Fungal metabolites of *Chaetomium* spp to control durian root rot pathogen cause by *Phytophthora* sp.

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN AGRICULTURE

FACULTY OF AGRICULTURAL TECHNOLOGY

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

2021

KMITL-2021-AG-D-064-030

หัวข้อวิทยานิพนธ์	สารออกฤทธิ์จากเชื้อรา *Chaetomium* sppในการควบคุมโรครากเน่าของทุเรียนที่เกิดจากเชื้อรา *Phytophthora* sp.
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บทคัดย่อ

ทดสอบประสิทธิภาพของเชื้อราต่อต้าน *Chaetomium Brasiliense, Chaetomium cupreum* CC3003 และ*Chaetomium cochliodes* CTh02 ในการควบคุมเชื้อรา *Phytophthora palmivora* DD01 เชื้อราสาเหตุของโรครากเน่าโคนเน่าในทุเรียน ด้วยวิธีเลี้ยงเชื้อบนอาหารร่วม สารสกัดจากเชื้อรา และ nano particles จากเชื้อรา *Chaetomium* spp ในห้องปฏิบัติการพบว่า *Ch. cochliodes* CTh02 มีประสิทธิภาพในการยับยั้งการเจริญเส้นใยและการสร้างสปอร์สูงที่สุด ด้วยวิธีเลี้ยงเชื้อบนอาหารร่วม และพบว่าหลังจากการเลี้ยงเชื้อบนอาหารร่วมหนึ่งเดือนเชื้อราต่อต้านทั้งสามชนิดสามารถเจริญเติบโตยับยั้งเชื้อรา *P. palmivora* DD01 และในการทดสอบประสิทธิภาพของสารสกัดจากเชื้อรา *Chaetomium* spp. ในการควบคุมเชื้อรา *P. palmivora* DD01 พบว่า สารสกัดจากเชื้อรา *Ch. cupreum* CC3003 ที่สกัดด้วยสาร ethyl acetate ประสิทธิภาพในการยับยั้งเชื้อรา *P. palmivora* DD01 ได้สูงที่สุด มีค่า ED_{50} เท่ากับ 60.07 ppm รองลงมา คือสารสกัดจากเชื้อรา *Ch. cochliodes* CTh02 ที่สกัดด้วย methanol และ สารสกัดจากเชื้อรา *Ch. brasiliense* ที่สกัดด้วย ethyl acetate โดยมีค่า ED_{50} เท่ากับ 25 และ 182.05 ppm ตามลำดับ สำหรับการทดสอบประสิทธิภาพ nano - particles จากเชื้อรา *Chaetomium* spp ที่ความเข้มข้น 15 ppm พบว่าการใช้ nano particles จากเชื้อรา *Ch. cupreum* ที่สกัดด้วย ethyl acetate มีประสิทธิภาพในการยับยั้งเชื้อรา *P. palmivora* DD01 ได้สูงที่สุด มีค่า ED_{50} เท่ากับ 11.01 ppm รองลงมา คือ nano particles จากเชื้อรา *Ch. brasiliense* ที่สกัดด้วย hexane และสารสกัดจากเชื้อรา *Ch. cochliodes* CTh02 ที่สกัดด้วย ethyl acetate โดยมีค่า ED_{50} เท่ากับ 8.68 และ 13.83 ppm ตามลำดับ เมื่อนำ nano - particles จากเชื้อรา *Chaetomium* spp มาทดสอบหาประสิทธิภาพในการกระตุ้นภูมิคุ้มกันโรคเน่าของทุเรียน จากเชื้อรา *P. palmivora* DD01 พบว่ามีการสร้าง scopoletin ซึ่งมีลักษณะเป็นสารสีฟ้าเรืองแสงที่ปรากฏบนแผ่น TLC และมีค่า Rf 0.75 เช่นเดียวกับสารมาตรฐาน (standard)

Thesis	Fungal metabolites of *Chaetomium* spp to control durian root rot pathogen cause by *Phytophthora* sp.
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Year	2021
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ABSTRACT

Phytophthora palmivora DD01 was isolated from root rot of durian var Monthong and proved to be pathogenic isolate. *P. palmivora* DD01 including the antagonistic fungi, *Chaetomium brasiliense, Chaetomium cupreum* CC3003 and *Chaetomium cochliodes* CTh02 were confirmed species level using morphological and molecular phylogenetic. Testing those antagonistic fungi to control *P. palmivora* DD01 causing root rot of durian were done by dual culture method. Crude extracts and nano particles tests were antifungal evaluated. Dual culture test showed that *Ch. cochliodes* CTh02 gave significantly highest inhibition against *P. palmivora* DD01. The crude extracts from antagonistic fungi with hexane, ethyl acetate and methanol were tested to inhibit *P. palmivora* DD01. Crude methanol from *Ch. cupreum* CC3003 gave significantly highest inhibition of the tested pathogen, *P. palmivora* DD01 which the ED_{50} of 60.07 ppm and followed by crude methanol extract from *Ch. cochliodes* CTh02 and crude ethyl acetate from *Ch. brasiliense* which the ED_{50} of 25 and 182.05 ppm, respectively. Antifungal biological activities of nano particles from antagonistic fungi were investigated. The results showed nano particles from *Ch. cupreum* CC3003 expressed antifungal activity againtst *P. palmivora* DD01 at the concentration of 15 ppm which the ED_{50} of 11.01 ppm, and followed by nano particles from *Ch. brasiliense* and nano particles from *Ch. cochliodes* CTh02 which the ED_{50} values were 8.68 and 13.83 ppm, respectively. The nano particles from *Chaetomium* spp were tested for their efficiency to induce plant immunity for durian root rot caused by *P. palmivora* DD01 resulted to produce scopoletin appeared a fluorescent blue compound in TLC as same as the standard which the Rf 0.75.

ACKNOWLEDGEMENTS

I would not have successfully executed this project without the support and guidance of various people at the King Mongkut's Institute of Technology Ladkrabang (KMITL).

Firstly, I would like to thank Professor Dr. Kasem Soytong for his unrelenting support and supervision when writing my Ph.D. thesis and it is truly an honor. I appreciate his advice, ideas, moral support, and patience in guiding me through this project. Thank you for his enthusiasm for the study of plant pathology and organic production. His knowledge in the field of biotechnology in plant pathology, in particular, is inspirational. I acknowledge the opportunity to provide me to grow up in this field of expertise. His ability to solve complex challenges astonishes me. I appreciate his continuous support and guidance, which kept me motivated throughout the writing and editing of the articles included in this thesis.

I extend my sincere gratitude to Ms. Jiaojiao Song and Ms. Rungrat Vareeket for kindly help and support during my study and when I am wringing my thesis. Besides, I would like to thank you for being available to give additional information for my thesis.

Lastly, I would like to thank my family for dedication, unqualified love, and sustenance, sense of humor, tolerance, hopefulness, and guidance are more valuable than I can ever imagine.

Rujira Tongon

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CHAPTER 1

Introduction

1.1. Statement and significance

Durian (*Durio zibethinus*) is crowned the "King of Fruits" in the tropics. The durian is prized for its aroma and flavor, though detractors consider the durian's strong aroma offensive. Durian is products rich in health-related food components as they are good sources of health benefiting B-complex groups of vitamins; a rare feature for fruits, such as niacin, riboflavin, pantothenic acid (vitamin B5), pyridoxine (vitamin B-6) and thiamin (vitamin B-1), antioxidant vitamin-C and also contains a good amount of minerals like manganese, copper, iron and magnesium (Ho and Bhat 2015). Durian is a seasonal fruit and is commercially cultivated in many tropical countries mainly in Thailand, Malaysia, Indonesia and Philippines. The most serious diseases of durian are caused by *Phytophthora* spp., especially *P. palmivora. P. palmivora* causes seedling dieback, leaf blight, root rot, trunk cankers and postharvest fruit rots (Lim, 1998). Members of the genus *Phytophthora* are among the most serious threats to agriculture production, causing injurious diseases in several pant host such as cacao, oil palm, pomelo, para rubber, citrus, cassava, olive and apricot. (Sundram *et al.,* 2008; Vanegtern *et al.,* 2015; Torres *et al.,* 2016; Hung *et al.,* 2015a; Promwee *et al.,* 2017; Chi *et al.,* 2020; Arutselvan *et al.,* 2020; Gharbi *et al.,* 2020; Türkölmez *et al.,* 2017). Wet environments condition is benefit *Phytophthora* species, which are soil-dwelling pathogens. In the absence of a suitable host, *Phytophthora* species produce resting spores that can persist for years in moist soil. The pathogen can be spreaded in splashing rain or irrigation water, in surface irrigation, and runoff water, and by movement of contaminated soil, equipments, or plant parts. Flooded and saturated soil favors the spread of *Phytophthora* to healthy plants (Judelson and Blanco, 2005)

*Phytophthor*a species are controlled by chemical fungicides such as phenylamides (metalaxyl and related compounds) that reported to be resistance to those fungicides (Erwin and Ribeiro, 1996). Disease management of *Phytophthora* diseases has developed to be eco-friendly control diseases as to reduce the application cost, and harm of fungicides. Biological control agents (BCAs) has become an importance research aspect and environmentally friendly agricultural control to consider among the most promising applications for sustainable agriculture to carry out all over the world (Naqvi, 2004; Sönmez and Mamay, 2018).

Chaetomium Kunze is a large genus of saprophytic ascomycetes with more than 350 recognized species (Zhang *et al.* 2012). *Chaetomium* species are reported to act as antagonists against various plant pathogens and commercial bio-product has been developed from potent strains of *Chaetomium* spp. (Soytong *et al.* 2001). Furthermore, the genus Chaetomium has produced over 200 metabolites with a diverse range of bioactivities, many of which have antifungal activity against plant pathogens. (Zhang *et al.* 2012). Inadequate understandings of the modes and abilities of *Chaetomium* species in controlling *Phytophthora* species, however, are major reason of the limited application of these high potential biological control agents in durian production.

1.2. Objectives

- To isolate and identify *Phytophthora* spp. causing root rots of durian, using morphology and molecular phylogeny.

- To prove pathogenicity of *Phytophthora* isolates.

- To control *Phytophthora* spp. causing root rots of durian using *Chaetomium* spp. and their crude extracts.

- To evaluate the phytoalexin induction through active metabolites nano particles constructed from *Chaetomium* species.

CHAPTER 2

REVIEW LITERATURE

2.1 Introduction of durian

2.1.1 Origin, botany and morphology structure of durian

Durian belongs to the Malvales order, Bombacaceae family, and Durio genus. *D. kutejensis, D. oxleyanus, D. graveolens, D. ducis, D. grandiflorus*, and *D. zibethinus* are six of the 29 species of this group that are edible. Durian is considered to have evolved on Borneo Island, where there are more Durio species than anywhere else (Reksodihardjo, 1962; Siti and Nakashima *et al.,* 2008). Durian was once a small crop in Southeast Asia, but it is now a major tropical fruit crop. The fruit is a loculicidal capsule with five segments and an oval, ovoid, cylindroidal, or ellipsoidal form (locules). The weight varies from 1–5 kg, and the stem is wide, measuring 1–1.2 cm in diameter. There are two sections of the stem. From the fruit to the abscission region, the lower section emerges from a flower pedicel. The flower peduncle develops the upper section, from the abscission region to the attachment point on the branch. Once the fruit ripens, it falls from the tree at the abscission area.

The rind is tough and fibrous on the exterior, brownish green to yellowish green on the inside, 0.3 to 1.5 cm thick, and coated with three to seven sides of pyramidal sharp and strong spines. A suture attaches the stem to the stylar end in the center of each locule. Early in flower growth, this is the dehiscense region, where each individual carpel's ovary wall joins the others to shape the locules. It is here that the fruit breaks or dehisces. Depending on the fertilization of the ovules, each locule comprises of one to five pulp units connected to the fruit axis. The aril, which grows from the funiculus of each seed and extends outward to cover the whole seed, is a cream-colored to dark yellow pulp (Amad and Nanthachai, 1994; Bhusiri, 1988; Polprasid, 1983).

The fruit will grow into a symmetrical form if pollination and fertilization are total, but it will have strange, distorted forms if pollination and fertilization are incomplete. With just one ovary, the fruit emerges from a single flower. Just one to three flowers on an inflorescence arising from main branches grow into mature berries.

Durian has two essential mechanisms that are often ignored. The peltate trichome on the surface of the spine is one example. The second is the formation of periderm and lenticel on the surface of the spine

as well as along the groove between the spines, especially along the suture, which gives the fruit its brown color when mature.

The seeds are large, measuring 2–3 cm broad and 5–7 cm long, are rough, and have a thin light brown skin that turns brown as the fruit matures. The aril surrounding the seed is usually thin (2–10 mm) when the seed grows well. The seed, on the other side, may be aborted and then shrivel, but the aril develops and forms a dense pulp, up to 3 cm thick. Each durian fruit contains 55–66% rind, 20–35% aril, and 5–15% seeds, according to the above-mentioned structure (Polprasid, 1983).

2.1.2 Durian Varieties in Thailand

There are 234 varieties registered with the government, with 60-80 varieties being cultivated commercially.

2.1.2.1 Monthong

Thailand's most important and well-known durian variety is Monthong. In Thai, it means "golden pillow." It bears huge, elongated, oval-cylindrical fruits with a stylar end that tapers. The large fruit has a prominent beak and is lobed, yellowish-brown, and weighs between 2 and 6 kg. The rind is thick and covered with sharp, pointed, thin, conical, densely packed spines, and the peduncle is thick and moderately long. Each fruit contains 10-15 arils as well as a large number of small, shrunken (aborted) seeds. There are typically three broad, thick, creamy, smooth, pale yellow arils in each locule. The pulp has a moderate odor and is of outstanding consistency, accounting for more than 30% of the edible part and showing few physiological anomalies. (Figure 2.1)

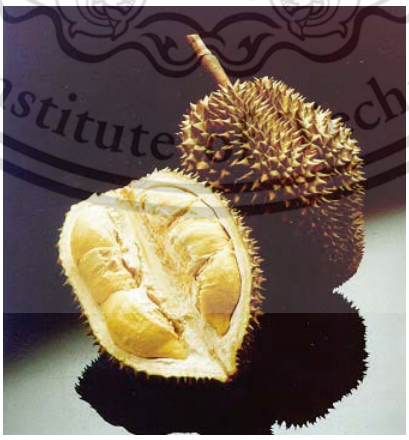

Figure 2.1 Durian var. Monthong (Source: Chung, 2017)

2.1.2.2 Cha-nee

In Thai, "Cha-nee" means "gibbon," and it is a cultivar of the Luang cultivar group. This is an early variety that bears fruit between the ages of 4-6 years. The fruit is oval to broad cylindrical, lobed, and greyish brown, weighing 2-4.5kg. The peduncle is thick and long, and the rind is brownish yellow, thin, and covered in blunt, broad, widely spaced spines. Three to four arils are found in each locule. The dense, fine-textured, firm, creamy smooth, soft, and flavorful pulp is bright yellow in color. The ripening of the flesh is consistent. Strong flesh fiber, recurrent physiological disorder, watery at full ripening point, poor fruit environment, and phytophthora and fruit borer susceptibility are some of the less desirable characteristics. (Figure 2.2)

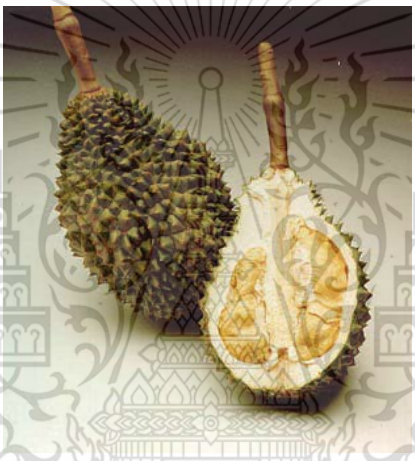

Figure 2.2 Durian var. Cha-nee (Source: Chung, 2017)

2.1.2.3 Kan Yao

In Thai, the word "Kan Yao" refers to a long stalk. A long, thick peduncle of 10-14 cm separates the fruit. The fruit is globose to lychee-shaped, greyish brown in color, rough, and has a moderately thick rind with small, sharp, straight, moderately dense spines. Each locule contains three to four broad, thick arils. The pulp is golden yellow, smooth, creamy, sweet, and fragrant. This cultivar has a low flesh fiber content and strong fruit setting characteristics. Each fruit is between 2 and 4.5 kilograms in weight. Large seeds and a high number of seeds/fruits are two characteristics of inferior fruit. (Figure 2.3)

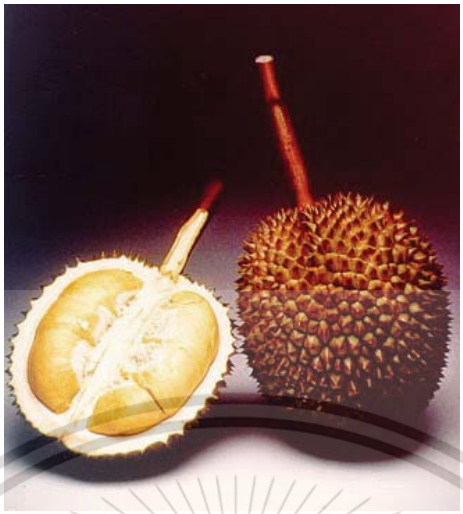

Figure 2.3 Durian var. Kan Yao (Source: Chung, 2017)

2.1.2.4 Gradum-Tong

In Thai, "Gradum-Tong" means "golden button." This cultivar bears fruit for 4-6 years after planting and is an early season variety in Thailand, with fruiting beginning in March. The fruit is large, weighing between 2 either 4 kilograms, and symmetrically or uniformly lobed. The rind is brownish green in color, small, and coated in tiny, sharp spines that are tightly packed. The peduncle of the fruit is relatively long. 3–4 large, dense arils/locules with yellow flesh are present. (Figure 2.4)

Figure 2.4 Durian var. Gradum-Tong (Source: Chung, 2017)

2.2 History, taxonomy and biology of *Phytophthora*

Phytophthora is a member of the Pythiaceae, Peronosporales, within the Oomycota, Stramenopile (Beakes *et al.* 2012; Thines, 2014). *Phytophthora* species have over 120 species, the majority of which are plant pathogens, that have been discovered and officially identified. Some *Phytophthora* species have a small host range, while others have a large one. *Phytophthora fragariae*, for example, infects a single host, while other species, such as *Phytophthora nicotianae*, *Phytophthora cinnamoni* and *Phytophthora palmivora*, can infect hundreds to thousands of plant species, whereas others can infect a handful to hundreds of hosts. While Oomycetes share many ecological and life-history characteristics with true fungi such as Basidiomycetes and Ascomycetes, their genetics, cell wall compositions, and biochemical pathways differentiate them from these fungi. Oomycetes have a diploid life history, while fungi have a haploid life history. Oomycetes' cell walls are made up of glucans rather than chitin, which is a typical part of fungi's cell walls. Members of the 6 Oomycota do not synthesize sterol, unlike fungi. As a consequence, fungicides that target chitin and sterol synthesis are ineffective against them. Furthermore, their zoospores are biflagellate, which is not typical of fungi (Kennedy and Duncan. 1995; Erwin and Ribiero. 1996).

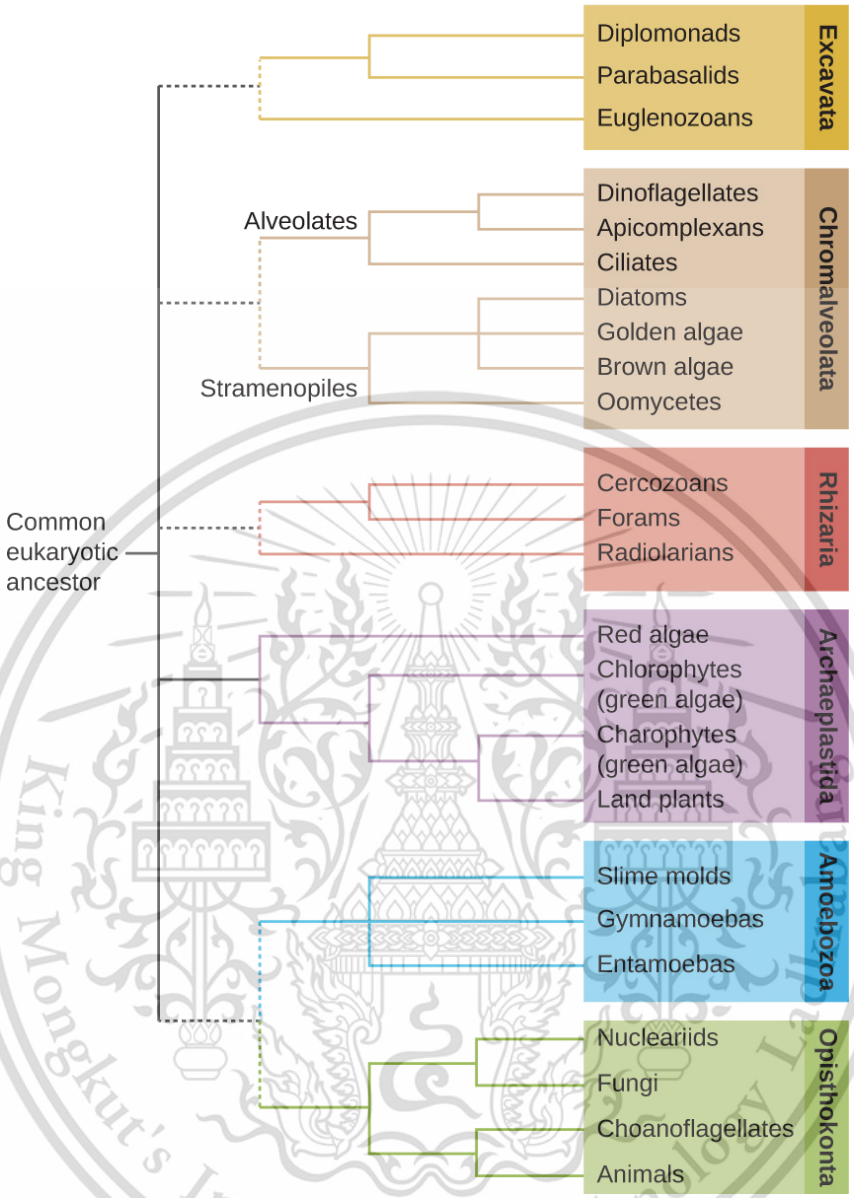

Figure 2.5 Eukaryotic supergroups (Source: OpenStax College, 2013)

2.2.1 Symptoms of Phytophthora Diseases

Pathogens of the Phytophthora genus can cause a variety of diseases and symptoms in a broad range of plant species. The most common illness signs are described in the following segment.

2.2.1.1 Root rot

Phytophthora root rot and damping off are very popular in seedlings of many plants. The wilting and yellowing of young seedlings are the first signals. Root rot presents itself in plants that are water stressed, chlorotic, and sometimes stunted in growth. And when there is adequate water, new leaves are

always tiny and light green to yellow in color, and wilting happens. Instead of the creamy white appearance of healthy roots, affected root tissue is fuzzy, water-soaked, and discolored to dark brown. Root rot causes a shortage of secondary and tertiary roots, as well as a lack of stable root tips.

2.2.1.2 Collar rot

Collar rot, commonly known as foot rot, happens often at or above ground level. The fungus also spreads upward from the base, rotting lower bark tissue and discoloring the lower stem. Gum exudation is normal in the infected areas. The damaged bark region is sometimes unusual in form and scale, and it starts as a water-soaked lesion that then dries, sunkens, and cracks in the bark that are typically dark brown in color. Above-ground symptoms entail wilting, leaf loss, and branch dieback, close to the symptoms induced by *P. capsici* in pepper. Bark and cortex tissues are often bloated and cracked, and they quickly separate from the underlying tissue. The disease may also propagate across the trunk, allowing the main roots or the trunk to girdle.

2.2.1.3 Tree canker

Many *Phytophthora* species may cause cankers on the stems of their host plants. Stripe canker (cinnamon), patch canker (durian), and trunk canker are some of the terms for these cankers (cocoa). Wet lesions on the bark surface, frequently near to the branch points at the lower end of the tree, are the first indicator of canker. Necrosis is also followed by bark discoloration and the exudation of a reddish brown resinous material. When the bark is removed, the cortical tissues and wood become dark and discolored, ranging from cream to reddish brown in color. The form of wood lesions is often irregular, but they are well described. Water and nutrient supply to linking branches is significantly limited by growing lesions, resulting in wilting. Dieback is more widespread in the crown if the lesion girdles the tree node, and the tree may lose all of its leaves.

2.2.1.4 Stem lesions

Phytophthora species are attacked to leaves and stems. *P. infestans*, for example, grows on potatoes and onions, while *P. nicotianae* grows on tobacco. In the early stages, the cortical tissue on the stem produces dry, dark-brown or black lesions. In the case of black shank on tobacco, lesions sometimes begin near the soil line and then extend upward, covering up to half the length of the stalk. Expanded lesions often girdle the stem, causing the upper branches and leaves to wilt and die.

2.2.1.5 Leaf blight

Leaf blight is caused by several species of *phytophthora*. *P. infestans* on potatoes and onions, *P. palmivora* on rubber and a range of tropical fruit types, including durian, and *P. colocasiae* on taro are only a few examples. The blights on leaves start off as tiny flecks, but after 3–5 days, they develop into large lesions. Infected tissue is initially waterlogged, but within a few days, it becomes necrotic (brown or black). A halo of light green tissue also covers the lesions. Spores occur as a white velvety development at the edges of lesions, usually on the leaf's underside. Phytophthora leaf blight is distinguished from other foliar diseases by its white development. As 1–4 sporangiophores spread from the stomata on the underside of the leaf, large numbers of sporangiospores are frequently developed. Sporangiospores may become airborne, causing the disease to propagate quickly.

2.2.1.6 Fruit rot

Fruit rot caused by Phytophthora species impacts a broad variety of plant species, including lemon, durian, cocoa, papaya, and chili pepper. Depending on the host, it occurs as water-soaked lesions with light-brown centers 3–5 days after infection. The lesions spread quickly and may cause a whole fruit to rot. White/grey mycelium can be located behind the advancing margin of the lesions while the humidity is strong. The fruit does not often fall to the ground and will mummify on the tree. Internal contamination is possible, as with *P. palmivora* in papaya, where mycelial development can be seen on the seeds after breaking open infected berries. Brown rot occurs as an expanding circular lesion on citrus with a dull-brown appearance. The diseased tissue stays solid as it darkens in appearance, which is characteristic of many phytophthora-caused fruit rots. A heavy odor emitting from the fruit is another sign of brown rot in citrus.

