สำนักหอสมุดกลาง พระจอมเกล้าลาดกระบัง

BIOLOGICAL CONTROL OF TOMATO FUSARIUM WILT BY USING *CHAETOMIUM* SPP.

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ใบรับรองวิทยานิพนธ์

หัวข้อวิทยานิพนธ์ การใช้ *Chaetomium* spp. ที่มีผลต่อการเจริญเติบโตและควบคุมโรคเหี่ยวของมะเขือเทศที่เกิดจากเชื้อฟิวซาเรียม

Biological control of tomato Fusarium wilt by using *Chaetomium* spp.

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ABSTRACT

Fusarium solani was proved to be cause wilt of tomato var Sida by morphology and molecular phylogenic identification on the basis of ITS1-5.8S-ITS2 ribosomal gene sequence acquisition and analyses, including pathogenicity test. The antagonistic fungi were also morphology and molecular phylogenic confirmation identified and confirmed as *Chaetomium brasiliense*, *Chaetomium cochliodes* and *Chaetomium globosum* when compared to genebank to those available in public database. *Ch. brasiliense*, *Ch. cochliodes* and *Ch. globosum* were tested against *Fusarium solani* by dual culture method. These antagonists expressed ability to inhibit the growth and spore production of *F. solani* causing wilt of tomato var sida.

Moreover, EtOAc crude extract of *Ch. brasiliense* expressed antifungal activity to inhibit spore production of *F. solani* which the ED_{50} value of 65.44 ppm, followed by crude methanol extract and hexane crude extract which the ED_{50} were 69.06 and 102.58 ppm., respectively. The methanol crude extract of *Ch. cochliodes* inhibit spore production of *F. solani* which the ED_{50} of 229 ppm, and followed by EtOAc crude extract and hexane crude extract which the ED_{50} value were 319 and 973 ppm, respectively. Hexane crude extract of *Ch. globosum* inhibited the spore production of *F. solani* at the ED_{50} of 200 ppm, and followed by crude EtOAc crude extract and methanol extract which the ED_{50} values of 314 and 378 ppm, respectively.

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Nano-particles at low concentration of *Chaetomium* spp gave a better inhibition of *F. solani* than crude extracts in all tests. As results, nano-CBH expressed highest antifungal activity against *F. solani* which the ED_{50} of 3.86 ppm, and followed by nano-CBH and nano-CBE which the ED_{50} values of 4.37 and 5.42 ppm, respectively. Nano-CCOM showed the highest spore inhibition of *F. solani* which the ED_{50} of 8.78 ppm, and followed by nano-CCOH and nano CCOE which the ED_{50} values were 9.21 and 9.70 ppm, respectively. However, nano CGM actively expressed antifungal activity of *F. solani* which the ED_{50} of 1.48 ppm, and followed by nano-CGH and nano CGE which the ED_{50} values were 3.41 and 3.48 ppm, respectively.

Crude extracts and nano-particles from *Ch. brasiliense, Ch.cochliodes* and *Ch. globosum* resulted to control wilt of tomato caused by *F. solani* in pot experiment. It is concluded that crude-CB, crude-CCO and crude CG gave significantly different in disease index and plant growth parameters when compared to the inoculated control. Nano-CB, nano- CCO and nano-CG treatments were significantly different than the non-inoculated control. The experiment revealed that either crude extract or nano-particle gave significantly reduction the disease index of tomato and gave better plant parameter than the inoculated control.

บทคัดย่อ

Fusarium solani ได้รับการพิสูจน์ว่าเป็นสาเหตุของโรคเหี่ยวของมะเขือเทศพันธุ์สีดา โดยการจัดจำแนกทางสัณฐานวิทยาและทางชีววิทยาระดับโมเลกุลโดยใช้ ITS1-5.8S-ITS2 ribosomal gene เพื่อจัดลำดับ DNA ของเชื้อรา รวมถึงการทดสอบประสิทธิภาพการเกิดโรค จากการจัดจำแนกทางสัณฐานวิทยาและทางชีววิทยาระดับโมเลกุลโดยใช้ ITS1-5.8S-ITS4 ribosomal gene เพื่อจัดลำดับ DNA ของเชื้อจุลินทรีย์ต่อต้าน และสัณฐานวิทยาของโมเลกุลและยืนยันว่าเป็น *Chaetomium brasiliense*, *Chaetomium cochliodes* และ *Chaetomium globosum* เมื่อเทียบกับยีนแบงก์จากที่มีอยู่ในฐานข้อมูล *Ch. brasiliense*, *Ch cochliodes* และ *Ch globosum* ได้รับการทดสอบกับ *Fusarium solani* โดยวิธีเพาะเชื้อร่วม เชื้อจุลินทรีย์ต่อต้าน เหล่านี้แสดงความสามารถในการยับยั้งการเจริญเติบโตและการสร้างสปอร์ ของ *F. solani* ที่เป็นสาเหตุโรคเหี่ยวของมะเขือเทศพันธุ์สีดา

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นอกจากนี้ crude extract จาก EtOAc จาก *Ch. brasiliense* แสดงประสิทธิภาพในการยับยั้งการผลิตสปอร์ของ *F. solani* ซึ่งมีค่า ED_{50} ที่ความเข้มข้น 65.44 ppm ตามด้วย crude เมทานอลและ crude hexaneที่ความเข้มข้น ED_{50} เท่ากับ 69.06 และ 102.58 ppm ตามลำดับ crude เมทานอลที่ได้จาก *Ch. cochliodes* แสดงการยับยั้งการผลิตสปอร์ของ *F. solani* ที่ ED_{50} ที่ความเข้มข้น 229 ppm และตามด้วย crude จาก EtOAc และ crude hexaneซึ่งมีค่า ED_{50} เท่ากับ 319 และ 973 ppm ตามลำดับ crude hexane จาก *Ch. globosum* แสดงประสิทธิภาพการยับยั้งการผลิตสปอร์ของ *F. solani* ที่ ED_{50} ที่ความเข้มข้น 200 ppm และตามด้วย crude EtOAc และ crude เมทานอลที่มีค่า ED_{50} ที่ความเข้มข้น เท่ากับ 314 และ 378 ppm ตามลำดับ

การใช้ nano-particles จาก *Chaetomium* spp ด้วยความเข้มข้นต่ำแสดงสามารถในการควบคุม *F. solani* ได้ดีกว่าการใช้ crude extract ในการทดสอบทั้งหมด ผลการทดลองพบว่า nano-CBH มีฤทธิ์ยับยั้ง *F. solani* สูงสุดที่ ED_{50} ที่ความเข้มข้น 3.86 ppm และตามด้วย nano-CBH และ nano-CBE ที่มีค่า ED_{50}ที่ความเข้มข้นเท่ากับ 4.37 และ 5.42 ppm ตามลำดับ Nano-CCOM แสดงความสมารถในการยับยั้งการสร้างสปอร์ของ *F. solani* สูงสุดที่ ED_{50} ที่ความเข้มข้น 8.78 ppm และตามด้วย nano-CCOH และ nano CCOE ซึ่งค่า ED_{50}ที่ความเข้มข้นเท่ากับ 9.21 และ 9.70 ppm ตามลำดับ อย่างไรก็ตามนาโน CGM แสดงประสิทธิภาพในการต่อต้านเชื้อรา *F. solani* ที่ค่า ED_{50}ที่ความเข้มข้น 1.48 ppm และตามด้วย nano-CGH และ nano CGE ซึ่งค่า ED_{50} ที่ความเข้มข้นเท่ากับ 3.41 และ 3.48 ppm ตามลำดับ

crude extract และการใช้ nano-particles จาก *Ch. brasiliense, Ch. cochliodes* และ *Ch. globosum* มีผลต่อการควบคุมโรคเหี่ยวของมะเขือเทศที่มีสาเหตุมาจากเชื้อ *F. solani* ในการทดลองในกระถาง สรุปได้ว่า crude-CB, crude-CCO และ crude CG มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับชุดทรีทเมนต์ที่ได้รับการปลูกเชื้อ นอกจากนี้ยังพบว่าการใช้ Nano-CB, nano-CCO และ nano-CG แสดงความแตกต่างอย่างมีนัยสำคัญกับการชุด ทรีทเมนต์ควบคุมที่ไม่ได้รับการปลูกเชื้อ จากการทดลองพบว่า crude extract หรือการใช้ nano-particles ช่วยลดดัชนีโรคของมะเขือเทศได้อย่างมีนัยสำคัญและส่งเสริมการเจริญเติบโตของพืชเมื่อเปรียบเทียบกับทรีทเมนต์ควบคุม

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CHAPTER 1

Introduction

1.1 Tomato wilt caused by *Fusarium solani*

Tomato (*Solanum lycopersicum* L.) is one of the most important crop in the world due to the high value of its fruits for fresh market consumption (Arici *et.al*, 2013). Tomato is a products rich in health-related food components as they are good sources of carotenoids (in particular, lycopene), ascorbic acid (vitamin C), vitamin E, folate, flavonoids and potassium (Beecher, 1998; Leonardi *et al.*, 2000). Regular consumption of tomatoes has been correlated with a reduced risk of various types of cancer (Franceschi *et al.*, 1994; Gerster, 1997; Weisburger, 1998) and heart diseases (Lavelli *et al.*, 2000; Pandey *et al.*, 1995). Tomato is an economically important vegetable crop, suffering from many fungal diseases (Ketelaar and Kumar, 2002). Fusarium wilt is one of the most serious disease in tomato throughout the world, especially in upland. This disease is caused by *Fusarium solani* leading to serious economic losses (Snyder and Hansen, 1940). *Fusarium solani* is an abundant saprophyte in soil and organic matter and occurs worldwide in the rhizosphere of many plant species. Plants infected by this soil-dwelling fungus show leaf yellowing and wilting that progress upward from the base of the stem. Initially, only one side of a leaf midrib, one branch, or one side of a plant would be affected. The symptoms soon spread to the remainder of the plant. Wilted leaves usually drop prematurely to minimize losses from *Fusarium* wilt, it is advisable to plant resistant varieties, and many resistant varieties are available. It is reported tomato wilt and pathogenic to other economic plants (Ajilogba *et al.*, 2013)

1.2 Biological control of tomato wilt

The use of hazardous chemical pesticides and synthetic fertilizers in agriculture has led to low soil fertility and reduced in yield, insect and pathogens become resistant to those chemical ones and cause environmental pollution as well, so research finding to develop alternative environmental friendly in practices for controlling insect and plant diseases. (Chet and Inbar, 1994; Cook, 1993; Monte, 2001).

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Biological control of is the use of natural or modified organisms, gene or gene products to effect of undesirable organisms such as plant pathogen to favor desirable organisms such as crops (Research Briefing, 1987). Bio control agents are known as antagonists and antagonism is the generalized mechanism that they use to reduce the survival or disease causing activities of plant pathogen. Antagonism is actively expressed opposition and includes antibiosis, competition and parasitism. Biological control of plant disease with antagonist is accomplished by destroying existing pathogen inoculum, excluding the pathogen from the host plant, or suppressing or displacing the pathogen after infection has occurred (Cook and Baker, 1983)

Recently, there have been many reports that antagonistic fungi can be used to control Fusarium wilt in tomato plant such as *Trichoderma asperellum, Chaetomium elatum* ChE0, *Chaetomium globosum* N0802, *Chaetomium lucknowense* CLT , *Trichoderma harzianum* PC01, *Emericella rugulosa* ER01, *Chaetomium cupreum* (Mahmoud *et.al.*, 2015; Soytong,2015; Charoenporn *et.al.*, 2010; Sibounnavong *et.al.*, 2011; Soytong, 1992). Moreover, the use of bioactive compound extracted from difference species of antagonistic fungi were reported to inhibit the growth of the Fusarium wilt disease such as Trichotoxin A50 extracted from *Trichoderma harzianum* PC01 and Chaetoglobosin C extracted from *Chaetomium globosum.* These compounds have been reported to elicit resistance or immunity in plants by inducing oxidative burst in plant cells for plant immunity (Soytong *et al.*, 2001).

1.3 Objectives

A.) To isolate and study morphology and molecular phylogeny to confirm species of *Fusarium solani* witch causing tomato wilt disease.

B.) To study morphology and molecular phylogeny to confirm species of *Chaetomium brasiliense, Chaetomium cochliodes* and *Chaetomium globosum.*

C.) To study the control of tomato wilt disease by using *Chaetomium* spp.

D.) To test the efficacy of biological active substance to inhibit *Fusarium solani*

E.) To test the biological substance from *Chaetomium* spp to inhibit tomato wilt caused by *Fusarium solani* in *vivo* test.

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CHAPTER 2

RITERTURE REVIEW

2.1 General of tomato

2.1.1 History of tomato

Jones (1930) reviewed that tomato belongs to genus *Lycopersicon*, especially *L.esculentum* that is grown for edible fruit. The genus *Lycopersicon* of the family Solanaceae is believed to originate in western south America, from the equator to about 30° latitude south. The species is native South America, especially Peru and the Galápagos Islands, being first domesticated in Mexico. In the mid-16th century, the tomato was introduced in to Europe, primarily featured in early herbals. It was grown for the beauty of its fruit but was not often eaten, except in Italy and Spain. The fruit was thought to be poisonous like its relative, the deadly nightshade. Although native to the New World, the tomato was introduced back into America from Europe in the 18th century, although its importance as a vegetable has occurred only in this century.

The botanical classification of the tomato had an interesting in history, firstly being placed in the genus *Solanum* along with the potato and being identified as *Solnum lycopersicon*. However, this designation was changed to *Lycopersicon esculentum*. *Lycopersicon* being derived from the Greek word meaning "wolf peach" and *esculentum* simply meaning edible. Although there are similar plant characteristics between potato and tomato plants, flower color (yellow for tomato and mostly white or violet for potato) and particularly the shape and manner of the opening of the pollen-bearing structures are the characteristics that separate two plants.

The designation of the tomato fruit as Moor's apple (Italian) or "love apple" (France) during the 16th century is unverified, but commonly believed. The color of the fruit first noted in Italy was yellow. By 18th century, the tomato has been used as an edible food, although it was still listed among the poisonous plants. In the 17th century, European took the tomato to China, South and South-East Asia. In 18th century to Japan (Siemonsma and Piluek, 1993)

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Wild tomato plant are still found from Equador or Chile as well as on the Galápagos Islands, although only two edible fruits, *Lycopersicon esculentum* (the common tomato in the wild cultivation today) and *Lycopersicon pimpinellifolium* (sometime cultivation under the name of currant tomato). Small fruit type *Lycopersicon esculentum* var. *cerasiforme*, cultivated under the name of cherry tomato, is wildly distributed as a wild plant in the tropic and subtropics.

2.1.2 Botanical name

Division: Anthophyta

Class: Dicotyledons

Family: Solanaceae

Genus: *Lycopersicon esculentum* Mill	Tomato
Lycopersicon pimpinellifolium (L.) Mill	Currant tomato
Lycopersicon esculentum var. *cerasiforme*	Cherry tomato

2.1.3 Fruit characteristic of tomato

The tomato fruit is a berry with 2 to 12 locules congaing many seed. The size and sharp of the fruit itself is affected by the extent of pollination, which in turn determine the number of seed filling of locules. There is substantial ranged in fruit characteristic among the many tomato varieties. Most of tomato varieties are red in color due to red carotenoid lycopene and more than 90% of the fresh weight of tomato fruit contains water and the availability of water of the plant can influence fruit size. As the tomato fruit develop, the percentage of fresh weight is a sucrose degree while starch and reducing sugar increase.

2.2 Fusarium wilt of tomato

The fungal species *Fusarium solani* (Mart.) (Teleomorph = *Nectria haematococca;* Rossman *et al.* 1999) belongs to the Ascomycetes and represents a diverse complex of over 45 phylogenetic and/or biological species (Zhang *et al.,* 2006; O'Donnell *et al.*, 2008). *Fusarium solani* is reported to cause tomato wilt (Ajilogba *et al.,* 2013). This species complex is widely distributed and comprised soil-borne saprotrophs that are among the most frequently isolated fungal species from soil and plant debris. Fusarium wilts affect and cause severe losses on most vegetables and flowers, several field crops, such as cotton and tobacco, plantation crops, such as

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banana, plantain, coffee, and sugarcane and a few shade trees. Fusarium wilts are most severe under warm soil conditions and in greenhouses (Agrios, 2005) Under conducive conditions, this fungus can cause serious plant diseases, infecting at least 111 plant species spanning 87 genera (Kolattukudy and Gamble, 1995), and has also been shown to cause disease in some animals (Summerbell, 2003).

2.2.1 Disease cycle

The mycelium is colorless at first, but with age it becomes cream-colored, pale yellow, pale pink, or somewhat purplish. The fungus produces three kinds of asexual spores. Microconidia, which have one or two cells, are the most frequently and abundantly produced spores under all conditions, even inside the vessels of infected host plants. Macroconidia are the typical "*Fusarium*" spores; they are three to five celled, have gradually pointed and curved ends, and appear in sporodochia-like groups on the surface of plants killed by the pathogen. Chlamydospores are one- or two celled, thick-walled, round spores produced within or terminally on older mycelium or in macroconidia. All three types of spores are produced in cultures of the fungus and probably in the soil, although only chlamydospores can survive in the soil for long. The pathogen is a soil inhabitant. Between crops it survives in infected plant debris in the soil as mycelium and in all its spore forms but, most commonly, especially in the cooler temperate regions, as chlamydospores. It spreads over short distances by means of water and contaminated farm equipment and over long distances primarily in infected transplants or in the soil carried with them. Usually, once an area becomes infested with *Fusarium*, it remains so indefinitely. When healthy plants grow in contaminated soil, the germ tube of spores or the mycelium penetrates root tips directly or enters the roots through wounds or at the point of formation of lateral roots. The mycelium advances through the root cortex intercellularly, and when it reaches the xylem vessels it enters them through the pits. The mycelium then remains exclusively in the vessels and travels through them, mostly upward, toward the stem and crown of the plant. While in the vessels, the mycelium branches and produces microconidia, which are detached and carried upward in the sap stream. Microconidia germinate at the point where their upward movement is stopped, the mycelium penetrates the upper wall of the vessel, and more microconidia are

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produced in the next vessel. The mycelium also advances laterally into the adjacent vessels, penetrating them through the pits. A combination of the processes discussed earlier, namely vessel clogging by mycelium, spores, gels, gums, and tyloses and crushing of the vessels by proliferating adjacent parenchyma cells, is responsible for the breakdown of the infected plant. When the leaves transpire more water than the roots and stem that can transport to them, the stomata close and the leaves become wilt and followed by death of the rest of the plant. The fungus invades all tissues of the plant extensively, reaches the surface of the dead plant, and there sporulates profusely. The spores may be disseminated to new plants or areas by wind, water. The disease cycle is shown in Figure 2.1

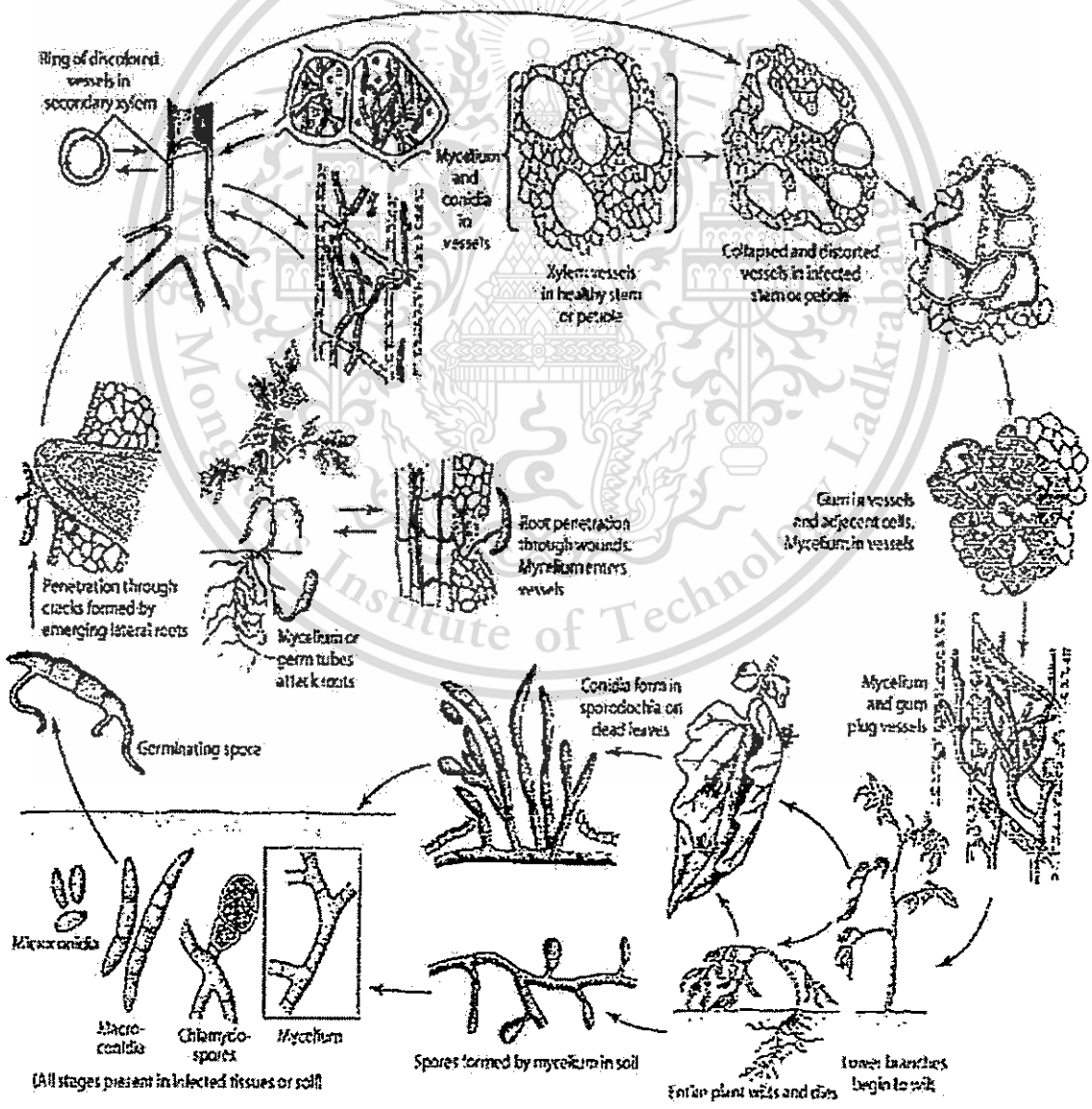

Figure 2.1 Disease and life cycle of *Fusarium* spp. causing wilt disease in plant (Agrios, 1997)

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2.3 Biological control

Biological control is a method of controlling pests such as insects, mites, weeds and plant diseases using other organisms or even natural plant and animals derived compounds (Flint and Dreistadt, 1998; Chanchaichaovivat *et al.*, 2007). Biological control agents of plant diseases are most often referred to as antagonists. Biological control of plant pathogens has provided a relatively recent strategy for integration with other control measures. Soytong *et al.* (1999) stated that the research and development of biological control agents for use against plant diseases have been undertaken for several years in both government and private sectors, as natural agents are needed to take the place of chemical fungicides. The problem associated with the use of hazardous chemicals for disease control has received increasing attention worldwide, due to the fact that pathogens become resistant to chemical fungicides and the resulting environmental pollution and ecological imbalances. Biological products are useful, not only for the protection against plant diseases but can also be used for curative effects of plant diseases. (Soytong *et al.*, 2001).

