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Full Length Article

Natural Product of Nano-Particles Constructed from *Chaetomium* spp. to Control Rice Blast Disease Caused by *Magnaporthe oryzae*

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Abstract

Nanoparticles containing active compounds derived from *Chaetomium cochliodes* (CTh05) was tested to control rice blast disease caused by *Magnaporthe oryzae* isolate PO1. The causal agent of rice blast on leaves of rice var. RD57 was isolated. *M. oryzae* and *C. cochliodes* were identified morphologically and confirmed by molecular phylogenetic. *M. oryzae* was demonstrated to be pathogenic, causing blast of rice var. RD57. Biculture tests demonstrated that *C. cochliodes* could suppress the growth of *M. oryzae*. The crude metabolites of *C. cochliodes* (CCoH, CCoE and CCoM) at the concentrations of 10–1000 ppm expressed antifungal activity against the *M. oryzae*, resulting in inhibited spore production in 12 days with effective dose (ED_{50}) values of 85, 144 and 374 ppm, respectively. The nanoparticles sizes of nano-CCoH, nano-CCoE and nano-CCoM were ranged between 567–611, `422–566 and 415–472 nm, respectively. Nanoparticles derived from *C. cochliodes* (nano-CCoH, nano-CCoE and nano-CCoM) at concentrations of 3–15 ppm significantly inhibited *M. oryzae* in 12 days with ED_{50} values of 9, 16 and 33 ppm, respectively. *In vivo* experiments revealed a reduction of 38% in blast disease after application of nanoparticles constructed from crude extract mixtures from *C. cochliodes* at 10 ppm for 30 days. Tricyclazole resulted in reduction of blast disease by 29%. Rice blast disease was decreased in 30 days after applying nano-CCoM at the concentration of 7 ppm (60% disease reduction), followed by nanoCCoE and nanoCCoH with disease reduction of 58 and 50% respectively, and tricyclazole resulted in a 56% reduction in rice blast.

Keywords: *Chaetomium* spp.; *Magnaporthe oryzae*; Nanoparticles; Rice blast

Introduction

Rice blast is a disease caused by *Magnaporthe oryzae* (Hebert) Barr. This disease is the first recorded of rice (*Oryza sativa* L.) and it was noted as rice fever disease in China as early as in 1637 (Wang and Valent 2009). Rice blast has spreaded through out in Asia, Latin America and Africa, and is now reported in over 85 countries worldwide (TeBeest *et al*. 2007). *M. oryzae* can infect all parts of the plant, which resulted in yield losses in many developing countries in recent years (Wang and Valent 2009). Rice blast has become the most common rice disease due to its wide distribution and high infection level under favourable conditions. Valent (2004) stated that the disease has already caused epidemics in all continents where rice is grown, and yield loss due to blast can be as high as 50% (Gnanamanickam 2009). The effective disease control strategies are needed to reduce or eliminate the use of chemical fungicides, which damaging to the surroundingenvironments and residue in agricultural products. Nanotechnology in agriculture has emerged as a new tool to create and re-structure the materials at the molecular level. Molecular nanotechnology involves in constructing organic materials into defined structures, atom by atom or molecule by molecule (Soutter 2013). The application of nanotechnology in agriculture has gained an interestingattention in recent years (Li *et al*. 2011). Researchers have actively investigated the synthesis of organic nanomaterials in various types andtested their biological properties (Elibol *et al*. 2003; Salata 2004). Nanotechnology in agriculture is being explored for crop production (Soutter 2013) and may bepotentially provided the solutions for various challenges faced in agriculture (Ditta 2012). Nanoparticles contain bioactive substances from natural products that can rapidly and effectively penetrate through plant cuticles and tissues and can increase

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the stability of active compounds to decrease leaching (Perlatti *et al*. 2013). Thus, it can provide an efficient pest management strategy in agriculture (Rai and Ingle 2012). These can be formulated in colloidal suspension or powder for application (Ditta 2012).

In recent year, the natural products from the fungus *Chaetomium* spp. reported to be antifungal activity against several plant pathogens (Soytong *et al*. 2001). Nanoparticles were constructed from crude hexane, ethyl acetate and methanol extracts of *Chaetomium globosum* KMITL-N0805, whichcoded as Nano-CGH, nano-CGE and nano-CGM, actively inhibited *Curvularia lunata* (Wakker) Boedijn in rice var. Sen Pidoa, with ED_{50} values of 1.21, 1.19 and 1.93 ppm, respectively (Tann and Soytong 2016). The findings are demonstrated *in vivo* tests that nano-CGH, nano-CGE and nano-CGM from*C. Globosum* can be controlled leaf spot of rice at 60 days; nano-CGH and nano-CGM decreased disease of 61.54% and nano-CGE decreased disease incidence by 53.83% (Tann and Soytong 2016). Furthermore, Tann and Soytong (2017) reported that nano-product derived from *C. cupreum* L.M Ames reduced the rice leaf spotranging from 41.7–58.3% compared to the non-treated controlin pot experiment.

The objective of the current research was to evaluate the nanoparticles constructed from natural products of *C. cochliodes* CTh05 for their biological activities against *M. oryzae*, which causes rice blast and their application for disease control.

