# Nano-particles constructed from *Chaetomium cochliodes* CTh05 against *Magnaporthe oryzae* causing rice blast

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**Running Head:** Nano-particles from *Chaetomium* against Rice Blast

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**ABSTRACT** The effective isolate of *Chaetomium cochliodes* CTh05 actively against *Magnaporthe oryzae* isolate PO1 causing rice blast were tested on rice var RD57. The symptoms of blast on rice var RD57 was isolated and confirmed by morphological and molecular phylogeny which identified as *Magnaporthe oryzae*. Morphology and phylogenic identification was also confirmed *C. cochliodes* CTh05. *Magnporthe oryzae* isolate PO1 proved to be aggressive isolate to cause blast of rice var. Rd57. Bi-culture test proved that *C. cochliodes* CTh05 can be suppressed the growth of *M. oryzae* PO1. The fungal metabolites extracted from *C. cochliodes* CTh05 (CCoE, CCoM and CCoH) expressed antifungal against *M. oryzae* isolate P01 (rice blast) with the $ED_{50}$ of 85, 144 and 374 ppm, respectively. Nano-particles derived from *C. cochliodes* CTh05 extracts (nanoCCoE, nanoCCoM and nanoCCoH) exhibited significantly antifungal activity against *M. oryzae* PO1 wih the $ED_{50}$ values of 9, 16 and 33 ppm, respectively. Further investigation is to formulate the nanoparticles from active compounds of *C. cochliodes* CTh05 for plant immunity and to be applied in the rice fields.

**IMPORTANCE** Rice blast is an economic important disease which caused by *Magnaporthe oryzae*. It causes yield loss and economic damage wherever rice is grown in Asia. It becomes the main disease due to wide distribution and most infection under favorable condition. The farmers have been applied several kinds of chemical fungicides that leading to the pathogen become resistant to those fungicides, and causes environmental pollution which the toxic chemicals residue in soil, water and surrounding environment. The significance of this research is to discover a new agricultural input as nanoparticles from *Chaetomium cochliodes* CTh05 to control the blast pathogen which will develop the nanoproduct to elicit immunity in rice, environmental protection and food safety.

**KEYWORDS:** Nano-particles, *Chaetomium cochliodes* CTh05, rice blast, *Magnaporthe oryzae*

**INTRODUCTION** Rice blast caused by *Magnaporthe oryzae* (Anamorph; *Pyricularia oryzae*). It is one of the first recorded as rice fever disease in China as early as 1637. Rice blast expanded through Asia, Latin America, and Africa and is now found in over 85 countries worldwide (1, 2). *M. oryzae* infects all parts of plants causing yield losses leading to shortage in many developing countries in recent years (1). Valent (3) stated that disease is epidemic in all continents wherever rice is grown and yield loss due to blast can be as high as 50% (4). The search for new effective disease control is needed to reduce or eliminate the chemical fungicides which causes the environment problem. Nanotechnology in agriculture becomes a new method to build up and restructure the materials at molecular level. Molecular nanotechnology involves constructing organic materials into defined structures, atom by atom or molecule by molecule (5). The application of nanotechnology in agriculture have increased attention in recent years (6). Researchers have actively investigated the synthesis of organic nanomaterials including different kinds of nanoparticle for biological properties (7, 8). The application of nanotechnology in agriculture are being explored (5) and could be helped the solution of overuse toxic chemicals faced by agriculture (9). Nanoparticles contain bioactive substances from natural products that can enable fast effective penetration through plant cuticles and tissues (10). It can provide efficient pest management in agriculture (11) and formulate to contain pesticides in colloidal suspension or powder to apply in agriculture (9), and increase the stability of active compounds (10). Application of natural products from *Chaetomium* spp. has been proven to be antifungal activity against several plant pathogens (12). Tann and Soytong (13) reported that nano particles constructed from *Chaetomium globosum* KMITL-N0805 expressed antifungal activity against *Curvularia lunata*, leaf spot of rice var. Sen Pidoa, and the tested nanoparticles can be controlled leaf spot of rice at 60 days. Furthermore, Tann and Soytong (14) reported nano-products derived from *Chaetomium cupreum* can

be applied to reduce the incidence of rice leaf spot. This research was conducted to evaluate nano-particles constructed from *Chaetomium cochliodes* CTh05 for biological activity against *Magnaporthe oryzae* causing rice blast

