponen and Trappe, 1998). Examination of foliose and crustose algae has revealed a wide range of alternative hosts for *Acrosiphonia sporophytes* (Sussmann and DeWreede, 2002). *Phialocephala fortinii* is a common root endophytic fungus with a wide geographic distribution which occurs in both xeric and hydric sites (Addy *et al.*, 2000).

Endophytic fungi also exhibit organ and tissue specificity as a result of their adaptation to different physiological conditions in plants (Rodrigue and Samuls, 1999).

Fluctuations in the bacterial profile were determined by different parameters (seasonal changes, plant organs, presence of phytoplasmas), revealing influences such as temperature (warmor cold according to the season) and in the organs examined (*e.g.* roots or stems) (Mocali *et al.*, 2003). In addition, more stressful environments drive the selection toward higher infection frequencies of endophytes in grasses (Dahl Jensen and Roulund, 2004). For instance, summer drought exerts a selection pressure on grass in favour of endophyte infection (Lewis *et al.*, 1997; Leyronas and Raynal, 2001). Dong *et al.* (2003) assessed the host range and strain specificity for endophytic colonisation with *Klebsiella pneumoniae* 342 (Kp342) on five host plants, inwhich Kp342was the most efficient coloniser of the plant apoplast. The monocots inoculated in this study were colonized endophytically in much higher numbers than the dicots. Cells of Kp342 congregate at lateral root junctions, suggesting that the cells enter the plant through cracks created by lateral root extensions. *Lasiodiplodia theobromae* is a cosmopolitan fungus with a worldwide distribution in the tropics and subtropics. A study of the genetic diversity and gene flow between populations of *L. theobromae* suggests predominant clonal reproduction with some genotypes widely distributed within a region (Mohali *et al.*, 2005).

Accordingly, older foliage is likely to harbor greater species diversity than younger foliage. Perennial species thus can be expected to harbor greater diversity than annuals, and plants with evergreen foliage are likely to harbor more diversity than deciduous or annual plants.

2.1.5 Mutualistic Symbiosis

Endophytic fungi and host plant are asymptomatic and may be mutualistic: plants provide live space, protect and feed endophytes. The metabolism of endophytes can produce plant-growth-regulatory, antimicrobial, antiviral or insecticidal substances to enhance the growth and competitiveness of the host in nature (Carroll, 1988). Some endophytic fungi are known as reliable sources of bioactive substances with agricultural and/or pharmaceutical potential, as exemplified by taxol (Stierle *et al.*, 1993; Wang *et al.*, 2000), subglutinol A and B (Lee *et al.*, 1995), and peptide leucinostatin A (Stroble and Hess, 1997). A plethora of competent endophytic fungi have already been discovered that are capable of providing different forms of fitness benefits to their associated host plants (Hamilton *et al.* 2012; Hamilton and Bauerle, 2012). For example, these organisms have demonstrated the capacity to produce a diverse range of biologically active secondary metabolites (Aly *et al.*, 2010; Debbab *et al.*, 2012; Gunatilaka 2006;

Kharwar *et al.*, 2011; Staniek *et al.*, 2008; Strobel and Daisy, 2003; Strobel *et al.*, 2004; Suryanarayanana *et al.*, 2009; Zhang *et al.*, 2006), occasionally including those similar to their associated host plants (Eyberger *et al.*, 2006; Kusari *et al.*, 2008, 2009a, b, c, 2011, 2012a), and induce host plant tolerance to environmental stress, herbivory, heat, salt, disease and drought (Arnold *et al.*, 2003; Márquez *et al.*, 2007; Porras-Alfaro and Bayman, 2011; Redman *et al.*, 2002; Rodriguez *et al.*, 2004, 2008; Rodriguez and Redman, 2008; Stone *et al.*, 2000; Waller *et al.*, 2005). Endophytic fungi are thus expected to be potential sources of new bioactive agents.

It is generally accepted that endophytic microbial communities play an important beneficial role in the physiology of host plants. Plants infected with endophytes are often healthier than endophyte-free ones (Waller *et al.*, 2005). This effect may be partly due to the endophytes'production of phytohormones (such as indole-3-acetic acid (IAA), cytokines, and other plant growth-promoting substances like vitamins) and/or partly owing to the fact that endophytes can enhance the hosts' absorption of nutritional elements such as nitrogen (Reis *et al.*, 2000; Lyons, 1990) and phosphorus,(Guo *et al.*, 2000; Gasoni and Gurfinkel, 1997; Malinowski *et al.*, 1999) and that they regulate nutritional qualities such as the carbon–nitrogen ratio (Raps and Vidal , 1998). For example, roots of Populus Esch5 explants were inoculated with *Piriformospora indica*, and there was an increase in root biomass, with the number of 2nd-order roots increasing significantly (Kaldorf *et al.*, 2005). Khan *et al.* (2012) investigated plant growth promoting activity of roots inhabiting endophytic fungi in order to evaluate their role in the survival of host plants under extreme sand dune environment of coastal regions. One hundred and twenty two fungal isolates were collected from the roots of 9 sand dune plants and were screened for growth promoting secondary metabolites. The results showed that 101 fungal isolates (82.7%) promoted plant height and shoot length of *Waito-C* rice, while 21 fungal isolates (17.2%) inhibited growth attributes. The fungal isolate *Gibberella fujikuroi* along with distilled water and Czapek broth medium were used as control during the experiment. It was concluded that a major proportion of endophytic fungi inhabiting sand dune plants produce metabolites, which are helpful in plant growth and development.

Endophyte fungi have diverse positive effects on their hosts. The latter include Balansia (Ascomycetes) (Bacon *et al.*, 1977; 1979), though reported elsewhere to be implicated in toxicosis of cattle and humans (Groger, 1972). Other PGPF fungi have been described to protect their hosts against insect pests, pathogens, and even domestic herbivores (Yan *et al.*, 2011). These

include *Aspergillus flavus* and *Penicillium sublateritium* (Webber, 1981), which live and feed on the host plant, and in turn, produce functional metabolites that enhance its fitness and resistance against stresses (Rana *et al.*, 1997). Last but not least, a number of species, including selected *Fusarium* sp. are reported to act as antagonists against plant pathogens. Accordingly, PGPF have commonly been used in practice as inocula to improve the growth of plants and suppress pathogens (Steinberg *et al.*, 1997; Cao *et al.*, 2002; Alabouvette *et al.*, 2009).

2.1.6 Secondary Metabolites of Fungal Endophytes

The number of secondary metabolites produced by fungal endophytes is larger than that of any other endophytic microorganism class. This may of course be a consequence of the high frequency of isolation of fungal endophytes from plants.Natural products from fungal endophytes have a broad spectrum of biological activity, and they can be grouped into several categories, including alkaloids, steroids, terpenoids, isocoumarins, quinones, phenylpropanoids and lignans, phenol and phenolic acids, aliphatic metabolites, lactones, *etc* (Zhang *et al.*, 2001).

Stierle *et al.* (1993) researched on taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew, *Taxus brevifolia*. The hyphomyceteous fungus produced taxol and related compounds when grown in a semi-synthetic liquid medium. Taxol was identified by mass spectrometry, chromatography and reactivity with monoclonal antibodies specific for taxol. Both [1-14C] acetic acid and L-[U-14C] phenylalanine served as precursors of [14C] taxol in fungal cultures.

Schulz *et al.* (2002) reviewed on how endophytic fungi serve as a source of novel biologically active secondary metabolites. Accordingly in course of the last 12 years, 6500 endophytic fungi were isolated from herbaceous plants and trees screened them for biological activities and have isolated and determined the structures of the biologically active compounds. The substances isolated were originated from different biosynthetic pathways belonging to diverse structural groups. The potential role of the endophyte and its biologically active metabolites in its association with its host has been investigated. Correlations were found between biological activity and biotope *e.g.* a higher proportion of the fungal endophytes in contrast to the soil isolates inhibited at least one of the test organisms for antialgal and herbicidal activities. It was seen that the fungal endophytes possess the exoenzymes necessary to colonize their hosts. Certain endophytic interactions associated with roots of the host may be mutualistic improving

growth of the host and supplying the mycobiont with enough nourishment to extensively colonize the host's roots. Further plant defense metabolites are higher in plants infected with endophytes. Hence the interaction fungal endophyte–plant host is characterized by a finely tuned equilibrium between fungal virulence and plant defence. Not only must the endophyte has to compete with epiphytes and pathogens but presumably also has to regulate metabolism of the host in their delicately balanced association. The utilization of a biotope such as that of the fungal endophyte is one aspect of intelligent screening and that fungi in different biotopes are still need to be exploited.

Kumala *et al.* (2007) were experimental in producing cytotoxic secondary metabolites from the fermentation broth of the endophytic fungus isolated from the fruits of *Brucea javanica. In vitro* cytotoxic assays were performed using leukemia cell line L1210. LC-MS analysis of the F4 fraction of n-butanol extracts of secondary metabolites revealed bruceocin and canthin–6 one compounds as cytotoxic constituents. These compounds were previously reported in the same host plant. Hence the present study could demonstrate the possibility of the endophytic fungi living symbiotically within the host plant producing cytotoxic secondary metabolites.

Wijeratne *et al.* (2008) isolated sesquiterpene, quinones and related metabolites from *Phyllosticta spinarum* a fungal strain endophytic in *Platycladus orientalis* of the Sonoran Desert. Five new metabolites (+)-(5*S*,10*S*)-4′ -hydroxymethylcyclozonarone (**1**), 3-ketotauranin (**3**), 3R-hydroxytauranin (**4**),12-hydroxytauranin (**5**), and phyllospinarone (**6**), together with tauranin (**2**) were isolated and the structures of these new compounds were determined on the basis of their 1D 37 and 2D NMR spectroscopic data and chemical interconversions. Tauranin showed activity when evaluated for inhibition of cell proliferation assay in a panel of five cancer cell lines that also induced apoptosis in PC-3M and NIH 3T3 cell lines during flow cytometry.

Lactones of endophytic origin have antiparasitic activity against *Plasmodium falciparum*. Three lactones were isolated from the culture medium of the endophytic fungus *Xylaria* sp. One was identified as (+)-phomalactone (**1**). The others were 6-(1-propenyl)-3, 4, 5, 6-tetrahydro-5-hydroxy-4Hpyran- 2-one (**2**) and 5-hydroxymellein (**3**). Compounds 1 and 2 were reported for the first time as constituents of *Xylaria*. Also this study was the first report showing the activity of these lactone compounds against a chloroquine-resistant *Plasmodium falciparum*

strain, Romero *et al.*, (2008).

Kusari *et al.*, (2009) isolated, identified and characterized an endophytic fungus, *Aspergillus fumigatus* from *Juniperus communis* and *L. Horstmann*, as a novel producer of deoxypodophyllotoxin and performed its *in vitro* antimicrobial assay against a panel of pathogenic bacteria. The study concluded the production of deoxypodophyllotoxin (found in the host) by the cultured endophyte which is an enigmatic observation. This demonstrates the horizontal transmission of genes from the host plant to its endophytic counterpart. It would be interesting to further study the deoxypodophyllotoxin production and regulation by the cultured endophyte as well as their scale up process for consistent and dependable production.

2.1.7 Tropical Endophytic Fungi

There have been many studies on endophytic fungi in grasses and woody plants in temperature regions. Several endophyte fungi studies have also recently been undertaken on tropical hosts.

Arnold *et al.* (2000) studied on fungal endophytes among tropical forests in Central Panama. Colonization patterns, richness, host preference and spatial variation in leaves of understorey tree species – *Heisteria concinna* and *Ouratea lucens* was studied. From 83 leaves, 418 endophyte morphospecies were isolated most of which were represented by a single isolate (59%). The studies suggest the evidence of host preference and spatial heterogeneity. Further the tropical endophytes are hyperdiverse and the extrapolating data estimates excluding them will underestimate fungal species diversity.

Arnold (2001) reviewed extensively on fungal endophytes in neotropical trees their abundance, diversity and their ecological implications. Accordingly tropical fungi are traditionally understudied and studies of endophytic fungi in tropical forests are yet in their infancy. Fungal endophytes may represent a ubiquitous, cryptic and ecologically interesting component of tropical 29 forests. Endophytes appear to be both ubiquitous and highly diverse in tropical forests. Further exploration of tropical endophytes will help to clarify the fungal diversity debate and will likely lend support to higher estimates of global fungal diversity. The study of tropical endophytes seems promising to enrich our understanding of plant-fungus interactions in tropical forests, tropical biodiversity and tropical ecology.

Taxonomically endophytes are, with few exceptions, ascomycetes or their

anamorphs (Carroll, 1988; Sridhar and Raviraja, 1995). Xylariaceous fungi are the most commonly isolated endophytes in tropical regions (Rodrigues and Petrini, 1997). They are also common endophytes in temperate zones (Stone and Petrini, 1997) but are less prevalent there. An investigation of the palm *Euterpe oleracea* in tropical Brazil yielded 13 species of *Xylariaceae* amongst the 21 dominant taxa (Rodrigues, 1994). *Xylaria cubensis* was the most important endophyte of *E. oleracea* leaves (frond blades) (Rodrigues, 1994) and the second most frequently isolated fungus from *Licuala ramsayi* leaves (Rodrigues and Samuels, 1990). *Xylaria* species can also be found as endophytes of palms in temperate regions, but at much lower frequencies (Taylor *et al.*, 1999). *Xylaria* anamorphs have also been isolated in the tropics from *Stylosanthes* (*Leguminosae*) (Pereira, Azevedo and Petrini, 1993), *Manilkara* (*Sapotaceae*) (Lodge, Fisher and Sutton, 1996), orchids (Bayman *et al.*, 1995, 1998) and *Heliconia* (*Heliconiaceae*) (Bills and Polishook, 1994).

Other genera commonly isolated from tropical plants are *Colletotrichum*, *Idriella*, *Phoma*, *Phomopsis* and *Phyllosticta* (Rodrigues and Petrini, 1997). Common saprobes such as *Cladosporium herbarum*, *Aureobasidium pullulans* and *Alternaria alternata* have been recorded as endophytes in both temperate and tropical areas, but they are less common in the latter (Rodrigues and Petrini, 1997).

Arnold and Lutzoni (2007) reviewed on diversity and host range of foliar fungal endophytes with reference to tropical arenas discussing them to be hotspots to explore the putative 30 hyperdiversity of tropical leaf endophytes, endophyte communities along the latitudinal gradient of Canadian arctic was compared to the lowland tropical forest of central Panama. Molecular sequence data from 1403 endophyte strains showed that endophytes increase in incidence, diversity and host breadth from arctic to tropical sites. Endophyte communities from higher latitudes constituted relatively few species whereas tropical endophyte assemblages are dominated by a small number of classes with a very large number of endophytic species. Leaves of tropical trees hence represent hotspots of fungal species diversity containing numerous species not yet recovered from other biomes. The challenge remains to recover and identify those elusive and rarely cultured taxa with narrower host ranges and to elucidate the ecological roles of these little known symbionts in tropical forests.

2.1.8 Endophytic Fungi Associated with Palms

Nowadays, many endophytic fungi associated with palms had reported, including temperature palms and tropic palms.

Frohlich *et al.* (2000) researched endophytic fungi from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia and got 75 fertile species in 48 genera and 60 sterile morphospecies including 10 *Xylaria* anamorphs, *Phomopsis* sp., *Phoma* sp., *Trichoderma* sp., *Colletotrichum* sp., *Pestalotiopsis palmarum*., *Lasiodiplodia* sp. *Hyphomycete* sp., *Nodulisporium* sp., *Dictyochaeta* sp., *Phyllosticta* sp., *Distocercospora* sp., *Verticillium* sp., *Coelomycete* sp., *Aspergillus niger*, *Beltraniella* spp., *Botrytis allii,* and so on. And, the endophyte communities of both palms were composed of a single, dominant xylariaceous species.

Taylor *et al.* (1999) isolated endophytic fungi associated with the temperate palm, *Trachycarpus fortune*, and the result showed that, endophytic fungi *Alternaria alternate, Oxydothis* sp., *Fusarium* sp., *Phoma* sp., *Phomopsis* sp., *Xylaria* sp., *Stagonospora* sp., *Glomerella cingulata, Guignardia cocogena*, and so on were got.

Frohlich (1997) noted that the endophyte assemblages of tropical palms showed more affinities to those of non-angiospermous and dicotyledonous hosts in tropical regions than to monocotyledonous temperate hosts. Temperate and tropical palms have many endophyte genera in common. Frohlich (1997) reported *Phomopsis* spp. and *Colletrotrichum* spp. As the second and fourth highest recorded genera respectively, isolated from *Licuala* spp. in Australia and Brunei. In other studies of palms there are also considerable overlaps of species and genera (Rodrigues and Samuels, 1990; Rodrigues, 1994).

Lumyong *et al.* (2009) reported fungal endophytes associated with the palms, *Calamus kerrianus* (rattan) and *Wallichia caryotoides* (taorang) were investigated at two sites within Doi Suthep-Pui National Park, Thailand. Endophytic fungi were isolated from different tissue types (petiole, leaf, lamina and leaf veins) during three periods of the year, rainy season (July-October, 1999), cold season (November1999-February 2000) and hot season (March-June 2000). Thirty-five endophytic fungi isolated included xylariaceous taxa (20 morphotypes), sterile mycelia, one unidentified and 13 mitosporic fungi including *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocaicola*, *Paecilomyces* sp.

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Pestalotiopsis sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phomopsis* sp., *Phyllosticta* sp., and *Sarcopodium* sp. The endophyte species, their relative frequency, isolate prevalence and diversity did not differ significantly between host species, tissue types, study sites and seasons.

Endophytic flora plays a vital role in the colonization and survival of host plants, especially in harsh environments, such as arid regions. This flora may, however, contain pathogenic species responsible for various troublesome host diseases (Chobba *et al.*, 2013). They study on investigating the diversity of both cultivable and non-cultivable endophytic fungal floras in the internal tissues (roots and leaves) of Tunisian date palm trees (*Phoenix dactylifera*). Accordingly, 13 isolates from root and leaf samples, exhibiting distinct colony morphology, were selected from potato dextrose agar (PDA) medium and identified by a sequence match search wherein their 18S–28S internal transcribed spacer (ITS) sequences were compared to those available in public databases. Additionally, total DNA from palm roots and leaves was further extracted and ITS fragments were amplified and led to the identification of *Alternaria*, *Cladosporium*, *Davidiella* (*Cladosporium* teleomorph), *Pythium*, *Curvularia*, and uncharacterized fungal endophytes. Both approaches confirmed that while the roots were predominantly colonized by *Fusaria* (members of the Nectriaceae family), the leaves were essentially colonized by *Alternaria* (members of the Pleosporaceae family). Overall, the findings of the present study constitute, to the authors' knowledge, the first extensive report on the diversity of endophytic fungal flora associated with date palm trees (*P. dactylifera*).

Fungal endophytes associated with the palms, *Calamus kerrianus* (rattan) and *Wallichia caryotoides* (taorang) were investigated at two sites within Doi Suthep-Pui National Park, Thailand. Endophytic fungi were isolated from different tissue types (petiole, leaf, lamina and leaf veins) during three periods of the year, rainy season (July-October, 1999), cold season (November 1999-February 2000) and hot season (March-June 2000). Thirty-five endophytic fungi isolated included xylariaceous taxa (20 morphotypes), sterile mycelia, one unidentified and 13 mitosporic fungi including *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocaicola*, *Paecilomyces* sp. *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phomopsis* sp., *Phyllosticta* sp., and *Sarcopodium* sp. The endophyte species, their relative frequency, isolate prevalence and diversity did not differ significantly between host species, tissue types, study sites and seasons (Lumyong *et al.*, 2009).

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Endophytic fungi were isolated from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia. Endophytes were very common in both species, with overall colonisation rates of 81±89%. Taking into account a lower sampling frequency in Australia, endophyte diversity was similar in the two *Licuala* species. The endophyte assemblages examined were very diverse, consisting of 75 fertile species and 60 sterile morphospecies. The endophyte communities of both palms were composed of a single, dominant xylariaceous species, approximately ten less common but equally ubiquitous species and a large number of species occurring at very low frequencies. Differences were observed between the endophytic mycotas of different palm tissues and of tissues of different ages. The results presented suggest that most of the endophytes entered the petiole via the leaf and that transmission of palm endophytes is likely to be horizontal (via airborne propagules) rather than vertical (via seed). Seasonal differences were not observed in Brunei. Increased sampling effort could be expected to yield more endophyte taxa in both species investigated (Frohlich, 1999).

Endophytic flora plays a vital role in the colonization and survival of host plants, especially in harsh environments, such as arid regions. This flora may, however, contain pathogenic species responsible for various troublesome host diseases. (Chobba *et al.*, 2013).The present study of Ines Ben Chobba *et al.* was aimed at investigating the diversity of both cultivable and non-cultivable endophytic fungal floras in the internal tissues (roots and leaves) of Tunisian date palm trees (*Phoenix dactylifera*). Accordingly, 13 isolates from both root and leaf samples, exhibiting distinct colony morphology, were selected from potato dextrose agar (PDA) medium and identified by a sequence match search wherein their 18S–28S internal transcribed spacer (ITS) sequences were compared to those available in public databases. These findings revealed that the cultivable root and leaf isolates fell into two groups, namely Nectriaceae and Pleosporaceae. Additionally, total DNA from palm roots and leaves was further extracted and ITS fragments were amplified. Restriction fragment length polymorphism (RFLP) analysis of the ITS from 200 fungal clones (leaves: 100; roots: 100) using *Hae*III restriction enzyme revealed 13 distinct patterns that were further sequenced and led to the identification of *Alternaria*, *Cladosporium*, *Davidiella* (*Cladosporium* teleomorph), *Pythium*, *Curvularia*, and uncharacterized fungal endophytes. Both approaches confirmed that while the roots were predominantly colonized by *Fusaria* (members of the Nectriaceae family), the leaves were essentially colonized by *Alternaria* (members of the Pleosporaceae family). Overall, the findings of the present study constitute, to

the authors' knowledge, the first extensive report on the diversity of endophytic fungal flora associated with date palm trees (*P. dactylifera*).

From research of Taylor *et al.* (1999), fungal endophytes associated with the palm, *Trachycarpus fortunei*, within and outside its natural geographic range were investigated. Endophytes were relatively common with colonization rates of 23±57% at the four sites sampled. The endophyte assemblages at the different sites were diverse with 75 fertile species of ascomycetes and mitosporic fungi. The assemblage composition at each site was similar and between seven and 13 species comprised 81±89% of the taxa present in relative frequencies of "1%. *Glomerella cingulata* and *Phomopsis* spp., were consistently dominant, and a large number of rare species were recorded. The diversity at each site was similar in number, but the abundance of isolates varied. The results obtained were comparable to those of previous studies of palm endophyte assemblages, but the assemblages showed more affinity with unrelated temperate hosts than with tropical palm hosts. Quantitative and qualitative differences in endophyte assemblages from old and young tissues were observed, and more isolates were recovered from old tissues independent of the age of the palm. The composition of the assemblage varied with several taxa being exclusively or more commonly isolated from old tissues (e.g. xylariaceous taxa, *Oxydothis* sp. nov.) or young tissues (e.g. *Stagonospora* spp., *Phoma multirostrata*). Some differences in the composition of the assemblage and in relative frequencies of various species were observed in trees and saplings. Significantly more isolates were recovered from the vein than intervein tissues, independent of leaf age or tree age. Tissue specificity was not exhibited by any taxa isolated from either leaf or petiole tissues, except for xylariaceous taxa in leaf tissues. Some other taxa showed a preference for leaf tissues or petioles, whereas others were equally distributed amongst all tissues. Endophyte assemblages of palms from continuous distributions were similar, but those from disjunct distributions (i.e. outside the natural geographic range of the palm, such as Australia and Switzerland), differed significantly from each other and from assemblages within continuous distributions.

2.2 Isolation of Endophytic Fungi

The method most commonly used to detect and quantify endophytic fungi is isolation from surface-sterilized host tissue. Surface sterilization of plant material usually entails treating the plant material with a strong oxidant or general disinfectant for a brief period, followed

by a sterile rinse to remove residual sterilant. Household chlorine bleach (NaOCl), usually diluted in water to concentrations of 2–10%, is the most commonly used surface sterilant. Ethanol (70–95%) is the most commonly used wetting agent; it has limited antibiotic activity and should not be used alone as a surface disinfectant (Schulz *et al.* 1993).

Other sterilants, not commonly used in endophyte studies, include silver nitrate, mercuric chloride, formalin, and ethylene or propylene oxide. Booth (1971) described methods and apparatus for surface sterilization of plant material using several of these substances. Silver nitrate (1%) commonly is used for surface sterilization of roots and stems of grasses. The silver nitrate can be precipitated following treatment by rinsing in 5% NaCl (Cunningham, 1981). Mercuric chloride (0.01% for 1 min) was used for surface sterilization of *Acer* leaves (Pugh and Buckley, 1971). It seldom is used now because of its residual toxicity and hazardous nature.

Serial washing often is used to remove soil from root tissues, to remove incidental spores from leaf surfaces, and to remove surface contamination in cases where a nontoxic method is desired. This is best accomplished using a large vessel so that the inflowing water vigorously agitates the sample (Booth, 1971). The serial washing method of Harley and Waid (1955) is relatively simple and can be used for study of fungi colonizing roots, shoots, and leaves (Mushin and Booth, 1987; Holdenreider and Sieber, 1992).

2.3 Biological Activity of Endophytic Fungi

In the 70's, endophytes were initially considered neutral, not causing benefits nor showing detriment to plants, but later on they started to be better studied. By that time it was possible to conclude that in many cases, they had an important role in medical, agricultural and industrial to use. Endophytic fungi are probably one of the major potential sources for new, useful metabolites (Dreyfuss and Chapela, 1994). There has been a great interest in endophytic fungi as potential producers of novel, biologically active products (Schulz *et al.*, 2002; Strobel and Daisy, 2003; Tomita, 2003; Urairuj *et al.*, 2003; Wildman, 2003).

Several studies on the use of bioactive compounds from endophytic fungi have been reported. Endophytic fungi are able to produce antimicrobial, anticancer such as Taxol (Walker and Croteau, 2001) and antimalarial activities (Wiyakrutta *et al.*, 2004). Study done by Woropong *et al.* (2001) showed that isolated endophytic fungi are able to produce mixture of volatile organic compounds that are lethal to human and plant pathogenic fungi and bacteria.

Some endophytic fungi from Chinese medicinal plants are also potential sources of diverse bioactive metabolites that may have potential for therapeutic purposes (Tan *et al.*, 2000; Tan and Zou, 2001). If these fungi could be utilized to produce the bioactive compounds of medicinal plants on large-scale fermentors, this would provide a new technology for producing many types of Traditional Chinese medicine. Some endophytic fungi have been found to produce similar medicinal compounds to that of the host. Proof of principal was realized when the anticancer drug taxol was found to be produced by endophytic fungi isolated from *Taxus brevifolia* (Strobel *et al.*, 1996). Screening of this diverse group of fungi that may produce valuable medicinal plant products is a promising approach for obtaining Traditional Chinese Medicine from plants on a commercial scale using microbes (Strobel, 2003; Strobel and Daisy, 2003).

Wiyakrutta *et al.* (2004) isolated endophytic fungi with antimicrobial, anticancer and antimalarial activities isolated from Thai medicinal plants. A total of 81 Thai medicinal plant species collected from forests were examined for the presence of endophytic fungi with biological activity. Of 582 pure isolates obtained, 360 morphologically distinct fungi were selected from which extracts were prepared and tested for biological activity. Extracts of 92 isolates could inhibit *Mycobacterium tuberculosis* by the microplate Alamar blue assay while extracts of 6 isolates inhibited *Plasmodium falciparum* as determined by the [3H] hypoxanthine incorporation method. Antiviral activity against Herpes simplex virus type 1 was observed in 40 isolates. Anticancer activity against human oral epidermoid carcinoma cells and breast cancer cells was also observed by sulphorhodamine B assay. Hence it was concluded that Thai medicinal plants inherits diverse endophytes possessing a potential source of novel bioactive compounds.

Effects of fatty acids and inhibitors of eicosanoid synthesis on the growth of a human breast cancer cell line in culture revealed results that Linoleic acid stimulated MDA-MB-231 cell growth with an optimal effect at a concentration of 0.75 μg/ml, whereas oleic acid produced growth stimulation at 0.25 μg/ml but was inhibitory at higher concentrations. Docosahexaenoic acid exhibited a dose-related inhibition of cell growth at concentrations ranging from 0.5 to 2.5 μg/ml and eicosapentaenoic acid was less effective. Similar inhibitory effects occurred with other saturated fatty acids, Rose and Connolly (1990).

Pimentel *et al.* (2011) reviewed on the use of endophytes for the production of

bioactive compounds and their use in biotransformation process. The role of endophytes on the production of anticancer, antimicrobial and antioxidant compounds illustrating their potential for human use was inferred. It also describes biotransformation as an auspicious method to obtain novel bioactive compounds from microbes. Biotransformation allows the production of regio and stereo selective compounds under mild conditions and that using endophytic fungi have been reviewed for e.g. biotransformation of grandisin by the endophytic fungus *Phomopsis* sp. to a tetrahydrofuran which showed trypanocidal activity.

Lotufo *et al.* (2005) evaluated the anticancer potential of 11 plants used in Bangladeshi folk medicine. The extracts were tested for cytotoxicity by the brine shrimp lethality assay, sea urchin eggs assay, hemolysis assay and MTT assay using tumor cell lines. The extract of *Oroxylum indicum* showed the highest toxicity on all tumor cell lines as well as on the sea urchin eggs. The extract of *Aegle marmelos* exhibited toxicity on all used assays but in a lower potency than *Oroxylum indicum*. The study concludes that only the extracts of *Oroxylum indicum*, *Moringa oleifera* and *Aegles marmelos* could be considered as potential sources of anticancer compounds among the tested plant extracts. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations.

Xu (2008) have found that the *C. militaris* extract can inhibit growth of MCF-7 human breast cancer cells in a dose and time-dependent manner. In addition to the apoptotic genes the levels of the methyltransferase gene *DNMT1* and *DNMT3a* transcripts were also suppressed in MCF-7 cells incubated with the *C. militaris* extract. Methylation in some tumor-suppressor genes may potentially lead to regained expression of these genes and subsequent inhibition of cancer cell growth and its extract inhibits human breast cancer cell growth through an apoptosis cascade by inducing pro-apoptotic and suppressing antiapoptotic marker gene expression. *C. militaris* extract reduced DNA methylation through the suppression of methyltransferase transcripts leading to the recovery of tumor-suppressor genes and eventually inhibiting tumor cell growth.

Banu and Kumar (2009) did preliminary screening of endophytic fungi from medicinal plants in India for antimicrobial and antitumor activity. 16 endophytic fungal isolates tested were found to exhibit antitumor activity in the yeast cell-based assay. The screening of antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, yeast and fungi

was carried out on 47 isopropanol extracts prepared from 121 isolates of endophytic fungi isolated from medicinal plants in India that includes *H. indicus*. Sensitivity was found to vary among the microorganisms. *Bacillus subtilis, Saccharomyces cerevisiae* and *Alternaria* sp. were susceptible to extracts from three, two and two isolates of endophytic fungi, respectively. None was found effective against *Salmonella typhimurium*. 16 endophytic fungal isolates tested were also found to exhibit antitumor activity in the yeast cell-based assay.

The natural and biological control of pests and diseases affecting cultivated plants has gained much attention in the past decades as a way of reducing the use of chemical products in agriculture.

Biological control has been frequently used in Brazil, supported by the development of basic and applied research on the field in South America as it can be found in several reviews (Lecuona, 1996; Alves, 1998; Melo and Azevedo, 1998). Vega *et al.*, (2008) studied fungal endophyte - mediated plant defense as a novel biological control mechanism against the coffee berry borer the most devastating pest of coffee throughout the world. A survey of fungal endophytes in coffee plants from Hawaii, Colombia, Mexico and Puerto Rico has revealed the presence of various genera of fungal entomopathogens including. *Acremonium, Beauveria, Cladosporium, Clonostachys,* and *Paecilomyces*. Two of these *B. bassiana* and *Clonostachys rosea* were tested against the coffee berry borer and were shown to be pathogenic.

Mohanta *et al.* (2008) studied the antimicrobial potentials of endophytic fungi inhabiting three Ethno-medicinal plants of Similipal Biosphere Reserve India. Nearly 60 fungal endophytes belonging to 14 genera were isolated out of which 31 endophytes (51.66%) were obtained as filamentous forms and 29 of them (48.33%) as yeast colonies. Species of *Curvularia, Fusarium, Alternaria* and *Penicillium* were isolated as dominant and host specific endophytes. Among the potent strains of about 13 isolates, 19.3% displayed both antibacterial and antifungal activity and 6.4% strain showed antimicrobial activity against all the test pathogens.

Antifungal products are vastly produced by majority of the endophytes. Griseofulvin-producing endophyte was first reported in fungus from *Abies holophylla* and was evaluated *in vivo* antifungal activity against plant pathogenic fungi. Based on nuclear ribosomal ITS1-5.8SITS2 sequence analysis, the fungus was identified and labeled as *Xylaria* sp. F0010. Two antifungal substances, griseofulvin and dechlorogriseofulvin were purified from liquid

cultures of *Xylaria* sp. and identified through mass and NMR spectral analyses. Compared to dechlorogriseofulvin, griseofulvin showed high *in vivo* and *in vitro* antifungal activity and effectively controlled the development of rice blast (*Magnaporthe grisea*), rice sheath blight (*Corticium sasaki*), wheat leaf rust (*Puccinia recondita*) and barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) (Park *et al.*, 2004).

Hundley (2005) did structure elucidation of bioactive compounds isolated from endophytes of *Alstonia Scholaris* and *Acmena graveolens*. In the present study, an endophyte of the genus *Xylaria* was isolated from a stem of *A. scholaris* its mycelia and exudate extracted and the extract assayed for growth inhibition of HeLa cancer cells *in vitro*. Several known compounds were isolated and identified based on NMR, infrared and mass spectral data. The compounds identified are 19, 20-epoxycytochalasin C; 19, 20-epoxycytochalasin D and xylobovide. Two other compounds, fusaric acid and dehydrofusaric acid were discovered in an endophyte of the Hypocreales family inhabiting the plant *A. graveolens*.

Antagonistic effect of endophytes against pathogens of some plants is also prominent. This effect has been studied in root rot pathogens of wheat. Four different endophyte species were isolated from Rye grasses, *Triticum* sp. and *Tall fescue*. All *Neotyphodium* and *Acremonium* sp. significantly affected the growth rate of five root-rot pathogens of wheat in PDA plates. Culture filtrates of endophytes have had some effect against these test fungi. On conducting the germination tests the pathogen has shown abnormal elongation of the hypha, lysis of the conidia and abnormal germ tubes Tunali and Marshall (1995).

Effect of Endophytic fungi - *Beaveria bassiana, Trichoderma koningii, Alternaria alternata, Phoma* sp., and *A. strictum* on the causal agents of maize seedling blight, stalk and root rot was elucidated. These endophytes were isolated from maize roots while the pathogens, *Fusarium oxysporum, Fusarium pallidoroseum, Fusarium verticillioides* and *Cladosporium* 35 *herbarum* were isolated from blighted maize plants. The *in vitro* assay of the endophytes against the pathogens showed reducing radial growth by 25 -75% and 53 - 80%, respectively. The *in vivo* studies revealed *T. koningii* as the best endophyte by reducing wilting rate to 25%. *T. koningii* and *A. Alternate* could be successfully formulated and applied as alternative fungicides in the management of maize wilt and seedling blight Orole and Adejumo (2009).

Rodrigues and Hasse (2000) performed antimicrobial assays with the secondary

metabolites produced by endophytic fungi from *Spondias mombin* (Anacardiaceae). Few of the isolated endophytes were chosen for preparation of culture broth extracts: *Guignardia* sp. *Phomopsis* sp. and *Pestalotiopsis guepinii*. Extracts were separated by chromatographic methods and tested for biological activities. The crude extracts were tested against 14 organisms including actinomycetes, Gram-negative and Gram-positive bacteria, yeast and filamentous fungi. All fungal extracts inhibited actinomycete growth. *Guignardia* sp. was active against *Escherichia coli, Staphylococcus aureus, Saccharomyces cerevisiae, Geotrichum* sp. and *Penicillium canadensis*. Culture extracts of *P. guepinii* were active against *S. cerevisae*, while strains of *Phomopsis* sp. showed a pronounced antifungal effect against *Cladosporium elatum, Mycotypha* sp. and *S. cerevisae*.

Different fungal species have been exploited as an alternative source of plant secondary metabolites. Endophytic fungi colonize plants internally without apparent adverse effects and do occur ubiquitously in plants. They are known to produce a number of important secondary metabolites including anticancer, antifungal, antidiabetic and immunosuppressant compounds *e.g.* paclitaxel, torreyanic acid, cytochalasins etc. have been isolated from endophytic sources. The discovery of Stierle and his co-workers, studies carried out by Strobel and Daisy had raised scope of using the endophytic fungus as a sustainable alternative source of important plant secondary metabolites. However our poor understanding of the evolutionary significance of these organisms and their dynamic interaction with their respective hosts results in failure of exploiting endophytic fungi in diverse arenas (Priti *et al.*, 2009).

2.4 General of Palm

Palms are woody monocotyledons in the family Arecaceae (alternative name Palmae) which is placed in the order Arecales. They are a natural group of plants with a characteristic appearance that enables most people to recognize them without great difficulty, although unrelated plants with a similar general appearance-such as Cycads, Cyclanths, Pandans, Cordylines-are often included with them by the inexperienced Palms are an ancient life form with fossil records from the late Cretaceous period. Being composed largely of durable materials, palms leave a relatively good fossil record and may have existed before this time. Palms are regarded as being 'princes' among plantsand indeed were labelled (such as 'Principes') by none other than the great Swedish botanist Carolus Linnaeus, the founder of the modern binomial

system of plant nomenclature (Jones, 1995).

2.4.1 Distribution of Palm

Palms are widely distributed in the well-watered zones of the world but are absent or rare in very dry or very cold regions. They are uncommon in the temperature zones but proliferate in the tropics. Not only do the vast majority of species occur here but palms are also a commonly encountered and sometimes dominant component of the vegetation. In the tropics thery can be found from the seashore to inland districts and to high altitudes.

Despite the wide distribution and frequency of palms in the world's tropics, there are very few large and widespread genera. In fact, most palm genera contain five or fewer species and monotypic genera are common. The climbing genus *Calamus* is the largest with 370 species with a Pantropical distribution. Many small ganera have a worldwide distribution – for example, *Borassus*, *Phoenix* and *Raphia* – while some larger ganera have proliferated in relatively restricted areas. For example, *Licuala* and *Pinanga* – each containing more than 100 species – are largely found between Malaysia and New Guinea, while the 100 species of *Chamaedora* are restricted to Central and South America. As a general rule there are more differences than similarities between palms of the Old World and those of the new world.

In the literature, the estimate of the number of species of palms varies from 2500 to 3500 in 210 to 236 genera. A more accurate modern estimation is probably about 2600 species in 200 genera. Variations in the tallies basically arise because of disagreement between botanists on the delimitations of species and genera. Whatever the number of species, palms are a highly significant and extremely diverse group and rank fourth of fifth in size in the monocotyledons. (Jones, 1995)

2.4.2 Palm Habtats

It has been estimated that more than two-thirds of the world's palm species grow in rainforests. Here they maybe emergent plants with their crowns well clear of the forest canopy; of intermediate size mingling with the canopy of the trees but not emerging; or growing as small understory plants in the generally shady, dull conditions of the rainforest floor. Climbing palms are also common in rainforests and while these have their roots anchored in the soil of the forest floor, their uppermost leaves mingle with the outer foliage of the canopy.

A significant number of palms grow in open habitats such as savanna grassland, open woodlands and sparse forests. Many of these are hardy species which are capable of withstanding periods of dryness and occasional fires. A characteristic of palms growing in open sites is that they are frequently found in extensive colonies, usually of a single species – for example, *Borassus*, *Livistona*, *Phoenix*, *Raphia*. By contrast, palms found in rainforest are not often in colonies and many different species may exist together in a small area – for example, *Pinanga*, *Licuala*.

Some palms that grow in open areas tend to favour wet sites such as marshes and swamps, or the margins of permanent streams, lakes or lagoons. Often the palms growing in these sites occur in dense colonies or thickets.(Raphia taedigera, Phoenix paludosa). Even sites subject to periodic inundation can support palms because their deep roots can tap ground water during the dry times. Although they may have excellent water-conserving, palms are absent from very dry habitats. Even those found in the desert – such as the Date Palm – will only survive where their roots can tap permanent underground water supplies.

A few palms have adapted to specialized habitats where they can compete successfully with other plants. These include situations along the seashore, in coastal estuaries, within the flood range of streams and even growing within the streams themselves. Few palms will tolerate snow but Nannorrhops ritchiana from the mountains of Afghanistan and species of Trachycarpus from the Himalayas are covered with snow regularly each winter. Some palms have adapted to unusual soil types and occur naturally on no other soil regime (Jones, 1995).

2.4.3 The Economic Importance of Palm

In the west, palms are an ornamental plant most familiar in tropical forest and gardens and elsewhere popular for indoor decoration. These same plants, however, contribuse significantly to the economies of many countries and are of prime importance in the daily lives of millions of people. The contributions these plants make to the woorld's economy, local economies and lifestyles is quite amazing and appreciated by every few people.