Materials and Methods

Isolation of pathogens and pathogenicity test

The blast specimens were collected from the symptomatic leaves of rice var. RD 57 in rice fields at the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand on 12 June 2017. Isolation was performed using a tissue transplanting technique (Abed-Ashtiani *et al*. 2016) and a pure culture was maintained in rice flour agar (RFA) (rice flour 25 g, yeast 2 g and agar 15 g) media. Morphological identification using a binocular compound microscope was done according to the work of Ou (1985). The pathogen was deposited as culture collection No. KMILT 001/2017 at the Biocontrol Research Unit, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Antagonistic fungus

C. cochliodes CTh05, used in the current study, has been reported by Phonkerd *et al*. (2008) to produce four dimeric spiro-azaphilones; (cochliodones A to D), two azaphilones; (chaetoviridines E and F) and an epi-chaetoviridin A, which expressed antimicrobial activity against malaria disease (*Plasmopara falciparum* Welch), tuberculosis (*Mycobacterium tuberculosis* Zopf) and cancer cell lines. *C. cochliodes* CTh05 was cultured in potato dextrose agar (PDA) and incubated at room temperature (27–30°C). The morphological identification was performed according to Arx *et al*. (1986) and Soytong (1989).

Molecular identification

The fungal genomic DNA was separately extracted from *M. oryzae* PO1 and *C. cochliodes* CTh05. Each fungus was cultured for 3 d in potato dextrose broth (PDB). The genomic DNA was done from freeze-dried mycelia using the modified cetyl trimethyl ammonium bromide (CTAB) method. The mycelia were cleaned with 25 m*M* ethylene diamine tetraacetic acid (EDTA) by centrifugation at 14 000 rpm at 4°C for 5 min, then, 100 mg of mycelia were crushed in liquid nitrogen, and lysed in CTAB buffer containing β-mercaptoethanol (2 μL). The lysate was extracted with an equal volume of chloroform/isoamyl alcohol (24:1) and then centrifuged at 14 000 rpm at 4°C, for 5 min then, 2 μL of Rnase (20 μg mL) was added to the aqueous phase and incubated at 37°C for 30 min. The samples were mixed with 50 μL of 10% CTAB and centrifuged. The pellets were washed twice with 70% and 95% ethanol and dissolved in 100 μL TE (Tris-EDTA) buffer at 37°C overnight. The quality and quantity of extracted DNA were monitored by electrophoresis on a 1% agarose gel. Quantification of the DNA was performed by comparing the intensity of the bands to known dilutions of lambda phage DNA. Polymerase chain reaction (PCR) was done to amplify the internal transcribed spacer (ITS) ribosomal DNA regions using the universal primers ITS1 and ITS4, according to the method of White *et al*. (1990).

The amplified products were sequenced and aligned with sequencing in the GeneBank by the basic local alignment search tool (BLAST) (Altschul *et al*. 1997) at the National Centre for Biotechnology Information (NCBI) database. The sequences of closely related organisms were downloaded to construct the phylogenetic trees, which were aligned through CLUSTALW using MEGA version 6.0 software (Tamura *et al*. 2007). The phylogenetic tree was done according to the neighbour-joining method.

Biculture test

The biculture test was conducted by following the method described by Soytong and Quimio (1989). The experimental design was done by completely randomized design (CRD) with four repeated experiments. *C. cochliodes* CTh05 and *M. oryzae* were transferred separately on agar plugs (0.3 cm diameter) to RFA at opposite sites in bi-culture plates. *C. cochliodes* CTh05 and the rice blast pathogen were separately cultured and each isolate on RFA served as the control. All plates were maintained at room temperature for 30 d. Data collection were recorded on colony diameter (cm), number of spores and were computed analysis of variance (ANOVA) by the Statistical Package for Social

Sciences (IBM SPSS Statistics, ver. 21.0) software (Titone *et al*. 2015). Significance was declared at $P \leq 0.05$ and 0.01.

Bioactivity tests of crude metabolites from *C. cochliodes* CTh05

Crude extracts from *C. cochliodes* CTh05 were cultured in PDB at room temperature (30°C) for 30 d. The dried fungal biomass culture was separatelyextracted with hexane, ethyl acetate and methanol following the method described by Phonkerd *et al*. (2008). The experiment was designed as a two-factor factorial experiment with a CRD and four replications. Factor A represented the crude extracts hexane, ethyl acetate and methanol, and factor B represented theconcentrations including 0, 10, 50, 100, 500 and 1000 ppm. A culture agar plug of 3 mm was transferred to the middle of RFA plate in each treatment and then incubated at room temperature (27–30°C) for 15 d. The data were presented as the colony diameter (cm), and the number of spores was determined using a hemocytometer. The data were analysed using ANOVA by SPSS software ver. 21.0 (Titone *et al*. 2015). Significance was declared at $P \leq 0.05$ and 0.01. The effective dose (ED_{50}) was done using the probit analysis program (Titone *et al*. 2015).

Bioactivity tests of crude metabolite nanoparticles from *C. cochliodes* CTh05

The morphological characteristics of the nano-CCoH, nano-CCOE, and nano-CCoM were viewed under a scanning electron microscope. The crude extracts from *C. cochliodes* CTh05, including crude hexane, ethyl acetate and methanol extracts, were separately incorporated into polylactic acid-based nanoparticles through electrospinning, following the method described by Dar and Soytong (2014) and Tann and Soytong (2016) to yield nanoparticles from crude hexane, ethyl acetate and methanol extrtacts of *C. cochliodes* CTh05, coded as nano-CCoH, nano-CCoE and nano-CCoM. The nanoparticle products were collected and stored in capped bottles after electrospinning. The characteristics of the nano-CCoH, nano-CCoE and nano-CCoM were observed by the naked eye and viewed under a scanning electron microscope and the properties were analysed by Fourier-transform infrared spectroscopy (FTIS).