## RESULTS

**Isolate of rice blast pathogen and *Chaetomium* sp.** The symptoms of blast was isolated and morphological identified as *Magnaporthe oryzae* isolate PO1 as seen in Fig. 1. Culture on PDA grown to full plate (9 cm) in10 days. Mycelia are septated and hyaline, producing conidiophores and 3 cells conidia. *C. cochliodes* CTh05 from previous experiment (15), it was cultured on PDA for 3 weeks and shows olive-green to brown, producing perithecia and subglobose asci, one ascus has eight ascospores (Fig. 2).

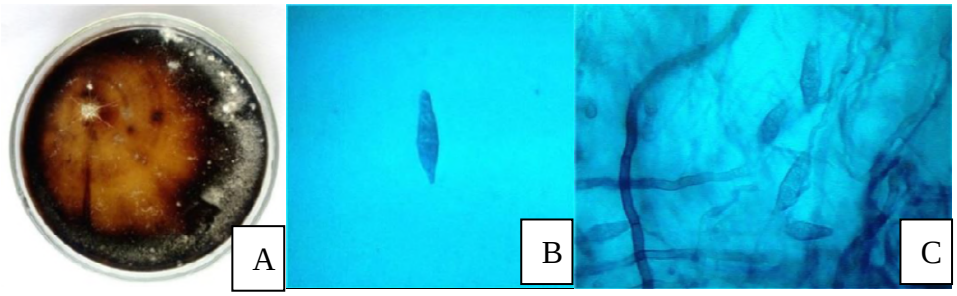


**FIG1** *Magnaporthe oryzae* PO1, A= pure culture in PDA; B= conidia; C = mycelium and conidia.

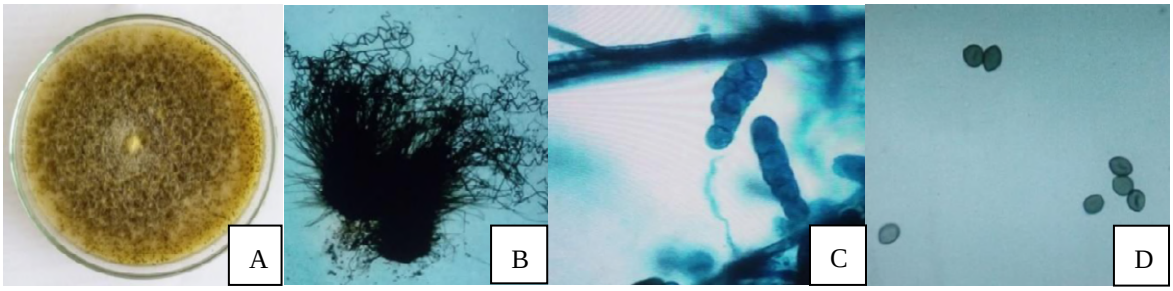


**FIG 2** *Chaetomium cochliodes* CTh05, A=Colony, B= Perithecia, C=Asci, D=Ascospores.

Molecular phylogenic identification was confirm species. Phylogenic tree was clearly demonstrated that the rice blast pathogen as *Magnporthe oryzae* compared to database from Genbank which closedly related to MF668691, MF669475, and followed by FN555115, FN555121 and KY825257. *Sordaria fimicola* KY930619 was out group (Fig. 3). Phylogenic confirmation of *Chaetomium cochliodes* CTh05 was clearly shown in phylogenic tree compared to *Colletotrichum gloeospoiodes* AY714052 as outgroup (Fig. 4). Data from Genbank can be reliable confirmed CTh05 as *Chaetomium cochliodes* when compared to HQ316565, and followed by KT895345, JN209868 and JN209864. *Magnporthe oryzae* isolate PO1 was conformed as the pathogenic isolate to rice var. Rd57 by pathogenicity test. The inoculated wounds showed the lesions as roundish to elongated, necrotic, gray spots about 1-2 mm in diameter within 5 days as seen in Fig. 5.

