International Journal of Agricultural Technology 2018 Vol. 14(7): 2207-2214
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Nano-particles from *Cheatomium brasiliense* against brown spot of rice

Vareeket, R.[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 10520, Thailand; [2]Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

Vareeket, R., Soytong, K., Kanokmedhakul, S. and Kanokmedhakul, K. (2018). Nano-particles from *Cheatomium brasiliense* against brown spot of rice. International Journal of Agricultural Technology 14(7): 2207-2214.

Abstract *Chaetomium brasiliense* was used in this study to control rice brown spot disease pathogen, *Drechslera oryzae*. The result of bi-culture test showed that *Ch. brasiliense* gave the hightest percentage of growth inhibition at 26.38% and had the highest spore inhibition at 23.81%. In crude extracts test, it was found that crude methanol extract obtained from *Ch. brasiliense* at 1000 ppm showed the highest inhibitory effect on colony growth and spore production. *Ch. brasiliense* gave a growth inhibition and spore inhibition rates at 83.50 and 99.78% respectively. The effective dose (ED_{50}) on growth and spore inhibition of *Ch. brasiliense* was 80.54 and 0.35 µg/ml, respectively. It was also found that Nano-particles obtained from crude methanol extract of *Ch. brasiliense* (nano CBM) at 10 ppm had the best inhibitory effect in terms of growth and spore inhibition. Nano CBM can inhibit the growth at 70.00% and spore production at 79.92%. The ED_{50} values for spore inhibition of *Ch. brasiliense* was 2.86 µg/ml.

Keywords: *Chaetomium brasiliense*, biological control, rice disease

Introduction

Rice (*Oryza sativa* L) is the most widely consumed staple food for a large part of the world's human population, especially in Asia and the West Indies. It is the grain with the third-highest worldwide production (FAOSTAT, 2017). *Drechslera oryzae* is a fungus that reports to cause brown leaf spot of rice. This is one of the important plant pathogen causing a widespread disease leading to yield losses. Brown spot of rice can infect the seedlings and mature plants. The symptom appeared as blight on seedling where grown from infected seeds (Rice Department, 2018).

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

This objective was to investgate the morphology of *Chaetomium brasilense* and *Drechslera oryzae*, pathogenicity test. Tesing crudec extraxts and nano-particles from *Chaetomium brasilense* to control brown leaf spot of rice var RD47 were also conducted.

Materials and methods

Isolation of pathogen and pathogenicity test

Drechslera oryzae causing brown leaf spot of rice were isolated form seed rice var. RD47. Rice seeds were soaked in sterilized water and then in 1% clorax for 3 min and then sterilized water. All seeds were moved to water agar and sub-cultured to PDA until get pure culture. Morphology was observed under compound microscope.

Morphology study of the *Drechslera oryzae*

Isolate of *Drechslera oryzae* was morphological identified. The characters of *D. oryzae* were determined under compound microscope.

Pathogenicity test

The experimental design was done by Completely Randomization with four repeated times. The pathogen isolate was proved for pathogenicity followed Koch's Postulate. The conidia suspension of 5×10^6 conidia/ml. was used for inoculation. The 15 days of rice seedlings var. RD47 were inoculated sprayed onto the wounded leaves (3 leaves/seedling). The inoculated leaves were covered with plastic sheets, then observed the infected leaves. The leaves with spraying sterilize distilled water were done to serve as controls.

Biological control of Drechslera oryzae

Strain of antagonist used for experiments

Chaetomium brasiliense was kindly provided by Assoc. Prof. Dr. Kasem Soytong.

Dual culture test

Ch. brasiliense was tested to control *D. oryzae*. Each fungus was separately cultured on PDA at room temperature for seven days. A 0.5 cm diameter sterilized cork borer was used to transfrred agar plugs from peripheral colony of each fungus to 9-cm diameter PDA plates, and put in the opposite site to each other. Control plates were transferred each fungus alone to PDA plates.

The testeds plates were incubated at room temperature for 30 days. Abnormal spores wewre observed under compound microscope and took photograph. Colony diameter, number of conidia of pathogenic fungus were collected. The growth or conidia inhibition of pathogen was calculated using formula below:

$$\text{Inhibition (\%)} = \frac{A-B}{A} \times 100 \quad (1)$$

A = colony diameter or conidia number of pathogen in control

B = colony diameter or conidia number of pathogen in control in dual culture plate

The data were statistically computed and means were compared by using Duncan's New Multiple Range Test at P=0.01 and P=0.05.

