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Bio-Formulation of *Chaetomium cochliodes* for Controlling Brown Leaf Spot of Rice

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Abstract *Chaetomium cochliodes* proved to be a new antagonistic fungus against brown leaf spot of rice var Pittsanulok 2 caused by *Drechslera oryzae.* It showed good inhibition of mycelial growth of 38.18 per cent and inhibited inoculum production of 71.55 per cent. Crude extracts from *Ch cochliodes* using hexane, ethyl acetate and methanol at 1,000 ppm could significantly inhibited the inoculum production of rice pathogen 93.85 per cent which ED_{50} value was 66.45 ppm when compared to the control (0 ppm). *Ch cochliodes* was formulated in different forms for applying to control brown leaf spot of rice. Biological products formulated from *Ch cochliodes* were tested to control brown leaf spot of rice caused by *D oryzae*. Result showed that bio-powder formulation gave significantly highest to control leaf spot and highest plant growth when compared to the non-treat control, followed by applying crude extract of *Ch cochliodes*, benlate and spore suspension of *Ch cochliodes*. Moreover, bio-powder formulation gave significantly increased in plant growth over 44 % and followed by crude extract of *Ch cochliodes*, spore suspension of *Ch cochliodes* and benlate.

Keywords: *Chaetomium cochliodes*, crude extract, *Drechslera oryzae*

Introduction

Rice (*Oryza sativa* L.) is one of economically stable crop in the world. During cultivation of rice, weed, pests and diseases are invaded to destroy the plants and low yield. Rice varieties are reported to infect with several plant pathogens eg *Trichoconis padwickii, Curvularia lunata, Fusarium semitectum, Drechslera oryzae, Sarocladium oryzae, Alternaria tenuis, Fusarium moniliforme, Nigrospora oryzae, Phoma* spp., *Cladosporium* spp. and *Pyricularia oryzae* (Thawat *et al*, 1997). The important diseases occur in susceptible variety of rice are rice blast caused by *Magnaporthe grisea* (*Pyricularia oryzae*) and brown leaf spot caused by *Drechslera oryzae* (*Helminthosporium oryzae*). Both diseases have been reported to seriously infected to susceptible variety of rice leading to low yield over 50 %. The

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traditional chemical fungicides have been used for years and some case the pathogens become resistant to those chemical fungicides. However, there are many researchers are reported to use the biological control agents to control those diseases. *Chaetomium cupreum, Chaetomium globosum* are reported to be antagonize *Pyricularia oryzae* cauaing rice blast and *Drechslera oryzae* (*Helminthosporium*) causing brown leaf spot of rice. Kanokmedhakul *et al.* (2001) stated that *Ch globosum* could produce antimycobacterial anthraquinone-chromanone compound and disktopiperazine alkaloid and antifungal Azaphilones from *Ch cupreum* (Phonkerd *et al.*, 2008) including bis-spiro-Azaphilones and azaphilones from *Ch cochliodes*. Thohinung *et al.*, (2010). The objective of research project was to evaluate bio-formulation of *Ch. cochliodes* to control *Drechslera oryzae* causing brown leaf spot of rice.

Materials and methods

Isolation of pathogen and pathogenicity test

Rice pathogens were isolated from rice seeds of Chainart 1, Chainart 2, Supanburi 2, Supanburi 2, Pitsanulok 2, Koekor 31, Koekor 39,Koekor 41 and Korkor 47 using moist chamber at room temperature, the signs of pathogens were transferred to water agar (WA) and then subcultured to potato dextrose agar (PDA) until get pure culture. The most frequency found pathogen would then prove for pathogenicity test followed the Koch's postulate method. Rice seedlings of 15 days were inoculated with spore suspension of pathogen at the inoculum concentration of $1x10^6$ spore/ml which inoculated to wounded lesions on leaves by sterilized needle. The infected areas of lesions are re-isolated to get pure culture of pathogen. Identification into species level was morphologically done under bi-nocular compoubd microscope followed instruction of Ellis (1971), Ou (1984), Domsch *et al.*(1980) ,Von Arx *et al.* (1986) ,Soytong and Quimio (1989), Soytong *et al* (2001) ,Pornsuriya *et al.* (2008) and Hoog G.S.(2000).