The nano-CCoH, -CCoE and -CCoM were tested for their abilities to inhibit the rice blast pathogen. The experiment was performed using a two-factor factorial CRD with four replications. Factor A represented the type of nanoparticles and factor B the concentrations (0, 3, 5, 7, 10 and 15 ppm). The experiment was repeated four times. The data presented as colony diameter (cm) and the number of spores. Statistical significance was determined using ANOVA by SPSS software, ver. 21.0 (Titone *et al*. 2015). Significance was declared at $P \leq 0.05$ and 0.01. The effective dose (ED50) was calculated using the probit analysis program (Titone *et al*. 2015).

***In vivo* nanoparticles constructed from *C. cochliodes* CTh05 against rice blast disease**

The experimental design was used as randomized complete block (RCB) with four replicates. The treatments were performed as follows: Treatment 1 was the non-inoculated control, Treatment 2 was the inoculated control, Treatment 3 was the nanoparticles from a crude extractmixture of *C. cochliodes* CTh05 at 10 ppm, and Treatment 4 was the chemical fungicide (tricyclazole) at the recommended rate of 2.25 g L^{-1}. The data were recorded as the fresh and dry weight of the stems at 90 d and computed analysis of variance. Mean comparison in each treatment was done by SPSS software, ver. 21.0, and significance was declared at $P \leq 0.05$ and 0.01. Plants were assigned a disease index at 7 d post-inoculation using a scale of 0–9 (modified from Xia *et al*. 1993) where 0 = no infection, 1 = small brown spot infection < 1 mm; 2 = small rounded spot infection < 2 mm; 3 = small spot infection with open centres < 3 mm; 4 = lesions with expanded open centres > 3 mm on < 10% of the leaf area; 5 = lesions with expanded open centres on 10–25% of the leaf area; 6 = lesions with expanded open centres on 26–50% of the leaf area; 7 = expanded lesions with open centres on 51–75% of the leaf area; 8 = expanded lesions with open centres on 76–90% of the leaf area; 9 = expanded lesions with open centres on > 90% of the leaf area.

***In vivo* nano-particles from *C. cochliodes* CTh05 against rice blast disease**

This experiment was designed as a RCBD with four replications. Nanoparticles derived from hexane, ethyl acetate and methanol crude extracts from *C. cochliodes* CTh05 were separately appliedat a concentration of 7 ppm to the rice seedlings inoculated with *M. oryzae* PO1. The treatments were done as follows: non-inoculated control (T1), inoculated with *M. oryzae* PO1 (T2), nano-CCoH (T3), nano-CCoE (T4), nano-CCoM (T5) and tricyclazole (T6). The disease index (DI) was evaluated as described above and disease reduction (DR) was calculated as follows:

$$\text{DR} = \text{averaged DI in treatment} - \frac{\text{averaged DI in inoculated control}}{\text{averaged DI in treatment}}$$

Results

Isolates of rice blast pathogen and *Chaetomium* spp.

The pathogen isolated from symptomatic leaves of rice var. RD57 was morphologically identified as *M. oryzae* isolate PO1 (Fig. 1). The fungus was cultured on RFA which covered the plate (9 cm) in 10 d. The mycelia were observed to be septate and hyaline, producing conidiophores and three-celled conidia. *C. cochliodes* CTh05, from a previous study by Phonkerd *et al*. (2008), was cultured on PDA for 3 weeks and was olive-green to brown, producing perithecia

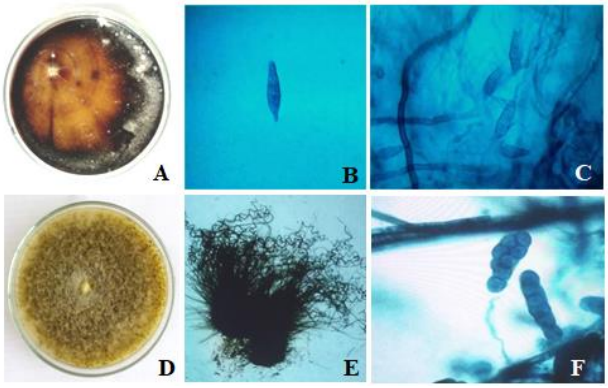

Fig. 1: *Magnaporthe oryzae* PO1, **A** = pure culture in RFA; **B** = conidium; **C** = mycelia and conidia and *Chaetomium cochliodes* CTh05, **D** = Colony, **E** = Perithecia, **F** = Asci and ascospores

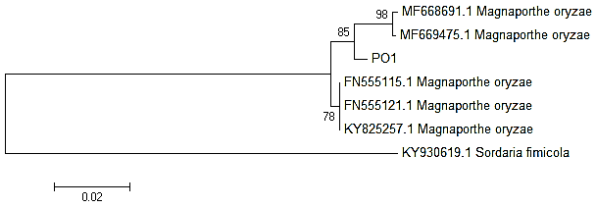

Fig. 2: Phylogenetic tree of *Magnporthe oryzae* from GenBank, including *Magnporthe oryzae* PO1, constructed based upon the distance-based analysis of the ITS1 and 5.8S regions of rDNA. The numbers at the branches indicate the percentage of bootstrap values after 1000 replications. The outgroup taxon is *Sordaria fimicola*