A surprisingly large number of products can be obtained from palms. For instance, the various parts of the widespread Coconut Palm can be used in more than 1000 ways and the Palmyra Palm in over 800 ways. The Date palm is not such a prolific relative by comparision and yet this species helps to keep millions of people alive by its tremendous production of energy-rich

dates in a climate where little else will grow. Numorous other species of palm are also of economic significance and contribute to human survival in various ways. Thus palms provide shelter, food and drink, clothing, fuel, fibre and medicine.

For example, every part of Coconut palm is used in some way even when the trees are blown over or felled for some reason, the trunk, the fibrous pith and the apical bud (cabbage) are all used (Jones, 1995).

- The meat of the nut can be cooked in more than a hundred different dishes or eaten raw or shredded, or dried and exported as desiccated coconut.
- the water, especially that of a green coconut, makes a refreshing drink or it can be blended with the meat to make coconut milk, cream or jam.
- the shells can be burnt, made into charcoal, or used as bowls, scoops and cups
- the fibre can be made into mats or woven into string and ropes or shredded and made into potting mix for nurserynplants
- the leaves can be used for thatching and the leaflets for weaving
- the wood for buiding
- the flowers for palm honey and the sap of the inflorescence for palm sugar, alcohol and the potent drink arrack
- coconut oil can be extracted from the kernels and dried kernels (copra) are an important component of this process as they can be stored and transported
- after oil extraction the remaining meaty material can be pressed into a cake suitable for animal feed
- coconuts are sold to tourists either whole or with designs carved into the husk
- the water of the green coconuts has growth-regulatory properties and is added to the growing media used in tissue culture and for raising orchid seedlings.

2.5 Anthracnose of Chili

Chili (*Capsicum annuum* L.) is an important tropical and subtropical cash crop (Anand *et al.*, 2007). Large areas are devoted to chili production in China, India, Mexico, Morocco, Pakistan, Turkey, and Thailand (Than *et al.*, 2008). A fungal disease that limits chili production in all these countries is anthracnose. Anthracnose, caused by several species of the fungus *Colletotrichum*, is a minor disease of pepper foliage but causes serious losses to pepper fruit. The disease was first reported in New Jersey, U.S.A., in 1890 and is currently found throughout North America, Asia, Australia and Africa (Hadden, 1989). The pathogenic fungi *C. capsici* were reported as chili anthracnose in Thailand (Sangchote *et al.*, 1998; Sangchote, 1999). This fungus is one of the major diseases for chili and causes severe damage on chili fruits in both pre and post harvest stages. And, these infections together account for more 50% of the crop losses (Pakdeevaraporn *et al.*, 2005). The fungus is both internally and externally seed-borne (Ramachandran *et al.*, 2007). The black wound found on infected fruits will expand very quickly under high moisture condition, especially in tropical countries. Fruiting bodies and spores of *C. capsici* will be abundantly produced on those black lesions.

2.6 Anthracnose of Coffee

Coffee is a tropical crop and according to FAO statistics production of green beans is around 4 million tons annually with sales of 6 to 12 billion dollars (González, 2000). There are many different diseases on coffee. Especially coffee anthracnose mainly has three kinds pathogen. Several species or strains of *Colletotrichum* have been reported from coffee. The organism pathogenic on green coffee berries is *C. coffeanum* while the other strains have been identified as *C.gloesporioides* (perfect stage = *Glomerella cingulata*) and in some instances *C. acutatum* (Hindorf, 1970, 1973a,b). There were over 20 % of disease incidence for leave and bean diseases in Arabica coffee in all visited plantations. *Colletotrichum coffeanum* causing anthracnose on leaves and beans before harvest is the most common found and destructive pathogen as seen from the result of pathogenicity test which confirmed *C.coffeanum* expressed virulent isolates to infect into coffee leaves and beans of Arabica variety (Vilavong and Kasem 2013). Symptoms: when leaves victims, mostly in the incidence of leaf margin, the upper and lower leaf surfaces showing irregular light brown to dark brown spots. Lesion central white, yellow edge, later gray, on which there are many small black dots (pathogen spores) are arranged

in concentric wheels pattern.

The general strategy of biological control is to use one living organism to control anthracnose (Druvefors, 2004). Recently, biological control has been developed as an alternative to synthetic fungicide treatment and considerable success has been achieved upon utilizing antagonistic microorganisms to control both pre harvest and postharvest diseases (Janisiewicz and Korsten, 2002). Metabolites from *Talaromyces muroii* EU18 acts as a new antagonist against *C. coffeanum* causing coffee anthracnose. The crude ethyl acetate and crude methanol extracted from *Talaromyces muroii* EU18 showed significantly antifungal activity against *C. coffeanum* (Soytong and Poeaim, 2015).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Isolation of Fungal Endophytes

3.1.1 Sample Collection

Plant samples were randomly collected from 10 species healthy palms at King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Species of palms were identified followed by Henderson *et al.* (1995), Hodel (1998), Langlois (1902) and Jones (1995). All the samples from healthy leaves, petioles and roots of randomly select plants were cut and taken to the laboratory, processed within 24 h. Ten species palms were collected as follows:

1. *Ptychosperma macarthuri* (MacArthur Palm);
2. *Rhapis humilis* (Slender Lady Palm);
3. *Wodyetia bifurcata* (Foxtail Palm);
4. *Chrysalidocarpus lotescens* ;
5. *Veitchia merrillii* (Manila Palm)
6. *Phoenix roebelenii ;*
7. *Rhapis Laosensis* (Thailand Lady Palm);
8. *Licula spinosa* ;
9. *Livistona chinensis* (Chinese Fan Palm);
10. *Mascarena Lagencuulis* (Bottle Palm).

3.1.2 Isolation of Endophytic Fungi

Plant specimens were thoroughly washed in running tap water for 5 min removed dust and debris and then air dried. The cleaned leaves, petioles and roots were surface sterilized with 75% ethanol 1 min and sodium hypochlorite (3%available chlorine) 3-5min and then were removed outer epidermal tissues and cuticle before cut under sterile conditions into small pieces of 3×3×3 mm. Briefly, fragments were cleaned in sterilized water and sterilized in 75% ethanol 30s and then cleaned in sterilized water again and placed on water agar (WA) medium, incubated

at room temperature waiting endophytic fungi grow out. The endophytic fungi growing out from the plant tissue were transferred into potato dextrose agar (PDA) plates and incubated for two to six days. Continuous plates were subcultured until get pure culture.

3.1.3 Identification of Endophytic Fungi

A total isolates of endophytic fungi were selected for morphological and molecular identifications.

3.1.3.1 Morphological Identification

The isolates of endophytic fungi were identified by the morphology of the fungal culture, including colony and medium color, colony characters, spore characters, mycelium characters, fruiting structures by following the standard mycological manuals (Ellis, 1971; Barnett and Hunter, 1987; Domsch and Games, 1993; Sutton, 1980; Nag, 1993). The sterile isolates were grown on PDA with decoction of host leaves medium to observe sporulation. For tentative identification, microscopic slides of each endophytic fungi were prepared and examined under binocular compound microscope for morphological identification.

3.1.3.2 Molecular Identification

In molecular identification, fungal genomic DNA were extracted and prepared from each endophytic fungus. Each endophytic fungus was cultured in PDB 3 days. The fungal mycelia were freeze-dried and the genomic DNA was extracted by the modified CTAB (Cetyl trimethyl ammonium bromide) method (Ausubel *et al.*, 1994). Briefly, the fungal mycelia were cleaned with 25mM EDTA by centrifugal machine. 100mg fungal mycelia were vigorously crushed in liquid nitrogen to make a fine powder and tansferred into an eppendorf tube. The cells were lysed in CTAB buffer and β-mercaptoethanol, mixed thoroughly and incubated at 65°C for 1h with mixing tubes every 15 min. And then, the lysate were extracted with an equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged at 14,000 rpm in a microcentrifuge for 5 min at 4°C. The aqueous phase were transferred to a new sterile tube and add 2µl Rnase (20µg/ml), incubated 30min at 37°C. Add 50µl 10% CTAB, mix thoroughly. Repeat add equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged at 14,000 rpm in a microcentrifuge for 5 min at 4°C. Remove aqueous top layer and transferred in to new eppendorf tube. Add an equal volum of cold isoproponal, mix thoroughly and let tubes sit at -20°C for 20min. The genomic DNA were precipitated in isopropanol and centrifuged at 4°C for 20 min at 14,000 rpm. The resulting pellets

were washed twice with 70% and 95% ethanol, air dried and dissolve in 100µl TE buffer at 37°C, over night. The quality and quantity of extracted DNA samples were monitored routinely by electrophoresis in a 1% agarose gel. Quantification was performed through comparison with known dilutions of lambda phage DNA. DNAs were stored at -20°C for further use.

Phylogenetic analyses of the endophytes were carried out by the acquisition of the ITS1-5.8S-ITS4 ribosomal gene sequencing. The ITS regions of the fungi were amplified with the universal ITS primers, ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3'), using the polymerase chain reaction (PCR). The used PCR conditions were as follows: initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 1 min., 56°C for 1min., 72°C for 2 min., and a final extension at 72°C for 5 min. The 25µl reaction mixture contained 2.5µl 10 × PCR buffer, 0.625µl each dNTP (1.25mM), 0.5µl $MgCl^2$, 1µl of each primer (20pmol/µl), 2 ng of DNA and 0.2µl of Taq DNA polymerase (1 U). The amplified products (5µl) were visualize on 1% (w/v) agarose gel to confirm the presence of a single amplified band.

The amplifications were purified by Amicon Ultra columns (Millipore, USA). The PCR products were electrophoresis in a 1% agarose gel, then, cut gel with DNA band under UV light and put into tubes. Add DB buffer malte gel at 50°C and loading to purification colume for centrifuge at 8500rpm, 1min, 4°C. Discard flow through, add wash buffer and centrifuge at 8500rpm, 1min, 4°C for dry samples. Transferred colume to new microcentrifuge tube and added elution buffer, stand for 5min. Centrifuge at 8500rpm, 1min, 4°C and electrophoresis in a 1% agarose gel for check DNA band. And then, purification products were sent to company for sequencing. The forward or the reverse primer (2 pmoles) also used in the sequencing reaction.

The amplified products were sequenced and aligned with the sequences in the GenBank by basic local alignment search tool (BLAST) analysis (Altschul *et al.*, 1997) in the National Center for Biotechnology Information (NCBI) databases to find out the sequence homology with closely related organisms. Sequences from the closely related organisms were downloaded to construct the phylogenetic trees. The closely related sequences obtained were aligned through CLUSTALW using MEGA version 6.0 software (Tamura *et al.*, 2007) and a phylogenetic tree was constructed by neighbor-joining method using the same software.

3.2 Bioactivity Test against Plant Pathogens (*Colletotrichum* spp.)

3.2.1 Isolation of Pathogens

Colletotrichum coffeanum causing coffee anthracnose in Arabica variety was isolated by tissue transplanting technique from coffee leaf with obvious symptoms. The disease leaves were cleaned with running tap water and after air-dry cut the advance margin of symptom between healthy tissue and diseased tissue to small pieces and then sterilized with sterilized water, 75% alcohol and sterilized water again. Then, were transferred onto WA medium and followed by potato dextrose agar (PDA) to obtain pure culture. *Colletotrichum coffeanum* was identified by morphological characteristic under binocular compound microscope.

Colletotrichum capsici causing chili anthracnose was isolated from chili fruit with obvious symptoms of anthracnose by moister chamber method. The disease fruit were placed into petri dish lined with sterile tissue paper and moistened with sterile distilled water, and, culture 2-3 days, after the stroma grow out, pycnidia were transferred to WA medium, followed by PDA to get the pure culture. *Colletotrichum capsici* also were identified by morphological characteristic under binocular compound microscope.

3.2.2 Pathogenicity Test

Pathogenicity test for *Colletotrichum coffeanum* causing coffee anthracnose was conducted using detached leaf method in the laboratory and performed pathogenicity followed Koch's Postulate. Select healthy leaves of coffee and washing in the running water and air-dried. A sterilized filter paper was placed in 9cm diameter sterilized petri dish and two sterilized glass slide were also put on the filter paper, and the filter paper were moistened by sterilized distilled water. Coffee leaves were wounded by sterilized needle and then placed on the glass slide in the petri dish then the spore surspention of *Colletotrichum coffeanum* was prepared at concentration is 1×10^{6} spores/ml. Spore surspention were sprayed on the surface of coffee leaves, including the wounded areas. And then, incubated for two weeks at room temperature. At the same time, sterilized water was also sprayed to coffee leaves as controls and incubated. Lesions on inocultated areas were obsereved on the coffee leaves, then re-isolated pathogen from lesion invaded with inoculated pathogen according to the above mentioned method and identified the re-isolates under microscope and get pure culture.

The pathogenicity test of *Colletotrichum capsici* causing chili anthracnose was

tested using detached fruit method, followed by above mentioned detached leaf method. Select healthy fruit of chili and washing in the running water and air-dried. Treatments were inoculated the spore surspention of *Colletotrichum capsici* at concentration is 5×10^6 spores/ml and placed in moist chamber in petri dish. Control was done by spray sterilized water to chilli fruits. And then, incubated for two weeks at room temperature. Lesions on inocultated areas were obsereved and re-isolated pathogen from lesion invaded with inoculated pathogen according to the above mentioned method and identified the re-isolates under microscope and get pure culture.

3.2.3 Bi-culture Antagonistic Test

Some isolates of endophytics were tested for antagonistic bi-culture. These isolates were tested to determine their bioactivity against plant pathogen *Colletotrichum capsici* causing anthracnose of chili and *Colletotrichum coffeanum* causing anthracnose of coffee. The experiment was conducted using a Completely Randomized Design (CRD) with 4 replications by the methods of Soytong (1992), Sibounavong *et al.* (2009) and Charoenporn *et al.* (2010). The antagonistic fungi and pathogen were separately cultured on PDA at room temperature (30-32 °C) for 7 days. And 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of cultures of the antagonistic fungi and pathogen and then transferred onto the same sterilized 9 cm-diameter PDA plates, an agar plug of the pathogen was placed on one side of the plate which opposited an agar plug of an antagonistic fungus. The single plug of antagonistic fungi and pathogen were transferred into two separate PDA plates as the controls. And then, all the plates were incubated at room temperature (30-32 °C) for 30 days. Data were collected regarding to diameter of colony (cm) and the number of conidia produced by the pathogen in the bi-culture plates and control plates. A haemcytometer was used to count the number of conidia of pathogens.

Percentage inhibition of pathogen colony growth and conidia production were calculated using the following formula:

$$\% \text{ inhibition} = (A-B) / A \times 100$$

Where, A is the diameter of colony or number of conidia produced by the pathogen on the control plates and B is the diameter of colony or number of conidia produced by the pathogen in the bi-culture plate.

Analysis of variance was statistically computed and treatment means were compared

using Duncan Multiple's Range Test (DMRT) at P = 0.05 and 0.01.

3.2.4 Bioactive Substances Extraction

The bioactive substances were extracted from endophytic fungi as crude extracts. The extraction was performed using the method of Kanomedhakul *et al.* (2003). Endophytic fungi were cultured in potato dextrose broth (PDB) at room temperature (28-30 °C) for 45 days. The fungal biomass of endophytics were removed from PDB, filtered through cheesecloth and air-dried overnight. Fresh weight and dry weight of fungal biomass were weighted. The fungal biomass was grounded with electrical blender, and placed in triangular flask. And then dissolved with equal volume hexane 5 days at room temperature, the biomass was separated by filtration through whatman filter paper. The solvent was evaporated in *vacuum* to yield crude hexane. The marc was further extracted with ethyl acetate (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. Each crude extract was weighted, and then kept in refrigerator at 4 °C until to use. Extraction method is shown in the chart as follows (Fig. 3.1):

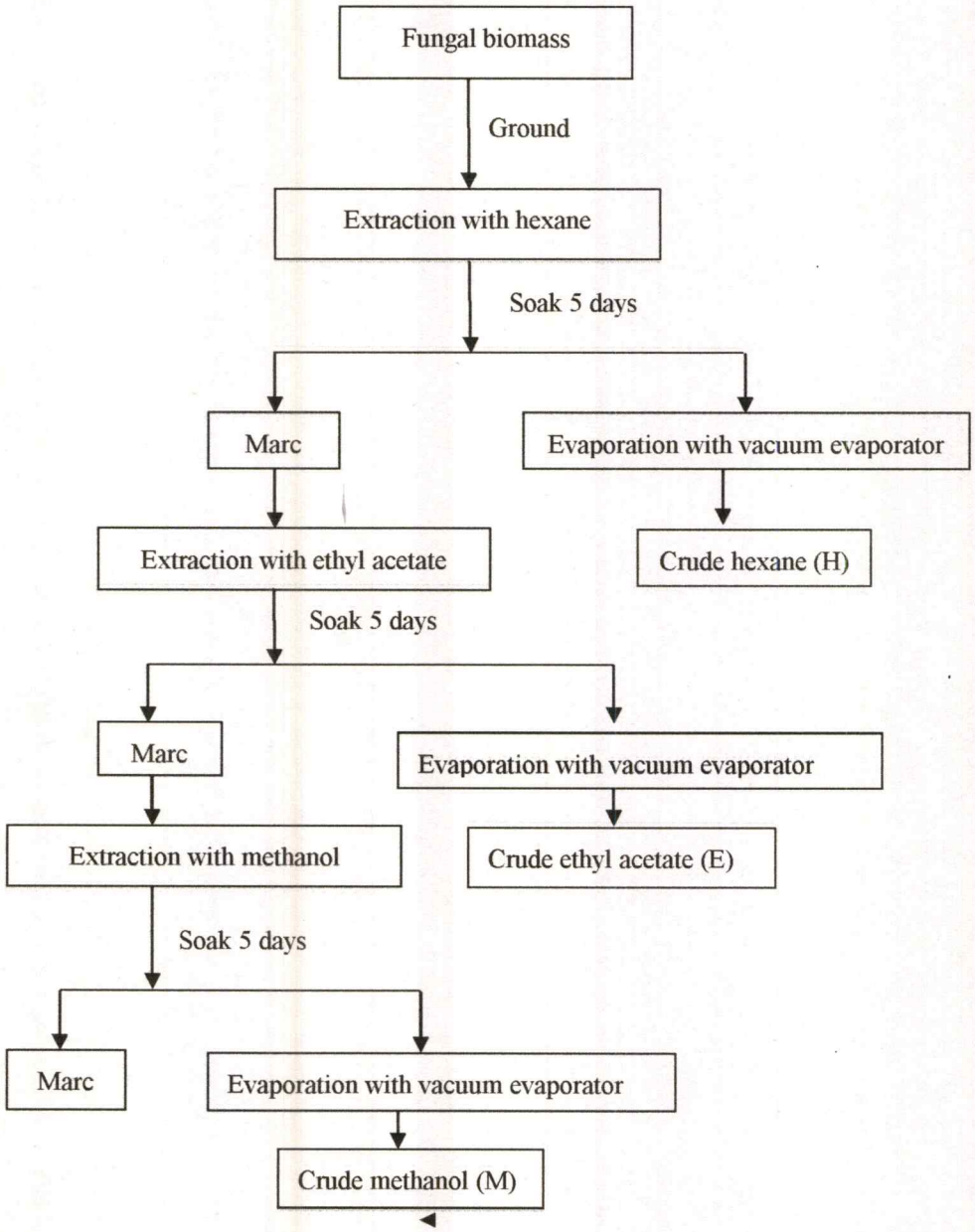


Fig. 3.1 Diagram of extraction method

3.2.5 Testing Efficacy of Biological Active Substances (crude extracts) against *Colletotrichum* spp.

The crude extracts of some species endophytic fungi were tested for inhibit the growth of *Colletotrichum coffeanum* and *colletotrichum capsici*. The experiment was conducted by using 3x6 factorials in Completely Randomized Design (CRD) with 4 replications. Factor A represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100, 500, and 1,000 ppm. Each crude extract was dissolved in one drop 2% dimethyl sulphite (DMSO), mixed into 30 ml potato

dextrose agar (PDA) before autoclaving at 121°C , 15 p for 30 minutes. The tested pathogen were cultured on PDA and incubated at room temperature for 7 days, and then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA(amending with each crude extracts) plate (5.0 cm diameter) in each concentration and incubated at room temperature (28°C-30°C) until the pathogen on the control plates growing full. Data were collected as colony diameter and the number of conidia. Percentage inhibition of pathogen colony growth and conidia production were calculated using the following formula:

$$\% \text{ inhibition} = (A-B) / A \times 100$$

Where, A is the diameter of colony or number of conidia produced by the pathogen in control plates and B is the diameter of colony or number of conidia produced by the pathogen in treatment plates.

Data were statistically computed analysis of variance and treatment means were compared using Duncan Multiple's Range Test (DMRT) at P = 0.05 and 0.01. The effective dose (ED_{50}) were calculated using probit analysis.

CHAPTER 4

RESULTS

4.1 Isolation of Fungal Endophytes

4.1.1 Sample Collection

Selection of host plants, from appropriate sites and obtaining fresh plant material is important for studying the occurrence and distribution of fungal endophytes. Plant samples were randomly collected from 10 species healthy palms at King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand (Fig. 4.1-4.10). Healthy leaves, petioles and roots were cut and taken to the laboratory, processed within 24 h.

Fig 4.1 *Ptychosperma macarthuri*

Fig 4.2 *Rhapis humilis*

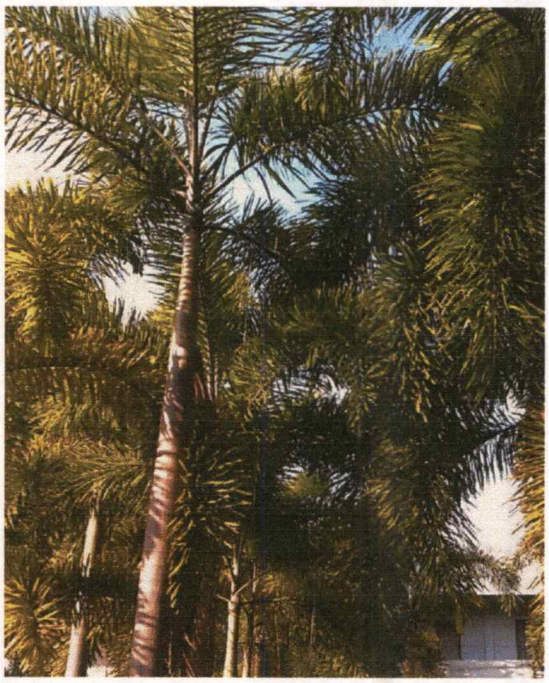


Fig 4.3 *Wodyetia bifurcate*

Fig 4.4 *Chrysalidocarpus lotescens*

Fig 4.5 *Veitchia merrillii*

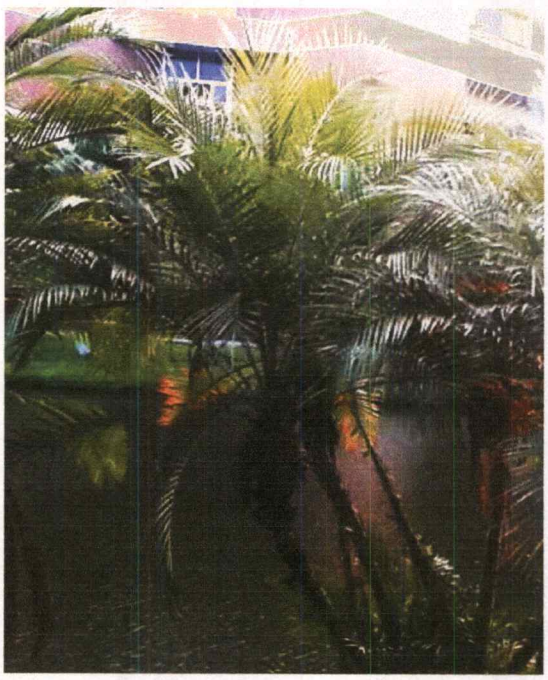


Fig 4.6 *Phoenix roebelenii*

Fig 4.7 *Rhapis Laosensis*

Fig 4.8 *Licula spinosa*

Fig 4.9 *Livistona chinensis*

Fig 4.10 *Mascarena Lagencuulis*

4.1.2 Isolation of Endophytic Fungi

From leaves, petioles and roots of 10 species palm trees in this study yielded 105 isolates (Table. 4.1). More isolates were obtained from *Wodyetia bifurcate* (Foxtail Palm) than other palms. And, Root of palms can harber more endophytics than leaves and petioles.

Table. 4.1 Number of isolates from leaf, petiole and root of 10 species palm trees.

Host Plant	Leaf samples	Petiole samples	Root samples	Total
Ptychosperma macarthuri	2	4	5	11
Rhapis humilis	3	2	3	8
Wodyetia bifurcate	5	4	8	17
Chrysalidocarpus lotescens	3	1	7	11
Veitchia merrillii	3	3	6	12
Phoenix roebelenii	5	3	5	13
Rhapis Laosensis	4	1	3	8
Licula spinosa	3	2	5	10
Livistona chinensis	1	2	3	6
Mascarena Lagencuulis	2	3	4	9
Total	31	25	49	105

4.1.3 Identification of Endophytic Fungi

4.1.3.1 Morphological Identification

The 105 isolates obtained from palm tissues in this study yielded 15 identified taxa (Table. 4.2). These identifiable cultures represented 60 (57.14%) of the total isolates. The remaining 45(42.86%) isolates did not sporulate and were grouped into Mycelia Sterilia fungi (MSF). The morphology characters of 15 identified endophytic fungi are as follows:-

1. *Cladosporium* spp.

Cladosporium spp. species were isolated as endophytic fungi from *Ptychosperma macarthuri* and *Wodyetia bifurcate*. The colonies range from olive or deep green to black color in PDA media. They are relatively slow-growing. Mycelium is immersed and superficial.

Conidiophores macronematous and micronematous, sometimes up to 350µm long but generally much shorter, 2-6 µm thick, phal to mid olivaceous brown, smooth or verruculose. Conidia formed in long branched chains, mostly 0-septate, ellipsoidal or limoniform, pale olivaceous brown, most commonly smooth but verruculose in some strains (Fig. 4.11).

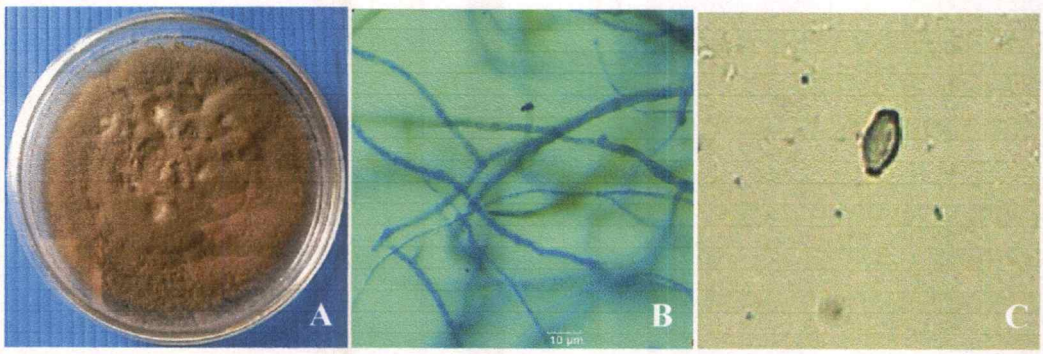


Fig. 4.11: *Cladosporium* spp. **A**: The colony culture on PDA 30 days **B:** mycelia (40X) C: Spore (40X).

2. *Phialophora* spp.

Phialophora spp. species were isolated from *Phoenix roebelenii* as endophytics. Colonies are slow-growing and white color with purple. Mycelium is partly superficial, partly immersed. No have Stroma. Setae and hyphopodia absent. Conidiogenesis is phialidic; phialides arising solitarily from vegetative hyphae or on branched conidiophores. Conidia aggregated in slimy heads, one-celled, straight or curved, ellipsoidal or oblong rounded at the ends, courless, smooth, 0-septate (Fig. 4.12).

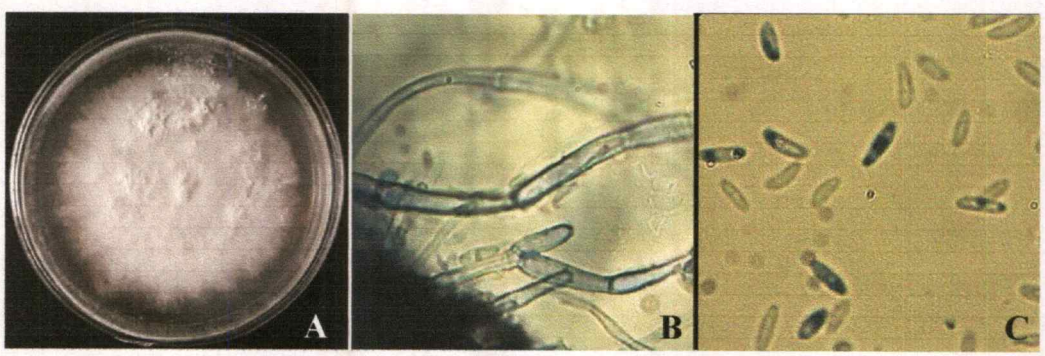


Fig. 4.12: *Phialophora* spp. **A**: The colony culture on PDA 30 days **B:** mycelia (40X) C: Spores (40X).

3. *Pestalotiopsis* sp1.

Pestalotiopsis sp1 was isolated from *Rhapis humilis* as endophytic fungi. Colonies grow fast with white yellow color. Acervulus is dark color and discoid or cushion-shaped, subepidermal. Conidiophores are short, simple; conidia dark, several-celled, with hyaline, pointed end cells, ellipsoid to fusoid, with two or more hyaline, apical appendages (Fig. 4.13).

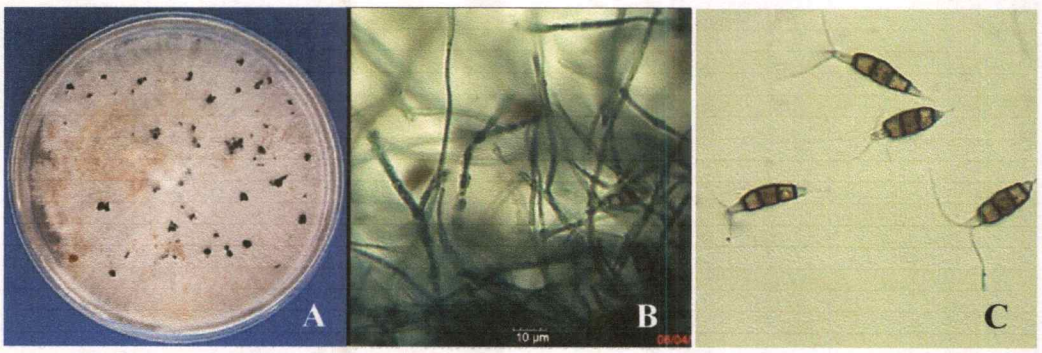


Fig. 4.13: *Pestalotiopsis* sp.1 **A:** The colony culture on PDA 30 days **B:** mycelia (40X) C: Spores (40X).

Pestalotiopsis sp2 was isolated as endophytic fungi from *Wodyetia bifurcate.* Colonies are slow-growing with white and grey felty aerial mycelium. Pycnidia brown to pale, in spots, erumpent to subsuperficial, globose to flattened, with small ostiole. Conidia hyaline, 2-celled, cylindrical, with 3 to 4 hyaline setate at one end (Fig. 4.14).

Fig. 4.14: *Pestalotiopsis* sp.2 **A:** The colony culture on PDA 30 days **B:** mycelia (40X) C: Spores (40X).

4. *Phoma* spp.

Phoma spp. species were isolated from *Rhapis humilis*, *Wodyetia bifurcate*, *Chrysalidocarpus lotescens*, *Veitchia merrillii* and *Phoenix roebelenii* as endophytic fungi. Colonies are fast growing on PDA with abundant floccose, whitish to olivaceous-grey aerial

mycelium; reverse uncoloured. Pycnidia abundantly produced in the centre of the colonies, olivaceous-brown, dark around the ostiole. Pseudosclerotia is absent; conidia oblong and two-celled (Fig. 4.15).

Fig. 4.15: *Phoma* spp. **A**: The colony culture on PDA 30 days **B:** mycelia (40X) C: Spores (40X).

5. *Nigrospora* spp.

Nigrospora spp. species were isolated as endophytic fungi from *Wodyetia bifurcate* and *Mascarena Lagencuulis*. The white woolly colonies grow fairly rapidly. At first, Colonies white with small, shining black conidia easily visible under a lower-power dissecting microscope, later brown when sporulation is abundant. Mycelium is all immersed or partly superficial. No have stroma. Setae and hyphopodia are absent. Conidiophores semi-macronematous, branched, flexuous, colourless to brown, smooth. Conidia solitary, with a violent discharge mechanism, acrogenous, simple, spherical or ellipsoidal, compressed dorsiventrally. Conidia are black, shining, smooth and 0-septate (Fig. 4.16).

Fig. 4.16: *Nigrospora* spp. **A**: The colony culture on PDA 30 days **B:** mycelia (40X) C: Spores (40X).

6. *Xylaria* spp.

Xylaria spp. species were isolated as endophytic fungi from *Wodyetia bifurcate*, *Veitchia merrillii* and *Phoenix roebelenii*. The colonies growing quite slow on PDA medium. Mycelium is white color. The stromata are finger-like. Ascocarps (fruitbodies) are black at the base but white and branched towards the top, didn't produce ascospore on PDA medium (Fig. 4.17).

Fig. 4.17: *Xylaria* spp. **A,B**: The colony culture on PDA **C:** mycelia (40X).

7. *Fusarium* sp1.

Fusarium sp1 species were isolated as endophytic fungi from *Chrysalidocarpus lotescens*. The colonies fast growing and moist, floccose, granulose, cream color. Aerial mycelium is floccose and whitish. Macro-conidia abundant and more-celled, slightly curved or bent at the pointed ends, typically canoe-shaped. Microconidia are 1-celled, ovoid or oblong. Chlamydospores often sparse, globose, intercalary, pale, formed singaly or in chains (Fig. 4.18).

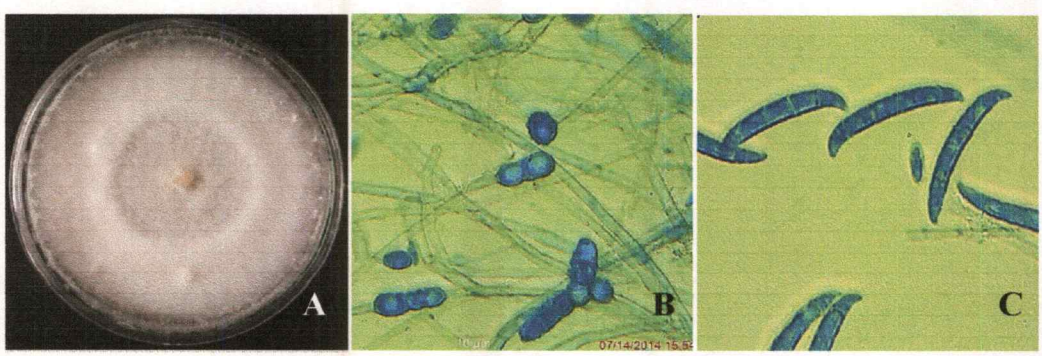


Fig. 4.18: *Fusarium* sp1. **A**: The colony culture on PDA 30 days **B:** mycelia and chlamydospores(40X) C: Macro-conidia and micro-conidia (40X).

***Fusarium* sp2.**

Fusarium sp2 species were isolated as endophytic fungi from *Rhapis Laosensis*, *Mascarena Lagencuulis*, *Livistona chinensis*, *Licula spinosa*, *Ptychosperma macarthuri* and *Rhapis humilis*. The colony is fast growing with discrete sporodochia and white-ochraceous colour. Aerial mycelium is floccose. Macro-conidia abundant and more-celled, slightly curved or bent at the pointed ends; central part straight, cylindrical, typically canoe-shaped. Phialides bearing micro-conidia very long (Fig. 4.19).

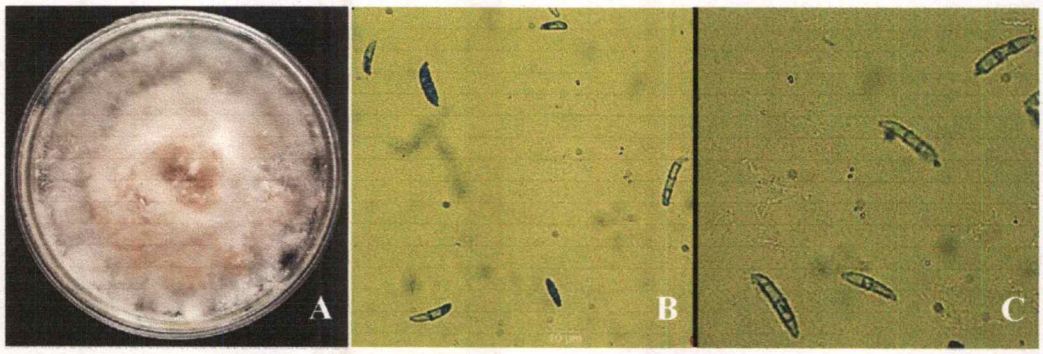


Fig. 4.19: *Fusarium* sp2. **A**: The colony culture on PDA 30 days **B,** C: macro-conidia and micro-conidia (40X).

8. *Rhizoctonia* spp.

Rhizoctonia spp. species were isolated as endophytic fungi from *Wodyetia bifurcate*, *Phoenix roebelenii*, *Licula spinosa* and *Livistona chinensis*. Colonies grow quite fast on agar surface. The mycelium is white at first and later becoming black. The culture didn't produce spores, but are composed of hyphae and sclerotia (hyphal propagules). The cells of mycelium long, septa of branches set off from the main hyphae (Fig. 4.20).

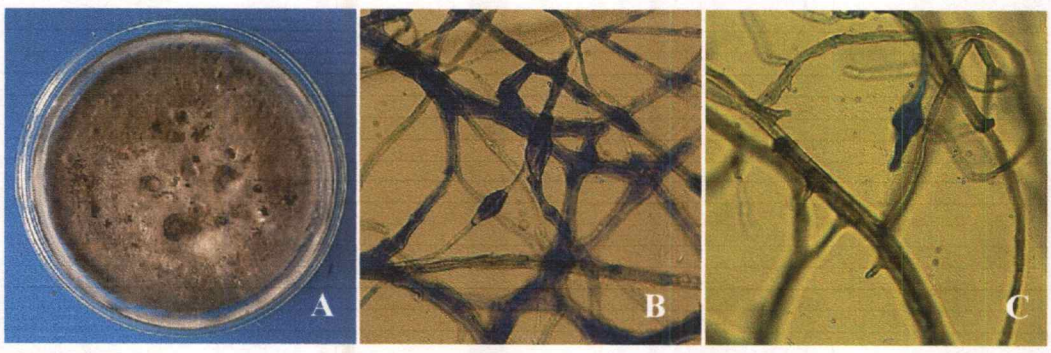


Fig. 4.20: *Rhizoctonia* spp. **A**: The colony culture on PDA 30 days **B,C:** mycelia (40X).

9. *Colletotrichum* spp.

Colletotrichum spp. species were isolated as endophytic fungi from *Ptychosperma macarthuri*, *Veitchia merrillii*, *Licula spinosa* and *Mascarena Lagencuulis*. The colony growth is slow, and mycelia initially white-grey and then become black-brown. Acervulus is disc-shaped, subepiidermal, typically with dark, spines at the edge or among the conidiophores. Conidia are 1-celled and ovoid or oblong (Fig. 4.21).

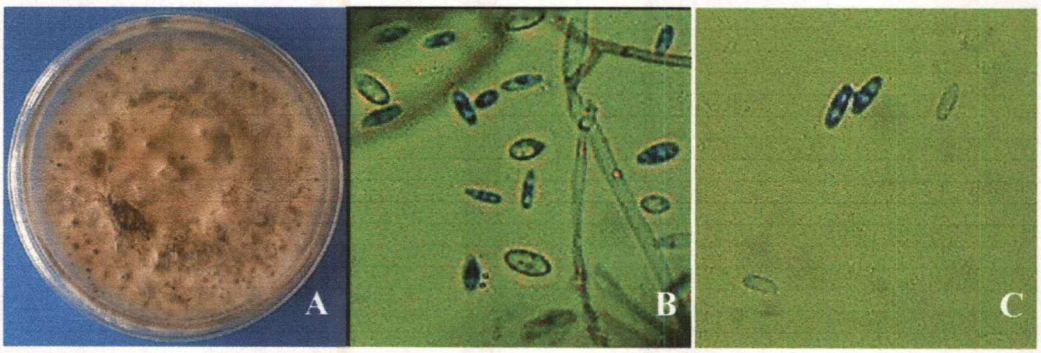


Fig. 4.21: *Colletotrichum* spp. **A**: The colony culture on PDA 30 days **B:** mycelia (40X) C: Spores (40X).

10. *Chaetomium globosum*

Chaetomium globosum was isolated as endophytic fungi from *Rhapis Laosensis.* Colonies are slow growing with little superficial mycelium and a dense olivaceous layer os ascomata. Phialoconidia are absent, ascomata dark brown or black colors, globose to subglobose; lateral hairs dark brown with paler tips, minutely roughened; terminal hairs dark olive brown with paler tips, wavy or loosely coiled and interwined. Spores pale greenish to dark olive-brown, flattened lemon-shaped, hardly apiculate (Fig. 4.22).