Test for control mechanism of Chaetomium cochliodes against rice pathogen

Bi-cultural antagonistic test was done using completely randomized design (CRD) with four replications. *Ch cochliodes.* The rice pathogen which proved for pathogenicity was co-cultured to PDA. The agar plug of antagonist and pathogen were transferred to PDA in opposite site to each other, incubated for 18 days at room temperature. Data were collected as coloby diameter (cm) and spore suspension using haemacytometer. Data were then transformed to

percent inhibition (PI) where PI = R1-R2/R1x100, R1 was colony diameter or number of spore in control plate and R2 was colony diameter or number of spore in co-cultured plates. The abnormal spore of pathogen was also observed under binocular compound microscope.

Bioactive compound from *Ch cochliodes* was tested for antifungal activity against rice pathogen. *Ch cochliodes* was cultured in potato dextrose broth (PDB) for 30 days then filtered and air dried at room temperature to get fungal biomass. The dried fungal biomass was ground and extracted in Hexane (1:1, v/v) at stationary for 3 days, and filtered through whatman filter paper No.4 to get culture filtrate. Culture filtrate was centrifuged using rotary vacuum evaporator to get crude hexane. The marc of fungal biomass was continued to extract by ethyl acetate (EtOAc) and methanol (MeOH).

Each crude extract of hexane, EtOAc and MeOH was separately dissolved in 2% DMSO)Dimethylsulfoxide(to test against rice pathogen. The experiment was done using two factors factorial experiment in CRD with four replications. Factor A represented crudecextract where A1 was crude hexane, A2 was crude ethyl acetate and A3 was crude methanol. Factor B represented fungal crude extracts ehich B1 was 0 ppm, B2 was 10 ppm, B3 was 50 ppm, B4 was 100 ppm, B5 was 500 ppm and B6 was 1,000 ppm. *Drechslera oryzae* was tested to inhibit by fungal crude extracts from hexane, ethyl acetate dissolved in 2 % DMSO (Dimethylsulfoxide) and methanol incorporated with PDA, then sterilized at 121 C, 15 lbs for 30 min. The culture agar plug of pathogen was transferred to the middle of PAD incorporated with each crude extract concentration, incubated at room temperature. Data were collected as colony diameter (cm) and number of spores then transformed to percentage of inhibition = R1-R2/R1x100; R1 = number of spores in control, R2 = number of spores in treated plates X 100. Data were computed for analysis of variance and treatment means were compared using Duncan Multiple Range Test (DMRT) at P = 0.005 and P = 0.01. The ED_{50} was computed by probit analysis program.

Bio-formulation of Chaetomium cochliodes testing to control brown leaf spot

The 15 rice seedlings were transferred to soil for 7 days before inoculation with spore suspension of pathogen at the concentration of $1x10^6$ spore/ml to wounded lesions in artificial rice field in greenhouse. The experiment was performed using randomized complete block design (RCBD) with four replications Treatments were done as follows:- T1 was inoculated control, T2 was non – inoculated control, T3 was bio-formulation from spore suspension ($1x10^6$) of *Ch cochliodes*, T4 was bio-powder form of *Ch*

cochliodes applied at 10 g/1Lof water, T5 was benlate-chemical fungicide applied at recommendation rate and T6 was mixed crude extract applied at 10 g/ L of water. All treatments were applied at every 15 days. Data were collected as disease incidence, plant height (cm), number of tillers, fresh and dried weight of plant and computed analysis of variance and treatment means were compared using Duncan Multiple Range Test (DMRT) at P = 0.005 and P = 0.01.