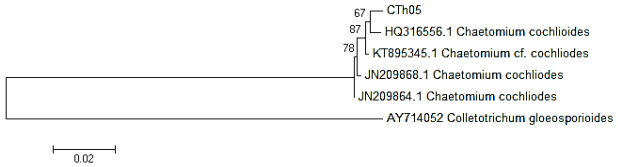

Fig. 3: Phylogenetic tree of *C. cochliodes* from GenBank, including *C. cochliodes* CTh05, constructed based upon the distance-based analysis of the ITS1 and 5.8S regions of rDNA. The numbers at the branches indicate the percentage of bootstrap values after 1000 replications. The outgroup taxon is *Colletotrichum gloeosporioides*

and subglobose asci; one ascus containing eight ascospores (Fig. 1).

Molecular phylogenic identification was performed to confirm the species. The phylogenetic treewasclearly identified the rice blast pathogen as *M. oryzae* MH590369, based upon the GeneBank database (Fig. 2). Data from the GeneBank reliably confirmed CTh105 as *C. cochliodes* MH590621 (Fig. 3). The pathogenicity of *M. oryzae* isolate PO1 to rice var. RD57 proved to be the blast pathogen. The inoculated wounds exhibited lesions inroundish to elongated grey necrotic spots, approximately 2–5 mm in diameter within 10 d.

Biculture test

M. oryzae isolate PO1 was inhibited by *C. cochliodes* CTh05. The colony diameter of *C. cochliodes* CTh05 averaged 4.4 cm in bicultureplate whereas the control platewas 9.0 cm. The colony growth inhibition was 52% after 10 d but when the incubation period was extended to 30 d, the colony grew over the pathogen and inhibition averaged above 90%.

Characterization of the nano-particles

The nanoparticles nano-CCoH, nano-CCoE and nano-CCoM, loaded with crude extracts from *C. cochliodes* CTh05, were visually characterized. Nano-CCoH, nano-CCoE and nano-CCoM were white, yellow and light yellow in colour, respectively (Fig. 4). Interestingly, the scanning electron images showed the range of particles size of nano-CCoH, nano-CCoE and nano-CCoM ranged between 567–611, 422–566 and 415–472 nm, respectively (Fig. 4).

Bioactivity test of crude metabolites from *C. cochliodes* CTh05

The results demonstrated that CCoE resulted in the highest spore inhibition of 88%, followed by CCoM and CCoH, which resulted in a spore inhibition of 81 and 68%, respectively, in 12 d at 1000 ppm (Table 1). The fungal metabolites of CCoH, CCoE and CCoM exhibited active antifungal activity against the *M. oryzae* isolate PO1 with ED_{50} values of 85, 144 and 374 ppm (Table 1).

Bioactivity tests of crude metabolite nanoparticles from *C. cochliodes* CTh05

The nano-CCoH, nano-CCoE and nano-CCoM at 15 ppm showed significantly inhibited spore production ($P \leq 0.01$) in comparison to the non-treated control (0 ppm). Nano-CCoE resulted in significantly ($P \leq 0.05$) greater spore inhibition in comparison to the non-treated control. Nano-CCoE inhibited spore production significantly ($P \leq 0.05$; 68%), followed by nano-CCoM (47%) and nano-CCoH (34%) in 12 d (Table 2). Nano-CCoE, nano-CCoM and nano-CCoH inhibited *M. oryzae* PO1 (rice blast) with ED_{50} values of 9.47, 16.51 and 33.41 ppm, respectively (Table 2). It was observed that the spores were abnormally shaped, and cells were broken after treatment with nanoparticles of *C. cochliodes* CTh05; in contrast, the spores were normally shaped in the non-treated control (Fig. 5).

***In vivo* nanoparticles constructed from *C. cochliodes* CTh05 against rice blast disease**

The result revealed that the blast incidence caused by the *M. oryzae* isolate PO1 was significantly reduced ($P \leq 0.01$) by 38%, after the application of nanoparticles from *C. cochliodes* CTh05, followed by the chemical fungicide

Table 1: The effects of fungal metabolites of *C. cochliodes* CTh05 against *M. oryzae* PO1 at 12 d

Metabolites	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	Number of spores (10^5)	Spore Inhibition (%)	ED_{50}(ppm)
CCoH	0	5.00[a]	-	20.75[a]	-	
	10	4.88[bc]	2.25[ij]	20.25[a]	2.56[j]	
	50	4.73[d]	5.25[gh]	17.25[b]	16.86[h]	374.43
	100	4.43[f]	11.25[e]	14.25[c]	31.23[g]	
	500	3.91[i]	21.75[b]	10.00[e]	52.18[de]	
	1000	3.68[j]	26.25 [a]	6.75[fg]	67.57[c]	
CCoE	0	5.00[a]	-	20.75[a]	-	
	10	4.94[ab]	1.00[j]	17.25[b]	16.70[h]	
	50	4.82[c]	3.50[i]	12.75[cd]	38.64[f]	85.87
	100	4.67[d]	6.50[g]	9.00[ef]	56.69[d]	
	500	4.02[h]	19.50[c]	5.00[gh]	76.01[b]	
	1000	3.64[j]	27.00[a]	2.50[h]	88.03[a]	
CCoM	0	5.00[a]	-	20.75[a]	-	
	10	4.86[c]	2.75[i]	18.75[ab]	9.68[i]	
	50	4.73[d]	4.00[hi]	14.00[c]	32.58[g]	144.23
	100	4.53[e]	9.25[f]	11.00[de]	46.95[e]	
	500	4.11[g]	17.75[d]	7.00[fg]	66.40[c]	
	1000	3.91[i]	21.75[b]	4.00[h]	80.74[b]	
C.V.(%)		0.74	7.22	10.79	6.27	-

