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Application of Nano-particles Derived from *Chaetomium elatum* ChE01 to Control *Pyricularia oryzae* causing Rice Blast

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Abstract *Pyricularia oryzae* causing rice blast was isolated and proved for pathogenicity. *Chaetomium elatum* ChE01 was proved to be antagonized *P. oryzae* in bi-culture antagonistic test which averaged inhibition of 60.40 % within 15 days. Fungal metabolites from *C. elatum* ChE01 were extracted and tested to inhibit *P. oryzae*. Results showed that crude ethyl acetate expressed antifungal activity against *P. oryzae* which the effective dose$_{50}$ (ED_{50}) was 231 ppm., followed by crude methanol and crude hexane which the ED_{50} were 460 and 2,122 ppm. respectively. It was shown that nano-CCE gave the highest inhibition *P. oryzae* which the ED_{50} was 8.25 ppm, and followed by nano-CEM and nano-CEH which the ED_{50} values were 11.21 and 65.52 ppm, respectively. Further research findings are investigated in pot and field experiments.

Keywords: Nano-particles, *Chaetomium*, Rice Blast

Introduction

The need to discover the alternative ways to safe human and environment as effective and efficient methods to control plant diseases is desired. One of the method is the application of nanotechnology. Nanotechnology is to build, re-structure, control and devise materials to be molecular level. A nanometer (nm) is one-billionth of a meter. Molecular nanotechnology applies to build the organic materials into molecule by molecule for agricultural application that is being studied (Li *et al.*, 2011). The scientists are actively studied the synthesis of organic nanoparticles are still having unusual properties like physical and biological ones (Elibol *et al.*, 2000; Salata, 2004). Application of nanotechnology in agriculture are being investigated (Soutter, 2012). Nanoparticles can be easily penetrate through plant cells (Perlatti *et al*., 2013).

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Bioactive compounds from *Chaetomium* species has been reported by Soytong *et al*. (2001; 2013) as antifungal agent against several phytopathogens. It is safe for environmentally friendly method to control plant diseases (Dar and Soytong, 2014). Emmanuel *et al.* (2013) reported crude extracts of *Chaetomium globosum* coud inhitbit Philippine strain of *P. oryzae*. Soytong *et al.* (2013) found that pure compounds of Chaetoglobosin C, chaetomanone A derived from *Chaetomium* sp. act as microbial elicitors to elicit tomatine in tomato leading to immunity against *Fusarium oxysporum* f sp *lycopersici* causing tomato wilt. Crude extracts and pure compound derived from *Chaetomium* sp were confirmed to be effectively inhibited several plant pathogens. Research finding is further investigated to be nano-particles of those metabolites or compounds to control plant pathogen effectively. Dar and Soytong (2014) developed nano-particles from *C. globosum* and *Chaetomium cupreum* to test for inhibition of several plant pathogens. Tann and Soytong (2016) reported that nano-CGH, nano-CGE, and nano-CGM from *C. 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.

C. elatum ChE01 was identified and studied its metabolites as reported by Thohinung *et al.* (2010) that it produced a new chaetoglobosin V, two new natural products, prochaetoglobosin III and prochaetoglobosin III(ed), six known chaetoglobosins B-D, F , and G and isochaetoglobosin D. All these pure compounds expressed cytotoxicity against the human breast cancer (IC_{50}) 2.54-21.29 microM and cholangiocarcinoma cell lines (IC_{50}) 3.41-86.95 microM. This isolate was proved to inhibit *P. oryzae* causing rice blast by using crude extracts and further research investigated those crude extracts to be nano-particles. Preliminary research finding found that nano-particles of *C. elatum* ChE01 actively expressed antifungal activitytest against *P. oryzae*. Nano-particles derived from *C. elatum* ChE01 as nano-CEH, nano-CEE and nano-CEM were significantly inhibited *P. oryzae* at low concentration (Song and Soytong, 2016). The objective was to prove further evaluation nano-particles derived from *C. elatum* ChE01 to inhibit rice blast caused by *P. oryzae* and control mechanism.

Materials and methods

Rice blast pathogen

The diseased samples were taken from leaves which appeared blast symptom and taken into laboratory. Tissue transplanting technique was done for isolation into pure culture. Pure cultures were morphologically identified.