Results

Isolation of pathogen and pathogenicity test

Rice pathogens were isolated from rice seeds of Chainart 1, Chainart 2, Supanburi 2, Supanburi 2, Pitsanulok 2, Koekor 31, Koekor 39,Koekor 41 and Korkor 47. It was found *Drechslera oryzae* which the most frequency appeared as seed borne fungi of rice var Pitsanulok 2, Koekor 31, Koekor 41 and Korkor 47. Pure culture showed brown color when mature, septate mycelia, porospores with many septate or cells on one conidia. *Chaetomium cochliodes* showed gray color colony when young and turned to olivaceous greenish gray to brown when mature on potato dextrose agar which released purple. Ascocarp become brown mature within 6 weeks, 120 x 85 µm, eight ascospores per ascus, ascus clavate, ascospore like lemon shape, 10 x 25 µm. (Fig.1).

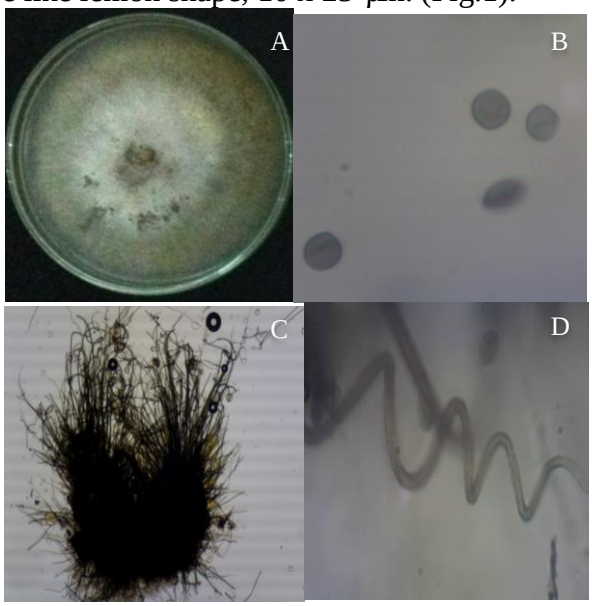

Fig. 1. *Chaetomium cochliodes* A : colony on PDA, B :ascomata, C : terminal hair, and D : ascospore

Test for control mechanism of Chaetomium cochliodes against rice pathogen

Bi-cultural antagonistic test

Result showed that *Ch cochliodes* could inhibit mycelial growth of *D oryzae* which averaged colony of 5.56 cm when compared to control plate of 9.00 cm. It could inhibit mycelia 38.17 per cent in 10 days. However, *Ch cochliodes* significantly inhibited spore production of *D. oryzae* 71.55 percent. It could show a control mechanism of lysis in hyphae of pathogen (Fig.2).

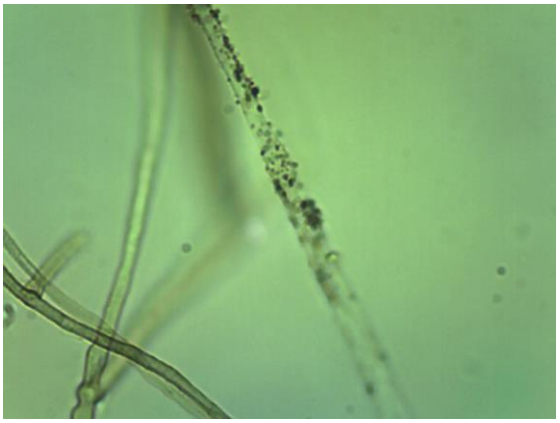

Fig. 2. Hyphe of *Drechslera oryzae* decomposed due to substances released from *Chaetomium cochliodes* in bi-culture antagonistic test

Table 1. *Chaetomium cochliodes* against *Drechslera oryzae* in bi-culture antagonistic test

	Drechslera oryzae		Inhibition [2] (%)	C.V. (%)
	control	Bi-culture		
Colony (cm)	9.00 a[1]	5.56 b	38.18	1.8
Spore number	29.06 a	8.05 b	71.55	26.8

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in bi-culture plates.