2.2.2 Life cycle

In wet environments, chlamydospores or oospores germinate and develop sporangia. Sporangia produce zoospores, which are single-celled swimming spores. Zoospores can swim in water on leaf surfaces or through waterlogged dirt, so they can dry out. When they swim across the earth, zoospores are drawn to plant roots, and when they reach one, they develop a cyst. Any *Phytophthora* species have the ability to encyst and invade both leaves and roots. Cysts germinate into small thread-like structures called hyphae, and the pathogen infects the plant by growing into the plant tissues to receive nutrients. The *Phytophthora* then develops more chlamydospores (asexually) or oospores (sexually) before producing sporangia and continuing the life cycle (Figure 2.6).

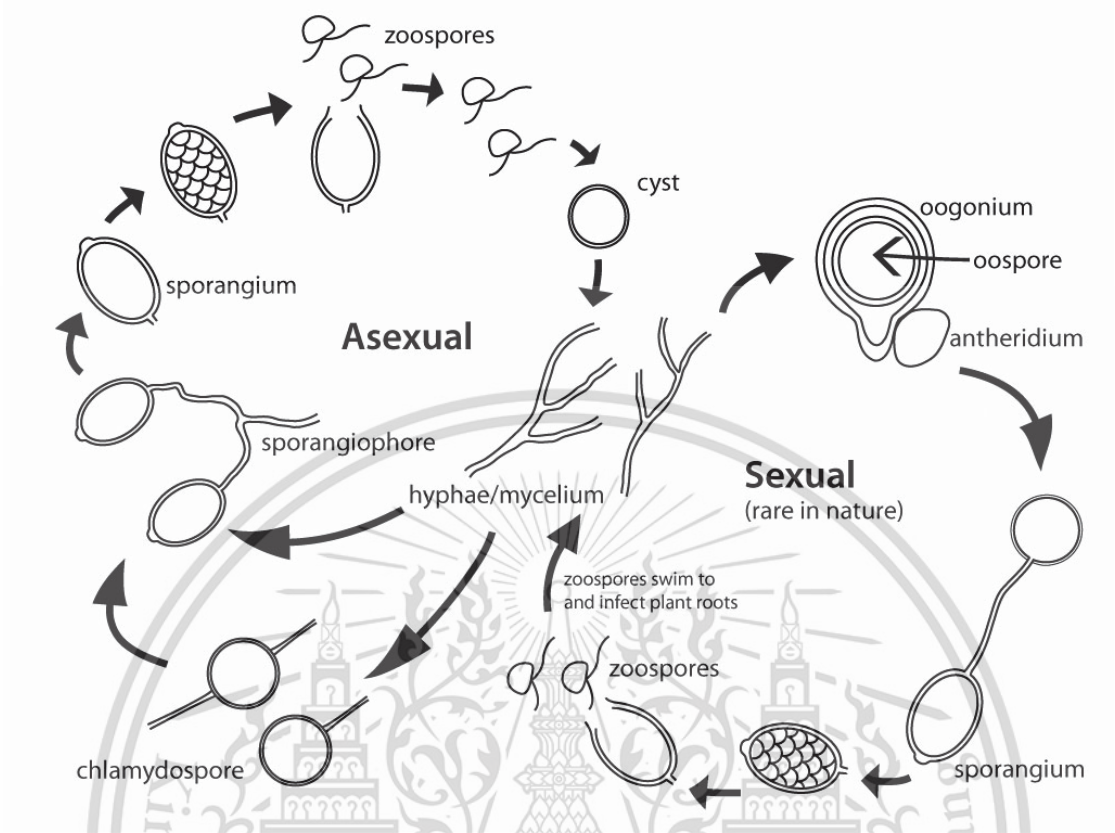

Figure 2.6 *Phytophthora* life cycle (Abad *et al.,* 2019)

2.3 Current status of durian production

The fruit of the genus *Durio* includes many tree species. As of 1987, 30 *Durio* species have been identified, at least nine of which produce edible fruit, with over 300 named varieties in Thailand and 100 in Malaysia. The only species available on the foreign market is *Durio zibethinus*, all other species are sold only in their native areas. (Morton, 1987). In Thailand, the durian is known as the "king of fruits" because of its heavy odor, and thorny rind (Heaton, 2006). Thailand sold 1.46 billion dollars worth of durians to foreign markets in 2019, a 54.6 percent increase from the previous year, with China and ASEAN countries responsible for 98 percent of exports. According to the Ministry of Agriculture and Cooperatives of Thailand, China imported 575,000 tons of durians from Thailand worth 14.7 billion yuan ($2.2 billion) in 2020, up 78 percent year on year. In 2020, fresh durians were the most exported fruit among the top nine of Thailand. Durians were the most common, accounting for 78 percent of all imports. About 90% of China's fresh durian comes from Thailand, according to reports (Global time, 2021).

2.4 Management of Phytophthora root rots in durian

Durian trees are grown in raised beds on flat or semi-flat land in Thailand to allow the roots to dry out after heavy rains. This allows the tree more tolerant of dry stress, which is needed for flowering and fruiting to occur. A successful drainage system often avoids disease transmission and decreases uneven fruit ripening and the occurrence of damp core and other fruit disorders. Prior to planting, change the pH of the soil and incorporate organic matter (OM). Liming within 30 cm of the soil surface at a distance of one meter, where the majority of the roots are supposed to grow, will increase the pH of the soil. Phytophthora species thrive in soils with a pH of less than 5.3. Furthermore, agricultural fertilizers, such as livestock waste (such as poultry dung), should be used on soils of less than 2% OM. Putting in irrigation systems. Until planting, nearly all durian farms in Thailand are equipped with sufficient irrigation facilities. Young durian plants that receive enough water are less vulnerable to Phytophthora infection. Premature leaf drop is one of the consequences of water stress in durian. Use tolerant clones from broad planting content (LPM). Plants 1.5 m tall and up are more tolerant of environmental stress, allowing for quicker establishment, rapid expansion, and disease tolerance. In terms of fruit size, yield, and tolerance to Phytophthora, clones from Malaysia ('D2,' 'D10,' 'MDUR 79,' and 'MDUR 88') and Thailand ('Cha-nee') are the strongest. The plants are put on top of the soil surface rather than in dig pits, and pulverized soil is applied to create a mound around them. This guarantees proper drainage at the plant's roots, stopping raindrops from splashing moist soil into the plant's leaves, trunks, and branches. It may establish a short-term, drier environment at the plant's root, avoiding disease infection. After-planting operations in the orchard. It is vital to preserve the health of durian trees by offering sufficient fertilization, irrigation, pruning, and other good management practices that will keep the trees healthy and immune to infection. Furthermore, the trees should not be able to grow more fruits than the plant can handle (Food and Fertilizer Technology Center for the Asian and Pacific Region, 2021).

2.5 Biological control

The search for effective biological control agents (BCAs) against *Phytophthora* spp. has been widely conducted in order to reduce plant production's reliance on noxious synthetic fungicide.

Antagonists are the most common term for biological control agents for plant diseases. Plant pathogen biological control is a relatively new strategy for integrating with other control measures. According to Phuwiwat and Soytong (1999), research and development of biological control agents for use against plant diseases has been ongoing in both the public and private sectors for several years, as natural

agents are needed to replace chemical fungicides. The problem of pathogens becoming resistant to chemical fungicides, as well as the resulting pollution and ecological imbalances, has gotten a lot of attention around the world. Biological products are beneficial not only for the prevention of plant diseases, but also for the treatment of plant diseases. (Soytong *et al.,* 2001)

2.5.1 *Chaetomium* spp. as the biological control agents

Chaetomium is a fungus genus with more than 300 species in the Chaetomiaceae family. *Chaetomium* is a dark-walled dematiaceous fungus. *Chaetomium* is a saprophytic fungus that lives in soils, cellulose, and plant matter. (von Arx *et al*. 1986 and Kirk *et al*. 2008). Several *Chaetomium* species have the potential to suppress the growth of bacteria and fungi through competition, mycoparasitism, antibiosis, or a combination of these mechanisms. More than 200 compounds have been isolated from *Chaetomium* spp., with a wide range of bioactive effects, and many of them have antifungal activity against plant pathogenic fungi. (Zhang and Yang 2007; Zhang *et al.*, 2012). Several *Chaetomium* species have been reported to act as antagonists against a variety of plant pathogens (Vannacci and Harman, 1987; Soytong *et al.* 2005; Tongon and Soytong, 2015, Soytong, 2015). According to Hani and Eman (2015), *Chaetomium* spp. can inhibit cancer cells in addition to plant pathogens. This is the result of the isolation and purification of two potential anticancer compounds from *Chaetomium globosum* isolated from Egyptian soil. The two compounds were tested against the Michigan Cancer Foundation-7 (MCF-7) breast cancer cell line and the Hepatocellular carcinoma, Human (HEPG-2) human liver carcinoma cell line, both of which showed inhibition of cell proliferation. HNMR and Mass spectroscopy revealed the structures of the two pure compounds as methyl 9-dihydro-8-trihydroxy-9-oxo-H-xanthene-1-carboxylate, a xanthone, and (E)-methyl 2-hydroxy-6, 6-dimethyl hept-3-enoate. Endophytic on *Ephedra fasciculata*, globosumones A and B were isolated from *Chaetomium globosum* (Mormon tea). Both compounds were tested in a panel of four cancer cell lines, including NCI-H460 (non-small cell lung cancer), MCF-7 (breast cancer), SF-268 (CNS glioma), and MIA Pa Ca-2 (pancreatic carcinoma), as well as normal human fibroblast cells, for their ability to inhibit cell proliferation (WI-38). The globosumones A and B were found to have a moderate level of activity (Bharat *et al.*, 2005).

There have also been reports of *Chaetomium* spp. antagonistic to various plant pathogens. *Fusarium oxysporum* f. sp. *lycopersici*, which causes tomato wilt, was controlled by *C. lucknowense* CL01, *C. elatum* ChE01, and their crude extracts (Sibounnavong *et al*. 2011). *Pythium aphanidermatum*, which causes pineapple root rots, was treated with crude extracts of *C. aureum, C. bostrychodes, C. cochliodes,* and *C. cupreum* (Pornsuriya *et al.* 2010). They can be used to control many soilborne diseases

of vegetable 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 has been successfully controlled by treated *Chetomium cupreum* CC3003, *Chaetomium globosum* CG05, and *Chaetomium lucknowense* CL01. *Chaetomium lucknowense* CL (C1) *Chaetomium cochiliodes* C1, C3, C4 and C5 significantly proved to inhibit or control *Ganoderma boninense* causing stem rot of oil palm in bi-culture test at 10 days (Soytong, 2014b)

Since 1989, *Chaetomiun* species have been screened in Thailand for use as antagonists. The potentials strains of *Chaetomiun* spp. have even been turned into a commercial product known as Ketornium®, which is widely used in Thailand and other Southeast Asian countries. In Thailand, the product has been effective in controlling *Phytophthora* spp. infected durian, black pepper, strawberry, mulberry, grape and orange (Soytong *et al.* 2001).

2.6 Phytoalexin in plant

2.6.1 General of Phytoalexin

Phytoalexins are natural products that plants secrete and accumulate in response to pathogen attack. They inhibit bacteria, fungi, nematodes, and insects, as well as having harmful effects on animals and plants (Braga, 1991). They are primarily lipophilic compounds that can cross the plasma membrane and function within the cell. Their toxicity in plants is caused by their acidic nature, high number of hydroxyl and substituents, according to Smith (1996).

According to Cavalcanti *et al.* (2005), phytoalexins inhibit seed germination and elongation of the germ tube, as well as reduce or inhibit mycelial development, by cytoplasmic granulation, disorganization of cellular contents, rupture of the plasma membrane, and inhibition of fungal enzymes.

Phytoalexins, according to Grayer and Kokubun (2001), are low-molecular-weight secondary metabolites that can't be added to antifungal proteins and peptides formed by plants until their molecular weight has increased. Stoessl *et al.* (1980) described phytoalexins as metabolic products of higher plants that are absent or present in negligible amounts in healthy tissues but accumulate in significant amounts in response to fungi or bacteria attack.

More than 300 forms of phytoalexins have been identified among various chemical compounds, including coumarins, diterpenes, flavonoids, alkaloids, phenolic compounds, luteolinidin, apigenidin, and apigeninidin, and these have been used as chemical markers in several studies. The rate

of phytoalexin accumulation is thought to be a key factor in pathogen infection establishment. (Harborne, 1999; Cavalcanti, 2005; Arruda *et al.*, 2016)

2.6.2 Elicitors

Elicitors are chemicals or bio-factors that can trigger physiological and morphological responses in target living organisms, as well as phytoalexin accumulation. Mainly, the term elicitor referred to molecules that could trigger the development of phytoalexins, but it is now used to refer to compounds that stimulate any form of plant protection (Thakur and Sohal, 2013). Elicitor molecules bind to special receptor proteins found on the membranes of plant cells. A receptor is a molecule of protein that receives chemical signals from the outside of a cell. A Ligand is a molecule that binds to a receptor. The receptors recognize the molecular pattern of elicitors, which stimulates intracellular protective signaling and contributes to an increase in the synthesis of secondary metabolites, phytoalexins, which minimize damage and increase tolerance to pests, pathogens, and environmental stresses (Darvill and Albersheim, 1984)

SAR (systemic acquired resistance) is a term used to describe a type of resistance that has developed over time. As a result, pathogenic infection defense responses occur not only at the infection site, but also in spatially separated tissues. SAR is a type of induced defense found in a wide range of plant species that provides potentially long-lasting protection against a wide range of microorganisms (Pajerowska-Mukhtar *et al.,* 2013).

Local acquired Resistance (LAR) is a term used to describe a type of acquired resistance that occurs locally. Local acquired resistance refers to induced resistance that is first localized around the point of plant necrosis caused by infection by the pathogen (or) by the chemical. Antifungal compounds and defense enzymes are found in high concentrations in LAR patients. LAR works against a wide variety of pathogens (Gowthami, 2018).

2.6.3 Scopoletin as plant Phytoalexin

Scopoletin is a phenolic coumarin and an important phytoalexin isolated from a variety of plants (Figure 2.7). Scopoletin accumulation has been linked to resistance to microbial attack as well as other stresses like mechanical injury and dehydration. In comparison to other related coumarins and coumarin glycosides, scopoletin appears to be the most important product rising in concentration in the infected plant. (Tal and Robeson, 1986; Tanaka *et al.,* 1983; Uritani, 1999; Buschmann *et al.*, 2000; Giesemann *et al.*, 2008). The presence of scopoletin was detected using thin layer chromatography

(TLC), and various biological activities were then elucidated through a series of studies. Different possible pathways for scopoletin biosynthesis have been reported over time, but all of these pathways have been described as present and functional in specific plant species (Kai *et al.,* 2006; Bayoumi *et al.,* 2008; Sun *et al.*, 2014).

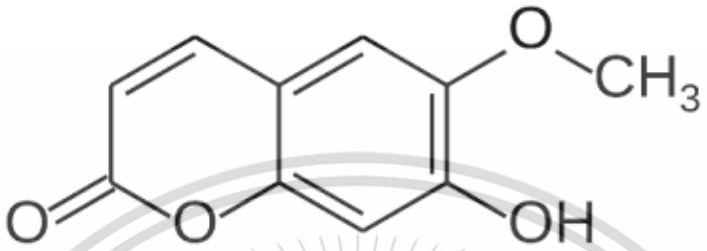

Figure 2.7 Scopoletin structure

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Isolation, identification and pathogenicity test

Phytophthora spp. causing root rot disease was isolated form durian root by tissue transplanting technique. Roots of durian 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 *Phytophthora* spp.

Isolate of *Phytophthora* spp. was morphological identified by culturing in potato dextrose agar (PDA) and incubated at room temperature for 7 days observation. The characters of *Phytophthora* spp. were determined under binocular compound microscope and the details of fungal morphology were recorded as mycelia structure, sporangium and oogonium.

3.1.2 Pathogenicity test

Pathogenicity test was conducted to determine the isolated fungus on 6 months of durian seedling var. Monthong. Sporangial suspension ($1x10^5$ sporagia/ml.) of *P. palmivora* isolate was prepared and inoculated to the soil and basal stem of the test plants at the amount of 10 ml./plant. Pathogenicity on the other parts of the plants was also done by inoculation the 0.5 cm. diameter of culture agar plugs into the detached leaves, twigs and fruits. The non-inoculated ones treated with sterile distilled water served as controls. Each was replicated four times. Percentage of disease incidence was measured as number of infected plants/ total number of tested plants x 100, and disease ratings was evaluated as 0= healthy plants, and 3= seriously infected plants (Soytong, 2010).

3.2 Molecular phylogeny identification based on DNA sequencing

All isolates of *Chaetomium* spp and *Phytophthora spp.* were reconfirmed species by DNA sequencing. Mycelial mass of each fungus was collected from a purified colony growth in PDB. DNA of isolates was extracted by CTAB method with some modification (Ausubel *et al.*, 1994; Prabha *et.al*

2013). The fungal mycelia were cleaned with 25mM EDTA by centrifugal machine. 100 mg fungal mycelia were diligently crushed in liquid nitrogen to make a fine powder and transferred into an eppendorf tube. The cells were lysed in CTAB buffer and β-mercaptoethanol, mixed carefully and incubated at 65°C for 1hr 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 in a microcentrifuge for 5 min at 4°C. The aqueous phase was transferred to a new sterile tube and add 2µl Rnase (20µg/ml), incubated for 30 min at 37°C and added 50 µl 10% CTAB, mix thoroughly, repeating by adding equal volume of 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 in to new eppendorf tube, added an equal volume of cold isoproponal, mixed well and let tubes standed at -20°C for 20min. The genomic DNAs 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. Quantification was performed through comparison with known dilutions of lambda phage DNA. DNAs were stored at -20°C for further use.

Phylogenetic analyses of the *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'), using the polymerase chain reaction (PCR) and phylogenetic analyses of the *Phytophthora 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'TCCGTAGGTGAACCTGCGG3') and ITS 2 (5'GCTGCGTTCTTCATCGAGC3') used PCR conditions were as follows: initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 56°C for 1min., 72°C for 2 min, and a final extension at 72°C for 5 min. The 25µl reaction mixture contained 2.5µl 10 × PCR buffer, 0.625µl each dNTP (1.25mM), 0.5µl $MgCl^2$, 1µl of each primer (20pmol/µl), 2 ng of DNA and 0.2µl of Taq DNA polymerase (1 U). The amplified products (5µl) were visualized on 1% (w/v) agarose gel to confirm the presence of a single amplified band.

The amplifications were purified by Amicon Ultra columns (Millipore, USA). The PCR products were electrophoresis in a 1% agarose gel, then, cut gel with DNA band under UV light and put into tubes. The following protocols were adding DB buffer malted gel at 50°C and loading to purification column for centrifuge at 8500rpm, 1min, 4°C, then discard flowed through, added wash buffer and centrifuged at

8500 rpm, 1min, 4°C for dry samples, transferred column to new microcentrifuge tube and added elution buffer, standed for 5min, centrifuge at 8500rpm, 1min, 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 forward or the reverse primers (2 moles) also used in the sequencing reaction.

The amplified products were sequenced and aligned with the sequences in the GenBank by basic local alignment search tool (BLAST) analysis (Altschul *et al.*, 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 closely related sequences obtained were aligned through CLUSTALW using MEGA version 6.0 software (Tamura *et al.*, 2012) and a phylogenetic tree was constructed by neighbor-joining method using the same software and compared with outgroup taxa.

3.3 Biological control of *Phytophthora* spp.

3.3.1 Strain of antagonistic fungi used for experiments

Chaetomium brasiliense (CB), *Chaetomium cupreum* CC3003 (CC) and *Chaetomium cochliodes* CTh02 (CCO) were kindly provided by Assoc. Prof. Dr. Kasem Soytong

3.3.2. Dual-culture test

The antagonistic fungi, *Ch. brasiliense* (CB), *Ch. cupreum* CC3003 (CC) and *Ch. cochliodes* CTh02 (CCO) were tested by using method of Soytong (1992). The experiment was designed as Completely Randomized Design (CRD) with four replications. *Phytophthora* spp 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 agar plug of *P. palmivora* 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

Ch. brasiliense (CB), *Ch. cupreum* CC3003 (CC) *and Ch. cochliodes* CTh02 (CCO) were extracted to tests their abilities to inhibit growth of *Phytophthora spp.* caused durian wilt.

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

The fungal antagonistic *Ch. brasiliense* (CB), *Ch. cupreum* CC3003 (CC) *and Ch. cochliodes* CTh02 (CCO) 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

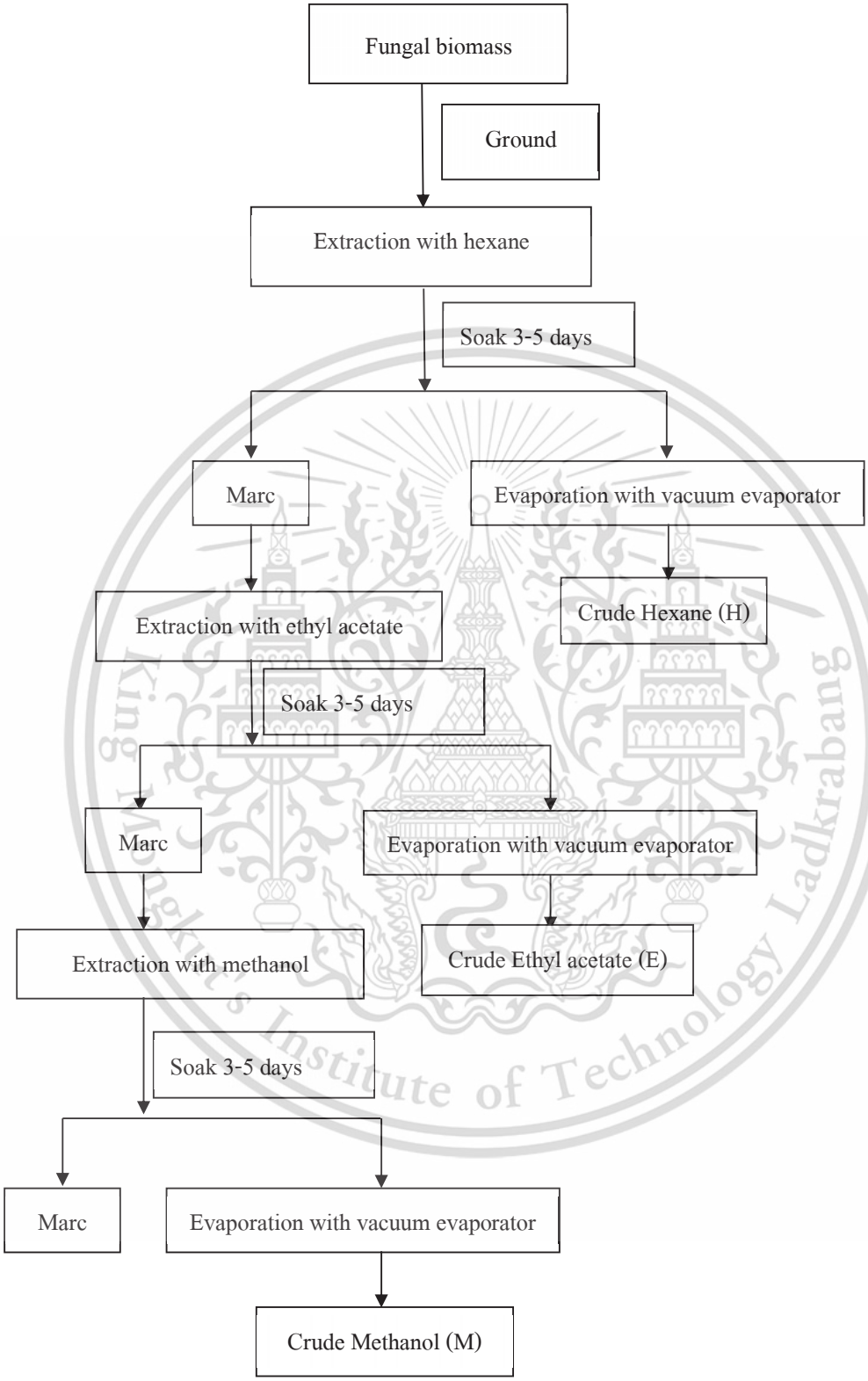

Figure 3.1 Protocol of extraction antagonistic fungi (Kanokmedhakul *et.al*., 2003)

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 *P. palmivora*

The crude extracts of *Ch. brasiliense* (CB), *Ch. cupreum* CC3003 (CC) *and Ch. cochliodes* CTh02 (CCO) were tested ability to inhibit the growth of mycelia and spore production of *P. palmivora.*

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. *P. palmivora* DD01 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 *P. palmivora* DD01 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 *P. palmivora*

The experiments were designed as 2 factors factorial in Completely Randomized Design (CRD) with four replications. Factors A represented different kinds of nano particles. Nano particles derived from *Ch. brasiliense* namely nano CBH, nano CBE, nano CBM*, Ch. cupreum* CC3003 (CC) namely nano CCH, nano CCE, nano CCM and *Ch. cochliodes* CTh02 namely nano CCOH, nano CCOE, nano CCOM. 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 *P. palmivora* DD01 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.

3.3.4 Testing nano particles for phytoalexin production in durian

Seedlings of durian var Monthong were inoculated with a sporangial suspension ($1x10^5$ sporangia/ml) of *P. palmivora* DD01 following cutting root tips before planting in a sterilized soil. The nano CB-M from *Ch. Brasiliense*, nano CC-E derived from *Ch. cupreum* CC3003 and nano CCO-E from *Ch. cochliodes* CTh02 at a concentration of 15 ppm was sprayed on the inoculated durian seedlings. Control plants were treated with sterile water (negative control) or scopoletin (positive control). Detection of phytoalexin in durian tissue extracts was carried out by thin layer chromatography (TLC) using 12% acetic acid. Fresh leaf samples (1 g.) were cleaned in tap water, ground, and soaked in 10 mL methanol before passing through a filter paper (Whatman No.4). The chromatogram was monitored under UV light (366 nm), and a single, blue fluorescent compound was characterized by comparison to the standard scopoletin (Sigma Co. Ltd) at Rf 0.75. The R_f value was calculated to compare with the scopoletin standard. The experiment was repeated three times. The R_f value was calculated as the equation

$$R_f = \frac{\text{distance spot travels}}{\text{distance mobile phase travels}}$$

Where: R_f – retention factor.