Antagonistic fungus

Chaetomium elatum ChE01 is derived from Biocontrol Reserch Unit, Department of Plant Production Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok. It was cultured on potato dextrose agar (PDA) at room temperature (27-30 C) for 3 weeks for studying morphological characters under binocular compound microscope.

Bi-culture antagonistic test

Treatments were bi-culture between pathogen and antagonist plate and pathogen alone which followed the method of Soytong and Quimio (1989). The agar culture of *C. elatum* ChE01 was cut by sterilized cock borer at peripheral colony and transferred to one side of PDA plate and the agar culture plug of pathogen was done the same and placed in opposite site at equal distance. Bi-culture plates were incubated at room temperature for 30 days.

Bioactivity test of crude extracts from Chaeomium elatum ChE01

Crude extracts from *C. elatum* ChE01 was done by followed the method of Phonkerd *et al*. (2008). Each crude extract with hexane, ethyl acetate and methanol was tested to inhibit the rice blast pathogen, *P. oryzae* at the concentrations of 0, 10, 50, 100, 500 and 1000 ppm. An agar plug of pathogen was moved the middle of potato dextrose agar (PDA) incorporated with each crude extract. The tested plates were incubated at room temperature (30 C) for 15 days.

Nano-particle testing for inhibition of rice blast pathogen

Nano –particles derived from Chaetomium sp was done by followed the method of Dar and Soytong (2014). The tested nano-CCH, nano CCE and nano-CEM) of *C. elatum* ChE01 was tested to inhibit *P. oryzae* causing rice blast with different concentrations (0, 3, 5, 7, 10 and 15 ppm.). A plug of rice blast pathogen was transferred onto the middle of each concentration plate containing PDA, then incubated at room temperature (30 C) for 15 days.

Statistical analysis

Bi-culture antagonistic test was set up using Completely Randomized Design (CRD) with four replications. The bioactivity tests were performed

using two factor factorial experiment in Completely Randomized Design with four replications. Data were collected as colony diameter (cm), number of spores and statistically computed analysis of variance. Treatment means were compared using DMRT at p = 0.05 and 0.01. The Effective dose at 50 % (ED_{50}) was computed by probit analysis program.

Results

Rice blast pathogen

Rice blast specimens were collected from rice-fields and brought into laboratory. Isolation was done by using tissue transplanting technique. Pure culture of *Pyricularia oryzae* wa cultured in rice flour agar (RFA) at room temperature (27-30 C) and morphological identified (Fig.1) and tested for pathogenicity.

Antagonistic fungus

Chaetomium elatum ChE01 offered from Biocontrol Research Laboratory, and culture on potato dextrose agar (PDA) for 3 weeks. It is an ascomycetous fungus which produces perithecia, asci and ascospores (Fig.2).

Bi-culture antagonistic test

Result showed that *C. elatum* ChE01 expressed antifungal activity against *P. oryzae* causing rice blast disease in bi-culture antagonistic test averaged 60.40 % within 15 days. The colony diameter in control plate was 9.00 while colony in bi-culture plate was averaged 3.56 cm. (Fig. 3). The colony of *C. elatum* ChE01 grew over the pathogen colony.

Bioactivity test of Crude extracts from antagonistic fungi

Crude extracts from *C. elatum* ChE01 were tested to inhibit *P. oryzae* by poisionous technique. Result showed that crude hexane, crude ethyl acetate and crude methanol at concentration 0f 1,000 ppm gave significantly highest inhibition of colony growth which averaged of 2.73, 1.48 and 2.13 cm., respectively (Table 1) while the control (o ppm) was 5.00 cm. However, crude ethyl acetate gave the most highly significant inhibited sporulation (3.93×10^6 spores), and followed by crude methanol and crude hexane which were 2.56×10^6 and 3.93×10^6spores), respectively. Moreover, crude ethyl acetate

expressed antifungal activity against *P. oryzae* which the effective dose$_{50}$ (ED$_{50}$) was 231 ppm and followed by crude methanol and crude hexane which the ED$_{50}$ were 460 and 2,122 ppm, respectively (Table 1).