Bioactive compound test

Chaetomium cochliodes was cultured in potato dextrose both (PDB) of 500 petri dishes (9cm dia.) for 30 days, then get the dried fungal biomass 20.5 g. The fungal biomass was ground in electrical blender and soaked into hexane 500 ml then placed in shaker for 72 h and filtered through whatman filter paper

No 4 to get the filtrate. The filtrate was then operated in rotary vacuum evaporator to get hexane crude extract of 0.79 g. The marc was further extracted through ethyl acetate and methanol followed the same method to get ethyl acetate crude and methanol crude extracts (Fig. 3).

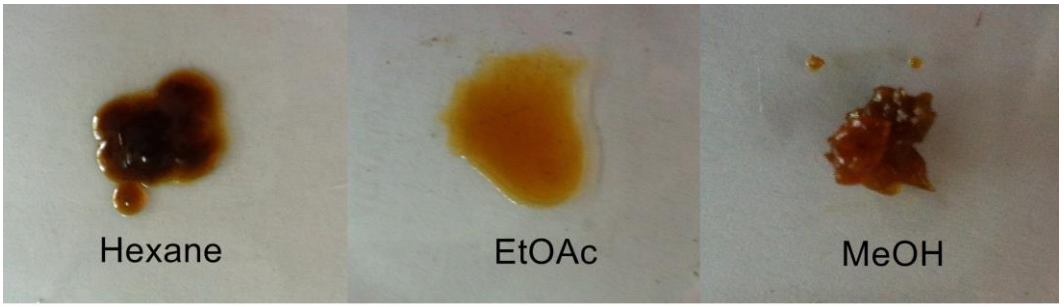

Fig.3. Hexane, ethyl acetate and methanol crude extracts from *Chaetomium cochliodes*

Result showed that hexane crude extract from *Chaetomium cochliodes* gave significantly highest inhibition of 54 % for the colony growth of *Drechslera oryzae* at the concentration of 1,000 ppm when compared to the control. Moreover, hexane crude extract from *Ch cochliodes* gave significantly highest inhibition of 93.85 % for the spore production of *D oryzae* at the concentration of 1,000 ppm when compared to the control which the ED_{50} was 66.45 ppm. Hexane crude extract from *Ch. cochliodes* could significantly inhibited colony growth of 54 % at concentration of 1,000 ppm and inhibited spore production of 90 %. Moreover, ethyl acetate and methanol crude extracts could significantly inhibit spore production of 82.37 and 93.35 %, respectively (Tables 2, 3 and 4). The ED_{50} values of crude hexane, ethyl acetate and methanol extracted from *Ch. cochliodes* could inhibit D oryzae at 66.45, 30.25 and 46.78 ppm, respectively (Table5). All crude extracts expressed abnormal spore of *D oryzae* (Fig.5, 6 and 7) and those abnormal spores lost pathogenicity.

Table 2. Crude extracts of *Chaetomium cochliodes* testing to inhibit *Drechslera oryzae* at 5 days

Crude extracts	Concentration (ppm)	Colonydiameter (cm)[1]	Growth inhibition (%)[2]
Crude hexane	0	5.00a	0.00e
	10	3.50c	30.00c
	50	2.47de	50.50ab
	100	2.87d	42.50b
	500	2.85d	43.00b
	1000	2.30e	54.00a
Crude ethyl acetate	0	5.00a	0.00e
	10	4.25b	15.00d
	50	3.82bc	23.50cd
	100	3.75c	25.00c
	500	3.52c	29.50c
	1000	3.52c	29.50c
Crude methanol	0	5.00a	0.00e
	10	5.00a	0.00e
	50	5.00a	0.00e
	100	4.25b	15.00d
	500	3.69c	26.00c
	1000	3.52c	29.50c
	C.V. (%)	6.08	20.42