CHAPTER 4

RESULTS

4.1 Isolation, identification and pathogenicity test

4.1.1 Morphology study of the *Phytophthora* spp.

Colonies of DD01 showed stellate pattern, comparatively slow growing, with aerial mycelia on PDA, white colour and reaching 9 cm diameter in 7-10 day at 29-32 °C. The isolate DD01 formed hyphae with lumpy branches and hyphal swellings, when sporangia were flooded in distilled water, zoospores were released directly. Sporangia are abundantly when grown on PDA, ovoid, ellipsoid shape and occurred in groups on sympodium or irregularly, were papillate and caducous with short pedicels. Mostly chlamydospores were globose in shape, abundantly from mycelia when incubated in dark condition.

Figure 4.1 Morphological characteristics of *Phytophthora palmivora* DD01. A= Colony of DD01 on PDA media, B= sporangia proliferation, C-E= sporangium, F=oogonium

4.1.2 Pathogenicity test

Pathogenicity test was conducted to determine the isolated fungus on 6 months of durian seedling var. Monthong showed die back symptoms within 10 days. The stem were significantly infected by the tested pathogen when compared to non-inoculated control which showed no symptoms (Figure 4.2).

Pathogenicity test was proved the pathogenic isolate, *P. palmivora* DD01 on detached leaves of durian var. Monthong after 3 days inoculation. The inoculated leaves of durain var. Monthong under moist chamber condition showed brown symptom which enlarge from the agar plug of the wounded leaves. The non-inoculated leaves of durian var Monthong showed leaves remained healthy (Figure 4.2).

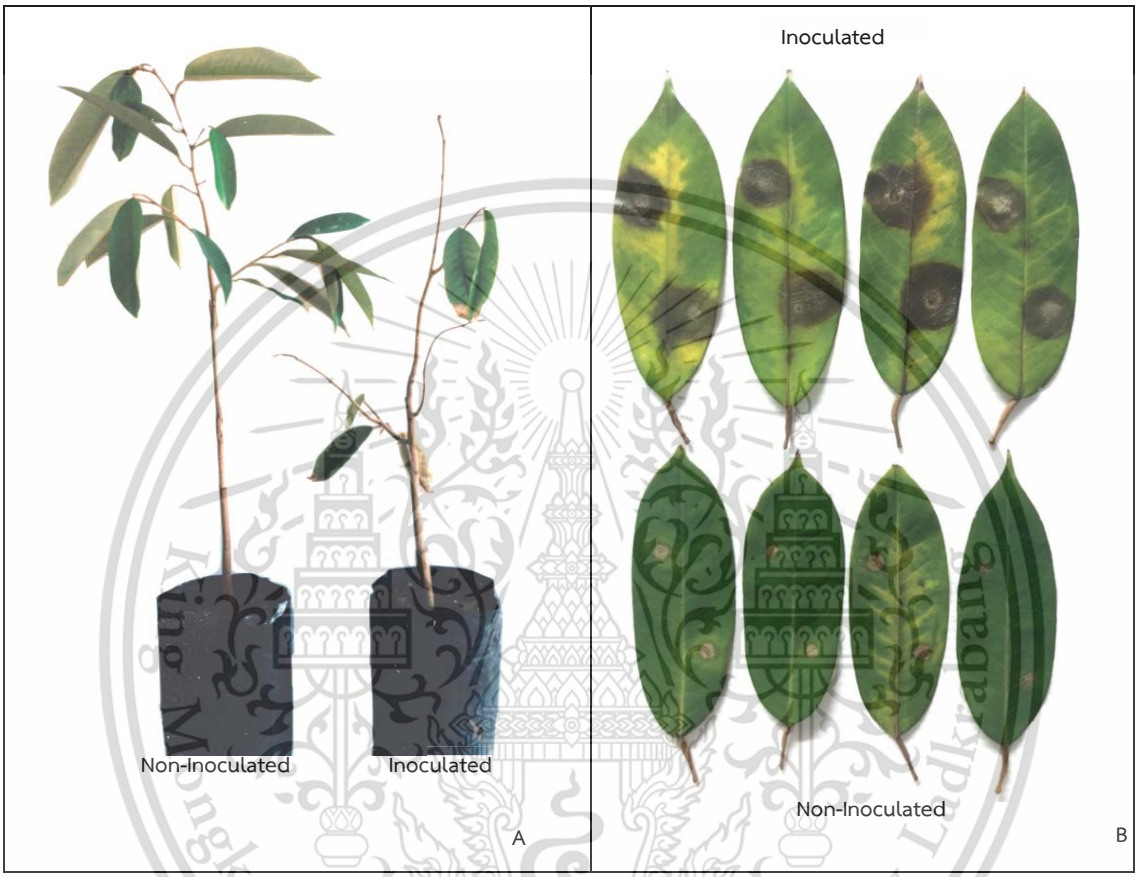

Figure 4.2 Pathogenicity test of *P. palmivora* DD01 caused durian root rot, A= Pathogenicity test of *P. palmivora* DD01 on durian seedling, B= Pathogenicity test of *P. palmivora* DD01 by detached leaves technique

4.2 Morphological of antagonistic fungi

Ch. 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).

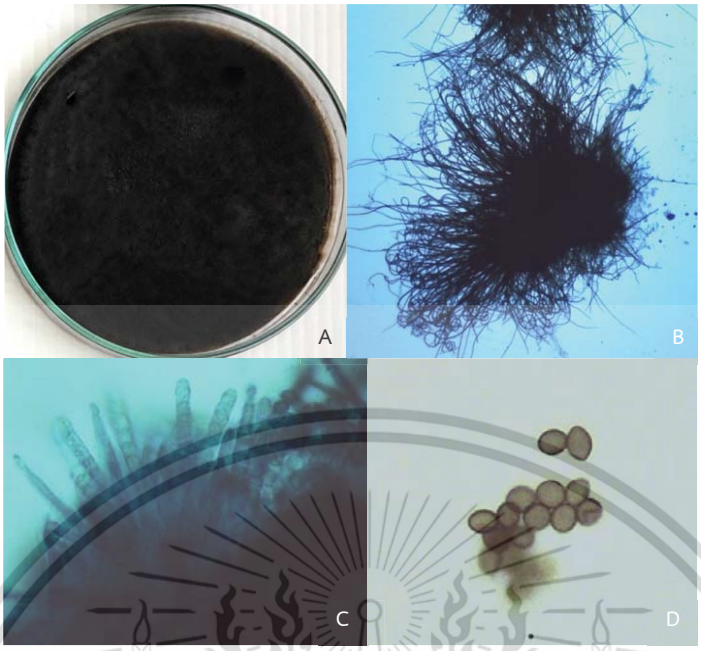

Figure 4.3 *Ch. brasiliense*, A= colony, B= ascocarps, C= asci and D= ascospores.

The culture of *Ch. cupreum* CC3003 was deep red colour, perithecia are ovate shape and copper colored with dimensions of 110–120 x 120–130 µm, each ascus contains 8 ascospores that are boat shaped. Hairs on the apex of the perithecium of *Ch. cupreum* are numerous, thin, septate lateral hairs with 1–2 spirals (Figure 4.4).

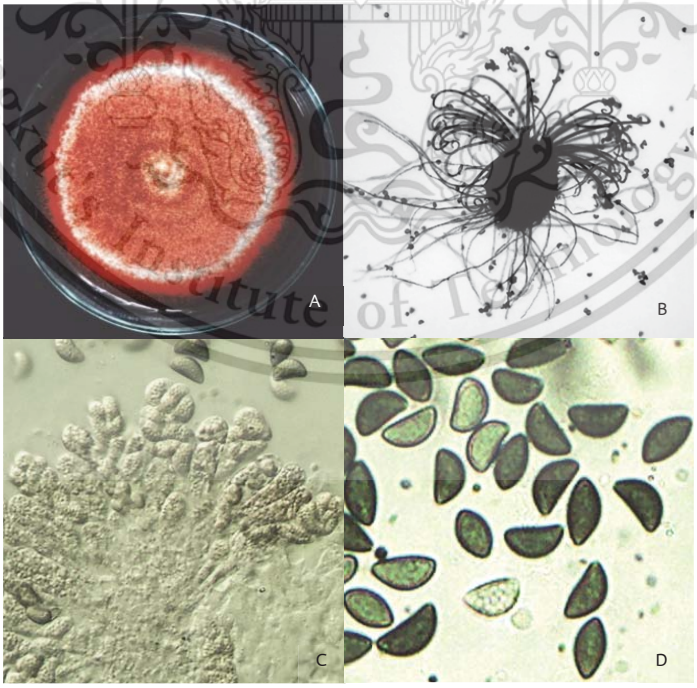

Figure 4.4 *Ch. cupreum* CC3003, A= pure culture, B= ascocarp, C= asci, F= ascospores

The culture of *Ch. cochliodes* CTh02 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 of the terminal hairs were spirally coiled in the upper part, with coils regularly tapering, and the asci was clavate in shape. Ascospore dark brown colour when mature 8.0×9.5-7.5×8.5 µm with an apical germ pore (Figure 4.5).

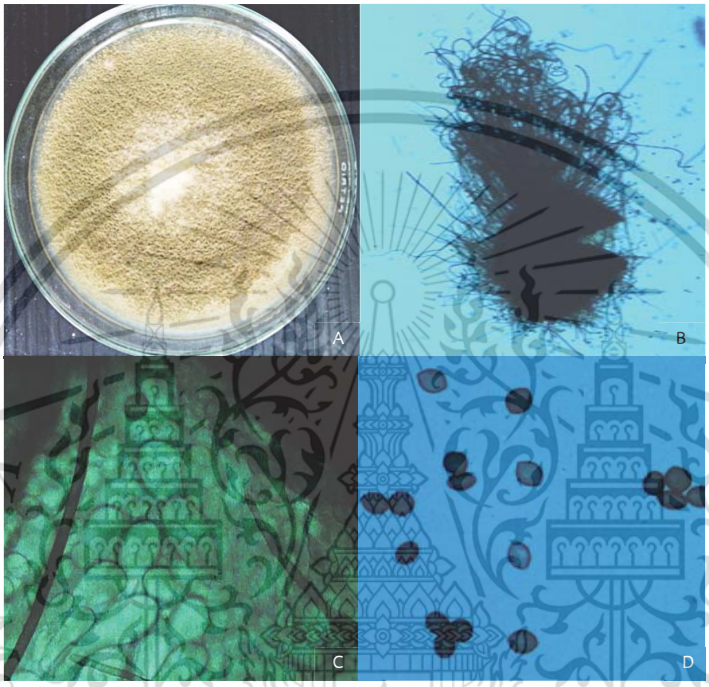

Figure 4.5 *Ch. cochliodes* CTh02, A= pure culture, B= ascocarp, C= asci, F= ascospores

4.3 Molecular phylogeny identification based on DNA sequencing

Ch. Brasiliense (CB), *Ch. cupreum* CC3003 (CC), *Ch. cochliodes* CTh02 (CCO) and *P. palmivora* were confirmed identification by molecular philogyny. Genomic DNA of these isolates were extracted using the standard method of Ausubel *et al.* (1994). The sequences were subjected to a preliminary BLAST analysis in NCBI databases, and sequence matches were used to determine their identities. Multiple sequence alignment was then carried out using ClustalW and phylogenetic tree was constructed using Mega X 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* (CB), the analysis showed that its nucleotide sequences shared 94% identity with those of number KT357683, KT357646 and KT 371339. *Ch. cupreum* CC3003 (CC) was identified, based on its morphology and the molecular analysis. The nucleotide sequences of *Ch. cupreum*

shared 99% identity with *Ch. cupreum* accession number AB509372, AB509368, AB509370, KF601371 and KU597363. The nucleotide sequences of *Ch. cochliodes* CTh02 (CCO) shared 97% identity with those of *Ch. cochliodes* accession number MN534819, MT279444, KT895345, JN209864 and MH590621 which compared to *Pythium takayamanum* as an outgroup (Figure 4.6). The nucleotide sequences of *P. palmivora* DD01 shared 88% identity to confirm with those of *P. palmivora* accession number MG956799, HQ659668, MH219826, MH219849, KP183963, MH219829, MH219866 and MH401200 which compared to *Sordaria tomentoalba* as an outgroup. (Figure 4.7).

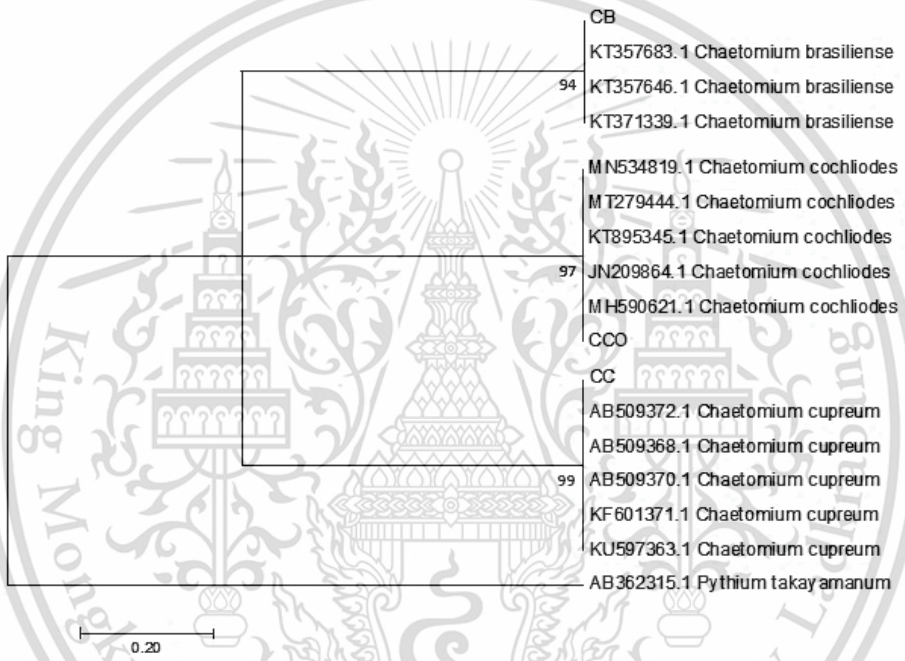

Figure 4.6 Phylogenetic tree to confirm and identified *Chaetomium* spp with related taxa inferred using a neighbor joining method with internal transcribed spacer (ITS) rDNA sequence. Bootstrap value based on 1,000 replications is shown above the branch.

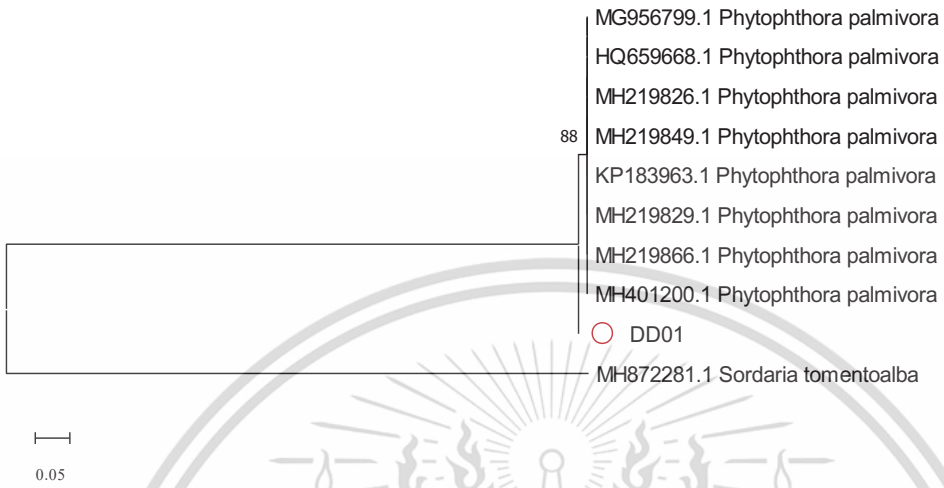

Figure 4.7 Phylogenic tree of *Phythophthora palmivora* from GenBank including *Phythophthora palmivora* strain DD01 constructed after distance based analyses of ITS1and ITS4 regions of rDNA. Numbers of the branches indicate percentage of bootstrap values after1000 replicates. The outgroup taxa is *Sordaria tomentoalba.*

4.4 Biological control of *Phytophthora spp.*

4.4.1 Dual-culture test

Ch. brasiliense, Ch. cupreum CC3003, *Ch. cochliodes* CTh02 were proved their abilities to inhibit plant pathogen *P. palmivora* DD01 causing disease of durian by using dual-culture tests.

The results showed that *Ch. brasiliense, Ch. cupreum* CC3003, *Ch. cochliodes* CTh02 gave significantly inhibition of *P. palmivora* DD01 which were 50.00%, 51.11% and 51.66%, respectively when compared to the control plate (Table 4.1). *Ch. brasiliense, Ch. cupreum* CC3003, *Ch. cochliodes* CTh02 showed percent inhibition of sporangial production which was 51.72%, 54.19% and 58.35% respectively. After 1 month *Ch. brasiliense, Ch. cupreum* CC3003, *Ch. cochliodes* CTh02 showed ability to grow over the colony *P. palmivora* as seen in Figure 4.8-4.10.

Table 4.1 Percent inhibition of *P. palmivora* in dual culture test.

Antagonist fungi	*P. palmivora*	
	% inhibition of colony	**% inhibition of sporangial**
Chaetomium brasiliense	50.00[a]	51.72[a]
Chaetomium cupreum	51.11[a]	54.19[a]
Chaetomium cochliodes	51.66[a]	58.35[a]
CV%	1.54	12.26

Figure 4.8 Dual- culture *Ch. brasiliense* aginst *P. palmivora*. A= *P. palmivora*, B= *Ch. brasiliense* Vs *P. palmivora*. C= *Ch. brasiliense*.

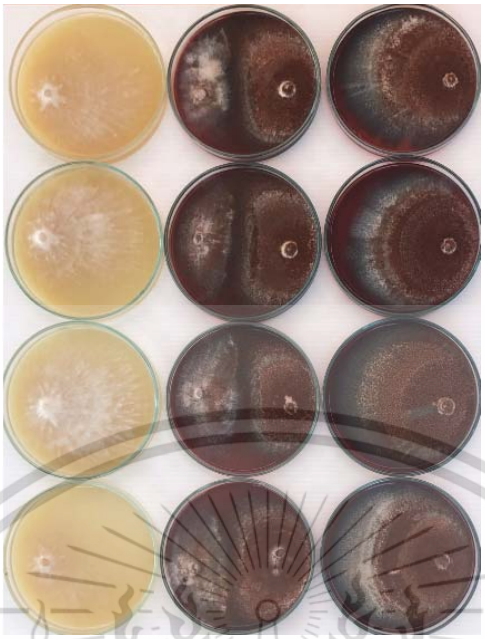

Figure 4.9 Dual- culture *Ch. cupreum* CC3003 aginst *P. palmivora*. A= *P. palmivora*, B= *Ch. cupreum* CC3003 Vs *P. palmivora*. C= *Ch. cupreum* CC3003.

Figure 4.10 Dual- culture *Ch. cochliodes* CTh02 aginst *P. palmivora*. A= *P. palmivora*, B= *Ch. cochliodes* CTH02 Vs *P. palmivora*. C= *Ch. cochliodes* CTh02.

4.4.2 Bioactivity test of metabolites extracts from *Chaetomium* spp against *P. palmivora*

Ch. brasiliense, Ch. cupreum CC3003, *Ch. cochliodes* CTh02 were extracted their bioactive substances as crude extracts and tested for their abilities to inhibit *P. palmivora* DD01. The dried fungal biomass of *Chaetomium* spp. were prepared and soaked in different solvents successively, including hexane, ethyl acetate and methanol. The filtrates were evaporated by vacuum evaporator to yield crude extracts including crude hexane, crude ethyl acetate and crude methanol, 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, crude ethyl acetate and crude methanol and nano particles from hexane, ethyl acetate and methanol were quite different: yellow, light brown, dark brown, orange or white color; oil, wax or solid textures (Figure 4.11-4.12).

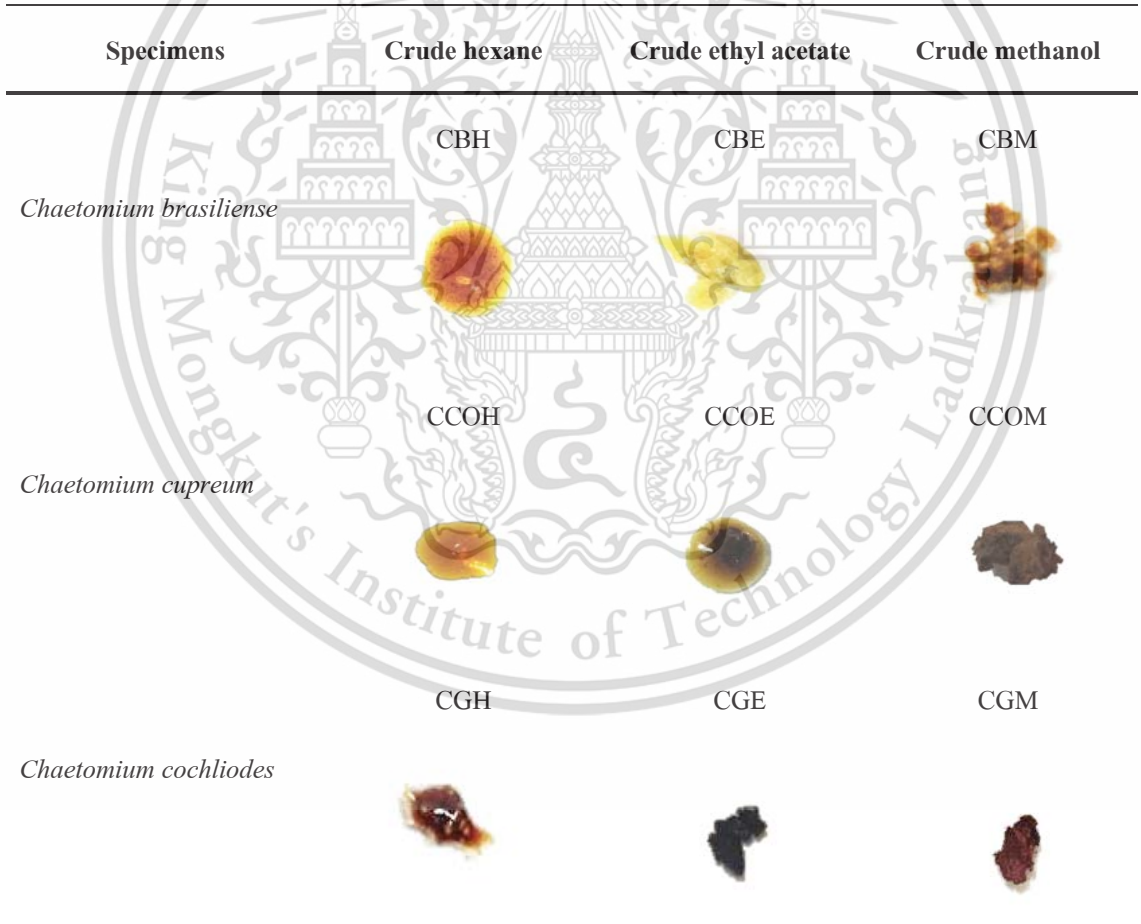

Figure 4.11 The characteristics of each crude extract

Specimens	Nano hexane	Nano ethyl acetate	Nano methanol
Chaetomium brasiliense	nano-CBH 	nano-CBE 	nano-CBM 
Chaetomium cochliodes	nano-CCOH 	nano-CCOE 	nano-CCOM 
Chaetomium cupreum	nano-CCH 	nano-CCE 	nano-CCM 

Figure 4.12 The characteristics of each nano particles.

4.4.2.1 Bioactivity test of crude extracts of *Chaetomium* spp against *P. palmivora* DD01

The crude extracts from *Ch. brasiliense, Ch. cupreum* CC3003, *Ch. cochliodes* CTh02 were used to test their ability to control the growth of *P. palmivora* DD01. Each crude extract was tested its inhibition against colony growth and sporangial production of pathogens with 6 concentrations (0, 10, 50, 100, 500, 1000 ppm) at room temperature. (Figure 4.13, Figure 4.14, Figure 4.15, Figure 4.16)

The crude extracts from *Ch. brasiliense* were tested their bioactivity against *P. palmivora*. The results showed that crude ethyl acetate extract gave highest inhibition of *P. palmivora* colony growth which was 82.75% at the concentration of 1,000 ppm with the ED_{50} values of 204.28 ppm when compared to the control. Crude ethyl acetate extract showed significantly highest inhibition for the sporangial production of *P. palmivora* as 97.72%, and the ED_{50} was 182.05 ppm, and followed by crude hexane extract which gave sporangial inhibition percent as 65.69% inhibition and ED_{50} value was 538.95 ppm. Crude methanol extract showed 65.23% and the ED_{50} was 722.08 ppm (Table 4.2, Figure 4.13, Figure 4.16).

The results crude ethyl acetate extract from *Ch. cupreum* CC3003 gave highest inhibition colony growth of *P. palmivora* DD01 as 90% at concentration of 500 ppm and 1,000 ppm with the ED_{50} values of 158.43 ppm when compared to the control and also showed significantly highest inhibition for the sporangial production of *P. palmivora* DD01 as 95.81% and 99.13% which ED_{50} values of 60.07 ppm, and followed by crude hexane extract which gave 90.00% inhibition and ED_{50} value was 97.21 ppm. Crude methanol of *Ch. cupreum* CC3003 showed 90% of colony inhibit and 98.33% of spore inhibition with ED_{50} value was 140.80 ppm (Table 4.3, Figure 4.14, Figure 4.16).

Crude methanol extract from *Ch. cochliodes* CTh02 gave highest inhibition colony growth and sporangial production *P. palmivora* DD01 as 90% and 93.18% at concentration of 1,000 ppm which ED_{50} values of 25 ppm, followed by crude methanol and crude hexane showed percent inhibition of *P. palmivora* DD01 as 90.00% and 63.50% and limited sporangial production of *P. palmivora* DD01 as 93.18% and 63.5% which ED_{50} value was 339 ppm and 163 ppm at concentration of 1,000 ppm (Table 4.4, Figure 4.15, Figure 4.16).