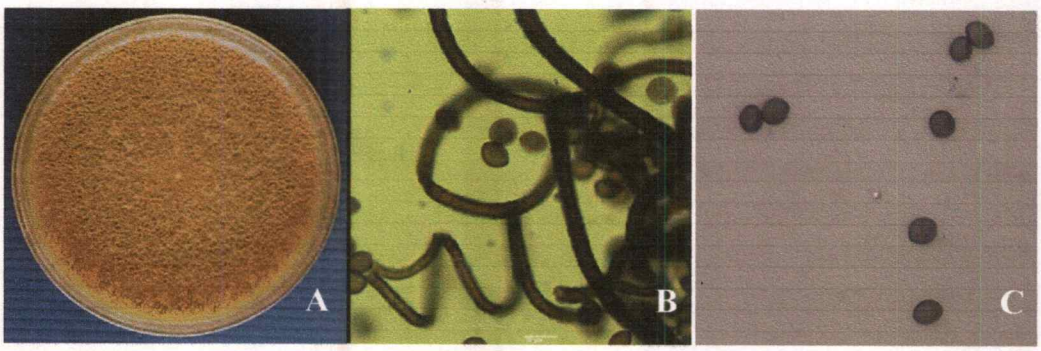


Fig. 4.22: *Chaetomium globosum* **A**: The colony culture on PDA 30 days **B,** C: Spores (40X).

11. *Chaetomium aureum*

Chaetomium aureum was isolated as endophytic fungi from *Livistona chinensis*. Colonies are slow growing with immersed mycelium and little superficial mycelium, red and white colors. Phialoconidia absent; ascomata red, globose; lateral hairs dark brown with paler tips, minutely roughened; terminal hairs dark olive brown with paler tips and interwined. Ascospores halfmoon-shaped with two apical germ pores and brown colour (Fig. 4.23).

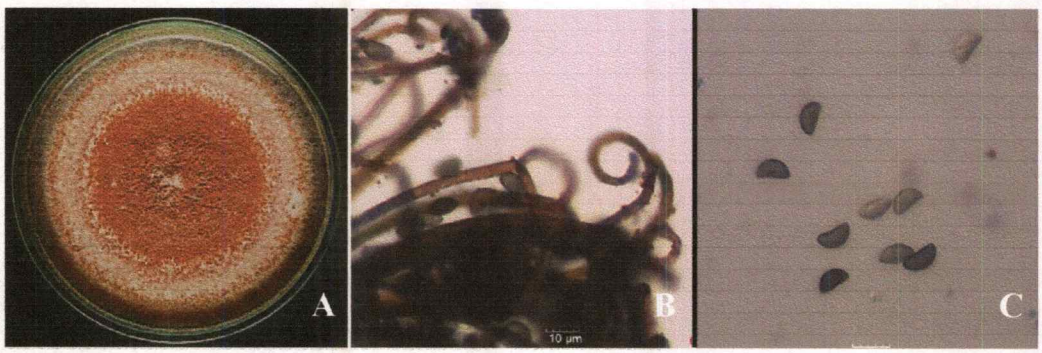


Fig. 4.23: *Chaetomium aureum* **A**: The colony culture on PDA 30 days **B:** Hairs (40X) C: Spores (40X).

12. *Gibberella* spp.

Gibberella spp. was isolated as endophytic fungi from *Livistona chinensis*. Colonies are slow-growing and white colours. The ascocarp is globose with smooth walls. Ascomata abundantly. The asci are globose or ovoid-shaped, lenticular, valves smooth or slightly roughened, with a broad equatorial furrow, with very low ridges but no wings (Fig. 4.24).

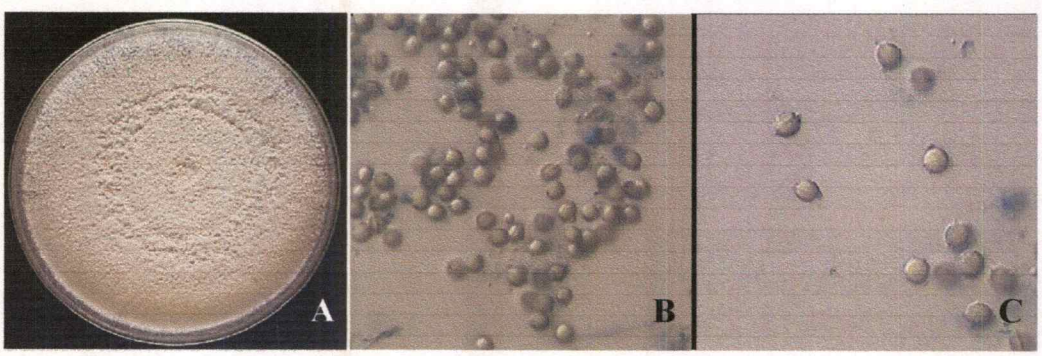


Fig. 4.24: *Giberrella* spp. **A**: The colony culture on PDA 30 days **B,**C: Spores (40X).

13. *Emericella* spp.

Emericella spp. was isolated as endophytic fungi from *Mascarena Lagencuulis.* Colonies are growing restrictedly, with few green conidial heads and abundant purple-brown ascomata formed in several layers. Ascomata surrounded by dark brown, globose hulle cells. Ascospores purple-red, lenticular, rugulose, with two sinuate equatorial crests (Fig. 4.25).

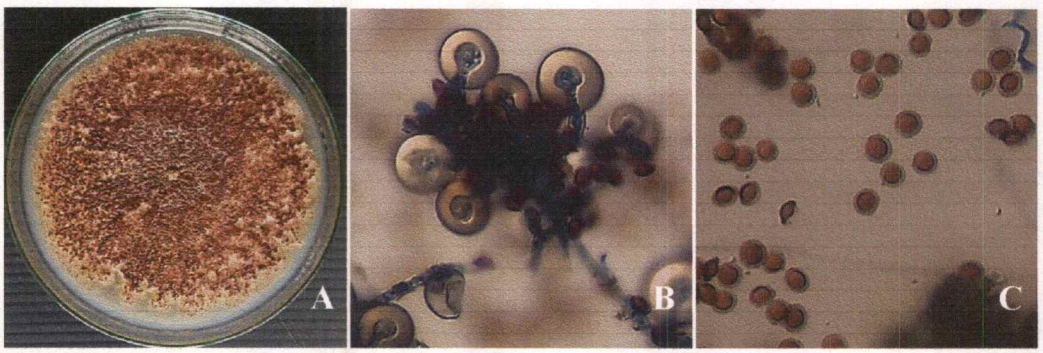


Fig. 4.25: *Emericella* spp. **A**: The colony culture on PDA 30 days **B:** ascospores (40X) C: Spores (40X).

14. *Aspergillas* spp.

Aspergillas spp. species were isolated as endophytic fungi from *Wodyetia bifurcate*; and *Veitchia merrillii.* Colonies are growing rather slow on PDA with creamy-yellow color. Mycelium partly immersed, partly superficial. Stroma none; Setae and hyphopodia absent. Vesicles small, variable in shape. Conidial heads globose and bright yellow. Conidia globose to subglobose, smooth-walled, uninucleate, the chains sometimes sliming down (Fig. 4.26).

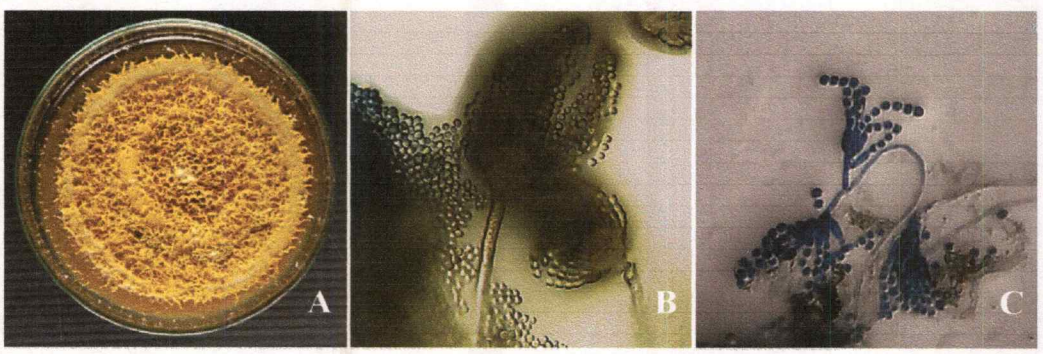


Fig. 4.26: *Aspergillas* spp. **A**: The colony culture on PDA 30 days **B:** conidial head (40X) C: Spores (40X).

15. *Curvalaria lunata*

Curvalaria lunata species were isolated as endophytic fungi from *Phoenix roebelenii.* Colonies are effuse, hairy and black color on PDA medium. Mycelium immersed in natural substrata. Conidia with hilum scarcely, remaining smooth-walled, dark brown color. Conidia predominantly 3-septate, the middle septum below the centre and the third cell strongly curved, tapering gradually towards the base (Fig. 4.27).

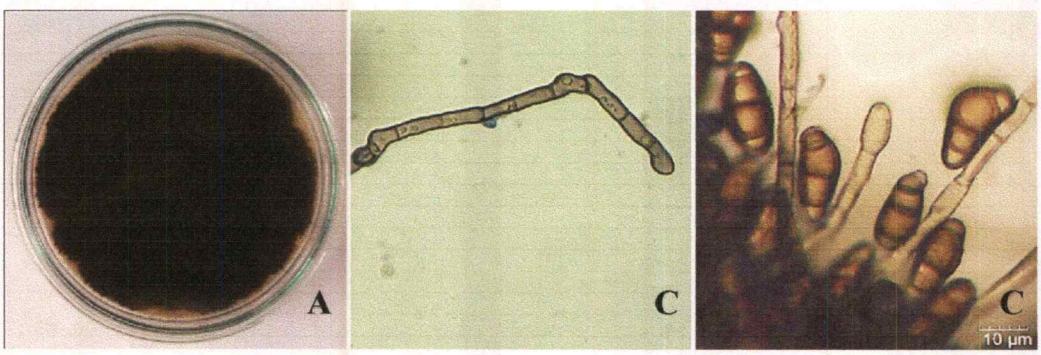


Fig. 4.27: *Curvalaria lunata* A: The colony culture on PDA 30 days **B:** mycelia (40X) C: Spores (40X).

Table. 4.2 Taxon of endophytic isolates.

Taxon	No.	Class and order	Family and Genus	Reference
Cladosporium spp.	3	Dothideomycetes; Capnodiales	Davidiellaceae; *Cladosporium*	Rosa *et al.*, 2012; Chobba *et al.* 2013
Phialophora spp.	4	Eurotiomycetes Chaetothyriales	Herpotrichiellaceae *Phialophora*	Rakotoniriana *et al.*, 2007
Pestalotiopsis spp.	4	Sordariomycetes Xylariales	Amphisphaeriaceae *Pestalotiopsis*	Frohlich, 1999
Phoma spp.	5	Dothideomycetes Pleosporales	Incertae sedis *Phoma*	Rosa *et al.*, 2012
Nigrospora spp	5	Sordariomycetes; Trichosphaeriales	Trichosphaeriaceae; *Nigrospora*	Cannon *et al.*, 2002
Xylaria spp.	9	Sordariomycetes; Xylariales	Xylariaceae; *Xylaria*	Nath *et al.*, 2012; Rakotoniriana *et al.*, 2007;
Fusarium spp.	13	Sordariomycetes Hypocreales	Nectriaceae *Fusarium*	Rosa *et al.*, 2012; Qadri *et al.*, 2013; Chobba *et al.*, 2013;
Rhizoctonia spp.	3	Agaricomycetes; Cantharellales	Ceratobasidiaceae; *Rhizoctonia*	Harvais and Hadley, 1967
Colletotrichum spp.	6	Sordariomycetes Glomerellales	Glomerellaceae *Colletotrichum*	Rosa *et al.*, 2012; Rakotoniriana *et al.*, 2007
Chaetomium globosum	1	Sordariomycetes Sordariales	Chaetomiaceae *Chaetomium*	Lu *et al.*, 2012
Chaetomium aureum	1	Sordariomycetes Sordariales	Chaetomiaceae *Chaetomium*	Pablo *et al.*, 2015;
Giberrella spp.	1	Sordariomycetes Hypocreales	Nectriaceae *Giberrella*	Qadri *et al.*, 2013
Emericella spp.	1	Eurotiomycetes Eurotiales	Trichocomaceae *Emericella*	Zhang, *et al.*, 2011
Aspergillas spp.	1	Eurotiomycetes Eurotiales	Trichocomaceae *Aspergillas*	Amin *et al.*, 2014;
Curvalaria lunata	3	Euascomycetes Pleosporales	Pleosporaceae *Curvalaria*	Chobba *et al.*, 2013; Amin *et al.*, 2014

4.1.3.2 Molecular Identification

Endophytic isolates 17-6 (*Nigrospora* spp.); 5r-1 (*Fusarium* spp.); 4r-3 (*Pestalotiopsis spp.*); 22s-2 (*Giberrella* spp.); 7s-1 (*Chaetomium globosum*) were selected to molecular identification. Genomic DNA of these isolates were extracted using the standard method of Ausubel *et al.* (1994). Fungal isolates were identified by sequencing the internal transcribed region (ITS) of rDNA using universal primers: ITS-1; 5′ -TCC GTA GGT GAA CCT GCG G-3′ and ITS-4; 5′ -TCC TCC GCT TAT TGATAT GC-3′ . The amplified products (5µl) were visualize on 1% (w/v) agarose gel to confirm the presence of a single amplified band. Visible, amplified bands at about 600-800 bp (Figure 4.28), the relative molecular mass fragment size is consistent with the intended purpose.

Furthermore, sequences were subjected to a preliminary BLAST analysis in NCBI databases, and sequence matches were used to determine their identities (Table. 4.3). Multiple sequence alignment was then carried out using ClustalW and phylogenetic tree was constructed using MEGA6.0 by neighbor-joining method. Phylogenetic analysis confirmed the relationships between endophytic fungal isolates 4r-3, 5r-1, 7s-1, 17-6, 22s-2 and related taxa (Fig. 4.29).

According to identification, which based on morphology and molecular phylogeny. The BLAST analysis showed that the nucleotide sequences of 17-6 (*Nigrospora* spp.), the analysis showed that its nucleotide sequences shared 99% identity with those of *Nigrospora sphaerica* accession numbers HQ608063, HQ608062 and KM893076. The fungal isolate 17-6 (*Nigrospora* spp.) was identified as *Nigrospora sphaerica*. For the isolate 5r-1 (*Fusarium* spp.), its nucleotide sequences shared 99% identity with those of *Fusarium falciforme* accession numbers JX624109 and KF255424. Thus, the isolate 5r-1 (*Fusarium* spp.) was identified as *Fusarium falciforme*, based on its morphology and the molecular analysis. The nucleotide sequences of isolates 4r-3 (*Pestalotiopsis* spp.) shared 92% identity with *Pestalotiopsis maculformans* accession number EU552146 and 91% identity with *Pestalotiopsis maculformans* accession number EU552147. Then, the fungal isolate 4r-3 (*Pestalotiopsis* spp.) was identified as *Pestalotiopsis maculformans*. The nucleotide sequences of isolates 22s-2 (*Giberrella* spp.) shared 99% identity with those of *Giberrella moniliformis* accession numbers EU567316, GQ916543 and EU828525. So, the fungal isolate 22s-2 (*Giberrella* spp.) was identified as *Giberrella*

moniliformis. The nucleotide sequences of isolates 7s-1 (*Chaetomium globosum*) shared 98% identity with those of *Chaetomium globosum* accession numbers KJ183693 and KT634076. The isolate 7s-1 (*Chaetomium globosum*) was identified as *Chaetomium globosum*, based on its morphology and the molecular analysis.

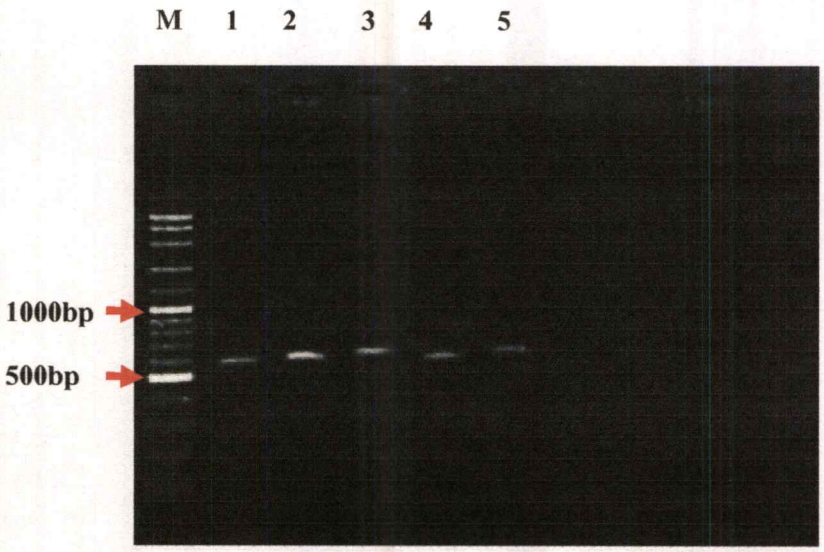


Fig. 4.28 Elecrophoresis results of rDNA-ITS sequnences bacterial recombinant PCR products.
M: marker; **1:** 17-6; **2:** 5r-1; **3:** 4r-3; **4:** 22s-2; **5:** 7s-1.

Table. 4.3 BLAST analysis of endophytic fungal isolates.

Isolates No.	Morphology identification	No. of bp analyzed	Close related species	GenBank accession number	Identity(%)
17-6	*Nigrospora* spp.	561	*Nigrospora sphaerica*	HQ608063 HQ608062 KM893076	99 99 99
5r-1	*Fusarium* spp.	518	*Fusarium falciforme*	JX624109 KF255424	99 99
4r-3	*Pestalotiopsis* spp.	585	*Pestalotiopsis maculformans*	EU552146 EU552147	92 91
7s-1	*Chaetomium globosum*	494	*Chaetomium globosum*	KJ183693 KT634076	98 98
22s-2	*Giberrella* spp.	504	*Giberrella moniliformis*	EU567316 GQ916543 EU828525	99 99 99

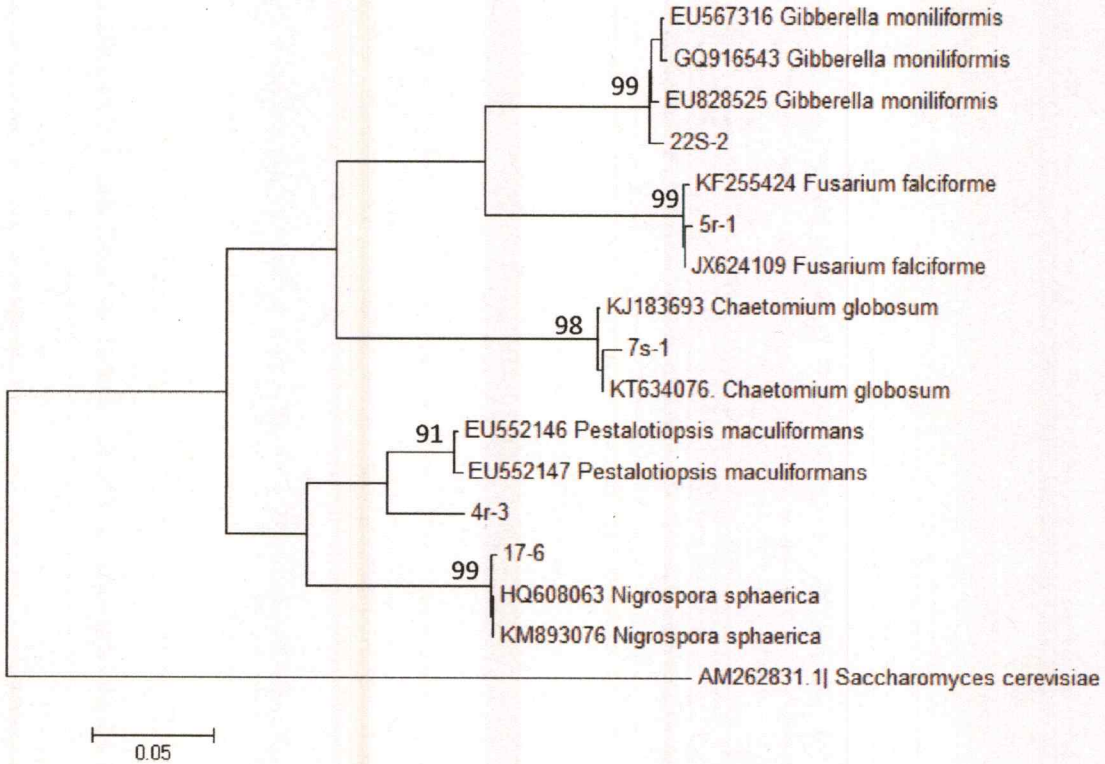


Fig. 4.29. Phylogenetic relationship between endophytic fungal isolates 4r-3, 5r-1, 7s-1, 17-6, 22s-2 and related taxa inferred using a neighbor joining method with internal transcribed spacer (ITS) rDNA sequence. Bootstrap value based on 1,000 replications is shown above the branch.

4.2 Bioactivity Test against Plant Pathogens (*Colletotrichum* spp.)

4.2.1 Isolation of Pathogens

Colletotrichum coffeanum was isolated from anthracnose of Arabica coffee leaves and identified from coffee leaves with obvious symptom of coffee anthracnose to pure culture (Fig. 4.30).

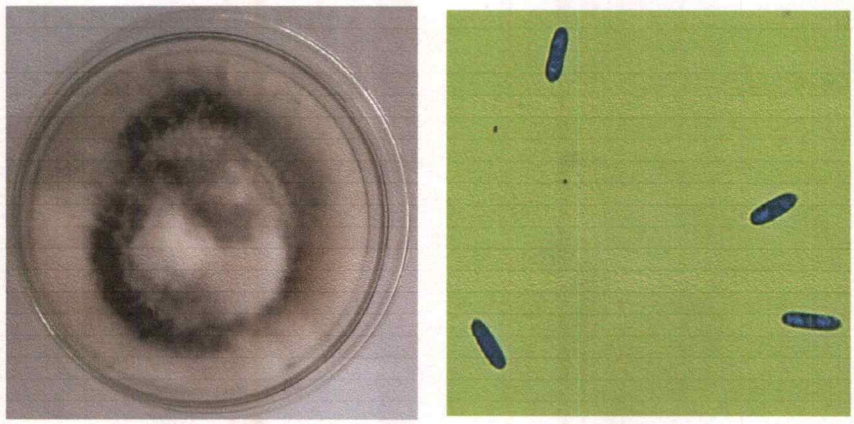


Fig. 4.30 *Colletotrichum coffeanum* pure culture and spores.

Colletotrichum capsici was isolated from anthracnose of chili fruit with obvious symptom and identified by morphological (Bills and Polishook, 1994) (Fig. 4.31).

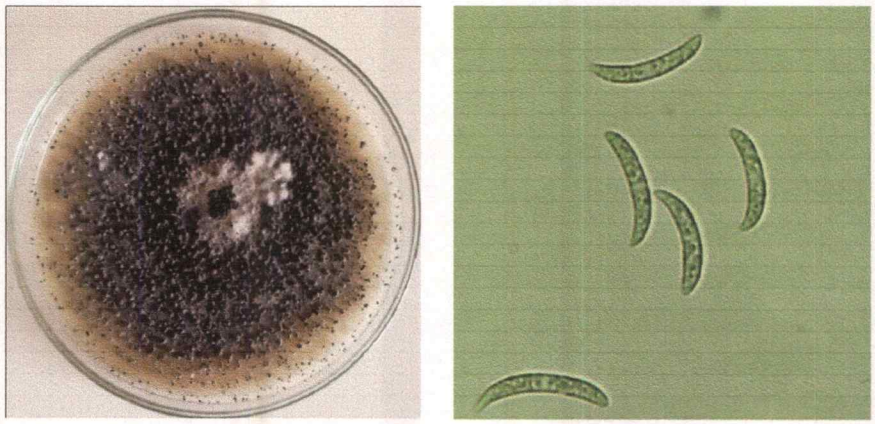
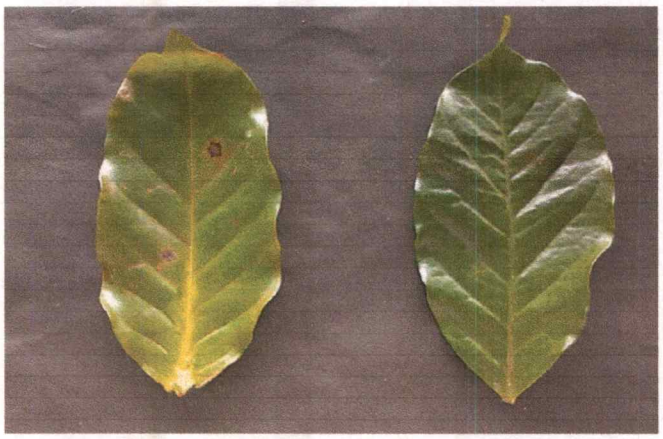


Fig. 4.31 *Colletotrichum capsici* pure culture and spores.

4.2.2 Pathogenicity Test

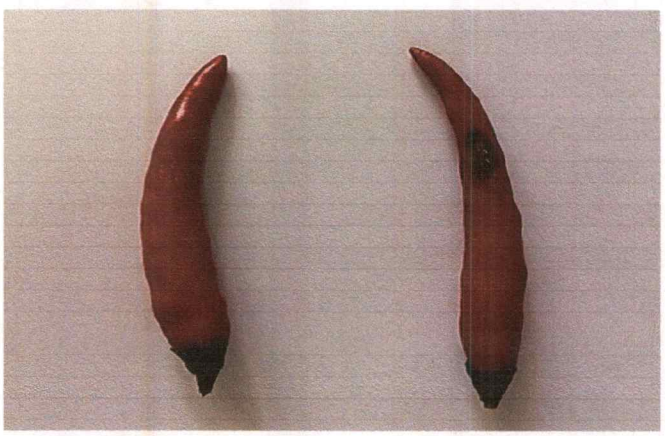
The isolate *Colletotrichum coffeanum* was confirmed pathogenic isolate from patyhogenecity test (Fig. 4.32). The result showed that pathogenic isolate could be infected in the coffee leaf and caused symtomp (around 1.5cm diameter) with the same symptom caused by *Colletotrichum coffeanum* causing leaf anthracnose on coffee leaves. Then, those leaves with symptom were used to re-isolated for ensure the pathogen and the result showed that the fungus that re-isolated from infected leaf was the same with the *Colletotrichum coffeanum* that inoculated at first time.



Inoculated one Non-inoculated control

Fig. 4.32. Pathogenicity test on coffee leaves.

The isolate *Colletotrichum capsici* was confirmed pathogenic isolate from pathogenecity test (Fig. 4.33). The result showed that pathogenic isolate could be infected in chili fruit and caused symptom (0.8 × 1.8) with the same symptom caused by *Colletotrichu capsici* causing fruit anthracnose on chili. Then, Those fruits with symptom also were used to re-isolated pathogen and the result showed that the fungus that re-isolated from infected fruit was same fungi with the *Colletotrichum capsici* that inoculated at first time.



Non-inoculated control Inoculated one

Fig. 4.33. Pathogenicity test on chili fruits.

4.2.3 Preliminary Screening of Bioactivities of Endophytic Fungi

4.2.3.1 Bi-culture Antagonistic Test against *Colletotrichum coffeanum*

Pestalotiopsis maculformans, Fusarium falciforme, Phialophora spp., *Nigrospora sphaerica* were proved their abilities to inhibit plant pathogen *Colletotrichum coffeanum* causing anthracnose of coffee by using bi-culture tests (Fig. 4.34).

Nigrospora sphaerica Vs *Colletotrichum coffeanum*

Pestalotiopsis maculformans Vs *Colletotrichum coffeanum*

Fusarium falciforme Vs *Colletotrichum coffeanum*

Phialophora spp. Vs *Colletotrichum coffeanum*

Antagonist plates Bi-culture plates Pathogen plates

Fig. 4.34 Bi-culture antagonistic tests against *Colletotrichum coffeanum*.

The results showed that all the endophytic fungi *Pestalotiopsis maculformans, Fusarium falciforme, Phialophora* spp., *Nigrospora sphaerica* gave significantly inhibition of *Colletotrichum coffeanum* which were 6.68cm, 5.89cm, 6.25cm, 5.66cm in colony diameter, respectively when compared to the control plate (Table 4.4). *Nigrospora sphaerica* showed higher inhibition percentage of colony diaemeter which was 37.02 % than *Fusarium falciforme* and *Phialophora* spp. which were 35.28 and 30.55%, respectively. But, *Pestalotiopsis maculformans* gave the lowest inhibition percentage of colony diameter which was 25.75% (Fig. 4.35).

Table. 4.4 Colony growth on antagonistic bi-culture tests.

Antagonist fungi	***Colletotrichum coffeanum***	
	Colony(cm)	% inhibition of colony
Control	9.00[a1/]	-
Nigrospora sphaerica	5.66[d]	37.02[a]
Fusarium falciforme	5.82[d]	35.28[a]
Pestalotiopsis maculformans	6.68[b]	25.75[c]
Phialophora spp.	6.25[c]	30.55[b]
CV%	1.77	4.57

1/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01

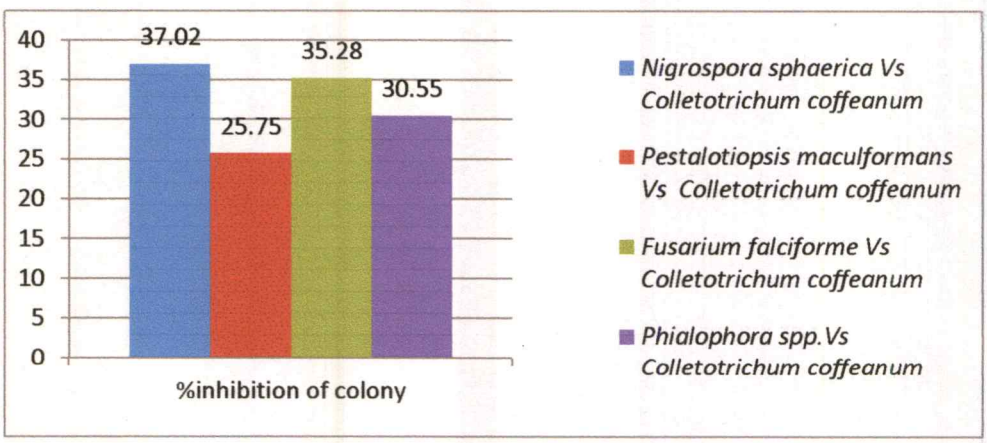


Fig. 4.35 Percentage inhibihion of colony diameter.

The number of spore producing of pathogens was counted by using Hemacytometer. The results showed that *Pestalotiopsis maculformans*, *Fusarium falciforme*, *Phialophora* spp., *Nigrospora sphaerica* significantly inhibited number of pathogen spores which were 0.87×10^6, 0.37×10^6, 1.12×10^6, 1.06×10^6 spores, respectively when compared to the control plate (Table 4.5). The pathogen spore production was inhibited 76.66, 89.99, 69.99 and 71.66%, respectively (Fig. 4.36). It is illustrated that all the tested endophytic fungi showed inhibition of spore production of *Colletotrichum coffeanum* and *Fusarium falciforme* gave the best inhibition of spore production.

Table. 4.5 Number of pathogen spores on antagonistic bi-culture tests.

Antagonist fungi	***Colletotrichum coffeanum***	
	Spores($\times10^6$)	%inhibition of number spores
Control	$3.75^{a2/}$	-
Nigrospora sphaerica	1.06^{b}	71.66^{ab}
Fusarium falciforme	0.37^{c}	89.99^{a}
Pestalotiopsis maculformans	0.87^{bc}	76.66^{ab}
Phialophora spp.	1.12^{b}	69.99^{b}
CV%	19.95	11.09

2/ Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01

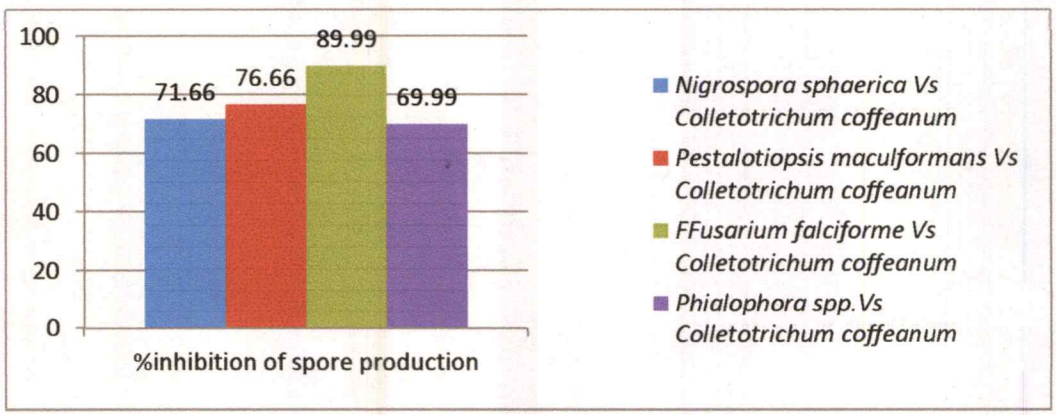


Fig. 4.36 Percentage inhibition of pathogen spore production.

Chaetomium globosum, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp. were also proved their abilities to inhibit plant pathogen *Colletotrichum coffeanum* causing anthracnose of coffee by using bi-culture tests (Fig. 4.37).

Fig. 4.37. Bi-culture antagonistic tests against *Colletotrichum coffeanum*.

The results showed that all the tested endophytic fungi (*Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp.) gave significantly inhibition of *Colletotrichum coffeanum* which were 6.15cm, 6.27, 5.91cm, 6.42cm in colony diameter, respectively when compared to the control plate (Table 4.6). *Giberrella moniliformis* showed higher inhibition percentage of colony diaemeter which was 34.27 % than *Chaetomium globosum*, *Chaetomium aureum* which were 31.38 and 30.27%, respectively. But, *Emericella* spp. gave the lowest inhibition percentage of colony diameter which was 28.61% (Fig. 4.38).

Table. 4.6 Colony growth on antagonistic bi-culture tests.

Antagonist fungi	***Colletotrichum coffeanum***	
	Colony(cm)	% inhibition of colony
Control	$9.00^{a1/}$	-
Chaetomium globosum	6.15^{c}	$31.38^{b1/}$
Chaetomium aureum	6.27^{bc}	30.27^{bc}
Giberrella moniliformis	5.91^{d}	34.30^{a}
Emericella spp.	6.42^{b}	28.61^{c}
CV%	1.83	4.92

1/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.05

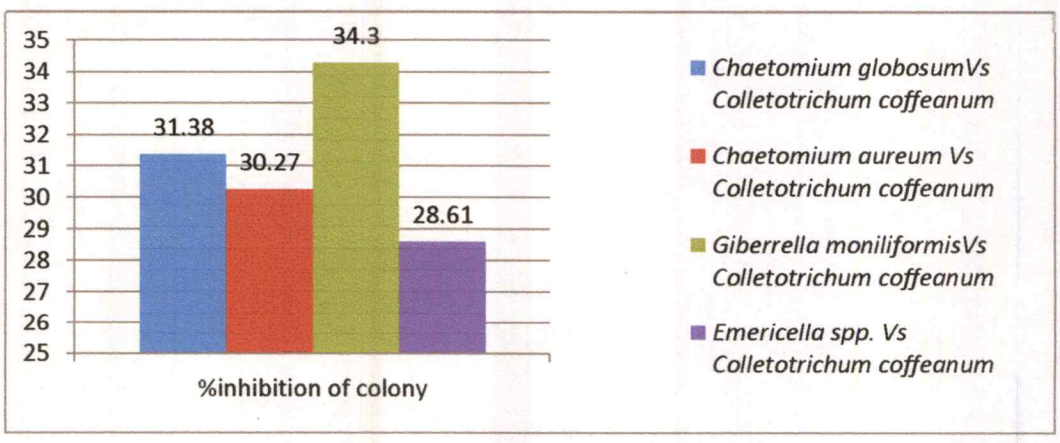


Fig. 4.38 Percentage inhibihion of colony diameter.

The number of spores that producing by the pathogen was counted by using Hemacytometer. The results showed that *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp. significantly inhibited the production of pathogen spores and the number of pathogen spores were 1.40×10^6, 1.21×10^6, 1.78×10^6, 2.43×10^6 spores, respectively when compared to the control plate (Table 4.7). The pathogen spore production was inhibited 73.56, 77.04, 66.70 and 53.08%, respectively (Fig. 4.39). It is illustrated that all the tested endophytic fungi showed inhibition of spore production of *Colletotrichum coffeanum* and *Chaetomium aureum* gave the best inhibition of pathogen spore production.

Table. 4.7 Number of pathogen spores on antagonistic bi-culture tests.

Antagonist fungi	***Colletotrichum coffeanum***	
	Spores($\times10^6$)	%inhibition of number spores
Control	5.15[a2/]	-
Chaetomium globosum	1.40[c]	73.56[a2/]
Chaetomium aureum	1.21[c]	77.04[a]
Giberrella moniliformis	1.78[bc]	66.70[a]
Emericella spp.	2.43[b]	53.08[b]
CV%	24.24	10.60

2/ Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.05

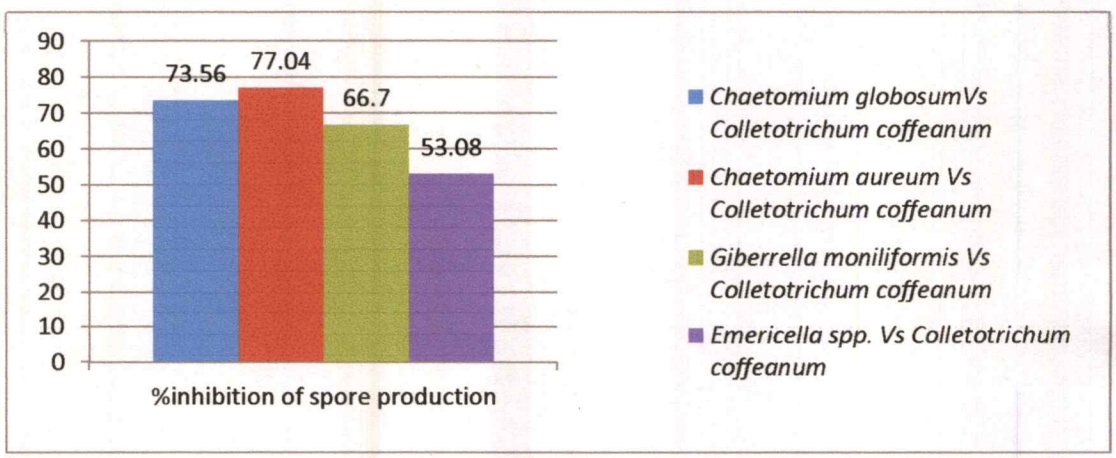


Fig. 4.39 Percentage inhibition of pathogen spore production.

4.2.3.2 Bi-culture Antagonistic Test against *Colletotrichum capsici*

Nigrospora sphaerica, Fusarium falciforme, Phialophora spp. *and Pestalotiopsis maculformans* were proved their abilities to inhibit plant pathogen *Colletotrichum capsici* causing anthracnose of chili by using bi-culture tests (Fig. 4.40).

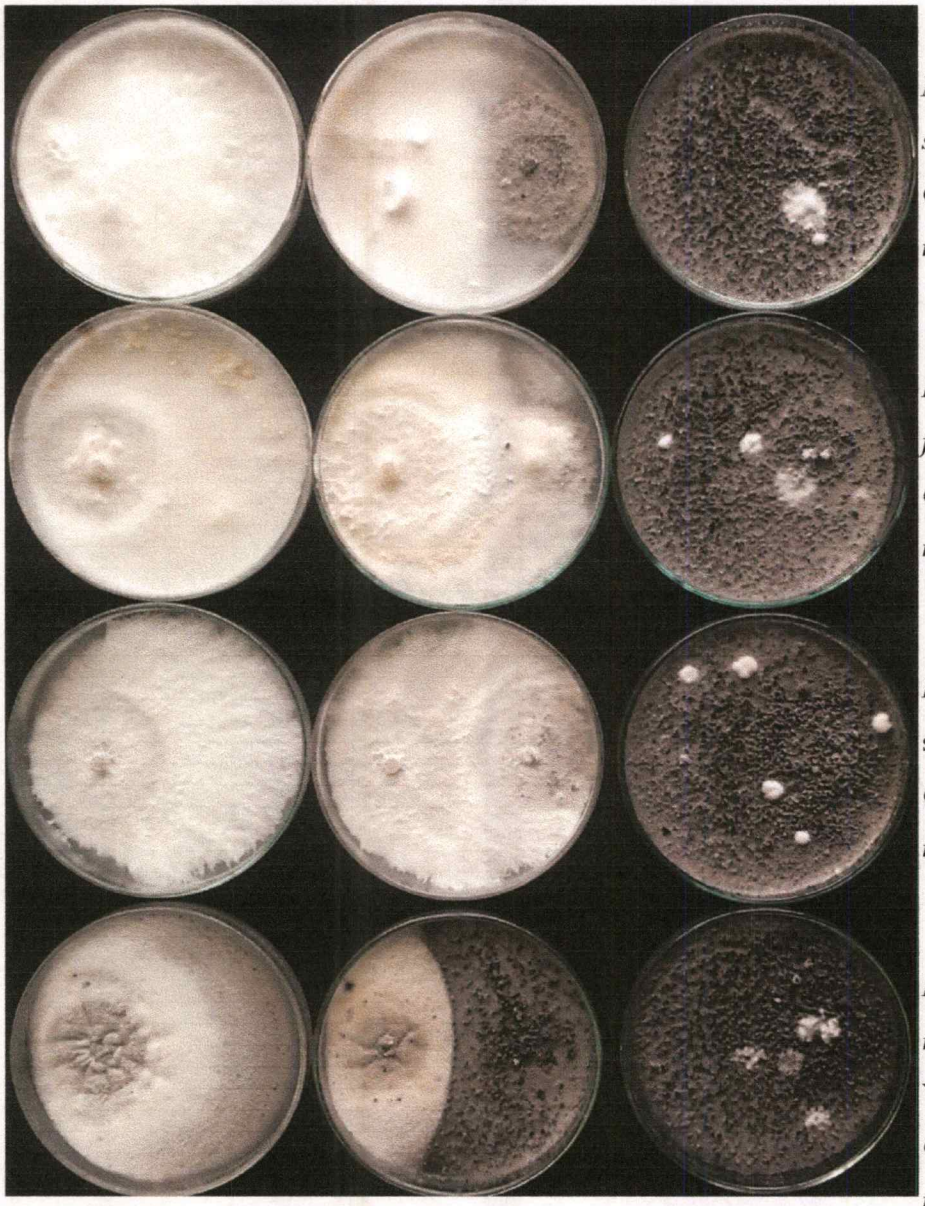


Fig. 4.40 Bi-culture antagonistic tests against *Colletotrichum capsici*.