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2] Inhibition (%) = R1-R2/R1x100 where R1was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 3. Growth inhibition of crude extracts from *Chaetomium cochliodes* to *Drechslera oryzae* at 5 days

Crude extracts	Concentration (ppm)	Mycelial fresh weight (g)	Inhibition (%)[3/]
Crude hexane	0	0.78a	0.00h
	10	0.27c	65.17f
	50	0.17de	78.91cde
	100	0.14def	81.78bcd
	500	0.14def	82.10bcd
	1000	0.08h	90.08a
Crude ethyl acetate	0	0.78a	0.00h
	10	0.71b	8.65g
	50	0.16def	0.00h
	100	0.14efg	82.05bcd
	500	0.14efg	82.05bcd
	1000	0.14efg	82.37bc
Crude methanol	0	0.77a	0.00h
	10	0.27c	65.34f
	50	0.16def	79.40cde
	100	0.18d	76.79e
	500	0.13fg	83.02b
	1000	0.12g	83.97b
	C.V. (%)	5.35	2.81

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition (%) = R1-R2/R1x100 ;where R1was mycelial fresh weight of pathogen in control and R2was mycelial fresh weight of pathogen in treated plate.

Table 4. Spore production inhibition of crude extracts from *Chaetomium cochliodes* to *Drechslera oryzae* at 5 days

Crude extracts	Concentration (ppm)	Number of spores	Inhibition (%)[2/]
Crude hexane	0	26.00a[1]	0.00j
	10	19.75b	24.14hi
	50	11.69de	54.99fg
	100	9.81f	62.33de
	500	3.06i	88.18ab
	1000	1.60i	93.85a
Crude ethyl acetate	0	25.56a	0.00j
	10	19.87b	21.63i
	50	9.00fg	63.14de
	100	6.06h	74.36c
	500	3.19i	85.32b
	1000	2.44i	88.18ab
Crude methanol	0	24.50a	0.00j
	10	17.25c	29.55h
	50	12.50d	48.96g
	100	10.37ef	57.60ef
	500	7.62gh	68.88cd
	1000	1.62i	93.35a
	C.V. (%)	7.79	6.97

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate.

Table 5. Effective dose (ED_{50}) of crude extracts from *Chaetomium cochliodes* to inhibit *Drechslera oryzae* at 5 days

Crude extracts	Concentration (ppm)	Growth imhibition[2/]	Mycelial inhibition[/]	Spore production inhibition (%)	ED_{50} (ppm)
Crude hexane	0	0.00e[1]	0.00h	0.00j	66.45
	10	30.00c	65.17f	4.14hi	
	50	50.50ab	78.91cde	54.99 g	
	100	42.50b	81.78bcd	62.33de	
	500	43.00b	82.10bcd	88.18ab	
	1000	54.00a	90.08a	93.85a	
Crude ethyl acetate	0	0.00e	0.00h	0.00j	30.25
	10	15.00d	8.65g	21.63i	
	50	23.50cd	0.00h	63.14de	
	100	25.00c	82.05bcd	74.36c	
	500	29.50c	82.05bcd	85.32b	
	1000	29.50c	82.37bc	88.18ab	
Crude methanol	0	0.00e	0.00h	0.00j	46.78
	10	0.00e	65.34f	29.55h	
	50	0.00e	79.40cde	48.96g	
	100	15.00d	76.79e	57.60ef	
	500	26.00c	83.02b	68.88cd	
	1000	29.50c	83.97b	93.35a	
	C.V.	20.42 %	6.75 %	6.97 %	

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition (%) = R1-R2/R1x100 where R1was colony growth or mycelial growth or number of pathogen spores in control, R2= colony growth or mycelial growth or number of pathogen spore in treated plate.

