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## Application of nano-particles from *Chaetomium globosum* to control leaf spot of rice

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Result showed that nano-CGH, nano-CGE and nano-CGM from *Chaetomium globosum* strain KMITL 0802 significantly inhibited *Curvularia lunata* causing leaf spots of rice, which the ED<sub>50</sub> values were 1.12, 1.19 and 1.93 ug.ml, respectively within 7 days. It is the first report of nano-particles from *Chaetomium globosum* to control *Curvularia lunata* causing leaf spot of rice. Further investigation is being done in the field trials.

**Keywords:** *Chaetomium globosum*; nano-particles; *Curvularia lunata*;

### Introduction

*Curvularia lunata* reported to caused leaf spot disease of rice which occurs mostly in tropical and subtropical areas which it is an facultative plant pathogen and the teleomorphic states in *Cochliobolus* and *Pseudocochliobolus* which is reported as a pathogen of several plants including leaf spots on rice under certain conditions. (Luna 2002). 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), *Fusarium oxysporum* f. sp. *Lycopersici* (Soyong et al., 1999a), *Phytophthora palmivora* (Pechprom and Soyong, 1996; Sodsart and Soyong, 1998), *Phytophthora parasitica* (Usuwat and Soyong, 1998). Screening *Chaetomium* spp as biological control agents has been carried out in Thailand since 1989, resulting in the development of a biological formulation from *Chaetomium cupreum* CC1-10 and *Chaetomium globosum* CG1-12. The product has now been developed into pellets and powder formulations and registered for a Patent Right No.6266, Intl. cl. 5 AO 1 N 25 / 12 in 1994 (Soyong, 1996). Objective of research findings were to isolate, identify leaf spot pathogen of rice, pathogenicity test, and to study the morphological characteristics of *Curvularia lunata* and *Chaetomium globosum*, and also testing the formulated nano-elicitor against *Curvularia lunata* causing leaf spot of rice.

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## **Materials and methods**

### ***Study on Morphology of Chaetomium globosum***

*Chaetomium globosum* which offered from Assoc. Prof. Dr. Kasem Soyong, were used to observe the growing colony on potato dextrose agar (PDA) media and then mycelia, ascocarp, ascospores and seta were observed under binocular compound microscope.

### ***Isolation of the pathogen, Curvularia lunata***

*Curvularia lunata* causing leaf spots of rice was isolated by tissue transplanting technique. The symptoms on leaves were properly cleaned with running tap water and after air-dried for a few minutes before cut the advance margin of symptom between healthy and diseased areas into small pieces and soaked sterilized water, and followed by 1% sodium hypochlorite (NaClO) for 3 min and then sterilized water again. All sectioned pieces were transferred onto water agar (WA) medium for firstly observation of appearing colonies and sub-cultured to PDA until get pure culture. Morphological identification was done by observation fungal characteristic under binocular compound microscope.

### ***Pathogenicity test.***

Pathogenicity test was done by following the method of Koch's Postulate. With this, Spore suspension of *Curvularia lunata* was prepared at concentration is  $1 \times 10^6$  spores/ml. and then sprayed on the wounded leaves (3 leaves/seedling). The inoculated leaves were then covered with plastic sheet and maintained to observe the infected leaves. The inoculated leaves with only spraying sterilize distilled water were done to serve as controls. Experiment was conducted using Completely Randomized Design (CRD) with four replication. Data were collected as lesion size (cm) and computed analysis of variance (ANOVA). Treatment means were compared using least significantly different test (LSD) at  $P=0.05$  and  $0.01$ .

### ***Testing nano-particles from Chaetomium globosum to control leaf spot of rice***

*Chaetomium globosum* strain 0805 was cultured in potato dextrose broth (PDB) at room temperature for 30 days. The fungal biomass was harvested by filtration through cheesecloth and air-dried overnight. Fresh and dried weight were weighted. The fungal biomass was then ground with electrical blender, and kept in flask. Extraction was done by added an equal volume hexane, and incubated in stationary phase for 5 days at room

temperature before separated to get filtrate through Whatman filter paper No.4. The filtrate was evaporated by using rotary vacuum evaporation to get crude hexane. The marc was further extracted with ethyl acetate and methanol consecutively to get crude ethyl acetate and crude methanol by using the same procedure as hexane and yield crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracts. Preparation of nano particles; nano-particle was done using the method of Dar and Soyong (2014) to get Nano-CGH, Nano-CGE and Nano-CGM.