Means followed by a common letter are not significantly different by DMRT at $P \leq 0.05$

Table 2: The effects of nano particles derived from *C. cochliodes* CTh05 against *M. oryzae* PO1 at 12 d

Nano-particles	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	Number of spores (10^5)	Spore Inhibition (%)	ED_{50} (ppm)
Nano-CCoH	0	5.00[a]	-	69.00[a]	-	
	3	4.96[ab]	0.75[e]	67.00[ab]	2.78[i]	
	5	4.88[bc]	2.25[de]	56.00[bcd]	18.98[f]	33.41
	10	4.82[cd]	3.50[cd]	50.75[cd]	26.63[e]	
	15	4.76[d]	4.75[c]	45.00[de]	34.87[cd]	
Nano-CCoE	0	5.00[a]	-	69.00[a]	-	
	3	4.93[ab]	1.25[e]	60.00[abc]	13.23[fg]	
	5	4.87[bc]	2.50[de]	49.00[cd]	28.99[de]	9.47
	10	4.78[d]	4.25[cd]	36.00[e]	47.99[b]	
	15	4.58[e]	8.25[b]	22.00[f]	68.26[a]	
Nano-CCoM	0	5.00[a]	-	69.00[a]	-	
	3	4.87[bc]	2.50[de]	64.00[ab]	7.36[gh]	
	5	4.77[d]	3.75[cd]	51.25[cd]	26.00[e]	16.51
	10	4.56[e]	8.75[b]	44.00[de]	36.40[c]	
	15	3.88[f]	22.25[a]	36.00[e]	47.97[b]	
C.V.(%)		1.18	25.04	11.03	13.91	-

Means followed by a common letter are not significantly different by DMRT at $P \leq 0.05$

treatment (tricyclazole), which reduced blast incidence by 29% when compared to the inoculated controlwith *M. oryzae* PO1 (Table 3). In the *in vivo* experiment, all treatments resulted in significant differences ($P \leq 0.01$) in plant height at 15 d post-treatment (Fig. 6). The application of nanoparticles of *C. cochliodes* CTh05 resulted in the greatest plant height (84.7 cm), which was significant at $P \leq 0.01$, followed by the chemical fungicide (tricyclazole), which resulted in a plant height of 74.9 cm; the non-inoculated control and the control inoculated with *M. oryzae* exhibited heights of 77.7 and 77.6 cm, respectively. The application of nanoparticles from *C. cochliodes* CTh05 and tricyclazole were not significant differences in stem fresh weight, 64.7 and 66.7 g, respectively, but they were significantly differed ($P \leq 0.01$) from the non-inoculated control and the inoculated with *M. oryzae*, 57.2 and 45.5 g, respectively. The root fresh weight showed the highest (79.4 g) in plants which treated with nanoparticles of *C. cochliodes* CTh05, followed by the non-inoculated control, the inoculated with *M. oryzae*, and the plants treated with tricyclazole treatments: 66.6, 58.0 and 44.2 g, respectively (Table 3). The nanoparticles of *C. cochliodes* resulted in significantly ($P \leq 0.01$) higher stem dry weight (14.1 g) compared with tricyclazole (11.1 g) and inoculation with *M. oryzae* alone 12.0 g (Table 3). The root dry weight showed the highest after treatment with tricyclazole (11.3 g), followed by the nanoparticles of *C. cochliodes* (10.5 g) and inoculated with *M. oryzae* isolate PO1 alone (6.4 g).

In vivo nano-particles from *C. cochliodes* CTh05 against rice blast disease

The results demonstrated that 15 d after the treatments, nano-CCoM resulted in ($P \leq 0.01$) greater plant height (43.0 cm) compared with nano-CCoH and nano-CCoE, which resulted in a plant height of 41.0 and 40.5 cm, respectively, and followed by tricyclazole 38.5 cm, meanwhile, the inoculated control showeda plant height of 38.6 cm (Table 4 and Fig. 7). However, 30 d after treatment, plant height did not significantly differ among plants treated with nano-

Table 3: Effect of crude extract mixture nano-particles on plant height, fresh and dry weight of stems and roots and disease reduction of blast in rice var. RD 57

Treatments	Plant height (15 d) (cm)	Plant height (30d) (cm)	Stem Fresh weight (g)	Root Fresh weight (g)	Stem Dry Weight (g)	Root Dry Weight (g)	Disease Reduction (%)
T1(Non-inoculated control)	62.0^{a}	77.74^{b}	57.23^{b}	66.55^{b}	17.81^{a}	8.82^{b}	-
T2 (Inoculated with *M. oryzae)*	62.6^{a}	77.58^{b}	45.53^{c}	58.03^{c}	12.01^{c}	6.42^{b}	-
T3(*M. oryzae* + nanoparticles of *C. cochliodes*)	63.3^{a}	84.67^{a}	64.70^{a}	79.41^{a}	14.05^{b}	10.51^{a}	37.5
T4 (*M. oryzae* + Tricyclazole)	62.1^{a}	74.91^{b}	66.66^{a}	44.20^{d}	11.12^{cd}	11.28^{a}	29.1
CV(%)	2.52	12.48	22.82	29.87	29.29	28.96	-