Biological activity of antagonist against pathogen (crude extract test)

Crude extracts from *Ch. brasiliense* was tested against *D. oryzae* The fungus was cultured in potato dextrose broth at room temperature for one month. The biomass was collected, ground with the electrical blender and dissolved with solvents. The solvents were then separtately evaporated *in vacuo* to yield crude hexane, crude ethyl acetate, and crude methanol extracts, respectively.

The crude extracts were assayed to inhibit the tested pathogen, *D. oryzae.* The experimental design was conducted by using two factors factorial experiment in Compleletly Randomization withy four repatedted times. Factor A1 was crude hexane, A2 crude ethyl acetate and A3 crude methanol. Factor B1 was 0 μg/ml(control), B2 = 10 μg/ml, B3 = 50 μg/ml, B4 = 100 μg/ml, B5 = 500 μg/ml and B6 = 1,000 μg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxide and added to PDA before autoclaved at 121°C (15 psi) for 20 minutes. A sterilized 3-mm diameter cork borer was used to remove agar plugs from peripheral colony to the center of 5 cm dia Petri dishes of PDA containing crude extract at each concentration. Incubation was done until grown full plates. The number of spore was collected and calculated the inhibition. The effective dose (ED_{50}) was calculated using Probit analysis.

Testing nano-particles from Chaetomium brasiliense to control brown leaf spot

Nano-particles were performed using the method of Dar and Soytong (2014) to get Nano-CBH, Nano-CBE and Nano-CBM. Two factors factorial experiment in Complately Randomized Design was conducted with four

replications . Factor A was Nano-CBH, Nano-CBE and Nano-CBM and factor B was 0, 1, 3, 5, 7 and 10µg/ml. Each Nano-particle was dissolved in 2% dimethyl sulfoxide, mixed into PDA medium, and autoclaved at 121^0C, 15 lbs/inch2 for 30 min. The colony of *D. oryzae* was cut at the peripheral colony with sterilized cock borer (3mm), transferred to the middle of PDA incorporated with nano-particles. The plates were incubated at room temperature until the pathogen in control growing full plate. Abnormal spores were observed under compound microscope. Colony diameter and the number of spores were recorded. Inhibition was computed and the effective dose (ED_{50}) was calculated using Probit analysis.

Results

Isolation of pathogen and pathogenicity test

Rice pathogen was isolated from rice seeds of RD 47. It was found *Drechslera oryzae*. Pure culture showed brown color when mature, septate mycelia, and conidia with many septates or cells on one conidia (Figure 1).

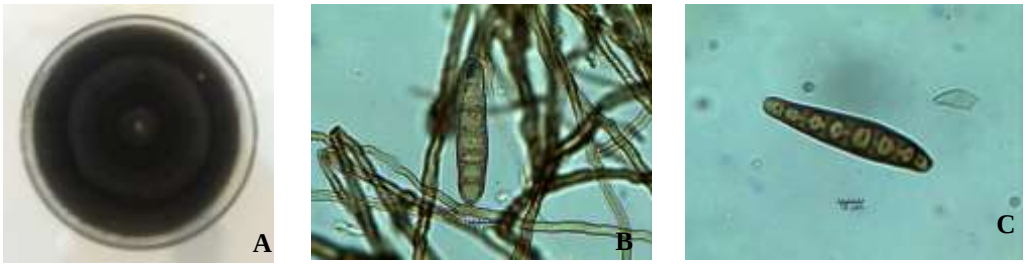

Figure 1. *Drechslera oryzae* on PDA medium after 7 days **(A),** *Drechslera oryzae* conidiophore (B) and *Drechslera oryzae* conidium **(C)**

Figure 2. Pathogenicity test of *Drechslera oryzae* on rice. The inoculated control (left) and inoculated leaves (right) after 7 days

The pathogenicity of the isolate was confirmed; the symptoms appear in from of minute light brown or brownish red spots and these leaf spots becomes dark brown with a surrounding halo region (Figure 2).

Biological control of Drechslera oryzae

Ch. brasiliense resulted to inhibit mycelial growth of *D. oryzae* which averaged colony was 6.62 cm when compared to control plate (9.00 cm). It significantlyt inhibited the mycelia growth of 26.38%. *Ch. brasiliense* alsp espressed significantly spore inhibition of *D. oryzae* 23.81% as seen in Figure 3, and Table 2].

