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Antifungal Activity of *Emericella nidulans* against *Pyricularia oryzae* causing Rice Blast

Jiaojiao Song[1*] K. Soytong[1] and S. Kanokmedhakul[2]

[1]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand; [2] Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

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Fungal metabolites of *Emericella nidulans* were tested against *Pyricularia oryzae*. The experiment was designed as two factor factorial experiment in Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts and factor B represented different concentrations of 0, 10, 50, 100, 500 and 1000 ppm. Result showed that EtoAC crude extract and methanol crude extract were the best inhibition of pathogen and were not significantly differed between treatments, and followed by hexane crude extract when compared to the non-treated control. In this study showed that all tested crude extracts affected the pathogen cells to become abnormal cells and possible loss of pathogenicity. Further research finding is to evaluate *Emericella nidulans* to control rice blast pathogen in pot experiment and possible in the field trial.

KeyWords: *Emericella nidulans*, antifungal activity, *Pyricularia oryzae*

Introduction

Rice blast, caused by the fungus *Magnaporthe oryzae* (*Pyricularia oryzae*), is one of the first recorded diseases of rice. It was known as rice fever disease in China as early as 1637 and was reported as *Imochi-byo* in Japan in 1704. As the rice production expanded through Asia, Latin America, and Africa over the last few centuries, the disease followed and is now found in over 85 countries worldwide. *M. oryzae* (*P. oryzae*) attacks all parts of the rice plant causing losses upwards of hundreds of millions of tons of rice grain annually. Such losses have led to rice shortages in many developing countries in recent years, making effective control of this devastating disease imperative for global food security and social well-being. (Wang G. L., 2009).

[*] **Corresponding Author:** Jiaojiao Song; **Email:** misssongjiaojiao@gmail.com

Rice blast is considered the principal disease of rice because of its wide distribution and high incidence under favorable conditions. Valent (2004) considered the disease as the world's chief disease of rice about which a lot has to be learned yet. The disease is distributed in about 85 countries in all continents where rice is cultivated. It is a potentially damaging disease in upland environment where drought and soil stress predispose the rice crop to severe attacks by the pathogen. Yield loss due to blast can be as high as 50% when the disease occurs in epidemic proportions. The damage to the rice crop is often influenced by environmental factors. Rice blast disease finds its place in biological terrorism because of the potential devastation it can cause to rice production (Gnanamanickam S.S., 2009).

Practices for controlling rice blast diseases are largely based on introduction of resistant varieties, cultivation management in fields and application of synthetic fungicides (Zeigler *et al.* 1994). Intensive use of chemical fungicides in control of plant pathogens may lead to harmful to the environment and human health. The need to reduce the use of noxious synthetic fungicides in agriculture production has led to a search for biological control agents against plant pathogens, which are safe for both the environment and human consumption.

Biological control, using microorganisms to suppress plant disease, provides a high potential alternative to the use of synthetic chemicals, is the most cost effective and safety to the environment (Sáenz-de-Cabezón. 2010; Narayanasamy. 2013). *Emericella nidulans* is reported to antagonize *F. oxysporum* f. sp. *lycopersici* (Sibounnavong *et al.*, 2009).

The antimicrobial activity of tajixanthone which is produced by *Emericella rugulosa* ER01 reported to be involved in the disease control mechanism of antagonistic fungus against the tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici*.

The objective was to test antagonistic ability of *Emericella nidulans* to control *Pyricularia oryzae* causing rice blast.

Materials and methods

Isolation and pathogenicity test

Disease samples of blast were collected from infested rice field soil in Thailand. The pathogen was isolated by tissue transplanting techniques using water agar (WA) and potato dextrose agar media until get pure culture. Pure culture of *Pyricularia oryzae* was morphological identified under compound microscope, maintained on PDA slants and deposited at the Biocontrol

Research Unit and Mycology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Pathogenicity test was done using detached leaf method which modified from Soytong and Quimio (1989).

Crude extract bioassay

Antagonistic fungus, *Emericella nidulans* which offered by Dr. Kasem Soytong, KMITL, Bangkok, Thailand was used and it was cultured in potato broth followed the method of Sibiunnavong and Soytong (2011). Crude extracts of *E. nidulans* were tested for inhibition of *P. oryzae*, a rice blast pathogen.

The experiment was performed using two factor factorial experiment in CRD with four replications. Factor A represented crude extracts where A1 = crude hexane, A2 = crude ethyl acetate and A3 = crude methanol. Factor B represented concentrations where B1 = 0 ppm (control), B2=10 ppm, B3 = 50 ppm, B4 = 100 ppm, B5 = 500 ppm and B6 = 1,000 ppm. Crude extract was dissolved in dimethyl sulfoxide (2%) before mixed to PDA, and autoclaved at 121℃ (15 psi) for 30 min. Then, the agar plugs from peripheral colony of the pathogen culture was transferred to the center of Petri dishes of PDA containing each crude extract concentration. All plates were incubated at room temperature.

Data were collected as colony diameter (cm) and number of spore. The effective dose (ED50) was calculated using Probit analysis.

Result and Discussion

Pyricularia oryzae was isolated and tested for pathogenicity to confirm the virulent isolate. This research finding showed that EtoAC crude extract and methanol crude extract were the best inhibition of pathogen and were not significantly differed between treatments, and followed by hexane crude extract when compared to the non-treated control.

The control mechanism may involve antibiosis which Moosophon *et al.* (2009) stated *Emericella rugulosa* is produced prenylxanthones, rugulox-anthones A to C, 14-methoxytajixanthone,-tajixanthone ethanoate, a bicycle(3.3.1)_nona-2, 6-diene derivative named rugulosone, shamixanthone, tajixanthone, 14-methoxytajixanthone-25-acetate, tajixanthone hydrate, tajixanthone methanoate, isoemericellin and ergosterol. Withthis, the bicycle (3.3.1)–nona-2,6-diene deriveative is shown to antimalarial and antimycobacterial activity and cytotoxicity against three cancer cell lines.

Further report was similar as Charoenpoen *et al*. (2010) who stated that crude hexane, crude ethyl acetate and crude methanol from *Ch. lucknowense* CLT inhibited *F. oxysporum* f. sp. *lycopersici*.

In this study, those crude extracts of *Emericella nidulans* expressed antifungal activity against *Pyricularia oryzae*, a rice blast pathogen. The previous reported by Sibounnavong and Soytong (2011) stated that the compound tajixanthone from *E. rugulosa* ER01 could inhibit the tested plant pathogen, *F. oxysporum* f. sp. *lycopersici*.

Moreover, Moosophon *et al*. (2009) reported that *E. rugulosa* ER01 produces tajixanthone which it is expected to imply antibiosis against *P. oryzae*, a rice blast pathogen. Moreover, in this study showed that all tested crude extracts affected the pathogen cells of *Pyricularia oryzae* and become abnormal cells and possible loss of pathogenicity.

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