The results showed that all the tested endophytic fungi (*Nigrospora sphaerica, Fusarium falciforme, Phialophora* spp. *and Pestalotiopsis maculformans*) gave significantly inhibition of *Colletotrichum capsici* which were 4.03, 3.82, 3.51, 6.22cm in colony diameter, respectively when compared to the control plate (Table 4.8). *Fusarium falciforme* showed higher inhibition percentage of colony diaemeter which was 60.97 % than *Nigrospora sphaerica, Phialophora* spp. which were 55.13 and 57.50%, respectively. But, *Pestalotiopsis maculformans* gave the lowest inhibition percentage of colony diameter which was 28.61% (Fig. 4.41).

Table. 4.8 Colony growth on antagonistic bi-culture tests.

Antagonist fungi	***Colletotrichum capsici***	
	Colony(cm)	% inhibition of colony
Control	9.00[a1/]	-
Nigrospora sphaerica	4.03[c]	55.13[b2/]
Fusarium falciforme	3.51[d]	60.97[c]
Pestalotiopsis maculformans	6.22[b]	30.83[b]
Phialophora spp.	3.82[cd]	57.50[ab]
CV%	4.47	5.78

1/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01

2/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.05

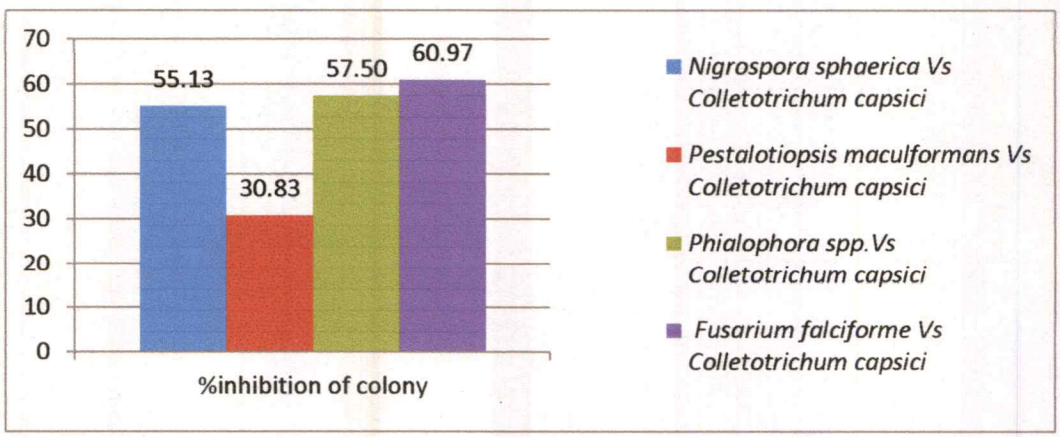


Fig. 4.41 Percentage inhibihion of colony diameter.

The number of spore producing of pathogens was counted by using Hemacytometer. The results showed that *Nigrospora sphaerica, Fusarium falciforme, Phialophora spp. and Pestalotiopsis maculformans* significantly inhibited the production of pathogen spores and the number of pathogen spores were 3.62×10^6, 2.50×10^6, 2.62×10^6, 9.50×10^6 spores, respectively when compared to the control plate (Table 4.9). The pathogen spore production was inhibited 67.58, 77.59, 76.28 and 13.61%, respectively (Fig. 4.42). It is illustrated that all the tested endophytic fungi showed inhibition of spore production of *Colletotrichum capsici* and *Fusarium falciforme* gave the best inhibition of spore production.

Table. 4.9 Number of pathogen spores on antagonistic bi-culture tests.

Antagonist fungi	***Colletotrichum capsici***	
	Spores($\times10^6$)	%inhibition of number spores
Control	11.00[a1/]	-
Nigrospora sphaerica	3.62[b]	67.58[b2/]
Fusarium falciforme	2.50[b]	77.59[a]
Phialophora spp.	9.50[a]	13.61[c]
Pestalotiopsis maculformans	2.62[b]	76.28[a]
CV%	18.91	11.09

1/ Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01

2/ Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.05

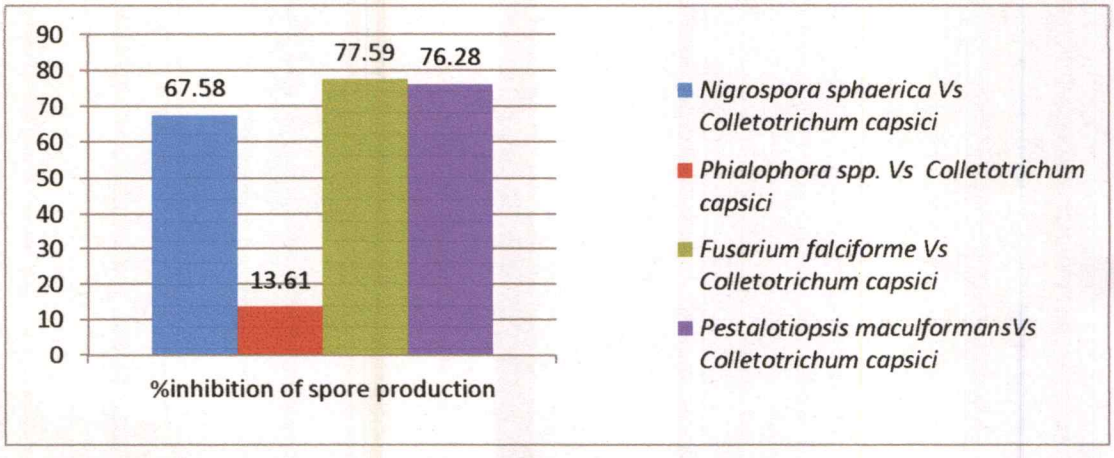


Fig. 4.42 Percentage inhibition of pathogen spore production.

Chaetomium globosum, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp. were also proved their abilities to inhibit plant pathogen *Colletotrichum capsici* causing anthracnose of coffee by using bi-culture tests (Fig. 4.43).

Fig. 4.43 Bi-culture antagonistic tests against *Colletotrichum capsici*.

The results showed that all the tested endophytic fungi (*Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp.) gave significantly inhibition of *Colletotrichum capsici* which were 4.93cm, 4.50, 5.35cm, 5.92cm in colony diameter, respectively when compared to the control plate (Table 4.10). *Chaetomium aureum* showed higher inhibition percentage of colony diaemeter which was 50.00 % than *Chaetomium globosum*, *Giberrella moniliformis* which were 45.16 and 40.55%, respectively. But, *Giberrella moniliformis* gave the lowest inhibition percentage of colony diameter which was 34.16% (Fig. 4.44).

Table. 4.10 Colony growth on antagonistic bi-culture tests.

Antagonist fungi	***Colletotrichum capsici***	
	Colony(cm)	% inhibition of colony
Control	9.00[a1/]	-
Chaetomium globosum	4.93[cd]	45.16[b2/]
Chaetomium aureum	4.50[d]	50.00[a]
Giberrella moniliformis	5.35[c]	40.55[c]
Emericella spp.	5.92[b]	34.16[d]
CV%	3.98	6.91

1/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01

2/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.05

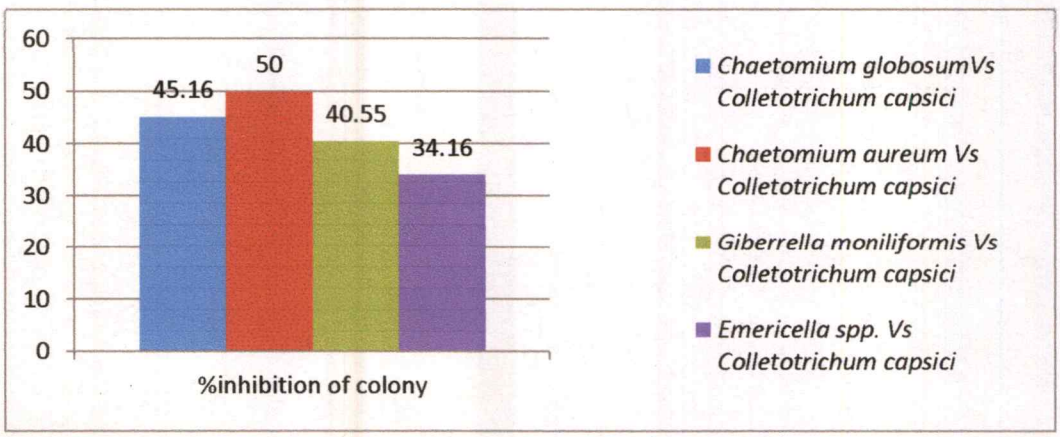


Fig. 4.44 Percentage inhibihion of colony diameter.

The number of spore producing by pathogens was counted by using Hemacytometer. The results showed that *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp. significantly inhibited the production of pathogen spores and the number of pathogen spores were 3.50×10^6, 3.18×10^6, 1.62×10^6, 4.25×10^6 spores, respectively when compared to the control plate (Table 4.11). The pathogen spore production was inhibited 49.77, 54.51, 77.35 and 40.00%, respectively (Fig. 4.45). It is illustrated that all the tested endophytic fungi showed inhibition of spore production of *Colletotrichum capsici* and *Giberrella moniliformis* gave the best inhibition of pathogen spore production.

Table. 4.11 Number of pathogen spores on antagonistic bi-culture tests.

Antagonist fungi	***Colletotrichum capsici***	
	Spores($\times10^6$)	%inhibition of number spores
Control	$7.00^{a1/}$	-
Chaetomium globosum	3.50^{b}	$49.77^{bc1/}$
Chaetomium aureum	3.18^{bc}	54.51^{b}
Giberrella moniliformis	1.62^{c}	77.35^{a}
Emericella spp.	4.25^{b}	40.00^{c}
CV%	21.24	10.85

1/ Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01

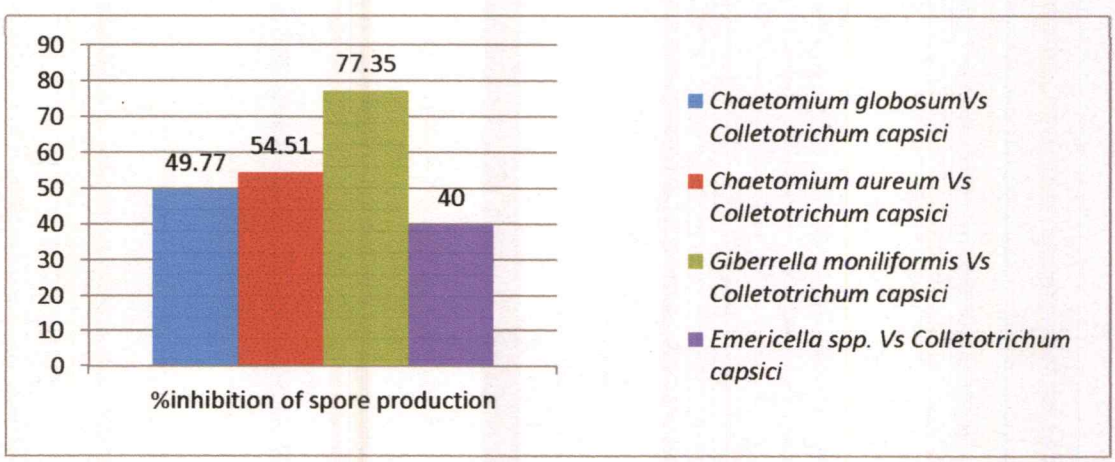


Fig. 4.45 Percentage inhibition of pathogen spore production.

4.2.4 Bioactive Substances Extraction

Nigrospora sphaerica, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* were extracted their bioactive substances as crude extracts and tested for their abilities to inhibit plant pathogens (*Colletotrichum coffeanum* and *Colletotrichum capsici*). The dried fungal biomass of endophytics were prepared and soaked in different solvents successively, including hexane, ethyl acetate (EtOAc) and methanol (MeOH). And the filtrates were evaporated by vacuum evaporator to yield crude extracts including crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH), respectively. The color and texture of crude Hexane, EtOAc and MeOH were quite different: cream, light brown, dark brown, red or red brown color; oil, wax or solid textures (Fig. 4.46).

The weight of crude Hexane, crude EtOAc and crude MeOH of each tested endophytic fungal also were collected and obtained the percentage of yield cude extracts (Table. 4.12). With this, the crude Hexane, crude EtOAc and crude MeOH of *Nigrospora sphaerica* yielded 0.37, 0.67 and 2.00%, respectively. The crude Hexane, crude EtOAc and crude MeOH from *Fusarium falciforme* yielded 1.74, 0.83 and 3.85%, respectively. The crude Hexane, crude EtOAc and crude MeOH from *Pestalotiopsis maculformans* yielded 1.18, 2.27 and 2.77%, respectively. The crude Hexane, crude EtOAc and crude MeOH from *Chaetomium globosum* yielded 0.52, 2.73 and 5.31%, respectively. The crude Hexane, crude EtOAc and crude MeOH from *Chaetomium aureum* yielded 1.00, 1.60 and 6.50%, respectively. The crude Hexane, crude EtOAc and crude MeOH from *Giberrella moniliformis* yielded 1.80, 3.05 and 2.94%, respectively. The obtained rate of crude MeOH ectracted from *Chaetomium aureum* and *Chaetomium globosum* are the highest yield (6.50, 5.31%) compared with *Nigrospora sphaerica* which was lowest obtained rate of crude extracts. Totally, the crude extrats from *Chaetomium aureum* had the highest obtained rate (9.10%) and followed by crude extrats from *Chaetomium globosum* and *Giberrella moniliformis*. Meanwhile, most crude hexane had the lowest obtained rate and crude MeOH had the highest obtained rate.

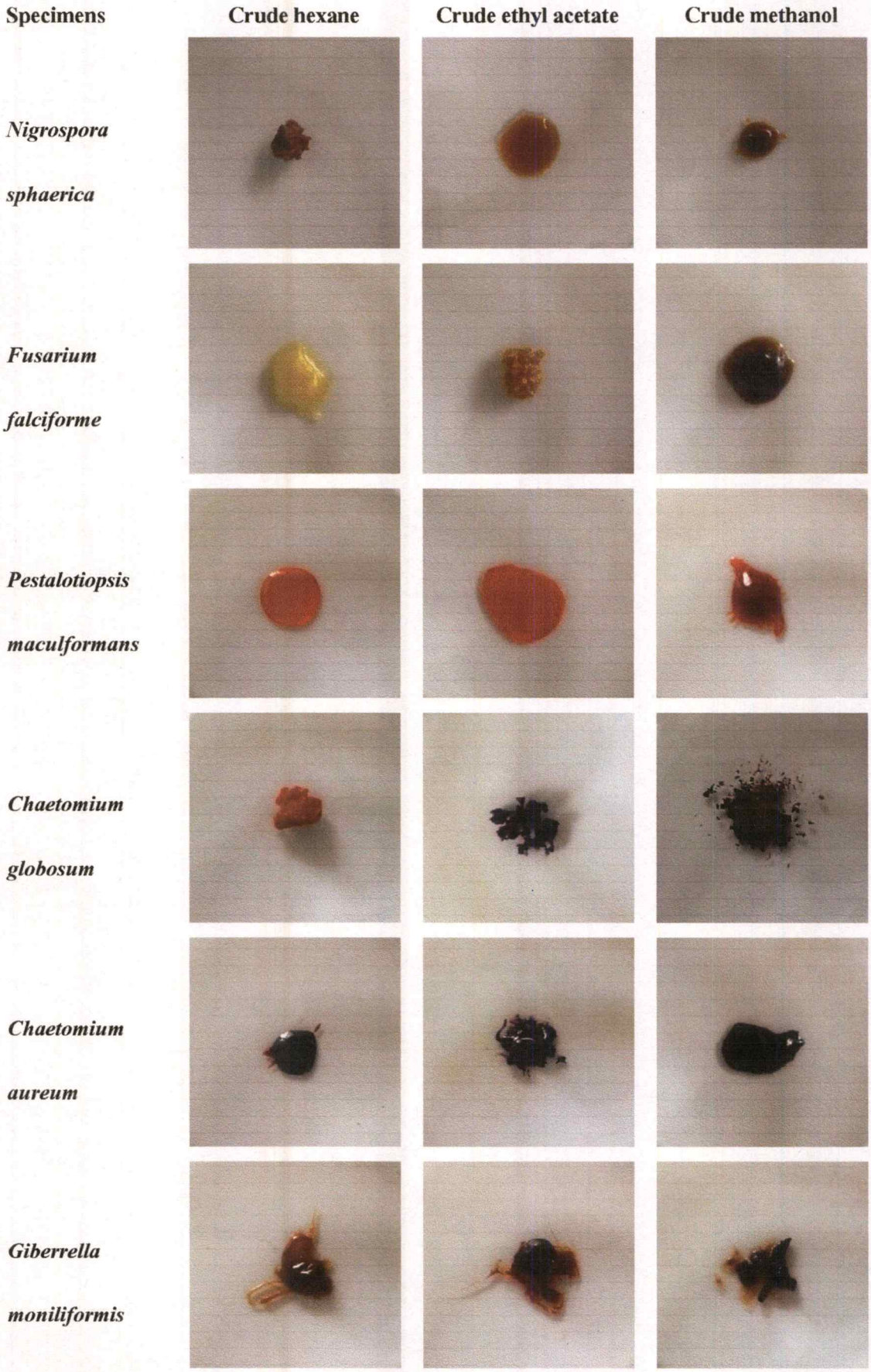


Fig. 4.46 The characteristics of each crude extract.

Table 4.12 Extraction of biological active substances.

Specimens	Fresh weight (g)	Dry weight (g)	Organic solvents			
			Crude Hexane(g)	Crude EtOAc(g)	Crude MeOH(g)	Total
Nigrospora sphaerica	2750	90	0.34 (0.37%)	0.60 (0.67%)	1.80 (2.00%)	2.74 (3.04%)
Fusarium falciforme	3670	250	4.36 (1.74%)	2.08 (0.83%)	9.62 (3.85%)	16.06 (6.42%)
Pestalotiopsis maculformans	5400	380	4.50 (1.18%)	8.65 (2.27%)	10.51 (2.77%)	23.66 (6.23%)
Chaetomium globosum	2200	150	0.78 (0.52%)	4.10 (2.73%)	7.96 (5.31%)	12.84 (8.56%)
Chaetomium aureum	2000	90	0.90 (1.00%)	1.44 (1.60%)	5.85 (6.50%)	8.19 (9.10%)
Giberrella moniliformis	2000	120	2.23 (1.80%)	3.67 (3.05%)	3.53 (2.94%)	9.43 (7.85%)

4.2.5 Testing Efficacy of Biological Active Substances (crude extracts) of Endophytic Fungi

The crude extracts from *Nigrospora sphaerica, Fusarium falciforme*, *Pestalotiopsis maculformans, Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* were used to test their ability to control the growth of plant pathogens (*Colletotrichum coffeanum* and *colletotrichum capsici*). Each crude extract was tested its inhibition against colony growth and spore production of pathogens with 6 concentritions (0, 10, 50, 100, 500, 1000ppm) at room temperature and the abnormal spores were also observed and compared with normal spores of pathogens (Figs. 4.47-4.60, Tables. 4.13-4.40).

4.2.5.1 Bioactivities Tests of Crude Extract against *Colletotrichum coffeanum*

The crude extracts from *Nigrospora sphaerica, Fusarium falciforme, Pestalotiopsis maculformans, Chaetomium globosum, Chaetomium aureum* and *Giberrella moniliformis* were selected to tested bioactivity against *Colletotrichum coffeanum* causing coffee anthracnose.

The results showed that crude methanol from *Nigrospora sphaerica* gave highest inhibition of *C. coffeanum* colony growth which was 54.00% at the concentration of 1,000 ppm when compared to the control (Table 4.13). Methanol crude extract from *Nigrospora sphaerica* showed significantly highest inhibition for the spore production of *C. coffeanum* as 72.18% at the concentration of 1,000 ppm, and the ED_{50} inhibited *C. coffeanum* spore production at concentration 143.65 ppm. Followed by crude ethyl acetate gave 69.99% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 404.53 ppm. Crude hexane showed 51.63% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 1092.44 ppm (Table 4.14).

Methanol crude extract from *Fusarium falciforme* gave significantly highest inhibition of *C. coffeanum* colony growth which was 63.00% at the concentration of 500 ppm when compared to the control (Table 4.15). Methanol crude extract from *Fusarium falciforme* showed significantly highest inhibition for the spore production of *C. coffeanum* as 76.74% at the concentration of 500 ppm, and the ED_{50} inhibited *C. coffeanum* spore production at concentration 161.14 ppm. Crude ethyl acetate gave 68.83% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 262.01 ppm; crude hexane showed 52.41% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 1054.88 ppm (Table 4.16).

Crude methanol from *Pestalotiopsis maculformans* gave significantly highest inhibition of 70.25% for the colony growth of *C. coffeanum* at concentration of 1,000 ppm when compared to the control, followed by crude ethyl acetate which inhibited 51.75% at concentration of 1000 ppm when compared to the control (Table 4.17). Methanol crude extract from *Pestalotiopsis maculformans* gave significantly highest inhibition for the spore production of *C. coffeanum* as 63.43% at concentration of 1000 ppm and the effective dose (ED_{50}) inhibited spore production of *C. coffeanum* at concentration of 231.21 ppm. Crude ethyl acetate gave 44.62% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 1460.24 ppm. Crude hexane showed 38.68% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of

4122.2 ppm (Table 4.18).

Methanol crude extract from *Chaetomium globosum* gave significantly highest inhibition of *C. coffeanum* colony growth which was 56.00% at the concentration of 1,000 ppm when compared to the control (Table 4.19). Methanol crude extract from *Chaetomium globosum* showed significantly highest inhibition for the spore production of *C. coffeanum* as 89.63% at the concentration of 1,000 ppm, and the ED_{50} inhibited *C. coffeanum* spore production at concentration 65.52 ppm. Followed by crude ethyl acetate gave 82.52% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 173.49 ppm; crude hexane showed 67.53% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 470.44 ppm (Table 4.20).

Hexane crude extract from *Chaetomium aureum* gave significantly highest inhibition of *C. coffeanum* colony growth which was 69.75% at the concentration of 1,000 ppm when compared to the control, followed by crude methanol which inhibited 64.50% at concentration of 50 ppm and 61.50% at concentration of 1000 ppm when compared to the control (Table 4.21). Methanol crude extract from *Chaetomium aureum* showed significantly highest inhibition for the spore production of *C. coffeanum* as 80.69% at the concentration of 50 ppm, and the ED_{50} inhibited *C. coffeanum* spore production at concentration 30.54 ppm. Crude ethyl acetate gave 75.83% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 126.84 ppm; crude hexane showed 66.23% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 420.24 ppm (Table 4.22).

Crude ethyl acetate from *Giberrella moniliformis* gave highest inhibition of *C. coffeanum* colony growth which was 59.00% at the concentration of 1,000 ppm when compared to the control (Table 4.23). Ethyl acetate crude extract from *Giberrella moniliformis* showed significantly highest inhibition for the spore production of *C. coffeanum* as 78.06% at the concentration of 1,000 ppm, and the ED_{50} inhibited *C. coffeanum* spore production at concentration 213.69 ppm. Followed by crude methanol gave 64.03% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 351.98 ppm. Crude hexane showed 47.61% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 2053.15 ppm (Table 4.24).

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

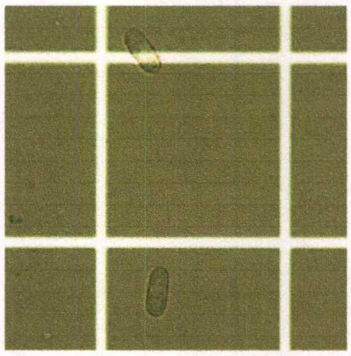
Crude Hexane

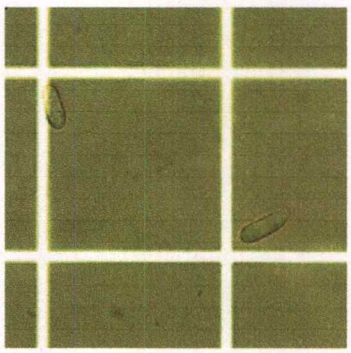
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude EtOAc

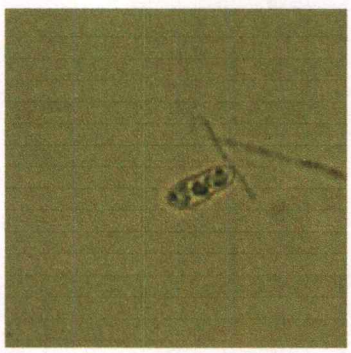
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

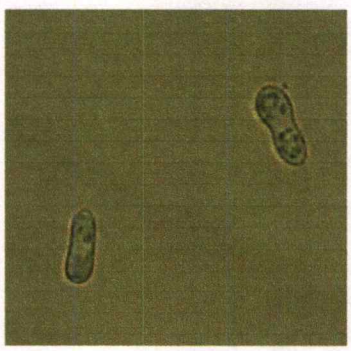
Crude MeOH





Normal Spores





Abnormal spores

Fig 4. 47 Crude extract test of *Nigrospora sphaerica* against *Colletotrichum coffeanum*.

Table 4.13 Crude extracts of *Nigrospora sphaerica* testing for growth inhibition of *Colletotrichum coffeanum* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	-
	10	4.97[a]	0.50[i]
	50	4.91[ab]	1.75[hi]
	100	4.77[cd]	4.50[fg]
	500	3.63[h]	27.25[b]
	1000	4.26[fg]	14.75[cd]
EtOAc	0	5.00[a]	-
	10	4.85[bc]	3.00[gh]
	50	4.75[d]	5.00[f]
	100	4.57[e]	8.50[e]
	500	4.33[f]	13.25[d]
	1000	4.19[g]	16.00[c]
MeOH	0	5.00[a]	-
	10	4.98[a]	0.25[i]
	50	4.87[b]	2.50[h]
	100	4.63[e]	7.25[e]
	500	3.58[h]	28.25[b]
	1000	2.29[i]	54.00[a]
C.V.(%)		1.35	10.65

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

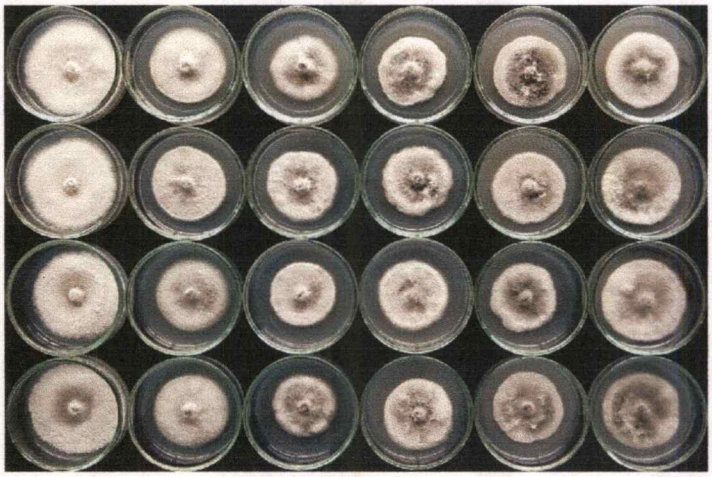
Table 4.14 Spore production inhibition of crude extracts from *Nigrospora sphaerica* to *Colletotrichum coffeanum* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	5.56 [a]	-	1092.44
	10	5.25 [ab]	5.42 [i]	
	50	4.62 [bc]	16.66 [g]	
	100	4.12 [cd]	25.46 [f]	
	500	3.25 [ef]	41.39 [d]	
	1000	2.68 [fg]	51.63 [c]	
EtOAc	0	5.56 [a]	-	404.53
	10	5.00 [ab]	10.05 [h]	
	50	4.06 [cd]	26.91 [f]	
	100	3.62 [de]	34.77 [e]	
	500	2.75 [f]	50.44 [c]	
	1000	1.68 [h]	69.99 [a]	
MeOH	0	5.56 [a]	-	143.65
	10	4.18 [cd]	24.53 [f]	
	50	3.68 [de]	33.58 [e]	
	100	2.68 [fg]	51.69 [c]	
	500	2.00 [gh]	64.02 [b]	
	1000	1.56 [h]	72.18 [a]	
C.V.(%)		9.73	7.98	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

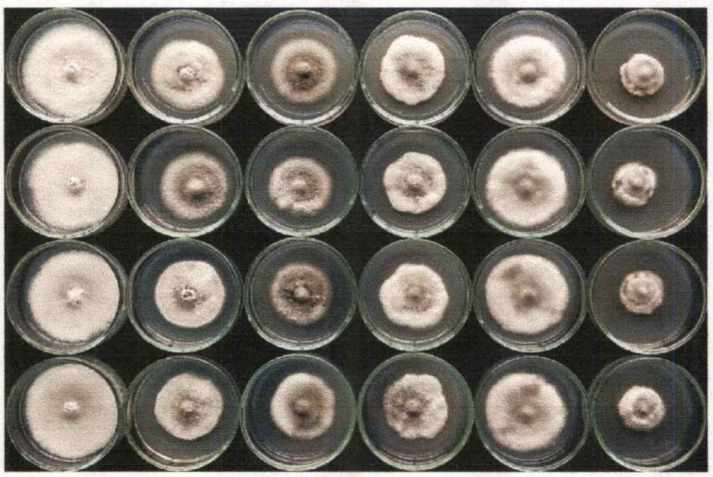
[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .



0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude Hexane



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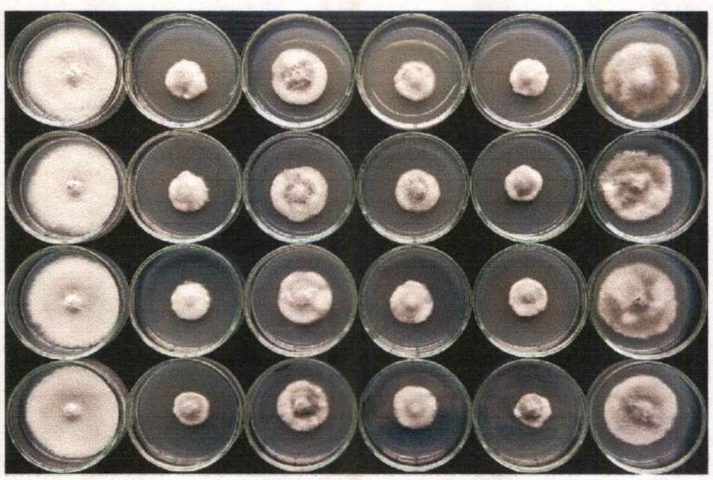
Crude EtOAc



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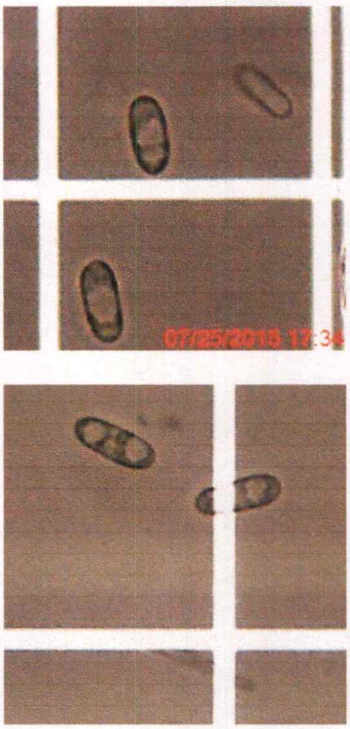
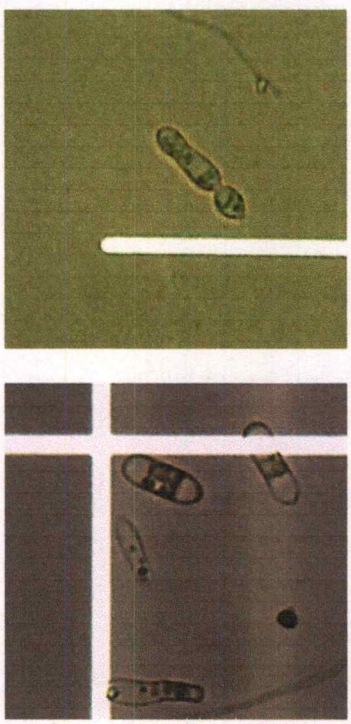
Crude MeOH



Normal Spores



Abnormal spores

Fig 4.48 Crude extract test of *Fusarium falciforme* against *Colletotrichum coffeanum*.

Table 4.15 Crude extracts of *Fusarium falciforme* testing for growth inhibition of *Colletotrichum coffeanum* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	-
	10	3.70[d]	26.00[g]
	50	3.45[ef]	31.00[ef]
	100	3.41[f]	31.75[e]
	500	3.44[ef]	31.00[ef]
	1000	3.87[c]	22.50[h]
EtOAc	0	5.00[a]	-
	10	3.48[ef]	30.25[ef]
	50	3.22[g]	35.50[d]
	100	3.21[g]	35.75[d]
	500	4.04[b]	19.00[i]
	1000	2.21[i]	55.75[b]
MeOH	0	5.00[a]	-
	10	1.89[j]	62.00[a]
	50	2.71[h]	45.75[c]
	100	2.19[i]	56.00[b]
	500	1.84[j]	63.00[a]
	1000	3.61[de]	27.75[fg]
C.V.(%)		2.43	4.75

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.16 Spore production inhibition of crude extracts from *Fusarium falciforme* to *Colletotrichum coffeanum* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	8.00^{a}	-	1054.88
	10	6.93^{ab}	13.15^{j}	
	50	6.68^{bc}	16.09^{j}	
	100	6.12^{bcd}	23.38^{i}	
	500	4.75^{efg}	40.85^{g}	
	1000	3.81^{gh}	52.41^{d}	
EtOAc	0	8.00^{a}	-	262.01
	10	5.75^{cde}	27.91^{hi}	
	50	5.37^{def}	32.51^{h}	
	100	4.31^{fg}	46.41^{ef}	
	500	4.00^{gh}	50.26^{de}	
	1000	2.50^{ij}	68.83^{b}	
MeOH	0	8.00^{a}	-	161.14
	10	4.93^{efg}	38.31^{g}	
	50	4.81^{efg}	39.96^{g}	
	100	3.12^{hi}	61.21^{c}	
	500	1.87^{j}	76.74^{a}	
	1000	4.62^{efg}	42.35^{fg}	
C.V.(%)		14.02	8.47	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

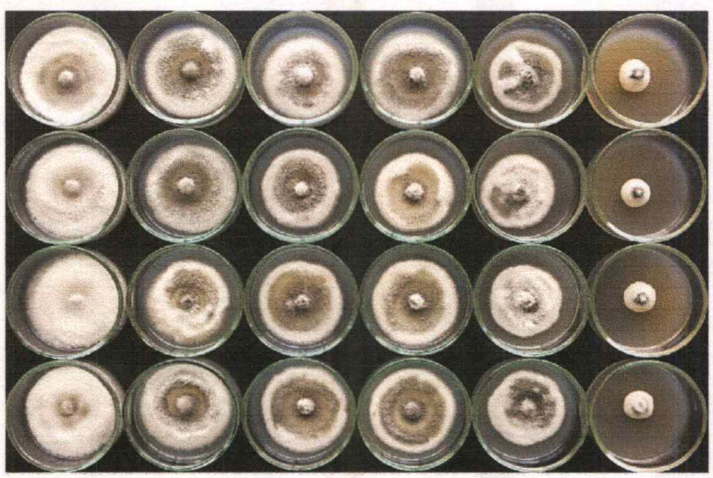
[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude Hexane

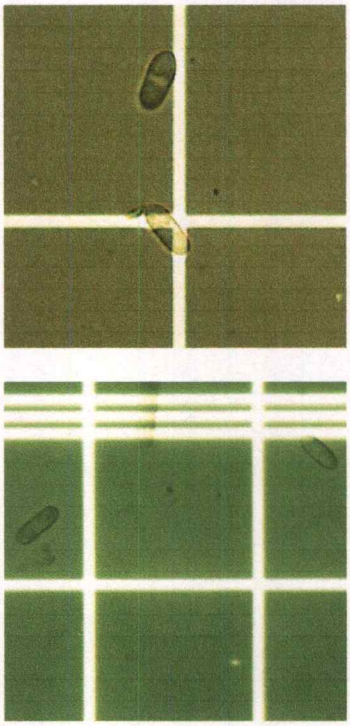
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude EtOAc

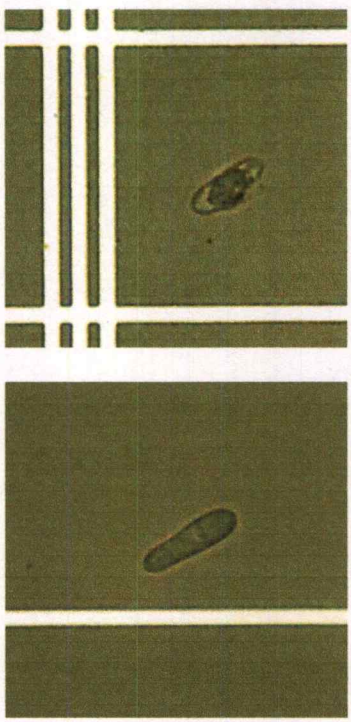


0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH



Normal Spores



Abnormal spores

Fig 4. 49 Crude extract test of *Pestalotiopsis maculformans* against *Colletotrichum coffeanum*.

Table 4.17 Crude extracts of *Pestalotiopsis maculformans* testing for growth inhibition of *Colletotrichum coffeanum* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00^{a}	-
	10	4.92^{a}	1.50^{j}
	50	4.58^{b}	8.00^{i}
	100	4.48^{bc}	10.25^{hi}
	500	3.96^{f}	20.75^{e}
	1000	3.71^{g}	25.75^{d}
EtOAc	0	5.00^{a}	-
	10	4.92^{a}	1.50^{j}
	50	4.37^{cd}	12.50^{gh}
	100	4.25^{de}	15.00^{fg}
	500	3.19^{h}	36.00^{c}
	1000	2.41^{i}	51.75^{b}
MeOH	0	5.00^{a}	-
	10	4.94^{a}	1.00^{j}
	50	4.31^{cd}	13.75^{fgh}
	100	4.12^{e}f	17.50^{ef}
	500	3.61^{g}	27.75^{d}
	1000	1.48^{j}	70.25^{a}
C.V.(%)		2.17	9.55

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.18 Spore production inhibition of crude extracts from *Pestalotiopsis maculformans* to *Colletotrichum coffeanum* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	6.50^{a}	-	4122.2
	10	6.25^{ab}	3.61^{i}	
	50	5.87^{abc}	8.96^{ghi}	
	100	5.43^{bcd}	15.54^{fgh}	
	500	5.12^{cde}	20.29^{fg}	
	1000	3.93^{fgh}	38.68^{cd}	
EtOAc	0	6.50^{a}	-	1460.24
	10	6.06^{abc}	6.53^{hi}	
	50	5.31^{bcd}	17.27^{fgh}	
	100	4.81^{def}	25.21^{ef}	
	500	3.75^{gh}	42.06^{cd}	
	1000	3.56^{ghi}	44.62^{cd}	
MeOH	0	6.50^{a}	-	231.21
	10	5.93^{abc}	8.25^{hi}	
	50	4.31^{efg}	33.70^{de}	
	100	3.31^{hij}	49.06^{bc}	
	500	2.62^{ij}	59.56^{ab}	
	1000	2.37^{j}	63.43^{a}	
C.V.(%)		12.89	19.01	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

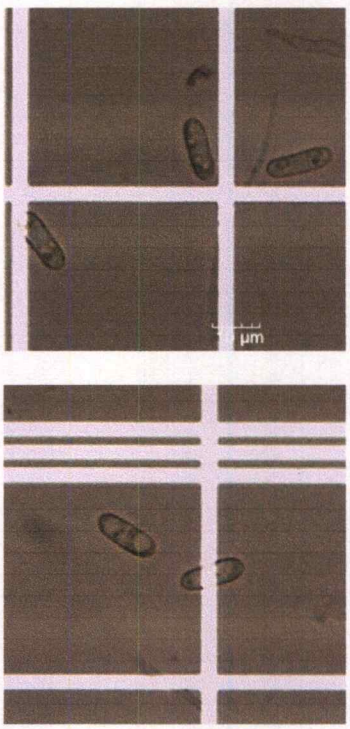
Crude Hexane

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude EtOAc

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH



Normal Spores

Abnormal spores

Fig 4. 50 Crude extract test of *Chaetomium globosum* against *Colletotrichum coffeanum*.