Figure 3. Bi-culture antagonistic test between *Drechslera oryzae* and *Chaetomium brasiliense*

Table 1. *Chaetomium brasiliense* against *Drechslera oryzae* in bi-culture tests

	Chaetomium brasiliense		**Growth Inhibition percent**	**C.V. (%)**
	Control	**Bi-culture**		
Colony growth(cm)	9.00[a1]	6.62[b]	26.38	0.55
Spore number (10^7/ml)	22.2[a]	16.90[b]	23.81	7.24

1/: Means four repeated experiments and followed by the same letter are not significantly differed by DMRT at P=0.01.

Biological activity of antagonist against pathogen

Crude ethyl acetate extract of *Chaetomium brasiliense* gave significantly highest inhibition of *Drechslera oryzae* which the ED_{50} of 0.24 μg/ml, and followed by crude hexane and methanol extracts which the ED_{50} of 0.32 and

0.35 µg/ml, respectively. Crude methanol extract at 1,000 ppm showed significantly highest spore inhibition of 99.78 %, and followed by crude hexane (99.77 %) and ethyl acetate extracts (99.60 %). Crude methanol extract significantly inhibited colony growth by 83.50 %, and followed by the crude ethyl acetate (82 %) and hexane extracts (74.50 %) as seen in Table 2.

Table 2. Crude extracts of *Chaetomium brasiliense* testing to inhibit *Drechslera oryzae*

Crude extracts	Concentration (ppm)	Colonydiameter (cm)	Growth inhibition (%)	ED_{50} (µg/ml)	Number of spore[1/]	Spore inhibition (%)	ED_{50} (µg/ml)
	0	5.00^{a1}	-		185.06^{a}	-	
	10	3.50^{c}	30.00^{k}		22.78^{bc}	87.65^{e}	
Crude	50	3.30^{de}	34.00^{ij}	93.09	20.35^{bcd}	88.99^{de}	0.32
hexane	100	2.80^{h}	44.00^{h}		12.23^{de}	93.34^{c}	
	500	1.53^{j}	69.50^{d}		0.76^{f}	99.58^{a}	
	1000	1.27^{k}	74.50^{c}		0.40^{f}	99.77^{a}	
	0	5.00^{a}	-		185.06^{a}	-	
	10	3.22^{e}	35.50^{i}		20.56^{bcd}	88.81^{e}	
Crude	50	2.47^{g}	50.50^{g}	85.77	18.13^{cd}	90.18^{d}	0.24
ethyl	100	2.12^{i}	57.50^{e}		12.76^{de}	93.09^{c}	
acetate	500	1.05^{l}	79.00^{b}		1.16^{f}	99.36^{a}	
	1000	0.90^{m}	82.00^{a}		0.73^{f}	99.60^{a}	
	0	5.00^{a}	-		185.06^{a}	-	
	10	4.07^{b}	18.50^{l}		29.32^{b}	84.13^{f}	
Crude	50	3.38^{cd}	32.50^{jk}	80.5	21.43^{bcd}	88.38^{e}	0.35
methanol	100	2.32^{h}	53.50^{f}		8.02^{ef}	95.66^{b}	
	500	1.27^{k}	74.50^{c}		2.18^{f}	98.79^{a}	
	1000	0.83^{m}	83.50^{a}		0.38^{f}	99.78^{a}	
	C.V. (%)	3.25			14.92		

1/: Means four repeated experiments and followed by the same letter are not significantly differed by DMRT at P=0.01.

Testing nano-particles from Chaetomium brasiliense to control brown leaf spot

The nano-CBH, nano-CBE, and nano-CBM at the concentration of 10 ppm inhibited spore production by 85.25%, 77.86%, and 79.92%, respectively. These nanoparticles expressed antifungal activity against *Drechslera oryzae* with ED_{50}values of 5.86, 4.92, and 2.86 µg/ml, respectively (Table 3).