2.3.1 *Chaetomium* spp as antagonistic fungus

Chaetomium is a genus of fungus which belongs to the Chaetomiaceae with more than 300 species worldwide. *Chaetomium* is a dematiaceous (dark-walled) mold. *Chaetomium*, a strictly saprophytic fungus normally found in soil, cellulose and plant debris. (von Arx *et al.*, 1986 and Kirk *et al.*, 2008). Several species of *Chaetomium* with the potential as biological control agents suppress the growth of bacteria and fungi through competition, mycoparasitism, antibiosis, or their various combinations (Zhang and Yang, 2007). Presently, more than 200 compounds with a wide range of bioactive effects have been isolated from *Chaetomium* spp and many of them exhibited antifungal activity against plant pathogenic fungi (Zhang *et al.*, 2012). Many species of *Chaetomium* have been reported to work as antagonists against several plant pathogens (Vannacci and Harman, 1987; Soytong *et al.*, 2005; Tongon and Soytong, 2015). *Chaetomium* spp not even play the role to inhibit only plant pathogens but also can inhibit cancer cells as a report of Hani and Eman (2015) that isolation and purification of two potential

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anticancer compounds from *Ch. globosum* isolated from Egyptian soil. The two compounds were tested against Michigan Cancer Foundation-7(MCF-7) breast cancer cell line and *Hepatocellular carcinoma*, Human (HEPG-2) a human liver carcinoma cell line and showed inhibition effect on the proliferation of two cell lines. The structures of the two pure compounds were elucidated by HNMR and Mass spectroscopy as methyl 9-dihydro-8-trihydroxy-9-oxo-H-xanthene-1-carboxylate as a member of xanthones and (E)-methyl 2-hydroxy-6, 6-dimethyl hept-3-enoate. The globosumones A and globosumones B that isolated from *Ch. globosum* endophytic on *Ephedra fasciculata* (Mormon tea). Both compounds were evaluated for inhibition of cell proliferation in a panel of four cancer cell lines, NCI-H460 (non-small cell lung cancer), MCF-7 (breast cancer), SF-268 (CNS glioma), and MIA Pa Ca-2 (pancreatic carcinoma), and normal human fibroblast cells (WI-38). The globosumones A and globosumones B were found to be moderately active. (Bharat *et al.,* 2005)

Chaetomium spp is the fungi and a worldwide distribution. Their potential in the biological control of plant diseases are well known. Their bio-control mechanisms include producing antibiotics and ergosterols compounds that can suppress different plant pathogens, especially those soil bone plant pathogenic fungi, stimulate growth of plants and induce resistance to the diseases. They can be used to control many soilborne pathogens of vegetables and fruits (Qian *et al.,* 2007) such as controlling spot blotch disease of wheat caused by *Cochliobolus sativus* (Aggarwal *et al.*, 2004), Ascochyta blight of chick pea (Rajkumar *et al.*, 2005) and root rot of tea cause by *Fusarium oxysporum* in Vietnam was has been successfully controlled by treated *Ch. cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01. *Ch. lucknowense* CL (C1) *Ch. cochiliodes* C1, C3, C4 and C5 which significantly proved to inhibit *Ganoderma boninense* causing stem rot of oil palm in bi-culture test at 10 days (Soytong, 2014a).

Di Pietro *et al.* (1992) reported that *Ch. globosum* are capable for antagonizing several plant pathogenic fungi through the production of antibiotic and mycoparasitism. The antifungal compound that produced by *Ch. globosum*, 2-(buta-1, 3 dienyl)-3-hydroxy-4-(penta-1,3-dienyl)-tetrahydrofuran (BHT) and the epidithiadiketopiperazine and chaetomin play an

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important role against damping-off disease caused by *Pythium ultimum*. Chaetoglobosin-C is antifungal compound from *Ch. globosum* gave significantly inhibited colony, sporangia, and oospores growth of *Phythothora parasitica* causing root rot of citrus (Quyet *et al.*, 2016). Treetong *et al.* (2000) reported that the rotiorinal is antibiotic substance from *Ch. cupreum* have ability to induce plant immunity at the concentration of 500 ppm gave the highest significant immunizing induction to *Phytophthora* root rot of citrus seedling.

Soytong *et al.* (2001) reported that the practical integrated biological control technology to control plant diseases has been successfully introduced to growers in China, Philippines, Russia, Thailand and Vietnam by using new broad spectrum biological fungicides from *Chaetomium* (Thailand Patent No. 6266, International Code: AO 1N 25/12 and registered as Ketomium® mycofungicide). Since 1989, the biological product has been developed and improved from 22-strains of *Ch. cupreum* CCOI-CC I0 and *Ch. globosum* CGOI-CG 12 in the form of pellet and powder formulation. The formulation has successfully been applied to infested field-soils with integrated with cultural control measures and organic amendments for the long-term protection of durian (*Durio zibethinus* L.) and black pepper (*Piper nigram* L.) caused by *P. palmivora* wilt of tomato (*Lycopersicon esculentum* L.) caused by *F. oxysporum* f. sp. *lycopersici* and basal rot of corn (*Zea mays* L.) caused by *Sclerotium rolfsi*. Crude extract from *Ch. globosum* N0802 were reported to exhibit successful against *F. oxysporum* f.sp. *lycopersici* causing tomato wilt disease both *in vitro* tested and controlled disease with promote growth of tomato in *in vivo* tested.

2.4 The plant immune system

In agricultural practice worldwide, plant diseases regularly cause severe crop losses that may devastate the staple of millions of people, thus causing famines, and collectively result in economic damage of country (Van *et al.*, 2008). Famous examples from the past are the Irish potato famine, caused by the oomycete pathogen *P. infestans* and the great Bengal famine when the rice pathogen *Helmintosporium oryzae* caused a food shortage that resulted in the death of

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two million of people (Padmanab, 1973). Since 70% of total calories consumed by human population come from only four of the six cultures previously mentioned (Raven *et al.*, 1999).

Understanding the dynamics of plant-microbes interactions has enormously and positively affected the management of plant diseases worldwide. Widening the knowledge about microorganisms and hosts creating a "pathosystem", Phytopathology has explained most of the successful/unsuccessful mechanisms of attack of pathogens and unraveled many of the pathways leading to plant resistance/susceptibility. Nowadays it is able to take advantage of this knowledge. In the last twenty years, the plant immune system has become a primary topic for Plant Science which inducing forms of resistance in plants through processes of immunization, or genetically engineering in order to express resistance factors to a particular pathogen, are not challenges anymore, but for plant defense (Stuiver and Custers, 2001).

Pathogen activities focus on colonization of the host and utilization of its resources, while plants are adapted to detect the presence of pathogens and to respond with antimicrobial defenses and other stress responses. The ability of a pathogen is to produce a disease in a host plant is usually the exception. This is because plants have an innate ability to recognize potential invading pathogens and to set up successful defenses. On the other hand, successful pathogens produce diseases because they are able to evade detection or suppress host defense mechanisms, or both (Borrás and Hidalgo, 2004).

Plants rely on an innate immune system to defend themselves. Plants lack mobile defender cells and a somatic adaptive immune system. Instead, they rely on the innate immunity of each cell and on systemic signals emanating from infection sites (Ausubel, 2005). The key for an effective activation of a defense response in the plant is a rapid detection of an external molecule as extraneous, a concept known in Immunology as non-self recognition. Non-self determinants are often referred as "elicitors" and are constituted by an array of compounds including different oligosaccharides, lipids, peptides and proteins (Montesano *et al.,* 2003). The broader definition of elicitor includes both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitors) (Boller,

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1995; Ebel and Cosio, 1994). Their recognition occurs directly, via receptor ligand interaction, and indirectly, via host-encoded intermediates (Da *et al.*, 2006).

Recognition of a potential pathogen results in several defense responses of the plant, like activation of enzymes and generation of reactive oxygen species (ROS) (Chai and Doke, 1987; Legendre *et al.,* 1993). These early events are followed by other defense responses including production of antimicrobial compounds, such as defense proteins and phytoalexins, and induction of a hypersensitive response (HR), a localized cell death around the site of infection able to stop the spread of the pathogen. Before introducing the different forms of resistance in plants, it's necessary to deepen the process at the basis of plant-pathogen recognition: the first and essential encounter between host and microbe molecules (Lancioni, 2008).

2.4.1 Plant-pathogen interactions: elicitors and receptors

Plant-pathogen communications rely on the interaction among a wide and heterogeneous world of molecules, distinguished between those produced by the pathogen, often referred as "elicitors", and those produced by the plant and responsible for the detection of the elicitors, called "receptors". At first this distinction may look simple but the nature of these molecules gives an idea of the complexity of this communication. Oligo and polysaccharides, enzymes and toxins, proteins and small peptides, fatty acids and gases: specific and non-specific interactions between these players determine and influence the outcome of the challenge among plants and pathogens. (Lancioni, 2008)

Originally the term elicitor was used to describe molecules able to induce the production of phytoalexins (a class of defense molecules in plants) but it is now commonly used for compounds stimulating any type of plant defense (Ebel and Cosio, 1994). They were first described in the early 1970 (Keen, 1975). Elicitors may be classified into two groups, "general elicitors" or "PAMPs", and "race specific elicitors" or "effectors", depending on the specificity of the defense response induced in the plant. While general elicitors are able to trigger defense both in host and non-host plants (Nürnberger, 1999), race specific elicitors induce defense responses leading to disease resistance only in specific host cultivars (Angelova *et al.*, 2006).

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CHAPTER 3

RESEARCH METHODOLOGY

3.1 Isolation, identification and pathogenicity test

Fusarium solani causing wilt disease was isolated form tomato root by tissue transplanting technique. Roots of tomato were properly cleaned with running tap water and after air-dried for a few minutes and cut it in small pieces and soaked in sterilized water, followed by 1% sodium hypochlorite (NaClO) for 3 min and then sterilized water again. All of the small piece roots were transferred onto water agar (WA) medium for firstly observation of appearing colonies and sub-cultured to PDA until get pure culture. Morphological identification was done by observation fungal characteristic under binocular compound microscope.

3.1.1 Morphology study of the *Fusarium* spp.

Isolate of *Fusarium* spp was morphological identified by culturing in potato dextrose agar (PDA) and incubated at room temperature for 14 day observation. The characters of *Fusarium* spp were determined under binocular compound microscope and the details of fungal morphology were recorded as mycelia structure, shape and size of macroconidia, microconidia, conidiophores and chamydospores.

3.1.2 Pathogenicity test

The experiment was designed by using Completely Randomized Design (CRD) with four replications. The mycelia of *F. solani* were removed into sterilized distilled water and conidia suspension which adjust to 1×10^6 conidia/ml by using haemacytometer. Seedling of tomato var. sida was grown in mix soil for 15 days. The root dip method was used to inoculate which followed the method of Bao *et al.* (2002), gently removed dirt and excess soil from roots of tomato var. sida seeding by using tap water. Root tips of seedlings were cut by sterilized scissors for 5 mm and dipped into conidia suspension for 30 seconds. Seedling roots in control were cut root tips and dipped into sterilized distilled water without inoculum. Before transfer to plastic pots that contained the sterilized soil which autoclaved at 121°C, 15lbs/inch^2 for 1 hour. Disease severity index (DSI) was scored by followed the modified method of Sibounnavong *et al.* (2012), as follows: 1= no symptom; 2= yellowing leaves and root rot 1-20%, 3= yellowing leaves and

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root rot 21- 40%, 4= yellowing leaves and root rot 41-60%, 5= yellowing leaves and root rot 61-80%, and 6= yellowing leaves and root rot 81-100% or die. Disease severity index (DSI) was analysed by using analysis of variance (ANOVA) and mean comparison was computed Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05.

3.2 Molecular phylogeny identification based on DNA sequencing

All isolates of *Chaetomium* spp and *Fusarium* spp were reconfirmed species by DNA sequencing. Mycelial mass of each fungus was collected from a purified colony growth in PDB. Genomic DNA was extracted by CTAB method with some modification (Doyle and Doyle, 1987; Doyle and Dickson, 1987) The fungal mycelia were cleaned with 25mM EDTA. 100 mg fungal mycelia were diligently crushed in liquid nitrogen to make a fine powder and transferred into sterile microcentrifuge tube. The cells were lysed in CTAB buffer and β-mercaptoethanol, mixed carefully and incubated at 65°C for 1 hr with mixing tubes every 15 min. The lysate were extracted with an equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged at 14,000 rpm for 5 min at 4°C. The aqueous phase was transferred to a new steriled tube, added 2 µl Rnase (20 µg/ml), incubated 30 min at 37°C and added 50 µl 10% CTAB, repeated by adding chloroform/isoamyl alcohol (24:1) and centrifuged at 14,000 rpm in a microcentrifuge for 5 min at 4°C, removed aqueous top layer and transferred to new eppendorf tube, added an equal volume of cold isoproponal, mixed well and let tubes standed at -20°C for 20 min. The genomic DNA were precipitated in isopropanol and centrifuged at 4°C for 20 min at 14,000 rpm. The pellets were washed twice with 70 and 95% ethanol, air dried and dissolved in 100 µl TE buffer at 37°C, overnight. The quality and quantity of extracted DNA samples were routinely monitored by electrophoresis in a 1% agarose gel. DNAs were stored at 20°C for further use.

Phylogenetic analyses of the *Chaetomium* spp were carried out by the acquisition of the ITS1-5.8S-ITS2 ribosomal gene sequencing. The ITS regions of the fungi were amplified with the universal ITS primers, ITS1 (5'TCCGTAGGTGAACCTGCTGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3') (White *et al.,* 1990) using the polymerase chain reaction (PCR) and phylogenetic analyses of the *Fusarium solani* was carried out by using primers, ITS1-5.8S-ITS2 ribosomal gene sequencing. The ITS regions of the fungi were amplified with the universal ITS primers, ITS1 and ITS 2 (5'GCTGCGTTCTTCATCGAGC3') (White *et al.,* 1990). The 25 µl reaction mixture contained 2.5 µl 10 × PCR buffer, 4 µl each dNTP (1.25mM), 0.5 µl

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$MgCl^2$ 1 µl of each primer (20 pmol/µl), 1 µl of DNA and 0.2 µl of Taq DNA polymerase (1 U). 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 1 min, 72°C for 2 min, and a final extension at 72°C for 5 min. The amplified products (5 µl) were visualized on 1% (w/v) agarose gel to confirm the presence of a single amplified band.

The amplifications were purified by vivantis GF-1 Ambiclean Kit. The following protocols were adding DB buffer malted gel at 50°C and loading to purification column for centrifuge at 8500 rpm, 1 min, 4°C, then discard flowed through, added wash buffer and centrifuged at 8500 rpm, 1 min, 4°C for dry samples, transferred to new microcentrifuge tube and added elution buffer, incubated for 5 min, centrifuge at 8500 rpm, 1 min, 4°C and electrophoresis was made in a 1% agarose gel for check DNA band. The purification products were sent to company for sequencing.

The amplified products were sequenced and aligned with the sequences in the GenBank by basic local alignment search tool (BLAST) analysis (Altschul *et al.*, 1990) 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 sequences 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.

3.3 Biological control of *Fusarium solani*

3.3.1 Strain of antagonistic fungi used for experiments

Chaetomium brasiliense (CB), *Chaetomium cochliodes* (CCO) and *Chaetomium globosum* (CG) were kindly provided by Assoc. Prof. Dr. Kasem Soytong

3.3.2. Dual-culture test

The antagonistic fungi, *Chaetomium brasiliense, Chaetomium cochliodes* and *Chaetomium globosum* were tested by using method of Soytong (1992). The experiment was designed as Completely Randomized Design (CRD) with four replications. *Fusarium solani* and *Chaetomium* spp were cut with 0.5 mm sterilized cork borer and one agar plug of each fungus were transferred to PDA plate at one side 4 cm from center of plate. For control treatment either

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agar plug of *F. solani* or *Chaetomium* spp was placed on PDA plate at 4 cm from center of the medium. The tested plates were incubated at room temperature. The data were collected as colony diameter, number of conidia of pathogenic fungus. Percentage of growth and conidia inhibition of pathogen was calculated using formula below:

$$\text{Inhibition (\%)} = \frac{A-B}{A} \times 100 \quad \text{——————} \quad (1)$$

A = colony diameter or conidia number of pathogen in control

B = colony diameter or conidia number of pathogen in control in dual culture plate

The data were statistically computed for analysis of variance (ANOVA) and mean comparison was computed by using Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05. The effective dose (ED_{50}) was computed by using probit analysis

3.3.3 Testing antagonistic substances

Chaetomium brasiliense, Chaetomium cochliodes and *Chaetomium globosum* were extracted to tests their abilities to inhibit growth of *Fusarium solani* caused tomato wilt.

3.3.3.1 Extraction antagonistic crude extracts and preparation of nano particle from *Chaetomium* spp.

The fungal antagonistic *Ch. brasiliense, Ch. cochliodes* and *Ch. globosum* were separately cultured in potato dextrose broth (PDB) and incubated at room temperature for 30 days. Biomass of each antagonistic fungus was removed from PDB by filtering thorough cheesecloth and air-dried at room temperature. Biomass was collected and weighted as fresh and dried. Dried biomass were ground with electric blender. Dried biomass of each antagonist was extracted by the method described by Kanokmedhakul *et.al* (2003) as show in Figure 3.1

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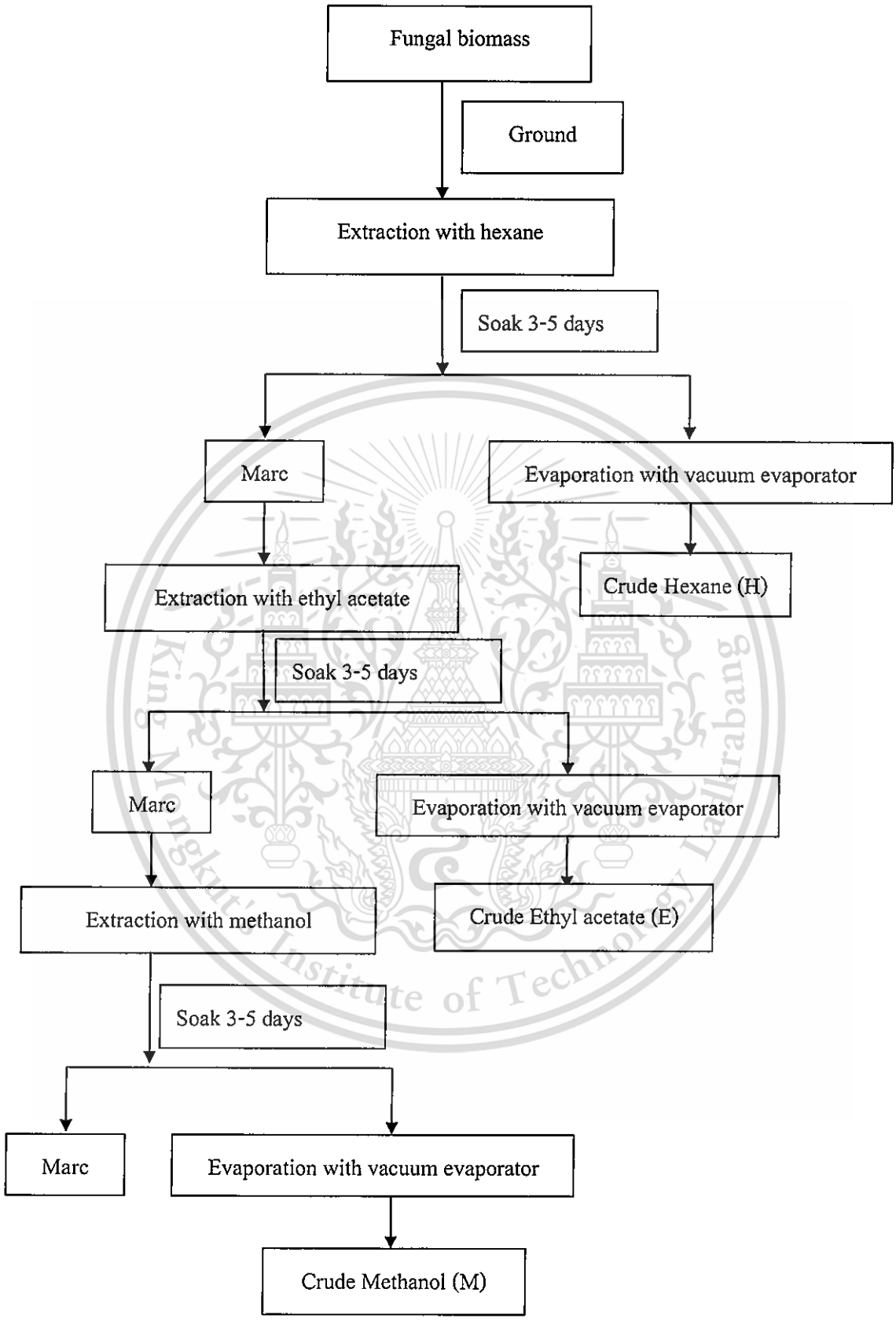

Figure 3.1 Protocol of extraction antagonistic fungi

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Each dried biomass was extracted with hexane (1:1 v/v) in 1000 ml of flask and incubation at room temperature. The ground marc was separated from the solvent by filtering through filter paper (Whatman No.4). The hexane filtrate was evaporated thought rotary vacuum evaporator to yield crude hexane extract. Further, the marc was then extracted with ethyl acetate (EtOAc) and methanol (MeOH), respectively using the same method as hexane. Finally, it yielded crude hexane, crude ethyl acetate and crude methanol of each antagonist. Preparation of nano particles was done using the method of Dar and Soytong (2014) to get nano particles of each *Chaetomium* spp.

3.3.3.2 Bioactivity test of crude extracts from *Chaetomium* spp against *F. solani*

The crude extracts of *Ch. brasiliense, Ch. cochliodes* and *Ch. globosum* were tested ability to inhibit the growth of mycelia and spore production of *F. solani.*

The experiments were designed as 2 factors factorial in Completely Randomized Design (CRD) with four replications. Factors A represented crude extracts which included of crude extracts from hexane, ethyl acetate (EtOAc) and methanol (MeOH). Factors B represented different concentrations of crude extracts as follows 0, 10, 50, 100, 500, 1,000 µg/ml. Each crude extracts were dissolved by 2% dimethyl sulfoxide (DMSO) and mixed with PDA before autoclaved at 121°C, 15lbs/inch^2 for 30 min. *F. solani* was cultured on PDA and incubated at room temperature for 5 days, then colony margin was cut by 0.5 mm sterilized cork borer. The agar plugs of *F.solani* was transferred into the middle of 5 cm of petri dish in different concentrations and incubated at room temperature for 5-7 days. Data were collected as colony diameter, number of conidia. Percentage of inhibition of mycelial growth and number of conidia was using calculated formula (1) above and data were statistically computed for analysis of variance (ANOVA) and mean comparison was computed by using Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05. The effective dose (ED_{50}) was computed by using probit analysis. The normal and abnormal spores were observed and compared under compound *microscope.*

3.3.3.3 Testing nano particles form *Chaetomium* spp against *F. solani*

The experiments were designed as 2 factors factorial in Completely Randomized Design (CRD) with four replications. Factors A represented different kinds of nano particles.

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Nano particles derived from *Chaetomium brasiliense* namely nano CBH, nano CBE, nano CBM , *Chaetomium cochliodes* namely nano CCH, nano CCE, nano CCM and *Chaetomium globosum* namely nano CGH, nano CGE, nano CGM.

Factor B represented concentrations of 0, 3, 5, 10, 15 µg/ml. Each nano particle was dissolved by 2% dimethyl sulfoxide (DMSO) and mixed with PDA before autoclaved at 121°C, 15lbs/inch^2 for 30 min. The pathogen was cultured on PDA and incubated at room temperature for 5 days, then colony margin was cut by 0.5 mm sterilized cork borer. The agar plugs of *F. solani* was transferred into the middle of 5 cm of petri dishes in deferent concentrations and incubated at room temperature for 5-7 days. Data were collected as colony diameter, number of conidia. Percentage of inhibition of mycelial growth and number of conidia was calculated using formula (1) above and data were statistically computed for analysis of variance (ANOVA) and mean comparison was calculated by Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05. The effective dose (ED_{50}) was computed by using probit analysis. The comparison of normal and abnormal spores were observed under compound microscope.

