International Journal of Agricultural Technology 2018 Vol. 14(7): 1961-1968
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Nano-particles from *Chaetomium lucknowense* to inhibit rice blast pathogen causded by *Pyricularia oryzae* in pot experiment

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

[1]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand; [2]Department of Chemistry, Faculty of Science, Khon Khan Unviersity, Khon Khan, Thailand.

Song, J. J., Soytong, K., Kanokmedhakul, S. and Kanokmedhakul, K. (2018). Nano-particles from *Chaetomium lucknowense* to inhibit rice blast pathogen causded by *Pyricularia oryzae* in pot experiment. International Journal of Agricultural Technology 14(7): 1961-1968.

Abstract Nano-particles derived from *Chaetomium lucknowense* proved to be antagonized *Pyricularia oryzae* causing rice blast disease var. RD57. Result showed that nano-ECL, nano-MCL and nano-HCL expressed antifungal activities against *P. oryzae* (rice blast disease) at the ED_{50} values were 82, 114, 181ppm, respectively. In pot experiment, the nano-CL gave significantly better to control rice blast than the chemical fungicide (Tricyclazole) in rice var. RD57. Rice blast disease showed that nano-CL gave the highest reduction of 54 %, when compared to the chemical fungicide that the disease decreased 29.26 %. Application of nano-CL gave the highest plant strands of 87.62 cm when compared to Tricyclazole (74.91 cm). Nano-particles from *C. lucknowense* is being developed to be nano-elicitors for plant immunity.

Keywords: Chaetomium, nano-particles, rice blast

Introduction

Oryza sativa L. recognized as Asian rice. The origins of rice are numerously debated. (Harris *et al.,* 1996) found that genetic variation had expressed to all forms of Asian rice that occurred 8,200–13,500 years ago in China. Rice was first domesticated in the region of Yangtze valley in China (Normile, 1997). There are many pathogens that infected to rice and caused the yield loss, the blast disease seems to be the most important one caussed by *Pyricularia oryzae* (perfect stage: *Magnaporthe oryzae*).

It is pathogenic to rice which highly destructive to 30% yield loss worldwide (Skamnioti and Gurr, 2009). Moreover, *P. oryzae* isolates from rice are also reported to be a host-specific (OU, 1985). *Chaetomium* spp. are reported to antogonized to many phythopathogens (Soytong *et al.,* 2001). The application of chemical fungicides for disease control has been used for years and gradually caused pollution to surrounding environment. It is recorded that

[*]**Corresponding Author:** Soytong, K.; **Email:** ajkasem@gmail.com

the over use of fungicides can be detrimental affected to human health (Soytong, 2001). *Chaetomium* species are unique isolated from fertile soil and animal dungs (von Arx, 1986). It is reported to control *P. oryzae* (Soytong and Quimio, 1989), *Phytophora palmivora* causing root rot of Pomelo (Hung *et al.* 2015). *Colletotrichum gloeosporioides, Fusarium oxysporum* f. sp. *lycopersici* (Soytong *et al.,* 2001). The objective was to evaluate the constructed nano-particles from *Chaetomium lucknowense* to control rice blast disease caused by *P. oryzae*.

Materials and methods

Pathogen, antagonists and nano-partcles preparation

Pyricularia oryzae was isolated from rice var. RD57, identified and tested pathogenicity from previous experiment used in this study. The original isolate of*Chaetomium lucknowense* used in this study is reported to produce bioactive substances to be effectice against human (Thahinung *et al.,* 2010) and phytopathogen (Charoenporn *et al*., 2011). Bioactive substances extracted from *C. lucknowense* were done by following the method of Phokerd *et al*. (2008). The bioactive compound was constructed to be nano-particles by followiong the method of Dar and Soytong, 2014).

