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Antifungal Activity of *Chaetomium elatum* against *Pyricularia oryzae* Causing Rice Blast

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Bioactive substances and Nano-particles of *Chaetomium elatum* were examined for antifungal activity test against *Pyricularia oryzae* causing rice blast. *C. elatum* was tested for antagonistic to determine bioactivity against *P. oryzae* which isolated from rice var kor-khor 9 by bi-culture technique. The result showed that *C. elatum* inhibited the growth of *P. oryzae.*60.44%. The crude hexane, ethyl acetate and methanol of *C. elatum* were yielded and examined for bioactivity test against *P. oryzae* and crude ethyl acetate showed significantly highest growth inhibition of *P. oryzae*. The nano-particles of *C. elatum*, Nano-CEH, Nano-CEE and Nano-CEM were also tested to inhibit *P. oryzae* and the result showed that Nano-CEE gave highest inhibition of colony growth and spore production of *P. oryzae.*

Key words: Antifungal activity, *Chaetomium elatum*, *Pyricularia oryzae*

Introduction

As one of the most widely cultivated food crops in the word, annual total production of rice is estimated at 520 million tons. Since the first description of rice blast by Soong Ying-Shin in the early 17th century (Ou, 1987), blast has become the biggest constraint to stable rice production. Rice blast still remains a serious problem where rice is cultivated regardless of rice ecosystems. Management of rice blast is not a simple process. One or two methods alone cannot provide satisfactory blast management. The

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introduction of resistant varieties and proper cultural practices followed by timely application of fungicide will result in an ideal management of blast disease (Zeigler, 1994).

There are many reports about the application of potential antagonists to control plant pathogens. *Chaetomium* spp. are well known as producers of secondary metabolites with different bioactivities (Li *et al.* 2011; Zhang *et al.* 2012). Soytong (1989, 1992) indicated that strains of *C. cupreum* and *C. globosum* are able to control plant pathogens such as *Curvularia lunata*, *Pyricularia oryzae* and *Rhizoctonia oryzae* in vitro. The antagonism test of *Chaetomium elatum* ChE01 were done by Soytong (2015) to against *F. oxysporum* f.sp. lycopersici and it is demonstrated the antagonistic activity of *Ch. elatum* ChE01 to inhibit the conidial production of F. oxysporum f. sp. lycopersici. Bioactivities tests of crude extracts and pure compounds were proved as a control mechanism. All tested crude extracts of Ch. elatum ChE01 was significantly inhibited conidia production of F. oxysporum f. sp. lycopersici. The objective of research finding was to isolate the rice blast pathogen and tested for its pathogenicity, and tested Chaetomium elatum by bi-culture technique. Crude extracts and nano particles were also tested against P. oryzae caused rice blast.

Materials and methods

Study on Morphology of Chaetomium elatum

Chaetomium elatum is offered from Assoc. Prof. Dr. Kasem Soytong, and used in this study. It is cultured in potato dextrose agar (PDA) medium and observe the growing colony and other characteristics of mycelia, ascocarp, asci, ascospores, terminal and lateral hairs under binocular compound microscope.

Isolation and identification of plant pathogen

The plant pathogen *Pyricularia oryzae* causing rice blast was isolated from rice Kor-Khor 9 variety by tissue transplanting technique from leaf symptom. The diseased leaves were cleaned with running tap water and after air-dried, then cut the advance margin of symptom between healthy tissue and diseased tissue into small pieces and then sterilized with sterilized water, 10 % ethyl alcohol and sterilized water again. Then, transferred onto water agar (WA) medium, the hyphal tip was cut from colony and

transferred to potato dextrose agar (PDA) to obtain pure culture. The isolates were identified based on the morphological characters of the fungal pure culture under binocular compound microscope by following the standard mycological manuals (e.g. M.B. Ellis, 1971).