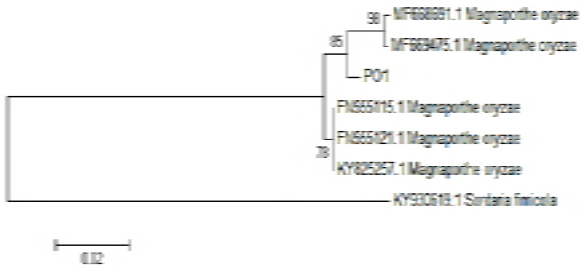


**FIG 3** Phylogenic tree of *Magnporthe oryzae* (PO1)

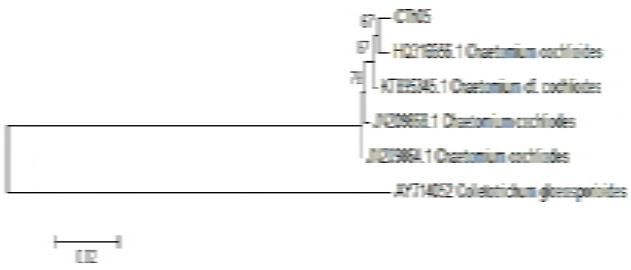


**FIG 4** Phylogenic tree of *Chaetomium cochliodes* CTh05

**FIG 5** Pathogenicity test on rice leaf
Left = Control
Right =Treatment

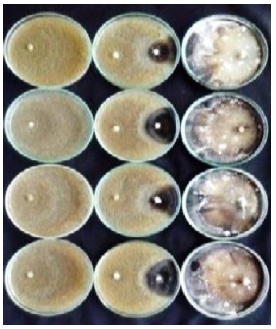

**FIG 6** Bi-culture test between *Magnaporthe oryzae* isolate PO1 and *Chaetomium cochliodes* CTh05, Left = Chaetomium culture, Middle = Bi-culture plates, and Right = Magnaporthe culture

**Bi-culture Test.** *M. oryzae* isolate PO1 was inhibited by *C. cochliodes* CTh05. The colony diameter of the antagonist averaged of 4.35 cm while the control was 9.00 cm, the colony growth inhibition was 51.66 % in 10 days. Thereafter incubation period was extended to 30 days and the colony inhibition averaged over 90 % (Fig. 6).

**Bioactivity test of fungal metabolites from *Chaetomium cochliodes* CTh05.** Result showed that CCoE gave significantly highest spore inhibition of 88 %, followed by CCoM and CCoH with spore inhibition of 80.74 and 67.57 %, respectively at 1,000 ppm in 12 days. The fungal metabolites CCoE, CCoM and CCoH showed antifungal activity against *M. oryzae* isolate PO1 for spore inhibition with the $ED_{50}$ values of 85, 144, 374 ppm (Table 1). Interestingly, at 15 ppm nanoCCoH, nanoCCoE and nanoCCoM gave significantly inhibited spore production. NanoCCoE gave significantly inhibited spore production of 68 % while nanoCCoM and nanoCCoH at 47 and 34 %, respectively in 12 days. The nanoparticles, nanoCCoE, nanoCCoM and nanoCCoH showed effective antifungal activity against *M. oryzae* PO1 (rice blast) with $ED_{50}$ values of 9.41, 16.51 and 33.41 ppm, respectively (Table 2). It observed that the spores were abnormally shape and broken cells after treated with nanoparticles of *C. cochliodes* CTh05 and spores were normal shape in the non-treated control (Figure 7).

**TABLE 1** Fungal metabolites of *Chaetomium cochliodes* CTh05 against *Magnaporthe oryzae* PO1 at 12 days

| metabolites | Concentration (ppm) | Colony diameter (cm)[/2,3] | Growth inhibition (%)[/2,3] | Number of spores [/2,3]($10^5$) | Spore Inhibition (%)[/2,3] | $ED_{50}$(ppm) |
|---|---|---|---|---|---|---|
| CCoH | 0 | 5.00[a] | - | 20.75[a] | - | 374.43 |
| | 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] | |
| | 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] | - | 85.87 |
| | 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] | |
| | 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] | - | 144.23 |
| | 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] | |
| | 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 | - |

[1]/Average of four replications. Means followed by a common letter are not significantly different at P=0.05 level;
[2]/Average of four replications. Means followed by a common letter are not significantly different at P=0.01 level;
[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