Biological activity of nano-particles against Pyricularia oryzae

Testing biological activity was followed the method of Charoenporn *et al*. (2011). Two factors experiment were conducted with four replications, and the factor A was nano-particles derived from nano-particles derived from *C. lucknowense* (nano-HCL, nano-ECL, nano-MCL) and factor B was the concentrations of 0,1,3,5 and 10 ppm.

Evaluation of nano-particles to control rice blast in pot experiment

The 15 days of rice seedings var. RD57 were planted in sterilized clay soil in pot experiment for 15 days before treatment. Treaments were set up as follows:- treatment 1(T1) was inoculated with *P. oryzae*, treatment 2 (T2) was inoculated with *P. oryzae* and treated with nano-CL, and treatment 3 (T3) was inoculated with *P. oryzae* and treated with chemical fungicide,Tricyclazole. All treatments were done at the same manner of inoculated the pathogen through wounds, which made by sterilized syrinse at 0.5 mm. dia. One wound was

inoculated with inoculum suspension of 1 ml. Each treatment was done as mentioned above at every 15 days.

Experimental design and staistical analysis

The experiment for biological activity was set up as 3 x 5 factorial experiment in Completely Randomized Design (CRD). Colony diameter (cm) and spore number were collected and computed inhibition percentage. The effective dise (ED_{50}) was computed by probit analysis. The pot experiment was performed by using Randomized Completely Block Design (RCBD) with four repeated experiments. Disease index(DI) was scored and computed disease reduction (%), plant height(cm), plant fresh and dry weight (g) were statistical analyzed. Means in each experiment were compared by using Duncan's New Multiple Range Test (DMRT) at P=0.05 and 0.01.

Results

Efficacy of nano-particles derived from Chaetomium lucknowense against Pyricularia oryzae

Result showed that the nano-particles derived from *C. lucknowense* namely nano-HCL, nano-ECL and nano-MCL expressed biological activity against *P. oryzae* causing rice blast disease. It was significantly highest spore inhibition whren treated at the concentration of 10 ppm with nano-ECL and nano-MCL which inhibited the spore production at 81.93 and 81.80 %, respectively, and followed by nano-HCL which was 77.26 %. The ED_{50} values of nano-ECL, nano-MCL and nano-HCL actively inhibited the sporulation of *P. oryzae* at the concentration of 82, 114, 181 ppm., respectively as seen in Table 1 and Figure 1).

Evaluation of nano-particles to control rice blast in pot experiment

The nano-particles derived from*C. lucknowense* gave a good result to inhibit *P. oryzae* causing blast disease in pot experiment. The treated nano-CL gave significantly better to control rice blast disease than the chemical fungicide, Tricyclazole in rice var. RD57 after 20 days. It was not significantly differed in plant height of all treatments, when started the experiment which inoculated with *P. oryzae*, treated with nano-CL, and and treated with chemical fungicide,Tricyclazole. Plant height parameters at 20 days were 62, 65 and 62 cm., repectively. The application of nano-CL at 40 days gave the highest plant

growth of 87 cm when compared to inoculated with *P. oryzae* and treated with chemical fungicide,Tricyclazole which were 77 and 74 cm., respectively as seen in Table 2. It is noted that the rice blast disease showed the highest disease reduction of 54 % when compared to the chemical fubgicide that the disease reduction of 29.26 % (Table 2).

Table 1. Testing of nano-particles from *Chaetomium lucknowense* against *Pyricularia oryzae*