Means followed by a common letter are not significantly different by DMRT at $P \leq 0.05$

Table 4: Effect of nanoparticles derived from hexane, ethyl acetate and methanol extraction plant height and disease reduction (%) on rice var. RD 57

Treatments	15 (d)	30 (d)	Disease reduction (%)
T1 (Non-inoculated control)	38.70^{c}	54.50^{c}	-
T2 (Inoculated with *M. oryzae*)	38.60^{c}	53.75^{c}	-
T3 (Nano-CCoH)	41.00^{ab}	62.25^{b}	50.2
T4 (Nano-CCoE)	40.50^{b}	76.25^{a}	57.5
T5 (Nano-CCoM)	43.00^{a}	77.75^{a}	59.8
T6 (Tricyclazole)	38.50^{c}	75.00^{a}	55.5

Means followed by a common letter are not significantly different by DMRT at $P \leq 0.05$

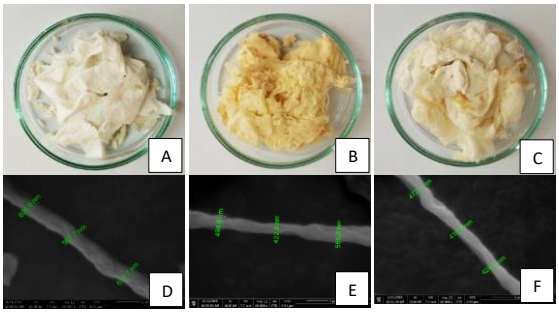

Fig. 4: Nanoparticles from *C. cochliodes* CTh05 (upper part: **A**= nano-CCoH, **B** = nano-CCoE, **C** = nano-CCoM) and scanning electron microscopy of nano-particles (lower part: **D** = nano-CCoH, **E** = nano-CCoE and **F** =nano-CCoM)

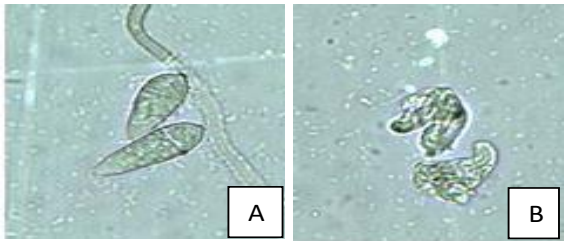

Fig. 5: Normal spores of the rice blast pathogen (**A**) and abnormal spores (**B**) of the *M. oryzae* isolate PO1 after treatment with nanoparticles derived *C. cochliodes* CTh05

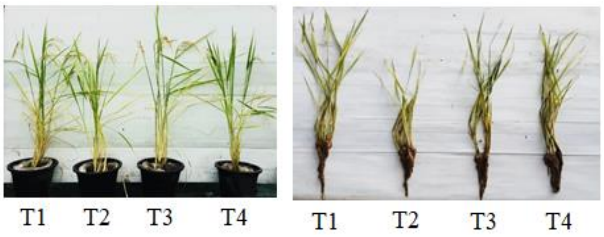

Fig. 6: Testing the capacity for nanoparticles derived from *C. cochliodes* CTh05 to inhibit rice blast, T1 = non-inoculated control, T2 = inoculated with *M. oryzae*, T3 = *M. oryzae* + nanoparticles of *C. cochliodes*, and T4 = *M. oryzae* + Tricyclazole

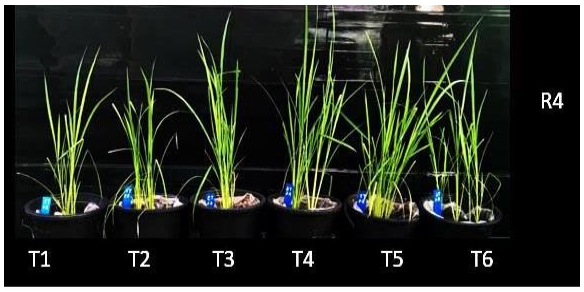

Fig. 7: Testing the capacity of nanoparticles derived from *C. cochliodes* CTh05 in inhibiting rice blast 15 days after treatments, non-inoculated control (T1), inoculated with *M. oryzae* (T2), nano-CCoH (T3), nano-CCoE (T4), nano-CCoM (T5) and tricyclazole (T6)

CCoM, nano-CCoE and tricyclazole; for these groups, plant heights were 77.8, 76.3 and 75.0 cm, respectively and followed by the nano-CCoH (62.3 cm) treatment. The inoculated control had a plant height of 53.8 cm. The blast incidence was reduced after treatment with nano-CCoM (60%) more than nano-CCoE (58%), tricyclazole (56%) or nano-CCoH (50%).