Pathogenicity test

The plant pathogen *Pyricularia oryzae* was performed pathogenicity test followed Koch's Postulate by the modify methods of Charles *et al.* (2013). With this, Experiment was conducted using Completely Randomized Design (CRD) with four replications. Rice seedlings were inoculated with *P. oryzae* at the 4-5 leaf stage (21-day-old seedlings, 3 leaves/seedling) in the evening using a low-pressure spray bottle with a suspension of conidia 2 x 10^5 spores/ml. Inoculum preparation was done as described by (Thinlay and Finckh, 2000) and then covered with plastic sheet and maintained to observe the infected leaves. The inoculated leaves with only spraying sterilize distilled water were done to serve as controls. Disease index was assessed seven to ten days after inoculation based on the (IRRI, 1996) standard evaluation scale of 0 – 9 where: 0= no lesions; 1= small, brown, specks of pinhead size; 3 = small, roundish to slightly elongated, necrotic, gray spots about 1-2 mm in diameter; 5 = typical blast lesions infecting < 10% of the leaf area; 7 = typical blast lesions infecting 26-50% of the leaf area; 9 = typical blast lesions infecting >51% leaf area and many dead leaves (Windarsih *et al*., 2014).

Bi-culture antagonistic tests

Chaetomium elatum was tested for antagonistic to determine bioactivity against *Pyricularia oryzae* by bi-culture method. The experiment was conducted using a Completely Randomized Design (CRD) with 4 replications by the methods of Soytong (1992), Sibounnavong *et al*. (2009) and Charoenporn *et al.* (2010). The antagonistic fungi and pathogen were separately cultured on PDA at room temperature (30- 32 ℃) for 7 days. And 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of cultures of the antagonistic fungi and pathogen and then transferred onto the same sterilized 9 cm-diameter PDA plates, an agar plug of the pathogen was placed on one side of the plate which opposited an agar plug of an antagonistic fungus. The single plug of antagonistic fungi and pathogen were transferred into two separate PDA

plates as the controls. And then, all the plates were incubated at room temperature (30-32 ℃) for 30 days. Data were collected regarding to diameter of colony (cm) and the number of conidia produced by the pathogen in the bi-culture plates and control plates. A haemcytometer was used to count the number of conidia of pathogen.

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

Data were statistically computed analysis of variance (ANOV) and treatment means were compared using DMRT at P = 0.05 and 0.01.

Crude Extraction of Bioactivity Substances

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

Bioactivity test of crude extracts from C. elatum against Pyricularia oryzae causing rice blast.

The crude extracts of *C. elatum* were tested for inhibition of *P. oryzae*. The experiment was conducted by using 3x6 factorials in Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100, 500, and 1,000 ppm. Each crude extract was dissolved in one drop 2% dimethyl

sulphite (DMSO), mixed into 30 ml potato dextrose agar (PDA) before autoclaving at 121℃ , 15 p for 30 minutes. The tested pathogen were cultured on PDA and incubated at room temperature for 7 days, and then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA(amending with each crude extracts) plate (5.0 cm diameter) in each concentration and incubated at room temperature (28℃-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:

% inhibition = (A-B) / A ×100

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

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

Testing nano-particles from C. elatum to control P. oryzae.

Preparation of nano particles of *C. elatum*;-nano-particles were done using the method of Dar and Soytong (2014) to get Nano-CEH, Nano-CEE and Nano-CEM.

The nano-particles of *C. elatum*, Nano-CEH, Nano-CEE and Nano-CEM were tested to inhibited *P. oryzae* causing rice blast. Experiment was designed by using two factors factorial experiment in CRD with four replications. Factor A represented Nano-CEH, Nano-CEE and Nano-CEM and factor B represented concentrations at 0, 1, 5 and 10 µg/ml. Each Nano-particle was dissolved in one drop 2% dimethyl sulfoxide (DMSO), and then mixed into 30ml PDA medium before autoclaving at 121℃,15lbs/inch2 for 30 min. The culture of *P. oryzae* was cut at the edge of colony with sterilized cock borer (3mm). Agar plug of pathogen was transferred to the middle of PDA media in plate (5.0mm diameter) incorporated with each nano-particles. The transferred plates were incubated at room temperature until the pathogen in control plates growing full. Abnormal and normal spores of pathogen from each treatment were observed under binocular compound microscope and taken photograph for comparison. The data were collected as colony diameter and the number of

spores that counted by using Haemacytomete. Percentage of inhibition was computed and the effective dose (ED50) was then calculated using probit analysis.

Results and Disscusion

Study on Morphology of Chaetomium elatum

C. elatum strain 0805 was cultured and morphological observation following Soytong and Quimio (1989). Ascoacrp, asci and ascospores were taken photograph under compound microscope (Figure 1).