The nano-particles, Nano-CGH, Nano-CGE and Nano-CGM were tested to inhibit *Curvularia lunata* causing leaf spots of rice. Experiment was designed by using two factors factorial experiment in CRD with four replications. Factor A represented Nano-CGH, Nano-CGE and Nano-CGM and factor B represented concentrations at 0, 1, 5 and 10 µg/ml. Each Nano-particle was dissolved in one drop 2% dimethyl sulfoxide (DMSO), and then mixed into 30ml PDA medium before autoclaving at 121°C, 15 lbs/inch<sup>2</sup> for 30 min. The culture of *Curvularia lunata* was cut at the edge of colony with sterilized cork borer (3mm). Agar plug of pathogen was transferred to the middle of PDA media in plate (5.0mm diameter) incorporated with each nano-particles. The transferred plates were incubated at room temperature until the pathogen in control plates growing full. Abnormal and normal spores of pathogen from each treatment were observed under binocular compound microscope and taken photograph for comparison. The data were collected as colony diameter and the number of spores that counted by using Haemocytometer. Percentage of inhibition was computed and the effective dose (ED<sub>50</sub>) was then calculated using probit analysis.

## Results

### *Study on Morphology of Chaetomium globosum*

*Chaetomium globosum* strain 0805 was cultured and morphological observation following Soyong and Quimio (1989). Ascomycetes, asci and ascospores were taken photograph under compound microscope (Figure 1).

### *Isolation, identification, and pathogenicity test of the pathogen, Curvularia lunata*

*Curvularia lunata* was isolated, identified and proved to be pathogenic isolate causing leaf spots of rice as seen in Table 2, Figure 2 and 3.

**Table.1.** Percent disease intensity of *Curvularialunata* in rice var Thadorkkam11

Treatments	DI
T1 Control	1
T2 <i>Curvularialunata</i>	4

<sup>1/</sup> Disease index (DI), 1= (0%)no symptoms, 2= (1-25%) small blighted spot and still healthy tissue, 3= (26-50%)dead cells in are of blighted spot 1-2 mm and turn brown color, 4= (51-75%)expanded lesion in oval shape 1-2 cm and cell death in the center of lesion and 5= (76-100%) diseased area over 20 % and finally death modified by Wilaiporn (2001).

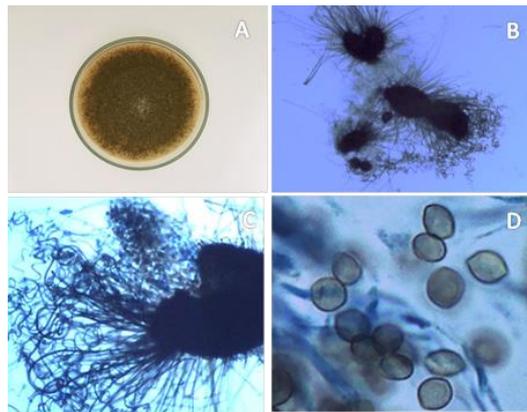
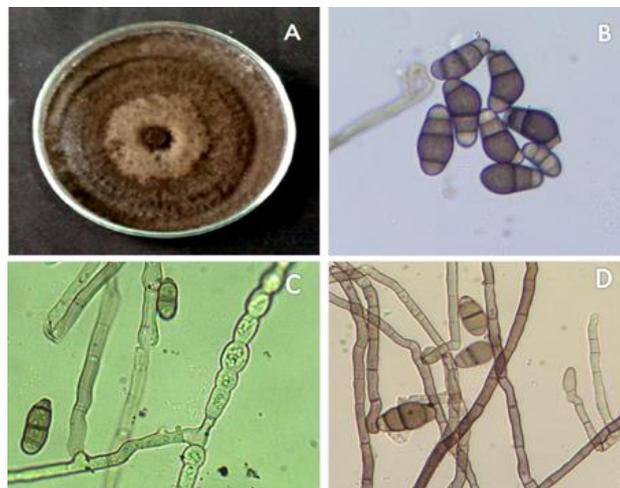
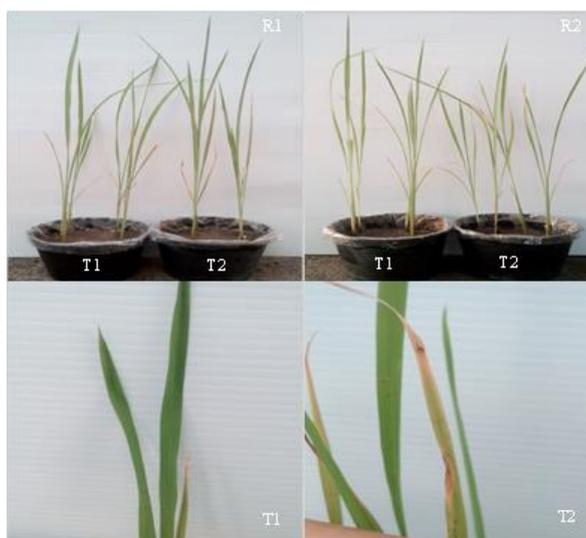