Table 4.2 Crude metabolites of *Chaetomium brasiliense* against *Phytophthora palmivora* DD01

Crude metabolites	Concentration (ppm)	Colony diameter (cm)[/2,3]	Growth inhibition (%)[/2,3]	ED_{50} (ppm)	Number of sporangial [/2,3](10^5)	Sporangial Inhibition (%)[/2,3]	ED_{50} (ppm)
CB-H	0	5.00[a]	-	770.60	32.25[a]	-	538.95
	10	5.00[a]	0.00[g]		29.00[b]	10.47[fg]	
	50	5.00[a]	0.00[g]		27.25[b]	15.34[fg]	
	100	5.00[a]	0.00[g]		21.25[d]	33.77[e]	
	500	3.29[c]	34.00[e]		13.75[e]	57.29[c]	
	1000	2.02[e]	57.75[c]		11.00[f]	65.69[b]	
CB-E	0	5.00[a]	-	204.28	32.25[a]	-	182.05
	10	5.00[a]	0.00[g]		28.50[b]	11.35[fg]	
	50	5.00[a]	20.00[f]		26.50[b]	17.69[f]	
	100	3.37[e]	32.5[e]		15.25[e]	47.44[d]	
	500	1.25[f]	75.00[b]		10.00[f]	96.25[a]	
	1000	0.86[g]	82.75[a]		0.50[g]	97.72[a]	
CB-M	0	5.00[a]	-	-	32.25[a]	-	722.08
	10	5.00[a]	0.00[g]		29.25[ab]	9.04[g]	
	50	5.00[a]	0.00[g]		27.75[b]	13.77[fg]	
	100	5.00[a]	0.00[g]		21.00[d]	34.33[e]	
	500	4.12[b]	17.5[f]		21.50[d]	33.11[e]	
	1000	2.65[d]	47.00[d]		11.25[f]	65.23[b]	
C.V. (%)		3.96	9.52		7.13	14.92	

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

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

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

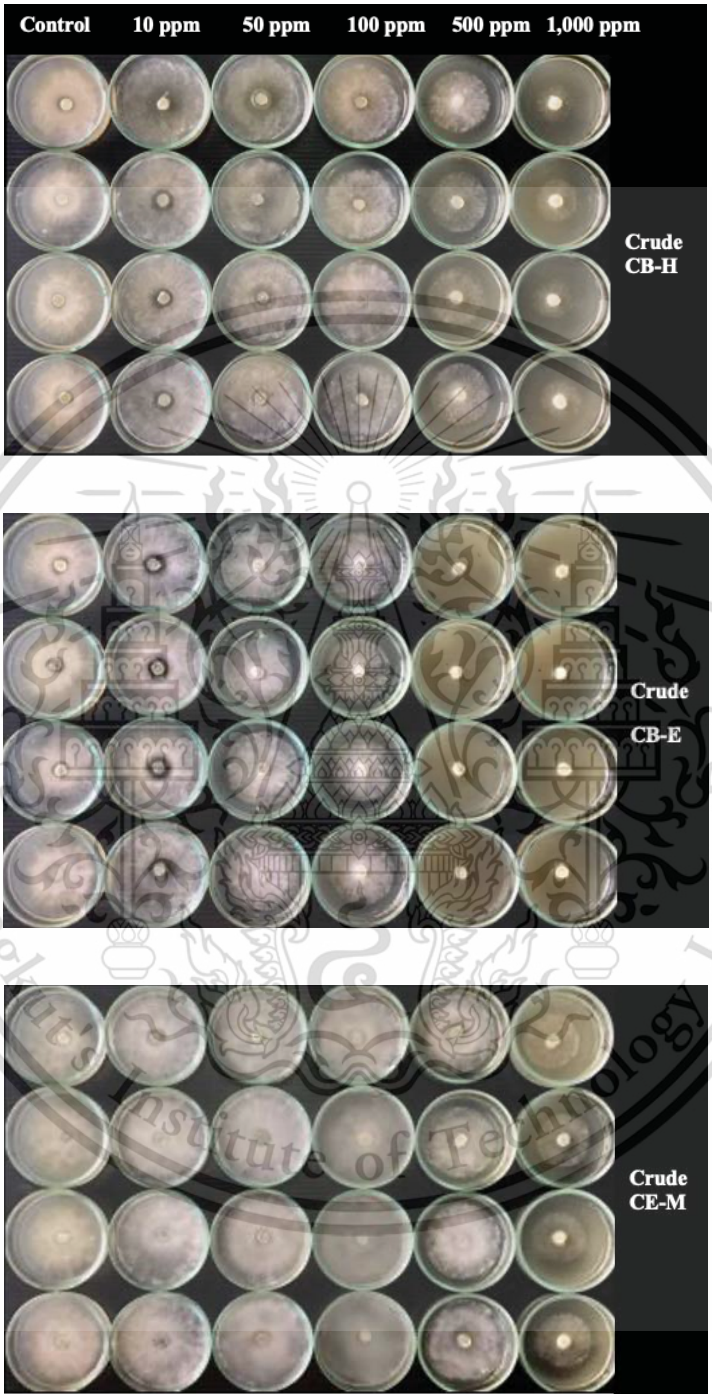

Figure 4.13. Fungal metabolites testing *Ch. brasiliense* against *P. palmivora* DD01

Table 4.3 Crude metabolites of *Chaetomium cupreum* CC3003 against *Phytophthora palmivora* DD01

Crude metabolites	Concentration (ppm)	Colony diameter (cm)[2,3]	Growth inhibition (%)[2,3]	ED_{50} (ppm)	Number of sporangial [2,3](10^5)	Sporangial Inhibition (%)[2,3]	ED_{50} (ppm)
	0	5.00[a]	-		31.00[c]	-	
	10	5.00[a]	0[f]		27.0[ab]	13.03[e]	
CC-H	50	5.00[a]	0[f]	411.60	22.00[c]	29.05[cd]	97.21
	100	5.00[a]	0[f]		20.75[c]	33.03[cd]	
	500	0.50[f]	90.00[a]		2.25[e]	92.48[a]	
	1000	0.50[f]	90.00[a]		0.50[e]	98.33[a]	
CC-E	0	5.00[a]	-		31.00[c]	-	
	10	2.32[c]	53.50[d]		10.75[d]	65.26[b]	
	50	1.97[d]	60.50[c]	158.43	10.00[d]	67.59[b]	60.07
	100	1.52[e]	69.50[b]		8.25[d]	72.90[b]	
	500	0.50[f]	90.00[a]		1.25[e]	95.81[a]	
	1000	0.50[f]	90.00[a]		0.25[e]	99.13[a]	
	0	5.00[a]	-		31.00[e]	-	
	10	5.00[a]	0[f]		28.75[a]	7.10[ef]	
CC-M	50	5.00[a]	0[f]		28.75[ab]	24.72[d]	140.80
	100	4.87[a]	2.50[f]	482.44	19.25c	37.83[c]	
	500	2.57[b]	48.50[e]		9.50[d]	68.52[b]	
	1000	0.50[f]	90.00[a]		0.50[e]	98.33[a]	
C.V. (%)		4.25	7.06		15.49	14.20	

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

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

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

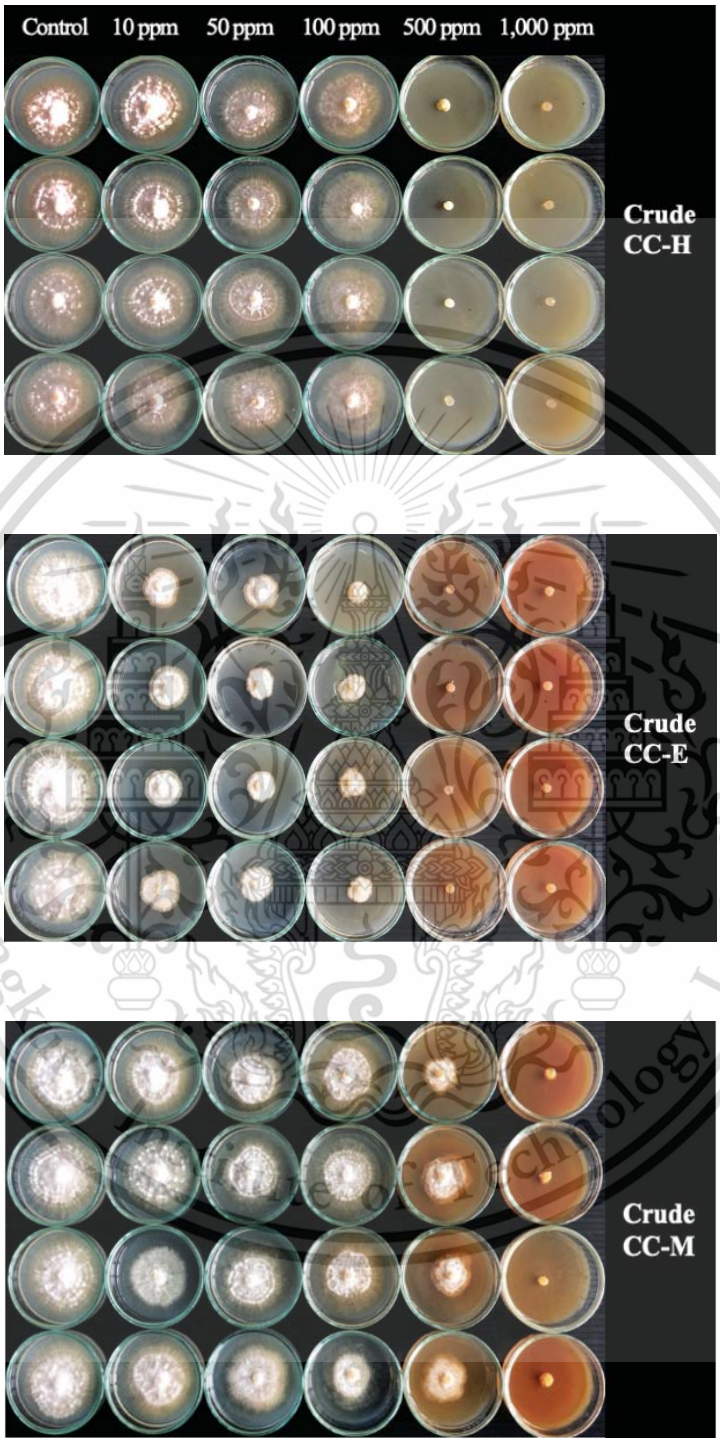

Figure 4.14. Inhibition of *P. palmivora* DD01 using crude extracts derived from *Ch. cupreum* CC3003

Table 4.4 Crude metabolites of *Chaetomium cochliodes* CTH02 against *Phytophthora palmivora* DD01

Crude metabolites	Concentration (ppm)	Colony diameter (cm)[/2,3]	Growth inhibition (%)[/2,3]	ED_{50} (ppm)	Number of sporangial [/2,3](10^5)	Sporangial Inhibition (%)[/2,3]	ED_{50} (ppm)
	0	5^{a1}	-		12.69^{a1}	-	
	10	5^{a}	-		10.15^{bc}	19.27^{ef}	
CCO-H	50	5^{a}	-	955	6.55^{ef}	46.96^{c}	
	100	5^{a}	-		4.75^{fg}	61.15^{b}	163
	500	5^{a}	-		3.75^{gh}	69.04^{b}	
	1000	1.82^{f}	63.50^{c}		1.69^{i}	86.48^{a}	
	0	5^{a}	-		12.69^{a}	-	
	10	5^{a}	-		11.54^{ab}	8.58^{fg}	
CCO-E	50	3.81^{c}	23.75^{ef}		9.67^{bcd}	23.07^{e}	
	100	2.62^{e}	47.50^{d}	402	8.52^{cd}	32.05^{de}	339
	500	1.67^{f}	66.50^{c}		3.89^{g}	68.72^{b}	
	1000	0.50^{h}	90.00^{a}		0.88^{i}	92.85^{a}	
	0	5^{a}	-		12.69^{a}	-	
	10	4.18^{b}	16.25^{f}		12.35^{a}	2.88^{g}	
CCO-M	50	3.43^{d}	31.25^{e}		10.27^{bc}	18.93^{ef}	
	100	2.46^{e}	50.75^{d}	240	7.95^{de}	37.17^{cd}	25
	500	1.08^{g}	78.25^{b}		1.91^{hi}	84.93^{a}	
	1000	0.50^{h}	90.00^{a}		0.82^{i}	93.18^{a}	
C.V. (%)		6.67	14.85		17.87	22.37	

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

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

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

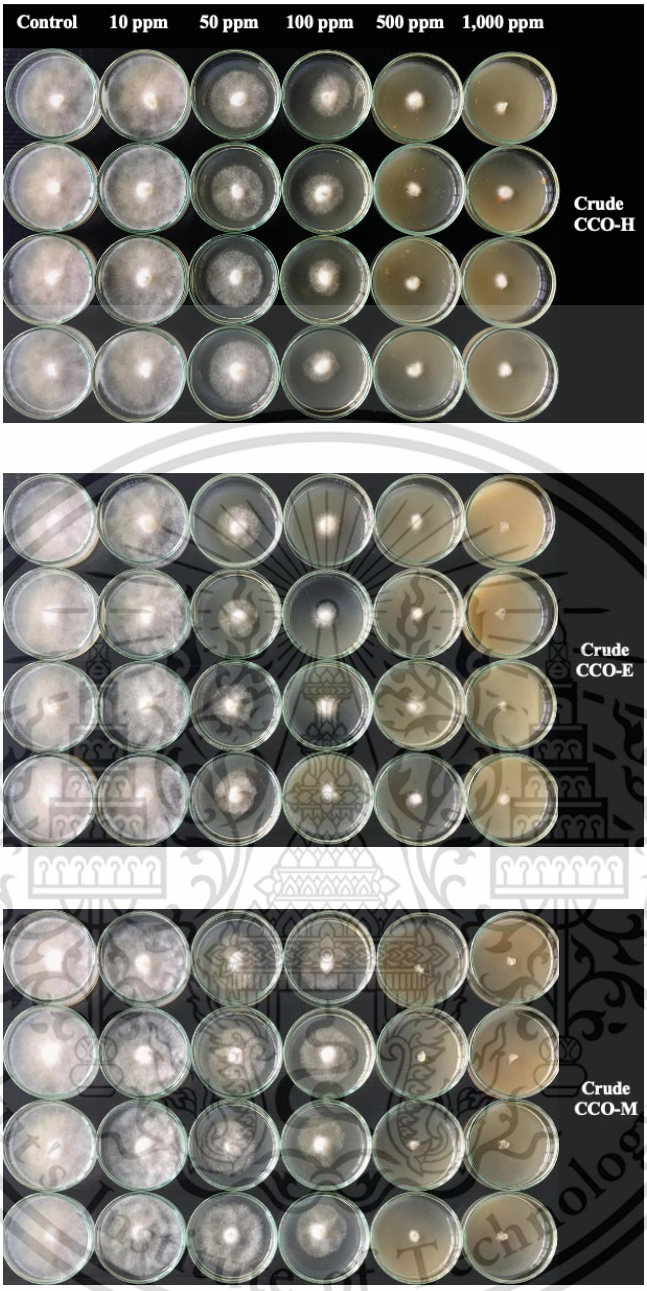

Figure 4.15. Inhibition of *P. palmivora* DD01 using crude extracts derived from *Ch. cochliodes* CTh02

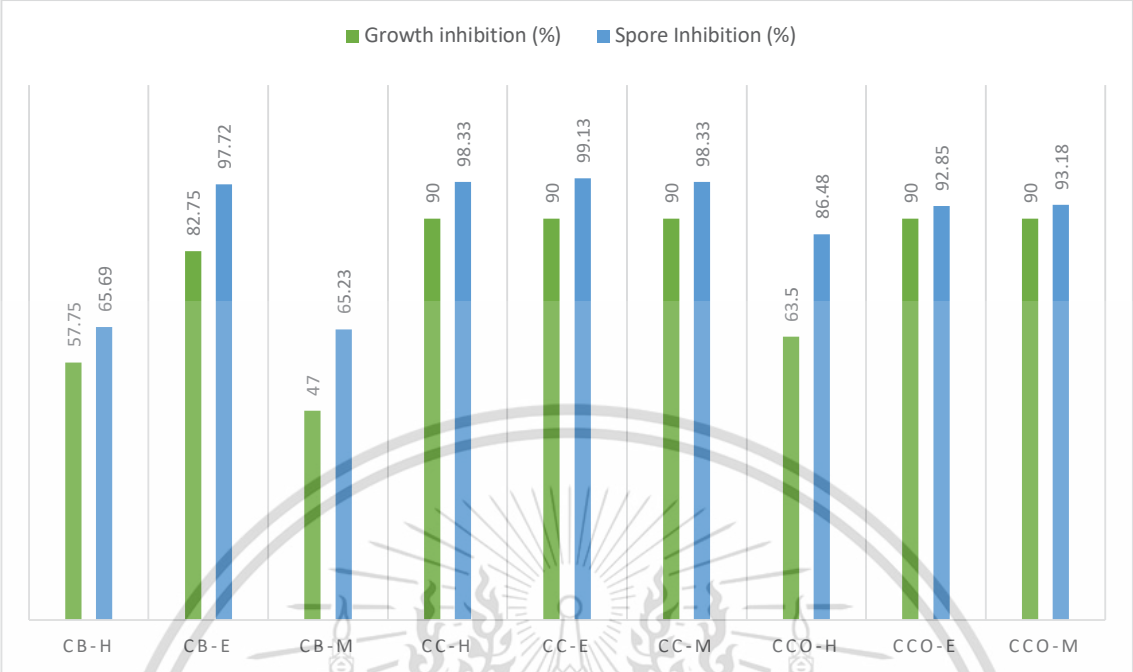

Figure 4.16 *P. palmivora* inhibition by crude extract from *Chaetomium* spp.

4.4.3 Testing nano particles form *Chaetomium* spp against *P. palmivora* DD01

The nano particles from *Ch. Brasiliense* (CB), *Ch. cupreum* CC3003 (CC), *Ch. cochliodes* CTh02 (CCO) were tested their abilities to inhibit *P. palmivora* DD01. 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 sporangial were observed and compared with normal spores of pathogens. (Figure 4.17, Figure 4.18, Figure 4.19, Figure 4.20)

The results showed that all nano particles from *Ch. brasiliense*, including Nano CB-H, CB-E, and CB-M, inhibited colony growth of *P. palmivora* DD01 by 90.00 % at a concentration of 15 ppm, with ED_{50} values of 1.25, 1.12, and 1.08 ppm, respectively. Nano CB-E and CB-M showed 100 % inhibition of *P. palmivora* sporangial production at a concentration of 10 ppm, with ED_{50} values of 12.20 and 10.77 ppm, respectively. Nano CB-H showed 100% inhibition of spore production at a concentration of 15 ppm, with ED_{50} values of 8.68 ppm. (Figure 4.16, Figure 4.20, Table 4.5)

Nano CC-E and CC-M inhibited colony growth of *P. palmivora* by 90.00 % at a concentration of 10 ppm, with ED_{50} values of 1.51 and 1.19 ppm, respectively, followed by nano CC-H, which inhibited colony growth of *P. palmivora* DD01 by 90.00 % at a concentration of 15 ppm, with ED_{50} 1.78 ppm. Nano CC-E inhibited *P. palmivora* DD01 sporangial production by 99.16 % at 15 ppm, with an ED_{50} of 11.01 ppm, and nano CC-H and CC-M inhibited *P. palmivora* DD01 sporangial production by 98.38 % and

98.33 %, respectively, with ED_{50} values of 13.03 and 16.48 ppm. (Figure 4.17, Figure 4.20, Table 4.6)

The nano particles from *Ch. cochliodes* CTh02 (CCO) including CCO-H, CCO-E and CCO-M expressed the highest colony inhibition percentage of *P. palmivora* DD01 90.00 % at a concentration of 5 ppm, with ED_{50} 24.91, 16.09 and 22.64 ppm. Nano CCO-H, CCO-E and CCO-M showed percent sporangial inhibition of *P. palmivora* DD01 over 95.00% at concentration of 5, 10, and 15 ppm, which ED_{50} values of 21.30, 13.83 and 19.48 ppm, respectively. (Figure 4.18, Figure 4.20, Table 4.7)

Table 4.5 Activity of nanoparticles of *Chaetomium brasiliense* against *Phytophthora palmivora* for 7 days

Nano particles	Concentration (ppm)	Colony diameter (cm)[/2,3]	Growth inhibition (%)[/2,3]	ED_{50} (ppm)	Number of sporangial [/2,3](10^5)	Sporangial Inhibition (%)[/2,3]	ED_{50} (ppm)
	0	5.00[a]	-		32.37[a]	-	
	1	2.75[b]	45.00[e]		13.25[b]	59.20[f]	
Nano	3	1.41[e]	71.75[cd]	1.25	3.50[d]	89.05[c]	8.68
CB-H	5	1.35[ef]	73.00[cd]		1.50[e]	95.42[b]	
	10	1.11[g]	77.75[bc]		0.50[e]	98.50[ab]	
	15	0.5[h]	90.00[a]		0.00[e]	100.00[a]	
	0	5.00[a]	-		32.37[a]	-	
Nano	1	2.60[bc]	47.50[c]		10.00[c]	69.12[e]	
CB-E	3	1.67[d]	66.50[d]	1.12	1.50[e]	95.40[b]	12.20
	5	1.17[fg]	76.50[bc]		0.25[e]	99.21[a]	
	10	1.00[g]	80.00[b]		0.00[e]	100.00[a]	
	15	0.5[h]	90.00[a]		0.00[e]	100.00[a]	
	0	5.00[a]	-		32.37[a]	-	
Nano	1	2.50[c]	50.00[e]		8.75[c]	72.94[d]	
CB-M	3	1.52[de]	69.50[d]		0.75[e]	97.79[ab]	
	5	1.12[fg]	77.50[bc]	1.08	0.25[e]	99.21[a]	10.77
	10	0.5[h]	90.00[a]		0.00[e]	100.00[a]	
	15	0.5[h]	90.00[a]		0.00[e]	100.00[a]	
C.V. (%)		8.74	7.90		13.90	2.71	

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

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

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

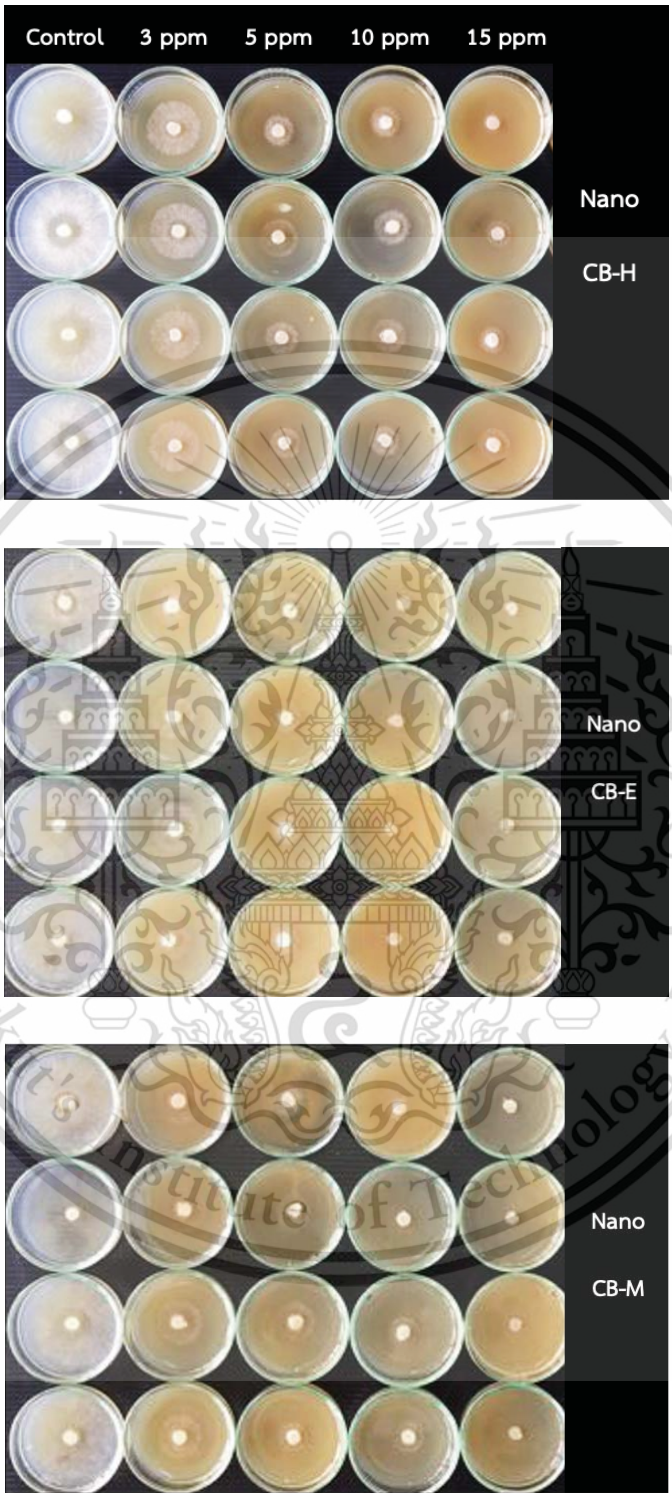

Figure 4.17 Testing nano particles from *Ch. brasiliense* against *P. palmivora* DD01

Table 4.6 Activity of nanoparticles of *Chaetomium cupreum* CC3003 against *Phytophthora palmivora* for 7 days

Nano particles	Concentration (ppm)	Colony diameter (cm)/[2,3]	Growth inhibition (%)/[2,3]	ED50 (ppm)	Number of sporangial /[2,3](10[5])	Sporangial Inhibition (%)/[2,3]	ED50 (ppm)
Nano CC-H	0	5.00[a1]	-		29.25[a]	-	
	3	2.31[b]	53.75[c]		4.00[b]	86.16[d]	
	5	1.25[c]	78.75[b]	1.78	1.50[cd]	94.81[bc]	13.03
	10	0.56[d]	88.75[a]		0.50[cd]	97.49[ab]	
	15	0.50[d]	90.00[a]		0.50[cd]	98.38[a]	
Nano CC-E	0	5.00[a]	-		29.25[a]	-	
	3	2.25[b]	55.00[c]		2.00[c]	93.08[c]	
	5	1.22[c]	75.50[b]	1.51	1.50[cd]	94.81[bc]	11.01
	10	0.50[d]	90.00[a]		0.50[cd]	98.36[a]	
	15	0.50[d]	90.00[a]		0.25[d]	99.16[a]	
Nano CC-M	0	5.00[a]	-		29.25[a]	-	
	3	2.31[b]	53.75[c]		4.50[b]	84.70[d]	
	5	0.56[d]	88.75[a]	1.19	1.50[cd]	94.83[bc]	16.48
	10	0.50[d]	90.00[a]		0.75[cd]	98.21[ab]	
	15	0.50[d]	90.00[a]		0.50[cd]	98.33[a]	
C.V. (%)		6.39	4.52		13.62	2.89	