Bioactive metabolites	conc. (ppm)	colony dia. (cm)	growth inhibition,%	spores(10^5 spores/ml)	spore inhibition,%	ED_{50} (ppm)
nano-HCL	0	5.00[a1]	-	47.63[a]	-	
	1	4.88[b]	2.50[c]	37.80[b]	20.52[f]	
	3	4.80[b]	4.00[c]	35.30[b]	25.82[f]	181
	5	4.72[b]	5.50[c]	26.15[c]	45.02[de]	
	7	4.80[b]	4.00[c]	20.20[cd]	57.67[c]	
	10	4.13[c]	17.50[a]	10.85[e]	77.26[b]	
nano-ECL	0	5.00[a]	-	47.63a	-	
	1	4.90[b]	2.00[c]	34.00b	28.55[f]	
	3	4.90[b]	2.00[c]	27.75c	41.75[de]	
	5	4.80[b]	4.00[c]	21.70c	54.46[d]	82
	7	4.80[b]	4.00[c]	17.45d	63.39[c]	
	10	4.67[b]	6.50[c]	8.60e	81.93[a]	
nano-MCL	0	5.00[a]	-	47.63[a]	-	
	1	4.85[b]	3.00[c]	37.40[b]	21.48[f]	
	3	4.85[b]	3.00[c]	31.45[b]	33.81[f]	
	5	4.80[b]	4.00[c]	22.35[c]	53.05[d]	114
	7	4.73[b]	5.50[c]	17.60[d]	62.97[c]	
	10	4.47[b]	10.50[b]	8.65[e]	81.80[a]	
	C.V. (%)	2.06		11.31		

[1] Means of four repeated experiment which followed by a common letter in each column were not significantly differed at DMRT = P 0.01.

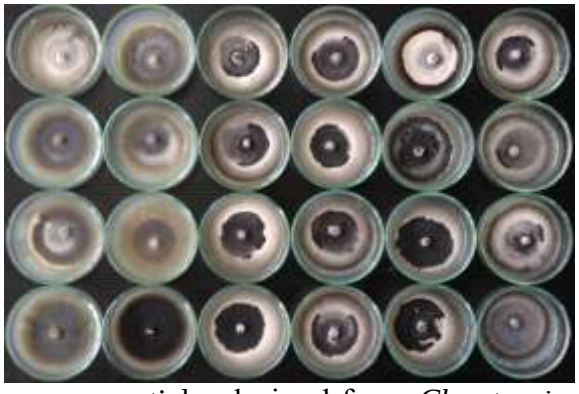

Figure 1. Testing nano-particles derived from *Chaetomium lucknowense* against *Pyricularia oryzae*

Table 2. Disease reduction and plant height of rice var. RD 57 treated with nano-particles derived from *Chaetomium lucknowense*

Treatments	plant height(20 day) cm.)[1]	plant height(40 days) (cm)[1]	Disease reduction, %
inoculated *P. oryzae*	62[a]	77[b]	-
***P. oryzae* + nano-CL**	65[a]	87[a]	54
***P. oryzae* + Tricyclazole**	62[a]	74[b]	29
C.V. (%)	2.52	12.48	

[1] Means of four repeated experiment which followed by a common letter in each column were not significantly differed at DMRT = P 0.01.

Moreover, the further result showed that Tricyclazole gave significanlty highest fresh weight of stems which was 66 g and followed by nano-CL (55 g) when compared to inoculated control with *P. oryzae* which was 45 g as seen in Table 3. Nano-CL was significantly differed in fresh root weight of 79.14 g when compared to the inoculated control with *P. oryzae* and Tricyclazole 58 and 44 g, respectively.

Table 3. Fresh and dry weight of rice var. RD57 treated with nano-particles derived from *Chaetomium lucknowense*

Treatments	fresh weight (g)	
	stems[1]	roots[2]
inocullated control with *P. oryzae*	45c	58b
***P. oryzae* + nano-CL**	55b	79a
***P. oryzae* + Tricyclazole**	66a	44c
C.V. (%)	22.82	29.87

[1] Means of four repeated experiment which followed by a common letter in each column were not significanly differed at DMRT = P 0.01.

Table 4. Dry weight of stems and roots in rice var. RD 57 treated with nano-particles derived from *Chaetomium lucknowense*

Treatments	dry weight (g)	
	stems[1]	roots[2]
inoculated control with *P. oryzae*	14b	6b
***P. oryzae* + nano-CL**	20a	12a
***P. oryzae* + Tricyclazole**	11bc	11a
C.V. (%)	29.29	28.96

[1] Means of four repeated experiment which followed by a common letter in each column were not significantly differed at DMRT = P 0.01.