Table 4.19 Crude extracts of *Chaetomium globosum* testing for growth inhibition of *Colletotrichum coffeanum* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00^{a}	-
	10	4.87^{ab}	2.50^{j}
	50	4.68^{bc}	6.25^{ij}
	100	4.28^{ef}	14.25^{gh}
	500	3.72^{h}	25.50^{e}
	1000	2.83^{j}	43.25^{c}
EtOAc	0	5.00^{a}	-
	10	4.82^{ab}	3.50^{j}
	50	4.34^{de}	13.00^{h}
	100	4.10^{fg}	18.00^{fg}
	500	3.33^{i}	33.25^{d}
	1000	2.73^{j}	45.25^{bc}
MeOH	0	5.00^{a}	-
	10	4.50^{cd}	10.00^{hi}
	50	4.01^{g}	19.75^{f}
	100	3.63^{h}	27.25^{e}
	500	2.52^{k}	49.50^{b}
	1000	2.20^{l}	56.00^{a}
C.V.(%)		2.68	9.57

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.20 Spore production inhibition of crude extracts from *Chaetomium globosum* to *Colletotrichum coffeanum* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	6.00 [a]	-	470.44
	10	5.68 [bcd]	4.85 [i]	
	50	4.81 [bcd]	18.80 [g]	
	100	4.12 [de]	30.15 [f]	
	500	3.43 [ef]	42.29 [e]	
	1000	1.93 [gh]	67.53 [c]	
EtOAc	0	6.00 [a]	-	173.49
	10	5.31 [abc]	11.60 [h]	
	50	4.68 [cd]	21.32 [g]	
	100	3.06 [f]	49.20 [d]	
	500	1.81 [gh]	70.03 [c]	
	1000	1.06 [hi]	82.52 [b]	
MeOH	0	6.00 [a]	-	65.52
	10	4.56 [cd]	23.43 [g]	
	50	3.50 [ef]	41.18 [e]	
	100	2.18 [g]	63.28 [c]	
	500	1.25 [hi]	79.39 [b]	
	1000	0 .62 [i]	89.63 [a]	
C.V.(%)		16.06	9.99	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

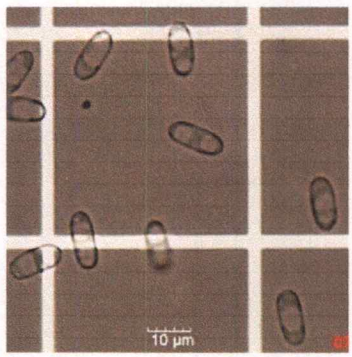
Crude Hexane

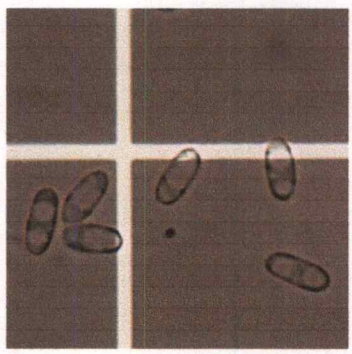
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude EtOAc

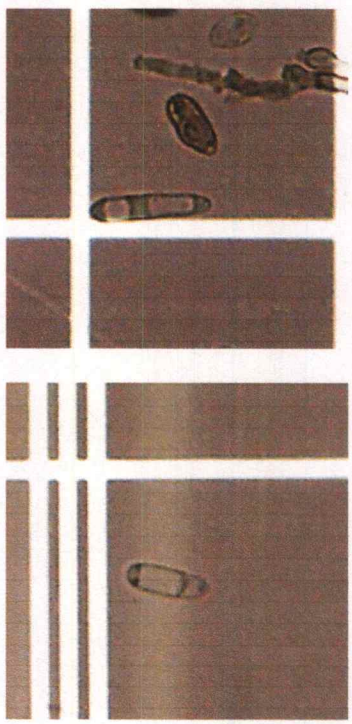
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH





Normal Spores



Abnormal spores

Fig 4. 51 Crude extract test of *Chaetomium aureum* against *Colletotrichum coffeanum*.

Table 4.21 Crude extracts of *Chaetomium aureum* testing for growth inhibition of *colletotrichum Coffeanum* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	-
	10	3.90[c]	22.00[hi]
	50	3.80[cd]	24.00[ghi]
	100	3.65[d]	27.00[g]
	500	3.39[e]	32.00[f]
	1000	1.51[i]	69.75[a]
EtOAc	0	5.00[a]	-
	10	3.92[c]	21.50[i]
	50	3.75[cd]	25.00[gh]
	100	3.25[e]	35.00[f]
	500	2.82[f]	43.50[e]
	1000	2.50[g]	53.00[c]
MeOH	0	5.00[a]	-
	10	4.36[b]	12.75[j]
	50	1.77[h]	64.50[b]
	100	3.81[cd]	23.75[ghi]
	500	2.55[g]	49.00[d]
	1000	1.92[h]	61.50[b]
C.V.(%)		2.77	5.60

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.22 Spore production inhibition of crude extracts from *Chaetomium aureum* to *Colletotrichum coffeanum* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	10.87^{a}	-	420.24
	10	10.18^{a}	6.28^{j}	
	50	9.18^{b}	15.52^{i}	
	100	8.06^{cd}	25.98^{g}	
	500	5.00^{f}	54.25^{d}	
	1000	3.68^{g}	66.23^{c}	
EtOAc	0	10.87^{a}	-	126.84
	10	8.81^{bc}	19.09^{h}	
	50	6.93^{e}	36.13^{f}	
	100	5.62^{f}	48.36^{e}	
	500	3.43^{gh}	68.46^{c}	
	1000	2.62^{hi}	75.83^{b}	
MeOH	0	10.87^{a}	-	30.54
	10	7.68^{de}	29.30^{g}	
	50	2.12^{i}	80.69^{a}	
	100	5.43^{f}	50.01^{e}	
	500	3.31^{gh}	69.55^{c}	
	1000	2.56^{hi}	76.52^{b}	
C.V.(%)		9.28	5.11	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

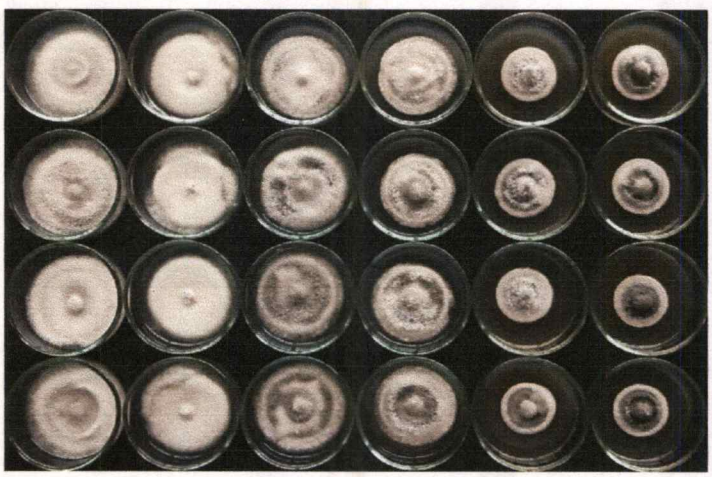
[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude Hexane

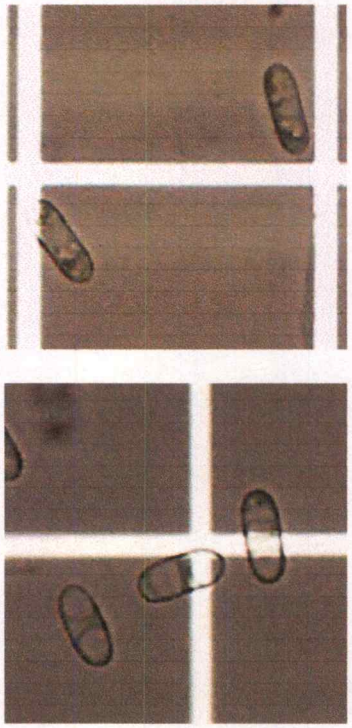
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude EtOAc

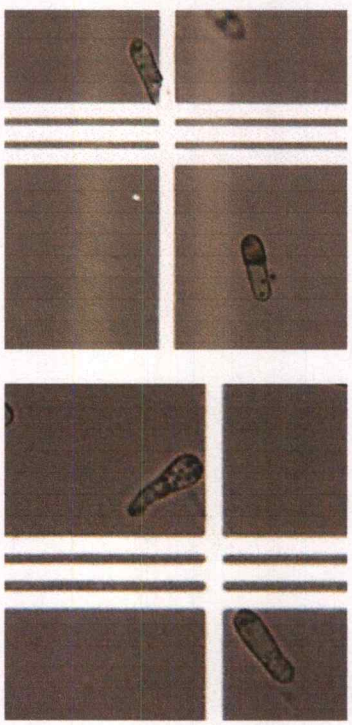


0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH



Normal Spores



Abnormal spores

Fig 4.52 Crude extract test of *Giberrella moniliformis* against *Colletotrichum coffeanum*.

Table 4.23 Crude extracts of *Giberrella moniliformis* testing for growth inhibition of *Colletotrichum coffeanum* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	-
	10	4.96[a]	0.75[k]
	50	4.18[cd]	16.25[gh]
	100	3.91[e]	21.75[f]
	500	3.40[f]	32.00[e]
	1000	3.17[g]	36.50[d]
EtOAc	0	5.00[a]	-
	10	4.33[c]	13.25[hi]
	50	4.10[de]	18.00[g]
	100	3.17[g]	36.50[d]
	500	2.93[h]	41.25[c]
	1000	2.04[j]	59.00[a]
MeOH	0	5.00[a]	-
	10	4.66[b]	6.75[j]
	50	4.37[c]	12.50[i]
	100	3.92[e]	21.50[f]
	500	2.87[h]	42.50[c]
	1000	2.61[i]	47.75[b]
C.V.(%)		2.53	7.94

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.24 Spore production inhibition of crude extracts from *Giberrella moniliformis* to *Colletotrichum coffeanum* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	5.37 [a]	-	2053.15
	10	5.18 [a]	3.42 [k]	
	50	4.62 [bc]	13.93 [i]	
	100	4.12 [cd]	23.17 [h]	
	500	3.81 [de]	29.10 [g]	
	1000	2.81 [gh]	47.61 [d]	
EtOAc	0	5.37 [a]	-	213.69
	10	4.62 [bc]	13.93 [i]	
	50	3.75 [de]	30.03 [g]	
	100	3.18 [fg]	40.75 [e]	
	500	2.18 [i]	60.05 [b]	
	1000	1.18 [j]	78.06 [a]	
MeOH	0	5.37 [a]	-	351.98
	10	4.93 [ab]	8.12 [j]	
	50	4.37 [bc]	18.59 [h]	
	100	3.50 [ef]	34.92 [f]	
	500	2.43 [hi]	54.88 [c]	
	1000	1.93 [i]	64.03 [b]	
C.V.(%)		9.81	9.27	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

According all, Crude methanol of *Pestalotiopsis maculformans* gave significantly inhibition of *Colletotrichum coffeanum* colony growth which was 70.25% at concentration 1000 ppm. Crude methanol of *Chaetomium globosum* gave highest significantly inhibition for the spore production of *Colletotrichum coffeanum* at concentration 1000 ppm with the ED_{50} at concentration 65.52 ppm. Crude methanol of *Chaetomium aureum* showed the lowest effect dose (ED_{50}) at concentration 30.54 ppm (Fig. 4.53, Table 4.25, 4.26).

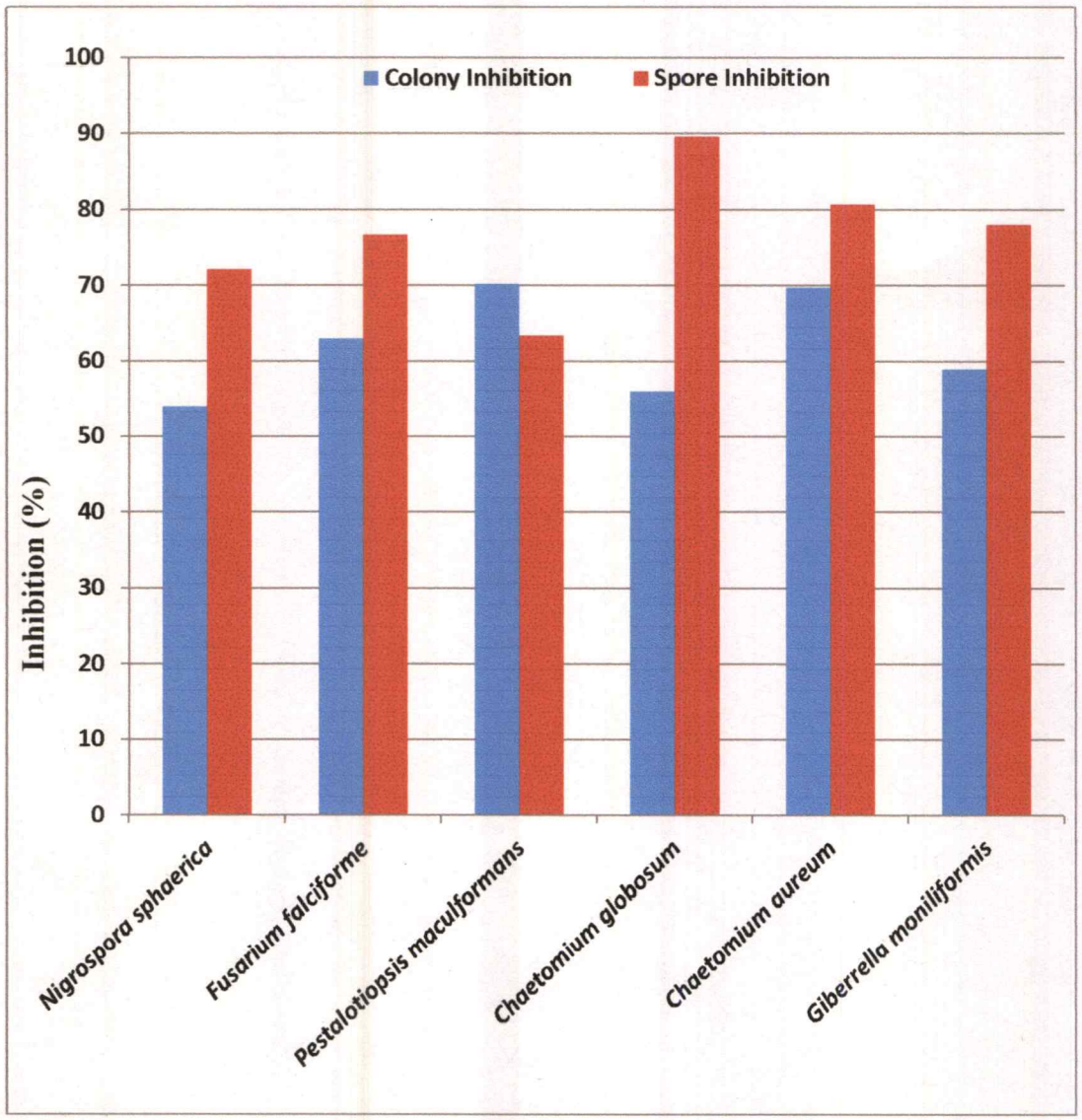


Fig. 4.53 Inhibition of *Colletotrichum coffeanum* by crude extracts of endophytics.

Table 4.25 Colony growth inhibition of *Colletotrichum coffeanum* at 7 days.

Crude extracts		Concentration (ppm)				
		10	**50**	**100**	**500**	**1000**
Nigrospora	**Hexane**	0.50	1.75	4.50	27.25	14.75
sphaerica	**EtOAc**	3.00	5.00	8.50	13.25	16.00
	MeOH	0.25	2.50	7.25	28.25	54.00
Fusarium	**Hexane**	26.00	31.00	31.75	31.00	22.50
falciforme	**EtOAc**	30.25	35.50	35.75	19.00	55.75
	MeOH	62.00	45.75	56.00	63.00	27.75
Pestalotiopsis	**Hexane**	1.50	8.00	10.25	20.75	25.75
maculformans	**EtOAc**	1.50	12.50	15.00	36.00	51.75
	MeOH	1.00	13.75	17.50	27.75	70.25
Chaetomium	**Hexane**	2.50	6.25	14.25	25.50	43..25
globosum	**EtOAc**	3.50	13.00	18.00	33.25	45.25
	MeOH	10.00	19.75	27.25	49.50	56.00
Chaetomium	**Hexane**	22.00	24.00	27.00	32.00	69.75
aureum	**EtOAc**	21.50	25.00	35.00	43.50	53.00
	MeOH	12.75	64.50	23.75	49.00	61.50
Giberrella	**Hexane**	0.75	16.25	21.75	32.00	36.50
moniliformis	**EtOAc**	13.25	18.00	36.50	41.25	59.00
	MeOH	6.75	12.50	21.50	42.50	47.75

Table 4.26 Spore production inhibition of *Colletotrichum coffeanum* at 30 days.

Crude extracts		Concentration (ppm)					ED_{50}(ppm)
		10	**50**	**100**	**500**	**1000**	
Nigrospora	**Hexane**	5.42	16.66	25.46	41.39	51.63	1092.44
sphaerica	**EtOAc**	10.05	26.91	34.77	50.44	69.99	404.53
	MeOH	24.53	33.58	51.69	64.02	72.18	143.53
Fusarium	**Hexane**	13.15	16.09	23.38	40.85	52.41	1054.88
falciforme	**EtOAc**	27.91	32.51	46.41	50.26	68.83	262.01
	MeOH	38.31	39.96	61.21	76.74	42.35	161.14
Pestalotiopsis	**Hexane**	3.61	8.96	15.54	20.29	38.68	4122.2
maculformans	**EtOAc**	6.53	17.27	25.21	42.06	44.62	1460.24
	MeOH	8.25	33.70	49.06	59.56	63.43	231.21
Chaetomium	**Hexane**	4.85	18.80	30.15	42.29	67.53	470.44
globosum	**EtOAc**	11.60	21.32	49.20	70.03	82.52	173.49
	MeOH	23.43	41.18	63.28	79.39	89.63	65.52
Chaetomium	**Hexane**	6.28	15.52	25.98	54.25	66.23	420.24
aureum	**EtOAc**	19.09	36.13	48.36	68.46	75.83	126.84
	MeOH	29.30	80.69	50.01	69.55	76.52	30.54
Giberrella	**Hexane**	3.42	13.93	23.17	29.10	47.61	2053.15
moniliformis	**EtOAc**	13.93	30.03	40.75	60.05	78.06	213.69
	MeOH	8.12	18.59	34.92	54.88	64.03	351.98

4.2.5.2 Bioactivities Tests of Crude Extract against *Colletotrichum Capsici*

The crude extracts from *Nigrospora sphaerica, Fusarium falciforme*, *Pestalotiopsis maculformans, Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* were selected to test bioactivity against *Colletotrichum capsici* causing chili anthracnose.

Results showed that crude ethyl acetate from *Nigrospora* spp. gave significantly highest inhibition of 52.25% for the colony growth of *C. capsici* at concentration of 1,000 ppm when compared to the control, followed by crude methanol which inhibited 48.75% at concentration of 1000 ppm when compared to the control (Table 4.27). Methanol crude extract from *Nigrospora* spp. gave significantly highest inhibition for the spore production of *C. capsici* as 87.26% at concentration of 500 ppm and the effective dose (ED_{50}) inhibited spore production of *C. capsici* at concentration of 41.51 ppm. Followed by, crude ethyl acetate gave 80.93% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 130.90 ppm. Crude hexane showed 78.36% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 259.56 ppm (Table 4.28).

The crude methanol from *Fusarium* spp. expressed highest inhibition percentage of *C. capsici* colony growth which was 34.50% at the concentration of 1,000 ppm when compared to the control (Table 4.29). Methanol crude extract from *Fusarium* spp. showed significantly highest inhibition for the spore production of *C. capsici* as 74.52% at the concentration of 1,000 ppm, and the ED_{50} inhibited *C.capsici* spore production at concentration 47.39 ppm. Crude hexane, crude ethyl acetate showed inhibition as 65.18, 68.03% respectively, and the ED_{50} at 491.57, 42.40 ppm, respectively (Table 4.30).

Methanol crude extract from *Pestalotiopsis maculformans* gave significantly highest inhibition of *C. capsici* colony growth which was 72.50% at the concentration of 1,000 ppm when compared to the control (Table 4.31). Methanol crude extract from *Pestalotiopsis maculformans* showed significantly highest inhibition for the spore production of *C. capsici* as 79.17% at the concentration of 1,000 ppm, and the ED_{50} inhibited *C.capsici* spore production at concentration 103.50 ppm. Followed by crude ethyl acetate gave 73.38% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 228.11 ppm; crude hexane showed 67.85% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 633.31ppm (Table 4.32).

Crude ethyl acetate from *Chaetomium globosum* gave significantly highest

inhibition of 75.00% for the colony growth of *C. capsici* at concentration of 1,000 ppm when compared to the control, followed by crude methanol which inhibited 40.50% at concentration of 1000 ppm when compared to the control (Table 4.33). Ethyl acetate crude extract from *Chaetomium globosum* gave significantly highest inhibition for the spore production of *C. capsici* as 85.89% at concentration of 1000 ppm and the effective dose (ED_{50}) inhibited spore production of *C. capsici* at concentration of 87.26 ppm. Followed by, crude methanol gave 81.52% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 121.49 ppm. Crude hexane showed 72.72% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 338.61 ppm (Table 4.34).

Methanol crude extract from *Chaetomium aureum* gave significantly highest inhibition of *C. capsici* colony growth which was 82.25% at the concentration of 1,000 ppm when compared to the control, followed by crude ethyl acetate which inhibited 77.00% at concentration of 1000 ppm when compared to the control (Table 4.35). Methanol crude extract from *Chaetomium aureum* showed significantly highest inhibition for the spore production of *C. capsici* as 77.91% at the concentration of 50 ppm, and the ED_{50} inhibited *C.capsici* spore production at concentration 33.06 ppm. Followed by crude ethyl acetate gave 73.78% inhibition at concentration of 1,00 ppm, and ED_{50} at concentration of 122.39 ppm; crude hexane showed 63.83% inhibition at concentration of 500 ppm, and ED_{50} at concentration of 330.85 ppm (Table 4.36).

The crude methanol from *Giberrella moniliformis* expressed highest inhibition percentage of *C. capsici* colony growth which was 70.00% at the concentration of 1,000 ppm when compared to the control (Table 4.37). Ethyl acetate crude extract from *Giberrella moniliformis* showed significantly highest inhibition for the spore production of *C. capsici* as 77.17% at the concentration of 1,000 ppm, and the ED_{50} inhibited *C.capsici* spore production at concentration 94.70 ppm. Crude hexane, crude methanol showed inhibition as 71.06, 63.90% respectively, and the ED_{50} at 231.78, 351.00 ppm, respectively (Table 4.38).

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

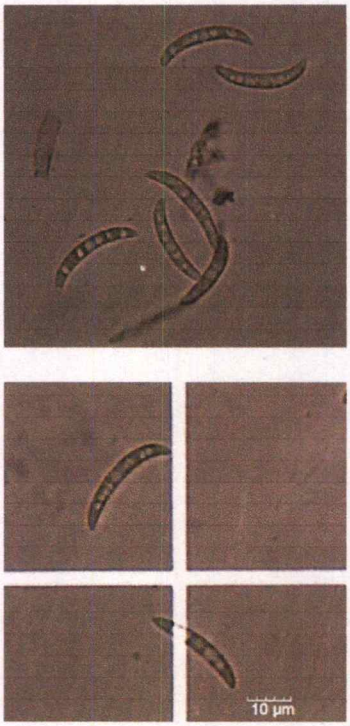
Crude Hexane

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

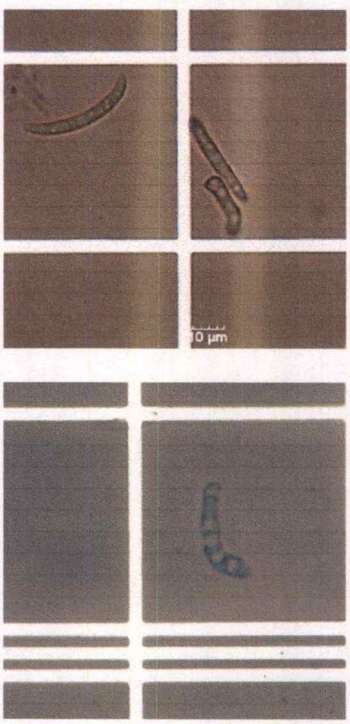
Crude EtOAc

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH



Normal Spores



Abnormal spores

Fig 4. 54 Crude extract test of *Nigrospora sphaerica* against *Colletotrichum capsici*.

Table 4.27 Crude extracts of *Nigrospora sphaerica* testing for growth inhibition of *Colletotrichum capsici* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	
	10	4.87[abc]	2.50[ij]
	50	4.77[bc]	4.50[ij]
	100	4.57[d]	8.50g[h]
	500	3.72[f]	25.50[e]
	1000	3.30[h]	34.25[c]
EtOAc	0	5.00[a]	
	10	4.93[ab]	1.25[j]
	50	4.72[c]	5.50[hi]
	100	4.55[d]	9.00[g]
	500	3.63[fg]	27.25[de]
	1000	2.38[j]	52.25[a]
MeOH	0	5.00[a]	
	10	4.89[ab]	2.00[ij]
	50	4.85[abc]	3.00[ij]
	100	4.10[e]	18.00[f]
	500	3.51[g]	29.75[d]
	1000	2.56[i]	48.75[b]
C.V.(%)		1.86	9.63

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.28 Spore production inhibition of crude extracts from *Nigrospora sphaerica* to *Colletotrichum capsici* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
	0	4.87^{a}		
	10	4.25^{b}	12.72^{j}	
Hexane	50	3.68^{bcd}	24.40^{hi}	259.56
	100	3.50^{cd}	28.16^{gh}	
	500	2.06^{gh}	57.84^{e}	
	1000	1.06^{ijk}	78.36^{bc}	
	0	4.87^{a}		
EtOAc	10	3.93^{bc}	19.18^{ij}	
	50	3.25^{de}	33.43^{g}	130.90
	100	2.81^{ef}	42.40^{f}	
	500	1.50^{hi}	69.39^{d}	
	1000	0.93^{ijk}	80.93^{ab}	
	0	4.87^{a}		
MeOH	10	3.62^{cd}	25.84^{hi}	
	50	2.50^{fg}	48.74^{f}	41.51
	100	1.31^{ij}	73.40^{cd}	
	500	0.62^{k}	87.26^{a}	
	1000	0.81^{ik}	83.50^{ab}	
C.V.(%)		10.77	7.38	

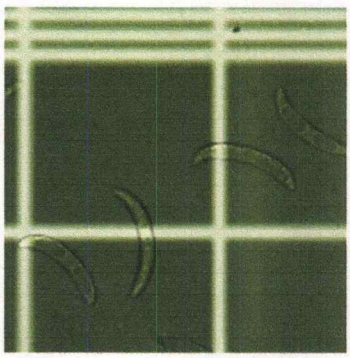
[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

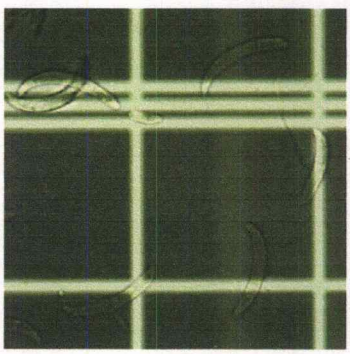
[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude Hexane

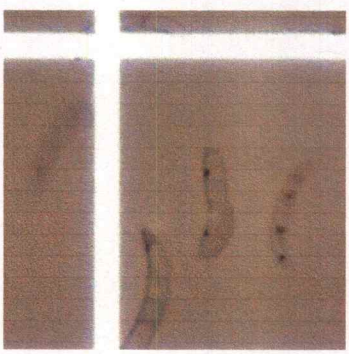




Normal Spores

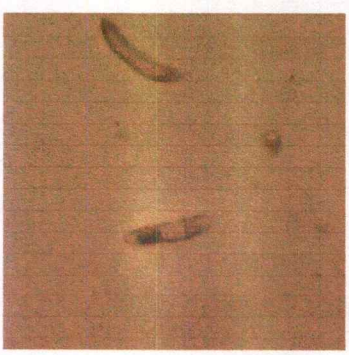
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude EtOAc



0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH



Abnormal spores

Fig 4. 55 Crude extract test of *Fusarium falciforme* against *Colletotrichum capsici*.

Table 4.29 Crude extracts of *Fusarium falciforme* testing for growth inhibition of *Colletotrichum capsici* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	
	10	4.73[b]	5.25[j]
	50	4.69[b]	6.00[ij]
	100	4.56[cd]	8.75[ghi]
	500	4.56[cd]	9.00[ghi]
	1000	4.61[bc]	7.75[hij]
EtOAc	0	5.00[a]	
	10	4.62[bc]	7.50[hij]
	50	4.49[cde]	32.50[e]
	100	4.38[ef]	10.00[fgh]
	500	4.44[de]	12.25[ef]
	1000	4.30[f]	11.00[fg]
MeOH	0	5.00[a]	
	10	4.41[ef]	11.75[efg]
	50	4.12[g]	17.50[d]
	100	3.96[h]	20.75[c]
	500	3.56[i]	28.75[b]
	1000	3.27[j]	34.50[a]
C.V.(%)		1.48	10.60

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.30 Spore production inhibition of crude extracts from *Fusarium falciforme* to *Colletotrichum capsici* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	14.56[a]		491.57
	10	10.81[b]	25.12[de]	
	50	10.00[bc]	32.23[d]	
	100	6.62[def]	54.45[bc]	
	500	5.06[ef]	65.18[ab]	
	1000	9.25[bcd]	36.06[d]	
EtOAc	0	14.56[a]		42.40
	10	4.68[ef]	68.03[a]	
	50	9.25[bcd]	35.86[d]	
	100	9.12[bcd]	36.26[d]	
	500	6.81[cdef]	53.56[bc]	
	1000	11.68[ab]	16.61[e]	
MeOH	0	14.56[a]		47.39
	10	10.00[bc]	30.54[d]	
	50	7.00[cde]	51.36[c]	
	100	4.81[ef]	67.01[a]	
	500	4.18[ef]	70.85[a]	
	1000	3.68[f]	74.52[a]	
C.V.(%)		17.63	12.52	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

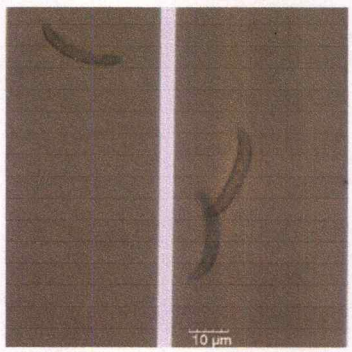
Crude Hexane

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

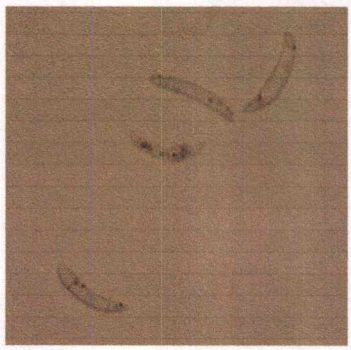
Crude EtOAc

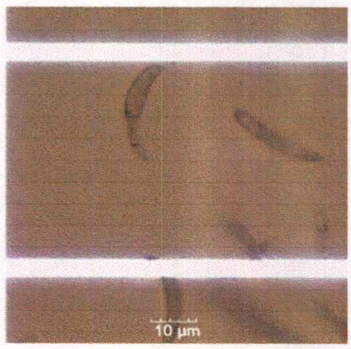
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH



Normal Spores





Abnormal spores

Fig 4. 56 Crude extract test of *Pestalotiopsis maculformans* against *Colletotrichum capsici*.

Table 4.31 Crude extracts of *Pestalotiopsis maculformans* testing for growth inhibition of *Colletotrichum capsici* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	
	10	4.85[ab]	2.50[h]
	50	4.76[b]	4.75[h]
	100	4.75[b]	5.00[h]
	500	4.07[d]	18.50[de]
	1000	2.88[g]	42.25[b]
EtOAc	0	5.00[a]	
	10	4.76[b]	4.75[h]
	50	4.48[c]	10.25[g]
	100	4.19[d]	16.00[ef]
	500	2.86[g]	42.75[b]
	1000	2.81[g]	43.75[b]
MeOH	0	5.00[a]	
	10	4.42[c]	11.50[g]
	50	4.38[c]	12.25[fg]
	100	3.88[e]	22.25[d]
	500	3.67[f]	26.50[c]
	1000	1.22[h]	75.50[a]
C.V.(%)		2.31	9.26

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.32 Spore production inhibition of crude extracts from *Pestalotiopsis maculformans* to *Colletotrichum capsici* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	9.87^{a}	-	633.31
	10	9.31^{ab}	5.27^{i}	
	50	8.37^{ab}	14.70^{h}	
	100	6.62^{cd}	32.67^{g}	
	500	5.68^{def}	42.23^{ef}	
	1000	4.12^{fgh}	58.48^{c}	
EtOAc	0	9.87^{a}	-	228.11
	10	8.12^{abc}	17.23^{h}	
	50	5.75^{def}	41.70^{ef}	
	100	5.31^{def}	46.27^{de}	
	500	4.62^{efg}	53.25^{cd}	
	1000	3.18^{ghi}	67.85^{b}	
MeOH	0	9.87^{a}	-	103.5
	10	7.93^{bc}	19.39^{h}	
	50	6.18^{de}	37.32^{fg}	
	100	4.87^{defg}	50.89^{d}	
	500	2.62^{hi}	73.38^{ab}	
	1000	2.06^{i}	79.17^{a}	
C.V.(%)		13.42	8.22	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

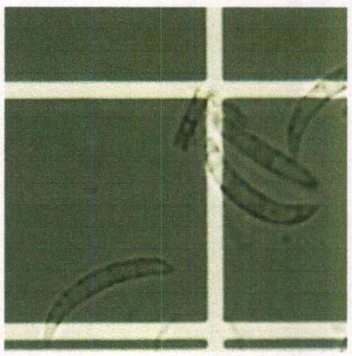
Crude Hexane

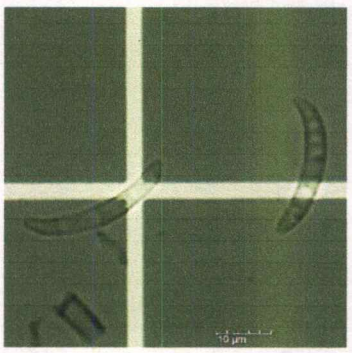
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude EtOAc

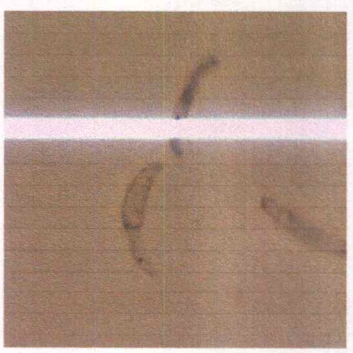
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH





Normal Spores



Abnormal spores

Fig 4. 57 Crude extract test of *Chaetomium globosum* against *Colletotrichum capsici*.

Table 4.33 Crude extracts of *Chaetomium globosum* testing for growth inhibition of *Colletotrichum capsici* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00^{a}	-
	10	4.95^{a}	1.00^{j}
	50	4.71^{b}	5.75^{i}
	100	4.46^{c}	10.75^{h}
	500	4.06^{e}	18.75^{f}
	1000	3.07^{i}	38.50^{b}
EtOAc	0	5.00^{a}	-
	10	4.88^{a}	2.25^{j}
	50	4.29^{d}	1.40^{g}
	100	3.86^{f}	22.75^{e}
	500	3.36^{gh}	32.75^{cd}
	1000	1.25^{j}	75.00^{a}
MeOH	0	5.00^{a}	-
	10	4.08^{e}	18.25^{f}
	50	3.81^{f}	23.75^{e}
	100	3.47^{g}	30.50^{d}
	500	3.25^{h}	35.00^{c}
	1000	2.97^{i}	40.50^{b}
C.V.(%)		1.91	6.76

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.34 Spore production inhibition of crude extracts from *Chaetomium globosum* to *Colletotrichum capsici* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	12.81^{a}	-	338.61
	10	11.68^{ab}	8.74^{l}	
	50	9.87^{de}	23.03^{i}	
	100	9.00^{e}	29.88^{h}	
	500	6.00^{g}	53.24^{e}	
	1000	3.50^{h}	72.72^{c}	
EtOAc	0	12.81^{a}	-	87.26
	10	11.06^{bc}	13.60^{k}	
	50	6.62^{g}	47.36^{f}	
	100	5.81^{g}	54.75^{e}	
	500	3.68^{h}	71.24^{c}	
	1000	1.81^{i}	85.89^{a}	
MeOH	0	12.81^{a}	-	121.49
	10	10.37^{cd}	18.97^{j}	
	50	7.87^{f}	38.64^{g}	
	100	6.68^{g}	47.77^{f}	
	500	4.50^{h}	64.90^{d}	
	1000	2.37^{i}	81.52^{b}	
C.V.(%)		7.53	4.31	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

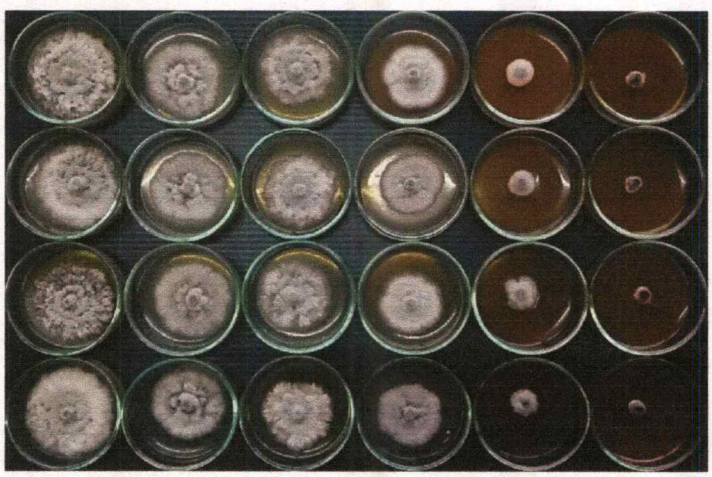
[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude Hexane

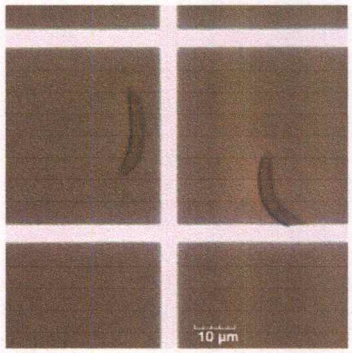
0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude EtOAc

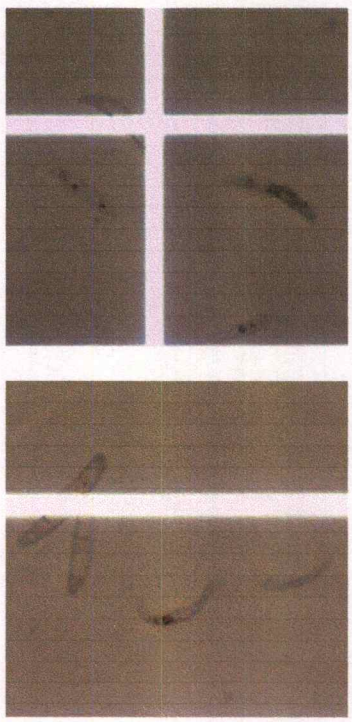


0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH



Normal Spores



Abnormal spores

Fig 4. 58 Crude extract test of *Chaetomium aureum* against *Colletotrichum capsici*.