3.3.3.4 Testing crude extracts and nano-particles from *Chaetomium* spp to inhibit *F. solani* causing crown and root rot of tomato

The experiment was designed in Completely Randomized Design (CRD) with four replications and eight treatments. Fifteen day seedling of tomato var. sida were inoculated with conidia suspension of *F. solani* at concentration 1×10^6 conidia/ml and planted into plastic pots that containing sterilized soil which autoclave at 121°C, 15lbs/inch^2 for 1 hour. Treatment were designed as follows:-

T1 = inoculated control,

T2 = non- treated control,

T3 = treated mixed crude extracts from *Chaetomium brasiliense* at concentration of 1,000 µg/ml, namely crude-CB

T4 = treated mixed crude extracts from *Chaetomium cochliodes* at concentration of 1,000 µg/ml, namely crude-CCO

T5 = treated mixed crude extracts from *Chaetomium globosum* at concentration of 1,000 µg/ml, namely crude-CCO

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T6 = treated with nano particles derived from mixture of CBH, CBE, CBM of *Chaetomium brasiliense* at concentration of 15 µg/ml, namely nano-CB

T7 = treated with nano particles derived from mixture of CCOH, CCOE, CCOM of *Chaetomium cochliodes* at concentration of 15 µg/ml, namely nano-CCO

T8= treated with nano particles derived from mixture of CGH,CGE, CGM of *Chaetomium globosum* at concentration of 15 µg/ml, namely nano-CG

Crude extracts and nano elicitors were treated on inoculated tomato seedlings var. sida every 15 days as described above.

Data were collected as plant fresh weight (g), plant dry weight (g), plant height (cm) and fruit weight (g). Data were statistically computed for analysis of variance (ANOVA) and mean comparison was computed by Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05.

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CHAPTER 4

RESULTS

4.1 Isolation, identification and pathogenicity test

4.1.1 Morphological identification

Fusarium solani was isolated from diseased plant parts, especially from roots of tomato var sida. The characteristics of colony were fast growing with aerial mycelium floccose, white or cream colour, reaching 9 cm diameter in 5-7 day at 29-32 °C. Conidiophores formed singly, slender and cylindrical shape. Macroconidia found abundant, moderately curved, blunt apical and pedicellate basal cells 3-5, septate, 24.7-38.2 × 3.5-5.9 µm. Microconidia usually were abundant with 1 septate, 8.8-18.6 × 3.0-5.2 µm. Chlamydospores were singly or in paired in terminal, lateral or more rarely intercalary positions, smooth wall, 2.5-4.3 × 2.6-4.9 µm (Figure 4.1)

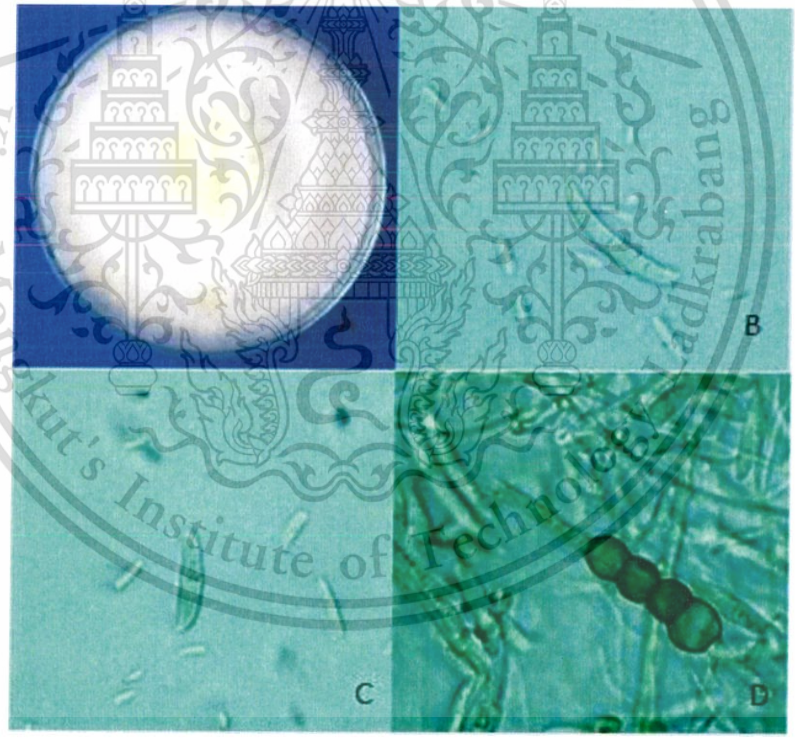

Figure 4.1 *Fusarium solani*, A= colony, B= macroconidia (40X), C = microconidia (40X) and D= clamydospores (40X).

4.1.2 Pathogenicity test

Pathogenicity test was conducted by dipping cut root into spore suspension of *F. solani* at the concentration of 1 x10^6 conodia/ml for 30 seconds which resulted tomato seedlings showed

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yellowing leaves and root rot 41-60 %. It was significantly differed when compared to the non-inoculated control (Figure 4.2, Table 4.1). As a result, it was similar to the work of Sibounnavong *et al.* (2012).

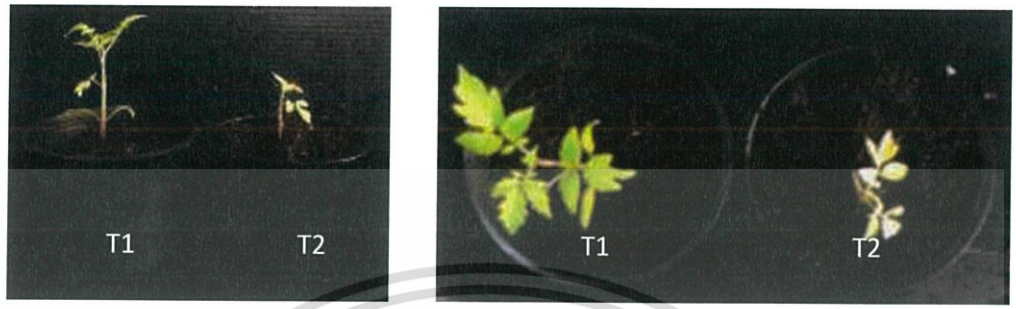

Figure 4.2 Pathogenicity test of *F. solani* caused tomato wilt, T1= dipped root with sterile water (control), T2=dipped root with spore suspension of *F. solani*.

Table 4.1. Percent disease index of *F. solani* in tomato.

Treatments	Disease index
T1= Control	1
T2= *Fusarium solani*	4

Disease index are as follows: 1= no symptom; 2= yellowing leaves and root rot 1-20%, 3= yellowing leaves and root rot 21- 40%, 4= yellowing leaves and root rot 41-60%, 5= yellowing leaves and root rot 61-80%, and 6= yellowing leaves and root rot 81-100% or die.

4.2 Morphological of antagonistic fungi

Chatomium brasiliense was cultured and observed morphological characters. Ascocarp, asci and ascospores were taken photograph under compound microscope. Culture was dark grey colour, perithecia globose, subglobose or broadly ovoid 101.5-229.0 × 230.5-398.5 µm, cylindrical asci. Ascospore was uniseriate, dark brown at maturity, broadly ovoid shape 6.4-7.5 × 4.0-5.0 µm, and smooth walled with central germ pore. (Figure 4.3)

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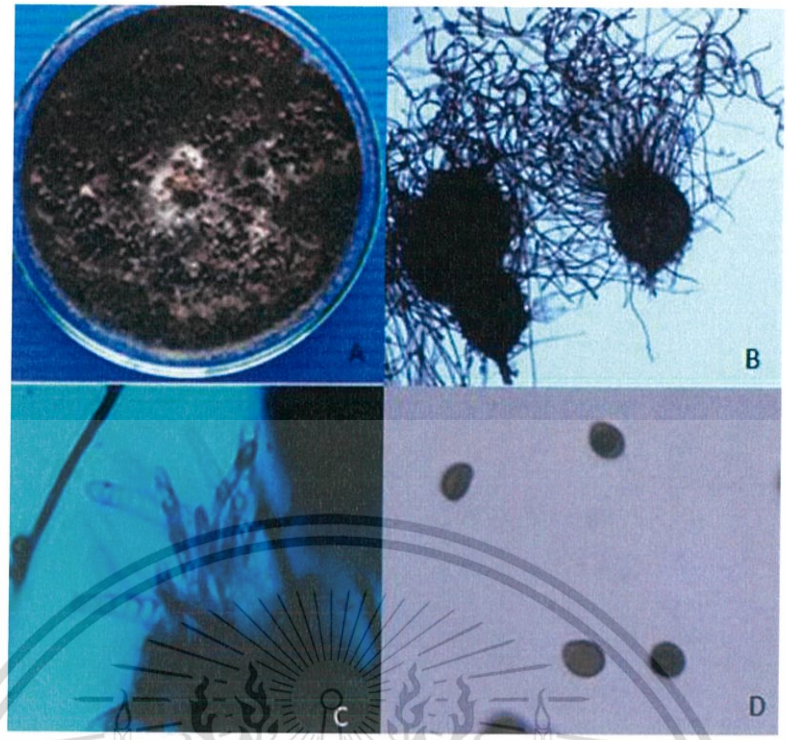

Figure 4.3 *Chaetomium brasiliense*, A= colony, B= ascocarps (10X), C= asci (40X) and D= ascospores (40X).

The culture of *Ch. cochliodes* was slow growing with olivaceous colour. Ascomata was superficial or subglobose with dark brown colour of ascomatal wall, 170.0 × 390.5 - 277.7 - 458.2 µm. Terminal hairs were verrucose and dark brown, the tips, spirally coiled in the upper part, with coils regularly tapering, asci was clavate shape. Ascospore dark brown colour when mature 8.0 × 9.5-7.5 × 8.5 µm with an apical germ pore. (Figure 4.4)

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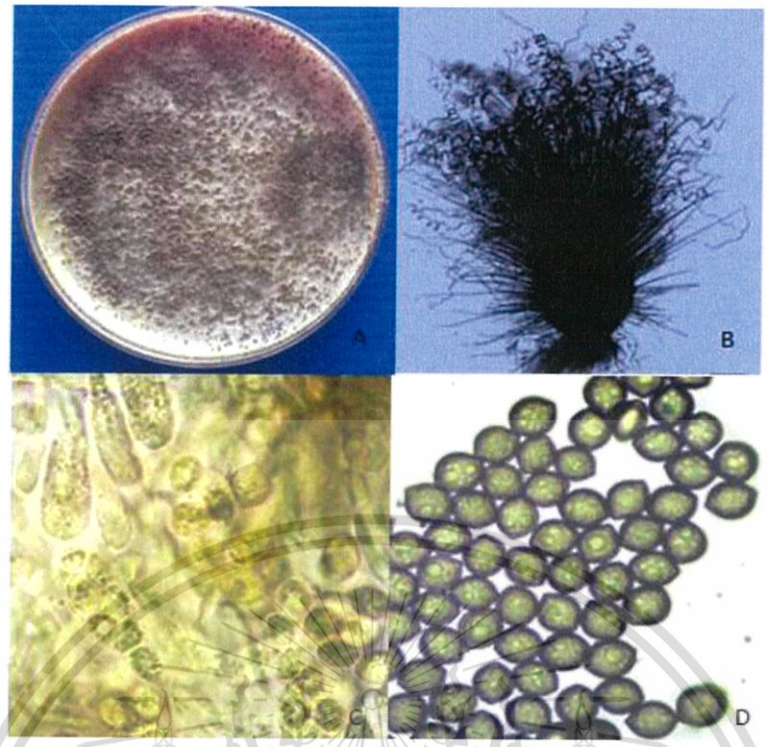

Figure 4.4 *Chaetomium cochliodes*, A= colony, B= ascocarps (10X), C= asci (40X) and D= ascospores (40X).

Colonies of *Ch. globosum* was slow growing with little superficial mycelium and a dense olivaceous layer on ascomata. Ascomata was dark brown or black colors, globose to subglobose, 230.0 - 370.0 × 210.0 - 350.0 µm, lateral hairs dark brown with paler tips, minutely roughened, terminal hairs dark olive brown with paler tips, wavy or loosely coiled and interwined. Ascopores was pale greenish to dark olive-brown, flattened lemon-shaped, 8.0 × 11.0 - 7.5 × 8.5 µm. (Figure. 4.5)

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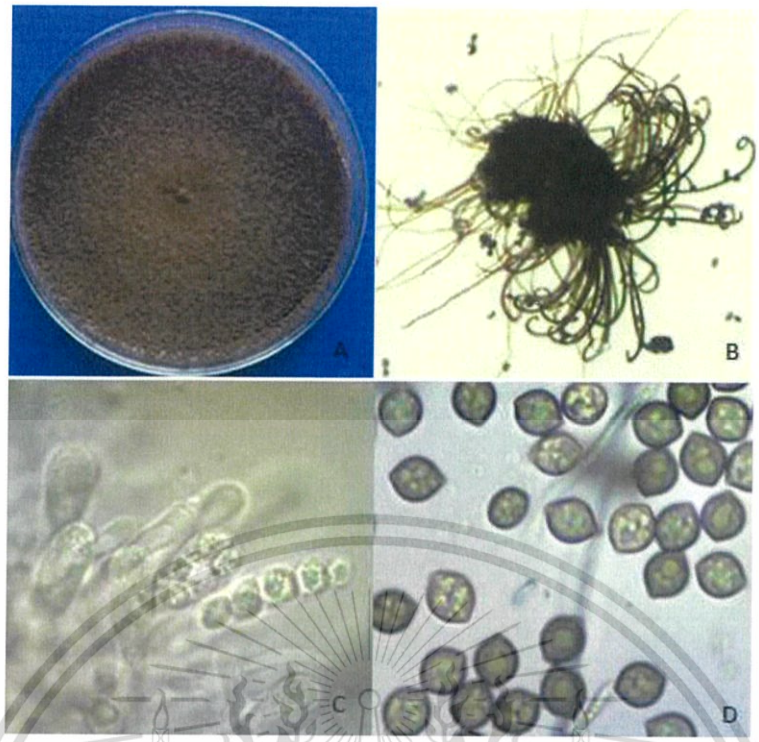

Figure 4.5 *Chaetomium globosum*, A= colony, B= ascocarps (10X), C= asci (40X) and D= ascospores (40X).

4.3 Molecular phylogeny identification based on DNA sequencing

After morphological studied of *Chaetomium* spp and *Fusarium* spp, both species were confirmed identification by molecular technique. The amplified products were visualized on 1% (w/v) agarose gel to confirm the presence of a single amplified band. Visible, amplified bands at about 650 bp for *Chaetomium* spp and 450 bp for *Fusarium* spp the relative molecular mass fragment size was consistent with the intended purpose.

The sequences were subjected to a preliminary BLAST analysis in NCBI databases, and sequence matches were used to determine their identities (Table 4.2). Multiple sequences alignment was then carried out using clustalW and phylogenetic tree was constructed using Mega 6.0 by neighbor-joining method. Phylogenetic analysis was confirmed all of fungal species.

Identification was done which based on morphology and molecular phylogeny. The BLAST analysis showed that the nucleotide sequences of *Ch. brasiliense* (b1), the analysis showed that its nucleotide sequences shared 99% identity with those of number JX966545, KT357646, KT 371339. *Ch. cochliodes* (co1) was identified, based on its morphology and the molecular analysis. The nucleotide sequences of *Ch. cochliodes* shared 99% identity with *Ch.*

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cochliodes accession number JN209864, HQ316556 and KT895345. The nucleotide sequences of *Ch. globosum* (g1) shared 96% identity with those of *Ch. globosum* accession number KC202936, KM030576, JF817309, HM016879 and KM822861 (Table 4.2). The amplified bands were used at about 500 bp for made phylogenic tree. As results, phylogenic tree was show that *Ch. brasiliense*, *Ch. cochliodes* and *Ch. globosum* were confirm the species level as show in figure4.6

The nucleotide sequences of *F. solani* shared 100% identity to confirm with those of *F. solani* accession number HQ833835, KF 494130, KF999012and KJ 584550 (Table 4.2). The phylogenic tree of *Fusariuum* spp confirmed the tested species as *Fusarium solani* at 99% similarity as compared to the different groups of *Fusarium poae* and *Fusarium oxysporum* (Figure 4.7).

Table 4.2 BLAST analysis of *Chaetomium* spp and *Fusarium* spp.

Isolates No.	Morphology identification	No. of bp analyzed	Close related species	GenBank accession number	Identity (%)
b1	*Ch. brasiliense*	544	*Ch. brasiliense*	JX966545	99
				KT357646	99
				KT371339	99
co1	*Ch. cochliodes*	553	*Ch. cochliodes*	JN209864	99
				HQ316556	99
				KT895345	99
g1	*Ch. globosum*	526	*Ch. globosum*	KC202936	96
				KM030576	96
				JF817309	96
				HM016879	96
				KM822861	96
s1	*F. solani*	453	*F. solani*	HQ833835	99
				KF494130	99
				KF999012	99
				KJ584550	99

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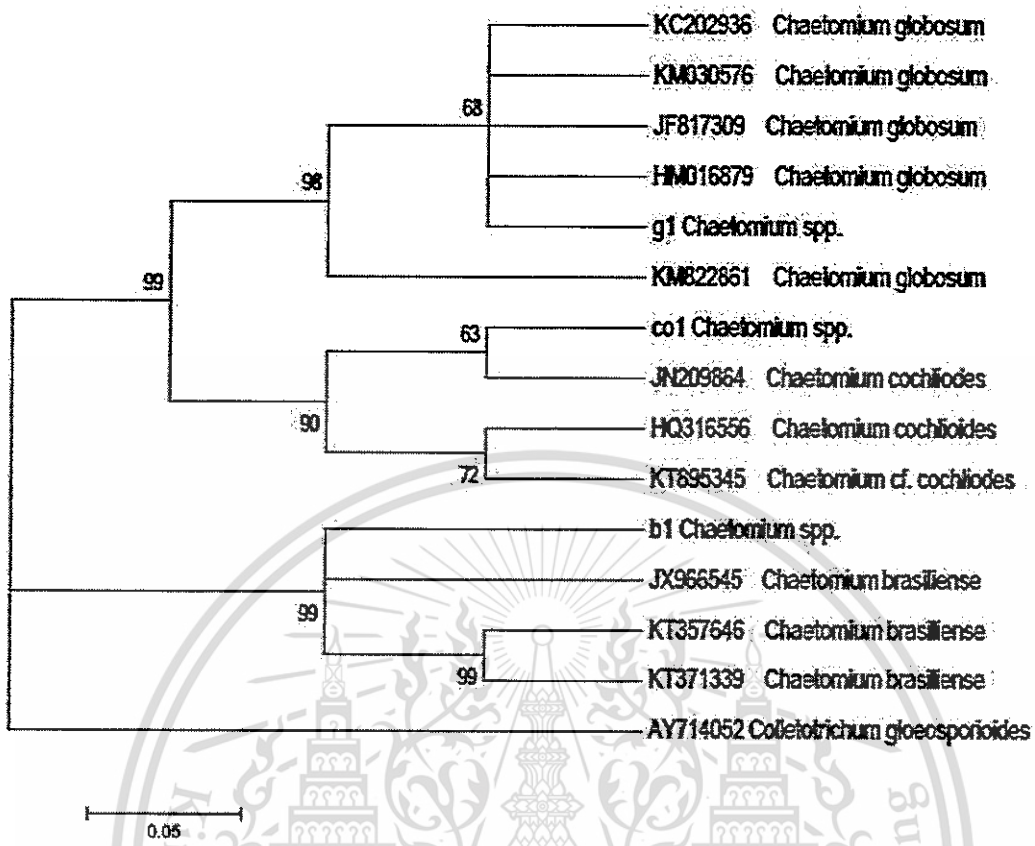

Figure 4.6 Phylogenetic tree to confirm and identified *Chaetomium* spp with related taxa inferred using a neighbor joining method with internal transcribed spacer (ITS) rDNA sequence. Bootstrap value based on 1,000 replications is shown above the branch.

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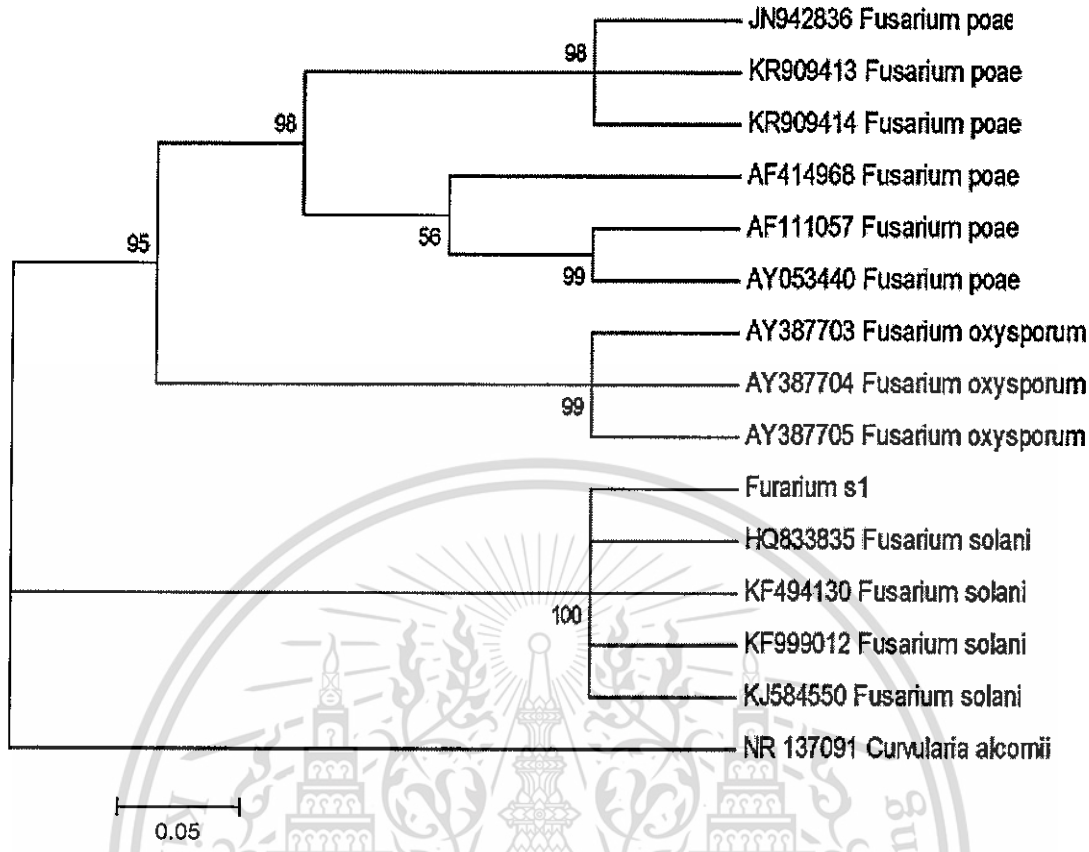

Figure 4.7 Phylogenetic tree to confirm and identified pathogen with related taxa inferred using a neighbor joining method with internal transcribed spacer (ITS) rDNA sequence. Bootstrap value based on 1,000 replications is shown above the branch.

4.4 Biological control of *Fusarium solani*

4.4.1 Dual-culture test

Ch. brasiliense, *Ch. cochliodes* and *Ch. globosum* were proved their abilities to inhibit plant pathogen *F. solani* causing disease of tomato by using dual-culture tests (Figure. 4.8).