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

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

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

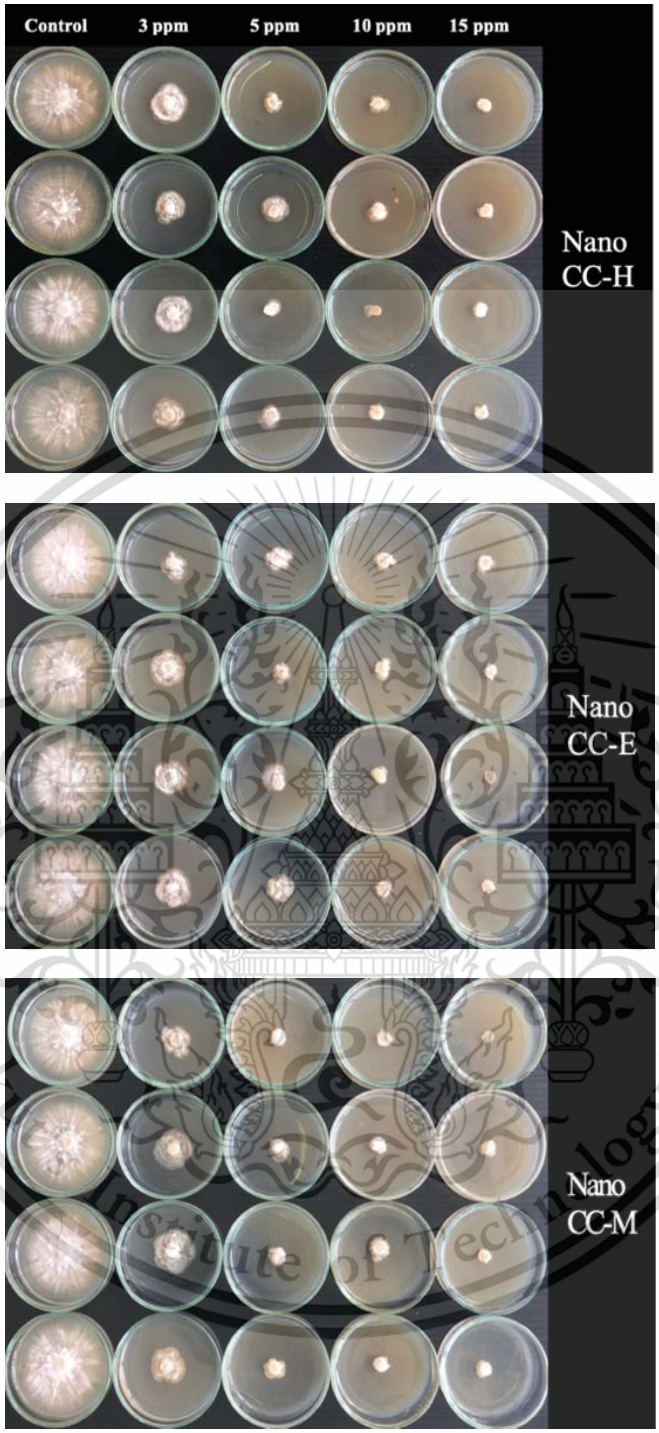

Figure 4.18 Testing nano particles from *Ch. cupreum* CC3003 against *P. palmivora* DD01

Table 4.7 Activity of nanoparticles of *Chaetomium cochliodes* CTh02 against *Phytophthora palmivora* for 7 days

Nano particles	Concentration (ppm)	Colony diameter (cm)[/2,3]	Growth inhibition (%)[/2,3]	ED50 (ppm)	Number of sporangial [/2,3](10^5)	Sporangial Inhibition (%)[/2,3]	ED50 (ppm)
Nano CCO-H	0	5.00^a	-		30.05^a	-	
	3	0.50^d	90.00^a		2.00^c	83.50^d	
	5	0.50^d	90.00^a	24.91	1.50^c	95.04^{ab}	21.30
	10	0.50^d	90.00^a		0.75^c	97.49^{ab}	
	15	0.50^d	90.00^a		0.50^c	98.38^a	
Nano CCO-E	0	5.00^a	-		30.05^a	-	
	3	2.34^b	53.00^c		5.00^b	93.29^b	
	5	0.50^d	90.00^a	16.09	1.50^c	94.99^{ab}	
	10	0.50^d	90.00^a		0.50^c	98.38^a	13.83
	15	0.50^d	90.00^a		0.50^c	98.38^a	
Nano CCO-M	0	5.00^a	-		30.05^a	-	
	3	1.56^c	68.75^b		2.00^c	93.45^b	
	5	0.50^d	90.00^a	22.64	1.25^c	95.85^{ab}	19.48
	10	0.50^d	90.00^a		0.75^c	97.49^{ab}	
	15	0.50^d	90.00^a		0.50^c	98.38^a	
C.V. (%)		1.01	0.47		14.19	3.74	

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

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

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

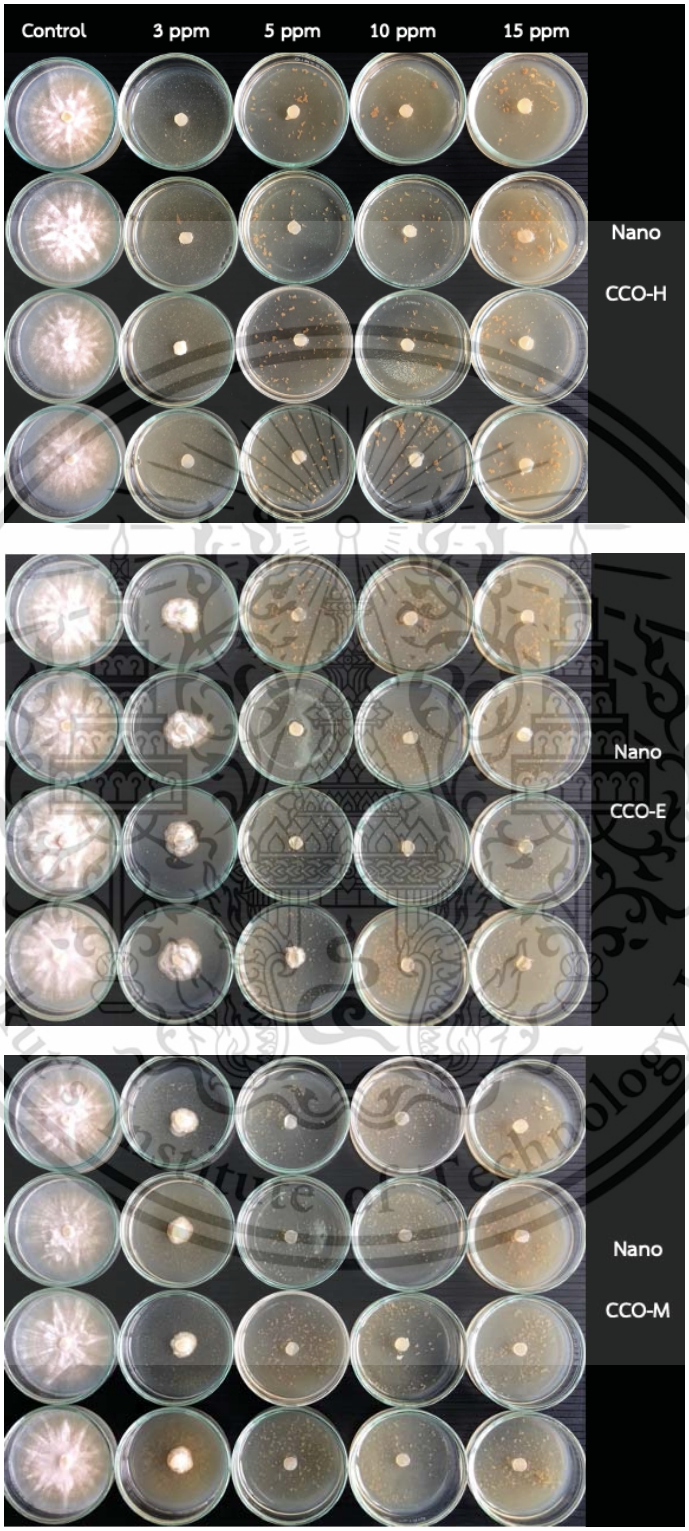

Figure 4.19 Testing nano particles from *Ch. cochliodes* against *P. palmivora* DD01

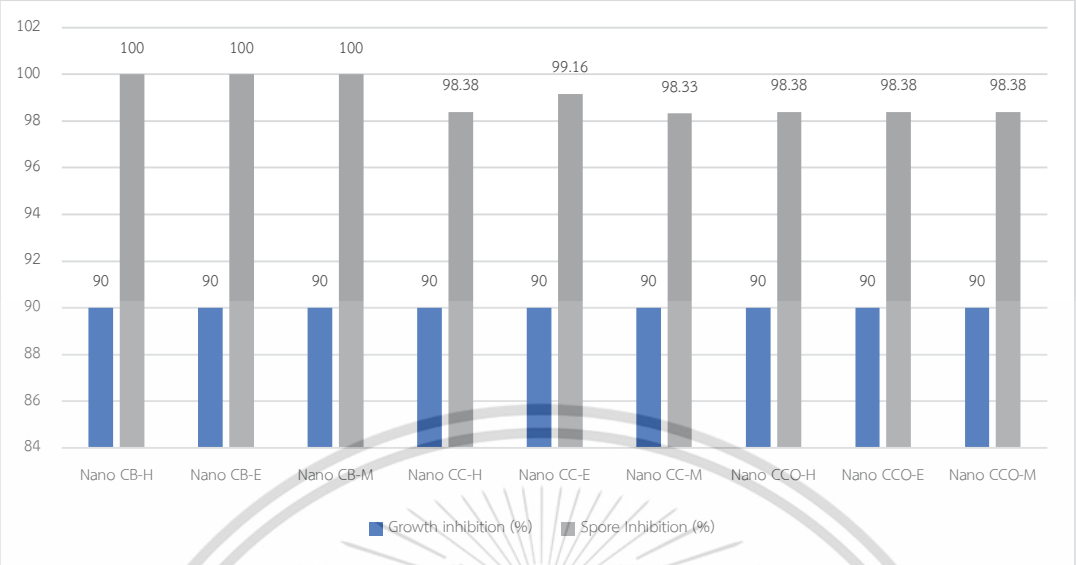

Figure 4.20 *P. palmivora* inhibition by nano particles from *Chaetomium* spp.

4.5 Testing nano particles for phytoalexin production in durian

The current research found that nano CB-H from *Ch. brasiliense*, nano CC-E derived from *Ch. cupreum* CC3003 and nano CCO-E from *Ch. cochliodes* CTh02 at a concentration of 15 ppm, used to treat seedlings of durian var Monthong inoculated with *P. palmivora* DD01 expressed a spot on TLC with an Rf value of 0.75 which proved to be scopoletin (Figure 4.21).

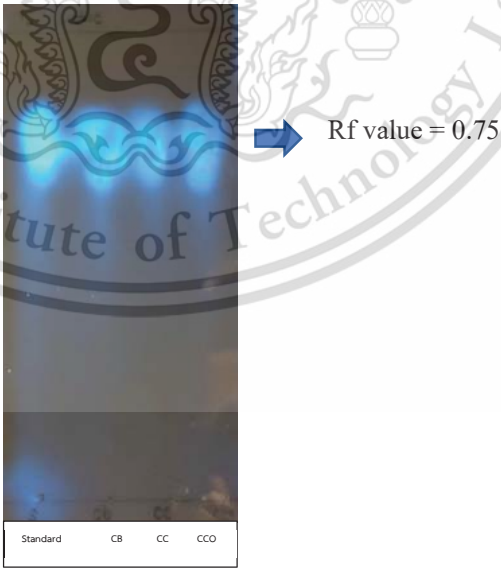

Figure 4.21 Phytoalexin investigation

CHAPTER 5

DISCUSSION

In this research isolated from soil and infected roots of durian var. Monthong by using batting technique. Colony are white colour, stellate pattern, papillate caducous sporangia with short pedicels, and occurring in groups of 5 – 15 on one sympodium. According to Erwin and Ribeiro (1996), these are typical characteristics of *P. palmivora*, which identified the occurrences of groups (up to 20) conspicuous papillate sporangia with short pedicels on a sympodium as the distinguishing characteristics of this species. The ITS sequences of these isolates also verified that they were *P. palmivora* that similar with research of Latifah *et al.* (2018) and Suksiri *et al.* (2018). *P. palmivora* isolate DD01 its high virulence for roots of durian in pathogenicity test suggested that this species is the causal agent, was similar with report of Pongpisutta *et al.* (2004); Torres *et al.* (2010) and Suksiri *et al.* (2018) stated that *P. palmivora* is often aggressive and damage to durian root in orchards and laboratory tested. Additionally, *P. palmivora* is known as plant pathogen with a wide host range, which infects various important crops such as oil palm, citrus, cacao, coconut, rubber and papaya in Southeast Asia (Drenth and Guest, 2004; Paterson, 2020; Chi *et al.,* 2020; Delgadillo-Durán *et al.,* 2020; Karyath Palliyath *et al.,* 2021; Deenamo *et al.,* 2018; Soesanto *et al.,* 2019).

Bio-control microorganisms' antagonistic activity is frequently demonstrated by pathogen growth, infection, or reproduction being inhibited (Alabouvette *et al.* 2011). In this study, *Chaetomium* spp., both as living organisms, crude extracts and nano particles, inhibited the development of *P. palmivora* in vitro.

Representing as antagonists, Chaetomium spp. inhibited 50 % colony growth; 50-58% sporangial production of *P. palmivora* DD01 in dual culture tests. The ability of *Chaetomium* spp. to inhibit fungal pathogen sporulation and mycelial growth is well documented. Many studies have shown that *Chaetomium* spp. inhibited the colony growth of various plant pathogens in vitro such as *Magnaporthe grisea* (Park *et al.*, 2005), *Phytophthora nicotianae* (Hung *et al.,* 2015b), *Pythium ultimum* (Di Pietro *et al.*, 1992), *Verticillium dahlia* (Zhang *et al.*, 2021), *Bipolaris sorokinian* (Kaur *et al.*, 2021). In this study *Chaetomium* spp showed ability to inhibit the colony growth of *P. palmivora* and grew over the colony of pathogen after one month in dual culture test. Antibiosis is a type of antagonism resulting from the production of secondary metabolites toxic to other microorganisms, whereas mycoparasitism relies on various lytic enzymes for degradation of the host's cell wall, causing death of the target organism, resulting in a decrease in inoculum density. (Sun *et al.* 2006, Alabouvette *et al.* 2011; Narayanasamy 2013). All of the antagonists used in this

research, on the other hand, were known to develop antibiotics with antifungal activity against various plant pathogens (Soytong *et al.* 2001; Kanokmedhakul *et al.* 2006).

All crude extracts of *Chaetomium* spp exhibited antifungal activities on mycelial growth and spore productions of *P. palmivora* DD01. The crude extract of *Ch. brasiliense, Ch. cupreum*, *Ch. cochliodes* showed strongly antifungal activities on mycelial growth and spore production of *P. palmivora* DD01 over 90% with ED_{50} values of 60.07-722.08 ppm. *Chaetomium* species are well-known for developing hundreds of metabolites with a range of bioactivities that help in their biological control activities and different species or even different isolates within a species can differently type of a metabolite (Sun *et al.* 2006; Zhang *et al*. 2012; Narayanasamy, 2013). Soytong and Kaewchai (2014) found that crude hexane extract from *Ch. cupreum* RY202 gave the highest inhibition of mycelial growth of *Rigidoporus microporus* with inhibition of 82.0% and crude ethyl acetate extract gave colony inhibition of 80 %. bioactive compound from *Ch. cupreum* named rotiorinol showed ability to inhibit the growth of *R. microporus* caused white root disease in rubber tree. Crude ethyl acetate extract and crude methanol extract from *Ch. globosum* EF18, isolated as endophytic fungus from Indian ginseng were found effective against *Sclerotinia sclerotiorum* at 80.83 % and 75.68% on 5th day of experiment (Kumar *et al.* 2013). Soytong (2014a) reported that crude hexane, ethyl acetate and methanol extract from *Ch. cochliodes* were proved to inhibit spore of *Drechslera oryzae* caused brown leaf spot of rice at 90.08%, 82.37% and 83.97 respectively.

The nano particles from *Ch. brasiliense, Ch. cupreum* and *Ch. globosum* were tested their abilities to inhibit *P. palmivora*. All of different kind of nano particles from *Chaetomium* spp showed highly performance inhibit colony growth and spore production over 90% event in used lowest concentration when compare with crude extract. The nano particles from *Ch. brasiliense,* including Nano CB-H, CB-E, and CB-M, inhibited colony growth of *P. palmivora* by 90.00% at a concentration of 15 ppm, with ED_{50} values of 1.25, 1.12, and 1.08 ppm, respectively and nano CB-H showed 100% inhibition of spore production at a concentration of 15 ppm, with ED_{50} values of 8.68 ppm which similar with report of Song *et al.,* (2020a) stated that nano particles containing active compounds derived from *Ch. cochliodes* (CTh05) was inhibited rice blast disease caused by *Magnaporthe oryzae* isolate PO1 with effective dose (ED_{50}) values of 85, 144 and 374 ppm, respectively in vivo tested. Tann and Soytong (2016a) also reported that Nano-CGH, nano-CGE, and nano-CGM from *Ch. globosum* KMITL-N0805 expressed antifungal activity (ED_{50} values of 1.21, 1.19, and 1.93ppm/mL, respectively) against *Curvularia lunata*, the causal agent of leaf spot disease of rice var. Sen Pidoa. 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.

Nano CC-E and CC-M inhibited colony growth of *P. palmivora* by 90.00 % at a concentration of 10 ppm, with ED_{50} values of 1.51 and 1.19 ppm, respectively. Nano CC-E inhibited *P. palmivora* spore production by 99.16 % at 15 ppm, with an ED_{50} of 11.01 ppm, and nano CC-H and CC-M inhibited *P. palmivora* spore production by 98.38 % and 98.33 %, respectively, with ED_{50} values of 13.03 and 16.48 ppm. This result similar with research of Tann and Soytong (2016b) stated that hexane-crude extract, EtOAc-crude extract and methanol-crude extract from *Ch. cupreum* CC3003 inhibited sporulation of *Curvularia lunata* with ED_{50} of 6.41, 0.83 and 7.81 ppm, respectively. Thongkham *et al.* (2017) found that nano-CCH, nano-CCE and nano-CCM derived from *Ch. cupreum* to inhibit mycelial growth and spore production of *Phytophthora* spp. showed the ED_{50} values of 3.49, 3.47 and 3.80 ppm, respectively. Moreover, Tongon and Soytong (2015) reported nano particles from *Ch. globosum* showed highly inhibitory effects on *C. lunata* causing leaf spots of rice with low ED_{50} values.

Nano CCO-H, CCO-E and CCO-M showed percent spore inhibition of *P. palmivora* over 95.00% at concentration of 5, 10, and 15 ppm and expressed colony inhibition percentage of *P. palmivora* 90.00 % at a concentration of 5 ppm. Udompongsuk *et al.* (2017) stated that nano particles derived from *Ch. cochliodes* showed ability exhibited antifungal activities against mycelial growth and sporangia formation of *Pythium* spp caused root rot disease of *Citrus reticulata* with 80-86% and 83-95%, respectively.

The current research found that nano CB-M from *Ch. Brasiliense*, nano CC-E derived from *Ch. cupreum* and nano CCO-E from *Ch. cochliodes* at a concentration of 15 ppm, used to treat seedlings of durian var Monthong inoculated with *P. palmivora* expressed a spot on TLC with an Rf value of 0.75 which proved to be scopoletin this study was similar with Song *et al.* (2020b) stated that rice leaves treated with nano *Chaetomium* spp produced Sakuranertin and Oryzalexin B on the thin layer chromatography (TLC). It was found that the nanoparticles act as elicitors to induce immunity in rice plants through the production of phytoalexin. Churngchow and Rattarasarn (2001) stated that the chromatogram of scopoletin was observed under UV light (366 nm), and a single, blue fluorescent compound.

CHAPTER 6

CONCLUSION

P. palmivora DD01 is proved to be the causal agents of durian root rot in Thailand. *Phytophthora* isolates DD01, which isolated from infected roots and soils of durian, and proved to be highly virulent for root infection in the pathogenicity tests. The analyses of ITS ribosomal DNA sequences and phylogeny of isolate DD01, the representative of the slow growth, confirmed it is *P. palmivora*.

The dual culture tests showed *Ch. cochliodes* gave significantly inhibition of *P. palmivora* which 51.66% and 58.35% for inhibited spore production. *Chaetomium* spp were grew over the colony of *P. palmivora* DD01 after one month.

The crude extracts of the tested *Chaetomium* spp. exhibited varyingly antifungal activities against *P. palmivora in vitro*. In generally, crude extract from *Ch. cupreum* gave highly significance to inhibit colony and spore production of *P. palmivora* ~90-99%.

Nano particles from *Chaetomium* spp were tested their abilities to inhibit *P. palmivora in vivo*. Mostly, all different kinds of nano particles from *Chaetomium* spp inhibited the colony growth of *P. palmivora* at 90% and highly significant inhibited the spore production at 90-100% at the concentration of 15 ppm, which provided lower of effective dose (ED_{50}) values than crude extract tests.

The investigation of phytoalexin in the infected durian var Monthong found that blue fluorescent spot on TLC is comparable to scopoletin as the standard which the Rf value of 0.75. The nano particles from *Chaetomium* spp would further be developed to be a new bioregulator for durian as natural product immune elicitor.

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APPENDIX

International Journal of Agricultural Technology 2018 Vol. 14(7): 2163-2170
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Nano-particles from *Chaetomium brasiliense* to control *Phytophthora palmivora* caused root rot disease in durian var Montong

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Tongon, R., Soytong, K., Kanokmedhakul, S. and Kanokmedhakul, K. (2018). Nano-particles from *Chaetomium brasiliense* to control *Phytophthora palmivora* caused root rot disease in durian var Montong. International Journal of Agricultural Technology 14(7): 2163-2170.

Abstract The antagonistic fungus, *Chaetomium brasiliense* was tested to control *Phytophthora palmivora* causing rot disease of Durian (*Durio zibithenus* L.) var Montong by crude extracts and Nano-particles derived from *Ch. brasiliense*. Crude extracts of antagonistic fungus was tested for antifungal biological activities. The crude extracts from antagonistic fungus with hexane, ethyl acetate and methanol were tested against *P. palmivora*. Crude ethyl acetate from *Ch. brasiliense* gave significantly against *P. palmivora* which the ED_{50} values was 17.46 µg/ml. Testing Nano-particles were tested for antifungal activities. The results showed nano - particles from *Ch. brasiliense* gave effectively significantly inhibition of colony growth and spore production which the ED_{50} values were 1.08 µg/ml, and 8.68 µg/ml respectively. Application of Nano - particles to control the *P. palmivora* causing root rot disease of durian in pot experiment was successfully done. The results showed nano-particles from *Ch. brasiliense* reduced the root rot disease on durain of 40%. The nano–particles from *Ch. brasilense* gave significiantly high plant growth which were 79.5 cm when compared to the non-trated control.

Keywords: Nano-particles, *Chaetomium brasiliense*, *Phytophthora palmivora*, biological control

Introduction

Durian (*Durio zibithenus* L) is originated from the region of Borneo and Sumatra, growing wild in the Malay peninsula, cultivated in a wide region from India to New Guinea four hundred years ago. It was across to Myanmar, and cultivated throug Thailand and South Vietnam (Morton, 2000). The problem for durian cultivation in Thailand faced the root rot which caused by *Phytophthora* spp. It can infect all stages of growing durian trees. The symptoms are appeared as root rot, leaves and stem blight, bark and fruit rot. Chemical fungicides have

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been used to control this disease and trened to lead the negative side effect to the environment. In addition, Phytophthora spp become resistant to those fungicide application eg. metalaxyl and related compounds (Erwin and Ribeiro, 1996). Tye eco-friendly management of *Phytophthora* diseases would be done to reduce the application cost. The application of biocontrol agents against *Phytophthora* rot has become an importance research aspect and is being carried out all over the world (Naqvi, 2004).

Biological control of plant pathogen is known as using antagonists against plant pathogens. The antagonism is the control mechanism to reduce the pathogen onocullum and disease incidence. Antagonism is expressed betweeb two organisms including antibiosis, competition and parasitism (Cook and Baker, 1983). However, thereare many reports indicated that the antagonists can express to against seversal plant pathogens eg such as *Chaetomium* spp; *and Emericella nidulans*. (Xu *et al.,* 2017; Hung, *et al.,* 2015a; Arjona-Girona *et al.,* 2018; Tran *et al.,* 2007; Song *et al.,* 2017).

The objective was to test the nano-particles derived from *Ch. brasiliense* to inhibit *Phytophthora* sp causing root rot in durian var Montong.

Materials and methods

Isolation of pathogen and testing pathogenicity

Phytophthora sp was isolated the durian diseased plant parts eg bark and root rot using tissue transplanting method. The collected diseased samples were washed with sterilized water, and and cut it into small pieces, soaked 1% clorox for 3 min. The piece samples of diseased part was moved to water agar (WA), then incubated at room temperature to observe growing colonies, them gentle moved to PDA to get pure culture. Morphology was identified and observed the characters of fungus under compound microscope. The pathogenicity was tested using detached leaves technique. The agar plugs of *Phytophthora* sp were transferred to wounded leaves. The non -inoculated leaves with steriled agar discs was done to serve as controls, and incubated at room temperature (27-30 °C) for seven days. The experiment was replicated four times. Disease incidence was calculated as number of infected plants/ total number of tested plants x 100, and disease ratings was evaluated as 0= healthy plants, and 3= seriously infected plants (Soytong, 2010).

Morphological study of Chaetomium brasilense

Chaetomium brasilense used in this reserch study is offered from Assoc. Prof. Dr. Kasem Soytong, Department of Plant Production Technology, Faculty

of Agricultural Technlogy, King Monkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. The culture was grown on patotao dextrose agar and observed morphological characters under compound microscope.

Dual culture test

The experimental design was used as Completely Randomization with four replications. Dual culture was done by followed the methods of Soytong (1992). *Ch. brasilense* and *Phytophthora* sp. were separately cultured on PDA at room temperature (27-30 ºC) for seven days. The 0.5 cm diameter of sterilized cork borer was cut at the peripheral colony in each culture and moved to PDA plates (9 cm). The agar plug of *Phytophthora* sp. was moved to PDA plate on one side of the plate that opposited the agar plug of *Ch. brasilense*. The plug of each fungus was transferred to PDA plate served as the controls. All plates were incubated at room temperature for 30 days. Colony diameter (cm) and conidia production in the dual culture plates and control plates were recorded. The number of conidia was counted by using haemacytometer.

