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Fungal Metabolites of *Chaetomium lucknowense* for Inhibition of a Rice Blast Pathogen, *Pyricularia oryzae*

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Pyricularia oryzae was isolated from rice blast and proved for pathogenicity in rice var. Koe-Ko 9. Bi-culture antagonistic test showed efficacy of *Chaetomium lucknowense* significanltly inhibited *P. oryzae* that can be seen clear zone between the pathogen and antagonist. Whenever, the bi-culture plates were incubated for over month, the colony of *Chaetomium* grown over the pathogen colony. The methanol crude extract and ethyl acetate crude extract of *C. lucknowense* were shown to be the most effective inhibition the growth of *Pyricularia oryzae* which significantly differed from the methanol crude extract when compared to the control. The effective of metabolites to control blast pathogen is being investigated.

Keywords: fungal metabolites, *Chaetomium lucknowense*, *Pyricularia oryzae*

Introduction

Oryza sativa (rice) is recognized as one of the most important crops in the world and it provides the main source of energy for more than half of the world population. It is the major food crop in India, China and the rest of Asia where 92% of the world's rice is grown (Gnanamanickam S.S., 2009).On December 16, 2002, the UN General Assembly declared the year 2004 the International Year of Rice. The declaration was sponsored by more than 40 countries. It is one of the three major cereal grains (maize, wheat and rice) that feed the growing population. Rice sustains about 3.5 billion people either partially or fully for caloric intake around the world, mostly in Asia. These achievements in increasing rice production have greatly helped in hunger alleviation in the world (Gnanamanickam S.S., 2009).

Biological control of plant pathogens has been shown to have potential to control many diseases in plantations. *Chaetomium*, *Penicillium Emericella* and

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Trichoderma species are biological control agents that have the potential to control plant diseases. Field trails have shown that *Chaetomium* formulated bioproducts have promise as broad spectrum mycofungicides to control many diseases (Soytong and Soytong, 1997). *Emericella nidulans* was proved to be antagonistic to *Colletotrichum gloeosporioides* (Penz) causing anthracnose of *Vanilla planifolia* by Talubnak and Soytong (2010). *Chaetomium* species are ubiquitous fungi most found in soils and death materials, with more than 350 species exist (Zhang *et al.* 2012). They decompose cellulose and other organic material. Some certain strains of these fungi can act as antagonists against plant pathogens. Hung *et al.* (2015a) presented in vitro evaluation of the capacities of *Chaetomium* spp. to control the *Phytophora palmivora* PHY02 causing root rot of Pomelo, *Chaetomium globosum*, *Chaetomium cupreum*, *Chaetomium lucknowense* as antagonists showed inhibition of mycelium growth and sporangial production of *P. palmivora* PHY02.

Application of chemical fungicides has been recognized to cause environmental pollution and leave chemical residues in the soil, water and agricultural products, and it is known that the continuous use of chemical fungicides leads to the development of resistance in some pathogens (Soytong, 1996). Biological control of plant pathogens is a recent successful strategy for disease control and has successfully been integrated with other control measures. Biological control methods can reduce the heavy use of chemical fungicides, improving the agro-ecosystem and maintaining a natural balance (Soytong *et al*., 1999). There are several reports on the potential use of biological control agents against plant pathogens. *Chaetomium* species are strictly saprobic antagonists and have been shown to be against several plant pathogens, e.g. *Botrytis cinerea* (Kohl *et al*., 1995), *Colletotrichum gloeosporioides* (Noiaium and Soytong, 1999), *Fusarium oxysporum* f. sp. *lycopersici* (Soytong *et al*., 1999a), *Phytophthora palmivora* (Pechprome and Soytong, 1996; Sodsa-art and Soytong, 1998), *P. parasitica* (Usuwan and Soytong, 1998), *Venturia inegualis* (Heye and Andrews, 1983). The screening of *Chaetomium* species as biological control agents has been carried out in Thailand since 1989, resulting in the development of a biological formulation from *C. cupreum* CC1-10 and *C. globosum* CG1-12. The product has now been developed into pellet and powder formulations and registered for a Patent Right No.6266, Intl. cl. 5 AO 1 N 25 / 12 in 1994 (Soytong, 1996).

Rice blast, caused by the fungus *Magnaporthe oryzae* (*Pyricularia oryzae*), is one of the first recorded diseases of rice (Wang G. L., 2009). Valent (2004) considered the rice blast disease as the worldwide which has distributed over 85 countries in all continents where rice is cultivated.