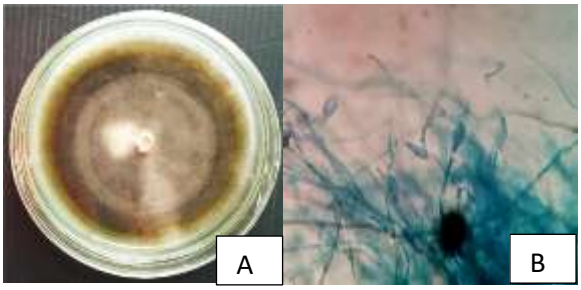

Figure 1. Rice blast pathogen *Pyriculria oryzae*, A = culture on PDA and B = Conidia

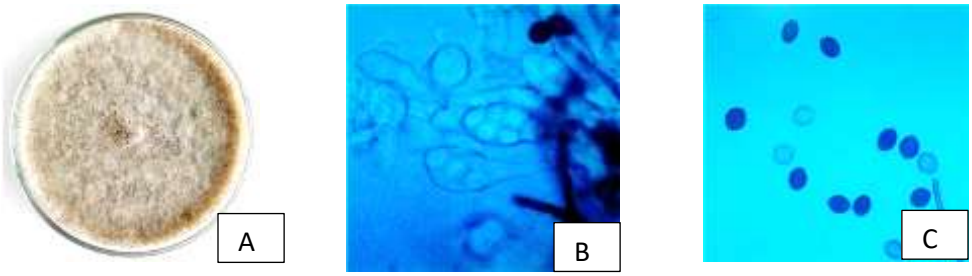

Figure 2. Antagonistic fungus *Chaetomium elatum*, A = culture on PDA, B = Asci, C = bi-culture test and D = ascospores

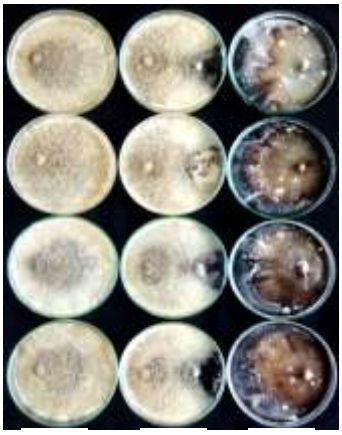

Figure 3. Bi-culture antagonistic test between *Chaetomium elatum* and *Pyricularia oryzae*, A = antagonist, B = Bi-culture and C = pathogen

Table 1. Crude extracts of *Chaetomium elatum* testing for growth inhibition of *Pyricularia oryzae* at 8 days, spore production inhibition at 15 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[/1]	Growth inhibition (%)	Number of spores [/1](10^6)	Spore Inhibition (%)[/2,3]	ED_{50}(ppm)
	0	5.00[a]	-	6.50[a]	-	
	10	4.73[b]	5.25[l]	6.25[ab]	3.61 [i] 2.25	
Crude	50	4.47[c]	10.50[k]	5.87[abc]	8.96[ghi]	2122
Hexane	100	4.27[d]	14.50[j]	5.43[bcd]	15.54[fgh]	
	500	3.67[f]	26.50[h]	5.12[cde]	20.29[fg]	
	1000	2.73[i]	45.25[e]	3.93[fgh]	38.68[cd]	
	0	5.00[a]	-	6.50[a]	-	
	10	4.36[cd]	12.75[jk]	5.93[abc]	8.25 [hi]	
Crude	50	3.57[f]	28.50[h]	4.31[efg]	33.70 [de]	
EtOAc	100	3.02[h]	39.50[f]	3.31[hij]	49.06[bc]	231
	500	2.00[k]	60.00[c]	2.62[ij]	59.56[ab]	
	1000	1.48[l]	70.25[b]	2.37[j]	63.43 [a]	
	0	5.00[a]	-	6.50[a]	-	
	10	4.64[b]	7.00[l]	6.06[abc]	6.53 [hi]	
Crude	50	4.12[e]	17.50[i]	5.31[bcd]	27.27 [ef]	
MeOH	100	3.23[g]	35.25[g]	4.81[def]	35.21 [de]	460
	500	2.13[j]	57.25[d]	3.75[gh]	42.06 [cd]	
	1000	1.48[m]	69.00[a]	2.56[ghi]	58.62[ab]	
C.V.(%)		1.70	4.23	12.89	19.01	

[1]/Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.
[2]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Nano-particle testing for inhibition of rice blast pathogen