The inhibition of colony growth or conidia production were calculated using the following formula:

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

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

Testing crude extracts of Chaetomium brasilense to against Phytophthora spp in vitro

Crude extracts of *Ch. brasilense* were evaluated to inhibit *Phytophthora* sp. The experimental design was conducted using factorial experiment in Completely Randomization, and repeated four times. Factor A was crude hexane, crude ethyl acetate and crude methanol. Factor B was 0, 10, 50, 100, 500, and 1,000 µg/ml. Each crude extract was poured to 2% dimethyl sulphite and mixed to 30 ml potato dextrose agar, then autoclaved at 121°C,15 lbs/inch^2 for 35 minutes. *Phytophthora* sp colony was cut at peripheral colony with sterilized cock borer (0.5 mm). Agar plug of pathogen was transferred to the middle of PDA media in plate incorporated with each and incubated at room temperature (27-30°C) until the pathogen growing full plate. Data were recorded as colony diameter, number of conidia. The pathogen colony growth and conidia production were calculated the inhibition using the above formula. Data were statistically computed analysis of variance. Means were compared using Duncan Multiple's Range Test. The effective dose was computed using probit analysis.

In vitro testing nano-particles from Chaetomium brasilense to control root rot disease in durian var Montong

Chaetomium brasilense was cultured in potato dextrose broth (PDB) at room temperature (27-30 C) for 30 days. The biomass was filtered through cheesecloth and air-dried. Fresh and dried biomass were weighted. The biomass was ground in electrical blender, then extracted by adding the same volume of hexane, and kept in stationary phase for 7 days at room temperature. The filtrate was get it through Whatman filter paper, evaporated in rotary vacuum evaporator to yield crude hexane. The remaining marc was consecutively extracted with ethyl acetate and methanol to get crude extracts using the same procedure as hexane to yield crude ethyl acetate (EtOAc) and crude methanol (MeOH). The nano- particles were done by followed the method of Dar and Soytong (2014), to yield Nano-CBH, Nano-CBE and Nano-CBM. Those nano-particles were evaluated to inhibit *Phytophthora* sp. The experimental design was used two factors factorial experiment in Completely Randomization. The experiment was repeated four times. Factor A was Nano-CBH, Nano-CBE and Nano- CBM. Factor B was 0, 1, 5 and 10 μg/ml. Each Nano-particle was dissolved in 2% dimethyl sulfoxide, and mixed to PDA before autoclaved at 121°C,15 lbs/inch^2 for 30 min. The culture of *Phytophthora* sp was cut at peripheral colony with sterilized cock borer (0.5 mm). Agar plug of *Phytophthora* sp was moved to the middle of PDA mixed with each nano-particles. All plates were incubated at room temperature until the pathogen in control growing full plate. The pathogen from each treatment were observed abnormal spores under compound microscope. Data were stistaically computed. The effective dose was calculated using probit analysis.

Results

Pathogenicity test

Pathogenicity test was conducted by detached leaves method which resulted durian leave var Montong showed brown hydrolysis expand around agar plug of pathogen. In control, leaves remained healthy as seen in Fig.1.

Dual culture test

Ch. brasiliense was proved it ability to inhibit plant pathogen *P. palmivora* causing disease of durian by using dual culture tests. The results showed that *Ch. brasiliense* gave significantly growth inhibition of *P. palmivora* which were 58.33% and showed significantly inhibited the spore production of pathogen of 88.82% (Table 1).

Figure1. Pathogenicity test of Phytophthora sp on Durian leaves

Testing crude extracts of Chaetomium brasilense to against Phytophthora spp in vitro

The crude extracts from *Ch. brasiliense* were used to test their abilities to control the growth of *P. palmivora*. The results showed that crude ethyl acetate (EtOAc) extract gave highest inhibition of *P. palmivora* colony growth which was 57.75% at the concentration of 1,000 µg/ml with the ED_{50} values of 204.28 µg/ml when compared to the control. Crude hexane and crude ethyl acetate (EtOAc) extract and showed significantly highest inhibition for the spore production of *P. palmivora* as 100% at the concentration of 1,000 µg/ml. Crude methanol extract gave the best to inhibit the trsed pathogen wghivh the ED50 was 5.94 ppm, and followed by crude ethyl acetate and crude hexane extracts which the ED50 values were 17.46 and 28.56 µg/ml., respectively (Table 2).

Table 1. Colony growth and number of spore on antagonistic dual-culture tests

Antaginistic fungi	*P. palmivora*			
	Colony diameter (cm)	Growth inhibition (%)	Spore number (10^4/ml)	Spore inhibition (%)
Control	9.00[a1]	-	27.43[a]	-
Ch. brasiliense	3.75[b]	58.33[b]	5.31[b]	88.82[a]
C.V. (%)	7.90		19.39	

[1] Means of four repeated experiments. Means followed by the same letters are not significantly differed by DMRT at P=0.01.

In vitro testing nano-particles from Chaetomium brasilense to control Phytohthora sp causing durian rot var Montong

Result from nano-particles showed high efficacy antifungal activity of nanoparticles from *Ch. brasilense* against *P. palmivora* The result of Nano particles from *Ch. brasilense* was showed that Nano-CBH, Nano-CBE and Nano-CBM gave highly significant inhibited the colony growth of *P. palmivora* as 90.00% which the ED_{50} values of 1.25, 1.12, 1.08 µg/ml., respectively and gave highly significant inhibition for the spore production of *P. palmivora* as 100% which the ED_{50} values of 8.68, 12.20 and 10.77ppm (Table 3).

Table 2. Crude extracts of *Ch. brasilense* testing for growth inhibition of *P. palmivora*

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth Inhibition(%)	ED_{50} (µg/ml)	Number of spores (10^7)	Inhibition (%)	ED_{50} (µg/ml)
Hexane	0	5.00^{a}	-		1.475^{a}	-	
	10	5.00^{a}	0.00^{g}		0.49^{bc}	63.19^{de}	
	50	5.00^{a}	0.00^{g}	770.60	0.53^{bc}	63.87^{de}	28.56
	100	5.00^{a}	0.00^{g}		0.39^{cd}	72.13^{cde}	
	500	3.29^{c}	34.00^{e}		0.17^{efg}	87.90^{abc}	
	1000	2.02^{e}	57.75^{c}		0.00^{g}	100^{a}	
Ethyl Acetate	0	5.00^{a}	-		1.475^{a}	-	
	10	5.00^{a}	0.00^{g}		0.63^{b}	55.85^{e}	
	50	5.00^{a}	20.00^{f}		0.32^{cde}	78.17^{bcd}	
	100	3.37^{e}	32.5^{e}	204.28	0.31^{cde}	78.29^{bcd}	17.46
	500	1.25^{f}	75.00^{b}		0.00^{g}	100^{a}	
	1000	0.86^{g}	82.75^{a}		0.00^{g}	100^{a}	
Methanol	0	5.00^{a}	-		1.475^{a}	-	
	10	5.00^{a}	0.00^{g}		0.48^{bc}	65.88^{de}	
	50	5.00^{a}	0.00^{g}		0.39^{cd}	71.85^{bcd}	
	100	5.00^{a}	0.00^{g}	-	0.23^{efg}	83.51^{bcd}	5.93
	500	4.12^{b}	17.5^{f}		0.13^{efg}	91.40^{ab}	
	1000	2.65^{d}	47.00^{d}		0.02^{fg}	97.91^{a}	
C.V. (%)		3.96			29.34		

[1] Means of four repeated experiments. Means followed by the same letters are not significantly differed by DMRT at P=0.01.

Table 3. Nano particle extracts of *Ch. brasilense* testing for growth inhibition of *P. palmivora*

Nano particles	Concentration (ppm)	Colony diameter(cm)[1]	Growth inhibition (%)	ED_{50} (µg/ml)	Number of spores (10^7)	Inhibition (%)	ED_{50} (µg/ml)
Nano-CBH	0	5.00^{a}	-		0.56^{a}	-	
	1	2.75^{b}	45.00^{e}		0.22^{b}	57.45^{b}	
	3	1.41^{e}	71.75^{cd}	1.25	0.00^{c}	100^{a}	8.68
	5	1.35^{ef}	73.00^{cd}		0.00^{c}	100^{a}	
	7	1.11^{g}	77.75^{bc}		0.00^{c}	100^{a}	
	10	0.5^{h}	90.00^{a}		0.00^{c}	100^{a}	
Nano-CBE	0	5.00^{a}	-		0.56^{a}	-	
	1	2.60^{bc}	47.50^{e}		0.32^{b}	36.67^{c}	
	3	1.67^{d}	66.50^{d}	1.12	0.00^{c}	100^{a}	12.20
	5	1.00^{g}	80.00^{b}		0.00^{c}	100^{a}	
	7	1.17^{fg}	76.50^{bc}		0.00^{c}	100^{a}	
	10	0.5^{h}	90.00^{a}		0.00^{c}	100^{a}	
Nano-CBM	0	5.00^{a}	-		0.56^{a}	-	
	1	2.50^{c}	50.00^{e}		0.28^{b}	47.95^{bc}	
	3	1.52^{de}	69.50^{d}	1.08	0.00^{c}	100^{a}	10.77
	5	1.12^{fg}	77.50^{bc}		0.00^{c}	100^{a}	
	7	0.5^{h}	90.00^{a}		0.00^{c}	100^{a}	
	10	0.5^{h}	90.00^{a}		0.00^{c}	100^{a}	
C.V. (%)		8.74			51.16		

[1] Means of four repeated experiments. Means followed by the same letters are not significantly differed by DMRT at P=0.01.

Discussion

Morphological study was done in previous experiment to prove identification of *P. palmivora* which cultured on potato dextrose agar. The colony is white, slow growing, non septate mycelia, sporangia produce readily and abundantly. The identity in morphological characteristics are consistent with descriptions of *Phytophthora palmivora* (Erwin and Ribiero, 1996).

The results showed that crude ethyl acetate (EtOAc) extract from *Ch. brasilense* gave highest inhibition of *P. palmivora* colony growth which was 57.75% at the concentration of 1,000 µg/ml with the ED_{50} values of 204.28 ppm when compared to the control. As similar in the previous report, found that crude extract of *Ch. globosum* CG05, *Ch. cupreum* CC3003, *Ch. lucknowense* CL01 showed ability to inhibit mycelial growth and spore production of *P. palmivora* PHY02 in laboratoty test (Hung *et al.,* 2015b). This result is also similar reported by Hung *et al.*, (2015a) found that crude extracts of these *Chaetomium* species exhibited antifungal activities against mycelial growth of *P. nicotianae*, with effective doses of 2.6~101.4 µg/ml.

It was found that nano-particles derived from *Ch. brasilense* was actively inhibited the *P. palmivora*. Nano-CBH, Nano-CBE and Nano-CBM gave highly significant inhibition of the colony growth of *P. palmivora* at 90.00% and gave highly significant inhibition for the spore production of *P. palmivora* at 100%. This result is similar reported by Thongkham (*et al.,* 2017) found that nano-particles derived from *Ch. cupreum* to inhibit mycelial growth and spore production of *Phytophthora* spp causing root rot in durian. This study was also similar to Dar *et al*. (2013) that nano particles of *Ch. globosum* and *Ch. cuperum* were proved to againt *F. oxysporum* f.sp. *lycopersici* and *Colletotrichum capsici*.

Acknowledgement

I would like to acknowledge the financial support from Thailand Research Fund (Grant No RTA5980002).

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(Received: 28 August 2018, accepted: 27 October 2018)

International Journal of Agricultural Technology 2021Vol. 17(2):753-766
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Application of *Chaetomium cochliodes* CTh02 to against durian root rot cause by *Phytophthora palmivora* RT01

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Tongon, R. and Soytong, K. (2021). Application of *Chaetomium cochliodes* CTh02 to against durian root rot cause by *Phytophthora palmivora* RT01. International Journal of Agricultural Technology 17(2):753-766.

Abstract The durian is cultivated in tropical regions which are caused root rot by *Phytophthora palimovora* RT01. *P. palmivora* RT01 causing root rot of durian var. monthong was proved to be pathogenic isolate and confirmed morphological and phylogenetic identification. *Chaetomium cochliodes* CTh02 proved to be antagonized *P. palmivora* RT01 causing root rot of durain var. monthong through dual culture evaluation. Crude hexane, crude ethyl acetate and crude methanol extracts of *Ch. cochliodes* CTh02 significantly inhibited colony growth of *P. palmivora* RT01 at the ED_{50} values of 955, 402 and 240 ppm., respectively. Morevover, Crude methanol extracts gave significantly highest inhibited sporangia of *P. palmivora* RT01 which ED_{50} value of 25 ppm and followed by crude hexane, crude ethyl acetate extracts which the ED_{50} values of 163, and 339 ppm, respectively. The control mechanism *Ch. cochliodes* CTh02 against *P. palmivora* RT01 may possible act as antibiosis.

Keywords: *Chaetomium cochliodes*, *Phytophthora*, Durian root rot

Introduction

The pathogenicity of *Phytophthora palmivora* has infected a wide host range in Durian and Cocoa (Chee, 1969; 1974; Chan and Lim, 1986). Thailand is the leading producer and export durian in the world. Nowadays durian get high demand especially from China that makes durian become an importance economic crop. The most importance problem for durian cultivation is root rot disease cause by *Phytophthora* spp., especially *P. palmivora*. Withthis, Prommate *et al.* (2019) reported that *Phytophthora* rot of durian isolated from durian plantation is identified as *P. palmivora*. *Phytophthora* spp. can damage durian trees in any phase of cultivation, the symptoms of disease appear by the rot of root, leaves blight, stem blight and fruit rot. The application of chemical fungicides found the negative side effect to the environment and cause pathogen resistance. *Phytophthora* species are controlled by chemical

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fungicides such as phenylamides (metalaxyl and related compounds) that reported to be resistance to those fungicides (Erwin and Ribeiro, 1996). Disease management of *Phytophthora* diseases has developed to be eco-friendly control diseases as to reduce the application cost, and harm of fungicides. Biological control agents (BCAs) have become an importance research aspect and environmentally friendly agricultural control to consider among the most promising applications for sustainable agriculture to carry out all over the world (Naqvi, 2004; Sönmez and Mamay 2018). BCAs are increasingly investigated to control plant disease. Soytong (2010) reported that Chaetomium-biological fungicide was proved to be effective against *Phytophthora palmivora* causing stem and root rot of Durian (*Durio zebithenus* L.). *Chaetomium cupreum* strain CC6 and *Chaetomium globosum* strain CG7 significantly inhibited the growth of *P. palmivora* causiong durian rot. *Ch. globosum* and *Ch. aureum* were also reported to inhibit the growth of *Phytophthora infestans Fusarium culmorum* and *P. palmivora* causing pomelo root rot (Linkies *et al.*, 2020; Hung *et al.*, 2015). The research finding was aimed to evaluate the efficiency of *Chaetomium cochliodes* CTh02 to control *P. palmivora* RT01 causing root rot of durian var. monthong.

Materials and methods

Morphological study of Chaetomium cochliodes CTh02 and Phytophthora palmivora RT01

The islate of *Ch. cochliodes* CTh02 was reported by Phonkerd *et al.* (2008) to produce chaetoviridins E, chaetochalasin A which inbibited *Plasmodium falciparum* (malaria disease), and produced chchliodones C, chaetoviridins F, chaetochalasin A to inhibit Mycobacterium tuberculosis. Pure culture of *Ch. cochliodes* CTh02 was sub-cultured from holotype at Biocontrol Research Unit, Faculty of Agricultural Technology, King Mongkut's Institute of Technolopgy Ladkrabang (KMITL) into potato dextrose agare (PDA). *P. palmivora* was isolated from root rot of durian from Chantaburi province, characteristic under binocular compound microscope.

Molecular phylogenetic confirmation

Chaetomium cochliodes CTh02

DNAs was extracted by CTAB (cetyltrimethyl ammonium bromide) which modified the method of Doyle and Doyle (1987). DNA extract was then diluted in 50 µl TE buffer, stored at -20°C. T1/T2 primer pairs were used and

multiplied a 700 bp segments of the β-tubulin gene. The reaction was conducted in 5 µl consisted of 1 µl genomic DNA, 0.5 µl dNTPs, 1 µl of each primer, 0.2 µl Taq DNA polymerase in 2.5 µl PCR buffer. PCR products were seperated by 1.0% agarose gel in 1X TAE buffer. It PCR was purified using the PureLinkTM Quick Gel Extraction Kit (Invitrogen). The nucleotide sequences were obtained and searched from database to compare in the Genbank by BLAST. Nucleotide sequences of the related species based on β-tubulin gene that retrieved from GenBank. *Fusarium oxysporum* from GenBank accession No. MK 962470 was an outgroup. The sequences were assembled by BioEdit and aligned ClustalX. Phylogenetic relationship tree was constructed by performing heuristic search under Neighbor-joining (NJ).

***Phytophthora palmivora* RT01**

DNA was extracted using 800 µl of modified digestion buffer of 10 mM Tris/HCl pH 8.0, 50 mM EDTA, 0.5% SDS, 0.5% Triton X-100, 0.5% Tween 20, and 2 µl of 20 mg proteinase K/ml which poured to a 2 ml micro-tube contained the frozen ground fungal biomass, incubated at 55°C for 30 min. The sample was added 800 µl chloroform/isoamyl alcohol (24:1, v/v), and centrifuged for 10 min at ~10,000×*g*. The supernatant was removed to a 2 ml tube and added chloroform/isoamyl alcohol. The precipitated DNA pellets were washed with 1 ml 70% (v/v) ethanol and 1 ml absolute ethanol and resuspended in 100 µl warm (55°C) TE buffer (10 mM Tris/HCl, pH 8.0, 1 mM EDTA). The pellets were dissolved, and the DNA solution was cooled to 27°C, 2 µl 20 mg. Ribonuclease A/ml (Sigma, USA) was added and incubated at 37°C for 30 min. DNA was extracted using the commercial DNeasy Plant Mini Kit and eluted in 50 µl of TE buffer. The sequences were retrived from Genbank by BLAST. *Aspergillus niger* from GenBank accession No. LC496500 was an outgroup. BioEdit and aligned ClustalX were used sequencing. Phylogenetic tree was constructed by performing heuristic search under Neighbor-joining (NJ).

Pathogenicity test

P. palmivora RT01 was tested pathogenicity using Kock's postulate method. The isolate was cultured on PDA for 7 days, sterilized cork borer of 0.3 cm was cut at peripheral colony and moved to the wounded leaves of durian var monthong, then incubated in moist chamber at 29-30 C for a few days to observe the lesion. The infected lesion was re-isolated *P. palmivora* by tissue transplating technique. The non-inoculated wounds on leaf were treated with only PDA agar plug served as controls. Each experiment was replicated four times.

Dual culture test between Chaetomium cochliodes CTh02 and Phytophthora palmivora RT01

Dual culture was evaluated using Completely Randomized Design (CRD) with four replications. *P. palmivora* RT01 and *Ch. cochliodes* CTh02 were separately cultured on PDA at room temperature (28-30 C) for 7 days. The agar plug (0.5 cm diameter) of each isolate was cut by sterilized cork borer from the actively growing edges and was transferred to 9 cm diameter PDA plate by placing an agar plug of the *P. palmivora* pathogen one side of the PDA plate and opposited an agar plug of *Ch. cochliodes* CTh02. The agar plugs on PDA plates either *P. palmivora* RT01 or *Ch. cochliodes* CTh02 were separatedly transferred to PDA served as the controls. The dual culture plates were incubated at room temperature (28-30 C°) for periodically observation within 30 days. The colony diameter (cm.) was recorded and the number of sporangia was counted by haemacytometyer. Inbibition (%) was calculated as the colony diameter or number of sporangia in control plate - the colony diameter or number of sporangia in dual culture plates/ the colony diameter or number of sporangia in control plate X 100. Analysis of variance (ANOVA) was computed to all data and treatment means were comprised with Duncan's New Multiple Range Test (DMRT) at P= 0.05 and 0.01.

Testing biological activity of fungal metaboliotes from Chaetomium cochliodes CTh02 against Phytophthora palmivora RT01

The extraction method was followed the protocal of Kanokmedhakul *et al*. (2006). *Ch. cochliodes* CTh02 was cultured in sterilized potato dextrose broth (PDB), and incubated at room temperature (28-30 C°) for 45 days. The dried biomass of *Ch. cochliodes* CTh02 was yielded by removing from PDB, filtered through cheesecloth and air-dried overnight. The dried biomass was ground with electrical blender and placed in flask with equal volume of hexane for 5 days at room temperature and filtered through Whatman filter paper No.4. The filtrate was then evaporated in vacuum evaporator to yield a crude hexane. The marc was further consequency extracted with ethyl acetate, and methanol by using the same procedure as hexane. The crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracts were yielded. The crude metabolites *Ch. cochliodes* CTh02 were evaluated to inhibit *P. palmivora* RT01, The 3×6 factorial experiment in CRD with four replications was performed. Factor A was crude metabolites (hexane, ethyl acetate and methanol extracts), and factor B was concentrations (0, 10, 50, 100, 500 and 1,000 ppm). Each crude metabolite was dissolved a drop of 2% dimethyl sulfoxide (DMSO)

and mixed to 30 ml PDA before autoclaved at 121℃,15 Psi for 20 mins. 5 mm diameter agar plug of *P. palmivora* RT01 was transferred to the middle of PDA amened with each concentration of crude metabolite, incubated at room temperature (28-30 C°) until *P. palmivora* RT01 in control grew in full plate. The colony diameter was recorded and the number of sporangia was counted using haemacytometer. Data were statistically computed ANOVA and treatment means were compared by DMRT at P = 0.05 and 0.01. The effective dose (ED_{50}) was computed by probit analysis.

Results

Morphological study of Phytophthora palmivora RT01

The characteristics of colony were fast growing with aerial mycelium floccose, white colour, reaching 9 cm diameter in 5-7 days. Sporangia are papillate and ovoid with the widest part close to the base. Sporangiophores are proliferated, and oogonium with a single antheridium (Figure 1).

Figure 1. *Phytophthora pamivora* RT01, A=colony on PDA media, B= sporangia proliferation (40X), C=sporangium, D=oogonium

Morphological study of Chaetomium cochliodes CTh02

The culture of *Ch. cochliodes* was slow growing with olivaceous colour. Perithecium was superficial or subglobose with dark brown colour. Terminal hairs were verrucose and dark brown, and the tips, spirally coiled in the upper part, with coils regularly tapering, asci are clavate shape. Ascospores are dark brown colour when mature, then ascal wall disappers (Figure 2).

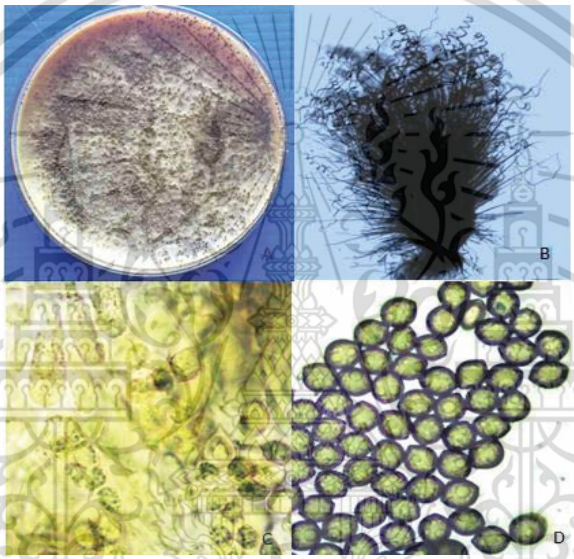

Figure 2. *Chaetomium cochliodes* CTh02, A= colony, B= ascocarps (10X), C= asci (40X) and D= ascospores (40X)

Molecular phylogenetic confirmation

Chaetomium cochliodes CTh02 was confirmed by molecular phylogenetic to species level. PCR prducts were sequenced in the tub2 gene for the identification of the species level. *Ch. cochliodes* CTh02 was comparted to *Ch. cochliodes* from GenBank accession No MH590621, HQ326556, KT895345, JN209864, JN209868 with 1q00 % similarity (Figure 3). Phylogenetic tree of *Chaetomium cochliodes* CTh02 was constructed by Neighbor-joining method based on the 5' region of the tub2 gene.

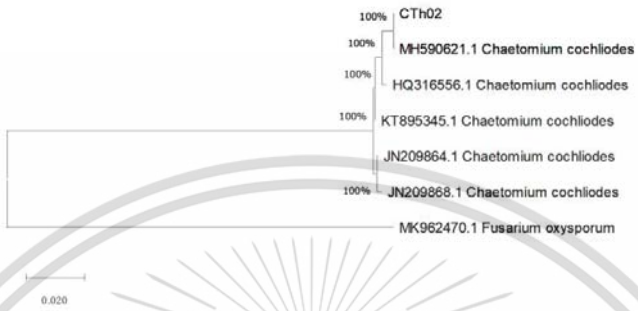

Figure 3. Phylogenetic tree of *Chaetomium cochliodes* CTh02

Phylogenetic tree of *Phytophthora pamivora* RT01 showed similarity cpmparison to *Phytophthora pamivora* which retrived from Genbank accession No. KR920754, KR920758, KR920759, HQ237479, MH219852, MH2!9839, MH219857. The *Aspergillus niger* with Genbank accession No. LC496500 was outgroup (Figure 4).

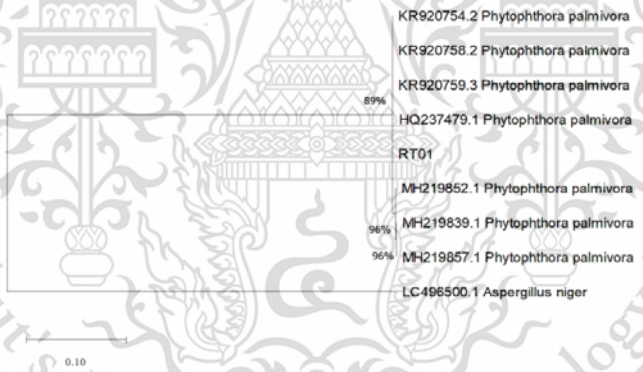

Figure 4. Phylogenetic tree of *Phytophthora palmivora* RT01

Pathogenicity test

Pathogenicity test was proved the pathogenic isolate, *P. palmivora* RT01 on detached leaves of durian var. monthong after 3 days inoculation. The inoculated leaves of durain bvar. Monthong under moist chamber condition showed brown symptom which enlargeg from the agar plug of the wounded leaves. The non- inoculated leaves of durian var monthong showed leaves remained healthy (Figure 5).