The tested nano-CL was significantly highest stem drty weight (20 g) and follwed by inoculated control with *P. oryzae*, nano-CE, Tricyclazole which were 14 and11 g., respectively. Nano-CL and Tricyclazole were not significantly differed which root dry weight were12 and 11 g respectively, when compared to the inoculated control with *P. oryzae* which was 6 g as seen in Table 4.

Discussion

Morphological study was done in previous experiment to prove identification of *P. oryzae* which cultured on Rice Flour Agar. The colony is geryish white, slow growing, septate mycelia, conidiaare pyriform shape, 3 septa in a cell, 17-23 × 8-11 micron that similar reports by Bussaban, *et al* (2005) and Wang (2016). Skamnioti *et al.* (2019) stated that the causing agent of blast disease that widely distributed and is destructive to rice production of 30% yield loss. It is interesting that Ou (1985) reported that isolate of *P. oryzae* from rice is mostly host-specific.

It was found that nano-particles derived from *C. lucknowense* gave actively inhibited the rice blast pathogen. As results, nano-ECL, nano-MCL and nano-HCL were actively against *P. oryzae* which the ED_{50} values were 82, 114, and 181ppm, rtespectively. As similar in the previous report found that that nano-particles of *C. elatum* was actively against *P. oryzae* causing rice blast (Song, *et al*, 2016). This result is similar reported by Tann and Soytong (2016) found that Nano-CGH, nano-CGE, and nano-CGM derived *Chaetomium globosum* gave antifungal activity against *Curvularia lunata* (leaf spot disease of rice var. Sen Pidoa), which the ED_{50} values of 1.21, 1.19, and 1.93 ppm/mL, respectively). It was also found in pot experiment that the nano-particles derived from *C. lucknowense* resulted to control the rice blast disease.

Application of nano-CL gave better disease control than chemical fungicide, Tricyclazole. Similar report was stated by Tann and Soytong (2016) tested in a pot experiment showed that nano-CGH, nano-CGE, and nano-CGM derived *Chaetomium globosum* resulted to decrease leaf spot of rice var. Sen Pidoa in pot experiment. Result showed that the nano-CL gave better plant strands than the inoculated control. Tann and Soytong (2016) also reported similar result that the nano-CGH, nano-CGE, and nano-CGM derived *C. globosum* significantly increased the height of the rice plant when compared to the non-treated control.

The research finding is confirmed that *C. elatum* produces bioactive metabolites against the teated pathogen, *P. oryzae* as a control mechanism and the crude extracts of *C. lucknowense* gave significantly inhibited the tested pathogen in

higher rate of application when compared to nano-particles derived from *C. lucknowense*. Similar discussion searched from Vilavong and Soytong (2017) stated that using nano-rotiorinol, dericed from *Chaetomium cupreum*, nano-trichotoxin derived from *Trichoderma harzianum* and ascospore suspension of *C. cupreum* resulted to reduce coffee anthracnose of 46.23, 42.71 and 18.59 %, respectively. It is needed to develop new approach of non-toxic agricultural inputs to be used instead of the chemical fungicides. As known, the chemical fungicides are ready recognized to cause human diseases, pollution, residue in the soil, water and agriculture products. It is proved that continuous chemical fungicide application will let the pathogen resistant to chemical fungicides (Soytong *et al*, 2001).

As result in pot experiment the tested nano-particles from *C. lucknowense* significantly controlled rice blast disease better than the chemical fungicide. Similar report from Vilavong and Soytong (2017) that bio-formulation of *C. cupreum* in powder form, nano- rotiorinol, from *C. cupreum* and nano-trichotoxin from *T. harzianum* gave a good control of coffee anthracnose.