Figure 1. *Chaetomium globosum* , A= colony, B-Ascocarps, C= Ascocarp and asci and D-ascospores



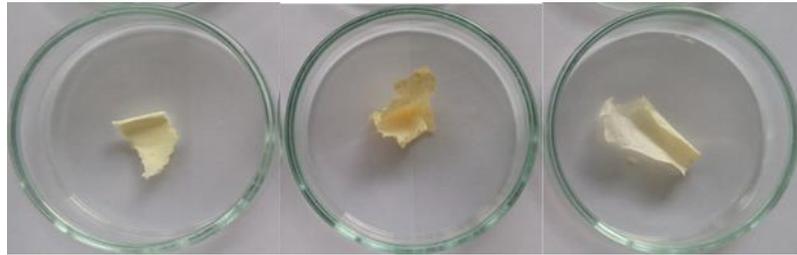
**Figure 2** *Curvularia lunata* causing leaf spots of rice , A- colony, B=conidia, C= mycelia and conidia and D= conidiophores and conidia



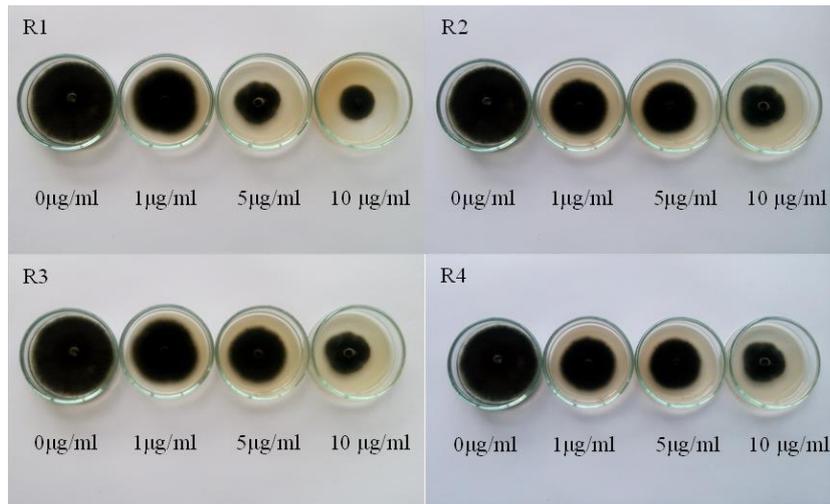
**Figure 3.** Pathogenicity test of *Curvularia lunata* causing leaf spots of rice, T1 = Sprayed by sterile water (Control) and T2 = Sprayed spore suspension of *Curvularia lunata*

### ***Testing nano-particles from Chaetomium globosum to control leaf spot of rice***

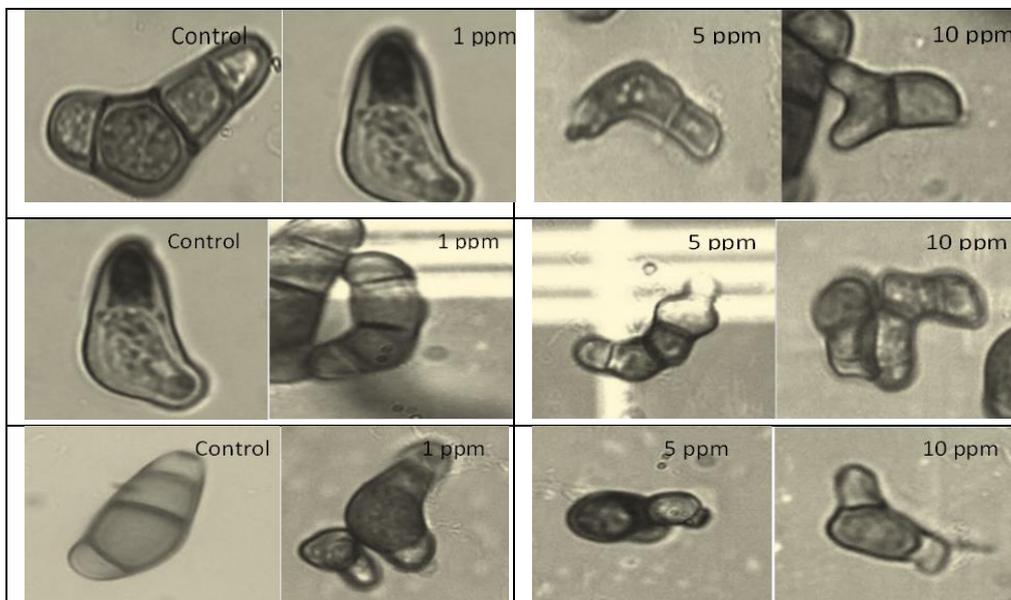
Characteristics of nano-CGH, nano-CGE and nano-CGM were seen in Figure 4. Result showed high efficacy antimicrobial activity of Nano-CGH, Nano-CGE and Nano-CGM from *Chaetomium globosum* strain 0805 against *Curvularia lunata* causing leaf spots of rice which the ED<sub>50</sub> values were 1.12, 1.19 and 1.93 µg/ml, respectively within 7 days (Table 2). It was not clearly seen in the tested plates on colony inhibition but when counted the number of conidia which served as pathogen inoculum that it was significantly differed when compared to the control (0 µg/ml) for 7 days. However, Nano-CGH, Nano-CGE and Nano-CGM at the concentration of 10 µg/ml inhibited the colony growth of 27, 25 and 52 %, respectively within 7 days (Figure 5). Nano-CGH, Nano-CGE and Nano-CGM at the concentration of 10 µg/ml were inhibited the spore production of 75, 52 and 70 %, respectively. The effects of Nano-CGH, Nano-CGE and Nano-CGM on *Curvularia lunata* were clearly demonstrated at all tested concentrations leading to broken the pathogen cells which become abnormal cells and lost of pathogenicity (Figures 6).