Table 4.35 Crude extracts of *Chaetomium aureum* testing for growth inhibition of *colletotrichum Capsici* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	
	10	4.41[b]	11.75[g]
	50	4.30[b]	14.00[g]
	100	3.98[c]	20.25[f]
	500	3.85[cd]	23.00[ef]
	1000	1.58[f]	68.25[c]
EtOAc	0	5.00[a]	
	10	3.99[c]	20.00[f]
	50	3.75[d]	25.00[e]
	100	3.33[e]	33.25[d]
	500	1.37[g]	74.00[b]
	1000	1.15[h]	77.00[b]
MeOH	0	5.00[a]	
	10	3.76[d]	24.75[e]
	50	3.76[d]	24.75[e]
	100	3.22[e]	35.50[d]
	500	1.51[fg]	69.75[c]
	1000	0.88[i]	82.25[a]
C.V.(%)		2.78	5.01

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.36 Spore production inhibition of crude extracts from *Chaetomium aureum* to *Colletotrichum capsici* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	4.50^{a}		330.85
	10	3.81^{ab}	15.00^{i}	
	50	3.00^{c}	33.34^{g}	
	100	2.06^{de}	54.26^{e}	
	500	1.62^{efg}	63.83^{cd}	
	1000	1.75^{ef}	61.27^{d}	
EtOAc	0	4.50^{a}		122.39
	10	3.37^{bc}	25.17^{h}	
	50	2.68^{cd}	40.19^{f}	
	100	1.18^{fg}	73.78^{ab}	
	500	1.75^{ef}	61.11^{d}	
	1000	1.37^{efg}	69.75^{bc}	
MeOH	0	4.50^{a}		33.06
	10	2.68^{cd}	40.19^{f}	
	50	1.00^{g}	77.91^{a}	
	100	1.68^{efg}	62.58^{d}	
	500	3.18^{bc}	29.55^{h}	
	1000	3.37^{bc}	25.20^{h}	
C.V.(%)		13.07	6.71	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

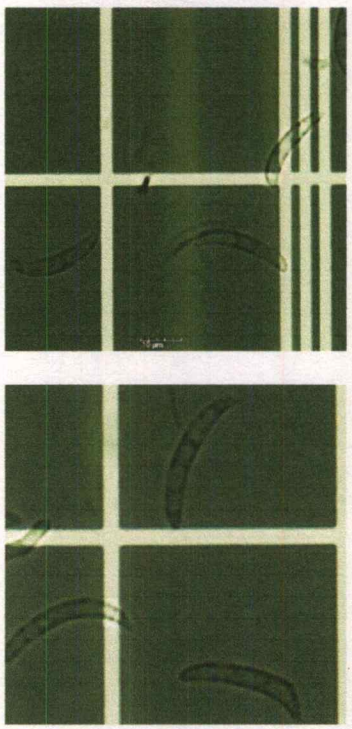
Crude Hexane

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

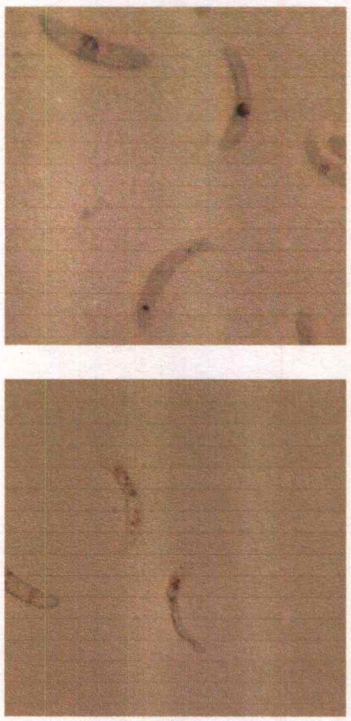
Crude EtOAc

0ppm 10ppm 50ppm 100ppm 500ppm 1000ppm

Crude MeOH



Normal Spores



Abnormal spores

Fig 4. 59 Crude extract test of *Giberrella moniliformis* against *Colletotrichum capsici*.

Table 4.37 Crude extracts of *Giberrella moniliformis* testing for growth inhibition of *Colletotrichum capsici* at 7 days.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth inhibition(%)[2,3]
Hexane	0	5.00[a]	
	10	4.62[c]	7.50[j]
	50	4.46[d]	10.75[i]
	100	4.11[e]	17.75[h]
	500	3.81[fg]	23.75[fg]
	1000	3.48[h]	30.25[e]
EtOAc	0	5.00[a]	
	10	4.81[b]	3.75[k]
	50	3.91[f]	21.75[g]
	100	3.71[g]	25.75[f]
	500	2.92[i]	41.50[d]
	1000	2.51[j]	49.75[c]
MeOH	0	5.00[a]	
	10	4.68[bc]	6.25[jk]
	50	3.89[f]	22.00[g]
	100	2.93[i]	41.25[d]
	500	1.76[k]	64.75[b]
	1000	1.50[l]	70.00[a]
C.V.(%)		1.98	5.66

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4.38 Spore production inhibition of crude extracts from *Giberrella moniliformis* to *Colletotrichum capsici* at 30 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Number of spores [1] (10^6)	Inhibition(%) [2,3]	ED_{50}(ppm)
Hexane	0	11.18^{a}		231.78
	10	8.81^{bc}	21.20^{ij}	
	50	7.18^{cde}	36.15^{gh}	
	100	6.31^{de}	43.70^{efg}	
	500	5.31^{efg}	52.59^{def}	
	1000	3.25^{gh}	71.06^{ab}	
EtOAc	0	11.18^{a}		94.70
	10	8.18^{bcd}	26.96^{hi}	
	50	7.00^{cde}	37.28^{gh}	
	100	5.00^{efg}	55.52^{cd}	
	500	3.18^{gh}	71.58^{ab}	
	1000	2.56^{h}	77.17^{a}	
MeOH	0	11.18^{a}		351.00
	10	9.68^{ab}	13.31^{j}	
	50	5.68^{ef}	49.34^{def}	
	100	4.06^{fgh}	63.90^{bc}	
	500	6.37^{de}	43.22^{fg}	
	1000	5.12^{efg}	54.58^{cde}	
C.V.(%)		15.60	11.14	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[3]/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

According all, Crude methanol of *Chaetomium aureum* gave significantly inhibition of *Colletotrichum capsici* colony growth which was 82.25% at concentration 1000 ppm. Crude methanol of *Nigrospora sphaerica* gave highest significantly inhibition for the spore production of *Colletotrichum capsici*at which was 87.26 at concentration 500 ppm with the ED_{50} at concentration 41.51 ppm. Crude methanol of *Chaetomium aureum* showed the lowest effect dose (ED_{50}) at concentration 33.06 ppm (Fig. 4.60, Table 4.39, 4.40).

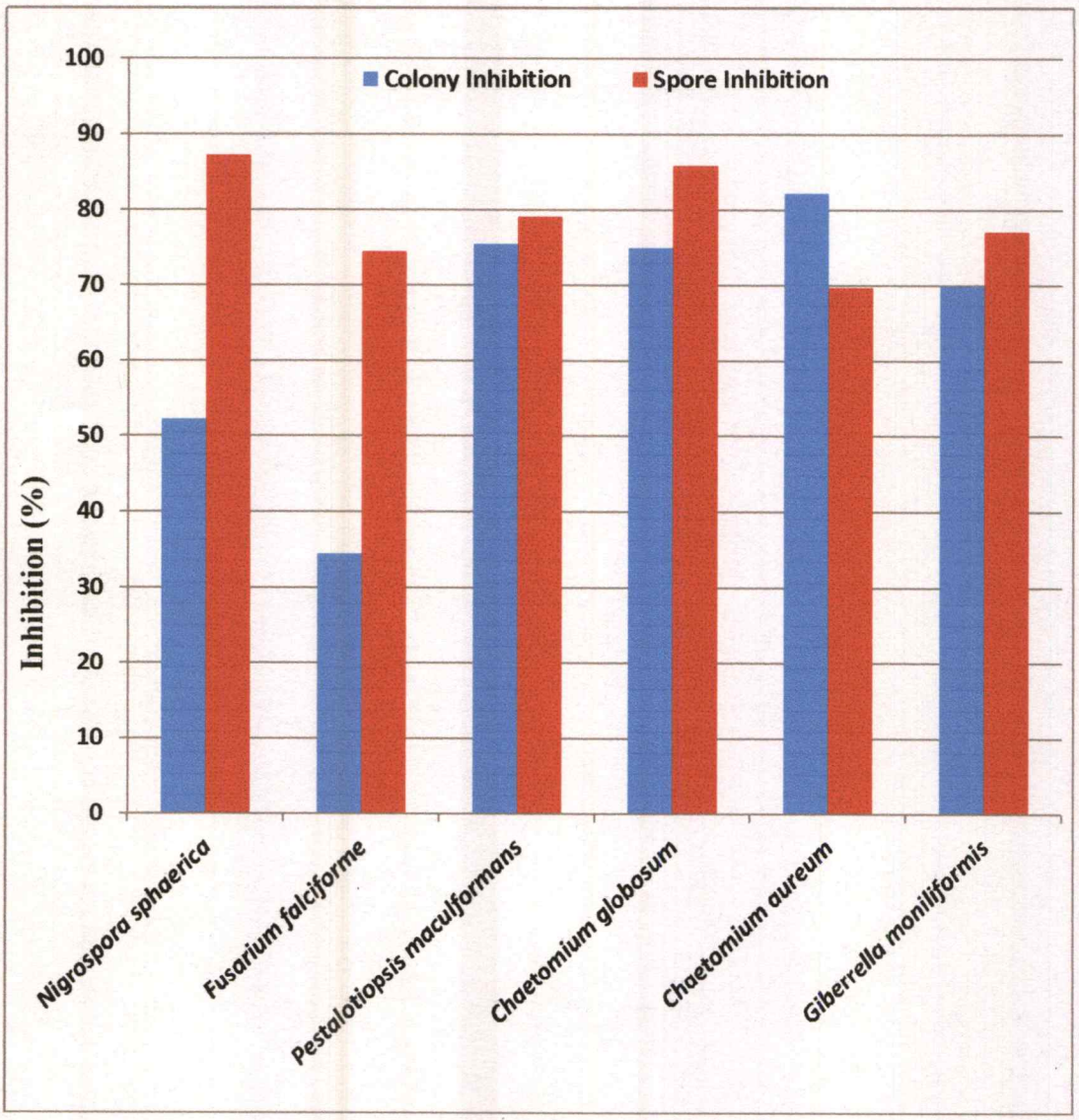


Fig. 4.60 Inhibition of *Colletotrichum capsici* by crude extracts of endophytics.

Table 4.39 Colony growth inhibition of *Colletotrichum capsici* at 5 days.

Crude extracts		Concentration (ppm)				
		10	**50**	**100**	**500**	**1000**
Nigrospora sphaerica	**Hexane**	2.50	4.50	8.50	25.50	34.25
	EtOAc	1.25	5.50	9.00	27.25	52.25
	MeOH	2.00	3.00	18.00	29.75	48.75
Fusarium falciforme	**Hexane**	5.25	6.00	8.75	9.00	7.75
	EtOAc	7.50	32.50	10.00	12.25	11.00
	MeOH	11.75	17.50	20.75	28.75	34.50
Pestalotiopsis maculformans	**Hexane**	2.50	4.75	5.00	18.50	42.25
	EtOAc	4.75	10.25	16.00	42.75	43.75
	MeOH	11.50	12.25	22.25	26.50	75.50
Chaetomium globosum	**Hexane**	1.00	5.75	10.75	18.75	38.50
	EtOAc	2.25	1.40	22.75	32.75	75.00
	MeOH	18.25	23.75	30.50	35.00	40.50
Chaetomium aureum	**Hexane**	11.75	14.00	20.25	23.00	68.25
	EtOAc	20.00	25.00	33.25	74.00	77.00
	MeOH	24.75	24.75	35.50	69.75	82.25
Giberrella moniliformis	**Hexane**	7.50	10.75	17.75	23.75	30.25
	EtOAc	3.75	21.75	25.75	41.50	49.75
	MeOH	6.25	22.00	41.25	64.75	70.00

Table 4.40 Spore production inhibition of *Colletotrichum capsici* at 30 days.

Crude extracts		Concentration (ppm)					ED_{50}(ppm)
		10	**50**	**100**	**500**	**1000**	
Nigrospora	**Hexane**	12.72	24.40	28.16	57.84	78.36	259.56
sphaerica	**EtOAc**	19.18	33.43	42.40	69.39	80.93	130.90
	MeOH	25.84	48.74	73.40	87.26	83.50	41.51
Fusarium	**Hexane**	25.12	32.23	54.45	65.18	36.06	491.57
falciforme	**EtOAc**	68.03	35.86	36.26	53.56	16.61	42.40
	MeOH	30.54	51.36	67.01	70.85	74.52	47.39
Pestalotiopsis	**Hexane**	5.27	14.70	32.67	42.23	58.48	633.31
maculformans	**EtOAc**	17.23	41.70	46.27	53.25	67.85	228.11
	MeOH	19.39	37.32	50.89	73.38	79.17	103.5
Chaetomium	**Hexane**	8.74	23.03	29.88	53.24	72.72	338.61
globosum	**EtOAc**	13.60	47.36	54.75	71.24	85.89	87.26
	MeOH	18.97	38.64	47.77	64.90	81.52	121.49
Chaetomium	**Hexane**	15.00	33.34	54.26	63.83	61.27	330.85
aureum	**EtOAc**	25.17	40.19	73.78	61.11	69.75	122.39
	MeOH	40.19	77.91	62.58	29.55	25.20	33.06
Giberrella	**Hexane**	21.20	36.15	43.70	52.59	71.06	231.78
moniliformis	**EtOAc**	26.96	37.28	55.52	71.58	77.17	94.70
	MeOH	13.31	49.34	63.90	43.22	54.58	351.00

CHAPTER 5

DISCUSSION

Totally, 105 isolates were obtained from leaves, petioles and roots of palm trees and the most islates were obtained from roots. Sixty isolates belonging to 15 identified species based on morphology characters (Table 4.2) and 45 isolates belonging to mycelia sterilia fungi which is morphological fungal types, but not forming true spores. Fifteen identified species including *Cladosporium* spp.; *Phialophora* spp.; *Pestalotiopsis* spp.; *Phoma* spp.; *Nigrospora* spp.; *Xylaria* spp.; *Fusarium* spp.; *Rhizoctonia* spp.; *Colletotrichum* spp.; *Chaetomium globosum*; *Chaetomium aureum*; *Gibberella* spp.; *Emericella* spp.; *Aspergillus* spp. and *Curvularia lunata*. All endophytic fungi in this study had isolated from other plants from previous study. Rosa *et al.* (2012) study evaluates the diversity of microbial community associated with healthy *E. purpurea* clones and their ability to produce defense compounds. They recovered and identified thirty-nine fungal endophytes through the molecular methods in 15 distinct phylotypes, which were closely related to species of the following genera *Ceratobasidium*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Glomerella*, and *Mycoleptodiscus*. Crude extracts of fungal isolates were also tested for antifungal and insecticidal biological activities. The results suggest that the symbiosis between the endo-phytic fungal community and micropropagated clones of *E. purpurea* was re-established after acclimatization to soil and the endophytic fungi produced compounds against phytopathogenic fungi. Fungal endophytes associated with the palms, *Calamus kerrianus* (rattan) and *Wallichia caryotoides* (taorang) were investigated at two sites within Doi Suthep-Pui National Park, Thailand. Thirty-five endophytic fungi isolated included xylariaceous taxa (20 morphotypes), sterile mycelia, one unidentified and 13 mitosporic fungi including *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp.,*Guignardia cocaicola*, *Paecilomyces* sp. *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phomopsis* sp., *Phyllosticta* sp., and *Sarcopodium* sp. (Lumyong *et al.*, 2009). Endophytic fungi were isolated from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia. *Xylaria* spp., *Phomopsis* spp., *Pestalotiopsis* spp., *Nodulisporium* spp., *Colletotrichum* sp. and *Distocercospora* sp. were obtained as endophytic fungi (Frohlich, 1999). The diversity of endophytic fungi in leaves, stems and roots from transgenic (Bt) and its isoline

(non-Bt) cotton was evaluated during different plant developmental stages to investigate possible non-target effects of genetically modified cotton on endophytic fungal communities. A total of 17 genera of endophytic fungi were isolated. The most frequently isolated species were *Phomopsis archeri* from leaves and stems and *Phoma destructiva* from roots (Vieira *et al.*, 2011). Endophytic fungi were isolated from living symptomless leaves of 12 tree species from two locations in the Iwokrama Forest Reserve, Guyana. Species of *Colletotrichum*, *Nodulisporium*, *Pestalotiopsis* and *Phomopsis* were most frequently isolated, *Nigrospora* sp. also were identified as endophytic fungi (Cannon *et al.*, 2002). The occurrence of *Xylaria* species as endophytes in tropical plants is well documented (Bacon *et al.*, 1994; Bayman *et al.*, 1997; Bussaban *et al.*, 2001; Clay 1988; Fro¨hlich *et al.*, 2000; Photita *et al.*, 2001; Tomita, 2003). Cladistic analyses in the study of Promputtha *et al.* (2007) also show that more than one species of *Xylaria* can coexist within living leaf tissues of *M. liliifera.* In this study, most *Xylaria* species are endophytic in origin. The study of (Qadri *et al.*, 2013) was conducted to characterize and explore the endophytic fungi of selected plants from the Western Himalayas for their bioactive potential. A total of 72 strains of endophytic fungi were isolated and characterized morphologically as well as on the basis of ITS1-5.8S-ITS2 ribosomal gene sequence acquisition and analyses. Samples of *Platanus orientalis* were found to harbor only *Fusarium* spp. representing 4 different strains. Strangely, strains belonging to other genera could not be obtained from any of the samples of this plant. *Artemesia annua* also possessed several strains of *Fusarium* as endophytes (5/22) whereas almost half of the endophytes of *Withania somnifera* (4/9) were also *Fusarium* spp. Harvais and Hadley (1967) Extensive isolation of root endophytes from *Orchis {Daciylorchis) pitrpurella* and other British orchids yielded a variety of strains of *Rhizoctonia* and of other fungi. *R. repens,* a common orchid endophyte, occurred in several host species and habitats. *R. solani* was uncommon, being obtained only from *Orchis piirpiirella* and *Coeloglossum viride* in certain situations. Most *Rhizoctonia* strains were obtained from only one plant and one habitat and the evidence indicated that there was no specificity between host and endophyte. Fungal endophytes were isolated from leaves of *Centella asiatica* (Apiaceae) collected at Mangoro (Rakotoniriana *et al.*, 2007). The most common endophytes were the non-sporulating species 1 (isolation frequency IF 19.2%) followed by *Colletotrichum* sp.1 (IF 13.2%), *Guignardia* sp. (IF 8.5%), *Glomerella* sp. (IF 7.7%), an unidentified ascomycete (IF 7.2%), the nonsporulating species 2 (IF 3.7%) and *Phialophora* sp. (IF 3.5%). Endophytic fungi from the Chinese medicinal plant *Actinidia macrosperma* were

isolated and identified for the first time (Lu *et al.*, 2012). In total, 17 fungal isolates were obtained. Five different taxa were represented by 11 isolates (*Acremonium furcatum*, *Cylindrocarpon pauciseptatum*, *Trichoderma citrinoviride*, *Paecilomyces marquandii*, and *Chaetomium globosum*). In Pablo *et al.* (2015) study, 154 endophyte isolates were selected from a collection of 546 fungi tested in a preliminary confrontation assay. These isolates were then tested against *F. circinatum* in an in vitro antagonism experiment. Four different types of indicators (length of the central axis of the colony of the pathogen, the shape coefficient, percentage inhibition of radial growth and percentage inhibition zone) were used to detect and quantify the antagonistic activity directed towards the pathogen by the endophytes. In total, 138 endophytes displayed antagonistic activity towards *F. circinatum* in the dual cultures of the in vitro experiment. In the field test, the endophytes *Chaetomium aureum* and *Alternaria* sp. reduced the area under disease progress curve (AUDPC) for the *P. radiata* seedlings, indicating that they may therefore be suitable for use as biological control agents (BCAs) of the disease. Qadri *et al.* (2013) conducted to characterize and explore the endophytic fungi of selected plants from the Western Himalayas for their bioactive potential. A total of 72 strains of endophytic fungi were isolated. The fungi represented 27 genera of which two belonged to Basidiomycota, each representing a single isolate, while most of the isolates comprised of Ascomycetous fungi. *Gibberella moniliformis*, *Chaetomium globosum*, *Alternaria* spp., *Fusarium* spp., *Cladosporium cladosporioides* and so on were collected and identified based on morphology and molecular methods. Chemical investigation of the endophytic fungus *Emericella* sp. (HK-ZJ) isolated from the mangrove plant Aegiceras corniculatum led to isolation of six isoindolones derivatives termed as emerimidine A and B and emeriphenolicins A and D, and six previously reported compounds named aspernidine A and B, austin, austinol, dehydroaustin, and acetoxydehydroaustin, respectively (Zhang *et al.*, 2011). In this paper, the first isolation, structural elucidation and biological evaluation of novel isoindolone derivatives from the fungal endophyte *Emericella* sp. (HK-ZJ) was reported. Amin *et al.* (2014) isolate and identify fungal endophyte from clones cocoa resistant VSD M.05 and clones cocoa susceptible VSD M.01. A total of 10 isolates of fungal endophytewere isolated from clones cocoa resistent VSD M.05. The isolates belonged to 6 genera namely: *Curvularia* sp., *Fusarium* sp., *Geotrichum* sp., *Aspergillus* sp., *Gliocladium* sp., *Colletotrichum* sp.. The fungal endophyte were isolated of clones cocoa susceptible VSD M.01, that as 4 genera identified as *Aspergillus* sp., and *Gliocladium* sp.. The present study of Chobba *et al.* (2013) investigating the diversity of both

cultivable and non-cultivable endophytic fungal floras in the internal tissues (roots and leaves) of Tunisian date palm trees (*Phoenix dactylifera*). Accordingly, 13 isolates from both root and leaf samples, exhibiting distinct colony morphology, were identified by a sequence match search where in their 18S–28S internal transcribed spacer (ITS) sequences were compared to those available in public databases. The cultivable root and leaf isolates mostly fell into *Alternaria* spp., *Fusarium* spp., *Curvularia* spp., *Cladosporium* spp. and *Pythium* spp..

Mycelia sterilia fungi is considerably prevalent in endophyte studies (Lacap *et al.*, 2003). In this study, mycelia sterilia had the highest relative frequency (42.86%). *Fusarium* which are frequently identified as endophytes (Qadri *et al.*, 2013) was the second most frequent endophytic group. Followed by *Xylaria* spp.; *Colletotrichum spp.*; *Phoma* spp. and *Nigrospora* spp. These were the dominant genera or order of endophytic fungi found in this study, similar to the findings reported previously for many tropical endophytic fungi (Rosa *et al.*, 2012; Amin *et al.*, 2014; Frohlich, 1999). All endophytic fungi in this study had isolated from other plants from previous study. *Fusarium* was the common species that isolated as endophytic fungi from many plants. Rosa *et al.* (2012) isolated *Fusarium* spp. from healthy *E. purpurea*. Lumyong *et al.* (2009) isolated endophytic fungi associated with the palms, *Calamus kerrianus* (rattan) and *Wallichia caryotoides* (taorang), *Platanus orientalis* were found to harbor only *Fusarium* spp. *Artemesia annua* also possessed several strains of *Fusarium* as endophytes (5/22) whereas almost half of the endophytes of *Withania somnifera* (4/9) were *Fusarium* spp. Xylariaceous fungi, as endophytic fungi, have most frequently been isolated in the previous studies of palms (Rodrigues & Samuels, 1990; Rodrigues, 1994; Rodrigues & Petrini, 1997; Frohlich *et al.*, 2000) and other plant hosts in tropical regions (Dreyfuss and Petrini, 1984; Pereira *et al.*, 1993; Fisher *et al.*, 1995; Lodge *et al.*, 1996). Many species of *Colletotrichum* spp. had defined to be pathogen, but also many species were reported as endophytic fungi. Frohlich, (1999) obtained *Colletotrichum* sp. as endophytic fungi from *Licuala* sp. palms; Rosa *et al.* (2012) isolated endophytic fungi from *E. purpurea*, also obtained many species of *Colletotrichum* spp.. The diversity of endophytic fungi in leaves, stems and roots from transgenic (Bt) and its isoline (non-Bt) cotton was evaluated (Vieira *et al.*, 2011). The most frequently isolated species were from roots was *Phoma* spp.. Fungal endophytes associated with the palms were investigated and *Phoma* sp., were obtained as endophytic fungi (Lumyong *et al.*, 2009). Many researchers had isolated *Nigrospora* spp. also from many species of plants. Cannon *et al.* (2002) isolated endophytic fungi from living symptomless leaves of 12

tree species from two locations in the Iwokrama Forest Reserve, Guyana and, *Nigrospora* sp. also were identified as endophytic fungi. The phytochemical and biological properties of the endophytic fungus *Nigrospora sphaerica* were examined and Nigrosphaerin A, a new isochromene derivative, along with nineteen known compounds was isolated and identified. Four compounds showed good *in vitro* antileukemic activity; three compounds showed moderate *in vitro* antileishmanial activity; one compound showed good antifungal activity. (Metwaly, 2014).

In this study, 8 endophytes isolates were selected tested in a preliminary confrontation assay including *Pestalotiopsis maculformans, Fusarium falciforme, Phialophora* spp., *Nigrospora sphaerica*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp.. These isolates were tested against plant pathogens *Colletotrichum coffeanum* causing anthracnose of coffee and *Colletotrichum capsici* causing anthracnose of chili by using in an in vitro antagonism experiment bi-culture tests. In the dual culture experiments, all the antagonists gave significantly different from the control. For *Colletotrichum coffeanum, Nigrospora sphaerica* and *Giberrella moniliformis* gave higer significantly inhibition percentage in colony diaemeter which was 37.02, 34.27 %, respectively. But, *Pestalotiopsis maculformans* gave the lowest inhibition percentage of colony diameter which was 25.75%. *Fusarium falciforme* and *Chaetomium aureum* gave the best inhibition of spore production which was 89.99, 77.04%, respectively. But, *Emericella* spp. gave the lowest inhibition percentage of spore prooduction which was 53.08%. For *Colletotrichum capsici, Fusarium falciforme* and *Chaetomium aureum* gave higer significantly inhibition percentage in colony diaemeter which was 60.97, 34.27 %, respectively. But, *Pestalotiopsis maculformans* gave the lowest inhibition percentage of colony diameter which was 30.83%. *Fusarium falciforme* and *Giberrella moniliformis* gave the best inhibition of spore production which was 89.99, 77.04%, respectively. But, *Phialophora* spp. gave the lowest inhibition percentage of spore prooduction which was 13.61%. According all, *Pestalotiopsis maculformans, Fusarium falciforme, Nigrospora sphaerica*, *Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* 6 species showed higher inhibition of *Colletotrichum coffeanum* and *Colletotrichum capsici. Emericella* spp. and *Phialophora* spp. gave the lower inhibition against *Colletotrichum coffeanum* and *Colletotrichum capsici*, respectively. *Fusarium falciforme* showed the best inhibition against pathogen *Colletotrichum coffeanum* and *Colletotrichum capsici*. This study were similar to the study of Qadri *et al.* (2013) who reported that endophytic fungus, *Fusarium*

tricinctum inhibited several phytopathogens significantly. Meca *et al.* (2010) also reported that some strains of *Fusarium tricinctum* are known to produce different enniatins which have strong biological activities including antifungal properties.

Nigrospora sphaerica, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* were extracted their bioactive substances as crude extracts and tested for their abilities to inhibit plant pathogens (*Colletotrichum coffeanum* and *Colletotrichum capsici*) with 6 concentritions (0, 10, 50, 100, 500, 1000ppm) at room temperature. For *Colletotrichum coffeanum*, *Nigrospora sphaerica*, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* gave highest inhibition of colony growth which was 54.00, 63.00, 70.25, 56.00, 69.75 and 59.00%, respectively at crude methanol (1000ppm), crude methanol (500ppm), crude methanol (1000ppm), crude methanol (1000ppm), crude hexane (1000ppm) and crude ethyl acetate (1000ppm), respectively. *Pestalotiopsis maculformans* gave significantly inhibition of colony growth. *Nigrospora sphaerica*, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* gave highest inhibition for the spore production of *C. coffeanum* which was 72.18, 76.74, 63.43, 89.63, 80.69and 78.06%, respectively at crude methanol (1000ppm), crude methanol (500ppm), crude methanol (1000ppm), crude methanol (1000ppm), crude methanol (50ppm) and crude ethyl acetate (1000ppm), respectively. Crude methanol of *Chaetomium globosum* gave highest significantly inhibition for the spore production of *C. coffeanum* with the ED_{50} at concentration 65.52 ppm, followed by, crude methanol of *Chaetomium aureum* and *Giberrella moniliformis* gave significantly inhibition for the spore production of *C. coffeanum* with the ED_{50} at concentration 30.54 and 213.69 ppm. For *Colletotrichum capsici*, *Nigrospora sphaerica*, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* gave highest inhibition of colony growth which was 52.25, 34.50, 72.50, 75.00, 82.25 and 70.00%, respectively at ethyl acetate (1000ppm), crude methanol (1000ppm), crude methanol (1000ppm), crude ethyl acetate (1000ppm), crude methanol (1000ppm) and crude ethyl acetate (1000ppm), respectively. *Chaetomium aureum* gave significantly inhibition of colony growth. *Nigrospora sphaerica*, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Chaetomium globosum*, *Chaetomium aureum* and *Giberrella moniliformis* gave highest inhibition for the spore production of *C. capsici* which was 87.26, 74.52, 79.17, 85.89, 77.91and 77.17%, respectively at crude

methanol (500ppm), crude methanol (1000ppm), crude methanol (1000ppm), crude ethyl acetate (1000ppm), crude methanol (50ppm) and crude ethyl acetate (1000ppm), respectively. Crude methanol of *Nigrospora sphaerica* gave highest significantly inhibition for the spore production of *C. coffeanum* with the ED_{50} at concentration 41.51 ppm, followed by, crude methanol of *Chaetomium globosum* and *Chaetomium aureum* gave significantly inhibition for the spore production of *C. coffeanum* with the ED_{50} at concentration 87.26 and 33.06 ppm. Totally, *Chaetomium globosum, Chaetomium aureum* showed significantly inhibition of pathogen *Colletotrichum coffeanum* and *Colletotrichum capsici*. This result similar with the research of Kumar *et al.* (2013) which reported that extracts of *Chaetomium globosum* EF18, isolated as endophytic fungus from Withania somnifera, were found effective against *Sclerotinia sclerotiorum*. Ethyl acetate and methanol extracts were more effective than hexane extract showing >80% growth inhibition. Bioactive compound (antibiotic Sch 210971, m/z 445 and λ max 290) having antifungal activity against *S. sclerotiorum* has been isolated in pure from the ethyl acetate extract following bioassay guided fractionation. Apart from this compound other fractions of polar to medium polarity were also found effective. Fraction no. VIII from VLC (Vacuum liquid chromatography) column of ethyl acetate extract was most active having IC_{50} value 35.4 µg/ml. In Pablo *et al.* (2015) study, endophyte isolates were selected from a collection of 546 fungi tested in a preliminary confrontation assay and tested against *F. circinatum* in an in vitro antagonism experiment. In total, 138 endophytes displayed antagonistic activity towards *F. circinatum* in the dual cultures of the in vitro experiment. In the field test, the endophytes *Chaetomium aureum* and *Alternaria* sp. reduced the area under disease progress curve (AUDPC) for the *P. radiata* seedlings, indicating that they may therefore be suitable for use as biological control agents (BCAs) of the disease. Fungi of the *Chaetomium* species, which belong to the family Chaetomiaceae, are the largest genus of saprophytic ascomycetes, with more than 350 *Chaetomium* species. Up to now, more than 200 metabolites with a wide range of bioactivities have been isolated from the genus *Chaetomium*, but compared with its richness of species, more bioactive secondary metabolites might be found in this fungus (Zhang *et al.,* 2012). Nigrosphaerin A, a new isochromene derivative (**1**), was isolated from the endophytic fungus *Nigrospora sphaerica* and chemically identified as 3-(3,4-dihydroxyphenyl)-4,6,8-trihydroxy-1H-isochromen-1-one-6-O-β-d-glucopyranoside. In addition nineteen known compounds (**2–20**) were isolated from the same fungus and chemically identified. Compounds (**1–3**, **5**, and **7–16**) were isolated for the first time from this fungus. *In*

vitro antileukemic, antileishmanial, antifungal, antibacterial and antimalarial activities of (**1–20**) were examined. Compounds **5**, **7**, **9** and **10** showed good antileukemic activity against HL60 cells with IC_{50} values of 0.03, 0.39, 0.2 and 0.4 μg/mL, respectively and against K562 cells with IC_{50} values of 0.35, 0.35, 0.49 and 0.01 μg/mL, respectively. Compounds **3**, **4** and **6** showed moderate antileishmanial activity with IC_{50} values of 30.2, 26.4 and 36.4 μg/ml, respectively. Compound **7** showed moderate antifungal activity against *Cryptococcus neoformans* with IC_{50} value of 14.8 μg/mL (Metwaly, 2014). The study of Qadri *et al.* (2013) was conducted to characterize and explore the endophytic fungi of selected plants from the Western Himalayas for their bioactive potential. All the endophytes were evaluated for antimycotic activity against a panel of seven important plant pathogens. 6 isolates (DEF3, WEF1, WEF2, Art, Art2 and Art9) were found highly active inhibiting five of the seven plant pathogens which belonging genera, *Talaromyces sp., Giberella sp., Cochliobolus sp., Fusarium sp.* and *Alternaria sp.* Among these were also the isolates, Art, Art2 and Art 9, the only strains active against *C. albicans*.

CHAPTER 6

CONCLUSION

Fifteen species of endophytic fungi were isolated and identified from leaves, petioles and roots of 10 species palm trees which belong to *Fusarium* spp., *Xylaria* spp., *Cladosporium* spp., *Phialophora* spp., *Pestalotiopsis* spp., *Rhizoctonia* spp., *Colletotrichum spp.*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella* spp., *Emericella* spp., *Aspergillas* spp., *Curvalaria lunata*, *Phoma* spp. and *Nigrospora* spp.. 45 unidentified isolates were group to mycelia sterilia fungi which was morphological fungal types, but not forming true spores. Mycelia sterilia fungi had the highest relative frequency (42.86%) in this study and *Fusarium* was the second most frequent endophytic group, followed by *Xylaria* spp.; *Colletotrichum spp.*; *Phoma* spp. and *Nigrospora* spp.

According to identification, which based on morphology and molecular phylogeny on the basis of ITS1-5.8S-ITS4 ribosomal gene sequence acquisition and analyses, endophytic isolates 17-6 (*Nigrospora* spp.); 5r-1 (*Fusarium* spp.); 4r-3 (*Pestalotiopsis spp.*); 22s-2 (*Giberrella* spp.); 7s-1 (*Chaetomium globosum*) were identified as *Nigrospora sphaerica*, *Fusarium falciforme*, *Pestalotiopsis maculformans*, *Giberrella moniliformis* and *Chaetomium globosum* when compared to those available in public database.

Eight isolates *Pestalotiopsis maculformans, Fusarium falciforme, Phialophora* spp., *Nigrospora sphaerica*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella moniliformis* and *Emericella* spp. were selected to test in preliminary screening of bioactivities experiments against plant pathogens *Colletotrichum coffeanum* isolated from coffee leaves with anthracnose disease and *Colletotrichum capsici* isolated from chili fruit with anthracnose symptoms by bi-culture method. All of these endophytes showed inhibition against pathogens. *Nigrospora sphaerica* gave highest significantly inhibition percentage in colony growth of *Colletotrichum coffeanum* and *Fusarium falciforme* gave highest significantly inhibition percentage in colony growth of *Colletotrichum capsici; Fusarium falciforme* showed the best inhibition for the spore production of both *Colletotrichum coffeanum* and *Colletotrichum capsici*.

Sixth species were selected from the results of preliminary screening of bioactivities experiments used to extract biological activity substances, which were *Nigrospora sphaerica*,

Fusarium falciforme, Pestalotiopsis maculformans, Chaetomium globosum, *Chaetomium aureum* and *Giberrella moniliformis*. Crude methanol from *Nigrospora sphaerica* gave highest inhibition of *C. coffeanum* colony growth and spore production, also showed highest inhibition of spore production of *C. capsici.* Crude ethyl acetate of *Nigrospora sphaerica* gave significantly highest inhibition of colony growth of *C. capsici*. But, crude hexane of *Nigrospora sphaerica* showed lower inhibition of colony growth and spore production against both *C. coffeanum* and *C. capsici*. Methanol cude extract from *Fusarium falciforme* gave significantly highest inhibition of colony growth and spore productionof both *C. coffeanum* and *C. capsici*. But, crude ethyl acetate extract of *Fusarium falciforme* also showed good inhibition to the spore production of *C. coffeanum* and *C. capsici*. Crude methanol extract from *Pestalotiopsis maculformans* gave significantly highest inhibition of colony growth and spore productionof both *C. coffeanum* and *C. capsici*, followed by crude ethyl acetate extract, also showed higher inhibition of colony growth and spore production of pathogens. Methanol crude extract from *Chaetomium globosum* gave significantly highest inhibition of *C. coffeanum* colony growth and spore production, but ethyl crude extract showed highest inhibition of *C. capsici* colony growth and spore production. Hexane crude extract from *Chaetomium aureum* gave significantly highest inhibition of *C. coffeanum* colony growth, but, methanol crude extract showed highest inhibition of *C. coffeanum* spore production and *C. capsici* colony growth and spore production. Crude ethyl acetate extract from *Giberrella moniliformis* gave highest inhibition of *C. coffeanum* colony growth and spore production, and also showed highest inhibition of *C. capsici* spore production; the crude methanol extract from *Giberrella moniliformis* expressed highest inhibition percentage of *C. capsici* colony growth.

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APPENDIX

PUBLICATIONS

Song JiaoJiao, Pongnak Wattanachai and Soytong Kasem (2015). Biological activity of endophytic fungi associated with palm trees. Journal of Agricultural Technology Vol. 11(2): 567-579.

Song JiaoJiao, Pongnak Wattanachai and Soytong Kasem (2015). Biological activity of endophytic fungi from palm trees against chili anthracnose caused by Colletotrichum capsici. Journal of Agricultural Technology. 11(8): 1819-1832.

Song JiaoJiao, Pongnak Wattanachai and Soytong Kasem (2016). Isolation and identification of endophytic fungi from 10 species palm trees. Journal of Agricultural Technology. 12(2): 349-363.

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Publications

International Conferences

①. 2013, 28-29 November, attend **2nd International Conference on Integration of Science and Technology for Sustainable Development** as an oral presentation entitled "Antifungal activity of limonoids from Harrisonia perforate against some plant pathogenic fungi", held at KMITL, Bangkok, Thailand.

②. 2014, 27-28 November, attend **3rd International Conference on Integration of Science and Technology for Sustainable Development** as a poster presentation entitled "Diversity of endophytic fungi associated with palm trees", held at Champasack Grand Hotel, Pakse, Champasack, Lao PDR.

③. 2015, 27-28 November, attend **4th International Conference on Integration of Science and Technology for Sustainable Development** as an oral presentation entitled "Biological activity of endophytic fungi from palm trees against chili anthracnose caused by *Colletotrichum capsici*", held at Center for Woman and Development- CWD Hotel, Hanoi, Vietnam.

International Publications:

①. Song Jiao Jiao and Yan Xiao Hui (2013). **Antifungal activity of limonoids from *Harrisonia perforate* against some plant pathogenic fungi.** Journal of Agricultural Technology Vol. 11(2): 567-579.

②. Song JiaoJiao, Pongnak Wattanachai and Soytong Kasem (2015). Biological activity of endophytic fungi associated with palm trees. Journal of Agricultural Technology Vol. 11(2): 567-579.

③. Song JiaoJiao, Pongnak Wattanachai and Soytong Kasem (2015). Biological activity of endophytic fungi from palm trees against chili anthracnose caused by Colletotrichum capsici. Journal of Agricultural Technology. 11(8): 1819-1832.