**TABLE 2** Nano particles derived from *Chaetomium cochliodes* CTh05 against *Magnaporthe oryzae* PO1 at 12 days

| Nano-particles | Concentration (ppm) | Colony diameter (cm)[/1] | Growth inhibition (%)[/1,3] | Number of spores [/2,3]($10^5$) | Spore Inhibition (%)[/1,3] | $ED_{50}$ (ppm) |
|---|---|---|---|---|---|---|
| Nano-CCoH | 0 | 5.00[a] | - | 69.00[a] | - | 33.41 |
| | 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] | |
| | 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] | - | 9.47 |
| | 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] | |
| | 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] | - | 16.51 |
| | 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] | |
| | 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 | - |

[1]/Average of four replications. Means followed by a common letter are not significantly different at P=0.05 level.
[2]/Average of four replications. Means followed by a common letter are not significantly different at P=0.01 level.
[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

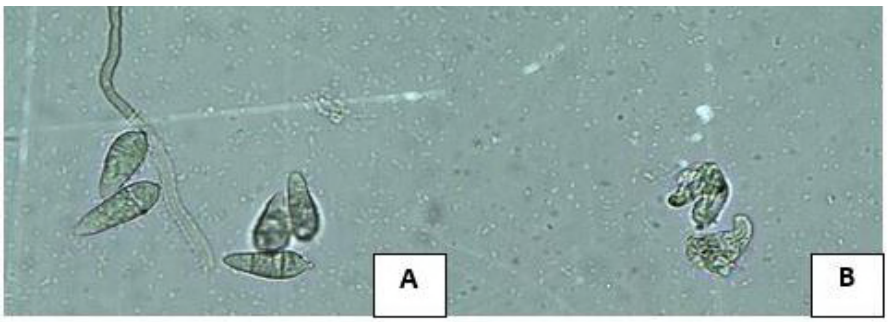


**FIG 7** Normal spores of rice blast pathogen (A) and abnormal spores (B) of *Magnaporthe oryzae* isolate PO1 after treated with nano-particles derived *Chaetomium cochliodes* CTh05 (B).

**DISCUSSION**

The symptoms of blast on rice var RD57 was isolated and confirmed morphological and phylogenic identified as *Magnaporthe oryzae. Magnporthe oryzae* isolate PO1 proved to be aggressive isolate to cause blast of rice var. RD57. *Chaetomium cochliodes* CTh05 was confirmed by morphology and phylogenic identification and it was actively effective against *Magnaporthe oryzae* isolate PO1 causing rice blast. Bi-culture test proved that *C. cochliodes* CTh05 can suppress the growth of *M. oryzae* PO1. Previous report of Soytong (16) stated that *C. cochliodes* proved to be antagonized *Drechslera oryzae* (brown leaf spot of rice var Pittsanulok 2). It significantly inhibited colony growth and spores of tested pathogen in bi-culture test. Similar report stated that *Chaetomium cupreum* CC3003 inhibited *C. -lunata* (leaf spot of rice) in bi-culture test (14). The fungal metabolites of *C. cochliodes* CTh05 (CCoE, CCoM and CCoH) expressed antifungal activity against *M. oryzae* isolate PO1 (rice blast) to inhibit spore production which the $ED_{50}$ of 85, 144, 374 ppm. Soytong (16) reported that metabolites from *C. cochliodes* gave significant inhibition on the spore production of *D. oryzae* (brown leaf spot of rice) that the ED50 value was 66.45 ppm. Tann and Soytong (14) reported that crude hexane, EtOAc and MeOH extracts of *C. cupreum* C3003 inhibited spore production of *C. lunata* (leaf spot of rice) with the $ED_{50}$ values of 6.41, 0.83 and 7.81 ppm, respectively.

The secondary metabolites from *C. cochliodes* CTh05 had been reported as the azaphilone types, cochliodones C and D, chaetoviridines E and F, *epi*-chaetoviridine A, and chaetoviridine A. The chaetoviridines E showed antimalarial activity against *Plasmodium falciparum*. Whereas, cochliodones C, chaetoviridines E and F expressed antimycobacterial activity against *Mycobacterium tuberculosis.* Moreover, chaetoviridines E and F exhibited cytotoxicity against the KB, BC1, and NCI-H187 cell lines (15). This research finding the fungal metabolites from *C. cochliodes* CTh05 actively against *M. oryzae* P01 causing rice blast pathogen.