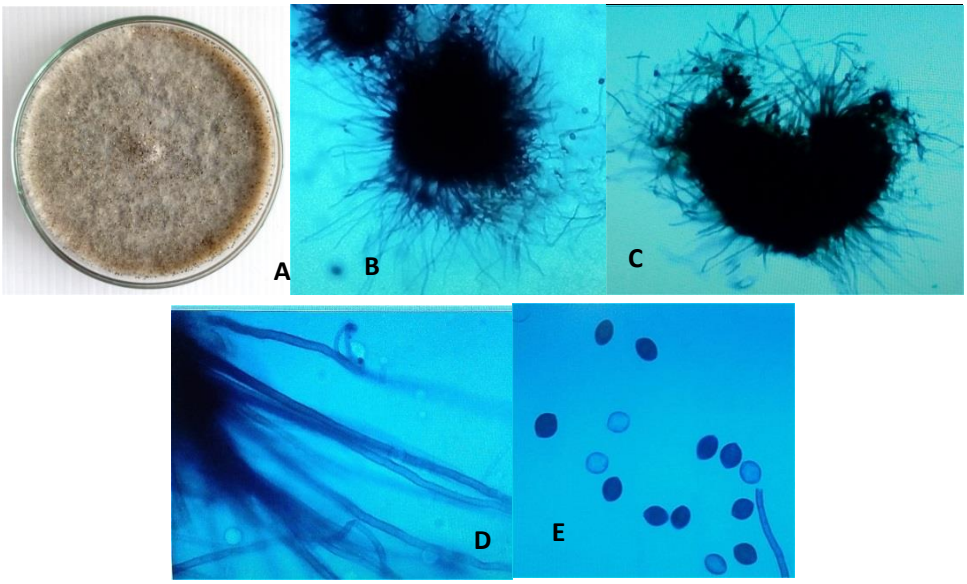

Fig.1 *Chaetomium elatum* , A= colony, B, C=Ascocarps, D= terminal hairs; E,F= ascospores

Isolation, Identification and Pathogenicity test of Pyricularia oryzae

Pyricularia oryzae were isolated and identified from rice leaf blast of Kor-Khor 9 with obvious symptom of rice blast and get pure culture (Fig.2). The isolate *Pyricularia oryzae* (KK9-2) was confirmed pathogenic isolate from pathogenecity test. The result showed that isolate KK9-2 could infected in the rice leaf of Kor-Khor 9 and caused symptom as DI=3(Fig. 3, Table 1). As a result, Soytong and Quimio (1989) reported that Pyricularia oryzae can be infected rice var IR 44 which cause blast symptom.

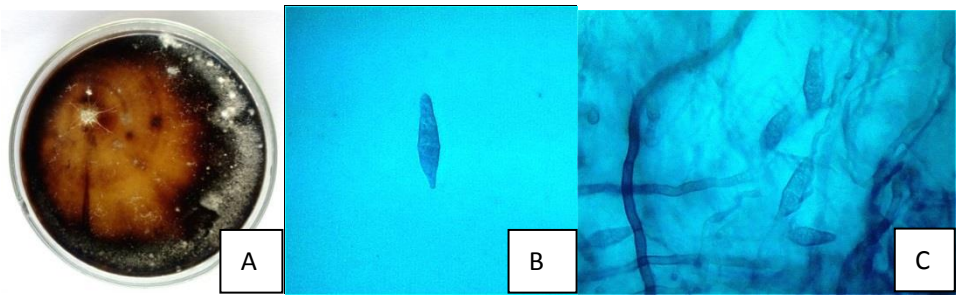

Fig.2 *Pyricularia oryzae* , A= pure culture in PDA; B= conidia; C = mycelium and conidia.

Control Treatment

Fig. 3 Pathogenicity test on rice leaf

Table 1 Pathogenicity test on rice leaf

Treatments	DI
Control	1
Pyricularia oryzae	3

Disease index (DI) 0= no lesions; 1= small, brown, specks of pinhead size; 3 = small, roundish to slightly elongated, necrotic, gray spots about 1-2 mm in diameter; 5 = typical blast lesions infecting < 10% of the leaf area; 7 = typical blast lesions infecting 26-50% of the leaf area; 9 = typical blast lesions infecting >51% leaf area and many dead leaves