Result showed that nano-CCE at 15 ppm gave highly significant inhibited colony growth of *P. oryzae* (3.00 cm) and followed by nano-CEM and

nano-CEH 3.40 and 3.65 ppm., respectively. Nano-CEH gave significantly better inhibited sporulation of *P. oryzae* (3.43 X 10^6 spores) than nano-CEE (1.25 X 10^6 spores) and nano CEM (1.81 X 10^6 spores). Morever, nano-CEE gave the highest inhibition *P. oryzae* which the ED_{50} was 8.25 ppm, and followed by nano-CEM and nano-CEH which the ED_{50} values were 11.21 and 65.52 ppm, respectively (Table 2). Nano-CEE at 15 ppm gave highest significantly in spore inhibition of *P. oryzae* (79.39 %) and followed by nano-CEE at concentration of 5 ppm (41.18 %) and nano-CEH at 14 ppm (42.29%).

Table 2. Nano particles of *Chaetomium elatum* ChE01 testing for growth inhibition of *Pyricularia oryzae* at 8 days, spore production inhibition at 15 days and effective dose (ED_{50}) values.

Crude extracts	Concentration (ppm)	Colony diameter (cm)[/1]	Growth inhibition (%)[/2]	Number of spores [/1](10^6)	Spore Inhibition (%)[/2]	ED_{50}(ppm)
	0	5.00[a]	-	6.00 [a]	-	
	3	4.79[b]	4.00[i]	5.68 [bcd]	4.85 [i]	
Nano-	5	4.60[de]	8.00[fg]	4.81 [bcd]	18.80 [g]	65.52
CEH	10	4.00[g]	10.00[f]	4.12 [de]	30.15 [f]	
	15	3.65[h]	20.00[d]	3.43 [ef]	42.29 [e]	
	0	5.00[a]	-	6.00 [a]	-	
	3	4.64[cd]	7.00[jh]	4.56 [cd]	23.43 [g]	
Nano-	5	4.30[f]	14.00[e]	3.50 [ef]	41.18 [e]	
CEE	10	3.60[h]	28.00[c]	2.18 [g]	63.28 [c]	8.25
	15	3.00[j]	40.00[c]	1.25 [hi]	79.39 [b]	
	0	5.00[a]	-	6.00 [a]	-	
	3	4.75[bc]	5.00[hi]	5.31 [abc]	11.60 [h]	
Nano-	5	4.50[e]	10.00[f]	4.68 [cd]	21.32 [g]	
CEM	10	3.95[g]	21.00[d]	3.06 [f]	49.20 [d]	11.21
	15	3.40[i]	32.00[b]	1.81 [gh]	70.03 [c]	
C.V.(%)		1.17	7.17	12.89	19.01	

[1]/Average of four replications. Means followed by a common letter 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.

Discussion

P. oryzae causing rice blast was isolated and proved for pathogenicity. Te Beest (2007) stated that rice blast, caused by a fungus, *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae***)** and it infected rice to appear the symptom on leaves, stems, peduncles, panicles, seeds, and roots. *C. elatum* ChE01 used in this experiment as the same isolate that reported by Thohinung *et al.* (2010) which that it produced antimicrobial activity eg. chaetoglobosin V, prochaetoglobosin III and prochaetoglobosin III against the human breast cancer and cholangiocarcinoma cell lines. These compounds may possible actively prove as antibiosis in bi-culture antagonistic test that *C. elatum* ChE01 may possible release to be antagonize *P. oryzae*. Moreover, fungal metabolites from *C. elatum* ChE01 were extracted and tested to inhibit *P. oryzae.* The crude ethyl acetate gave better antifungal activity against *P. oryzae* than crude methanol and crude hexane. Similar results was reported by Sibounnavong *et al*. (2012); Soytong *et al.* (2013) which reported that crude extracts from *Chaetomium* spp. actively inhibited *Fusarium oxysporum* f sp *lycopersici* causing tomato wilt. The research finding revealed that nano-CCE gave the highest inhibition *P. oryzae* which the ED50 was 8.25 ppm, and followed by nano-CEM and nano-CEH which the ED_{50} values were 11.21 and 65.52 ppm, respectively. Tan and Soytong (2016) reported that Nano-CGH, nano-CGE, and nano-CGM derived from *Chaetomium globosum* KMITL-N0805 gave a good control *Curvularia lunata* causing leaf spot disease of rice var. Sen Pidoa. Moreover, the effect of nanoparticles showed to be broken the pathogen cell and lost pathogenicity.

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