It is concluded that *C. lucknowense* is proved to be effectively against rice blast disease caused by *P. oryzae*. Its metabolites could be inhibited the pathogen inoculum and reduced the disease incidence of rice blast. Research and development on nanotechnology for crop protection is done by construction of nano-particles from the crude extracts and pure compound of *C. lucknowense* would become a new strategy for rice disease protection. Further research finding is to develop these natural product to be used in crop production.

Acknowledgement

I would like to acknowledge the King Mongkut's Institute of Technology Ladkrabang (KMITL) to offer a PhD Scholarship and research fund is supported by Faculty of Agricultural Technology, KMITL and further research fund [KREF126004] supported by KMITL, Bangkok, Thailand. The financial support from Thailand Research Fund (Grant No RTA5980002) is also gratefully acknowledged.

References

Bussaban, B., Lumyong, S., Lumyong, P., Seelanan, T., Park, D. C., McKenzie, E. H. C., Hyde, K. D. (2005). Molecular and morphological characterization of *Pyricularia* and allied genera. Mycologia. 97:1002-1011.

Charoenporn, C., Kanokmedhakul, S., Lin, F. C., Poeaim, S. and Soytong, K. (2011). Evaluation of bio-agent formulations to control Fusarium wilt of tomato. African Journal of Biotechnology. 9:5836-5844.

Dar, Joselito and Soytong, K. (2014). Construction and characterization of copolymer nanomaterials loaded with bioactive compounds from *Chaetomium* species. International Journal of Agricultural Technology. 10:823-831.

Hung, P. M., Pongnak, W., Soytong, K. and Poeaim, S. (2015). Efficacy of Chaetomium Species as Biological Control Agents against *Phytophthora nicotianae* Root Rot in Citrus. International Journal of Agricultural Technology. 10:833-844.

Normile, Dennis (1997). Yangtze seen as earliest rice site. Science. 275: 309-310.

Phonkerd, N., Kanokmedhakul, S., Kanokmedhakul, K. soytong, K., Prabpai, S. and Kongsearee, P. (2008). Bis-sprio-azaphilones and azaphilones from the fungi *Chaetomium cochliodes* Vth01 and *Ch. cochlioides* Cth05. Tetrahedron. 64:9636-9645.

Skamnioti, Par and Gurr, Sarah J. (2009). Against the grain: safeguarding rice from rice blast disease. Trends in Biotechnology. 27:141-150.

Soytong, K. and Quimio, T. (1992). Antagonism of *Chaetomium cupreum* to *Pyricularia oryzae*: A case study in biocontrol of rice blast disease. Journal of Plant Protection in the Tropics. 9:17-23.

Soytong, K. (2014). Bio-formulation of *Chaetomium cochliodes* for controlling brown leaf spot of rice. International Journal of Agricultural Technology. 10:321-337.

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

Tann, H. and Soytong, K. (2016). Effects of nanoparticles loaded with *Chaetomium globosum* KMITL-N0805 extracts against leaf spot of rice var. Sen Pidoa. Malaysian Applied Biology Journal. 45:1-7.

Ou, S. H. (1985). Rice diseases. CAB International; Wallingford, UK.

Vilavong, S. and Soytong, K. (2017). Application of A New Bio-Formulation of *Chaetomium cupreum* For Biocontrol of *Colletotrichum gloeosporioides* Causing Coffee Anthracnose on Arabica Variety in Laos Agrivita 39.

Von Arx, J. A., Guarro J. and Figueras, M. J. (1986). The Ascomycete Genus *Chaetomium*. Nova Hedwigia Beihefte, Lubrecht & Cramer Ltd.

Wang, X. W. (2016) Diversity and taxonomy of *Chaetomium* and chaetomium-like fungi from indoor environments. State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences. 84:145-224.

(Received: 12 September 2018, accepted: 31 October 2018)