The objective was to test antagonistic ability of Chaetomium lucknowense

to control *Pyricularia oryzae* causing rice blast by using bi-culture antagonistic test and fungal metabolite test.

Materials and methods

The tested pathogen

Pyricularia oryzae from previous experiment was used for the experiment. It was proved to be the virulent pathogenic isolate causing blast symtomp of vrice var Kor Ko9.

Bi-culture antagonistic test

Chaetomium lucknowense was tested 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). The antagonistic fungi and pathogen was separately cultured on PDA with rice flour media 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 was transferred into two separate PDA plates as the controls. Plates were incubated at room temperature (30-32 ℃) for 30 days. Data were collected as diameter of colony (cm) and the number of conidia produced by the pathogen in the bi-culture plates and control plates. A haemcytometer was used to count the number of conidia of pathogen.

Percentage inhibition of pathogen colony growth and conidia production were calculated using the following formula: % inhibition = (A-B) / A ×100.

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

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

Antifungal activity of Chaetomium lucknowense against Pyricularia oryzae

The crude extracts of *Chaetomium lucknowense* was tested for inhibition of *P. oryzae.* The experiment was conducted by using 3×6 factorial in

Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts (including hexane extract, ethyl acetate extract and methanol extract) and factor B represented the different concentrations of crude extracts: 0, 10, 50, 100, 500 and 1,000 ppm. Each crude extract was dissolved in one drop 2% dimethyl sulfoxide (DMSO), and then mixed into 30ml RFA medium before autoclaving at 121℃, 15 lbs for 30 mins. The tested pathogen was cut at the edge of colony with sterilized suction tubes (3mm), and the agar plug of pathogen was transferred to the middle of RFA medium (amending with each crude extracts) plate (5.0mm diameter) in each concentration and incubated at room temperature until the pathogen on the control plates growing full. Observation of abnormal spores and normal spore of pathogen from each treatment were observed under compound microscope and taken photograph for comparison. The data were collected as colony diameter and the number of conidia.

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

Result and Discussion

Bi-culture antagonistic test

Results showed that the antagonistic *Chaetomium lucknowense* CL was significantly inhibited the tested pathogen, *Pyricularia oryzae*. As Soytong (2014) reported *Chaetomium lucknowense* CL that proved to be inhibited *Ganoderma boninense* in bi-culture test at 10 days and observed for longer days of incubation period in bi-culture antagonistic plates, *Chaetomium* grown over and gave a much control of *G. boninense* as the appearance of clear zone or inhibition zone. In this study, it proved that the antagonistic *Chaetomium* can grow over the colony of *P. oryzae*. It is similar demonstrated that *Chaetomium* sp. as the biological agent gave a good control of *Thielaviopsis* Bud Rot of Bottle Palm (*Hyophorbe lagenicaulis*) in Thailand (Soytong *et al*., 2005). Chareon *et al* (2010) reported that *C. lucknowense* CLT gave higher significant inhibition of the mycelial growth of the pathogen, *Fusarium oxysporum* f sp *lycopersici* in bi-culture test.

Antifungal activity of Chaetomium lucknowense against Pyricularia oryzae

In this study, it showed that methanol crude extract and ethyl acetate crude extract of *C. lucknowense* gave the most effective inhibition the growth

of *Pyricularia oryzae* which significantly differed from the methanol crude extract when compared to the control. The research finding was similar reported by Chareon *et al.* (2010) who stated that the ED50 of *Chaetomium* extracted with hexane was 157 µg/ml which gave the highest inhibition of conidial production of *Fusarium oxysporum* f. sp. *lycopersici* (tomato wilt). Crude hexane from *Chaetomium lucknowense* CLT gave ED50 values of 192 µg/ml and clearly showed that antagonistic metabolites could be deformed and break the pathogen cells.

Sibounnavong *et al*. (2011) stated that Chaetoglobosin-C was isolated from *C. luckowense* effectively controlled the most virulent isolate of *Fusarium oxysporum* f. sp. *lycopersici* NKSC02 causing wilt of tomato . Chaetoglobosin-C from *C. lucknowense* showed greater antifungal activity against *F. oxysporum* f. sp. *lycopersici*.

The research finding is being studied on development of microbial nano-particles from *C. lucknowense* for plant immunity, especially in rice.

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