Discussion

In this study, *C. cochliodes* CTh05 demonstrated activity against the *M. oryzae* isolate PO1, which causes rice blast. The fungal isolate that caused blast symptoms on rice var. RD57 was morphologically and molecularly identified as *M.*

oryzae. Morphology and molecular techniques confirmed the identity of *C. cochliodes* CTh05. *M. oryzae* isolate PO1 found to be a virulent isolate causing blast of rice var. RD57. Biculture tests showed that *C. cochliodes* CTh05 inhibited the growth of *M. oryzae* PO1. A previous report by Soytong (2014) stated that *C. cochliodes* proved to be antagonistic to *Drechslera oryzae* (brown leaf spot of rice var. Pittsanulok 2). *C. cochliodes* CTh05 significantly inhibited colony growth and spore production of the tested pathogen in biculture tests, as reported by Tann and Soytong (2017) for *C. cupreum* CC3003 inhibiting *C. lunata* (leaf spot of rice).

The fungal metabolites of *C. cochliodes* CTh05 (CCoH, CCoE and CCoM) expressed antifungal activity against *M. oryzae* isolate PO1 and inhibited spore production with ED50 values of 374, 85, 144 ppm. Soytong (2014) reported that metabolites from *C. cochliodes* suppressed the spore production of *D. oryzae* (brown leaf spot of rice) significantly, with an ED50 value of 66.45 ppm. Tann and Soytong (2017) reported that the hexane-crude extract, ethyl acetate-crude extract and methanol-crude extract of *C. cupreum* C3003 inhibited the spore production of *C. lunata* (leaf spot of rice) with ED_{50} values of 6.41, 0.83 and 7.81 ppm, respectively. *C. cochliodes* CTh05, which was tested in the current study, was previously reported to produce chaetoviridines E, chaetochalasin A and 24(R)-5a, 8a-epidioxyergosta-6-22-diene-3b-ol which those compounds inhibited *Plasmodium falciparum*. The cochliodones C, chaetoviridines E, chaetoviridines F and chaetochalasin A exert antimycobacterial activity. Moreover, chaetoviridines E and F inhibitedcytotoxicity against the KB, BC1 and NCI-H187 cell lines (Phonkerd *et al*. 2008). The current study revealed that the fungal metabolites from *C. cochliodes* CTh05 suppressed *M. oryzae* PO1, which causes rice blast.

Nanoparticles derived from *C. cochliodes* CTh05 (nano-CCoH, nano-CCoE and nano-CCoM) demonstrated to inhibit *M. oryzae* PO1 with ED_{50} values of 9, 16 and 33 ppm, respectively. *C. cochliodes* CTh05 has been found to produce the active compoundsagainst human pathogens (Phokerd *et al*. 2008). Those active compounds may possible serve as a control mechanism.

The crude extracts derived from *C. cochliodes* CTh05, CCoH, CCoE and CCoM found to inhibit *M. oryzae* at ED_{50} values of 374, 85 and 144 ppm, respectively. The nanoparticles of nano-CCoH, nano-CCoE and nano-CCoM gave high potential at low application, with ED_{50} values of 33, 9 and 16 ppm, respectively. It is suggested that applying nanoparticles would be less costly than metabolites. A similar report by Tann and Soytong (2016) stated that nano-CGH, nano-CGE and nano-CGM derived from *C. globosum* KMITL-N0805 exhibited antifungal activity against *C. lunata* (leaf spot of rice) with ED_{50} values of 1.21, 1.19, and 1.93 ppm, respectively.

The current study revealed that the spores of *M. oryzae* exhibited abnormal morphology after treatment with nanoparticles from *C. cochliodes* CTh05. It was also reported by Tann and Soytong (2017) that nanoparticles from *C. globosum* can disrupt and distort the spores of *C. lunata* and cause a loss of pathogenicity. Singh *et al*. (2015) reported that nanotechnology in agriculture is being revolutionized by innovative new techniques for disease control. The current research revealed that nanoparticles constructed from fungal metabolites are promising for plant disease control. Dar and Soytong (2014) reported that the fungal metabolites from *C. globosum* and *C. cupreum*, generated to be nanomaterials at the averaged size of 241 and 171 nm, respectively. Tann and Soytong (2016) also reported that nanoparticles from *C. globosum* can disrupt and distort the pathogen cells and cause a loss of pathogenicity. Nano-CCoH, nano-CCoE and nano-CCoM constructed from *C. cochliodes* CTh05 may help to increase the efficiency of application at lower doses to inhibit rice blast pathogen as stated by Sharon *et al*. (2010).

In the current work, blast incidence in rice seedlings treated with nano-particles constructed from a crude extract mixture at 10 ppm was reduced by 38%, and followed by the chemical fungicide treatment (tricyclazole) which reduced blast incidence by 29%. Similar results were reported by Soytong (2014), who stated that bioproducts from *C. cochliodes* CTh05 can control the rice leaf spot caused by *C. lunata*, but at a high application rate of 50 g 20 L^{-1} of water.

Conclusion

In vivo evaluation of nano-CCoM, nano-CCoE and nano-CCoH constructed from crude hexane, ethyl acetate and methanol extracts of *C. cochliodes* CTh05 reduced blast incidence at concentrations of 7 ppm. It suggested that application of nanoparticles constructed from each crude extract (nano-CCoM, nano-CcoE and nano-CCoH) reduced blast incidence at a lower rate of application than nanoparticles derived from a crude extract mixture from *C. cochliodes* CTh05. Further research should investigate these nanoparticles as elicitors for rice immunity to blast disease.