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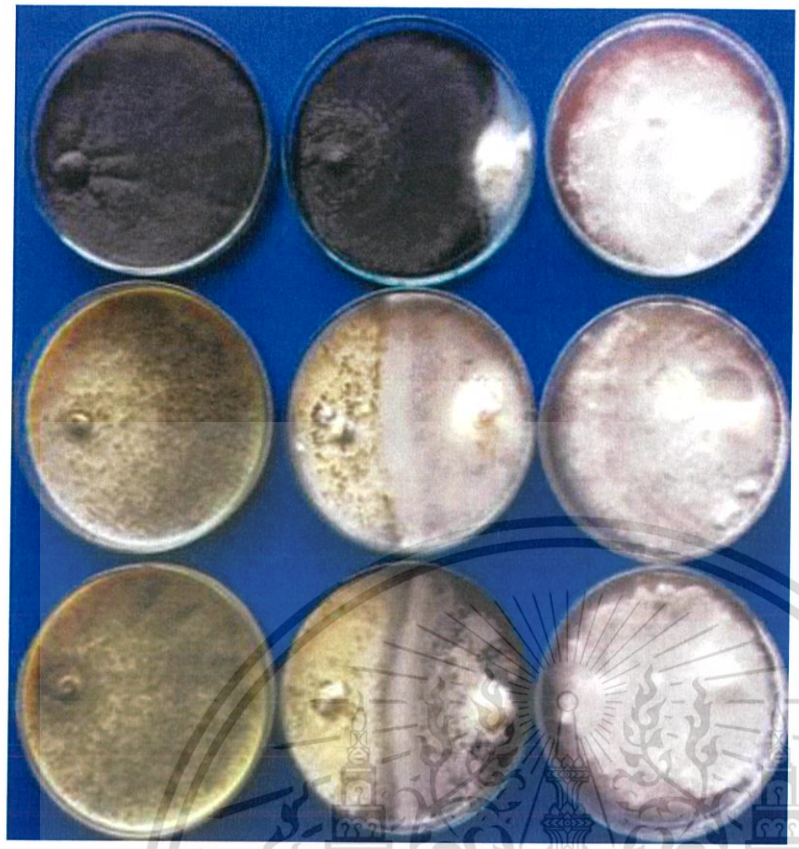

Chaetomium brasiliense

Vs *Fusarium solani*

Chaetomium cochliodes

Vs *Fusarium solani*

Chaetomium globosum

Vs *Fusarium solani*

Antagonistic plates Bi-culture plates Pathogen plates

Figure 4.8 Dual- culture *Chaetomium* spp aginst *Fusarium solani*.

The results showed that *Ch. brasiliense*, *Ch. cochliodes* and *Ch. globosum* gave significantly inhibition of *F. solani* which were 2.35 cm, 5.00 cm, 5.00 cm in colony diameter, respectively when compared to the control plate (Table 4.3). *Ch. brasiliense* showed higher inhibition percentage of colony diameter which was 68.68% than *Ch. globosum* and *Ch. cochliodes*, which were 51.67% and 47.65%, respectively. After 3 month *Ch. cochliodes* and *Ch. globosum* showed ability to grow over the colony of *F. solani* as seen in Figure 4.8.

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Table 4.3 Colony growth on dual-culture antagonistic tests.

Antagonist fungi	*Fusarium solani*	
	Colony(cm)	% inhibition of colony
Control	9.00[a 1/]	-
Chaetomium brasiliense	2.35[d]	73.85[a]
Chaetomium cochliodes	5.50[b]	38.88[c]
Chaetomium globosum	5.00[c]	44.35[b]
CV%	1.02	1.37

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

The number of spores that producing by the pathogen was counted by using Hemacytometer. The results showed that *Ch. brasiliense*, *Ch. globosum* and *Ch. cochliodes* significantly inhibited the production of pathogen spores and the number of pathogen spores were 1.60×10^6, 2.40×10^6, 2.60×10^6 spores, respectively when compared to the control plate (Table 4.4). The pathogen's spore production was inhibited 68.68, 51.67 and 47.65%, respectively. It was illustrated that all the tested antagonistic fungi showed inhibition of spore production of *F. solani* and *Ch. brasiliense* gave the best inhibition of pathogen's spore production.

Table 4.4 Number of pathogen spores on antagonistic dual-culture tests.

Antagonist fungi	*Fusarium solani*	
	Spores($\times10^6$)	%inhibition of number spores
Control	5.03[a2/]	-
Chaetomium brasiliense	1.60[c]	68.68[a]
Chaetomium cochliodes	2.60[b]	47.65[b]
Chaetomium globosum	2.40[b]	51.67[b]
CV%	16.15	16.99

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

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4.4.2 Bioactivity test of crude extract from *Chaetomium* spp against *F. solani*

Ch. brasiliense, *Ch. cochliodes* and *Ch. globosum* were extracted their bioactive substances as crude extracts and tested for their abilities to inhibit *F. solani*. The dried fungal biomass of *Chaetomium* spp. were prepared and soaked in different solvents successively, including hexane, ethyl acetate (EtOAc) and methanol (MeOH). The filtrates were evaporated by vacuum evaporator to yield crude extracts including crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH), respectively. After get each crude extract of *Chaetomium* spp, they were selected to preparation of nano particles by using the method of Dar and Soytong (2014) to get nano particles of each crude extract from *Chaetomium* spp. The color and texture of crude Hexane, EtOAc, MeOH and nano particles from hexane, EtOAc and MeOH were quite different: yellow, light brown, dark brown, orange or white color; oil, wax or solid textures (Figure 4.9-4.10).

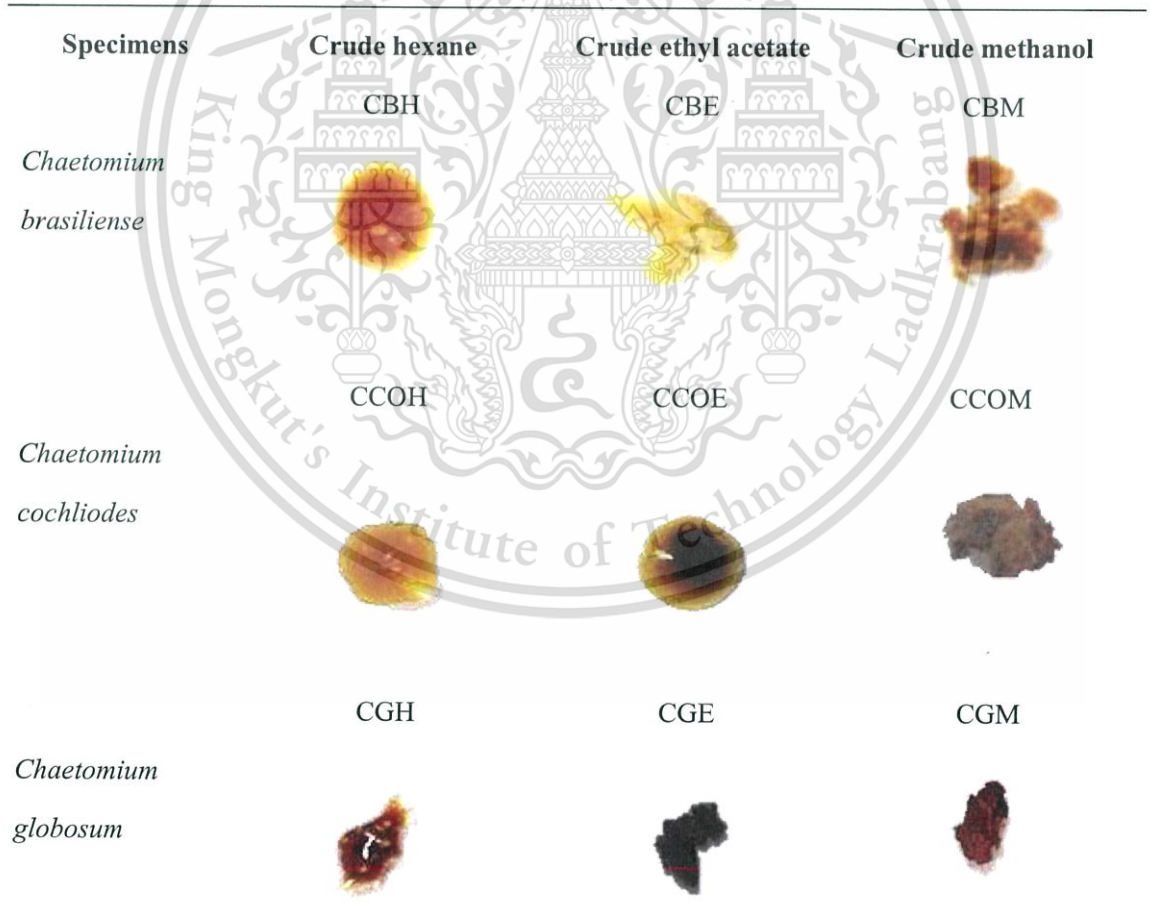

Figure 4.9 The characteristics of each crude extract

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Specimens	Crude hexane	Crude ethyl acetate	Crude methanol
Chaetomium brasiliense	nano-CBH 	nano-CBE 	nano-CBM 
Chaetomium cochliodes	nano-CCOH 	nano-CCOE 	nano-CCOM 
Chaetomium globosum	nano-CGH 	nano-CGE 	nano-CGM 

Figure 4.10 The characteristics of each nano particles.

4.4.2.1 Bioactivity test of crude extracts of *Chaetomium* spp against *F. solani*

The crude extracts from *Ch. brasiliense, Ch. cochliodes* and *Ch. globosum* were used to test their ability to control the growth of *F. solani*. Each crude extract was tested its inhibition against colony growth and spore production of pathogens with 6 concentrations (0, 10, 50, 100, 500, 1000 ppm) at room temperature and the abnormal spores were also observed and compared with normal spores of pathogens. (Figure 4.14 Figure 4.16, Figure 4.18)

The crude extracts from *Ch. brasiliense* were tested their bioactivity against *F. solani*. The results showed that crude ethyl acetate (EtOAc) extract gave highest inhibition of *F. solani* colony growth which was 73.00% at the concentration of 1,000 ppm with the ED_{50} values of 264.87 ppm when compared to the control (Table 4.5, Figure 4.11). Crude ethyl acetate (EtOAc) extract showed significantly highest inhibition for the spore production of *F. solani* as 97.06%, and the ED_{50} was 65.44 ppm, and followed by crude methanol (MeOH) extract which

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gave 96.43% inhibition and ED_{50} value was 69.06 ppm. Crude hexane extract showed 70.63% and the ED_{50} was 102.58 ppm (Table 4.6).

Methanol crude extract from *Ch. cochliodes* gave significantly highest inhibition of *F. solani* colony growth as 40.00% with the ED_{50} values of 1924.43 ppm when compared to the control (Table 4.7, Figure 4.13). Crude ethyl acetate extract from *Ch. cochliodes* showed significantly highest inhibition for the spore production of *F. solani* as 97.06% with the ED_{50} was 319.45 ppm. Crude methanol extract gave 96.43% inhibition with ED_{50} was 229.08 ppm and crude hexane extract showed 70.63% inhibition with ED_{50} was 973.76 ppm (Table 4.8).

Crude hexane extract from *Ch. globosum* gave significantly highest inhibition of 64.75% for the colony growth of *F. solani* with the ED_{50} values of 851.01 ppm, followed by crude methanol extract which inhibited 52.00% (Table 4.9, Figure 4.15). Crude hexane extract from *Ch. globosum* gave significantly highest inhibition for the spore production of *F. solani* as 93.29% with ED_{50} of 200.05 ppm. Crude methanol extract gave 79.75% inhibition with the ED_{50} was 378.47 ppm. Crude ethyl acetate extract showed 72.08% inhibition with ED_{50} was 314.16 ppm (Table 4.10).

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Table 4.5 Crude extracts of *Ch. brasilense* testing for growth inhibition of *F.solani* at 7 days

Crude extracts	Concentration (ppm)	Colony diameter(cm)[1/]	Growth inhibition(%)[2/,3/]	ED_{50}(ppm)
Hexane	0	5^{a}	-	2001.99
	10	5^{a}	0^{h}	
	50	4.98^{ab}	0.50^{h}	
	100	4.98^{ab}	0.50^{h}	
	500	3.43^{e}	31.25^{d}	
	1,000	2.37^{f}	52.00^{c}	
EtOAc	0	5^{a}	-	264.87
	10	4.61^{b}	7.75^{g}	
	50	4.41^{c}	11.75^{f}	
	100	3.73^{d}	25.25^{e}	
	500	1.62^{g}	67.50^{b}	
	1,000	1.34^{h}	73.00^{a}	
MeOH	0	5^{a}	-	481.47
	10	5^{a}	0^{h}	
	50	5^{a}	0^{h}	
	100	4.95^{ab}	10.00^{h}	
	500	2.37^{f}	52.50^{e}	
	1,000	1.41^{h}	71.75^{a}	
C.V. (%)		1.87	6.95	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

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

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Table 4.6 Spore inhibition of crude extracts from *Ch. brasilense* to *F. solani* at 7 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	6.60[a]	-	102.58
	10	5.73[ab]	13.23[h]	
	50	5.52[b]	16.53[h]	
	100	3.55[de]	45.45[ef]	
	500	2.22[g]	65.78[cd]	
	1,000	1.91[gh]	70.63[e]	
EtOAc	0	6.60[a]	-	65.44
	10	5.23[b]	20.33[h]	
	50	4.18[cd]	36.11[fg]	
	100	3.26[ef]	50.22[e]	
	500	1.06[h]	83.75[b]	
	1,000	0.23[i]	97.06[a]	
MeOH	0	6.60[a]	-	69.06
	10	5.59[ab]	15.25[h]	
	50	4.45[cd]	31.83[g]	
	100	4.35[cd]	33.67[g]	
	500	2.62[fg]	59.95[d]	
	1,000	0.18[i]	96.43[a]	
C.V. (%)		12.82		

[1/] Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2/] Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[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 .

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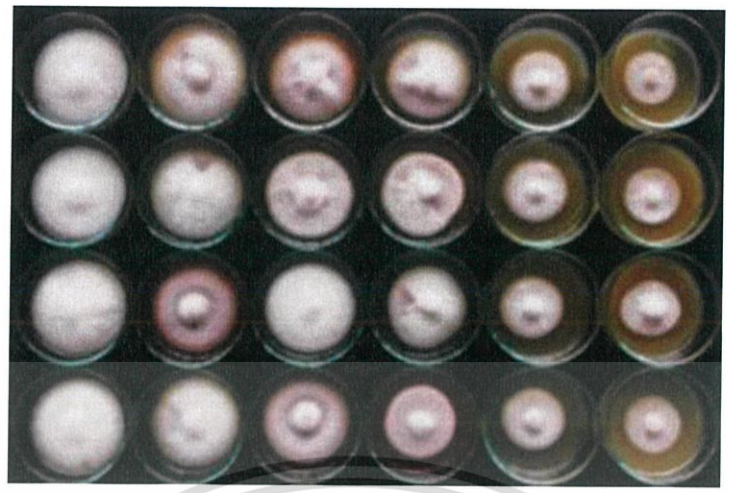
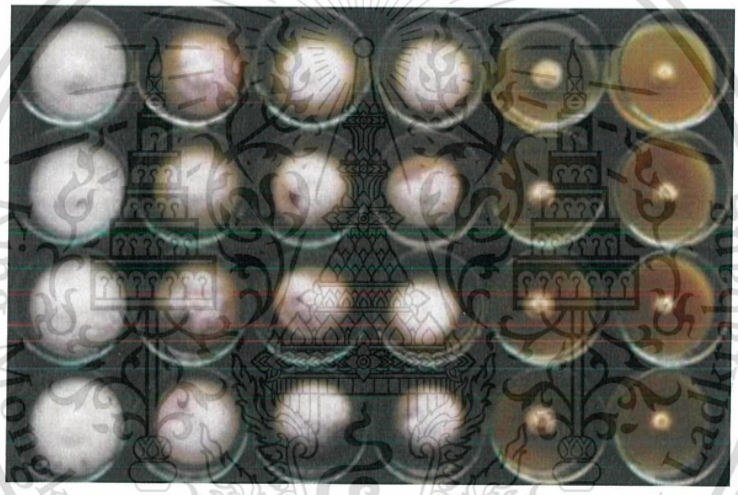
Crude Hexane



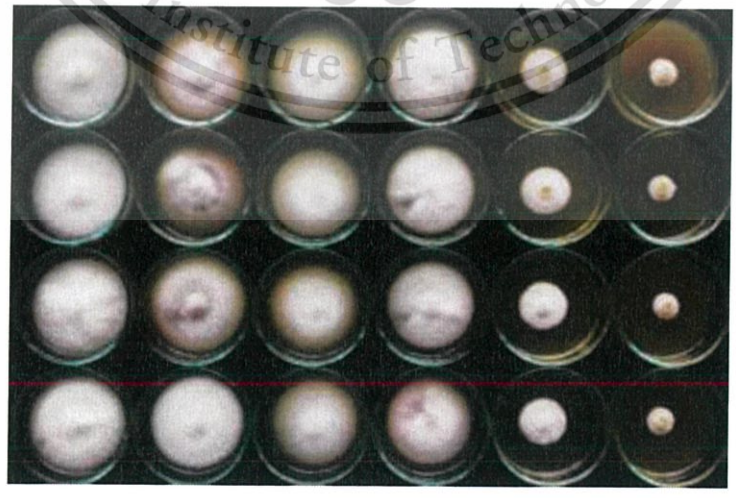
0 ppm 10 ppm 50 ppm 100 ppm 500 ppm 1000 ppm

Crude EtOAc



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

Crude MeOH



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

Figure 4.11 Crude extract test of *Ch. brasilense* inhibit colony growth of *F. solani*

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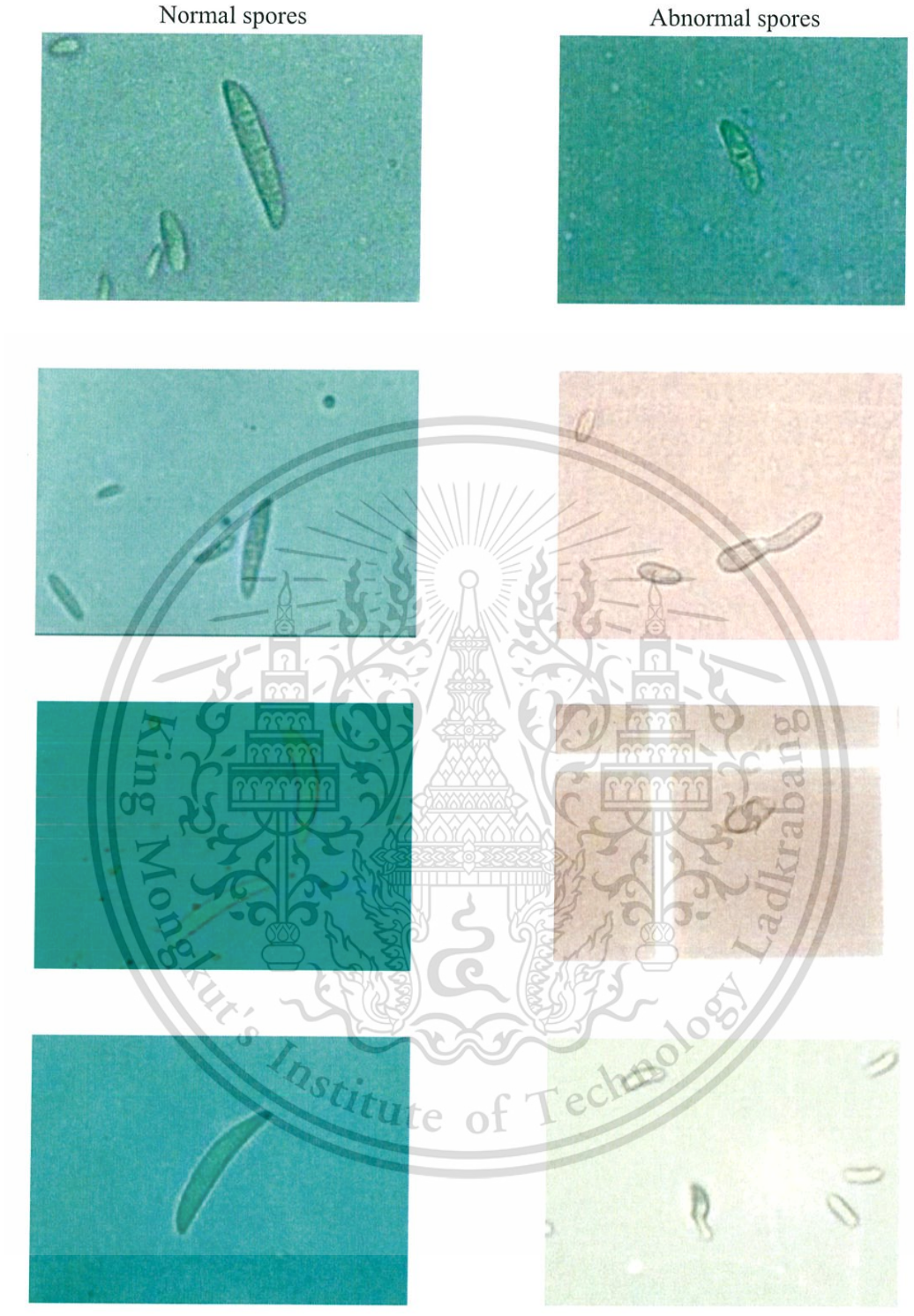

Figure 4.12 Comparison of normal spores and abnormal spores after test with crude extract from *Ch. brasilense* at concentration 1,000 ppm.

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Table 4.7 Crude extracts of *Ch. cochliodes* testing for growth inhibition of *F. solani* at 7 days

Crude extracts	Concentration (ppm)	Colony diameter(cm)[1/]	Growth inhibition(%)[2/,3]	ED_{50}(ppm)
Hexane	0	5^{a}	-	-
	10	4.95^{ab}	1.50^{gh}	
	50	4.92^{ab}	1.50^{gh}	
	100	4.63^{c}	7.25^{f}	
	500	3.73^{f}	25.25^{c}	
	1,000	3.50^{g}	30.00^{b}	
EtOAc	0	5^{a}	-	-
	10	4.95^{a}	10.00^{h}	
	50	4.12^{e}	17.50^{d}	
	100	4.12^{e}	17.50^{d}	
	500	3.61^{fg}	27.75^{bc}	
	1,000	3.10^{h}	38.00^{a}	
MeOH	0	5^{a}	-	-
	10	4.95^{a}	10.00^{h}	
	50	4.80^{b}	4.00^{g}	
	100	4.26^{d}	14.75^{e}	
	500	3.66^{f}	26.75^{c}	
	1,000	3.00^{h}	40.00^{a}	
C.V. (%)		2.14	13.08	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

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

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Table 4.8 Spore inhibition of crude extracts from *Ch. cochliodes* to *F. solani* at 7 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	6.60[a]	-	973.76
	10	5.49[bc]	13.23[h]	
	50	5.17[bcd]	16.53[h]	
	100	3.55[de]	45.45[ef]	
	500	2.22[g]	65.78[cd]	
	1,000	1.91[gh]	70.63[c]	
EtOAc	0	6.60[a]	-	319.45
	10	5.23[b]	20.33[h]	
	50	4.18[cd]	36.11[fg]	
	100	3.26[ef]	50.22[e]	
	500	1.06[h]	83.75[b]	
	1,000	0.23[i]	97.06[a]	
MeOH	0	6.60[a]	-	229.08
	10	5.59[ab]	15.25[h]	
	50	4.45[cd]	31.83[g]	
	100	4.35[cd]	33.67[g]	
	500	2.62[fg]	59.95[d]	
	1,000	0.18[i]	96.43[a]	
C.V. (%)		7.66	18.24	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[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 .