Figure 5. Pathogenicity test of *Phytophthora palmivora* RT01 A=non inoculated, B= inoculated

Dual culture test between Chaetomium cochliodes CTh02 and Phytophthora palmivora RT01

Ch. cochliodes CTh02 was proved its abilities to inhibit the growth of *P. palmivora* RT01 using dual culture test (Figure 6). Result showed that *Ch. cochliodes* CTh02 expressed antifugal activity against *P. palmivora* RT01 causing root ror of durian var. monthong which significantly inhibited colony growth and sporangial production of 60.11 and 68.55%, respectively.

Figure 6. *Chaetomium cochliodes* CTh02 inhibited colony growth of *Phytophthora palmivora* RT01 in dual culture test, A= *P. palmivora* RT01 alone, B= *P. palmivora* RT01 against *Ch. cochliodes* CTh02, and C= *Ch. cochliodes* CTh02 alone

Testing biological activity of fungal metaboliotes from Chaetomium cochliodes CTh02 against Phytophthora palmivora RT01

The active metabolites from *Ch. cochliodes* CTh02 were proved their abilities to inhibit the growth of *P. palmivora* RT01 causing root rot of durian var. monthong. Results demonstrated that crude ethyl acetate (EtOAc) extract and crude methanol showed significantly highest inhibited the colony of *P. palmivora* RT01 of 90.00% at the concentration of 1,000 ppm when compared to the control (Table 1, Figure 7). Meanwhile, ED_{50} values of crude ethyl acetate and crude methanol were 402 and 240 ppm. Crude hexane, crude ethyl acetate and crude methanol extracts expressed significantly highest inhibited sporangia of *P. palmivora* RT01 as 86.48%, 92.85% and 93.18% (Table 2) which ED_{50} values of 163, 339 and 25 ppm, respectively.

Table 1. Fungal crude metabolites testing *Chaetomium cochliodes* CTh02 against *Phytophthora palmivora* RT01 for 7 days

Crude metabolites	Concentrations (ppm)	Colony diameter (cm)	Growth inhibition (%)[/2]	ED_{50}(ppm)
	0	5^{a1}	-	
	10	5^{a}	-	
Hexane	50	5^{a}	-	955
	100	5^{a}	-	
	500	5^{a}	-	
	1,000	1.82^{f}	63.50^{c}	
	0	5^{a}	-	
	10	5^{a}	-	
EtOAc	50	3.81^{c}	23.75^{ef}	
	100	2.62^{e}	47.50^{d}	402
	500	1.67^{f}	66.50^{c}	
	1,000	0.50^{h}	90.00^{a}	
	0	5^{a}	-	
	10	4.18^{b}	16.25^{f}	
MeOH	50	3.43^{d}	31.25^{e}	
	100	2.46^{e}	50.75^{d}	240
	500	1.08^{g}	78.25^{b}	
	1,000	0.50^{h}	90.00^{a}	
C.V. (%)		6.67	14.85	

[1/]Means of four repeated experiments which followed by the same etter are not significantly differed by DMRT at P=0.05. [2]Inhibition (%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

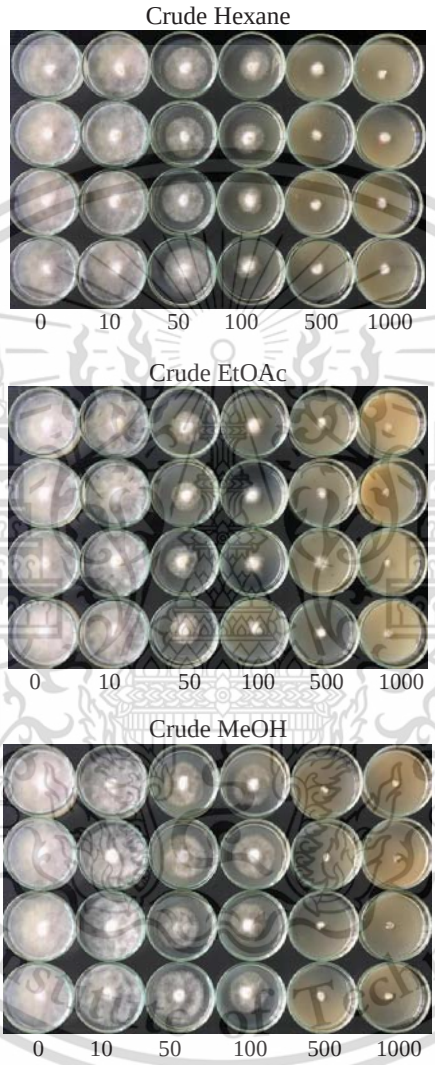

Figure 7. Fungal metabolites testing *Chaetomium cochliodes* CTh02 against *Phytophthora palmivora* RT01

Table 2. Sporangial inhibition of crude mertabolites from *Chaetomium cochliodes* CTh02 against *Phytophthora palmivora* RT01 for 7 days

Crude metabolites	Concentrations (ppm)	Number of sporagia (10^6) /1	Sporagia Inhibition (%)/2	ED_{50}(ppm)
Hexane	0	12.69^{a1}	-	163
	10	10.15^{bc}	19.27^{ef}	
	50	6.55^{ef}	46.96^{c}	
	100	4.75^{fg}	61.15^{b}	
	500	3.75^{gh}	69.04^{b}	
	1,000	1.69^{i}	86.48^{a}	
EtOAc	0	12.69^{a}	-	339
	10	11.54^{ab}	8.58^{fg}	
	50	9.67^{bcd}	23.07^{e}	
	100	8.52^{cd}	32.05^{de}	
	500	3.89^{g}	68.72^{b}	
	1,000	0.88^{i}	92.85^{a}	
MeOH	0	12.69^{a}	-	25
	10	12.35^{a}	2.88^{g}	
	50	10.27^{bc}	18.93^{ef}	
	100	7.95^{de}	37.17^{cd}	
	500	1.91^{hi}	84.93^{a}	
	1,000	0.82^{i}	93.18^{a}	
C.V. (%)		17.87	22.37	

/1Means of four repeated experiments which followed by the same etter are not significantly differed by DMRT at P=0.05. /2Inhibition (%) =R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Discussion

Phytophthora palmivora RT01 was morphological confirmation which the characteristics similar to report of Erwin and Ribeiro (1996). The molecular phylogenetic was confirmed to be *P. palmivora* as cpmpared to Genbank with the accession Nos.KR920754 and MH219852. *Chaetomium cochliodes* CTh02 was morphological and molecular phylogenetic identification which comfirmed that *Ch. cochliodes* CTh02 by 5' region of the compared to GenBank. Silmilarly, Wang *et al.* (2016a), stated that β-tubulin gene was more effective than the internal transcribed space gene sequence and the nucleotide sequences of tub2 gene were also useful to identify into species level of *Chaetomium* spp. (Wang *et al.*, 2016b).

Dual culture test *Ch. cochliodes* CTh02 expressed ability to inhibit colony growth and sporangial production of *P. palmivora* RT01 which similar reported by Kumar *et al.* (2020) who stated that *Ch. globosum* stain CG 5157 exhibited a broad spectrum antifungal activity against *Sclerotinia sclerotiorum*

at 73.80% and similar with report of Zhao *et al.* (2017) who statet that *Ch. globosum* CDW7 inhibited the growth of *Botrytis cinerea* (51.6%), *Phytophthora capsica* (55.8%), *Fusarium graminearum* (50.2%) and *S. sclerotiorum* (78.9%). Fungal metabolite extracts from *Ch. cochliodes* CTh02 against *P. palmivora* RT01 resulted that crude ethyl acetate and crude methanol extracts revealed the highest colony inhibition of *P. palmivora* RT01 and ED_{50} values were 402 and 240 ppm. It was similar to the study of Aggarwal *et al.* (2004) reported that crude extracts of from *Ch. globosum* Cg-2 inhibited the mycelial growth of *Cochliobolus sativus* over 87%. Crude hexane, ethyl acetate and methanol extrtacts were significantly highest inhibited sporangial production of *P. palmivora* RT01 from 80-93 %. It is similar reported by Tongon and Soytong (2016) who stated that crude extracts of *Chaetomium brasiliense* actively inhibited *Fusarium solani* and with the ED_{50} of 66 - 288 ppm. Moreover, Zhang *et al.* (2013) reported that *Ch. globosum* strain No.05 inhibited the northern corn leaf blight in maize.

Moreover, the control mechanism of *Ch. cochliodes* CTh02 against *P. palmivora* RT01 may possible act as antibiosis which Phonkerd *et al.* (2008) reported that *Ch. cochliodes* CTH02 produced chaetoviridins E, chaetochalasin A, chchliodones C, chaetoviridins F, chaetochalasin A like antibiotic substances against human pathogens. Similar report of Fatima *et al.* (2016) stated that the crude ethyl acetate extract of *Chaetomium* sp. NF15 showed promising antimicrobial activity against the clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The research finding is demonstrated that the active metabolites of *Ch. cochliodes* CTh02 gave significantly supressed *P. palmivora* RT01 causing root rot of duriamn var. monthong. Further research finging is towarded for phytoalexin induction of durian plants against *Phytophthora* rot using these active metabolites.

Acknowledgements

I would like to acknowledge the King Mongkut's Institute of Technology Ladkrabang and Faculty of Agricultural Technology, KMITL, Bangkok, Thailand for facilities and laboratory support.

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(Received: 20 December 2020, accepted: 25 February 2021)

Running title: Active metabolites' nanoparticles constructed from Chaetomium for plant disease control

Nanoparticles derived from active metabolites of *Chaetomium cupreum* CC3003 against *Phytophthora* rot of Durian

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Novelty Statement

The new discovery of microbial nanoparticles namely nano-CC-E, nano-CC-H and nano-CC-M constructed from *Chaetomium cupreum* CC3003 that can be induced plant immunity through phytoalexin production, scopoletin in the infected durian var. Monthong.

ABSTRACT

Phytophthora rot of durian (*Durio zibetinus* L.) is a serious disease wherever the crop has been planted and the disease control customarily uses chemical fungicides reported to be resistant by pathogen. Alternative non-chemical control strategies are being investigated to produce safe food. The main objective of this research was to test the activity of metabolites from *Chaetomium cupreum* CC3003 in the form of crude materials and nanoparticles to control and induce immunity to *Phytophthora palmivora* causing rot of durian var. Monthong. The results showed that *P. palmivora* proved to be pathogenic to durian var. Monthong. *C. cupreum* CC3003 acted as an antagonist and *P. palmivora* was

confirmed as the pathogen by morphological and molecular genetic identification. Effective doses (ED_{50}) of CC-E, CC-H and CC-M crude metabolites for spore inhibition were 60, 97 and 140 ppm, respectively. The research findings found that the diameters of nano CC-E, nano CC-H and nano CC-M were 534, 499 and 537 nm, respectively. The nano CC-E, nano CC-H and nano CC-M demonstrated antifungal activity against *P. palmivora* with ED_{50} of 11, 13 and 16 ppm, respectively. The nanoparticles at low concentrations were more effective than crude metabolites at high concentrations. Nano-CC-E used to treat seedlings of durian resulted in the production of scopoletin which served as an immunity agent or elicitor against rot disease of durian. It is concluded that active metabolites derived from *C. cupreum* significantly inhibited *P. palmivora* and induced immunity through phytoalexin production.

Keywords: *Chaetomium*, nanoparticles, phytoalexin, root rot, durian

INTRODUCTION

Durian (*Durio zibethinus* L.) is native fruit of Southeast Asian countries, and is one of the most famous fruit in the world. In Thailand, the durian fruit is considered as the "King of Fruits". Each durian tree produces around 15-800 fruits in every fruiting season (Subhadrabandhu and Shodal, 1997; Husin *et al*., 2018). Monthong variety genuinely means "golden pillow" in Thai. It's one of the popular varieties in Thailand and is characterized by triangular spikes, pale yellow fruit and a sweet test. *Phytophthora palmivora* (Butl.) is a destructive pathogen which infects various economic plants of over 2000 species including root rot in durian which is a serious problem leading to inferior quality and lower yield (Soytong, 2010). In 1996, Erwin and Ribeiro reported that

Phytophthora species are usually resistant to–the fungicide metalaxyl leading to disease control failure . Biological control of plant pathogens has been developed in recent years to reduce environmental harm, costly application of fungicides and decrease disease incidence caused by *Phytophthora* spp. (Palmieri *et al*., 2019).

Chaetomium spp. re potential biocontrol agents against different soil-borne pathogens. Many species of *Chaetomium* have demonstrated suppression of the growth of plant pathogens through competition, antibiosis and a combination of mechanisms (Shanthiyaa *et al*., 2013). Biological control by *Chaetomium* spp. was reported against *Melampsora puplicola*, *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium sporotrichioides* and *Colletotrichum gloeosporioides* (Jiang *et al*., 2017; Van Thiep and Soytong, 2015). *Chaetomium cupreum* CC3003 used in this research is reported to release azaphilones including rotiorinols A−C, stereoisomers named (−)-rotiorin and a known compound, rubrorotiorin. Rotiorinols A and C, (−)-rotiorin, and rubrorotiorin were reported to inhibit *Candida albicans* with IC_{50} values of 10.5, 16.7, 24.3, and 0.6 ppm., respectively (Kanokmedhakul *et al.,* 2006). Moreover, Tan and Soytong (2017) found that *C. cupreum* CC3003 inhibited *Cuvularia lunata* in bi-culture tests, and its metabolites including crude hexane, crude EtOAc and crude methanol extracts inhibited spore production of the tested pathogen with the ED_{50} of 6.4, 0.8 and 7.8 ppm, respectively.

Agricultural nanotechnology is being investigated for plant disease control to reduce the application of chemical fungicides which are harzadous to human beings, unbalance the agroecosystem and cause toxic residues in agricultural products. It has become a new tool to re-structure the materials at the atomic level including the formulation of organic materials as fine particles (Soutter 2012; Li *et al.,* 2011). Scienists have recently examined

the biological properties of organic nanomaterials (Elibol *et al*., 2003), applied incrop production (Soutter 2012; Ditta 2012). The bioactive substances from natural products can be constructed as nanoparticles that can easily penetrate through plant cells, and this increases the stability of effective compounds and decreases leaching from plant surface after application (Perlatti *et al.,* 2013). This technique can increase the efficacy plant disease management (Rai and Ingle 2012) by allowing formulation of disease control products in liquid or powder forms to apply to plants (Ditta 2012). Moreover, Tongon *et. al* (2018) reported that nanoparticles derived from *Chaetomium brasiliens*e inhibited *Phytophthora palmivora* with an ED_{50} of 1.08 ppm, and decreased the root rot disease of durian as well as increased plant growth parameters.

Phytoalexins are understood to be involved in plant defense (Ahuja *et al.,* 2012) and they can accumulate in healthy plant cells surrounding wounded or infected tissue (Deverall, 1982). Abiotic elicitors are capable of inducing phytoalexins in many crops (Angelova *et al.,* 2006; Yean *et al.*, 2009) while biotic elicitors are also reported to elicite phytoalexins (Liu *et al.,* 1995). Glazebrook *et al.,* (1994) stated that plants can produce phytoalexins after facing abiotic and biotic stress, and this process elicits the production of toxins which attack pathogens. Phytoalexins can help to delay pathogen maturation, interfer with metabolism, and prevent pathogen reproduction. It is important in plant defense to inhibit pathogen colonization. Gnonlonfin *et al.* (2012) stated that many plants produce coumarins with antimicrobial activities. A coumarin compound, scopoletin (6-methoxy-7-hydroxycoumarin), isolated from plant species was found to produce antifungal compound in tobacco plants against *Phytophthora* sp. The objectives of the current research were to

investigate the ability of crude metabolites, and nanoparticles constructed from *C. cupreum* CC3003 to inhibit *P. palmivora* DD01 and induce immunity to durian rot.

MATERIALS AND METHODS

Isolation of pathogen and pathogenicity test

Phytophthora sp. DD01 was isolated by using a baiting technique following the method described by Soytong (1989). Infested soil samples were placed in sterilized Petri dishes, sterile water was added, 1×1 cm pieces of durian leaves were added, and the dishes were incubated at room temperature. After 2 days of incubation, sporangia typical of *Phytophthora* spp. were observed under light microscopy and isolates were transfered to water agar (WA) in Petri dishes. The WA plates were maintained at room temperature (27-30C°), single colonies were sub-cultured to potato dextrose agar (PDA) and re-isolated until pure cultures were obtained, which were maintained in PDA for further experiments.

Pathogenicity tests were done using detached leaves and root inoculation of durian seedling var. Monthong. Healthy durian detached leaves were sterilized with 10% sodium hypochlorite., then wounded by a sterilized needle. Agar plugs of the pathogen were inoculated on the wound site of detached leaves. The controls were processed similarly using an agar plug without the pathogen. Root inoculation was done using 4-month-old durian seedlings planted in planting bags (size 6 inch). Sporangial suspensions ($1x10^5$ sporangia/ml) of the *P. palmivora* isolate were prepared and applied to the soil and basal stem of the test plants at the rate of 20 ml/plant. The experiment was repeated four times.

Disease incidence (%) was measured as the number of infected plants/ total number of tested plants x 100. Disease rating index was recorded as 0= healthy plants, and 3= seriously infected plants (Soytong, 2010).

Chaetomium antagonistic fungus

Chaetomium cupreum strain CC 3003 used in this study was previously reported to release rotiorinols A−C, (−)-rotiorin and rubrorotiorin which were found to inhibit *Candida albicans* (Kanokmedhakul *et al.,* 2006). The culture was morphologically identified according to Von Arx *et al.* (1986) and Soytong and Quimio (1989).

Morphological and molecular phylogenic identification

Phytophthora sp. DD01 was cultured on PDA and periodically observed morphologically. Agar containing the fungal sporangia and other structures of DD01 were cut into 1×1 cm piece and placed in sterilized Petri dishes containing sterile distilled water. Plates were incubated at 28-30 C° for 5 days before observation under a light microscope and photos were taken photos using MoticPlus 2.0 software. Genomic DNA of *Chaetomium* isolates were extracted using the CTAB method (Graham *et al*., 1994). Identification the pathogen at the molecular level used universal primers ITS 1 (5′TCC GTA GGT GAA CCT GCG G 3′) and ITS 4 (5′TCC TCC GCT TAT TGA TAT GC 3) to amplify the internal transcribed spacer (ITS) rDNA region of isolate DD01, under previously described PCR conditions (Ferrer *et al.,* 2001).

Bi-culture test

Phytophthora sp. DD01 was cultured in PDA for 7 days and 0.3 cm diameter discs were cut from the periphery of colonies and placed opposite a disc of the antagonist at the opposite edge of 9 cm diameter PDA plates. Bi-culture plates were incubated and periodically observed for 30 days. Colony growth and sporangia of *Phytophthora* sp. DD01 were observed and data were recorded from bi-culture and control plates. Sporangia were counted by haemacytometer. Data were calculated included the colony growth and sporangial inhibition as follows: Inhibition (%) = 100 × (A − B)/A, where A = sporangial number or colony size of *Phytophthora* sp. DD01 in control plates; B = sporangial number or colony size of *Phytophthora* sp. DD01 in bi-culture plates. Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at P = 0.05 and 0.01 were computed to compare treatment means.

Testing crude metabolites from *Chaetomium cupreum* CC3003

***Chaetomium cupreum* CC3003** was cultured in potato dextrose broth (PDB) medium for 30 days at room temperature (27-30 C°), then the fungal biomass was dried at room temperature and crude metabolites were obtained following the methods of Kanokmedhakul *et al.* (2006). The dried fungal biomass of *C. cupreum* CC3003 was ground into fine powder using an electric grinder. It was extracted by hexane (1:1 v/v) for 72 hr, then passed through Whatman No. 4 filter paper to separate the marc and hexane filtrate. Crude hexane extract was obtained using a rotary vacuum evaporator. The marc was soaked in ethyl acetate (1:1 v/v) for 72 hr.), and filtered then evaporated to get crude

ethyl acetate extract. Marc from ethyl acetate was further extracted in methanol (1:1 v/v) to yield crude methanol extract.

Each crude extract was tested for inhibitory activity against *P. palmivora* DD01 in two factor factorial experiments using a Completely Randomized Design (CRD); the experiment was repeared four times. Agar plugs of *P. palmivora* were placed on PDA plates (5 cm in diameter) in which each crude extract was incorporated at concentrations of 0, 10, 50, 100, 500, and 1,000 ppm. Each crude extract was dissolved in 2% dimethyl sulfoxide (DMSO). All tested crude extract concentrations were autoclaved at 121°C (15 psi) for 30 min. The agar plugs (0.3) cm of *C. cupreum* CC3003 were transferred to the middle of plates containing each tested sample concentration, and incubated for 7 days. Data were statistically computed by analysis of variance of the colony growth and sporangia number, and inhibition percentage using the above formulae. Colony growth and sporangia inhibition were used to compute the effective dose ED_{50} by probit analysis through SPSS Statistics ver. 23.0 software (IBM Co., Armonk, NY, USA).

Evaluation of nanoparticles derived from *Chaetomium cupreum* against *Phytophthora palmivola*

Nanoparticles were derived from crude extracts from *C. cupreum* CC3003 by using an electrospinning machine following the method of Dar and Soytong (2014) to get 3 samples of nanoparticles as follows: nano CC-H (from crude hexane), nano CC-E (from crude ethyl acetate) and nano CC-M (crude methanol). Each nanoparticle was observed under Scanning Electron Microscope (SEM). The nanoparticles of nano CC-H, nano CC-E and nano CC-M were tested for antimicrobial activity against *P. palmivora* DD01 (root rot

of durian). The research used two factor factorial experiments arranged in a CRD and was performed four times. Treatment combinations were expressed as factor A (nanoparticles of CC-H, CC-E and CC-M), and factor B (concentrations of 0, 3, 5, 10 and 15 ppm). One drop of 2% dimethyl sulfoxide (DMSO) was used to dissolve each nanoparticle and then added to 30 ml PDA, then autoclaved at 121°C for 30 min. A pure culture of *P. palmivora* DD01 was cut by sterilized cock borer (0.5 mm) at the periphery of the colony, then these agar plugs were transferred to the middle of PDA mixed with each nanoparticle. The tested plates were maintained at room temperature (27-30 C°) and incubated until the tested pathogen completely covered control plates. The normal and abnormal structures of the tested pathogen were observed under a compound binocular microscope. The collected data were statistically analysed using analysis of variance for colony size and sporangia number, then treatment means were compared using DMRT. The inhibition was computed as in previous experiments, and the effective dose (ED50) was calculated using probit analysis (SPSS Statistics ver. 23.0, IBM Co., Armonk, NY, USA).

Testing nano-CCE for phytoalexin production in durian

Seedlings of durian var Monthong were inoculated with a sporangial suspension ($1x10^5$ sporangia/ml) of *P. palmivora* DD01 following cutting root tips before planting in a sterilized soil mixture of loamy soil:organic compost at the ratio of 9:1. The nano CC-E at a concentration of 15 ppm was sprayed on the inoculated durian seedlings. Control plants were treated with sterile water (negative control) or scopoletin (positive control). Detection of phytoalexin in durian tissue extracts was carried out by thin layer chromatography (TLC) using 12% acetic acid. Fresh leaf samples (1 g.) were cleaned in tap water, ground, and

soaked in 10 mL methanol before passing through a filter paper (Whatman No.4). The chromatogram was monitored under UV light (366 nm), and a single, blue fluorescent compound was characterized by comparison to the standard scopoletin (Sigma Co. Ltd) at Rf 0.75. The R_f value was calculated to compare with the scopoletin standard. The experiment was repeated three times. The *R*f value was calculated as (Equation 1):

$$R_f = \frac{\text{distance spot travels}}{\text{distance mobile phase travels}} \quad (1)$$, Where: R_f – retention factor.

RESULTS

Isolation of pathogen

The root rot pathogen of durian var. Monthong was isolated by a baiting technique. A pure culture of the fungal isolate was morphologically identified by observation under a compound microscope. Pure cultures grew very fast on potato dextrose agar and the colony covered the plate in 3 days. Agar discs (1 x 1 cm) of the culture were cut and placed into sterile water and observed within 24 hr. Spherical sporangia, sporangiophore proliferation, zoospores released from pores of papulae were observed. Oogonia are round, and possessed amphigynous antheridia (Fig. 1).

Molecular phylogeny of *Phytophthora palmivola*

The phylogenetic tree showed a cluster of *Phytophthora palmivola* in the same clade with sequences of *P. palmivola* MG956799, HQ659668, MH219826, MH219849, KP813963, MH219829, MH219866, MH401200 from the Genbank database supported by an 88 % bootstrap value with *Sordaria tometoalba* MH872281 as an outgroup (Fig. 2). All

isolates were deposited at the Biocontrol Research Unit, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Pathogenicity test

The inoculated leaves of durian var. Monthong with *P. palmivora* DD01showed brown rot symptoms within 7 days. The leaves were significantly infected by the tested pathogen when compared to non-inoculated control which showed no symptoms (Fig. 1).

Chaetomium antagonistic fungus

Chaetomium cupreum CC 3003 was cultured on PDA for 3 weeks and colonies displayed yellow to orange or red to rust exudates. Ascomata superficial, ostiolate, subglobose or ovate with brown walls of *textura angularis* in the surface view. Terminal hairs usually arcuate, with apeces incurved, circinate to coiled. Lateral hairs flexuous or apically incurved. Asci fasciculate, clavate, with 8 biseriate or irregularly arranged ascospores, evanescent. Ascospores brown when mature, more or less inequilateral, fusiform, elongate fusiform, navicular, reniform, lunate or limoniform, sometimes bilaterally flattened, with one or two apical germ pores. Asexual stage unknown (Fig. 4).

Molecular phylogeny of *Chaetomium cupreum* CC3003

Molecular phylogeny confirmed identification at the species level. The phylogenetic tree clearly identified *Chaetomium* sp. based upon the GeneBank database. Data from the GeneBank reliably confirmed CC3003 as *Chaetomium cupreum* (Fig. 5).

Bi-culture test

The results showed that *C. cupreum* strain CC 3003 significantly inhibited *P. palmivora* DD01 causing root rot of durian by over 80% in 3 weeks as seen in Fig. 6. The colony of *Chaetomium* grew over the pathogen colony in 4 weeks after incubation.