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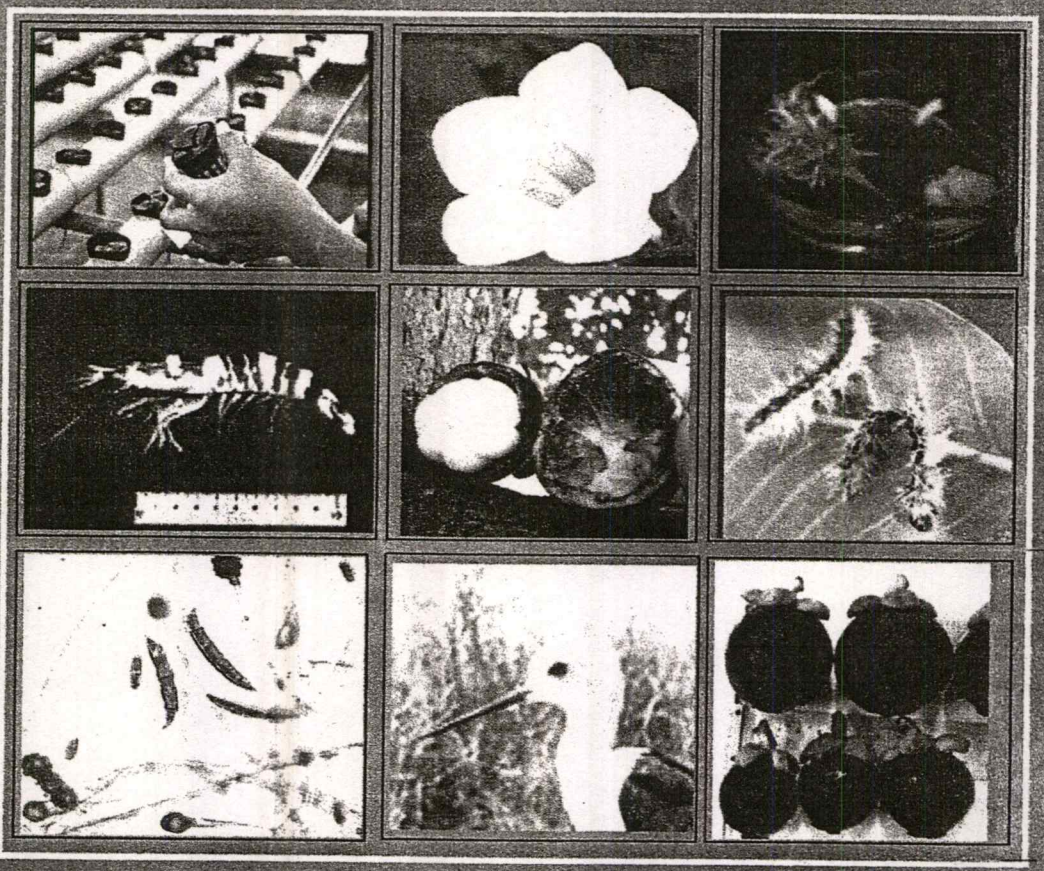

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Biological activity of endophytic fungi associated with palm trees

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Song, JiaoJiao , Pongnak Wattanachai and Soytong Kasem (2015). Biological activity of endophytic fungi associated with palm trees. Journal of Agricultural Technology Vol. 11(2): 567-579

Endophytic fungi were isolated from leaves, petioles and roots of palm trees in King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, and tested for biological activity test. A total of 60 isolates were obtained and morphological identification. *Phoma exigua.*, *Fusarium chamydosporum*, *Phialophola* spp. and *Nigrospora* spp. were examined for bi-culture antagonisict test against *Colletotrichum coffeanum* causing coffee Anthracnose. The result showed that all of these 4 species of endophytics exptressed the antifungal activity inhibited colony growth and spore production of *colletotrichum coffeanum*, which were 6.68cm, 5.89cm, 6.25cm, 5.66cm, in colony diadmeter and 0.875×10^6, 0.375×10^6, 1.125×10^6, 1.0625×10^6 in spore numbers. And, the inhibition percentage of colony growth were 25.75%, 35.28%, 30.55%, 37.02%, respectively, the inhibition percentage of spore production were 76.66%, 89.99%, 69.99%, 71.66% respectively. *Nigrospora spp.* showed the best inhibition percentage of colony growth, and, *Fusarium chamydosporum* gived the best inhibition percentage of spore production.

Key words: endophytic fungi, palm trees, *Colletotrichum coffeanum*, bi-culture antagonistic test

Introduction

Endophytes are contained within the plant without disease and plant tissues remain entire and functional. A wide range of plants have now been examined for endophytes, and endophytes have been found in nearly all of them. An enormous number of different fungi can be isolated from plants growing in their native habitat. Most of the fungi are uncommon and narrowly distributed, taxonomically and geographically. However a few fungi are widely

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distributed with the host, suggesting a long standing, close and mutually beneficial interaction.

Endophytic fungi have attracted great attention in the past few decades due to its ability to produce novel secondary metabolites for medical, agricultural and industrial use. And they are also considered as an outstanding source of bioactive compounds due to its ability to occupy any plants at any environments (Strobel and Daisy, 2003).

Palm trees are a botanical family of perennial lianas, shrubs, and trees. They are in the family Arecaceae (Due to historical usage, the family is alternativel called Palmae or Palmaceae). They are flowering plants, the only family in the monocot order Arecales. Roughly 202 genera with around 2600 species are currently known, most of them restricted totropical, subtropical, and warm temperate climates. Most palms are distinguished by their large, compound, evergreen leaves arranged at the top of an unbranched stem. Palms are one of the best known and most widely planted tree families. They have held an important role for humans throughout much of history. Many common products and foods come from palms. They are often used in parks and gardens that are in areas that do not have heavy frosts.

Nowadays, many endophytic fungi associated with palms had reported, including temperature palms and tropic palms (Rodrigues and Samuels, 1990; Fröhlich and Hyde, 2000; Hyde *et al.*, 2000). Frohlich *et al.* (2000) reported endophytic fungi from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia and got 75 fertile species in 48 genera and 60 sterile morphospecies including 10 *Xylaria* anamorphs, *Phomopsis* sp., *Phoma* sp., *Trichoderma* sp., *Colletotrichum* sp., *Pestalotiopsis palmarum*., *Lasiodiplodia* sp. *Hyphomycete* sp., *Nodulisporium* sp., *Dictyochaeta* sp., *Phyllosticta* sp., *Distocercospora* sp., *Verticillium* sp., *Coelomycete* sp., *Aspergillus niger*, *Beltraniella* spp., *Botrytis allii*. The endophyte communities of both palms were composed of a single, dominant xylariaceous species.

Several studies on the use of bioactive compounds from endophytic fungi have been reported. Endophytic fungi are able to produce antimicrobial, anticancer such as Taxol (Walker and Croteau, 2001) and antimalarial activities (Wiyakrutta *et al.*, 2004). Study done by Woropong *et al.* (2001) showed that isolated endophytic fungi are able to produce mixture of volatile organic compounds that are lethal to human and plant pathogenic fungi and bacteria.

The natural and biological control of pests and diseases affecting cultivated plants has gained much attention in the past decades as a way of reducing the use of chemical products in agriculture. Vega *et al.*, (2008) studied fungal endophyte - mediated plant defense as a novel biological control

mechanism against the coffee berry borer the most devastating pest of coffee throughout the world, A survey of fungal endophytes in coffee plants has revealed the presence of various genera of fungal entomopathogens including *Acremonium, Beauveria, Cladosporium, Clonostachys,* and *Paecilomyces*. Two of these *B. bassiana* and *Clonostachys rosea* were tested against the coffee berry borer and were shown to be pathogenic.Antifungal products are vastly produced by majority of the endophytes. Griseofulvin-producing endophyte was first reported in fungus from *Abies holophylla* and was evaluated *in vivo* antifungal activity against plant pathogenic fungi (Park *et al.*, 2004).

The purpose of this reseach focused on isolation of endophytic fungi from palm trees and their bioactivity against *Colletotrichum coffeanum* causing anthracnose of Arabica coffee using bi-culture antagonist test.

Materials and methods

Collection of plant samples

Plant samples were randomly collected from 10 species healthy palms:- Ptychosperma macarthuri (MacArthur Palm); Elaeis guineenis (African Oil Palm); Rhapis humilis (Slender Lady Palm); Wodyetia bifurcata (Foxtail Palm); Chrysalidocarpus lotescens; Cocos nucifera (Coconut); *Rhapis Laosensis* (Thailand Lady Palm); *Licula grandis* (Fan Palm); *Livistona chinensis* (Chinese Fan Palm) and *Mascarena Lagencuulis* (Bottle Palm) at King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand. All the samples were randomly selected plants of healthy leaves, petioles and roots then cut off and taken to laboratory and processed within 24 h.

Isolation and identification of endophytic fungi

Plant specimens were thoroughly washed in running tap water for 5 minutes to remove dust and debris and then air dried. The cleaned leaves, petioles and roots were surface sterilized with 75% ethanol 1 min and 10% sodium hypochlorite 3-5min, and then, cut into small pieces(3×3×3mm) under sterile conditions (petioles and roots were removed outer epidermal tissues and cuticle before cut into small pieces). Briefly, fragments were cleaned in sterilized water and sterilized with 75% ethanol 30s and then cleaned in sterilized water again and placed on water agar(WA) medium, incubated at room temperature. The endophytic fungi growing out from the plant tissue were transferred into potato dextrose agar (PDA) plates and incubated for two to six days for observation. Continuous plates were subculture until get pure culture.

All the isolates were identified by the morphology of the fungal culture, the mechanism of spore production and characteristics of the spores . The sterile isolates were grown on PDA with decoction of host leaf medium to observe sporulation. For tentative identification, microscopic slides of each endophytic fungi were prepared and examined under binocular compound microscope for morphological identification.

Isolation of pathogen and pathogenicity test

The plant pathogen *Colletotrichum coffeanum* causing coffee anthracnose in Arabica variety were isolated by tissue transplanting technique from the leaf with obvious symptoms. The disease leaves were cleaned with running tap water and after air-dry cut the advance margin of symptom between healthy tissue and diseased tissue to small pieces and then sterilized with sterilized water, 75% alcohol and sterilized water again. Then, transferred onto WA medium and followed by potato dextrose agar (PDA) to obtain pure culture. *Colletotrichum coffeanum* were identified by morphological characteristic under binocular compound microscope.

The pathogen was tested for pathogenicity using detached leaf method in the laboratory. Select healthy leaves of coffee and washing in the running water and air-dried. A sterilized filter paper was placed in 9cm diameter sterilized petri dish and two sterilized microslide were also put on the filter paper, and the filter paper were moistened by sterilized distilled water. Coffee leaves were wounded by sterilized needle and then placed on the microslide in the petri dish then the spore surspention of *Colletotrichum coffeanum* was prepared at concentration is 1×10^6 spores/ml. Spore surspention were sprayed on the surface of coffee leaves including the wounded areas, incubated for two weeks at room temperature. At the same time, sterilized water was also sprayed to coffee leaves as controls and incubated. Lesions on inocultated areas were obsereved on the coffee leaves, then re-isolated pathogen from lesion invaded with inoculated pathogen according to the above mentioned method and identified the re-isolates under microscope and get pure culture.

Bi-culture antagonistic tests

Some isolates of endophytics were tested for antagonistic bi-culture. These isolates were tested to determine their bioactivity against *Colletotrichum coffeanum.* The experiment was conducted using a Completely Randomized Design (CRD) with 4 replications by the methods of Soytong (1992), Sibounnavong *et al.* (2009) and Charoenporn *et al.* (2010). The antagonistic fungi and pathogen were separately cultured on PDA at room temperature (30-

32 °C) for 7 days. And 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of cultures of the antagonistic fungi and pathogen and then transferred onto the same sterilized 9 cm-diameter PDA plates, an agar plug of the pathogen was placed on one side of the plate which opposited an agar plug of an antagonistic fungus. The single plug of antagonistic fungi and pathogen were transferred into two separate PDA plates as the controls. And then, all the plates were incubated at room temperature (30-32 °C) for 30 days. Data were collected regarding to diameter of colony (cm) and the number of conidia produced by the pathogen in the bi-culture plates and control plates. A haemcytometer was used to count the number of conidia of pathogen.

Percentage inhibition of pathogen colony growth and conidia production were calculated using the following formula: % inhibition = (A-B) / A ×100. Where, A is the diameter of colony or number of conidia produced by the pathogen on the control plates and B is the diameter of colony or number of conidia produced by the pathogen in the bi-culture plate.

Analysis of variance was statistically computed and treatment means were compared using Duncan Multiple's Range Test (DMRT) at P = 0.05 and 0.01.

Results and discussion

Isolation and identification of endophytic fungi

Ten species of endophytic fungi were found including *Cladosporium* spp., *Phialophora* spp., *Pestalotiopsis* spp., *Phoma* spp., *Phomopsis* spp., *Nigrospora* spp., *Xylaria* spp., *Fusarium* spp., *Colletotrichum* spp. and *Rhizoctonia* spp., and other 20 isolates belonging to mycelia sterilia fungus .(Table 1)

A total of 65 isolates, 41.5% isolates isolated from leaves, 21.5% isolates isolated from petioles and 37.0% isolates isolated from roots. The leaf of palm can habor more endophytic fungi than petiole and root. And in this study showed higher frequency (30.7%) of sterilia fungus were isolated from palm trees. Four species of endophytic fungi were selected and tested antagonists by bi-culture method as follows: *Phoma exigua*, *Fusarium chamydosporum*, *Phialophora* spp. and *Nigrospora* spp. (Fig.1-4).

Table 1. Isolates of endophytic fungi from different parts of 10 palm trees

Endophytic fungi	Isolates			Total
	Leaf	*Petiole*	*Root*	
Cladosporium spp.	-	2	1	3 (4.6%)
Phialophora spp.	1	-	3	4 (6.2%)
Pestalotiopsis spp.	1	-	1	2 (3.1%)
Phoma spp.	2	1	2	5 (7.7%)
Phomopsis spp.	3	1	1	5 (7.7%)
Nigrospora spp.	3	-	2	5 (7.7%)
Xylaria spp.	3	1	-	4 (6.2%)
Fusarium spp.	2	1	4	7(10.8%)
Colletotrichum spp.	1	-	2	3 (4.6%)
Rhizoctonia spp.	2	3	2	7(10.8%)
Sterilia fungus	9	5	6	20(30.7%)
Total	27 (41.5%)	14 (21.5%)	24 (37.0%)	

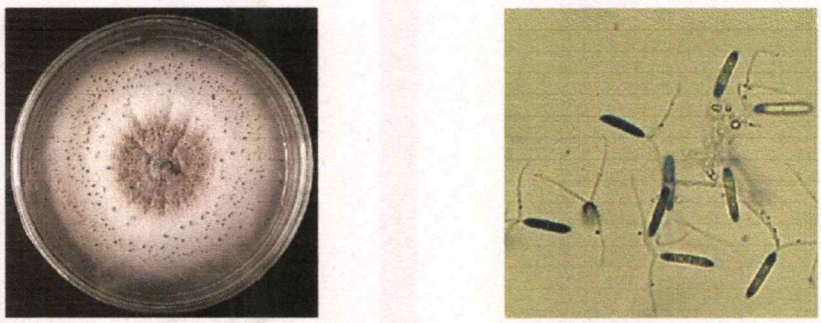


Fig. 1. *Phoma exigua* pure culture and spore

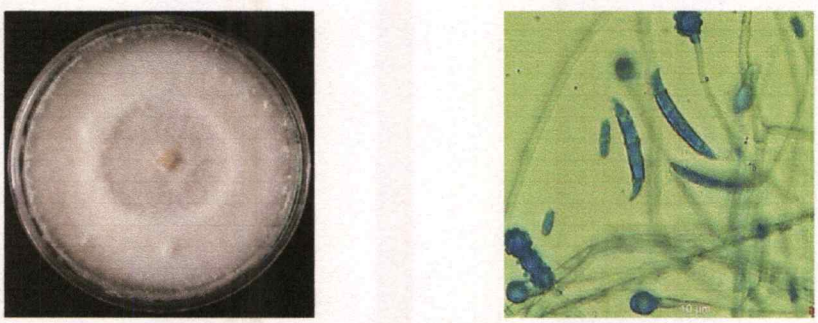


Fig. 2. *Fusarium chamydosporum* pure culture and spore

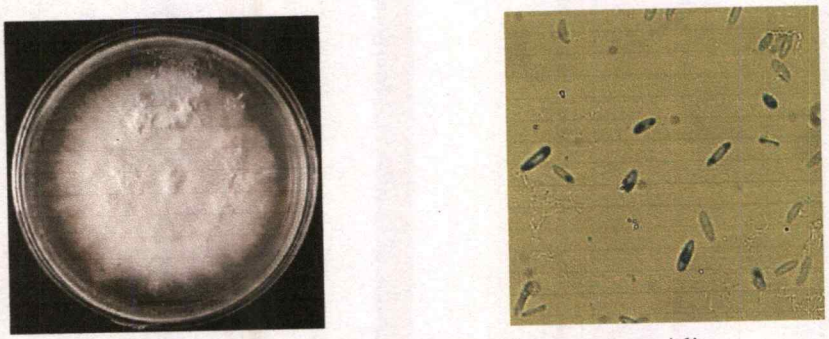


Fig. 3.*Phialophora spp.* pure culture and conidia

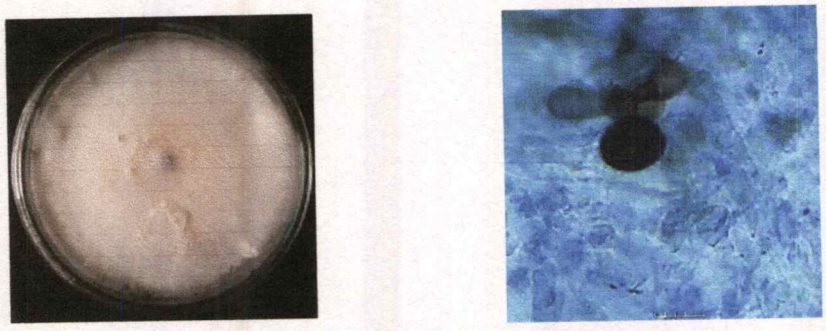


Fig.4. *Nigrospora spp.* pure culture and sporangiospore

Isolation of pathogen and pathogenicity test

Colletotrichum coffeanum were isolated from anthracnose of Arabica coffee leaves and identified from coffee leaves with obvious symptom of coffee anthracnose to pure culture (Fig.5). The isolate was confirmed pathogenic isolate from patyhogenecity test (Fig. 6). The result showed that pathogenic isolate could be infected in the coffee leaf and caused symtomp with the same symptom caused by *Colletotrichum coffeanum* causing leaf anthracnose on coffee leaves.

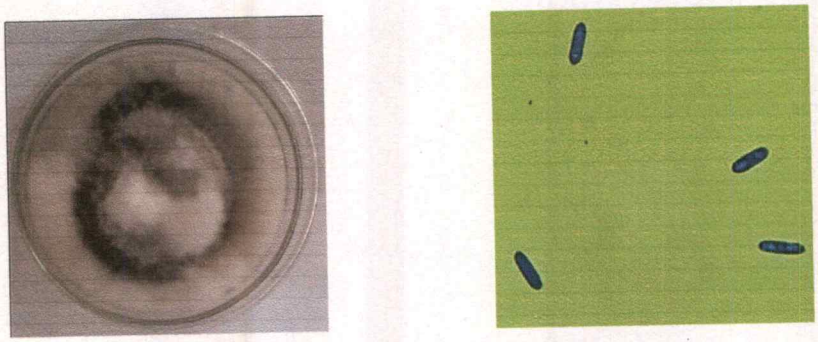
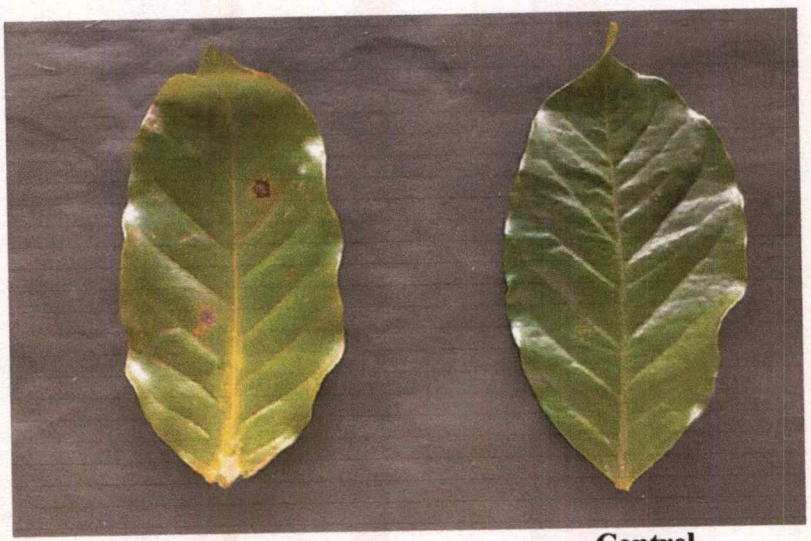


Fig.5 *Colletotrichum coffeanum* pure culture and spore



Treatment **Control**

Fig. 6. Pathogenicity test on coffee leaves

Bi-culture antagonistic tests

Phoma exigua, *Fusarium chamydosporum*, *Phialophora* spp. and *Nigrospora* spp. were proved their abilities to inhibit the growth of *Colletotrichum coffeanum* by using bi-culture tests. (Fig.7).

Fig. 7. Bi-culture antagonistic tests

The results showed that all the endophytics *Phoma exigua*, *Fusarium chamydosporum*, *Phialophora* spp. and *Nigrospora* spp. gave significantly inhibition of *Colletotrichum coffeanum* which were 6.68cm, 5.89cm, 6.25cm, 5.66cm in colony diameter, respectively when compared to the control plate (Table 2). *Nigrospora* spp. showed higher inhibition percentage of colony

diaemeter which was 37.02 % than *Fusarium chamydosporum*, *Phialophora* spp. which were 35.28% and 30.55%, respectively. But, *Phoma exigua* gave lowest inhibition percentage of colony diameter which was 25.75% (Fig. 8). This result was similar to the study from Luiz H. Rosa *et al.* (2012) who reported that the extracts of endophytic fungi, *Fusarium* spp. was able to inhibit the phytopathogens, *Colletotrichum acutatum, C. fraga- riae, and C. gloeosporioides* and endophytic *Nigrospora* spp also can inhibited some plant pathogen, *Colletotrichum* spp.

Table. 2. Colony growth on antagonistic bi-culture tests

Antagonist fungi	***Colletotrichum coffeanum***	
	Colony(cm)	% inhibition of colony
Control	$9.00^{a1/}$	-
Nigrospora spp.	5.66^{d}	37.02^{a}
Fusarium chamydosporum	5.82^{d}	35.28^{a}
Phoma exigua	6.68^{b}	25.75^{c}
Phialophora spp.	6.25^{c}	30.55^{b}
CV%	1.77	4.57

1/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01

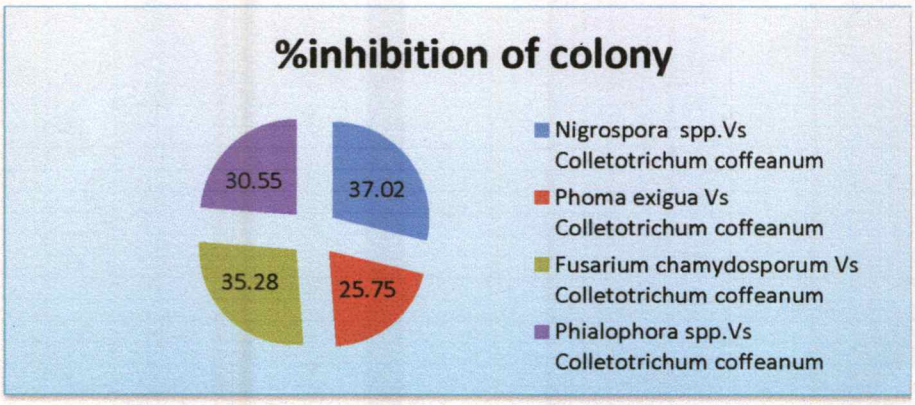


Fig. 8 Percentage inhibihion of colony diameter

The number of spores that producing by the pathogen was counted by using Hemacytometer. The results showed that *Phoma exigua*, *Fusarium chamydosporum*, *Phialophora* spp. and *Nigrospora* spp. significantly inhibited

number of pathogen spores which were 0.87×10^6, 0.37×10^6, 1.12×10^6, 1.06×10^6 spores, respectively when compared to the control plate (Table 3). The pathogen spore production was inhibited 76.66 %, 89.99%, 69.99% and 71.66% respectively (Fig.9). It is illustrated that all the tested endophytic fungi showed inhibition of spore production of *Colletotrichum coffeanum* and *Fusarium chamydosporum* gave the best inhibition of spore production. Meca *et al.* (2010) reported that some strains of *Fusarium tricinctum* are known to produce different enniatins which have strong biological activities including antifungal properties. This study were similar to the study of Masroor Qadri *et al.* (2013) who reported that endophytic fungus, *Fusarium tricinctum* inhibited several phytopathogens significantly.

Table. 3. Number of pathogen spores on antagonistic bi-culture tests

Antagonist fungi	*Colletotrichum coffeanum*	
	Spores($\times10^6$)	% inhibition of number spores
Control	$3.75^{a2/}$	-
Nigrospora spp.	1.0625^{b}	71.66^{ab}
Fusarium chamydosporum	0.375^{c}	89.99^{a}
Phoma exigua	0.875^{bc}	76.66^{ab}
Phialophora spp.	1.125^{b}	69.99^{b}
CV%	19.95	11.09

2/ Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01

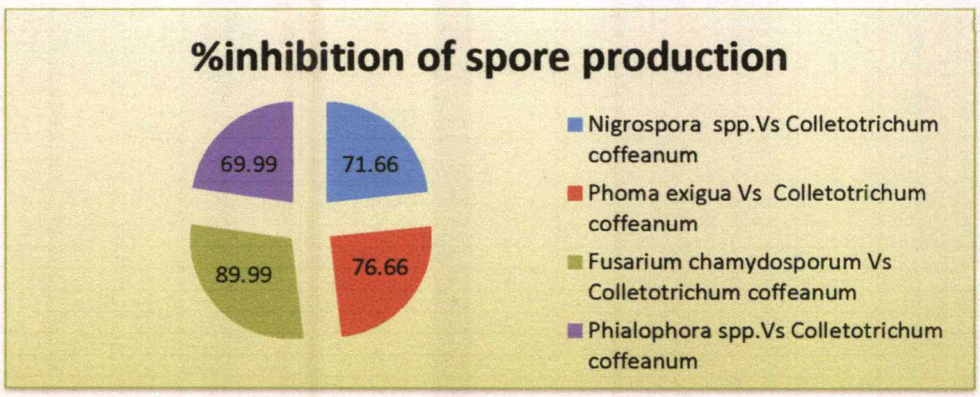


Fig. 9 Percentage inhibition of pathogen spore production

Conclusion

In this study revealed that endophytic fungi isolaed from palms can grow in any parts of palm trees. The tested endophytic fungi (Phoma *exigua*, *Fusarium chamydosporum*, *Phialophora* spp. and *Nigrospora* spp.) were proved biological activity against *Colletotrichum coffeanum* causing coffee anthracnose. The results illustrated that *Nigrospora* spp. showed the best inhibition of colony growth, *Fusarium chamydosporum* gave the best inhibition of spore production of *Colletotrichum coffeanum.*

Acknowledgement

I would like to thank the Faculty of Agriculture Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand for supporting me to study master degree and thanks to Dr. Kasem and Dr. Wattanachai who sincerely guiding my research. And thanks to Sir Jojo helping me solve the problems during this research.

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Biological activity of endophytic fungi from palm trees against chili anthracnose caused by *Colletotrichum capsici*

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Song J.J., Pongnak W. and Soytong K. (2015). Biological activity of endophytic fungi from palm trees against chili anthracnose caused by *Colletotrichum capsici*. Journal of Agricultural Technology. 11(8): 1927-1940.

Endophytic fungi are those living inside the host plant without causing any apparent negative effect on host plant. Endophytic fungi from palm trees in King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, were isolated and identified by morphology. Crude hexane, ethyl acetate and methanol. of endophytic *Fusarium* spp. isolated from *Mascarena Lagencuulis* and *Nigrospora* spp. isolated from *Chrysalidocarpus lotescens* were yielded and examined for bioactivity test against *Colletotrichum capsici* causing chili anthracnose. The results showed that crude methanol from *Nigrospora* spp., and *Fusarium* spp. gave the highest inhibition of colony growth of *C. capsici*, which were 48.75% and 34.50%, respectively at concertration of 1000 ppm. Crude methanol from *Nigrospora* spp. gave significantly highest inhibition of spore production of *C. capsici* as 87.26% at concentration of 500 ppm and crude methanol from *Fusarium* spp. showed highest inhibition of spore production as 74.52% at concentration of 1000 ppm. The methanol crude extract from *Nigrospora* spp. expressed the ED_{50} value of 41.51 ppm and ethyl acetate crude extract from *Fusarium* spp. expressed the ED_{50} of 42.40 ppm to inhibit *C. capsici*. The research findings are reported that the metabolites from *Nigrospora* spp. and *Fusarium* spp. inhibited *C. capsici*.

Key words: Endophytic fungi; palm trees; bioactivity test; chili; *Colletotrichum capsici*

Introduction

Endophytes are microorganisms that live within plants for at least a part of their life cycle without causing any visible manifestation of disease (Bacon and white, 2000). A wide range of plants have now been examined for endophytes, and endophytes have been found in nearly all of them, including trees, grass, algae and herbaceous plants. An enormous number of different fungi can be isolated from plants growing in their native habitat. Most of the fungi are uncommon and narrowly distributed, taxonomically and geographically. However a few fungi are widely distributed with the host, suggesting a long standing, close and mutually beneficial interaction. Endophytic fungi have attracted great attention in the past few decades due to its ability to produce novel secondary metabolites for medical,

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agricultural and industrial use. And they are also considered as an outstanding source of bioactive compounds due to its ability to occupy any plants at any environments (Strobel and Daisy 2003). Endophytic fungi are asymptomatic and may be mutualistic; plants protect and feed endophytes, which produce plant-growth-regulatory, antimicrobial, antiviral or insecticidal substances to enhance the growth and competitiveness of the host in nature (Carroll, 1988). Some endophytic fungi are known as reliable sources of bioactive substances with agricultural and/or pharmaceutical potential, as exemplified by taxol (Stierle *et al.*, 1993; Wang *et al.*, 2000), subglutinol A and B (Lee *et al.*, 1995), and peptide leucinostatin A (Stroble and Hess, 1997). Endophytic fungi are thus expected to be potential sources of new bioactive agents.

Several studies on the use of bioactive compounds from endophytic fungi have been reported. Endophytic fungi are able to produce antimicrobial, anticancer such as Taxol (Walker and Croteau 2001) and antimalarial activities (Wiyakrutta *et al.* 2004). Study done by Woropong *et al.* (2001) showed that isolated endophytic fungi are able to produce mixture of volatile organic compounds that are lethal to human and plant pathogenic fungi and bacteria.

The natural and biological control of pests and diseases affecting cultivated plants has gained much attention in the past decades as a way of reducing the use of chemical products in agriculture. Vega *et al.*, (2008) studied fungal endophyte - mediated plant defense as a novel biological control mechanism against the coffee berry borer the most devastating pest of coffee throughout the world, A survey of fungal endophytes in coffee plants has revealed the presence of various genera of fungal entomopathogens including *Acremonium, Beauveria, Cladosporium, Clonostachys,* and *Paecilomyces*. Two of these *B. bassiana* and *Clonostachys rosea* were tested against the coffee berry borer and were shown to be pathogenic.Antifungal products are vastly produced by majority of the endophytes. Griseofulvin-producing endophyte was first reported in fungus from *Abies holophylla* and was evaluated *in vivo* antifungal activity against plant pathogenic fungi. (Park *et al.*, 2004).

Nowadays, many endophytic fungi associated with palms had reported, including temperature palms and tropic palms (Rodrigues and Samuels 1990; Fröhlich and Hyde 2000; Hyde *et al.* 2000). Jane Frohlich *et. al.*(2000) reported endophytic fungi from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia and got 75 fertile species in 48 genera and 60 sterile morphospecies including 10 *Xylaria* anamorphs, *Phomopsis* sp., *Phoma* sp., *Trichoderma* sp., *Colletotrichum* sp., *Pestalotiopsis palmarum.*, *Lasiodiplodia* sp. *Hyphomycete* sp., *Nodulisporium* sp., *Dictyochaeta* sp., *Phyllosticta* sp., *Distocercospora* sp., *Verticillium* sp., *Coelomycete* sp., *Aspergillus niger*, *Beltraniella* spp., *Botrytis allii.* The endophyte communities of both palms

were composed of a single, dominant xylariaceous species.

Chili (*Capsicum* spp.) is the fourth important vegetable in the world and the first in Asia, with world production in 2006 at approximately 25.9 million t for fresh chili and 2.8 million t for dry chili (FAOSTAT, 2008).Anthracnose, caused by *Colletotrichum* spp., is a serious problem for chili production in the tropics and subtropics worldwide. The pathogenic fungi *C. capsici* were reported as chili anthracnose in Thailand (Sangchote *et al.*, 1998; Sangchote, 1999). This fungus is one of the major diseases for chili and causes severe damage on chili fruits in both pre and post harvest stages. And, these infections together account for more 50% of the crop losses (Pakdeevaraporn *et al.*, 2005).The fungus is both internally and externally seed-borne (Ramachandran *et al.*, 2007). The black wound found on infected fruits will expand very quickly under high moisture condition, especially in tropical countries. Fruiting bodies and spores of *C. capsici* will be abundantly produced on those black lesions.

Use of chemical for control plant disease is one of the most commonly used strategy usually what farmers followed, but nowadays people are more aware of various side effects caused by pesticide residues present in food and water, and also may lead to environmental pollution. There is needed to research biological product for control plant disease.

The aim of this research focused on preliminary test for bioactivity substances from endophytic fungi associated with palm trees, *Mascarena Lagencuulis* and *Chrysalidocarpus lotescens* to inhibit *C. capsici* causing anthracnose of chili.

Material and methods

Isolation and identification of endophytic fungi

Endophytic fungi were isolated from palm trees, *Mascarena Lagencuulis* and *Chrysalidocarpus lotescens* at King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok, 10520, Thailand. Plant specimens were randomly selected from healthy leaves, petioles and roots of palm trees and taken to laboratory and processed within 24 h. All the samples were thoroughly washed in running tap water for 5 minutes to remove dust and debris and then air dried. The cleaned leaves, petioles and roots were surface sterilized with 75% ethanol 1 min and 10% sodium hypochlorite 3-5min, and then, cut into small pieces(3×3×3mm) under sterile conditions (petioles and roots were removed outer epidermal tissues and cuticle before cut into small pieces). Briefly, fragments were cleaned in sterilized water and sterilized with 75% ethanol 30s and then cleaned in sterilized water again and placed on water agar (WA) medium, incubated at room temperature. The endophytic fungi growing out from the plant tissue were transferred into potato dextrose agar (PDA) plates and incubated for

two to six days for observation. Continuous plates were subculture until get pure culture.

All isolates were identified by the morphology of the fungal culture, the mechanism of spore production and characteristics of the spore. The isolates were grown on PDA with decoction of host leaf medium to observe sporulation. For tentative identification, microscopic slides of each endophytic fungus was prepared and examined under binocular compound microscope for morphological identification.

Isolation of pathogen and pathogenicity test

The plant pathogen *C. capsici* causing chili anthracnose were isolated by tissue transplanting technique from chili fruit with obvious symptoms and performed pathogenicity test followed Koch's Postulate. The disease chili were cleaned with running tap water and after air-dry cut the advance margin of symptom between healthy tissue and diseased tissue to small pieces and then sterilized with sterilized water, 75% alcohol and sterilized water again. Then, transferred onto WA medium and followed by potato dextrose agar (PDA) to obtain pure culture. *C. capsici* were identified by morphological characteristic under binocular compound microscope.

Then, the isolates were tested for pathogenicity using detached fruit method in the laboratory. Select healthy fruit of chili and washing in the running water and air-dried. A sterilized filter paper was placed in 9cm diameter sterilized petri dish and two sterilized glass slide were also put on the filter paper, and the filter paper were moistened by sterilized distilled water. Chili fruits were wounded by sterilized needle and then placed on the glass slide in the petri dish then the spore suspension of *C. capsici* was prepared at concentration was 5×10^6 spores/ml. Spore suspension were sprayed on the surface of chili fruits including the wounded areas, incubated for two weeks at room temperature. At the same time, sterilized water was also sprayed to chili fruits as controls and incubated. Lesions on inoculated areas were observed on the coffee leaves, then, re-isolated pathogen from lesion invaded with inoculated pathogen according to the above mentioned method and identified the re-isolates under microscope and get pure culture.

Crude Extraction of Bioactivity Substances

The bioactive compounds were extracted from endophytic fungi as crude extracts. The extraction was performed using the method of Kanomedhakul *et al.* (2003). Endophytic fungi were cultured in potato dextrose broth (PDB) at room temperature (28-30℃) for 45 days. The fungal biomass of endophytics were removed from PDB, filtered through cheesecloth and air-dried overnight. The fungal biomass were grounded with electrical blender, and placed in triangular flask. And then dissolved

with equal volume hexane 5 days at room temperature, the biomass were separated by filtration through whatman filter paper. The solvent was evaporated in *vacuum* to yield crude hexane. The marc was further extracted with ethyl acetate (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. Each crude extract was weighted, and then kept in refrigerator at 4 C until to use.

Bioactivity against C. capsici

The crude extracts were tested for inhibition of *C. capsici*. The experiment was conducted by using 3x6 factorials in Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100, 500, and 1,000 ppm. Each crude extract was dissolved in one drop 2% dimethyl sulphite (DMSO), mixed into 30 ml potato dextrose agar (PDA) before autoclaving at 121C , 15 p for 30 minutes. The tested pathogen were cultured on PDA and incubated at room temperature for 7 days, and then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA(amending with each crude extracts) plate (5.0 cm diameter) in each concentration and incubated at room temperature (28°C-30°C) until the pathogen on the control plates growing full. Data were collected as colony diameter and the number of conidia. Percentage inhibition of pathogen colony growth and conidia production were calculated using the following formula:

% inhibition = (A-B) / A ×100

Where, A is the diameter of colony or number of conidia produced by the pathogen in control plates and B is the diameter of colony or number of conidia produced by the pathogen in treatment plates.

Data were statistically computed analysis of variance and treatment means were compared using Duncan Multiple's Range Test (DMRT) at P = 0.05 and 0.01. The effective dose (ED_{50}) will be calculated using probit analysis.

Results and Discussion

Isolation and identification of endophytic fungi

Two isolates of endophytic fungi were tested for bioactivity against *C. capsici*. They were *Nigrospora* spp. (Fig. 3) from *Chrysalidocarpus lotescens* (Fig. 1) and *Fusarium* spp. (Fig. 4) from *Mascarena Lagencuulis* (Bottle palm) (Fig. 2).

Fig. 1 *Chrysalidocarpus lotescens*

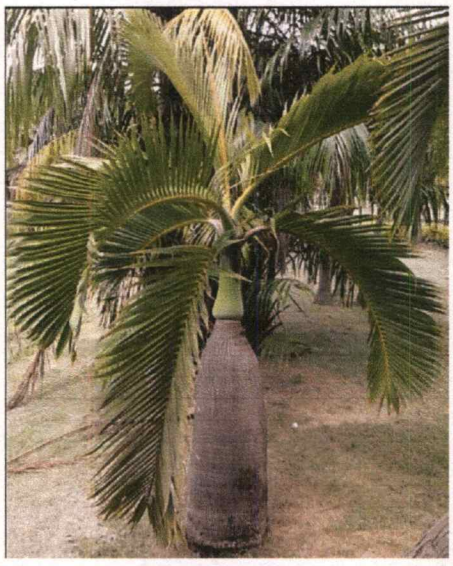
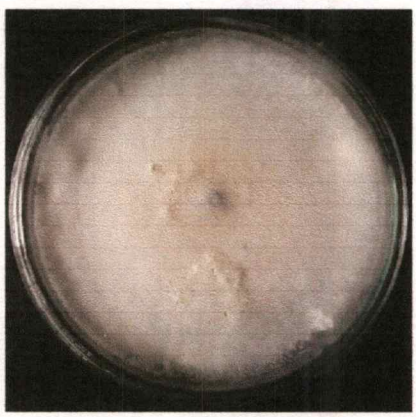


Fig. 2 *Mascarena Lagencuulis* (Bottle palm)



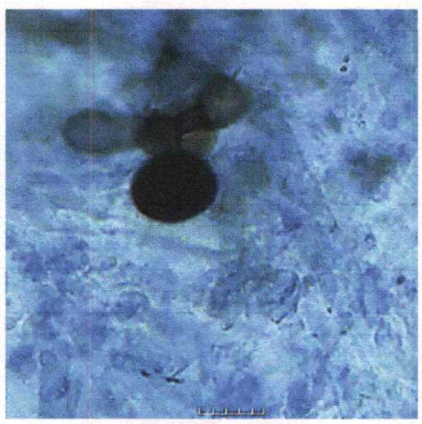
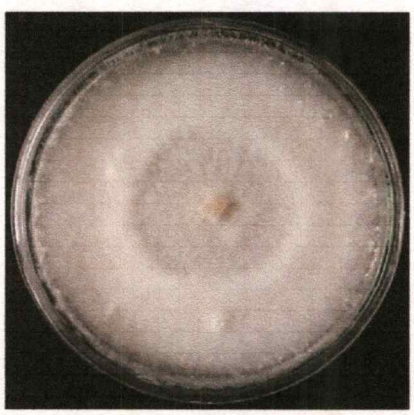


Fig. 3 *Nigrospora* spp. pure culture and spore



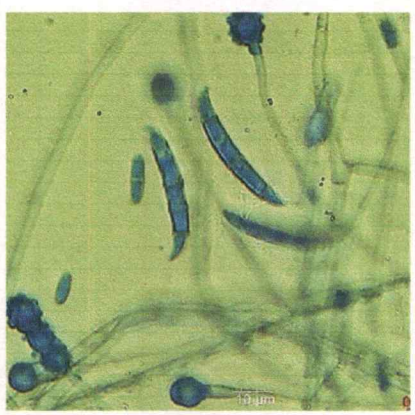


Fig. 4 *Fusarium* spp. pure culture and spores

Isolation of pathogen and pathogenicity test

Colletotrichum capsici were isolated from anthracnose of chili fruit with obvious symptom and identified by morphological (Fig.5). The isolate was confirmed pathogenic isolate from pathogenecity test (Fig. 6). The result showed that pathogenic isolate could be infected in chili fruit and caused symptom with the same symptom caused by *C. capsici* causing fruit anthracnose on chili.