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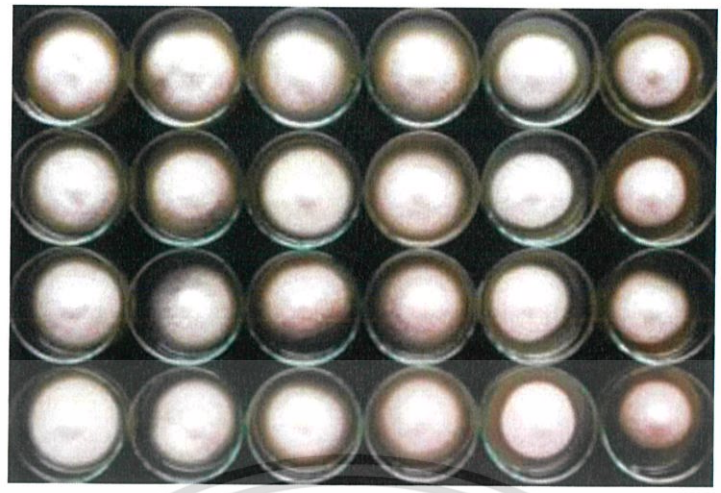
Crude Hexane



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

Crude EtOAc

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

Crude MeOH

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

Figure 4.13 Crude extract test of *Ch. cochliodes* aginst *F. solani*

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Figure 4.14 Comparison of normal spores and abnormal spores after test with crude extract from *Ch. cochliodes* at concentration 1,000 ppm.

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Table 4.9 Crude extracts of *Ch. globosum* testing for growth inhibition of *F. solani* at 7 days

Crude extracts	Concentration (ppm)	Colony diameter(cm)[1/]	Growth inhibition(%)[2/,3/]	ED_{50}(ppm)
Hexane	0	5[a]	-	851.01
	10	5[a]	-	
	50	5[a]	-	
	100	5[a]	-	
	500	3.82[c]	23.50[e]	
	1,000	1.76[g]	64.75[a]	
EtOAc	0	5[a]	-	-
	10	5[a]	-	
	50	5[a]	-	
	100	5[a]	-	
	500	3.72[d]	25.50[d]	
	1,000	3.40[e]	32.00[c]	
MeOH	0	5[a]	-	909.91
	10	5[a]	-	
	50	5[a]	-	
	100	5[a]	-	
	500	3.92[b]	21.00[f]	
	1,000	2.39[f]	52.00[b]	
C.V. (%)		4.39	11.46	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

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

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Table 4.10 Spore inhibition of crude extracts from *Ch. globosum* to *F. solani* at 7 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	6.52[a]	-	200.05
	10	5.81[bc]	11.01[ij]	
	50	4.88[ef]	25.13[fg]	
	100	4.75[f]	27.23[f]	
	500	2.52[h]	61.27[d]	
	1,000	0.44[k]	93.29[a]	
EtOAc	0	6.52[a]	-	314.16
	10	5.58[bc]	12.85[ij]	
	50	5.10[def]	21.84[fgh]	
	100	4.74[f]	27.34[f]	
	500	2.91[gh]	55.40[de]	
	1,000	1.82[i]	72.08[c]	
MeOH	0	6.52[a]	-	378.47
	10	6.04[b]	7.28[j]	
	50	5.43[cd]	16.59[hi]	
	100	5.23[de]	19.83[gh]	
	500	3.08[g]	52.65[e]	
	1,000	1.32[j]	79.75[b]	
C.V. (%)		4.41	11.66	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[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 .

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Crude Hexane

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

Crude EtOAc

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

Crude MeOH

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

Figure 4.15 Crude extract test of *Ch. globosum* against *F. solani*

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Figure 4.16 Comparison of normal spores and abnormal spores after tested with crude extract from *Ch. globosum* at concentration 1,000 ppm.

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4.4.2.2 Testing nano particles from *Chaetomium* spp against *F. solani*

The nano particles from *Ch. brasiliense, Ch. cochliodes* and *Ch. globosum* were tested their abilities to inhibit *F. solani*. Each nano particles was tested against colony growth and spore production of pathogens with 5 concentrations (0, 3, 5, 10, 15 ppm) at room temperature and the abnormal spores were observed and compared with normal spores of pathogens.(Figure 4.19, Figure 4.21, Figure 4.23)

Results showed that nano- CBM from *Ch. brasiliense* gave significantly highest inhibition of 90.00% for the colony growth of *F. solani* at concentration of 15 ppm which the ED_{50} values of 6.85 ppm when compared to the control, followed by Nano CBH which inhibited 72.25% and nano- CBE gave inhibited 53.50% (Table 4.11, Figure 4.18). Nano-CBM from *Ch. brasiliense* gave significantly highest inhibition for the spore production of *F. solani* as 98.76% at concentration of 15 ppm and the effective dose (ED_{50}) was 3.86 ppm, followed by nano-CBH which gave 86.83% and ED_{50} was 4.37 ppm. Nano-CBE showed 85.44% inhibition and ED_{50} was 5.42 ppm (Table 4.12).

The nano-CCOM from *Ch. cochliodes* expressed the highest colony inhibition percentage of *F. solani* which was 71.25% which the ED_{50} values of 12.52 ppm when compared to the control, followed by Nano-CCOH which inhibited 58.00 % and nano-CCOE gave 36.50 % inhibition (Table 4.13, Figure 4.20). Nano-CCOM gave significantly highest inhibition for the spore production of *F. solani* as 84.34% and the effective dose (ED_{50}) was 8.78 ppm, followed by nano-CCOH which gave 79.24% inhibition and ED_{50} was 9.21 ppm. Nano-CCOE showed 73.39% inhibition and ED_{50} was 9.70 ppm (Table 4.14).

The resulted of Nano particle from *Ch. globosum* was showed that Nano-CGH inhibited colony growth of *F. solani* as 90.00% which the ED_{50} values of 7.59 ppm. Nano -CGE inhibited colony growth of *F. solani* as 90.00% which the ED_{50} values of 8.68 ppm and nano-CGM inhibited colony growth of *F. solani* as 90.00% which the ED_{50} values of 3.78 ppm (Table 4.15, Figure 4.22). The resulted showed that nano-CGH, nano-CGM and nano-CGM gave the highest ability to inhibit spore production of *F. solani* as 99.39%, 99.54% and 99.69% respectively and ED_{50} were 3.41, 3.48 and 1.48 ppm respectively (Table 4.16).

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Table 4.11 Growth inhibition and ED_{50} of nano-CBH, nano-CBH, nano-CBM agianst *F. solani* at 7 days

Nano particles	Concentration (ppm)	Colony diameter(cm)[1/]	Growth inhibition(%)[2/,3/]	ED_{50}(ppm)
Nano-CBH*	0	5^{a}	-	11.30
	3	4.52^{ab}	9.50^{gh}	
	5	4.33^{cd}	13.25^{fg}	
	10	4.08^{cd}	18.25^{ef}	
	15	1.38^{h}	72.25^{b}	
Nano-CBE	0	5^{a}	-	10.03
	3	4.50^{bc}	10.00^{gh}	
	5	4.02^{e}	19.50^{e}	
	10	2.37^{f}	52.50^{d}	
	15	2.32^{f}	53.50^{d}	
Nano-CBM	0	5^{a}	-	6.85
	3	4.70^{a}	6.00^{h}	
	5	3.88^{e}	22.25^{e}	
	10	1.76^{g}	64.75^{c}	
	15	0.50^{i}	90.00^{a}	
C.V. (%)		5.16	12.76	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.[3/]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.* Nano particles derived from *Chaetomium brasiliense* namely nano CBH, nano CBE, nano CBM ,

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Table 4.12 Spore inhibition and ED_{50} of nano particles from *Ch. brasiliense* against *F. solani* at 7 days

Crude extracts	Concentration (ppm)	Number of spores [1/] (10^6)	Inhibition(%) [2/,3/]	ED_{50} (ppm)
Nano-CBM*	0	89.75[a]	-	4.37
	3	55.75[c]	37.93[fg]	
	5	37.87[d]	57.79[e]	
	10	30.75[e]	65.81[d]	
	15	11.87[g]	86.83[b]	
Nano-CBM	0	89.75[a]	-	5.42
	3	63.75[b]	29.06[h]	
	5	53.50[c]	40.43[f]	
	10	20.25[f]	77.52[c]	
	15	12.75[g]	85.44[b]	
Nano-CBM	0	89.75[a]	-	3.86
	3	57.62[c]	35.85[g]	
	5	30.37[e]	66.21[d]	
	10	12.37[g]	86..23[b]	
	15	1.12[h]	98.76[a]	
C.V. (%)		7.88	4.41	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [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 .* Nano particles derived from *Chaetomium brasiliense* namely nano CBH, nano CBE, nano CBM

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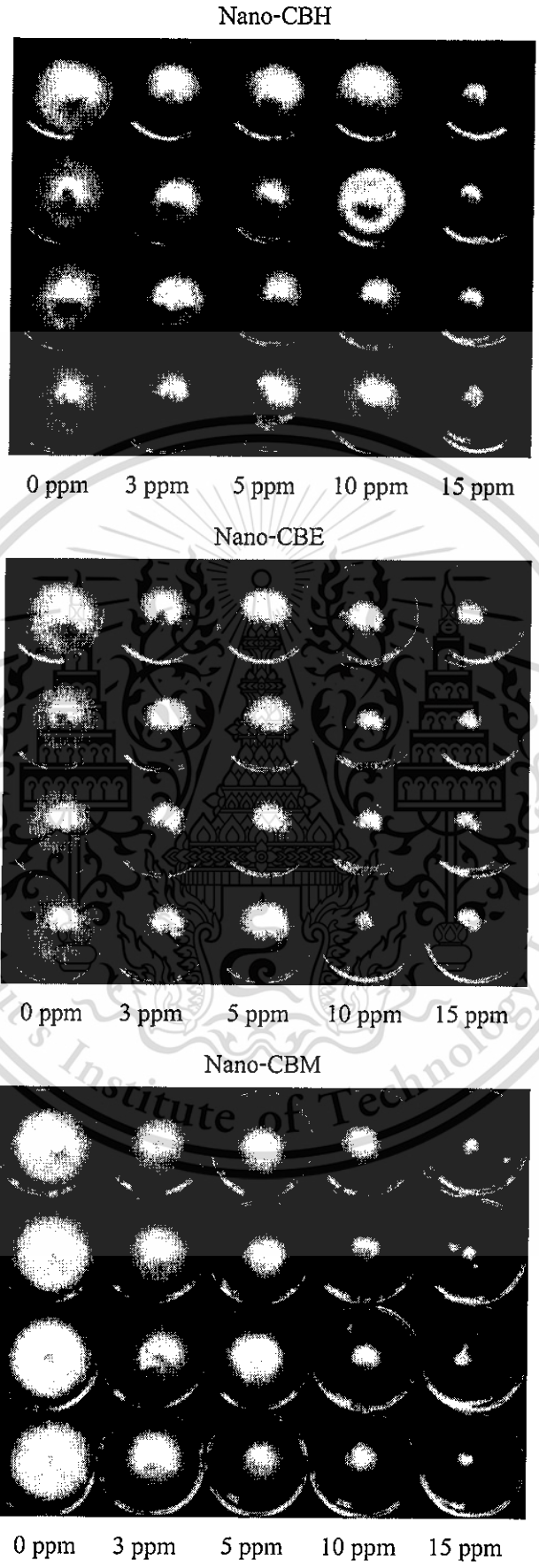

Figure 4.17 Testing nano particles of *Ch. brasiliense* against *F. solani*

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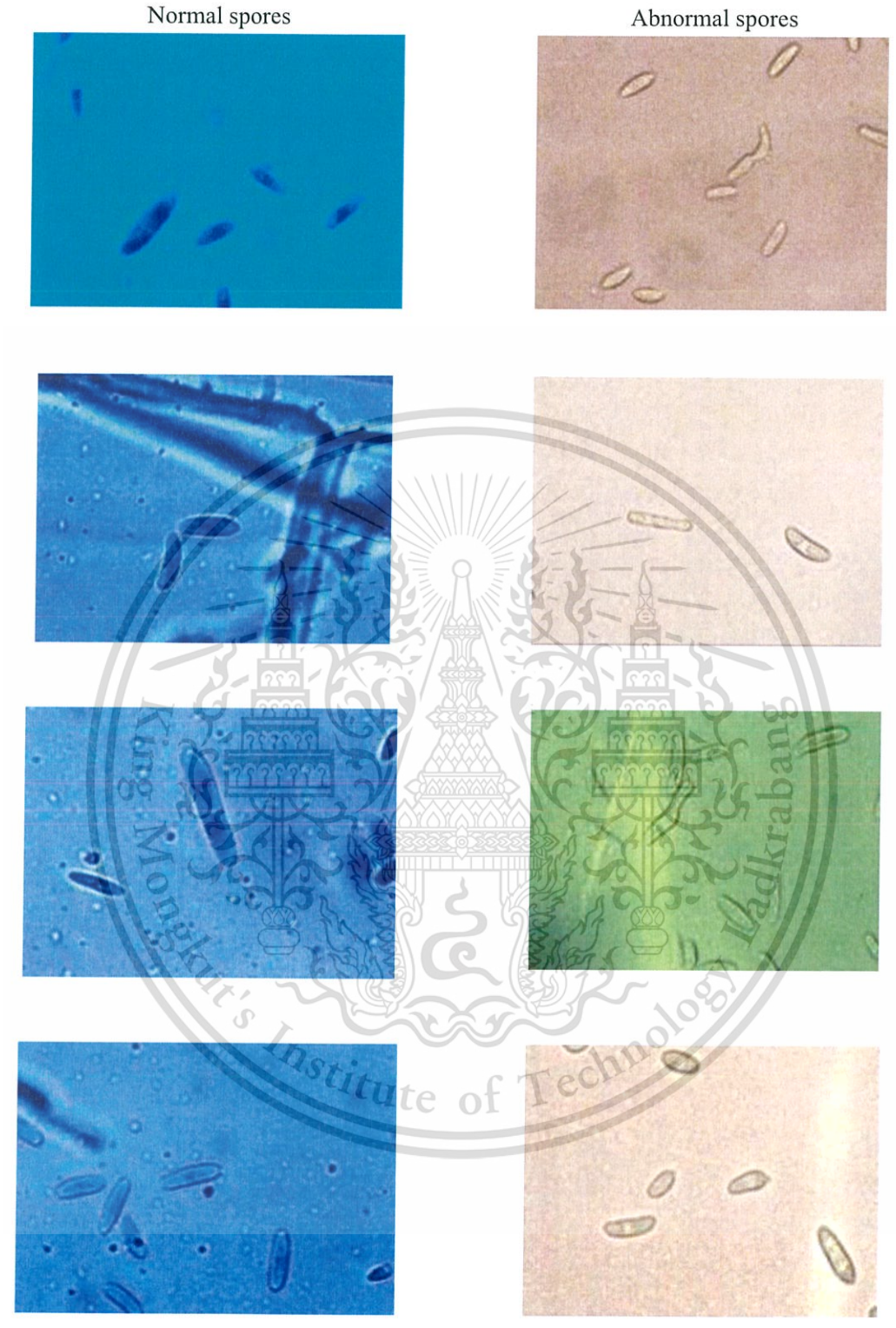

Figure 4.18 Comparison of normal spores and abnormal spores after tested with nano particles from *Ch. brasiliense* at concentration 15 ppm.

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Table 4.13 Growth inhibition and ED_{50} of nano-particles agianst *F. solani* at at 7 days

Nano particles	Concentration (ppm)	Colony diameter(cm)[1/]	Growth inhibition(%)[2/,3/]	ED_{50}(ppm)
	0	5[a]	-	
Nano-	3	5[a]	-	
CCOH*	5	4.26[b]	14.75[f]	9.06
	10	3.67[d]	26.50[d]	
	15	2.09[f]	58.00[b]	
	0	5[a]	-	
Nano-	3	5[a]	-	
CCOE	5	5[a]	-	-
	10	3.92[c]	21.50[e]	
	15	3.17[e]	36.50[c]	
	0	5[a]	-	
Nano-	3	5[a]	-	
CCOM	5	5[a]	-	12.52
	10	3.93[c]	21.25[e]	
	15	1.43[g]	71.25[a]	
C.V. (%)		3.17	15.89	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [3/]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.* Nano particles derived from *Chaetomium cochliodes* namely nano CCH, nano CCE, nano CCM.

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Table 4.14 Spore inhibition and ED_{50} of nano particles from *Ch. cochliodes* against *F. solani* at 7 days

Crude extracts	Concentration (ppm)	Number of spores [1/](10^6)	Inhibition(%)[2,3]	ED_{50} (ppm)
	0	87.75[a]	-	
Nano-	3	83.37[ab]	5.01[h]	
CCOH*	5	76.62[c]	12.65[f]	9.21
	10	39.62[d]	54.87[e]	
	15	18.25[f]	79.24[b]	
	0	87.75[a]	-	
Nano-	3	83.55[ab]	4.85[h]	
CCOE	5	79.00[bc]	10.00[g]	9.70
	10	38.75[d]	55.88[e]	
	15	23.37[e]	73.39[c]	
	0	87.75[a]	-	
Nano-	3	82.75[ab]	5.74[h]	
CCOM	5	79.37[bc]	9.60[g]	8.78
	10	35.75[d]	59.28[d]	
	15	13.75[f]	84.34[a]	
C.V. (%)		5.44	5.76	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [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. * Nano particles derived from *Chaetomium cochliodes* namely nano CCH, nano CCE, nano CCM.

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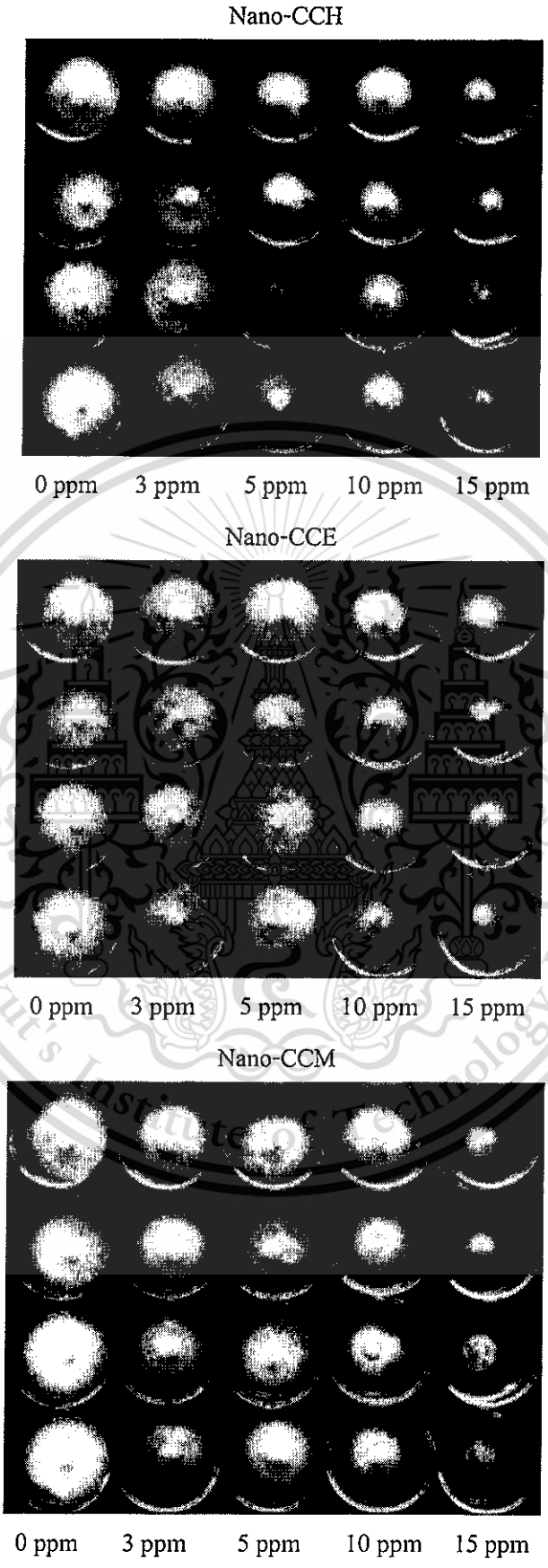

Figure 4.19 Testing nano particles from *Ch. cochliodes* against *F. solani*

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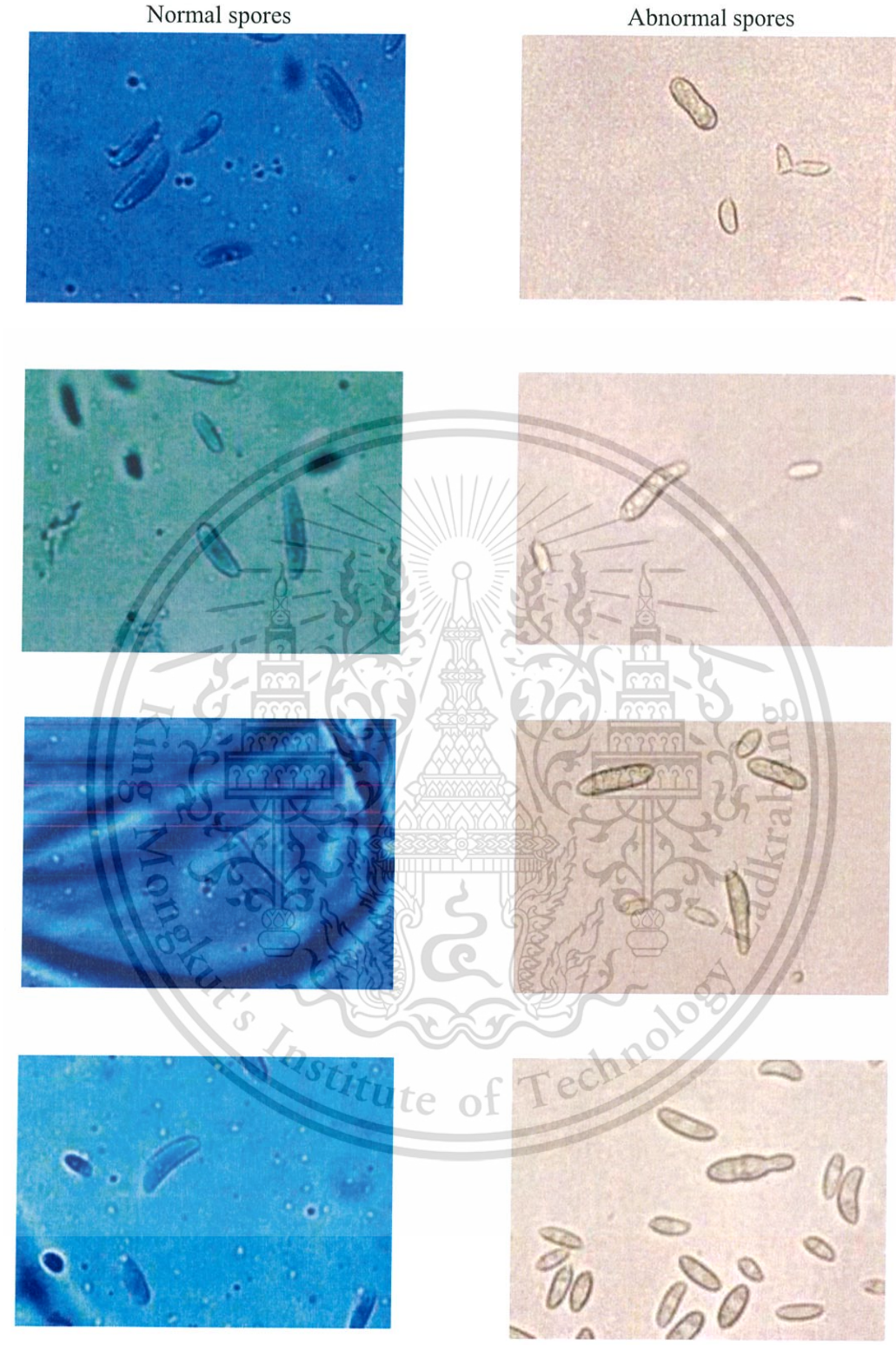

Figure 4.20 Comparison of normal spores and abnormal spores after testing with nano particles from *Ch. cochliodes* at concentration 15 ppm.