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References

Abed-Ashtiani F, J Kadir, A Nasehi, SR Hashemian-Rahaghi, G Vadamalai, SK Rambe (2016). Characterisation of *Magnaporthe oryzae* isolates from rice in Peninsular Malaysia. *Czech J Genet Plant Breed* 52:145–156

Altschul SF, TL Madden, AA Schaffer, JH Zhang, Z Zhang, W Miller, DJ Lipman (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acids Res* 25:3389–3402

Arx JAV, J Guarro, MJ Figueras (1986). *The Ascomycete Genus Chaetomium*, Vol. 84. Beihefte zur Nova Hedwigia. Lubrecht & Cramer, Berlin, Germany

Dar J, K Soytong (2014). Construction and characterization of copolymer nanomaterials loaded with bioactive compounds from *Chaetomium* species. *Intl J Agric Technol* 10:823–831

Ditta A (2012). How helpful is nanotechnology in agriculture? *Adv Nat Sci Nanosci Nanotechnol* 3; Article 033002

Elibol OH, DD Morisette, D Akin, JP Denton, R Bashir (2003). Integrated nanoscale silicon sensors using top-down fabrication. *Appl Phys Lett* 83:4613–4615

Gnanamanickam SS (2009). *Biological Control of Rice Diseases: Progress in Biological Control*, Vol. 8. Springer, Dordrecht, The Netherlands

Li M, Q Huang, Y Wu (2011). A novel chitosan- poly (lactide) copolymer and its submicron particles as imidacloprid carriers. *Pest Manage Sci* 67:831–836

Ou SH (1985). *Rice Diseases*. Commonwealth Mycological Institute, London, UK

Perlatti B, PLD Souza Bergo, MFGFD Silva, JB Fernandes, MR Forim (2013). Polymeric nanoparticle-based insecticides: a controlled release purpose for agrochemicals. *In*: *Insecticides – Development of Safer and More Effective Technologies*. Trdan S (Ed.). IntechOpen, London, UK

Phonkerd N, S Kanokmedhakul, K Kanokmedhakul, K Soytong, S Prabpai, P Kongsearee (2008). Bis-spiro-azaphilones and azaphilones from the fungi *Chaetomium cochliodes* VTh01 and *C. cochliodes* CTh05. *Tetrahedron* 64:9636–9645

Rai M, A Ingle (2012). Role of nanotechnology in agriculture with special reference to management of insect pests. *Appl Microbiol Biotechnol* 94:287–293

Salata OV (2004). Applications of nanoparticles in biology and medicine. *J. Nanobiotechnol*., 2; Article 3

Sharon M, AK Choudhary, R Kumar (2010). Nanotechnology in agricultural diseases and food safety. *J Phytol* 2:83–92

Singh S, BK Singh, SM Yadav, AK Gupta (2015). Applications of nanotechnology in agricultural and their role in disease management. *Res J Nanosci Nanotechnol* 5:1–5

Soutter W, 2013. *Nanotechnology in Agriculture*. Manchester, UK

Soytong K (2014). Bio-formulation of *Chaetomium cochliodes* for controlling brown leaf spot of rice. *Intl J Agric Technol* 10:321–337

Soytong K (1989). Antagonism of *Chaetomium cupreum* to *Pyricularia oryzae*: a case study to biocontrol of a rice blast disease. *Thai Phytopathol* 9:28–33

Soytong K, TH Quimio (1989). Antagonism of *Chaetomium globosum* to the rice blast pathogen, *Pyricularia oryzae*. *Kasetsart J Nat Sci* 23:198–203

Soytong K, S Kanokmedhakul, V Kukongviriyapan, M Isobe (2001). Application of *Chaetomium* species (Ketomium) as a new broad spectrum biological fungicide for plant disease control: A review article. *Fung Divers* 7:1–15

Tamura K, J Dudley, M Nei, S Kumar (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599

Tann H, K Soytong (2017). Biological control of brown leaf spot disease caused by *Curvularia lunata* and field application method on rice variety IR66 in Cambodia. *Agrivita J Agric Sci* 39:111–117

Tann H, K Soytong (2016). Effects of nanoparticles loaded with *Chaetomium globosum* LMITL0805 extracts against leaf spot of rice var Sen Pidoa. *Malays Appl Biol J* 45:1–7

TeBeest DO, C Guerber, M Ditmore (2007). Rice blast. *In: The Plant Health Instructor*. APSnet, St. Paul, Minnesota, USA

Titone P, G Mongiano, L Tamborini (2015). Resistance to neck blast caused by *Pyricularia oryzae* in Italian rice cultivars. *Eur J Plant Pathol* 142:49–59

Valent B (2004). Plant disease: Underground life for rice foe. *Nature* 431:516–517

Wang GL, B Valent (2009). *Advances in Genetics, Genomics and Control of Rice Blast Disease.*, Springer, New York, USA

White TJ, T Bruns, S Lee, J Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: *PCR Protocols: A Guide to Methods and Applications, A Laboratory Manual*, pp: 315–322. Innis MA, DH Gelfand, JJ Sninsky, TJ White (eds). Academic Press, New York, USA

Xia JQ, JC Correll, FN Lee, DD Rhoads, MA Marchetti (1993). DNA fingerprinting to examine variation in the *Magnaporthe grisea (Pyricularia grisea)* population in two rice fields in Arkansas. *Phytopathology* 83:1029–1035