Nano-particles derived from *C. cochliodes* CTh05 (nanoCCoE, nanoCCoM and nanoCCoH) showed significant antifungal activity against *M. oryzae* PO1 with $ED_{50}$ values of 9, 16 and 33 ppm, respectively. Similar report of Tann and Soytong (13) stated that nano-CGH, nano-CGE, and nano-CGM derived from *Chaetomium globosum* KMITL-N0805 showed antifungal activity against *Curvularia lunata* (leaf spot of rice) with $ED_{50}$ values of 1.21, 1.19, and 1.93 ppm, respectively.

Dar and Soytong (17) stated that the fungal metabolites from *Chaetoimum globosum* and *Chaetoimum cupreum* which were reacted with polylactic acid and electropun at 25-30 kv, nanomaterial from *C. globosum* was 241 nanometers,and *C. cupreum* was 171 nanometers. Furthermore, the research finding showed abnormal spores of *M. oryzae* after treated with nanoparticles from *C. cochliodes* CTh05 which was also reported by Tann

and Soytong (13) that nanoparticles from *C. globosum* can be disrupted and distorted the pathogen cells, and loss of pathogenicity. With this, Singh *et al.* (18) stated that nanotechnology in agriculture is revolutionized by innovating new techniques to control diseases. In this research findings revealed that nano-particles constructed from fungal metabolites gave the ability for plant disease control. As said by Sharon *et al*. (19) that nanotechnology is helped in agriculture and reduced environmental pollution by applying nano particles with the ability to increase the efficiency of pesticide application at lower dose.

## MATERIALS AND METHODS

**Isolation of pathogen and pathogenicity test.** The blast specimens were collected from blast symptom on leaves of rice var. RD 57. Isolation was done using tissue transplanting technique. Pure culture was maintained in rice flour agar (RFA) medium. Morphological identification was done under binocular compound microscope and followed the work of Ou (20). The pathogen is deposited at culture collection, Biocontrol Research Unit, Faculty of Agricultural Technology, KMITL, Bangkok, Thailand.

**Antagonistic fungus.** *Chaetomium cochliodes* CTh05 used in this study which is deposited at culture collection, Biocontrol Research Unit, Faculty of Agricultural Technology, KMITL, Bangkok, Thailand. that reported to be produced antimicrobial activity against malaria (*Plasmopara falciparum*) and tuberculosis (*Mycobacterium tuberculosis*) as well as cancer cell lines (15). This isolate was cultured and incubated in potato dextrose agar (PDA) at room temperature (27-30 C), and morphological identified according to von Arx (21) and Soytong (22).

**Molecular identification.** The genomic DNA were separately extracted from *Magnaporthe oryzae* PO1 and *Chaetomium cochliodes* CTh05. Each fungus was cultured in potato dextrose broth (PDB) media for 3 days. The mycelia were freeze-dried, genomic DNA was extracted by modified CTAB (Cetyl trimethyl ammonium bromide) method, cleaned with 25mM EDTA by centrifugation. 100 mg of mycelia were crushed in liquid nitrogen. The fungal cells were lysed in CTAB buffer, β-mercaptoethanol, then incubated at 65 ℃ for 1h in mixing tubes every 15 min. The lysate were extracted with an equal volume of chloroform/isoamyl alcohol (24:1), then centrifuged at 14,000 rpm for 5 min at 4 ℃. The aqueous phase was added 2 µl Rnase (20 µg/ml), and incubated 30 min at 37 ℃, then mixed with 50 µl 10% CTAB. The genomic DNA was precipitated in isopropanol and centrifuged at 4 ℃ for 20 min at 14,000 rpm. The resulting pellets were washed twice with 70% and 95% ethanol, air dried and dissolved in 100 µl TE buffer at 37 ℃, overnight. The quality and quantity of extracted DNA were monitored by electrophoresis in a 1% agarose gel. Quantification was performed with comparison to the known dilution of lambda phage DNA. Polymerase chain reaction (PCR) was performed by ITS ribosomal DNA regions which amplified by PCR using the universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (23). The 25 µl reaction mixture contained 2.5 µl 10 × PCR buffer, 0.625 µl each dNTP (1.25mM), 0.5 µl $MgCl_2$, 1 µl of each primer (20pmol/µl), 2 ng of DNA and 0.2 µl of Taq DNA polymerase (1 U) were performed. PCR condition for the ITS regions were programed with initial denaturation at 95 ℃ for 5 min, followed by 35 cycles of 95 ℃ for 1 min., 56 ℃ for 1 min., 72 ℃ for 2 min., and a final extension at 72 ℃ for 5 min. The amplified products (5 µl) were visualized on 1% (w/v) agarose gel for the presence of a single amplified band. The amplified products were sequenced and aligned with comparison to the sequences in the GenBank by basic local alignment search tool (BLAST) analysis (24) at National Center for Biotechnology Information (NCBI) databases. The sequences of closely related organisms were downloaded to construct the phylogenetic trees, aligned through CLUSTALW using MEGA version 6.0 software (25) and a phylogenetic tree was designed by neighbor-joining method using the same software.