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Table 4.15 Growth inhibition and ED_{50} of nano-particles against *F. solani* at 7 days

Nano particles	Concentration (ppm)	Colony diameter(cm)[1/]	Growth inhibition(%)[2/,3/]	ED_{50}(ppm)
	0	5[a]	-	
Nano-	3	4.71[b]	5.75[h]	
CGH*	5	3.56[c]	28.75[g]	7.59
	10	2.02[f]	59.50[d]	
	15	0.50[i]	90.00[a]	
	0	5[a]	-	
Nano-	3	4.69[b]	6.00[h]	
CGE	5	3.67[c]	26.50[g]	8.68
	10	2.73[e]	45.25[e]	
	15	0.50[i]	90.00[a]	
	0	5[a]	-	
Nano-	3	3.23[d]	35.25[f]	
CGM	5	1.73[g]	65.25[e]	3.78
	10	0.77[h]	84.50[b]	
	15	0.50[i]	90.00[a]	
C.V. (%)		3.08	4.29	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [3/]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates. *Nano particles derived from *Chaetomium globosum* namely nano CGH, nano CGE, nano CGM.

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Table 4.16 Spore inhibition and ED_{50} of nano particles from *Ch. globosum* against *F. solani* at 7 days

Crude extracts	Concentration (ppm)	Number of spores [1/] (10^6)	Inhibition(%) [2/,3/]	ED_{50} (ppm)
	0	82.37[a]	-	
Nano-	3	50.87[b]	38.24[g]	
CGH*	5	17.75[d]	78.48[e]	3.41
	10	6.12[e]	92.56[c]	
	15	0.50[g]	99.39[a]	
	0	82.37[a]	-	
Nano-	3	52.62[b]	36.13[h]	
CGE	5	17.25[d]	79.07[e]	3.48
	10	7.50[e]	90.91[d]	
	15	0.37[g]	99.54[a]	
	0	82.37[a]	-	
Nano-	3	19.87[c]	75.88[f]	
CGM	5	7.50[e]	90.90[d]	1.48
	10	3.12[f]	96.21[b]	
	15	0.25[g]	99.69[a]	
C.V. (%)		4.51	1.61	

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [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 . * Nano particles derived from *Chaetomium globosum* namely nano CGH, nano CGE, nano CGM.

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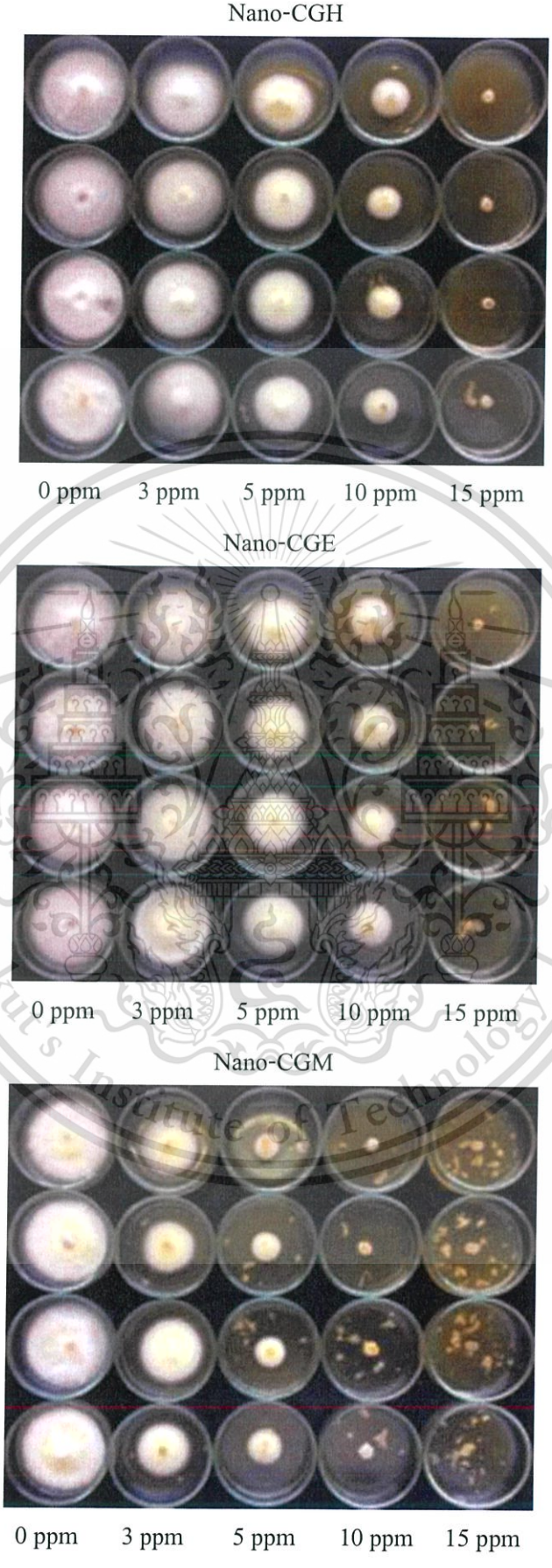

Figure 4.21 Testing nano particles from *Ch. globosum* against *F. solani*

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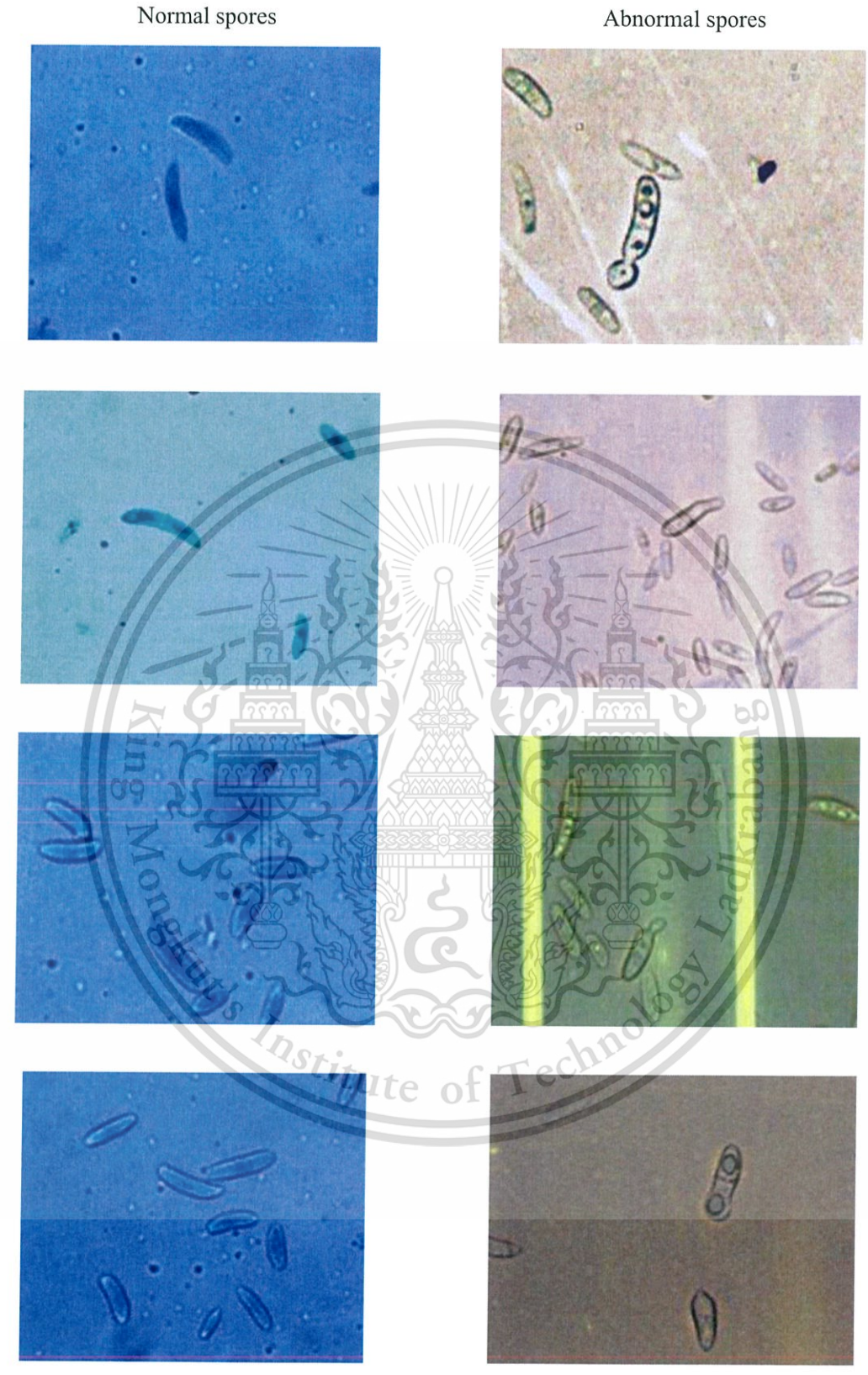

Figure 4.22 Comparison of normal spores and abnormal spores after testing with nano particles from *Ch. globosum* at concentration 15 ppm.

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4.4.2.3 Testing crude extracts and nano-particles from *Chaetomium* spp to inhibit *F. solani* causing crown and root rot of tomato

Crude extracts and nano-particles from *Chaetomium* spp were tested for their abilities to control crown and root rot of tomato cause by *F. solani* (Figure 4.24-Figure 4.28). Result showed that crude-CG gave significantly highest plant growth of 102.00 cm, followed by nano-CG , nano-CCO, crude-CB and crude-CCO which were 95.87, 93.25, 92.50 and 93.25 cm respectively (Table 4.18, Figure 4.26). The resulted showed that nano-CB, nano-CG , nano-CCO , crude-CG , crude-CB and crude-CCO gave significantly highest root lenght of 46.00, 45.00, 41.75, 40.62, 40.38 and 35.75 cm., respectively when compared to the inoculated control and non-inoculated one (Table 4.18, Figure 4.27). The result of stem fresh weight showed that crude-CB gave significantly highest fresh stem weight of 66.56g, followed by crude-CCO, nano-CCO, nano-CB, nano-CG, non-inoculated and crude CG which were 64.25, 64.13, 59.52, 53.70, 51.34 and 49.74 cm, respectively when compared to the inoculated control of 12.91 g and resulted of dried stem weight showed that crude-CB gave significantly highest of 16.75 g and followed by nano-CCO, crude-CCO, nano-CB, and nano-CG which were 16.61, 16.18, 15.74, 14.12 g, respectively when compared to the inoculated control which was 0.35 g (Table 4.19).

Crude-CB expressed highest fresh weight of 4.20 g and followed by nano-CG, crude-CG, nano-CCO, crude-CCO, nano-CB which were 4.13, 4.09, 3.83, 3.61, 3.23 g, respectively when compared to the inoculated control which was 1.07 g and non-inoculated was with 2.88 g. Dried weight of roots resulted that crude-CB expressed highest dried weight of tomato root which was 2.17 g., and followed by nano-CCO, nano-CG, crude-CG, and crude-CCO which were 2.04, 1.92, 1.85 and 1.85 g., respectively, when compared to the treated inoculated control which was ~~with~~ 0.31 g and non- inoculated one was 1.67 g. Moreover, nano-CG gave significantly highest in yields as 75.48 g when compared to the inoculated control of 22.71 g (Table 4.18). After three month of disease reduction showed that crude-CG gave highest percent on disease reduction as 83.33% when compared with non-inoculated control. Crude-CB, nano-CCO and nano-CG gave percent of disease reduction as 57.14%, and followed by nano-CB and crude-CCO which were 53.84 and 50.00 %, respectively (Table 4.17).

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Table 4.17 Disease after testing crude extracts and nano-particles from *Chaetomium* spp to inhibit *F. solani* causing crown and root rot of tomato var sida in pot experiment

Treatments	Disease index			Disease
	1 month	2 month	3 month	Reduction (%)
T1 inoculated	$3^{1/a}$	3.25^{a}	5.5^{a}	-
T2 non- inoculated	1^{c}	1^{c}	1^{f}	-
T3 crude-CB	2^{b}	2^{b}	3.5^{b}	57.14^{b}
T4 crude-CCO	2^{b}	2^{b}	2.25^{e}	50.00^{d}
T5 crude-CG	2^{b}	2^{b}	3^{d}	83.33^{a}
T6 nano-CB	2^{b}	2^{b}	3.25^{c}	53.84^{c}
T7 nano-CCO	2^{b}	2^{b}	3.5^{b}	57.14^{b}
T8 nano-CG	2^{b}	2^{b}	3.5^{b}	57.14^{b}
C.V. (%)	0.03	1.03	1.11	1.34

Disease index are as follows: 1= no symptom; 2= yellowing leaves and root rot 1-20%, 3= yellowing leaves and root rot 21- 40%, 4= yellowing leaves and root rot 41-60%, 5= yellowing leaves and root rot 61-80%, and 6= yellowing leaves and root rot 81-100% or die. $^{1/}$Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

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Table 4.18 Plant height and root length after testing crude extracts and nano-particles from *Chaetomium* spp to inhibit *F. solani* causing crown and root rot of tomato var sida in pot experiment

Treatments	Plant height (cm)	Root length (cm)
T1 inoculated	58.52$^{d/1}$	10.50^{b}
T2 non- inoculated	78.25^{c}	23.50^{ab}
T3 crude-CB	92.50^{abc}	40.38^{a}
T4 crude-CCO	92.25^{abc}	35.75^{a}
T5 crude-CG	102.00^{a}	40.62^{a}
T6 nano-CB	84.75^{bc}	46.00^{a}
T7 nano-CCO	93.25^{abc}	41.75^{a}
T8 nano-CG	95.87^{ab}	45.00^{a}
C.V. (%)	11.36	39.98

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

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Table 4.19 Stem root and fruit weight of tomato var sida caused by *F. solani in vivo* test

Treatments	Stems		Roots		Fruit weight (g)
	Fresh weight (g)	Dried weight (g)	Fresh weight (g)	Dried weight (g)	
T1 inoculated	12.91$^{g1/}$	0.35^{e}	1.07^{d}	0.31^{e}	22.71^{h}
T2 non- inoculated	51.34^{e}	12.31^{d}	2.88^{c}	1.67^{cd}	38.06^{g}
T3 crude-CB	66.56^{a}	16.75^{a}	4.20^{a}	2.17^{a}	63.65^{c}
T4 crude-CCO	64.25^{b}	16.18^{ab}	3.61^{ab}	1.85^{bc}	49.29^{e}
T5 crude-CG	49.74^{f}	11.78^{d}	4.09^{a}	1.85^{bc}	39.88^{f}
T6 nano-CB	59.52^{c}	15.74^{b}	3.23^{bc}	1.55^{d}	67.34^{b}
T7 nano-CCO	64.13^{b}	16.61^{a}	3.83^{ab}	2.04^{ab}	53.22^{d}
T8 nano-CG	53.70^{d}	14.12^{c}	4.13^{a}	1.92^{b}	75.48^{a}
C.V. (%)	3.42	3.72	13.27	7.64	2.16

$^{1/}$ Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05.

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Figure 4.23 Growth of tomato var sida *in vivo* test at 30 days

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Figure 4.24 Growth of tomato var sida at 60 days

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Figure 4.25 Growth of tomato var sida at 90 days

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Figure 4.26 Stem parts of tomato var sida at 90 days

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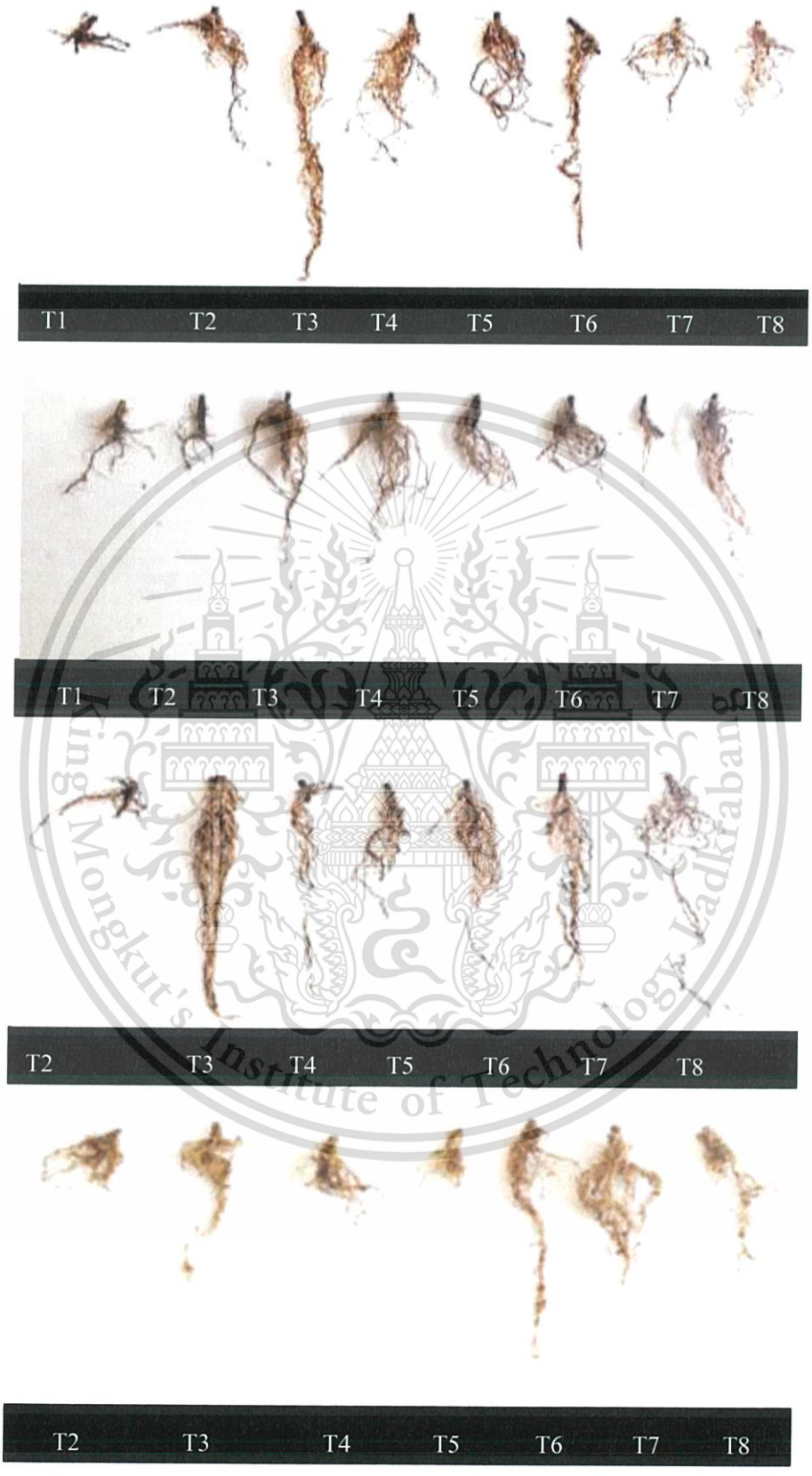

Figure 4.27 Root length of tomato var sida at 90 days

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CHAPTER 5

DISCUSSION

Fusarium solani was isolated from diseased plant tissues of tomato and ensured by pathogenicity test which also reported by Sibounnavong *et al.* (2011). The morphological identification of pathogen was done by culture into potato dextrose agar (PDA) and observed under microscope. The colony of *Fusarium solani* are fast growing, white or cream colour, reaching 9 cm diameter in 5-7 day at 29-32 °C. Macroconidia found abundant, moderately curved, blunt apical and pedicellate basal cells 3-5 , septate, 24.7-38.2×3.5-5.9 µm. Microconidia usually are abundant with 1 septate, 8.8-18.6 × 3.0-5.2 µm. Chlamydospores are singly or in paired in terminal, lateral or more rarely intercalary positions, smooth wall. This is similar to the reported of Domsch and Gams (1993) who stated that colony of *F. solani* are fast growing, cream colour. Macroconidia produced in variable quantities on shorter, branched conidiophores which soon form sporodochia, usually moderately curved, blunt apical and indistinctly pedicellate basal cells, mostly indistinctly 3 septate, 28.0-42.0 × 4.0-6.0 µm. Microconidia usually abundant, produced on elongate, sometime verticillate, 8.00-16.00 × 2.0-5.0 µm. Chlamydospores are produced singly or in pairs, in terminal, lateral, or more rarely positions, hyaline, smooth or rough walled, 6.0-10.0 µm.

Fusarium solani was confirmed by morphological and molecular phylogeny identification which using ITS sequence with the length of the complete ITS1, 5.8s and ITS 2 including of a small portion of 18S rDNA and small portion of 28s rDNA. The phylogenic tree presented cluster of Fusarium wilt into *F. solani*. The result was supported by Romberg *et al.* (2007) who stated that the rDNA region analyses have been successfully to identificaty as *F. solani*. Moreover, the phylogenic tree is clearly reconfirmed as *F. solani* which similar reports of Suga *et al.* (2000); Devi *et al.* (2012); Li *et al.* (2014) and Chen *et al.* (2017) that confirmed this region is used to confirm the species of *F. solani*.

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Chaetomium spp was cultured in PDA media for observing character of colony and its morphology under microscope. The culture of *Ch. brasiliense* is dark grey colour, perithecia globose, subglobose or broadly ovoid 101.5-229.0×230.5-398.5 µm, cylindrical asci. Ascospore uniseriate, dark brown at maturity, broadly ovoid shape 6.4-7.5×4.0-5.0 µm, and smooth walled with central germ pore as also reported by Doveri (2013). Perithecia is globose, subglobose or broadly ovoid, 100–220 µm diam., dark grey, membranous, entirely hairy with hairs denser around the ostiole. Peridium is thin, pseudoparenchymatous, a textura angularis of dark brown, thick-walled, polygonal cells, 5–15 × 4–11 µm. Terminal hairs are straight at their base, long spirally coiled at the apex, without secondary coiled branches, up to 6 µm diam at the base, 4–6 µm diam upwards, dark brown, strongly encrusted, thick-walled, densely septate, with roundish, slightly inflexed ends. Lateral and basal hairs are somewhat narrower, up to 4 µm diam., pale brown, enlarged at the base, septate, verruculose, attenuate towards the apex, straight or flexuous. Paraphyses were not observed. Asci unitunicate, nonamyloid, cylindric, fasciculate. Ascospores uniseriate, hyaline at first, dark grey at maturity, broadly ovoid in frontal view, bilaterally flattened and ellipsoidal or narrowly ovoid in side view, 6.5–7.5 × 6–6.5 × 4–5 µm, smooth, comparatively thin-walled, with an inconspicuous apiculus and a small, central germ pore at the pointed end. *Ch. cochliodes* is slow growing with olivaceous colour. Ascomata superficial or subglobose with dark brown colour of ascomatal wall, 170.0×390.5-277.7-458.2 µm. Terminal hairs verrucose and dark brown, the tips, spirally coiled in the upper part, with coils regularly tapering, asci is clavate shape. Ascospore dark brown colour when mature 8.0×9.5-7.5×8.5 µm with an apical germ pore. Similarly with the research of Wang *et al.* (2016) that stated colonies of *Ch. cochliodes* on media without aerial hyphae, usually without coloured exudates, but occasionally producing yellowish ochreous exudates diffusing into the medium, reverse uncoloured, but grey olivaceous under ascomata. Ascomata superficial, ostiolate, greenish olivaceous in reflected light owing to ascomatal hairs, ellipsoid or subglobose,270.0–450.0 µm high, 165.0–380.0 µm diam. Ascomatal wall brown, composed of hypha-like cells, textura

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intricata in surface view. Terminal hairs verrucose, dark brown, erect in the lower part, 3.5–6.0 µm near the base, tapering and fading towards the tips, spirally coiled in the upper part, with coils regularly tapering in diameter to appear as an elongated cone, occasionally with coiled branches. Lateral hairs brown, flexuous, undulate or coiled, tapering and fading towards the tips. Asci fasciculate, fusiform or clavate, spore-bearing part 23.0–32.0 × 13.0–15.0 µm, stalks 28.0–46.0 µm long, with eight biseriate ascospores, evanescent. Ascospores olivaceous brown when mature, limoniform, usually biapiculate at both ends, bilaterally flattened, (8.0–) 9.0–10.0 (–11.0) × (7.0–) 7.5–8.5 × 5.0–6.0(–6.5) µm, with an apical germ pore. Its morphological characters are as follows: colonies of *Ch. globosum* is slow growing with little superficial mycelium and a dense olivaceous layer on ascomata. Ascomata dark brown or black colors, globose to subglobose, 160.0-310.0×130.0-270.5 µm, lateral hairs dark brown with paler tips, minutely roughened, terminal hairs dark olive brown with paler tips, wavy or loosely coiled and interwined. Ascopores pale greenish to dark olive-brown, flattened lemon-shaped, 8.0×11.0-7.5×8.5 µm and this resulted is similar with Kunze (1817) that stated Ascomata superficial, ostiolate, greenish olivaceous or slightly dark olivaceous buff to grey in reflected light owing to ascomatal hairs, globose, ellipsoid, ovate or obovate, 160–300 µm high, 135–250 µm diam. Ascomatal wall brown, composed of hypha-like or amorphous cells, textura intricata in surface view. Terminal hairs abundant, finely verrucose, brown, tapering and fading towards the tips, 3–5 µm diam near the base, flexuous, undulate to loosely coiled with erect or flexuous lower part, usually unbranched. Lateral hairs brown, flexuous, fading and tapering towards the tips. Asci fasciculate, fusiform or clavate, with eight biseriate ascospores, evanescent. Ascospores olivaceous brown when mature, limoniform, usually biapiculate, bilaterally flattened, (8.0–) 8.5 –10.5(–11.0) × 7.0 –8.0 (–8.5) × 5.5–6.5(–7.0) µm, with an apical germ pore.