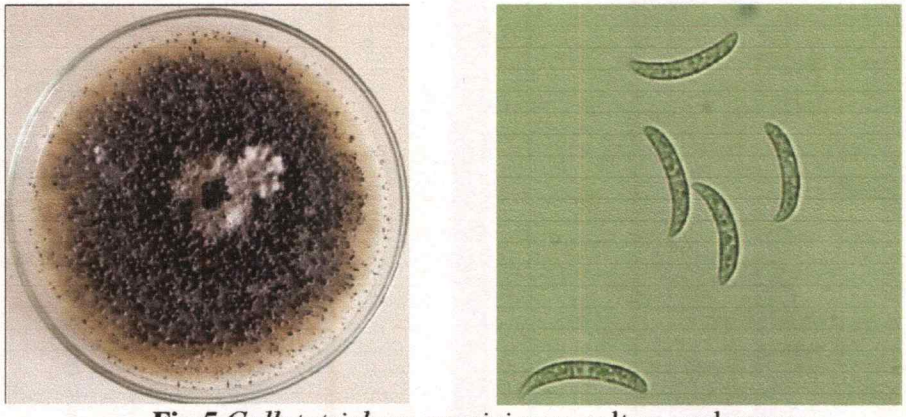
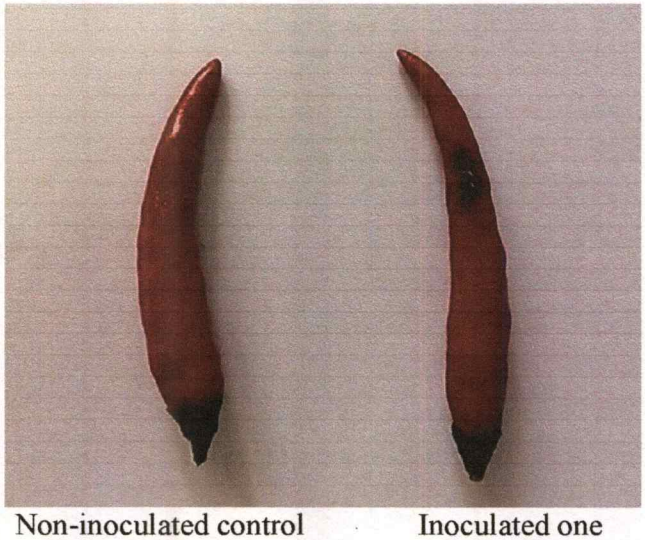


Fig.5 *Colletotrichum capsici* pure culture and spores



Non-inoculated control Inoculated one

Fig. 6. Pathogenicity test on chili fruits

Crude Extraction of Bioactivity Substances

The pure cultures of *Nigrospora* spp. and *Fusarium* spp. were cultured in PDB for 45days. Each fungal biomass was separately extracted to get crude hexane, crude ethyl acetate and crude methanol. With this, the crude

hexane, crude ethyl acetate and crude methanol from *Nigrospora* spp. yielded 0.37, 0.67 and 2%, respectively. The crude hexane, crude ethyl acetate and crude methanol from *Fusarium* spp. yielded 1.74, 0.83 and 3.85 %, respectively (Table. 1)

Table 1. Extraction of biological active substances from fungal biomass

endophytes	Fresh weight (g)	Dry weight (g)	Yield[1]			
			Crude Hexane(g)	Crude EtOAc(g)	Crude MeOH(g)	Total
Nigrospora sp.	2750	90	0.34 (0.37%)	0.60 (0.67%)	1.80 (2%)	2.74 (3.04%)
Fusarium sp.	3670	250	4.36 (1.74%)	2.08 (0.83%)	9.62 (3.85%)	16.06 (6.42%)

Bioactivity against C. capsici

Endophytic fungi *Nigrospora* spp. and *Fusarium* spp. were selected to yield metabolite as crude extracts and examined for bioactivity test against anthracnose of chili caused by *C. capsici*. The results showed that crude methanol from *Nigrospora* spp. gave significantly highest inhibition of 48.75% for the colony growth of *C. capsici* at concentration of 1,000 ppm when compared to the control, followed by crude hexane which inhibited 43.25% at concentration of 100 ppm (Table 2). Methanol crude extract from *Nigrospora* spp. gave significantly highest inhibition for the spore production of *C. capsici* as 87.26% at concentration of 500 ppm and the effective dose (ED_{50}) inhibited spore production of *C. capsici* at concentration of 41.51 ppm. Followed by, crude ethyl acetate gave 80.93% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 130.90 ppm. Crude hexane showed 78.36% inhibition at concentration of 1,000 ppm, and ED_{50} at concentration of 259.56 ppm. (Table 3) This result was similar with Zhao J. H. *et al.* (2012) who reported that four antifungal secondary metabolites were isolated from endophytic fungi *Nigrospora sp.* and antifungal assay showed clear inhibition of the growth of 8 plant pathogenic fungi in vitro.

The crude methanol from *Fusarium* spp. expressed highest inhibition percentage of *C. capsici* colony growth which was 34.50% at the concentration of 1,000 ppm. (Table 4). Methanol crude extract from *Fusarium* spp. showed significantly highest inhibition for the spore production of *C. capsici* as 74.52% at the concentration of 1,000 ppm, and the ED_{50} inhibited *C.capsici* spore production at concentration 47.39 ppm. Crude hexane, crude ethyl acetate showed inhibition as 65.18, 68.03% respectively, and the ED_{50} at 491.57, 42.40 ppm, respectively (Table 5). Meca *et al.* (2010) reported that some strains of *Fusarium tricinctum* are known to produce different enniatins which have strong biological activities including antifungal properties. This study were similar to the study of

Masroor Qadri *et al.* (2013) who reported that endophytic fungus, *Fusarium tricinctum* inhibited several phytopathogens significantly.

Table 2 Crude extracts of *Nigrospora* spp. testing for growth inhibition of *Colletotrichum capsici* at 7 days

Crude extracts	Concentration (ppm)	Colony diameter (cm)/1	Growth inhibition(%)/2
Crude Hexane	0	5.00[a]	
	10	4.41[c]	11.75[g]
	50	4.43[c]	11.25[g]
	100	2.83[h]	43.25[b]
	500	3.47[f]	30.50[d]
	1000	3.28[g]	34.25[c]
Crude EtOAc	0	5.00[a]	
	10	4.93[a]	1.25[i]
	50	4.72[b]	5.5[h]
	100	4.55[c]	9.00[g]
	500	3.63[e]	27.25[e]
	1000	3.44[f]	31.00[d]
Crude MeOH	0	5.00[a]	
	10	4.89[a]	2.00[i]
	50	4.85[ab]	3.00[hi]
	100	4.10[d]	18.00[f]
	500	3.51[ef]	29.75[de]
	1000	2.56[i]	48.75[a]
C.V.(%)		1.78	7.92

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 3 Spore production inhibition of crude extracts from *Nigrospora* spp. to *Colletotrichum capsici* at 30 days and effective dose (ED_{50}) values

Crude extracts	Concentration (ppm)	Number of spores /1 (10^6)	inhibition(%)/2	ED_{50}
Crude Hexane	0	4.87^{a}		259.56
	10	4.25^{b}	12.72^{j}	
	50	3.68^{bcd}	24.40^{hi}	
	100	3.50^{cd}	28.16^{gh}	
	500	2.06^{gh}	57.84^{e}	
	1000	1.06^{ijk}	78.36^{bc}	
Crude EtOAc	0	4.87^{a}		130.90
	10	3.93^{bc}	19.18^{ij}	
	50	3.25^{de}	33.43^{g}	
	100	2.81^{ef}	42.40^{f}	
	500	1.50^{hi}	69.39^{d}	
	1000	0.93^{ijk}	80.93^{ab}	
Crude MeOH	0	4.87^{a}		41.51
	10	3.62^{cd}	25.84^{hi}	
	50	2.50^{fg}	48.74^{f}	
	100	1.31^{ij}	73.40^{cd}	
	500	0.62^{k}	87.26^{a}	
	1000	0.81^{jk}	83.50^{ab}	
C.V.(%)		10.77	7.38	

1/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

2/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treat

Table 4 Crude extracts of *Fusarium* spp. testing for growth inhibition of *Colletotrichum capsici* at 7 days

Crude extracts	Concentration (ppm)	Colony diameter (cm)/1	Growth inhibition(%)/2
Crude Hexane	0	5.00^{a}	
	10	4.73^{b}	5.25^{j}
	50	4.69^{b}	6.00^{ij}
	100	4.56^{cd}	8.75^{ghi}
	500	4.56^{cd}	9.00^{ghi}
	1000	4.61^{bc}	7.75^{hij}
Crude EtOAc	0	5.00^{a}	
	10	4.62^{bc}	7.50^{hij}
	50	4.49^{cde}	32.50^{c}
	100	4.38^{ef}	10.00^{fgh}
	500	4.44^{de}	12.25^{ef}
	1000	4.30^{f}	11.00^{fg}
Crude MeOH	0	5.00^{a}	
	10	4.41^{ef}	11.75^{efg}
	50	4.12^{g}	17.50^{d}
	100	3.96^{h}	20.75^{c}
	500	3.56^{i}	28.75^{b}
	1000	3.27^{j}	34.50^{a}
C.V.(%)		1.48	10.60

1/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

2/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 5 Spore production inhibition of crude extracts from *Fusarium* spp. to *Colletotrichum capsici* at 30 days and effective dose (ED_{50}) values

Crude extracts	Concentration (ppm)	Number of spores /1 (10^6)	inhibition(%)/2	ED_{50}
Crude Hexane	0	14.56^{a}		491.57
	10	10.81^{b}	25.12^{de}	
	50	10.00^{bc}	32.23^{d}	
	100	6.62^{def}	54.45^{bc}	
	500	5.06^{ef}	65.18^{ab}	
	1000	9.25^{bcd}	36.06^{d}	
Crude EtOAc	0	14.56^{a}		42.40
	10	4.68^{ef}	68.03^{a}	
	50	9.25^{bcd}	35.86^{d}	
	100	9.12^{bcd}	36.26^{d}	
	500	6.81^{cdef}	53.56^{bc}	
	1000	11.68^{ab}	16.61^{e}	
Crude MeOH	0	14.56^{a}		47.39
	10	10.00^{bc}	30.54^{d}	
	50	7.00^{cde}	51.36^{c}	
	100	4.81^{ef}	67.01^{a}	
	500	4.18^{ef}	70.85^{a}	
	1000	3.68^{f}	74.52^{a}	
C.V.(%)		17.63	12.52	

1/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

2/Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

Conclusion

As results, it showed that the tested endophytic fungi, *Nigrospora* spp. from *Chrysalidocarpus lotescens* and *Fusarium* spp. from *Mascarena Lagencuulis* were proved biological activity against *C. capsici* causing chili anthracnose. The results demonstrated that crude methanol of *Nigrospora* spp. showed the best inhibition of colony growth and spore production of *C. capsici* at concentration of 1000 ppm. Both crude ethyl acetate and crude methanol of endophytic fungus *Fusarium* spp. expressed significantly inhibition of spore production of *C. capsici* with low effective dosé (ED_{50}) values as 42.40 and 47.39 ppm, respectively.

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Isolation and identification of endophytic fungi from 10 species palm trees

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Endophytic fungi are those living inside the host plant without causing any apparent negative effect on host plant. Endophytic fungi from palm trees in King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, were isolated and identified by morphology. 105 isolates were isolated from leaves, petioles and roots of 10 species healthy palms. Among them, 45 unidentified isolates were group to mycelia sterilia fungi. Other 60 isolates were identified as 15 species belong to *Fusarium* spp., *Xylaria* spp., *Cladosporium* spp., *Phialophora* spp., *Pestalotiopsis* spp., *Rhizoctonia* spp., *Colletotrichum spp.*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella* spp., *Emericella* spp., *Aspergillus* spp., *Curvalaria lunata*, *Phoma* spp. and *Nigrospora* spp. by morphological characters.
Key words: Endophytic fungi, Identification, palm trees

Introduction

Endophytes are microorganisms that live within plants for at least a part of their life cycle without causing any visible manifestation of disease (Bacon and white, 2000). A wide range of plants have now been examined for endophytes, and endophytes have been found in nearly all of them, including trees, grass, algae and herbaceous plants. Evidence of plant-associated microorganisms found in the fossilized tissues of stems and leaves has revealed that endophyte-plant associations may have evolved from the time higher plants first appeared on the earth (Redecker *et al.*, 2000). Hawksworth and Rossman estimated that nearly one million species of endophytes may exist in the unexplored plants (Strobel and Daisy, 2003; Arnold, 2005). Endophytes microorganisms were discovered including fungi, bacteria, and actinomycetes. And fungal endophytes are the most frequently encountered endophytes (Staniek *et al.*, 2008). It have great promise with diverse potential for exploitation (Li *et al.*, 2012; Staniek *et al.*, 2008). An enormous number of different fungi can be isolated from plants growing in their native habitat. Most of the fungi are uncommon and narrowly distributed, taxonomically and geographically. However a few

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fungi are widely distributed with the host, suggesting a long standing, close and mutually beneficial interaction. Ever since the discovery of the rich diversity of the endophytic fungi, their population dynamics, their role in improving plant growth, plant health (Hallmann *et al.*, 2007), their distribution in the plant, the metabolites they secrete and their potency to produce novel compounds within the plants (Tan and Zou, 2001), have formed an important aspect of present day research.sources of bioactive substances with agricultural and/or pharmaceutical potential, as exemplified by taxol (Stierle *et al.*, 1993; Wang *et al.*, 2000), subglutinol A and B (Lee *et al.*, 1995), and peptide leucinostatin A (Stroble and Hess, 1997). Endophytic fungi are thus expected to be potential sources of new bioactive agents.

Nowadays, many endophytic fungi associated with palms had reported, including temperature palms and tropic palms (Rodrigues and Samuels 1990; Fröhlich and Hyde 2000; Hyde *et al.* 2000). Jane Frohlich *et. al.*(2000) reported endophytic fungi from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia and got 75 fertile species in 48 genera and 60 sterile morphospecies including 10 *Xylaria* anamorphs, *Phomopsis* sp., *Phoma* sp., *Trichoderma* sp., *Colletotrichum* sp., *Pestalotiopsis palmarum.*, *Lasiodiplodia* sp. *Hyphomycete* sp., *Nodulisporium* sp., *Dictyochaeta* sp., *Phyllosticta* sp., *Distocercospora* sp., *Verticillium* sp., *Coelomycete* sp., *Aspergillus niger*, *Beltraniella* spp., *Botrytis allii.* The endophyte communities of both palms were composed of a single, dominant xylariaceous species.

The aim of this research focused on isolate endophytic fungi from leaves, petioles and roots of palm trees and identified by morphological characters.

Material and methods

Plant Sample Collection

Plant samples were randomly collected from 10 species healthy palms at King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand. Species of palms were identified followed by Andrew Henderson *et al.* (1995), Donald R. Hodel (1998), Langlois, Arthur C (1902) and David L.Jones (1995). All the samples from healthy leaves, petioles and roots of randomly select plants were cut and taken to the laboratory, processed within 24 h. 10 species palms were collected as follows:

1. *Ptychosperma macarthuri* (MacArthur Palm);
2. *Rhapis humilis* (Slender Lady Palm);
3. *Wodyetia bifurcata* (Foxtail Palm);
4. *Chrysalidocarpus lotescens* ;
5. *Veitchia merrillii* (Manila Palm)

6. *Phoenix roebelenii;*
7. *Rhapis Laosensis* (Thailand Lady Palm);
8. *Licula spinosa ;*
9. *Livistona chinensis* (Chinese Fan Palm);
10. *Mascarena Lagencuulis* (Bottle Palm).

Isolation of Endophytic Fungi

Plant specimens were thoroughly washed in running tap water for 5 minutes remove dust and debris and then air dried. The cleaned leaves, petioles and roots were surface sterilized with 75% ethanol 1 min and sodium hypochlorite (3%available chlorine) 3-5min and then were remove outer epidermal tissues and cuticle before cut under sterile conditions into small pieces of 3×3×3 mm. Briefly, fragments were cleaned in sterilized water and sterilized in 75% ethanol 30s and then cleaned in sterilized water again and placed on water agar (WA) medium, incubate at room temperature waiting endophytic fungi grow out. The endophytic fungi growing out from the plant tissue were transferred into potato dextrose agar (PDA) plates and incubate for two to six days . Continuous plates were subculture until get pure culture.

Identification of Endophytic Fungi

The isolates of endophytic fungi were identified by the morphology of the fungal culture, including colony and medium color, Colony characters, Spore characters, Mycelium characters, Fruiting structures by following the standard mycological manuals (e.g. M.B. Ellis, 1971; H.L. Barnett and Barry B. Hunter, 1987; K.H. Domsch and W, Games, 1993; Sutton, 1980; Nag Raj, 1993). The sterile isolates were grown on PDA with decoction of host leaves medium to observe sporulation. For tentative identification, microscopic slides of each endophytic fungi were prepared and examined under binocular compound microscope for morphological identification.

Results and Discussion

From leaves, petioles and roots of 10 species palm trees in this study yielded 105 isolates (Table. 1). More isolates were obtained from *Wodyetia bifurcate* (Foxtail Palm) than other palm trees. And, Root of palms can harber more endophytics than leaves and petioles.

Table. 1 Number of isolates from leaf, petiole and root of 10 species palm trees

Host Plant	Leaf samples	Petiole samples	Root samples	Total
Ptychosperma macarthuri	2	4	5	11
Rhapis humilis	3	2	3	8
Wodyetia bifurcate	5	4	8	17
Chrysalidocarpus lotescens	3	1	7	11
Veitchia merrillii	3	3	6	12
Phoenix roebelenii	5	3	5	13
Rhapis Laosensis	4	1	3	8
Licula spinosa	3	2	5	10
Livistona chinensis	1	2	3	6
Mascarena Lagencuulis	2	3	4	9
Total	31	25	49	105

Total 105 isolates obtained from palm tissues in this study yielded 15 identified taxa (Table. 2). These identifiable cultures represented 60 (57.14%) of the total isolates. The remaining 45(42.86%) isolates did not sporulate and were grouped into Mycelia Sterilia fungi (MSF). The morphology characters of 15 identified endophytic fungi (Fig. 1) are as follows:-

1. *Cladosporium* spp.

The colonies range from olive or deep green to black color in PDA media. They are relatively slow-growing. Mycelium immersed and superficial. Conidiophores macronematous and micronematous, sometimes up to 350µ long but generally much shorter, 2-6 µ thick, phal to mid olivaceous brown, smooth or verruculose. Conidia formed in long branched chains, mostly 0-septate, ellipsoidal or limoniform, pale olivaceous brown, most commonly smooth but verruculose in some strains.

Habit: *Ptychosperma macarthuri*; *Wodyetia bifurcate*

Luiz H. Rosa *et al.* (2012) study evaluates the diversity of microbial community associated with healthy *E. purpurea* clones and their ability to produce defense compounds. They recovered and identified thirty-nine fungal endophytes, which were closely related to species of the following genera *Ceratobasidium*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Glomerella*, and *Mycoleptodiscus*.

2. *Phialophora* spp.

Colonies slow-growing and white color with purple. Mycelium partly superficial, partly immersed. Stroma none. Setae and hyphopodia absent. Conidiogenesis phialidic; phialides arising solitarily from vegetative hyphae or on branched conidiophores. Conidia aggregated in slimy heads,

one-celled, straight or curved, ellipsoidal or oblong rounded at the ends, courless, smooth, 0-septate.

Habit: *Phoenix roebelenii*

Fungal endophytes associated with the palms, *Calamus kerrianus* (rattan) and *Wallichia caryotoides* (taorang) were investigated at two sites within Doi Suthep-Pui National Park, Thailand. Thirty-five endophytic fungi isolated included xylariaceous taxa (20 morphotypes), sterile mycelia, one unidentified and 13 mitosporic fungi including *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp.,*Guignardia cocaicola*, *Paecilomyces* sp. *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phomopsis* sp., *Phyllosticta* sp., and *Sarcopodium* sp. (Saisamorn Lumyong *et al.*, 2009).

3. *Pestalotiopsis* sp1.

Colonies grow fast with white yellow color. Acervuli dark, discoid or cushion-shaped, subepidermal. Conidiophores short, simple; conidia dark, several-celled, with hyaline, pointed end cells, ellipsoid to fusoid, with two or more hyaline, apical appendages.

Habit: *Rhapis humilis*

***Pestalotiopsis* sp2.**

Colonies slow-growing, with white and grey felty aerial mycelium. Pycnidia brown to pale, in spots, erumpent to subsuperficial, globose to flattened, with small ostiole. Conidia hyaline, 2-celled, cylindrical, with 3 to 4 hyaline setate at one end.

Habit: *Wodyetia bifurcate*

Endophytic fungi were isolated from three unidentified *Licuala* sp. palms in Brunei Darussalam and from three *L. ramsayi* palms in Australia. *Xylaria* spp., *Phomopsis* spp., *Pestalotiopsis* spp., *Nodulisporium* spp., *Colletotrichum* sp. and *Distocercospora* sp. were obtained as endophytic fungi (Jane Frohlich, 1999).

4. *Phoma* spp.

Colonies grow fast on PDA with abundant floccose, whitish to olivaceous-grey aerial mycelium; reverse uncoloured. Pycnidia abundantly produced in the centre of the colonies, olivaceous-brown, dark around the ostiole. Pseudosclerotia absent; conidia oblong, two-celled.

Habit: *Rhapis humilis*; *Wodyetia bifurcate*; *Chrysalidocarpus lotescens*; *Veitchia merrillii; Phoenix roebelenii*

The diversity of endophytic fungi in leaves, stems and roots from transgenic (Bt) and its isoline (non-Bt) cotton was evaluated during different plant developmental stages to investigate possible non-target effects of genetically modified cotton on endophytic fungal communities. A total of 17 genera of endophytic fungi were isolated. The most frequently isolated species were *Phomopsis archeri* from leaves and stems and *Phoma destructiva* from roots (P. D. de Souza Vieira *et al.*, 2011).

5. *Nigrospora* spp.

The white woolly colonies grow fairly rapidly. Colonies at first white with small, shining black conidia easily visible under a lower-power dissecting microscope, later brown when sporulation is abundant. Mycelium all immersed or partly superficial. Stroma none. Setae and hyphopodia absent. Conidiophores semi-macronematous, branched, flexuous, colourless to brown, smooth. Conidia solitary, with a violent discharge mechanism,acrogenous, simple, spherical or ellipsoidal, compressed dorsiventrally. Black, shining, smooth, 0-septate.

Habit: *Wodyetia bifurcate*; *Mascarena Lagencuulis*

Endophytic fungi were isolated from living symptomless leaves of 12 tree species from two locations in the Iwokrama Forest Reserve, Guyana. Species of *Colletotrichum*, *Nodulisporium*, *Pestalotiopsis* and *Phomopsis* were most frequently isolated, *Nigrospora* sp. also were identified as endophytic fungi. (Paul F. Cannon *et al.*, 2002).

6. *Xylaria* spp.

The colonies grow quite slow on PDA medium. Mycelium white color. The stromata are finger-like. ascocarps (fruitbodies) are black at the base but white and branched towards the top, Didn't produce ascospore on PDA medium.

Habit: *Wodyetia bifurcate*; *Veitchia merrillii*; *Phoenix roebelenii*

The occurrence of *Xylaria* species as endophytes in tropical plants is well documented (Bacon CW *et al.*, 1994; Bayman P *et al.*, 1997; Bussaban B. *et al.*, 2001; Clay K, 1988; Fro¨hlich J *et al.*, 2000; Photita W *et al.*, 2001; Tomita F, 2003). Cladistic analyses in the study of Itthayakorn Promputtha *et al.* (2007) also show that more than one species of *Xylaria* can coexist within living leaf tissues of *M. liliifera.* In this study, most *Xylaria* species are endophytic in origin.

7. *Fusarium* sp1.

The colonies fast growing and moist, floccose, granulose, cream color. Aerial mycelium floccose and whitish. Macro-conidia abundant and more-celled, slightly curved or bent at the pointed ends, typically canoe-shaped. Microconidia 1-celled, ovoid or oblong. Chlamydospores often sparse, globose, intercalary, pale, formed singaly or in chains.

Habit: *Chrysalidocarpus lotescens*

***Fusarium* sp2.**

The colonies fast growing with discrete sporodochia and white-ochraceous .colour. Aerial mycelium floccose. Macro-conidia abundant and more-celled, slightly curved or bent at the pointed ends; central part straight, cylindrical, typically canoe-shaped. Phialides bearing micro-conidia very long.

Habit: *Rhapis Laosensis*; *Mascarena Lagencuulis*; *Livistona chinensis*; *Licula spinosa*; *Ptychosperma macarthuri*; *Rhapis humilis*

The study of (Masroor Qadri *et al.*, 2013) was conducted to characterize and explore the endophytic fungi of selected plants from the Western Himalayas for their bioactive potential. A total of 72 strains of endophytic fungi were isolated and characterized morphologically as well as on the basis of ITS1-5.8S-ITS2 ribosomal gene sequence acquisition and analyses. Samples of *Platanus orientalis* were found to harbor only *Fusarium* spp. representing 4 different strains. *Artemesia annua* also possessed several strains of *Fusarium* as endophytes (5/22) whereas almost half of the endophytes of *Withania somnifera* (4/9) were also *Fusarium* spp.

8. *Rhizoctonia* spp.

Colonies grow quite fast on agar surface. The mycelium at first are white and later becoming black. The culture didn't produce spores, but are composed of hyphae and sclerotia (hyphal propagules). The cells of mycelium long, septa of branches set off from the main hyphae.

Habit: *Wodyetia bifurcate*; *Phoenix roebelenii*; *Licula spinosa*; *Livistona chinensis*

Harvais G. and Hadley G. (1967) Extensive isolation of root endophytes from *Orchis {Daciylorchis) pitrpurella* and other British orchids yielded a variety of strains of *Rhizoctonia* and of other fungi. *R. repens*, a common orchid endophyte, occurred in several host species and habitats. *R. solani* was uncommon, being obtained only from *Orchis piirpiirella* and *Coeloglossum viride* in certain situations.

9. *Colletotrichum* spp.

The colony growth slow, and mycelia initially white-grey and then become black-brown. Acervuli disc-shaped, subepiidermal, typically with dark, spines at the edge or among the conidiophores. Conidia 1-celled, ovoid or oblong.

Habit: *Ptychosperma macarthuri*; *Veitchia merrillii*; *Licula spinosa*; *Mascarena Lagencuulis*

Fungal endophytes were isolated from leaves of *Centella asiatica* (Apiaceae) collected at Mangoro (E. F. Rakotoniriana *et al.*, 2007). The most common endophytes were the non-sporulating species 1 (isolation frequency IF 19.2%) followed by *Colletotrichum* sp.1 (IF 13.2%), *Guignardia* sp. (IF 8.5%), *Glomerella* sp. (IF 7.7%), an unidentified ascomycete (IF 7.2%), the nonsporulating species 2 (IF 3.7%) and *Phialophora* sp. (IF 3.5%).

10. *Chaetomium globosum*

Colonies slow growing with little superficial mycelium and a dense olivaceous layer os ascomata. Phialoconidia absent; ascomata dark brown or black, globose to subglobose; lateral hairs dark brown with paler tips, minutely roughened; terminal hairs dark olive brown with paler tips, wavy or loosely coiled and interwined. Spores pale greenish to dark olive-brown, flattened lemon-shaped, hardly apiculate.

Habit: *Veitchia merrillii; Ptychosperma macarthuri*; *Rhapis Laosensis*

Endophytic fungi from the Chinese medicinal plant *Actinidia macrosperma* were isolated and identified for the first time (Yin Lu *et al.*, 2012). In total, 17 fungal isolates were obtained. Five different taxa were represented by 11 isolates (*Acremonium furcatum*, *Cylindrocarpon pauciseptatum*, *Trichoderma citrinoviride*, *Paecilomyces marquandii*, and *Chaetomium globosum*).

11. *Chaetomium aureum*

Colonies slow growing with immersed mycelium and little superficial mycelium, red and white colors. Phialoconidia absent; ascomata red, globose; lateral hairs dark brown with paler tips, minutely roughened; terminal hairs dark olive brown with paler tips and interwined. Ascospores halfmoon-shaped with two apical germ pores and brown colour.

Habit: *Livistona chinensis*

In Pablo Martínez-Álvarez *et al.* (2015) study, 154 endophyte isolates were selected from a collection of 546 fungi tested in a preliminary confrontation assay. In total, 138 endophytes displayed antagonistic activity towards *F. circinatum* in the dual cultures of the in vitro experiment. In the field test, the endophytes *Chaetomium aureum* and *Alternaria* sp. reduced the area under disease progress curve (AUDPC) for the *P. radiata* seedlings, indicating that they may therefore be suitable for use as biological control agents (BCAs) of the disease.

12. *Gibberella* spp.

Colonies slow-growing and white colours. The ascocarp is globose with smooth walls. Ascomata abundantly. The asci are globose or ovoid-shaped, lenticular, valves smooth or slightly roughened, with a broad equatorial furrow, with very low ridges but no wings.

Habit: *Livistona chinensis*

Masroor Qadri *et al.* (2013) conducted to characterize and explore the endophytic fungi of selected plants from the Western Himalayas for their bioactive potential. A total of 72 strains of endophytic fungi were isolated. *Gibberella moniliformis*, *Chaetomium globosum*, *Alternaria* spp., *Fusarium* spp., *Cladosporium cladosporioides* and so on were collected and identified based on morphology and molecular methods.

13. *Emericella* spp.

Colonies growing restrictedly, with few green conidial heads and abundant purple-brown ascomata formed in several layers. Ascomata surrounded by dark brown, globose hulle cells. Ascospores purple-red, lenticular, rugulose, with two sinuate equatorial crests.

Habit: *Mascarena Lagencuulis*

Chemical investigation of the endophytic fungus *Emericella* sp. (HK-ZJ) isolated from the mangrove plant Aegiceras corniculatum led to

isolation of six isoindolones derivatives termed as emerimidine A and B and emeriphenolicins A and D, and six previously reported compounds named aspernidine A and B, austin, austinol, dehydroaustin, and acetoxydehydroaustin, respectively (Zhang, G.J. *et al.*, 2011). In this paper, the first isolation, structural elucidation and biological evaluation of novel isoindolone derivatives from the fungal endophyte *Emericella* sp. (HK-ZJ) was reported.

14. *Aspergillus* sp.

Colonies growing rather slow on PDA with creamy-yellow color. Mycelium partly immersed, partly superficial. Stroma none; Setae and hyphopodia absent. Vesicles small, variable in shape. Conidial heads globose and bright yellow. Conidia globose to subglobose, smooth-walled, uninucleate, the chains sometimes sliming down.

Habit: *Wodyetia bifurcate*; *Veitchia merrillii*

Nur Amin *et al.* (2014) isolate and identify fungal endophyte from clones cocoa resistant VSD M.05 and clones cocoa susceptible VSD M.01. A total of 10 isolates of fungal endophytewere isolated from clones cocoa resistent VSD M.05. The isolates belonged to 6 genera namely: *Curvularia* sp., *Fusarium* sp., *Geotrichum* sp., *Aspergillus* sp., *Gliocladium* sp., *Colletotrichum* sp.. The fungal endophyte were isolated of clones cocoa susceptible VSD M.01, that as 4 genera identified as *Aspergillus* sp., and *Gliocladium* sp..

15. *Curvalaria lunata*

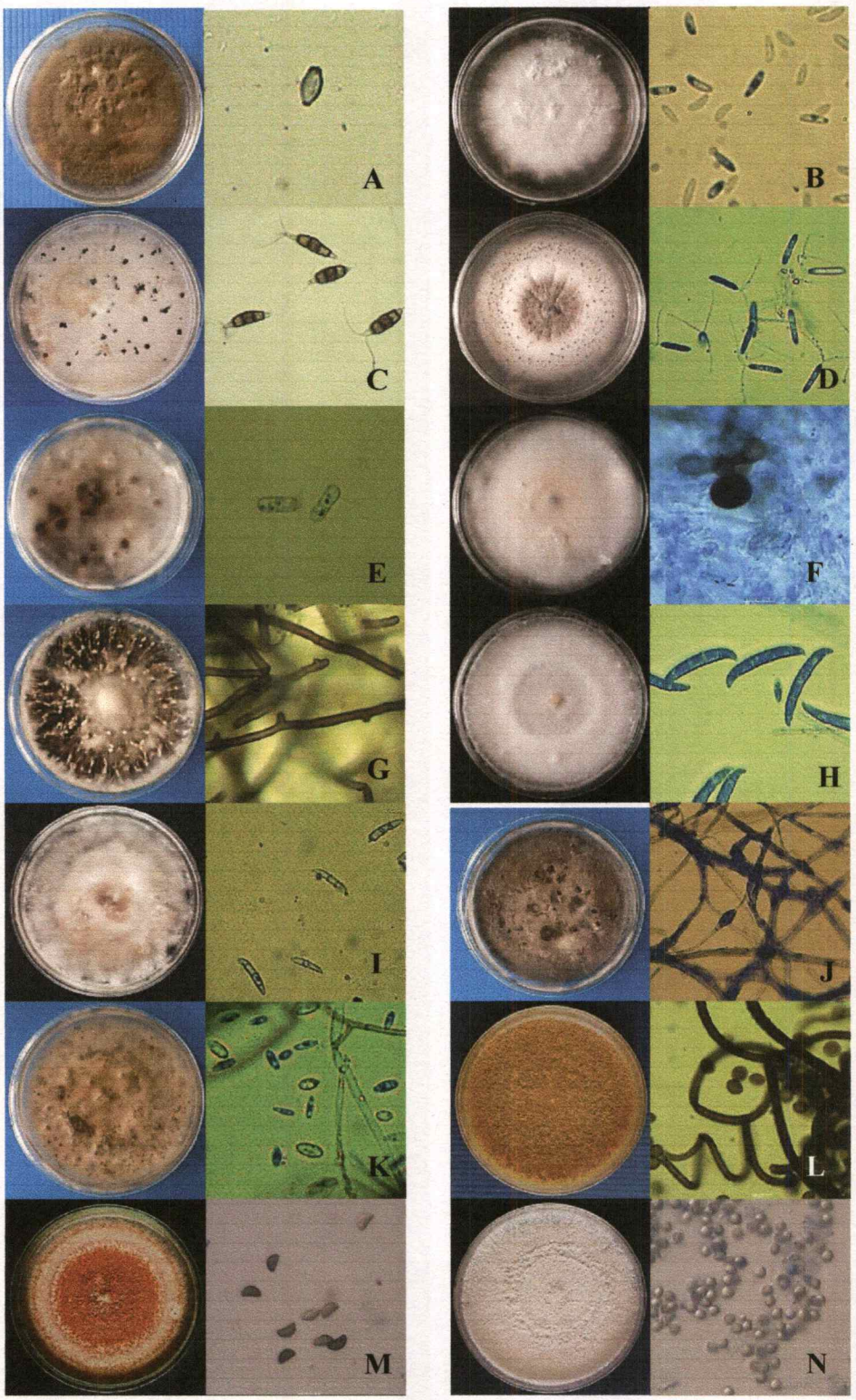
Colonies effuse, hairy and black color on PDA medium. Mycelium immersed in natural substrata. Conidia with hilum scarcely, remaining smooth-walled, dark brown color. Conidia predominantly 3-septate, the middle septum below the centre and the third cell strongly curved, tapering gradually towards the base.

Habit: *Phoenix roebelenii*

The present study of Ines Ben Chobba *et al.* (2013) investigating the diversity of both cultivable and non-cultivable endophytic fungal floras in the internal tissues (roots and leaves) of Tunisian date palm trees (*Phoenix dactylifera*). Accordingly, 13 isolates from both root and leaf samples, exhibiting distinct colony morphology, were identified by a sequence match search where in their 18S–28S internal transcribed spacer (ITS) sequences were compared to those available in public databases. The cultivable root and leaf isolates mostly fell into *Alternaria* spp., *Fusarium* spp., *Curvularia* spp., *Cladosporium* spp. and *Pythium* spp..

Table. 4.2: Taxon of endophytic isolates

Taxon	Class and order	Family and Genus	Reference
Cladosporium spp.	Dothideomycetes ; Capnodiales	Davidiellaceae; *Cladosporium*	Luiz H. Rosa *et al.*, 2012; Ines Ben Chobba *et al.* 2013
Phialophora spp.	Eurotiomycetes Chaetothyriales	Herpotrichiellaceae *Phialophora*	E. F. Rakotoniriana *et al.*, 2007
Pestalotiopsis spp.	Sordariomycetes Xylariales	Amphisphaeriaceae *Pestalotiopsis*	Jane Frohlich, 1999
Phoma spp.	Dothideomycetes Pleosporales	Incertae sedis *Phoma*	Luiz H. Rosa *et al.*, 2012
Nigrospora spp	Sordariomycetes; Trichosphaeriales	Trichosphaeriaceae ; *Nigrospora*	Paul F. Cannon *et al.*, 2002
Xylaria spp.	Sordariomycetes; Xylariales	Xylariaceae; *Xylaria*	Archana Nath *et al.*, 2012; E. F. Rakotoniriana *et al.*, 2007;
Fusarium spp.	Sordariomycetes Hypocreales	Nectriaceae *Fusarium*	Luiz H. Rosa *et al.*, 2012; Masroor Qadri *et al.*, 2013; Ines
Rhizoctonia spp.	Agaricomycetes; Cantharellales	Ceratobasidiaceae; *Rhizoctonia*	Harvais G. and Hadley G., 1967
Colletotrichum spp.	Sordariomycetes Glomerellales	Glomerellaceae *Colletotrichum*	Luiz H. Rosa *et al.*, 2012; E. F. Rakotoniriana *et al.*, 2007
Chaetomium globosum	Sordariomycetes Sordariales	Chaetomiaceae *Chaetomium*	Yin Lu *et al.*, 2012
Chaetomium aureum	Sordariomycetes Sordariales	Chaetomiaceae *Chaetomium*	Pablo Martínez-Álvarez *et al.*, 2015;
Giberrella spp.	Sordariomycetes Hypocreales	Nectriaceae *Giberrella*	Masroor Qadri *et al.*, 2013
Emericella spp.	Eurotiomycetes Eurotiales	Trichocomaceae *Emericella*	Zhang, G.J. *et al.*, 2011
Aspergillus spp.	Eurotiomycetes Eurotiales	Trichocomaceae *Aspergillas*	Nur Amin *et al.* 2014;
Curvalaria lunata	Euascomycetes Pleosporales	Pleosporaceae *Curvalaria*	Ines Ben Chobba *et al.* 2013; Nur Amin *et al.* 2014


A
B
C
D
E
F
G
H
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L
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Fig. 1 Endophytic isolates

A: *Cladosporium* spp.; B: *Phialophora* spp.; C: *Pestalotiopsis* sp1.; D: *Pestalotiopsis* sp2.; E: *Phoma* spp.; F: *Nigrospora* spp; G: *Xylaria* spp.; H: *Fusarium* sp1; I: *Fusarium* sp2.; J: *Rhizoctonia* spp.; K: *Colletotrichum* spp.; L: *Chaetomium globosum*; M: *Chaetomium aureum*; N: *Giberrella* spp.; O: *Aspergillas* spp.; P: *Curvalaria lunata*; Q: *Emericella* spp.

Table. 3 Isolat Numbers of each species

Endophytic fungi	**Isolate from**			**total**
	leaf	*petiole*	*root*	
***Cladosporium* spp.**	-	2	1	3 (2.85%)
***Phialophora* spp.**	1	-	3	4 (3.80%)
***Pestalotiopsis* spp.**	1	-	1	4 (3.80%)
***Phoma* spp.**	2	1	2	5 (4.76%)
***Nigrospora* spp.**	3	-	2	5 (4.76%)
***Xylaria* spp.**	5	3	1	9 (8.57%)
***Fusarium* spp.**	3	4	6	13(12.38%)
***Rhizoctonia* spp.**	1	-	2	3 (2.85%)
***Colletotrichum* spp.**	2	1	3	6(5.71%)
Chaetomium globosum	-	1	-	1(1.05%)
Chaetomium aureum	-	1	-	1(1.05%)
***Giberrella* spp.**	-	1	-	1(1.05%)
***Emericella* spp.**	-	-	1	1(1.05%)
***Aspergillus* sp.**	-	-	1	1(1.05%)
Curvalaria lunata	3	-	-	3(2.85%)
Mycelia Sterilia	12	13	20	45(42.85%)
Total	33	27	45	105

In this study, mycelia sterilia had the highest relative frequency (42.86%) (Table.2). *Fusarium* spp. (12.38%) which are frequently identified

as endophytes (Masroor Qadri *et al.*, 2013) was the second most frequent endophytic group. Followed by *Xylaria* spp.; *Colletotrichum spp.*; *Phoma* spp. and *Nigrospora* spp. These were the dominant genera or order of endophytic fungi found in this study, similar to the findings reported previously for many tropical endophytic fungi (Luiz H. Rosa *et al.*, 2012; Nur Amin *et al.*, 2014; Jane Frohlich, 1999).

Conclusion

Fifteen species of endophytic fungi were isolated and identified from leaves, petioles and roots of 10 species palm trees which belong to *Fusarium* spp., *Xylaria* spp., *Cladosporium* spp., *Phialophora* spp., *Pestalotiopsis* spp., *Rhizoctonia* spp., *Colletotrichum spp.*, *Chaetomium globosum*, *Chaetomium aureum*, *Giberrella* spp., *Emericella* spp., *Aspergillus* spp., *Curvalaria lunata*, *Phoma* spp. and *Nigrospora* spp.. 45 unidentified isolates were group to mycelia sterilia fungi which was morphological fungal types, but not forming true spores. Mycelia sterilia fungi had the highest relative frequency (42.86%) in this study and *Fusarium* was the second most frequent endophytic group, followed by *Xylaria* spp.; *Colletotrichum spp.*; *Phoma* spp. and *Nigrospora* spp.

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