Bi-culture antagonistic tests

C. elatum was proved its abilities to inhibit the growth of *P. oryzae* by using bi-culture tests (Fig.4). The result showed that *C. elatum* gave significantly colony inhibition of *P. oryzae* when compared to the control. The number of spores that producing by the pathogen *P. oryzae* was counted by using Hemacytometer. The results showed that *C. elatum* significantly inhibited number of pathogen spores when compared to the control plate. There are several researchers reported that *C. elatulum* can control plant

pathogen *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato (*Lycopersicon esculentum* var Sida). (Sibounnavong, 2011; Soytong, 2015). And other species of *Chaetomium* spp. are also reported to believe antagonize to many plant pathogens. Sibounnavong (2011) reported that *Chaetomium lucknowense* showed greater antifungal activity against *F. oxysporum* f. sp. *lycopersici* NKSC02. The crude extract of endophyte *Chaetomium globosum* No.04 which isolated from the medicinal plant *Ginkgo biloba* showed significant growth inhibitory activity against the phytopathogenic fungi *Rhizopus stolonifer* and *Coniothyrium diplodiella*. (Guizhen Zhang *et al.* 2013). Charoenporn *et al.* (2010) reported that oil bio-agent formulation from the antagonistic fungi of *Chaetomium globosum* and *Ch. lucknowense* showed their biological ability to control tomato wilt. The efficacy of *Chaetomium globosum* as a biocontrol agent against the late blight pathogen *Phytophthora infestans* was evaluated in potato plants by Shanthiyaa *et al.* (2013). Among eight *Chaetomium* isolates evaluated *C. globosum* isolate Cg-6 showed greater inhibition to mycelial growth of *P. infestans in vitro*. Phung *et al.* (2015) examined the in vitro and in vivo effects of *Chaetomium globosum*, *Chaetomium lucknowense*, *Chaetomium cupreum* and their crude extracts as biological control agents in controlling *Phytophthora nicotianae* causing root rot in citrus, and the result showed that *Chaetomium* species and their crude extracts strongly inhibited the growth of *Phytophthora. nicotianae* KA1.

Bioactivity test of crude extracts from Chaetomium elatum against Pyricularia oryzae causing rice blast.

Antagonistic *C. elatum* was yielded metabolites as crude extracts and examined to control the growth of plant pathogen *P. oryzae*. The results showed that crude ethyl acetate from *C. elatum* gave significantly highest inhibition of the colony growth of *P. oryzae* when compared to the control, followed by crude methanol. All tested crude extracts, ethyl acetate, hexane and methanol crude extracts from *C. elatum* gave significantly inhibition of the colony growth and spore production of *P. oryzae*. It showed that antagonistic fungus *C. elatum* produce bioactive metabolites against rice blast caused by *P. oryzae*. This study was similar to the study of Soytong (2015) that antagonistic fungus of *C. elatum* was proved to antagonize *Fusarium. oxysporum* f.sp. *lycopersici*. Sibounnavong (2011) reported that antifungal substances Chaetoglobosin-C which purified from *C. elatum* showed greater antifungal activity against *F. oxysporum* f. sp. *lycopersici* NKSC02, with an effective dose (ED50) of 5.98 µg/ml.

Testing nano-particles from Chaetomium elatum to control Pyricularia oryzae.

Nano particles of *C. elatum* including nano-CEH, nano-CEE and nano-CEM (Fig. 6) were used to examine for controlling the growth of plant pathogen *P. oryzae*. Result showed high efficacy antimicrobial activity of Nano-CEH, Nano-CEE and Nano-CEM from *C. elatum* against *P. oryzae*. Nano-CEE from *C. elatum* gave significantly highest inhibition for the colony growth of *P. oryzae* at concentration of 1,000 ppm when compared to the control, followed by Nano-CEM. Nano-CEE from *C. elatum* also gave significantly highest inhibition for the spore production of *P. oryzae* Nano-CEM and Nano-CEE also inhibited spore production of rice blast pathogen, This study was similar to the study of Dar. *et al*. (2014) that nano particles of antagonistic fungus of *Chaetomium globosum* and *Chaetomium cuperum* were proved to be antagonized plant pathogens *Fusarium. oxysporum* f.sp. *lycopersici* and *Colletotrichum capsici*.

Conclusion

As the results, *C. elatum* is proved to act as biological activity against *P. oryzae* causing rice blast and nano particles designed from crude extracts of *C. elatum* also can control *P. oryzae* at low concentration. It is useful resource as nature product to inhibit the pathogen which causing rice blast. It is not only reduced the production loss of growers but also reduced to pollute the environment as compared the traditional method.

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