Chaetomium spp was done morphological identification and molecular phylogeny by using ITS sequence with the length of the complete ITS1-5.8s-ITS4 including of a small portion of 18S rDNA and small portion of 28s rDNA. *Ch. brasiliense* was confirmed by report of Hubka *et al.* (2011); Kaushik *et al.* (2014) and Huang, J.H. and Jiang, Z.D. (2015) that this region

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analyses is successfully done. The result to confirm of *Ch. cochliodes* that supported by Zámocký *et al.* (2016) and Wang *et al.* (2016) who used ITS sequence with the length of the complete ITS1-5.8S-ITS4 to identify from genus *Chaetomium* to *Ch. cochliodes* species. The morphological identification by using ITS sequence of *Ch. globosum* was confirmed into species which supported by Aggarwal *et al.* (2003); Cantrell *et al.* (2006); Mushtaq *et al.* (2009); Asgari and Zare1 (2011); Qi *et al.* (2011); and Zhang *et al.* (2017) stated that using ITS1-5.8S-ITS4 to identify *Ch. globosum.*

Dual culture test exhibited that *Ch. brasiliense, Ch.cochliodes* and *Ch.globosum* could control *F. solani* causing tomato crown and root rot. Based on the result *Ch. brasiliense* and *Ch. globosum* had ability to inhibit the growth of *F. solani* and the result was similar to the report of Tongon and Soytong (2015). Tongon and Soytong (2016) stated that *Ch. brasiliense* and *Ch. globosum* showed efficacies to inhibit colony growth of *F. solani* and *Curvularia lunata* causing leaf spot disease in rice and also *Ch. brasiliense, Ch. cochliode* and *Ch. globosum* that showed efficacies to inhibit conidial production of *F. solani* over 50% and this resulted similar to the report of Moya *et al.* (2016) who stated that *Chaetomium* spp showed high potential to inhibit *Drechslera teres* and *Bipolaris sorokiniana* causing foliar diseases of barley.

Crude extracts of *Chaetomium* spp were tested for antifungal activities. These isolates were tested against *F. solani* causing tomato crown and root rot. The result showed that crude ethyl acetate form *Ch. brasiliens*, methanol crude extract from *Ch. cochliodes* and crude hexane from *Ch. globosum* gave significant highest inhibit colony of *F. solani. Ch. brasiliense, Ch. cochliode* and *Ch. globosum* were shown highest inhibition for the spore production of *F. solani* by treated with crude ethyl acetate, crude ethyl acetate and crude hexane. Crude ethyl acetate of *Ch. brasiliense* gave highest significantly inhibition for the spore production of *F. solani* and followed by, crude methanol of *Ch. cochliodes*, and crude hexane of *Ch. globosum* also gave highest significantly inhibition for the spore production of *F. solani*. Soytong (2014) reported that crude hexane of *Ch. cochliodes* inhibited spore production of *Drechslera sorokiniana* causing spot blotch of wheat at concentration of 1,000 µg/ml (93.85 %) which ED_{50} was 66.45 ppm

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(Biswas *et al.* 2002) and similar to Sibounnavong (2012) who reported that *Ch. brasilense* CB01 and *Ch.cupreum* CC03 inhibited the spore production of *F. oxysporum* f.sp. *lycopersici* NKSC02 between 63-77%. Kumar *et al.* (2013) reported that extracts of *Ch. globosum* EF18 was found to be effective against *Sclerotinia sclerotiorum*. Ethyl acetate and methanol extracts were more effective than hexane extract growth inhibition. Moreover, this result was similar to the report of Sibounnavong (2012a) who stated that crude hexane, crude ethyl acetate and crude methanol from *Ch. brasilense* CB01 and *Ch. cupreum* CC03 inhibited *F. oxysporum* f.sp. *lycopersici* NKSC02 with the ED_{50} of 9.13, 18.10 and 1.63 μg/ml, while in this study, those crude extracts inhibited the conidial production of different isolate of *D. oryzae* with the ED_{50} of 0.58, 31.38, 2.37, 10.15 and 20.24 μg/ml which were lower than those from previous report.

The nano particles from *Ch. brasiliense, Ch. cochliodes* and *Ch. globosum* were used to test their abilities to inhibit the growth of *F. solani*. Results showed that nano-CBM gave significantly highest inhibition the colony growth of *F. solani* and gave significantly highest inhibition for the spore production of *F. solani*. Moreover, nano-CCOM expressed highest inhibition percentage of *F. solani* colony growth which when compared to the control and also gave significantly highest inhibition for the spore production of *F. solani* followed by nano-CCOH and Nano-CCOE This study was similar to the study of Dar *et al.* (2013) that nano particles of *Ch. globosum* and *Ch. cuperum* were proved to control *F. oxysporum* f.sp. *lycopersici* and *Colletotrichum capsici*. Nano-CEH, Nano-CEE and Nano-CEM from *Ch. elatum* against *Pyricularia oryzae*. Nano-CEE from *Ch. elatum* gave significantly highest inhibition for the colony growth of *P. oryzae* at concentration of 15 ppm when compared to the control, followed by nano-CEM and nano-CEE (Song *et al.* 2016). Fungal metabolites from *Chaetomium* spp. were tested against *P. palmivora* PHY02. Results showed that, methanol extract of *Ch. globosum* CG05 expressed strongest inhibitory effects on mycelial growth and sporangium formation of *P. palmivora* PHY02 with effective dose ED_{50} values of 26.5 μg/mL and 2.3 μg/mL, respectively (Hung *et al.* 2015)

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Testing crude extracts and nano-particles from *Chaetomium* spp to inhibit *F. solani* causing crown and root rot of tomato in pot experiment revealed that

crude-CG gave significantly highest plant growth, nano-CB, nano-CG, nano-CCO, crude-CG, crude-CB and crude-CCO gave significantly highest root length when compared to the inoculated control and non-inoculated one. Sem fresh weight showed that crude-CB gave significantly highest stem fresh and dried weight. Moreover, result of crude-CB expressed highest fresh root weight and dried root weight. Nano-CG gave significantly highest in yield in this study. This result was similar to the report to This research finding was similar result to Tann and Soytong (2016) that Nano-CGH, nano-CGE, and nano-CGM from *Ch. globosum* KMITL-N0805 expressed antifungal activity against *Curvularia lunata*, the causal agent of leaf spot disease of rice var. Sen Pidoa. Tests in a pot experiment showed that nano-CGH, nano-CGE, and nano-CGM could significantly control leaf spot of rice var. Sen Pidoa. Based on the disease severity index at 60 days after treatment, nano-CGH and nano- CGM resulted in higher disease reduction (61.54%) than nano-CGE (53.83%). From these findings, it can be concluded that nano-CGH, nano-CGE, and nano-CGM have the ability to decrease leaf spot disease of rice var. Sen Pidoa caused by *C. lunata.* Moreover, the research showed that all three types of nanoparticles significantly increased the height and number of tillers of the rice plant relative to the non-treated control.

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CHAPTER 6

CONCLUSION

Fusarium solani was isolated crown and root rot of tomato var sida and tested for pathogenicity. Identification was confirmed species by morphology and molecular phylogeny on the basis of ITS1-5.8S-ITS2 ribosomal gene sequence acquisition and analyses, the pathogen was reconfirmed as *F. solani* when compared to those available in public database. The morphology and molecular phylogeny to confirmed species of *Chaetomium* spp on the basis of ITS1-5.8S-ITS4 ribosomal gene sequence acquisition and analyses were reconfirmed as *Ch. brasiliense, Ch. cochliodes* and *Ch. globosum* when compared to those available in public database.

The result of dual culture showed that *Ch. brasiliense, Ch.cochliodes* and *Ch. globosum* had ability to inhibit the growth and spore production of *F. solani.*

The EtOAc crude extract of *Ch. Brasiliense* expressed antifungal activity to inhibit spore production against *F. solani* causing tomato wilt which the ED_{50} value of 65.44 ppm, followed by crude methanol extract and hexane crude extract with the ED_{50} were 69.06 and 102.58 respectively. The methanol crude extract of *Ch. cochliodes* inhibited spore production of *F. solani* with the ED_{50} of 229 ppm, and followed by EtOAc crude extract and hexane crude extract with the ED_{50} value were 319 and 973 ppm, respectively. Moreover, hexane crude extract of *Ch. globosum* inhibited the spore production of *F. solani* at the ED_{50} of 200 ppm, followed by crude EtOAc crude extract and methanol extract with the ED_{50} values of 314 and 378 ppm, respectively.

It revealed that nano-particles at low concentration of *Chaetomium* spp gave a better inhibition of *F. solani* than crude extract in all tests. As results, nano-CBH expressed highest antifungal activity against *F. solani* causing tomato wilt with the ED_{50} of 3.86 ppm, and followed by nano-CBH and nano-CBE which the ED_{50} values of 4.37 and 5.42 ppm, respectively. Nano-CCOM showed the highest spore inhibition of *F. solani* with the ED_{50} of 8.78 ppm, and followed by nano-CCOH and nano CCOE which the ED_{50} values were 9.21 and 9.70 ppm, respectively. However, nano CGM actively expressed antifungal activity of *F. solani* with the ED_{50} of 1.48 ppm, followed by nano-CGH and nano CGE which the ED_{50} values were 3.41 and 3.48 ppm, respectively.

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Crude extracts and nano-elicitors from *Ch. brasiliense, Ch.cochliodes* and *Ch. globosum* were tested to control tomato wilt caused by *F. solani* in pot experiment. It is concluded that crude-CB, crude-CCO and crude CG gave significantly different in disease severity index and plant growth parameters from the inoculated control. Nano-CB, nano- CCO and nano- CG treatments were also significantly different than the non-inoculated control. The experiment revealed that eight crude extracts or nano-particle applied to control crown and root rot tomato gave significantly reduction the disease incidence of tomato and gave better plant parameter than the inoculated control.

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2014, 27-28 November, attend 3rd **International Conference on Integration of Science and Technology for Sustainable Development** as a poster presentation entitled "Study on the growth of *Pleurotus giganteus*", held at Champasack Grand Hotel, Pakse, Champasack, Lao PDR.

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2015, 27-28 November, attend 4th **International Conference on Integration of Science and Technology for Sustainable Development** as an oral presentation entitled "Application of nano-particles from *Chaetomium globosum* to control leaf spot of rice", held at Center for Woman and Development- CWD Hotel, Hanoi, Vietnam.

2016, 26-27 November, attend 5th **International Conference on Integrating Science and Technology for Sustainable Development** as an oral presentation entitled "Fungal Metabolites from *Chaetomium brasilense* to Inhibit *Fusarium solani*", held at Cherry Queen Hotel, Southern Shan State, Myanmar

International Publication:

Tongon R. and Soytong K. (2015). Application of nano-particles from *Chaetomium globosum* to control leaf spot of rice. Journal of Agricultural Technology 11(8): 1919-1926.

Tongon R. and Soytong K. (2016). Fungal Metabolites from *Chaetomium brasilense* to Inhibit *Fusarium solani*. Journal of Agricultural Technology 12(7.1):1463-1472.

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Application of nano-particles from *Chaetomium globosum* to control leaf spot of rice

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Tongon R. and Soytong K. (2015). Application of nano-particles from *Chaetomium globosum* to control leaf spot of rice. Journal of Agricultural Technology 11(8): 1919-1926.

Result showed that nano-CGH, nano-CGE and nano-CGM from *Chaetomium globosum* strain KMITL 0802 significantly inhibited *Curvularia lunata* causing leaf spots of rice, which the ED_{50} values were 1.12, 1.19 and 1.93 ug.ml, respectively within 7 days. It is the first report of nano-particles from *Chaetomium globosum* to control *Curvularia lunata* causing leaf spot of rice. Further investigation is being done in the field trials.

Keywords: *Chaetomium globosum;* nano-particles*; Curvularia lunata;*

Introduction

Curvularia lunata reported to caused leaf spot disease of rice which occurs mostly in tropical and subtropical areas which it is an facultative plant pathogen and theteleomorphic states in *Cochliobolus* and *Pseudocochliobolus*which is reported as a pathogen of several plants including leaf spots on rice under certain conditions.(Luna 2002).There are several reports on the potential use of biological control agents against plant pathogens. *Chaetomium* species are strictly saprobic antagonists and have been shown to be against several plant pathogens, e.g. *Botrytis cinerea* (Kohl et al., 1995).,*Fusarium oxysporum* f. sp. *Lycopersici* (Soytong et al., 1999a), *Phytophthora palmivora* (Pechprome and Soytong, 1996; Sodsa-art and Soytong, 1998), *Phytophthora parasitica* (Usuwan and Soytong, 1998). Screening *Chaetomium* spp as biological control agents has been carried out in Thailand since 1989, resulting in the development of a biological formulation from *Chaetomium cupreum* CC1-10 and *Chaetomium globosum* CG1-12. The product has now been developed into pellets and powder formulations and registered for a Patent Right No.6266, Intl. cl. 5 AO 1 N 25 / 12 in 1994 (Soytong, 1996).Objective of research findings were to isolate, identify leaf spot pathogen of rice, pathogenicity test, and to study the morphological characteristics of *Curvularia lunata and Chaetomium globosum*, and also testing the formulated nano-elicitor against *Curvularia lunata* causing leaf spot of rice.

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Materials and methods

Study on Morphology of Chaetomium globosum

Chaetomiumglobosum which offered from Assoc. Prof. Dr. KasemSoytong, were used to observe the growing colony on potato dextrose agar (PDA) media and then mycelia, ascocarp, ascospores and seta were observed under binocular compound microscope.

Isolation of the pathogen,Curvularia lunata

Curvularia lunata causing leaf spots of rice was isolated by tissue transplanting technique. The symptoms on leaves were properly cleaned with running tap water and after air-dried for a few minutes before cut the advance margin of symptom between healthy and diseased areasinto small pieces and soaked sterilized water, and followed by 1% sodium hypochlorite (NaClO) for 3 min and then sterilized water again.All sectioned pieces were transferred onto water agar (WA) medium for firstly observation of appearing colonies and sub-cultured to PDA until get pure culture. Morphological identification was done by observation fungal characteristic under binocular compound microscope.

Pathogenicity test.

Pathogenicity test was done by following the method of Koch's Postulate. With this, Spore suspension of*Curvularialunata*was prepared at concentration is 1×10^6 spores/ml.and then sprayed on the wounded leaves(3 leaves/seedling). The inoculated leaves were then covered with plastic sheet and maintained to observe the infected leaves. The inoculated leaves with only spraying sterilize distilled water were done to serve as controls. Experiment was conducted using Completely Randomized Design (CRD) with four replication. Data were collected as lesion size (cm) and computed analysis of variance (ANOVA). Treatment means were compared using least significantly different test (LSD) at P=0.05 and 0.01.

Testing nano-particles from Chaetomiumglobosum to control leaf spot of rice

Chaetomium globosum strain 0805 was cultured in potato dextrose broth (PDB) at room temperature for 30 days. The fungal biomass was harvested by filtration through cheesecloth and air-dried overnight. Fresh and dried weight were weighted. The fungal biomass was then ground with electrical blender, and kept in flask. Extraction was done by added an equal volume hexane, and incubated in stationary phase for 5 days at room

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temperature before separated to get filtrate through Whatmanfilter paper No.4. The filtrate was evaporated by using rotary vacuum evaporation to get crude hexane. The marc was further extracted with ethyl acetate and methanol consecutivelyto get crude ethyl acetate and crude methanol by using the same procedure as hexane and yield crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracts. Preparation of nano particles;-nano-particle was done using the method of Dar and Soytong (2014) to get Nano-CGH, Nano-CGE and Nano-CGM.

The nano-particles, Nano-CGH, Nano-CGE and Nano-CGMwere testedto inhibited *Curvularia lunata* causing leaf spots of rice. Experiment was designed by using two factors factorial experiment in CRD with four replications. Factor A represented Nano-CGH, Nano-CGE and Nano-CGMand factor B represented concentrations at 0, 1, 5 and 10µg/ml. Each Nano-particle was dissolved in one drop 2% dimethyl sulfoxide (DMSO), and then mixed into 30ml PDA medium before autoclaving at 121°C,15lbs/inch^2 for 30 min. The culture of *Curvularia lunata* was cut at the edge of colony with sterilized cock borer (3mm). Agar plug of pathogen was transferred to the middle of PDA media in plate (5.0mm diameter) incorporated with each nano-particles. The transferred plates were incubated at room temperature until the pathogen in control plates growing full. Abnormal and normal spores of pathogen from each treatment were observed under binocular compound microscope and taken photograph for comparison. The data were collected as colony diameter and the number of spores that counted by using Haemacytomete. Percentage of inhibition was computed andthe effective dose (ED_{50}) was thencalculated using probit analysis.

Results

Study on Morphology of Chaetomium globosum

Chaetomium globosum strain 0805 was cultured and morphological observation following Soytong and Quimio (1989). Ascoacrp, as ci and ascospores were taken photograph under compound microscope (Figure 1).

Isolation, identification, and pathogenicity test of the pathogen,Curvularialunata

Curvularia lunata was isolated, identified and proved to be pathogenic isolate causing leaf spots of riceas seen in Table 2, Figure 2 and 3.

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Table.1. Percent disease intensity of *Curvularialunata* in rice var Thadorkkam11

Treatments	DI
T1 Control	1
T2 *Curvularialunata*	4

[1/] Disease index (DI), 1= (0%)no symptoms, 2= (1-25%) small blighted spot and still healthy tissue, 3= (26-50%)dead cells in are of blighted spot 1-2 mm and turn brown color, 4= (51-75%)expanded lesion in oval shape 1-2 cm and cell death in the center of lesion and 5= (76-100%) diseased area over 20 % and finally death modified by Wilaiporn (2001).

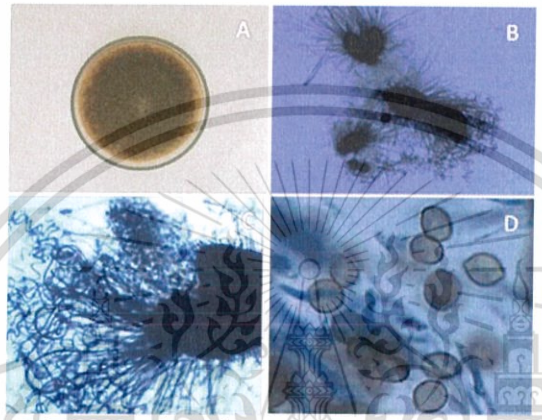

Figure 1. *Chaetomium globosum* , A= colony, B-Ascocarps, C= Ascocarp and asci and D-ascospores

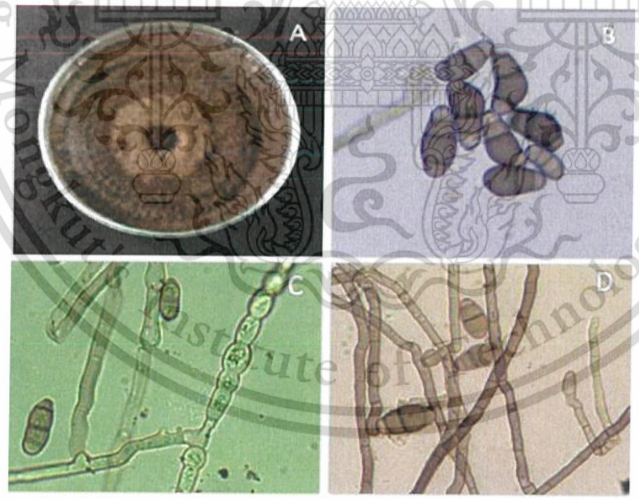

Figure 2 *Curvularia lunata* causing leaf spots of rice , A- colony, B=conidia, C= mycelia and conidia and D= conidiophores and conidia

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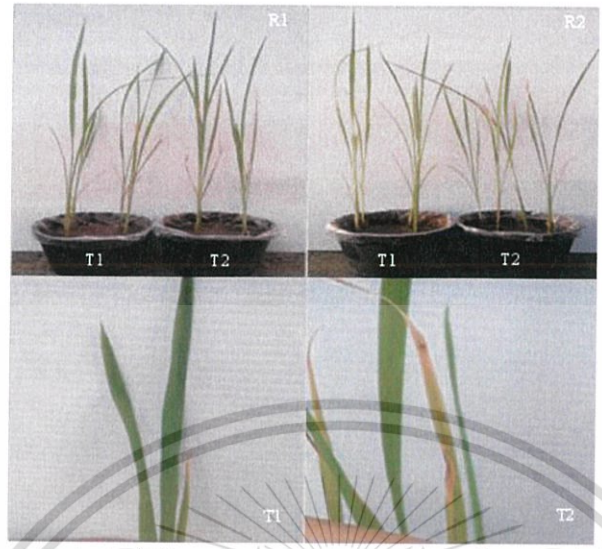

Figure 3. Pathogenicity test of *Curvularia lunata* causing leaf spots of rice, T1 = Sprayed by sterile water (Control) and T2 = Sprayed spore suspension of *Curvularia lunata*

Testing nano-particles from Chaetomiumglobosum to control leaf spot of rice

Characteristics of nano-CGH, nano-CGE and nano=CGM were seen in Figure 4. Result showed high efficacy antimicrobial activity of Nano-CGH, Nano-CGE and Nano-CGM from *Chaetomium globosum*atrain 0805 against *Curvularia lunata* causing leaf spots of rice which the ED_{50} values were 1.12, 1.19 and 1.93 ug.ml, respectively within 7 days (Table 2). It was not clearly seen in the tested plates on colony inhibition but when counted the number of conidia which served as pathogen inoculum that it was significantly differed when compared to the control (0 ug/ml) for 7 days. However, Nano-CGH, Nano-CGE and Nano-CGM at the concentration of 10 ug/ml inhibited the colony growth of 27, 25 and 52 %, respectively within 7 days (Figure 5). Nano-CGH, Nano-CGE and Nano-CGMat the concentration of 10 ug/ml were inhibited the spore production of 75, 52 and 70 %, respectively. The effects of Nano-CGH, Nano-CGE and Nano-CGMon *Curvularia lunata* were clearly demonstrated at all tested concentrations leading to broken the pathogen cells which become abnormal cells and lost of pathogenicity (Figures 6).