**Bi-culture test.** The test was done by followed the method of Soytong and Quimio (26). *C. cochliodes* CTh05 and rice blast pathogen were transferred each 0.3 m culture agar plug to PDA at opposite sites. *C. cochliodes* CTh05 and rice blast pathogen were separately cultured where each isolate on PDA served as the controls. All plates were incubated at room temperature for 30 days. Data were collected as colony diameter (cm) and sporulation and subjected to analysis of variance and compared using Duncan Multiple's Range Test (DMRT) at P=0.05 and 0.01.

**Fungal metabolites from *Chaetomium cochliodes* CTh05.** Biomass culture of *Chaetomium cochliodes* CTh05 was extracted with hexane, ethyl acetate and methanol using method described Phonkerd *et al.* (15) to give crude hexane (CCoH), ethyl acetate (CCoE) and methanol (CCoM) extracts. . The experiment was set up as two factor factorial experiment in CRD with four replications. Factor A represented crude extracts and factor B represented various concentrations of 0, 10, 50, 100, 500 and 1,000 ppm. The culture agar plug of 3 mm was transferred to the middle PDA plate in each treatment, then incubated at room temperature (27-30 C) for 15 days. Data were collected as colony diameter (cm) and counted number of spores using haemacytometer. Data were statistically computed analysis of variance (ANOVA). Treatment means were compared using DMRT at P=0.05 and 0.01. The effective dose ($ED_{50}$) was computed using probit analysis program.

**Nano-particles.** The crude extracts from *C. cochliodes* CTh05, CCoH, CCoE and CCoM were separately incorporated into polylactic acid (PLA)-based nanoparticles through electrospinning by followed the method of Dar and Soytong (17) to yield nanoCCoH, nanoCCoM and nanoCCoE, respectively. Nano CCoH, CCoM and CCoE were tested to inhibit the rice blast pathogen using two factorial in CRD with four replications. Factor A was nanoparticles, and factor B was 0, 3, 5, 7, 10 and 15 ppm. CRD was designed for the experiment which repeated four times. Data were collected as colony diameter (cm) and counted number of spores using haemacytometer, and statistically calculated analysis of variance (ANOVA). Means were compared using DMRT at P=0.05 and 0.01. The effective dose ($ED_{50}$) was calculate using probit analysis program.

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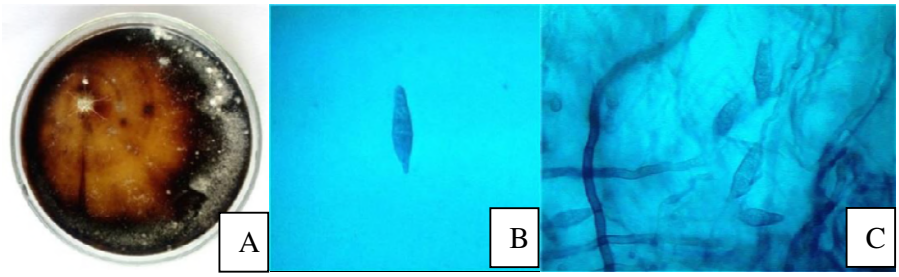