Table 6. Plant height of rice var Pitsanulok 2 after applying bio-formulation of *Chaetomium cochliodes*

Treatment	40 days /	Increase d (%)[2/]	55 days	Increase d (%)2/	70 days	Increase d (%)2/	85 days	Increase d (%)2/	100 days	Increase d (%)2/
Inoculated Control	8.00c	-	15.58c	-	21.00 b	-	33.91 b	-	47.66 e	-
Non-Inoculated Control	10.33b	22.56	20.16b	22.72	21.58 b	20.60	36.24 b	6.43	50.83 d	6.24
Spore suspention, *Ch. cochliodes*	11.83a b	26.65	21.08a b	26.09	29.25 a	28.21	47.66 a	28.85	59.91 c	20.45
Bio-powder *Ch. cochliodes*	13.41a	40.34	22.50a	30.76	30.91 a	32.06	50.83 a	33.29	70.83 a	32.71
Crude extract ofCh.cochliodes	13.16a	39.21	22.33a b	30.22	30.58 a	31.32	50.33 a	32.62	68.25 b	30.17
Benlate	12.00a b	33.33	20.58a b	24.29	29.91 a	29.79	48.91 a	30.67	59.50 c	19.90
C.V. (%)	9.40%		4.93%	-	3.40 %	-	5.58 %	-	1.58 %	-

[1] Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.
[2] increased percentage = plant height in each treatment – plant height in inoculated treatment /plant height in each treatment x 100

Table 7. plant height and disease index of rice var Pitsanulok 2 after applying bio-formulation of *Chaetomium cochliodes*

Treatment	40 days /	Per cent increased [2/]	55 days	Per cent increased	70 days	Per cent increased	85 days	Per cent increased	100 days	Per cent increased	Disease index[3]
Inoculated Control	3.00 b	-	6.50b	-	7.33c	-	7.50d	-	10.58 b	-	4.75a
Non-Inoculated Control	3.08 b	2.59	6.58b	1.21	9.08c	19.27	9.91c	24.32	11.17 b	5.28	1.00d
Spore suspention, Ch. cochliodes	3.58a	16.20	11.75 a	44.68	12.50 b	41.36	13.00 b	42.31	18.08 a	41.48	2.75c
Bio-powder Ch. cochliodes	3.16a b	5.06	12.16 a	46.54	18.66 a	60.72	19.08 a	60.50	19.16 a	44.78	2.50c
Crude extract ofCh.cochliodes	3.16a b	5.06	12.83 a	49.34	18.58 a	59.68	18.83 a	60.17	18.91 a	44.05	2.95c
Benlate	3.23a b	7.12	13.00 a	50.00	18.00 a	59.28	18.25 a	58.90	18.50 a	42.81	2.00b
C.V. (%)	6.78 %	-	13.31 %	-	7.65 %	-	7.09 %	-	6.79 %		7.70

[1] Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01. [2] increased percentage = plant height in each treatment – plant height in inoculated treatment /plant height in each treatment x 100 [3] Disease Index, 1= leaf spot 0 %, 2=leaf spot 1-25%,3=leaf spot 26-50%,4=leaf spot 51-75% and 5 =leaf spot over 75%.

Table 8. Plant growth parameters of rice var Pitsanulok 2 after applying bio-formulation of Chaetomium cochliodes