**Figure 4.** Characteristics of Nano-CGH, Nano-CGE and Nano-CGM



**Figure 5.** Testing nano-CGM from *Chaetomium globosum* against *Curvularia lunata*



**Figure 6.** Comparison between normal and abnormal conidia in various concentrations, upper = nano-CGH, middle = nano-CGM and lower parts = nano-CGM

**Table.2.** Colony diameter and number of spores *Curvularia lunata*

Nano-products	Concentrations (ug/ml)	Colony diameter(cm)	Number of conidia(x10 <sup>6</sup> )	Colony inhibition (%)	Conidia inhibition (%)	ED <sub>50</sub> (ppm)
Nano-CGH	0	5a	49.24a	0.00	0.00	
	1	4.43b	25.15c	11.25	48.11	1.21
	5	3.84c	18.90de	22.50	61.57	
	10	3.62c	12.75f	27.25	74.02	
Nano-CGE	0	5a	49.19a	0.00	0.00	
	1	3.33d	32.50b	33.25	33.87	1.19
	5	3.61c	28.55bc	27.75	41.87	
	10	3.73c	23.39cd	25.25	52.44	
Nano-CGM	0	5a	49.24a	0.00	0.00	
	1	2.93e	27.65bc	41.25	48.11	1.93
	5	2.83e	23.64cd	43.25	53.24	
	10	2.36f	15.00ef	52.75	70.08	
C.V. (%)		3.56	8.44	10.90	4.10	

Average of four replications. Means followed by a common letter are not significantly different by DMRT at P = 0.05

## Discussion

*Curvularia lunata* was isolated and proved to be aggressive isolate causing leaf spots of rice. With this, Soyong (2014) stated that many rice varieties are invaded by *Curvularia lunata* causing leaf spots which leading to low yield. *Chaetomium globosum* strain KMITL-N0802 was used in this study. This species is reported by Kanokmedhakul *et al.*(2001)who discovered new bioactive compounds which is antimycobacterial anthraquinone-chromanone compound against human pathogen. Nano-particles were designed from crude extracts of *Chaetomium globosum* KMITL0802 according to the method of Dar and Soyong (2014). The experiment showed that nano-CGH, nano-CGE and nano-CGM gave significantly inhibited spore production of *Curvularia lunata*. It is similar to the work of Dar and Soyong (2013) which reported *in-vitro testing* of nanomaterials derived *Chaetomium globosum* gave good result to inhibit *Fusariumoxysporum* f. sp. *lycopersici*. Moreover, similar report from other species of *Chaetomium*, eg.*Chaetomium cochliodes*reported as a new antagonistic fungus against brown leaf spot of ricevarPittsanulok 2 caused by *Drechsleraoryzae*by using hexane, ethyl acetate and methanol which ED<sub>50</sub>value was 66.45 ug/ml. It is observed in this research finding that all tested nano-materials could rapidly penetrate into pathogen ceels which leading to abnormal cells of pathogen as also reported by Dar and Soyong (2013). It is recommended that nano-materials may play the important role as plant immunity induction as reports by Soyong, *et al* (2014) who stated

microbial elicitor from *Chaetomium* can be induces immunity in chilli by production of phytoalexin namely capsidiol against anthracnose caused by *Colletotrichum capsici*. It is interesting that nano-*Chaetomium* would be expressed a new role for disease control.

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