**FIG1** *Magnaporthe oryzae* PO1, A= pure culture in PDA; B= conidia; C = mycelium and conidia.

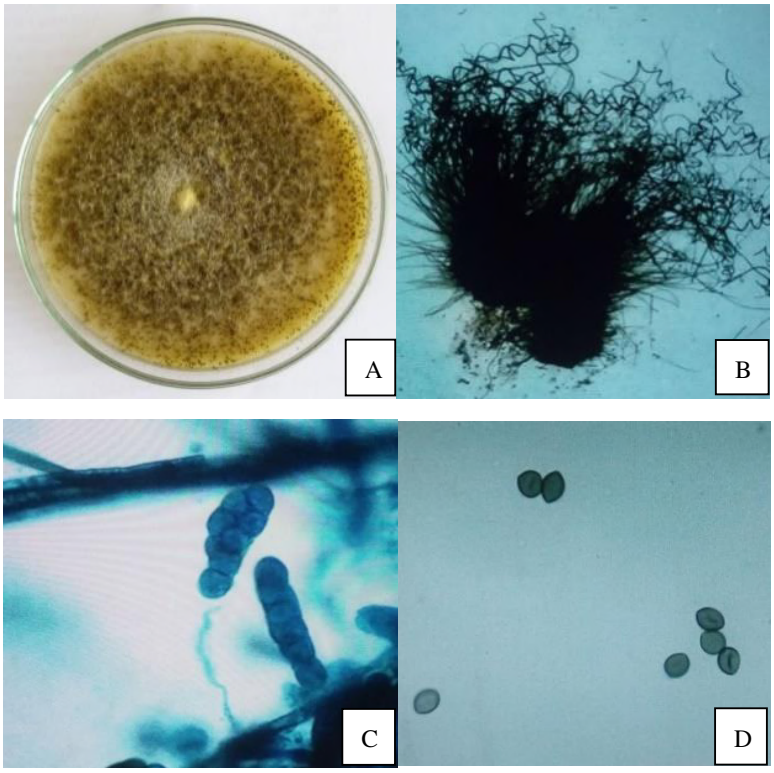


**FIG 2** *Chaetomium cochliodes* CTh05, A=Colony, B= Perithecia, C=Asci, D=Ascospores.

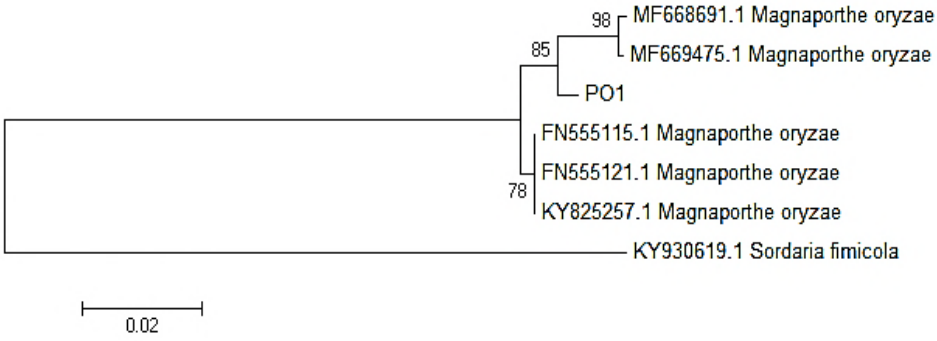


**FIG 3** Phylogenic tree of *Magnporthe oryzae* (PO1)

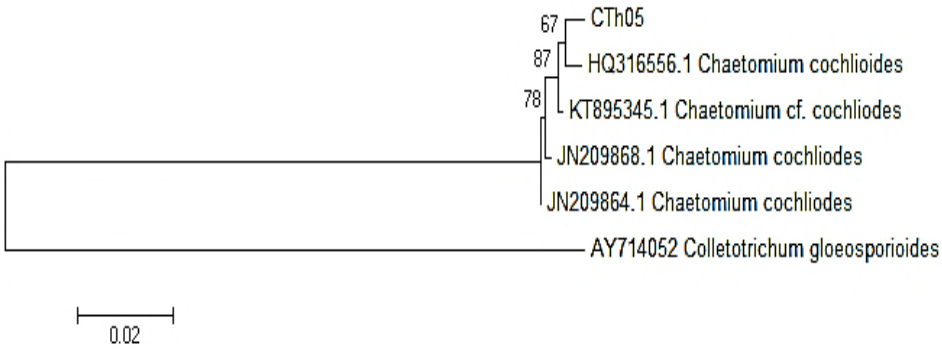


**FIG 4** Phylogenic tree of *Chaetomium cochliodes* CTh05

**FIG 5** Pathogenicity test on rice leaf
Left = Control
Right =Treatment

**FIG 6** Bi-culture test between *Magnaporthe oryzae* isolate PO1 and *Chaetomium cochliodes* CTh05, Left = Chaetomium culture, Middle = Bi-culture plates, and Right = Magnaporthe culture

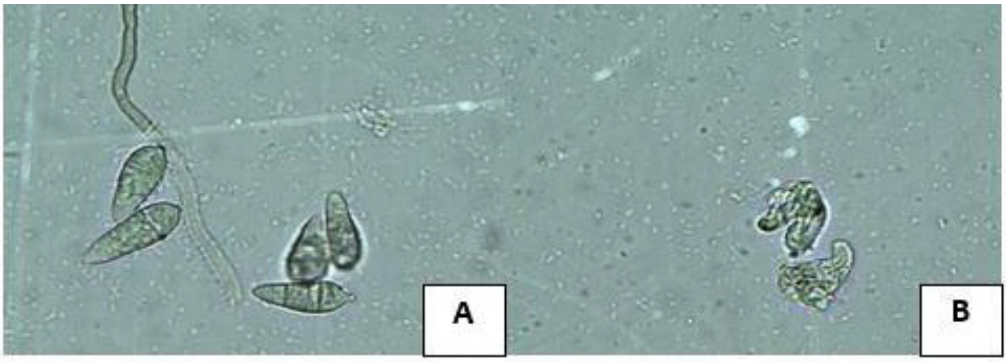


**FIG 7** Normal spores of rice blast pathogen (A) and abnormal spores (B) of *Magnaporthe oryzae* isolate PO1 after treated with nano-particles derived *Chaetomium cochliodes* CTh05 (B).

**TABLE 1** Fungal metabolites of *Chaetomium cochliodes* CTh05 against *Magnaporthe oryzae* PO1 at 12 days

| metabolites | Concentration (ppm) | Colony diameter (cm)[1,2,3] | Growth inhibition (%)[1,2,3] | Number of spores [1,2,3]($10^5$) | Spore Inhibition (%)[1,2,3] | $ED_{50}$(ppm) |
|---|---|---|---|---|---|---|
| | 0 | $5.00^{a}$ | - | $20.75^{a}$ | - | |
| | 10 | $4.88^{bc}$ | $2.25^{ij}$ | $20.25^{a}$ | $2.56^{j}$ | |
| CCoH | 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 | $3.64^{j}$ | $27.00^{a}$ | $2.50^{h}$ | $88.03^{a}$ | |
| | 0 | $5.00^{a}$ | - | $20.75^{a}$ | - | |
| | 10 | $4.86^{c}$ | $2.75^{i}$ | $18.75^{ab}$ | $9.68^{i}$ | |
| CCoM | 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 | - |

[1]/Average of four replications. Means followed by a common letter are not significantly different at P=0.05 level;
[2]/Average of four replications. Means followed by a common letter are not significantly different at P=0.01 level;
[3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

**TABLE 2** Nano particles derived from *Chaetomium cochliodes* CTh05 against *Magnaporthe oryzae* PO1 at 12 days

| Nano-particles | Concentration (ppm) | Colony diameter (cm)[1] | Growth inhibition (%)[1,3] | Number of spores [2,3]($10^5$) | Spore Inhibition (%)[1,3] | $ED_{50}$ (ppm) |
|---|---|---|---|---|---|---|
| Nano-CCoH | 0 | 5.00[a] | - | 69.00[a] | - | 33.41 |
| | 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] | |
| | 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] | - | 9.47 |
| | 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] | |
| | 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] | - | 16.51 |
| | 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] | |
| | 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 | - |

[1]/Average of four replications. Means followed by a common letter are not significantly different at P=0.05 level. [2]/Average of four replications. Means followed by a common letter are not significantly different at P=0.01 level. [3]/Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.