Treatment	Fresh plant weight (g)	Per cent increased 2/	Fresh root weight	Per cent increased 2/	Fresh panicle weight	Per cent increased 2/	Dried plant weight	Per cent increased 2/	Dried root weight	Per cent increased 2/	Dried panicle weight	Per cent increased 2/
Inoculated Control	201.25b	-	83.75 b	-	28.75 ab	-	31.25 b	-	9.50a	-	3.75b	-
Non-Inoculated Control	245.00b	17.86	61.25 b	26.87	23.75 b	17.39	31.75 b	1.57	9.50a	-	4.50b	16.67
Spore suspention, Ch. cochliodes	572.50a	64.85	310.00a	80.24	48.75 a	51.28	85.00 a	63.24	52.50a	81.90	15.50 ab	75.81
Bio-powder Ch. cochliodes	583.75a	65.52	187.50ab	67.33	35.00 ab	32.14	85.75 a	63.56	31.25a	69.60	11.50 ab	67.39
Crude extract ofCh.cochliodes	560.00a	64.06	170.00ab	63.97	46.25 a	48.65	71.75 ab	56.45	30.00a	68.33	16.50 a	77.27
Benlate	555.00a	63.74	195.00ab	68.59	30.00 ab	20.83	69.00 ab	54.71	32.00a	70.31	8.25ab	54.55
C.V. (%)	30.57 %	-	54.61 %		25.73 %		33.40 %		73.53%		51.97 %	

[1] Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.
[2] increased percentage = plant height in each treatment – plant height in inoculated treatment /plant height in each treatment x 100

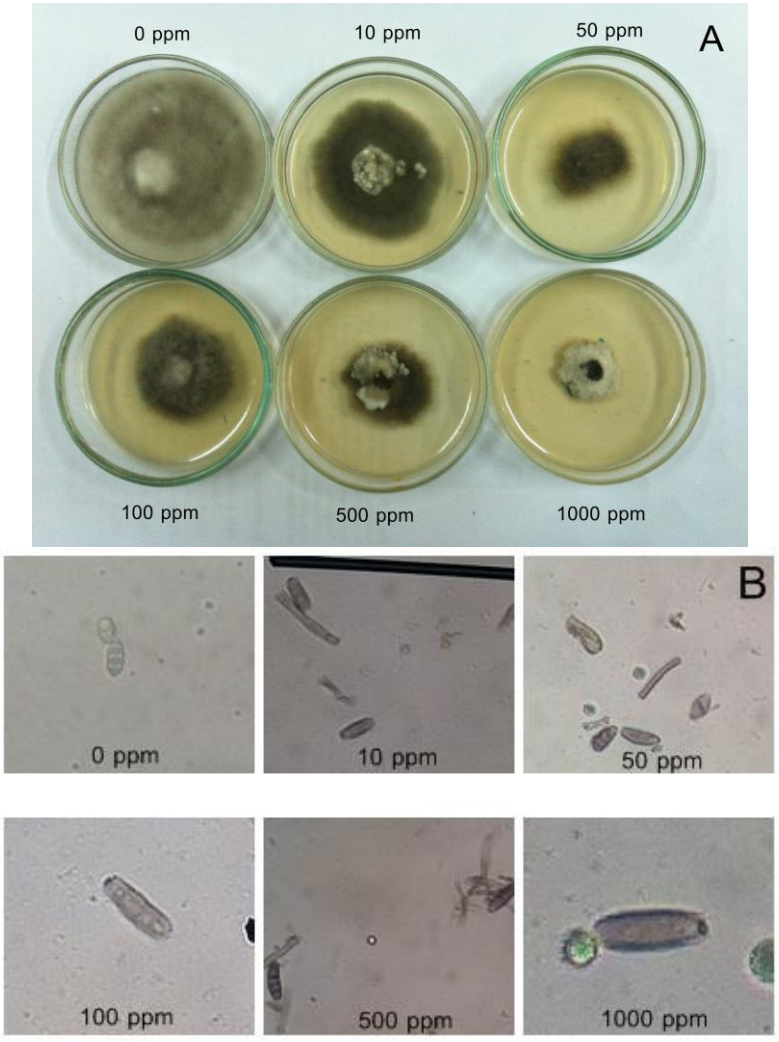

Fig. 5. Crude Hexane from *Chaetomium cochliodes* at various concentrations (A) and abnormal spores of *Drechslera oryzae* ; (B)