Table 3. Nano-particles of *Chaetomium brasiliense* testing to inhibit *Drechslera oryzae*

Crude extracts	Concentration (ppm)	Colonydiameter (cm)	Growth inhibition (%)/2	ED_{50} (µg/ml)	Number of spore	Spore inhibition (%)	ED_{50} (µg/ml)
	0	5.00^{a1}	-		5.45^{a}	-	
	1	1.91^{de}	61.75^{b}		2.37^{cde}	55.67^{efg}	
Nano-	3	2.09^{c}	58.25^{c}	-	1.62^{efg}	69.50^{bcde}	5.86
CBH	5	2.31^{b}	53.75^{f}		1.28^{fgh}	76.35^{abcd}	
	7	2.10^{c}	58.00^{f}		0.99^{gh}	81.73^{ab}	
	10	1.80^{ef}	64.00^{h}		0.77^{h}	85.25^{a}	
	0	5.00^{a}	-		5.45^{a}	-	
	1	2.07^{c}	58.50^{b}		2.89^{c}	46.71^{g}	
Nano-	3	2.05^{cd}	59.00^{d}	168.00	2.57^{cd}	52.36^{fg}	4.92
CBE	5	1.95^{cd}	61.00^{e}		2.03^{def}	62.50^{def}	
	7	1.97^{cd}	60.50^{g}		1.99^{def}	62.80^{def}	
	10	1.53^{g}	69.50^{h}		1.20^{gh}	77.86^{ab}	
	0	5.00^{a}	-		5.45^{a}	-	
	1	2.02^{cd}	59.50^{b}		3.96^{b}	25.60^{h}	
Nano-	3	2.00^{cd}	60.00^{cd}	237.00	2.35^{cde}	56.84^{efg}	2.86
CBM	5	1.68^{f}	66.50f		2.01^{def}	63.32^{def}	
	7	1.53^{g}	69.50^{f}		1.26^{fgh}	77.13^{def}	
	10	1.50^{g}	70.00^{h}		1.12^{gh}	79.92^{ab}	
	C.V. (%)	4.10			19.39		

1/: Means four repeated experiments and followed by the same letter are not significantly differed by DMRT at P=0.01.

Discussion

The brown spot of rice caused by *Drechslera oryzae* was isolteds and proved pathogenicityn as similar report of Tan and Soytong (2016b). *Ch brasiliense* proved to be antagonised the tested pathogen in bi-culture test was also similar result of Tan and Soytong (2016a). As a result, the crude hexane and methanol extracts from *Ch. brasiliense* actively against *D. oryzae* causing brown spot of rice var RD 47 which the ED_{50} values of 0.32 and 0.35 µg/ml, respectively. Tan and Soytong (2017) reported that bioformulation including crudec extrtacts from *Chaetomium cupreum* CC3003 gave a good control leaf spot of rice var. Sen Pidao in Cambodia. Each crude crude extract of *Ch. brasiliense* was performed to be nano-particles as nano-CBH, nano-CBE, and nano-CBM and tested to inhibit *D. oryzae* causing brown leaf spot of rice var RD47. As results, it showed that nanoparticles, nano-CBH, nano-CBE, and nano-CBM gave significantly inhiubited expressed *D. oryzae* at the ED_{50}values of 5.86, 4.92, and 2.86 µg/ml, respectively. It concluded that nanoparticles from *Ch. brasiliense* gave successfully inhibitred the tested pathogen. Further study would be applied the the fields and also tested to control the other plantb pathogens. As reports of Vilavong and Soytong (2017) stated that the

application of bio-formulation including nano-elicitor of *Ch. cupreum* could control *Colletotrichum gloeosporioides* causing Coffee Anthracnose on Arabica Variety in Laos.

Acknowledgement

I would express my sincerely thank Mr. Boonmee Ruengrat from Strong Crop Co. Ltd, Thailand through Association of Agricultural Technology in Southeast Asia (AATSEA) to offter my study for Ph. D. scholarship. This research project is preliminary presented as a part of Ph. D. thesis. The financial support from Thailand Research Fund (Grant No RTA5980002) is also gratefully acknowledged.

References

Joselito, D. 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.

Rice Department (2018). Rice Knowledge Bank. Retrieved by 5 September 2018. http://www.ricethailand.go.th/rkb/disease%20and%20insect/index.phpfile=content.php &id=113.htm.

Tann, H. and Soytong, K. (2016a). Bioformulations and nano product from *Chaetomium cupreum* CC3003 to control leaf spot of rice var. Sen Pidao in Cambodia. International Journal of Plant Biology. 7:6413.

Tann, H. and Soytong, K. (2016b). 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.

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

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 Journal of Agricultural Science. 39:303-310.

UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT). (2017). Retrieved by 10 September 2017. http://www.fao.org/faostat/en/#data/QC.

(Received: 30 August 2018, accepted: 21 October 2018)