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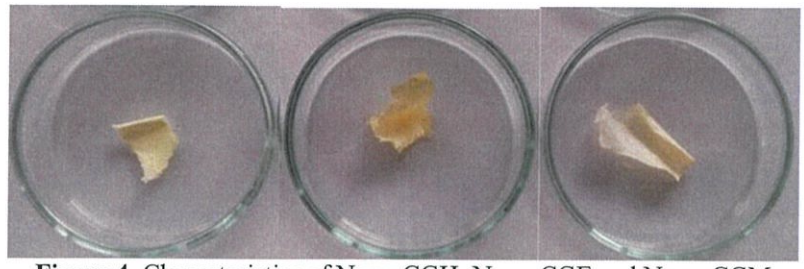

Figure 4. Characteristics of Nano-CGH, Nano-CGE and Nano-CGM

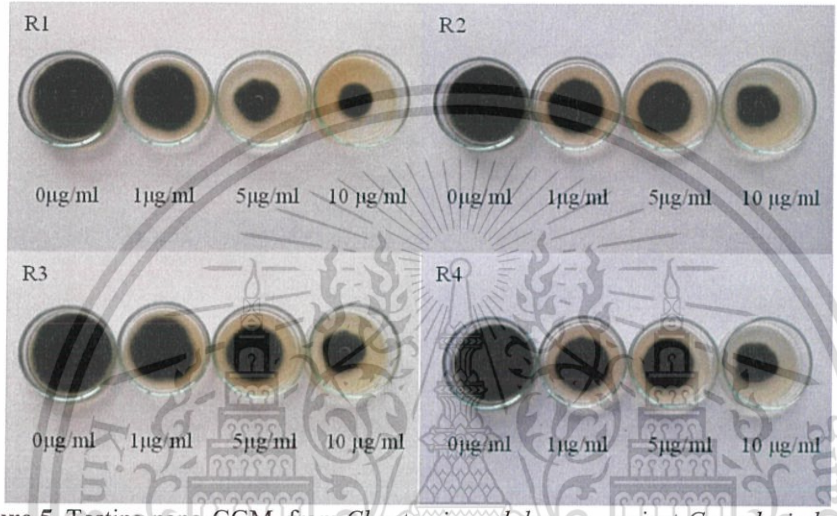

Figure 5. Testing nano-CGM from *Chaetomium globosum* against *Curvularia lunata*

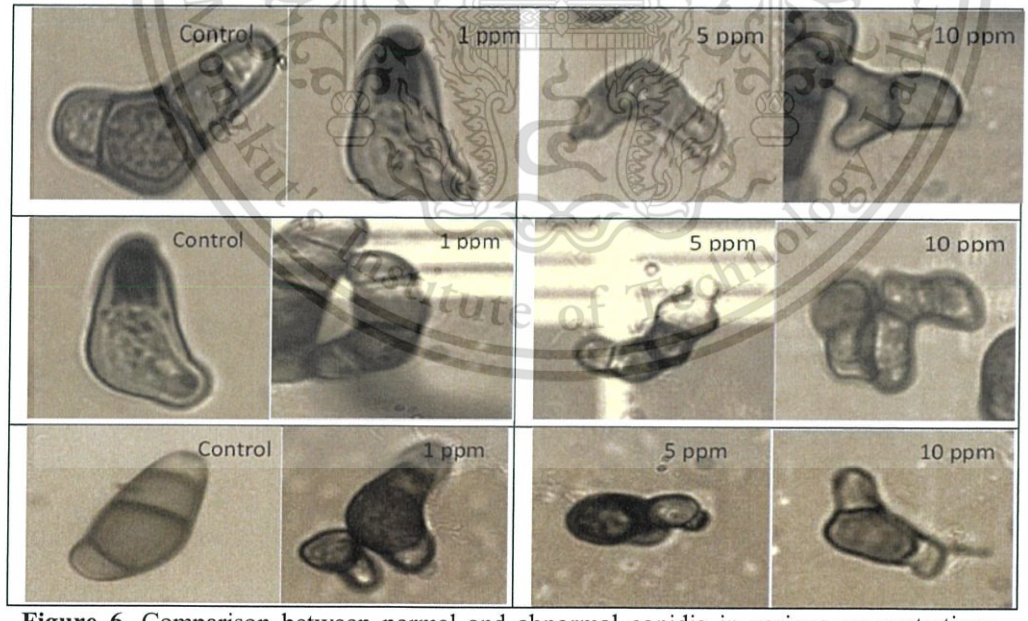

Figure 6. Comparison between normal and abnormal conidia in various concentrations, upper = nano-CGH, middle = nano-CGM and lower parts = nano-CGM

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Table.2. Colony diameter and number of spores *Curvularia lunata*

Nano-products	Concentrations (ug/ml)	Colony diameter(cm)	Number of conidia(x10^6)	Colony inhibition (%)	Conidia inhibition (%)	ED_{50} (ppm)
Nano-CGH	0	5a	49.24a	0.00	0.00	
	1	4.43b	25.15c	11.25	48.11	1.21
	5	3.84c	18.90de	22.50	61.57	
	10	3.62c	12.75f	27.25	74.02	
Nano-CGE	0	5a	49.19a	0.00	0.00	
	1	3.33d	32.50b	33.25	33.87	1.19
	5	3.61c	28.55bc	27.75	41.87	
	10	3.73c	23.39cd	25.25	52.44	
Nano-CGM	0	5a	49.24a	0.00	0.00	
	1	2.93e	27.65bc	41.25	48.11	1.93
	5	2.83e	23.64cd	43.25	53.24	
	10	2.36f	15.00ef	52.75	70.08	
C.V. (%)		3.56	8.44	10.90	4.10	

Average of four replications. Means followed by a common letter are not significantly different by DMRT at P = 0.05

Discussion

Curvularia lunata was isolated and proved to be aggressive isolate causing leaf spots of rice. With this, Soytong (2014) stated that many rice varieties are invaded by *Curvularia lunata* causing leaf spots which leading to low yield. *Chaetomium globosum* strain KMITL-N0802 was used in this study. This species is reported by Kanokmedhakul *et al.*(2001)who discovered new bioactive compounds which is antimycobacterial anthraquinone-chromanone compound against human pathogen. Nano-particles were designed from crude extracts of *Chaetomium globosum* KMITL0802 according to the method of Dar and Soytong (2014). The experiment showed that nano-CGH, nano-CGE and nano-CGM gave significantly inhibited spore production of *Curvularia lunata*. It is similar to the work of Dar and Soytong (2013) which reported *in-vitro testing* of nanomaterials derived *Chaetomium globosum* gave good result to inhibit *Fusariumoxysporum* f. sp. *lycopersici.* Moreover, similar report from other species of *Chaetomium*, eg.*Chaetomium cochliodes*reported as a new antagonistic fungus against brown leaf spot of ricevarPittsanulok 2 caused by *Drechsleraoryzae*by using hexane, ethyl acetate and methanol which ED_{50}value was 66.45 ug/ml. It is obsereved in this research finding that all tested nano-materials could rapidly penetrate into pathogen ceels which leading to abnormal cells of pathogen as also reported by Dar and Soytong (2013). It is recommended that nano-materials may play the important role as plant immunity induction as reports by Soytong, *et al* (2014) who stated

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microbial elicitor from *Chaetomium* can be induces immunity inchilliby production of phytoalexin namely capsidiolagainst anthracnose caused by *Colletotrichum capsici*. It is interested that nano-Chaetomium would be expressed a new role for disease control.

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Fungal Metabolites from *Chaetomium brasiliense* to Inhibit *Fusarium solani*

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Tongon R. and Soytong K. (2016). Fungal Metabolites from *chaetomium brasilense* to Inhibit *Fusarium solani*. International Journal of Agricultural Technology 12(7.1):1463-1472.

Tomato (*Solanum lycopersicum* L.) is one of among important cash crop nowaday. Several countries have consumed a lot of tomato consumtion. Tomato plants are always been attacked by several soil borne fungal pathogens, which cause serious disease such as wilt caused by *Fusarium solani* and other species. Fusarium species are soil fungi and have a worldwide distribution. In this research finding, tomato dry root rot and wilt were isolated and tesed for their pathogenicity. In vitro, the experiment was done by using the bio active compounds of *chaetomium brasiliense* to inhibit *Fusarium solani*. The different concentrations of bio active compounds of *chaetomium brasiliense* was separately ameneded into potato dextrose agar in each treatment and transferred the tested pathogen onto middle plates and the result showed that bio active compounds gave a potent to inhibit the growth of *Fusarium solani*. The ED50 for spore inhibition were 66, 288, and 140 µg/ml, respectively. Further investigation will be conducted in pot experiment.

Keywords: *Fusarium solani*, *chaetomium brasilense*, bio active compound

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown and consumed vegetables all over the world. Tomatoes and tomato products are rich in health-related food components as they are good sources of carotenoids (in particular, lycopene), ascorbic acid (vitamin C), vitamin E, folate, flavonoids and potassium (Beecher, 1998; Leonardi *et al.*, 2000). However, the tomato crop is usually attacked by many kinds of diseases such as Fusarium.

Fusarium solani is the most important soilborne fungal pathogens, which develop in both cultured and non-cultured soils, causing the symptoms of damping off and root rot diseases to wide range of vegetable and crop plants

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including tomato (Abu-Taleb *et al.*, 2011). *Fusarium solani* (Mart.) Appel and Wollenw is widely found in soil and constitutes one of the most important phytopathogens in agriculture. Symptoms include stunted growth with varying degrees of chlorosis, mottling and necrotic spotting on young foliage and cortical rot of tap root or discoloration of stele of the main lateral roots. In severely affected plants, the tap roots completely girdled and the crown was also rotted. Although plants are not often killed by the disease but it reduces the yield (Vawdrey and Petersone, 1988)

This resresch aimed to study morphological of *Chaetomium brasilense* and *Fusarium solani*, pathogenicity test of *Fusarium solani* and tested for the inhibition of pathogen using bioactive compound from *chaetomium brasilense*.

Materials and methods

Morphological study of Chaetomium brasilense

Chaetomium brasilense which offered from Assoc. Prof. Dr. Kasem Soytong, were used to observe the growing colony on potato dextrose agar (PDA) media and then mycelia, ascocarp, ascospores and seta were observed under binocular compound microscope.

Isolation pathogen, Fusarium solani

Fusarium solani causing wilt diseases were isolate form tomato root by tissue transplanting technique. Root of tomato were properly cleaned with running tap water and after air-dried for a few minutes and cut it in small pieces and soaked in sterilized water, and followed by 1% sodium hypochlorite (NaClO) for 3 min and then sterilized water again. All of the small pieces root were transferred onto water agar (WA) medium for firstly observation of appearing colonies and sub-cultured to PDA until get pure culture. Morphological identification was done by observation fungal characteristic under binocular compound microscope.

Pathogenicity test

The pathogenicity test of fusarium wilt (*Fusarium solani*) was conducted in vivo to 15 day tomato seedlings and carried out using a rootdip inoculation method. Tomato seedlings were uprooted gently and roots were washed with tap water to remove all soil (Bao J.R. *et al.*, 2001). The concentration of conidia in the suspension was determined using Haemacytometer to adjust the number of spores to $1x10^6$ conidia/ml. The 3-4 root tips will be cut and soaked into

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spore suspension for 30 seconds. Control plants were sown in soil and be treated with sterile distilled water. The disease severity was rated with follow the method of Sibounnavong (2012), as follows: 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21- 40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting 81-100% or die. The most virulent isolate was selected for further experiment.

Testing bio active compound of chaetomium brasilense to against Fusarium solani

The crude extracts of chaetomium brasilense were tested for inhibition of *Fusarium solani*. Experiment was designed by using two factors factorial experiment in 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 121°C , 15 psi for 30 minutes. The culture of *Fusarium solani* was cut at the edge of colony with sterilized cock borer (3mm). Agar plug of pathogen was transferred to the middle of PDA media in plate (5.0mm diameter) incorporated with each 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 (ED50) will be calculated using probit analysis.

Results and Discussion

Morphological study of Chaetomium brasilense

Chaetomium brasiliense was cultured and morphological observation. Ascocarp, asci and ascospores were taken photograph under compound

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microscope. Culture is dark gray color, perithecia globose, subglobose or broadly ovoid, cylindrical asci. This result is similar to report of Soytong and Quimio (1989) and Bell (2005). who stated that *C. brasiliense* is characterised by subglobose to ovate ascomata, undulate or spirally coiled terminal hairs, cylindrical asci, uniseriate, broadly ovoid, bilaterally flattened, comparatively small ascospores with an apical germ pore. It is reported that this species frequently grows on a variety of herbivore dungs (Ames, (1963) and Bell (2005) as seen in Fig.1.

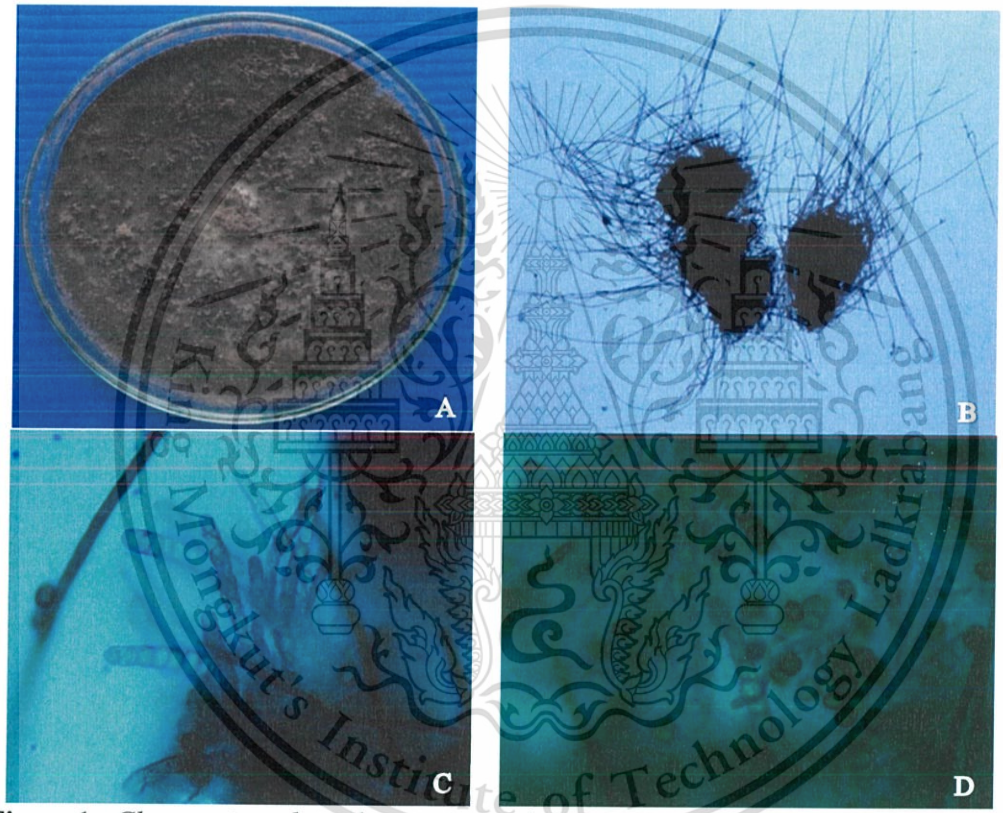

Figure1. *Chaetomium brasilense*, A= colony, B= ascocarps, C= asci and D= ascospores

Isolation of pathogen

Fusarium solani is isolated from disease plant parts, especially from roots of tomato var Sida. The characteristics are fast growing with discrete sporodochia and white-ochraceous colour, aerial mycelium floccose. Macro-conidia found abundant and more-celled, curved or bent at the pointed ends. It

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is morphological identification as *Fusarium solani* as also reported by Grunwald *et al.* (2003) and Hafizi *et al.* (2013) as seen in Fig. 2.

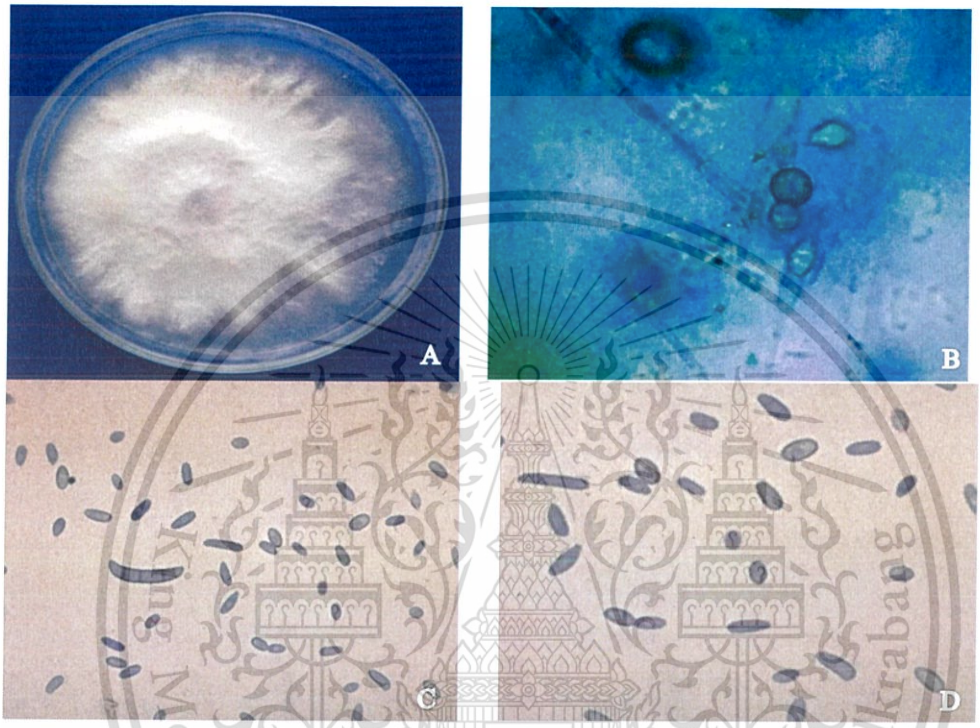

Figure2. *Fusarium solani*, A= colony, B= clamydospore, C= macrospore, D= microspore

Pathogenicity test

Pathogenicity test was conducted by dipping cut root into sopore suspension of *Fusarium solani* at the concentration of 1 x10^6 conodia/ml for 30 seconds which resulted tomato seedligs showed yellowing leaves and wilting 41-60 %. It was significantly differed when compared to the non-inoculated control (Fig.3, Table 1). As a result, this procedure for pathogenicity test is successfully done by Sibounnavong et al. (2012).

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Table 1. Percent disease index of *Fusarium solani* in tomato.

Treatments	Disease index
T1=Control	1
T2= *Fusarium solani*	4

Disease index are as follows: 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting 81-100% or die.

Figure3. Pathogenicity test of *Fusarium solani* caused tomato wilt, T1= dip root with sterile water (control), T2=dip root with spore suspension of *Fusarium solani*

Testing bioactive compound of chaetomium brasilense against Fusarium solani

Result showed high efficacy antimicrobial activity of active compound from *Chaetomium brasilense* against *Fusarium solani*. Hexane, ethylacetate and methanol cude extracts from *C. brasilense* gave significantly highest inhibition of *Fusarium solani* for colony growth which was 52.00, 73.00 and 71.75 per cent at the concentration of 1,000 ppm, when compared to the control (Table 2). All crude extracts from *C. brasilense* showed significantly highest inhibition for the spore production of *Fusarium solani* at the concentration of 1,000 ppm. The ED_{50} for spore inhibition were 66.66, 288.94, 140.88 µg/ml, respectively (Table 3, Fig. 4). This study was similar to the study of Sibounnavong et al.(2012) who reported that *Chaetomium brasilense* CB01 and *Chaetomium cupreum* CC03 gave effectively inhibition of *F. oxysporum* f. sp. *lycopersici* race 2 cuased wilt disease in tomato.

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Table 2 Crude extracts of *Chaetomium brasilense* testing for growth inhibition of *Fusarium solani* at 7 days

Crude extracts	Concentration (ppm)	Colony diameter(cm)[1/]	Growth inhibition(%)[2/,3]
Hexane	0	5^{a}	-
	10	5^{a}	0^{h}
	50	4.98^{ab}	0.50^{h}
	100	4.98^{ab}	0.50^{h}
	500	3.43^{e}	31.25^{d}
	1,000	2.37^{f}	52.00^{c}
EtOAc	0	5^{a}	-
	10	4.61^{b}	7.75^{g}
	50	4.41^{c}	11.75^{f}
	100	3.73^{d}	25.25^{e}
	500	1.62^{g}	67.50^{b}
	1,000	1.34^{h}	73.00^{a}
MeOH	0	5^{a}	-
	10	5^{a}	0^{h}
	50	5^{a}	0^{h}
	100	4.95^{ab}	10.00^{h}
	500	2.37^{f}	52.50^{c}
	1,000	1.41^{h}	71.75^{a}
C.V. (%)		1.87	6.95

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. , [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05., [3/]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

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Table 3 Spore production inhibition of crude extracts from *Chaetomium brasilense* to *Fusarium solani* at 7 days and effective dose (ED_{50}) values

Crude extracts	Concentration (ppm)	Number of spores [1/] (10^6)	Inhibition(%) [2,3/]	ED_{50}
Hexane	0	6.60^{a}	-	66.66
	10	5.73^{ab}	13.23^{h}	
	50	5.52^{b}	16.53^{h}	
	100	3.55^{de}	45.45^{ef}	
	500	2.22^{g}	65.78^{cd}	
	1,000	1.91^{gh}	70.63^{c}	
EtOAc	0	6.60^{a}	-	288.94
	10	5.23^{b}	20.33^{h}	
	50	4.18^{cd}	36.11^{fg}	
	100	3.26^{ef}	50.22^{e}	
	500	1.06^{h}	83.75^{b}	
	1,000	0.23^{i}	97.06^{a}	
MeOH	0	6.60^{a}	-	140.88
	10	5.59^{ab}	15.25^{h}	
	50	4.45^{cd}	31.83^{g}	
	100	4.35^{cd}	33.67^{g}	
	500	2.62^{fg}	59.95^{d}	
	1,000	0.18^{i}	96.43^{a}	
C.V. (%)		12.82		

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [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 .

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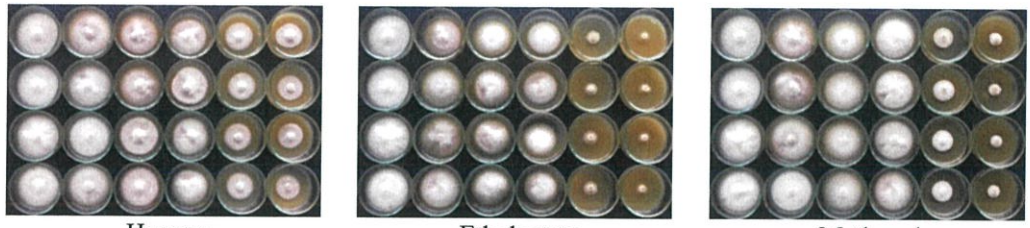

Figure 4 Testing bio active compound of *chaetomium brasilense* to against *Fusarium solani*

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