Testing crude metabolites from *Chaetomium cupreum* CC3003

The results showed that all tested crude metabolites significantly inhibited colony growth and sporangial production of *Phytophthora* sp. at a concentration of 1,000 ppm when compared to the controls. Crude CC-H, CC-E and CC-M at 1,000 ppm did not significantly inhibit colony growth by 90, 90 and 90 % and spore production by 98, 72 and 98 %, respectively. The effective dose (ED_{50}) of CC-E, CC-H and CC-M for spore inhibition were 411, 158 and 482 ppm, respectively (Table 1). Crude metabolites of CC-H, CC-E, CC-M expressed antifungal activity to inhibit the growth of *P. palmivora* (durian rot disease) with ED_{50} values of 97, 60 and 140 ppm, respectively. Moreover, the spore production of *P. palmivora* was inhibited by crude metabolites of CC-H, CC-E, CC-M with the ED_{50} values of 97, 60 and 140 ppm, respectively (Table 1).

Characterization of the nanoparticles

The nanoparticles nano CC-H, nano CC-E and nano CC-M, loaded with crude extracts from *C. cupreum* CC3003 were cream, light orange and light yellow in color, respectively (Fig. 7). Scanning electron images indicated that the particle size of nano CC-H, nano CC-E and nano CC-M averaged 534.1, 499.7 and 537.5 nm. (Fig. 7).

Evaluation of nanoparticles derived from *Chaetomium cupreum* against *Phytophthora palmivora*

Nanoparticles of *C. cupreum* CC3003 separately constructed using the electron spinning technique yielded nano CC-H (crude hexane), nano CC-E (crude ethyl acetate) and nano CC-M (crude methanol) as seen in Fig.8. All tested nanoparticles derived from *C. cupreum* CC3003 at concentrations of 3, 5, 10, and 15 ppm significantly inhibited colony growth and spore production when compared to the non-treated control (0 ppm). The highest tested concentration of 15 ppm gave the highest inhibition of colony growth and spore production. The nano-CC-E, nano-CC-H and nano-CC-M were actively antifungal against *P. palmivora* with the ED_{50} of 11, 13 and 16 ppm, respectively (Table 2). Morevoer, nano-CC-E, nano-CC-H and nano-CC-M measured under SEM showed sizes of 534, 499 and 537 nm. respectively.

Phytoalexin production

The current research found that nano-CCE derived from *C. cupreum* CC3003 at a concentration of 15 ppm used to treat seedlings of durian var Monthong inoculated with *P. palmivora* expressed a spot on TLC with an Rf value of 0.89 which proved to be scopoletin (Fig.9).

DISCUSSION

The fungal pathogen caused root rot disease in durian var. Monthong was identified morphologically and molecularly as *Phytophthora palmivora* DD01. Widmer (2014) stated that *P. palmivora* is a cosmopolitan pathogen causing rot of cacao, papaya, black pepper,

297 rubber, coconut, and citrus. *P. palmivora* is heterothallic with amphigynous antheridia and
298 spherical oogonia. Sporangia are papillate, varying in shape from ovoid-ellipsoid.
299 Chlamydospores are terminal and intercalary. *P. palmivora* DD01 found to be a virulent
300 isolate causing brown rot symptoms within 7 days. The leaves were significantly infected
301 by the tested pathogen. This was similarly reported by Tongon (2018). The inoculated
302 seedling roots with *P. palmivora* showed root rot and die back within 15 days when
303 compared to the non-inoculated seedlings of durian var. Monthong which exhibited no
304 symptoms. Those results were in accordance with Pechprome and Soytong (1996) who
305 stated that durian var Monthong stem and root rot was caused by *P. palmivora.*
306 Morphology and molecular techniques confirmed the identity of *C. cupreum* CC3003.
307 Biculture tests showed that *C. cupreum* CC3003 inhibited the growth of *P. palmivora.* The
308 research finding was similar to a report of Soytong and Quimio (1992) which found that *C.*
309 *cupreum* actively inhibited *Pyricularia oryzae* causing rice blast. Scanning electron images
310 indicated that the particle size of nano CC-H, nano CC-E and nano CC-M averaged 534.1,
311 499.7 and 537.5 nm.Song *et al.,* (2020) reported that nano-CCoH, nano-CCoE and nano-
312 CCoM from *Chaetomium cochliodes* (CTh05) ranged between 567–611, 422–566 and 415–
313 472 nm, respectively. The fungal metabolites of *C. cupreum* CC3003 (CC-H, CC-E, CC-M)
314 expressed antifungal activity against *P. palmivora* isolate DD01 highly inhibited colony
315 growth by 90% and spore production by 98, 72 and 98 %, respectively. The current
316 research was similar to that of Song and Soytong (2018) who found that crude extracts
317 from *Chaetomium* sp. gave the significantly highest sporulation inhibition of *Magnporthe*
318 sp. of 88 %, at 1,000 ppm. Nanoparticles of *C. cupreum* CC3003 separately constructed
319 using the electron spinning technique as report by Song *et al.,* (2020). Phytoalexin

production was done by using Thin layer chromatoraphy (TLC) and observed under UV light found blue fluorescent spot that similar as Power and Moore (1909) stated that the Rf value of paper chromatoraphy for scopoletin was 0.89 which *6%* AcOH and H_20-saturated isoamyl alcohol at the ratio of 1:1 expressed an Rf value of 0.84, and BuOH : AcOH : H_20_4 at the ration of 1:2:2 showed an Rf value of 0.89. Scopoletin was detected by fluorescence under an ultraviolet lamp. Einhellig *et al.* (1970) stated that when scopoletin was used to treat tobacco, sunflower and pigweed seedlings, scopoletin increased significantly in the tissue when compared with the control. Our research finding is consistent with Costet *et al.* (2002) who found that scopoletin accounted for the fluorescence after extraction by thin layer chromatography. As a result, nano-CCE constructed from *C. cupreum* CC3003 induced the test plant to produce scopoletin with activity against *P. palmivora* causing root rot of durian. Similarly, Sun *et al*. (2014) reported that scopoletin found in tobacco plants exhibited strong antifungal activity against *A. alternata* causing disease in tobacco.

CONCLUSIONS

CC-H, CC-E, CC-M are crude metabolites of *C. cupreum* CC3003 that inhibited the colony growth of *P. palmivora* with ED_{50} values of 97, 60 and 140 ppm, respectively, and inhibited the inocula production of the pathogen with the ED_{50} values of 97, 60 and 140 ppm, respectively. The constructed nano-CC-E, nano CC-H and nano CC-M from *C. cupreum* CC3003 significantly inhibited the inocula production of *P. palmivora* with the ED $_{50}$ of 11, 13 and 16 ppm. The nano CC-E constructed from *C. cupreum* CC3003 at a concentration of 15 ppm used to treated seedlings of durian var Monthong inoculated with *P. palmivora* clearly showed the production of scopoletin (Rf value 0.89) as a phytoalexin

produced by the seedlings. It was concluded that the active strain of *C. cupreum* CC3003 produced crude metabolites and the constructs of nanoparticles which inhibited inoculum production by *P. palmivora.* All tested nanoparticles derived from *C. cupreum* CC3003 more effectively inhibited the tested pathogen than the crude metabolites. It was noticed that the treatment of inoculated durian plants with nano-CCE induced scopoletin production.

ACKNOWLEDGEMENTS

I would like to thank the Association of Agricultural Technology in Southeast Asia (AATSEA) for providing some part of research facilities as it is a partly of Ph. D. dissertation at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

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Table 1. Crude metabolites of *Chaetomium cupreum* CC3003 against *Phytophthora palmivora*

Crude metabolites	Concentration (ppm)	Colony diameter (cm)[2,3]	Growth inhibition (%)[2,3]	ED_{50}(ppm)	Number of spores [2,3](10^5)	Spore Inhibition (%)[2,3]	ED_{50}(ppm)
CC-H	0	5.00^{a}	-	411.60	31.00^{c}	-	97.21
	10	5.00^{a}	0^{f}		27.0^{ab}	13.03^{e}	
	50	5.00^{a}	0^{f}		22.00^{c}	29.05^{cd}	
	100	5.00^{a}	0^{f}		20.75^{c}	33.03^{cd}	
	500	0.50^{f}	90.00^{a}		2.25^{e}	92.48^{a}	
	1000	0.50^{f}	90.00^{a}		0.50^{e}	98.33^{a}	
CC-E	0	5.00^{a}	-	158.43	31.00^{c}	-	60.07
	10	2.32^{c}	53.50^{d}		10.75^{d}	65.26^{b}	
	50	1.97^{d}	60.50^{c}		10.00^{d}	67.59^{b}	
	100	1.52^{e}	69.50^{b}		8.25^{d}	72.90^{b}	
	500	0.50^{f}	90.00^{a}		1.25^{e}	95.81^{a}	
	1000	0.50^{f}	90.00^{a}		0.25^{e}	99.13^{a}	
CC-M	0	5.00^{a}	-	482.44	31.00^{c}	-	140.80
	10	5.00^{a}	0^{f}		28.75^{a}	7.10 ^{ef}	
	50	5.00^{a}	0^{f}		28.75^{ab}	24.72^{d}	
	100	4.87^{a}	2.50^{f}		19.25c	37.83^{c}	
	500	2.57^{b}	48.50^{e}		9.50^{d}	68.52^{b}	
	1000	0.50^{f}	90.00^{a}		0.50^{e}	98.33^{a}	
C.V. (%)		4.25	7.06		15.49	14.20	

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

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

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

Table 2. Activity of nanoparticles of *Chaetomium cupreum* CC3003 against *Phytophthora palmivora*

Metabolites	Concentration (ppm)	Colony diameter (cm)[2,3]	Growth inhibition (%)[2,3]	ED_{50}(ppm)	Number of spores [2,3](10^5)	Spore Inhibition (%)[2,3]	ED_{50}(ppm)
Nano CC-H	0	5.00[a1]	-		29.25[a]	-	
	3	2.31[b]	53.75[c]		4.00[b]	86.16[d]	
	5	1.25[c]	78.75[b]	1.78	1.50[cd]	94.81[bc]	13.03
	10	0.56[d]	88.75[a]		0.50[cd]	97.49[ab]	
	15	0.50[d]	90.00[a]		0.50[cd]	98.38[a]	
Nano CC-E	0	5.00[a]	-		29.25[a]	-	
	3	2.25[b]	55.00[c]		2.00[c]	93.08[c]	
	5	1.22[c]	75.50[b]	1.51	1.50[cd]	94.81[bc]	11.01
	10	0.50[d]	90.00[a]		0.50[cd]	98.36[a]	
	15	0.50[d]	90.00[a]		0.25[d]	99.16[a]	
Nano CC-M	0	5.00[a]	-		29.25[a]	-	
	3	2.31[b]	53.75[c]		4.50[b]	84.70[d]	
	5	0.56[d]	88.75[a]	1.19	1.50[cd]	94.83[bc]	16.48
	10	0.50[d]	90.00[a]		0.75[cd]	98.21[ab]	
	15	0.50[d]	90.00[a]		0.50[cd]	98.33[a]	
C.V. (%)		6.39	4.52		13.62	2.89	

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

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

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

Fig. 1: *Phytophthora palmivola,* A= pure culture , B & C = sporangia and sporngial proliferation, D = sporangium, E= oogonium and anthridium, F= Oospore.

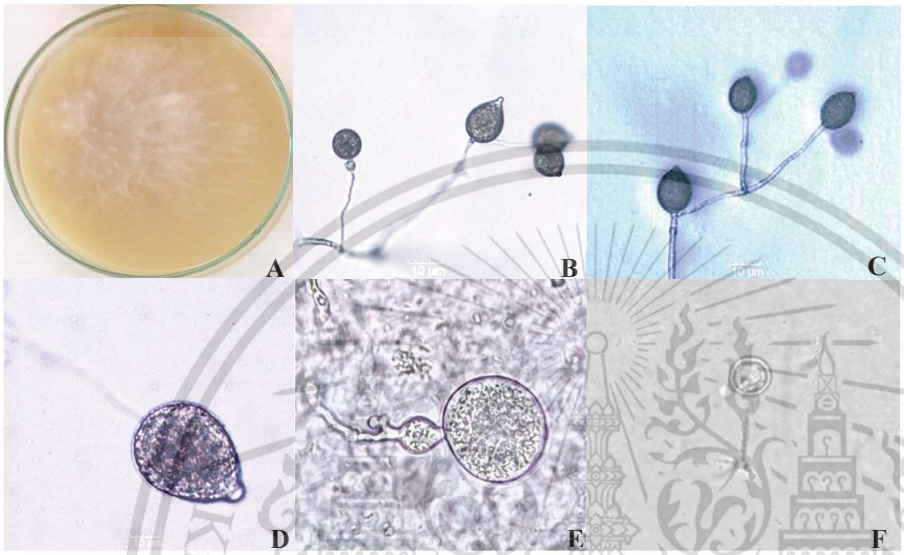

Fig. 2: Phylogenic tree of *Phythophthora palmivora* from GenBank including *Phythophthora palmivora* strain DD01 constructed after distance based analyses of ITS1and ITS4 regions of rDNA. Numbers of the branches indicate percentage of bootstrap values after1000 replicates. The outgroup taxa is *Sordaria tomentoalba.*

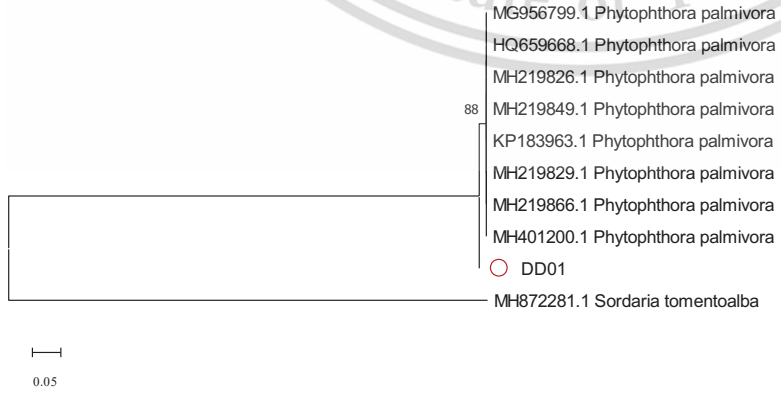

Fig. 3: Pathogenicity test of *Phythophthora palmivora* causing rot of durian var Monthong on leaves and seedings.

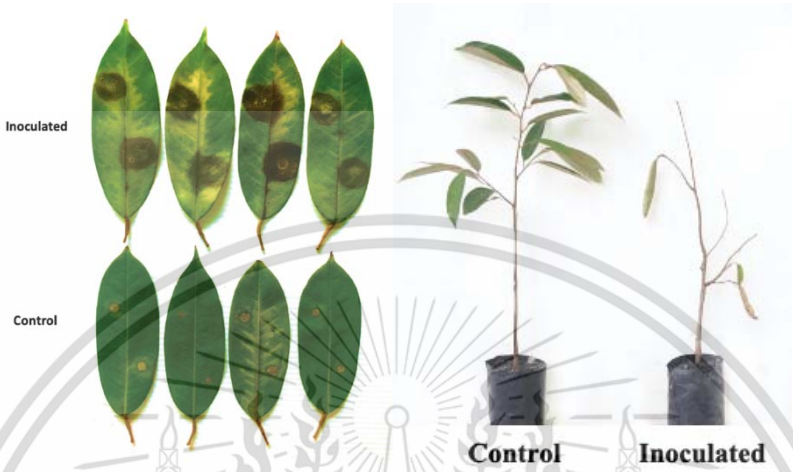

Fig. 4: *C. cupreum* strain Cc3003, A= pure culture, B= ascocarp, C-D= asci, E= terminal ascomatal hairs, F= ascospores

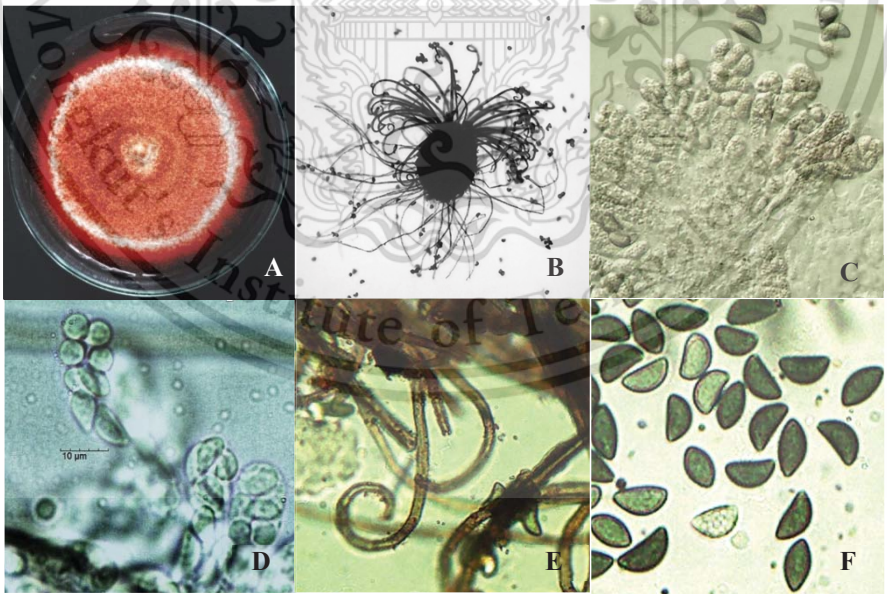

Fig. 5. Phylogenetic tree of *Chaetomium cupreum* from GenBank, including *Chaetomium cupreum* CC 3003, constructed based upon the distance-based analysis of the ITS1 and 5.8S regions of rDNA. The numbers at the branches indicate the percentage of bootstrap values after 1000 replications. The outgroup taxon is *Colletotrichu*m *queenslandicum*.

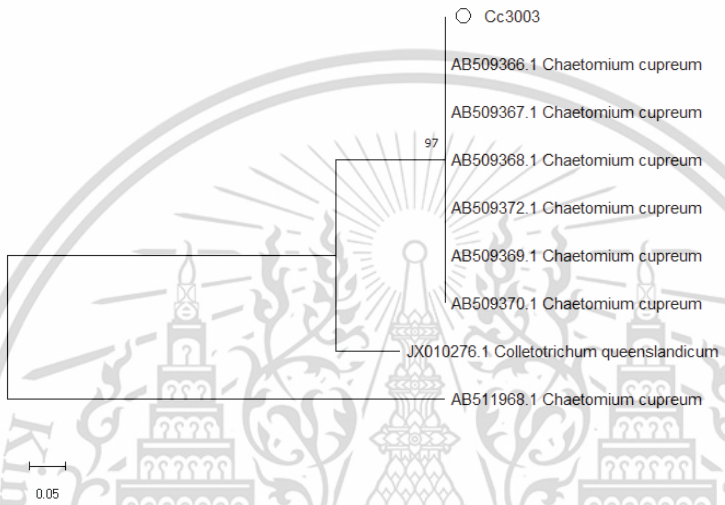

Fig. 6: *C. cupreum* strain CC 3003 vs *P. palmivora* DD01 (Left represents *P. palmivora*; middle represents *C. cupreum* strain CC 3003 vs *P. palmivora* and right is *C. cupreum* strain CC 3003)

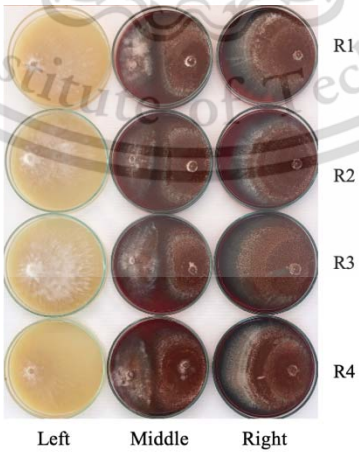

Fig. 7: Nanoparticles of *Chaetomium cupreum* CC3003

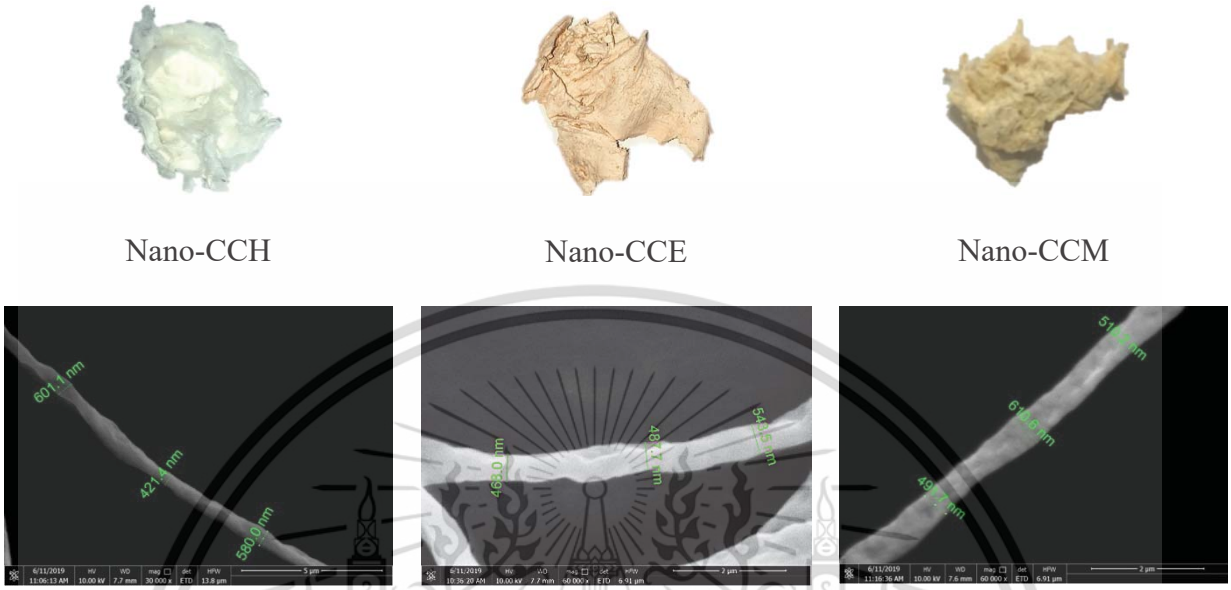

Fig. 9. Phytoalexin investigation

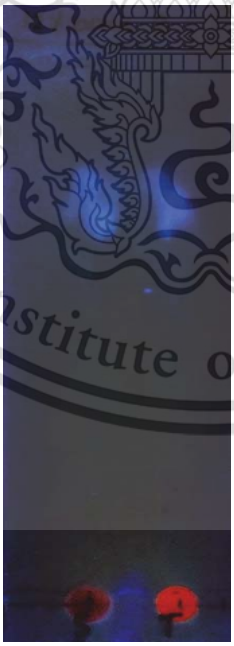

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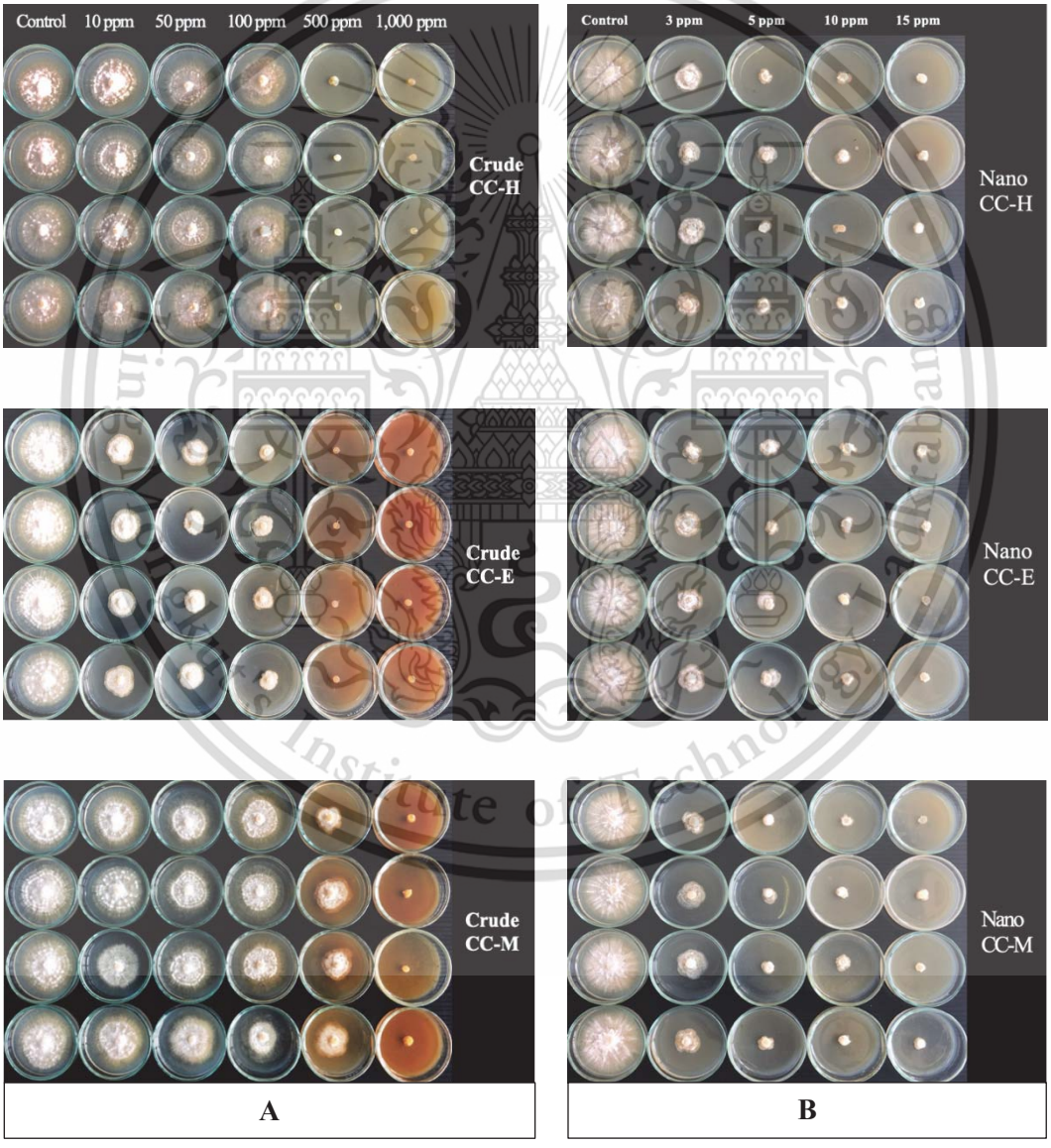
Fig. 8: Inhibition of *Phytophthora palmivora* DD1 using crude extracts (A) and nanoparticles (B) derived from *Chaetomium cupreum* CC3003. Note: Crude CC-H, Crude CC-E, and Crude CC-H represented crude extracts from hexane, ethyl acetate and methanol, and nano CC-H, nano CC-E and nano CC-M represented nanoparticles constructed from hexane, ethyl acetate and methanol crude extracts



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