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Bioactive Test of Metabolites from *Chaetomium cochliodes* against *Phytophthora* sp.

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Chaetomium cochliodes was tested ability to control durian disease cause by *Phytophthora* sp in vitro. The experiment was designed as two factor factorial experiment in Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts of *Chaetomium cochliodes* and factor B represented different concentrations of 0, 10, 50, 100, 500 and 1000 ppm. Result showed that hexane crude extract, methanol crude extract and EtoAC crude extract were the best inhibition sproe production of pathogen and were not significantly differed between treatments. For colony inhibition, the result showed that methanol crude extract and EtoAC crude extract were the best inhibit colony growth of *Phytophthora* sp. and followed by hexane crude extract. Further research finding is to evaluate *Chaetomium cochliodes* to control durian disease in pot experiment.

Keywords: bioactive test, *Chaetomium cochliodes*, *Phytophthora* sp.

Introduction

The durian has been known and consumed in Southeast Asia since prehistoric times, but has only been known to the western world for about 600 years. The earliest native reference to durian is the several bas relief panels of 9th-century Borobudur depicting durian as one of fruit offering for Javanese king, and also as one of the fruits sold in marketplace (Akhyari, 2015).

The most importance problem for durian cultivation in Thailand is the root rot disease caused by *Phytophthora* spp.. It 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. Chemical fungicides lead the negative side effect to the environment. In addition, the resistance of Phytophthora species to an important group of fungicides such as phenylamides (metalaxyl and related

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compounds) has become a serious problem in their chemical control (Erwin and Ribeiro, 1996). In order to develop eco-friendly management of *Phytophthora* diseases and to reduce the costly applications and harm of fungicides, application of bio-control agents against *Phytophthora* disease has become an importance research aspect and is being carried out all over the world (Naqvi, 2004).

Bio control agents are known as antagonists and antagonism is the generalized mechanism that they use to reduce the survival or disease causing activities of plant pathogen. Antagonism is actively expressed opposition and includes antibiosis, competition and parasitism (Cook and Baker, 1983). Recently, there have been many reports that antagonist fungi can used to control severial plsnt disease such as *Trichoderma asperellum, Chaetomium elatum* ChE0, *Chaetomium globosum* N0802, *Chaetomium lucknowense* CLT , *Trichoderma harzianum* PC01, *Emericella rugulosa* ER01, *Chaetomium cupreum* (Mahmoud *et.al*, 2015; Kasem, 2015; Charoenporn *et.al*,2010; Sibounnavong *et.al*,2011; Soytong ,1992).

The objective was to test testing ability of *Chaetomium cochliodes* to control durian disease cause by *Phytophthora* sp.

Materials and methods

Isolation pathogen, Phytophthora sp.

The roots of durian var. Mon thong were collected and brought to laboratory for isolation of the pathogen. Thus, root tissue pieces 1-2 cm. long were cut from the advancing edge of lesions, washed and surface-disinfected for 30 seconds in 10 % sodium hypochlorite, followed by three washings in sterilized distilled water. Disks were then blotted dry on sterilize paper towels and transferred to Petri dish containing water agar (WA) medium for firstly observation of appearing colonies and sub-cultured to PDA until get pure culture. Morphological identification was done by observation fungal characteristic under binocular compound microscope.

Pathogenicity test

Pathogenicity test will conduct to determine the isolate fungus on 15-months of durian seedling var. Mon thong. Sporangial suspension ($3x10^5$ sporagia/ml) of *Phytophthora* sp isolate will prepare and inoculate 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 will 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 will measure as number of infected plants/ total number of taste plants x 100, and disease ratings was evaluated as 0= healthy plants, and 3= seriously infected plants (Soytong, 2010).

Testing bio active compound of Chaetomium cochliodes to against Phytophthora sp.

The crude extracts of *Chaetomium cochliodes* were tested for inhibition of *Phytophthora* sp. Experiment was designed by using two factors factorial experiment in CRD with four replications. Factor A represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100, 500, and 1,000 ppm. Each crude extract was dissolved in one drop 2% dimethyl sulphite (DMSO), mixed into 30 ml potato dextrose agar (PDA) before autoclaving at 121 ℃ , 15 psi for 30 minutes. The culture of *Phytophthora sp* was cut at the edge of colony with sterilized cock borer (3mm). Agar plug of pathogen was transferred to the middle of PDA media in plate (5.0mm diameter) incorporated with each and incubated at room temperature (28 ℃-30 ℃) until the pathogen on the control plates growing full. Data were collected as colony diameter and the number of conidia. Percentage inhibition of pathogen colony growth and conidia production were calculated using the following formula:

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

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

Data were statistically computed analysis of variance and treatment means were compared using Duncan Multiple's Range Test (DMRT) at P = 0.05 and 0.01. The effective dose (ED50) will be calculated using probit analysis.

Results and Discussion

Phytophthora sp was isolated and tested for pathogenicity to confirm the virulent isolate. The result showed that hexane crude extract, methanol crude extract and EtoAC crude extract were the best inhibition sproe production of pathogen and were not significantly differed between treatments when compared to the non-treated control. For colony inhibition, the result showed that methanol crude extract and EtoAC crude extract were the best inhibit

colony growth of *Phytophthora* sp and followed by hexane crude extract when compared to the non-treated control.

Further report was similar as Tongon and Soytong (2016) stated that *Ch. brasiliense* and *Ch. globosum* showed efficacies to inhibit colony growth of *F. solani* and *Curvularia lunata* causing leaf spot disease in rice and also similar with Moya *et al.* (2016) who stated that *Chaetomium* spp showed high potential to inhibit *Drechslera teres* and *Bipolaris sorokiniana* causing foliar diseases of barley.

In this study, eatch crude extracts of *Chaetomium cochliodes* expressed antifungal activity against *Phytophthora* sp. The previous reported by Soytong (2014) reported that *Ch. cochliodes* proved to be a new antagonist against brown leaf spot of rice var Pittsanulok 2 caused by *Drechslera oryzae*. Soytong *et al*. (2000) the application of bio-products from *Chaetomium* can protect and cure Thielaviopsis bud rot of *Hyophorbe lagenicaulis* in Thailand and also similar with Kanokmedhakul *et al.* (1993) who stated that using the extracts from the *Chaetomium* spp to prevented the spore production of *F. oxysporum* f. sp. *lycopersici* which caused tomato wilt.

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