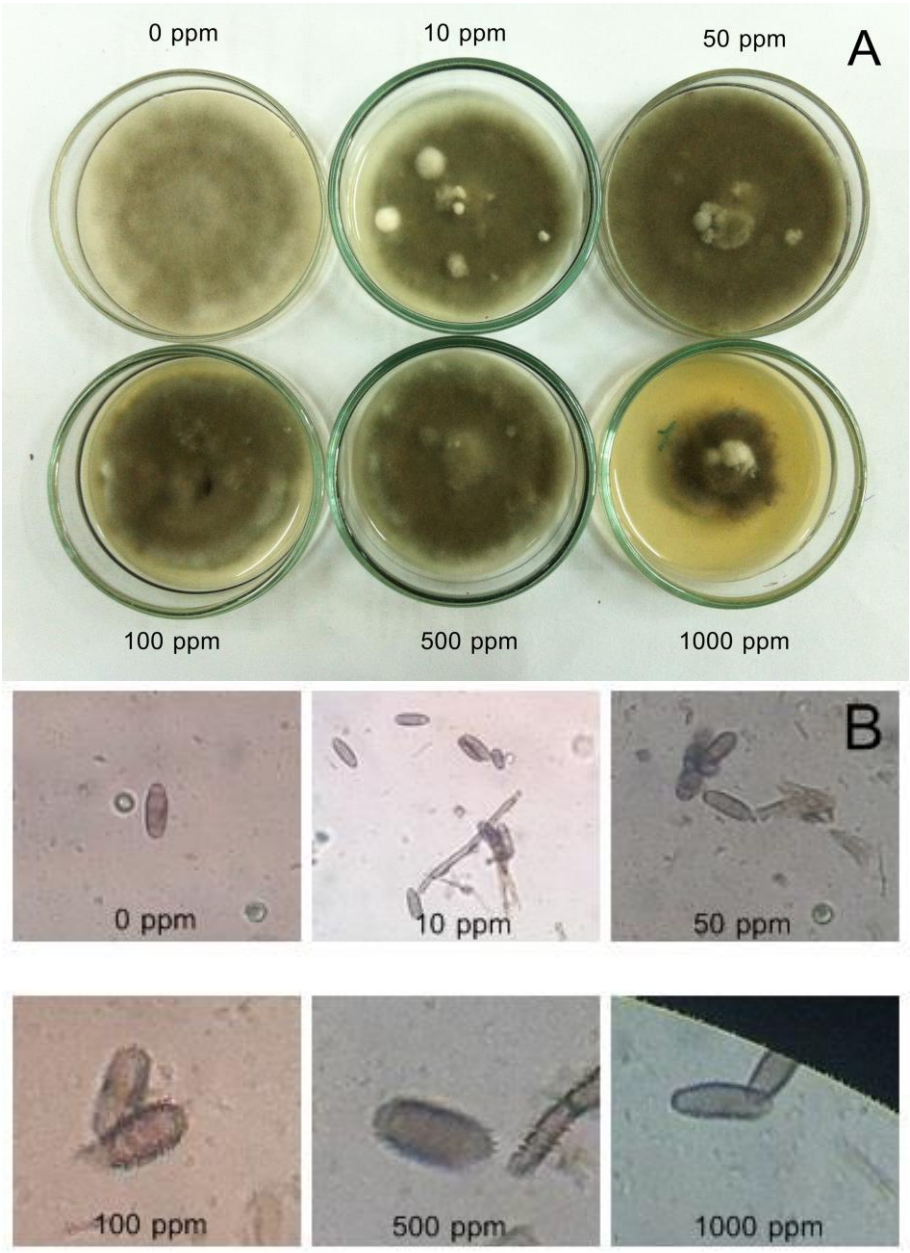

Fig. 6. Crude ethyl acetate from *Chaetomium cochliodes* at various concentrations (A) and abnormal spores of *Drechslera oryzae* ; (B)

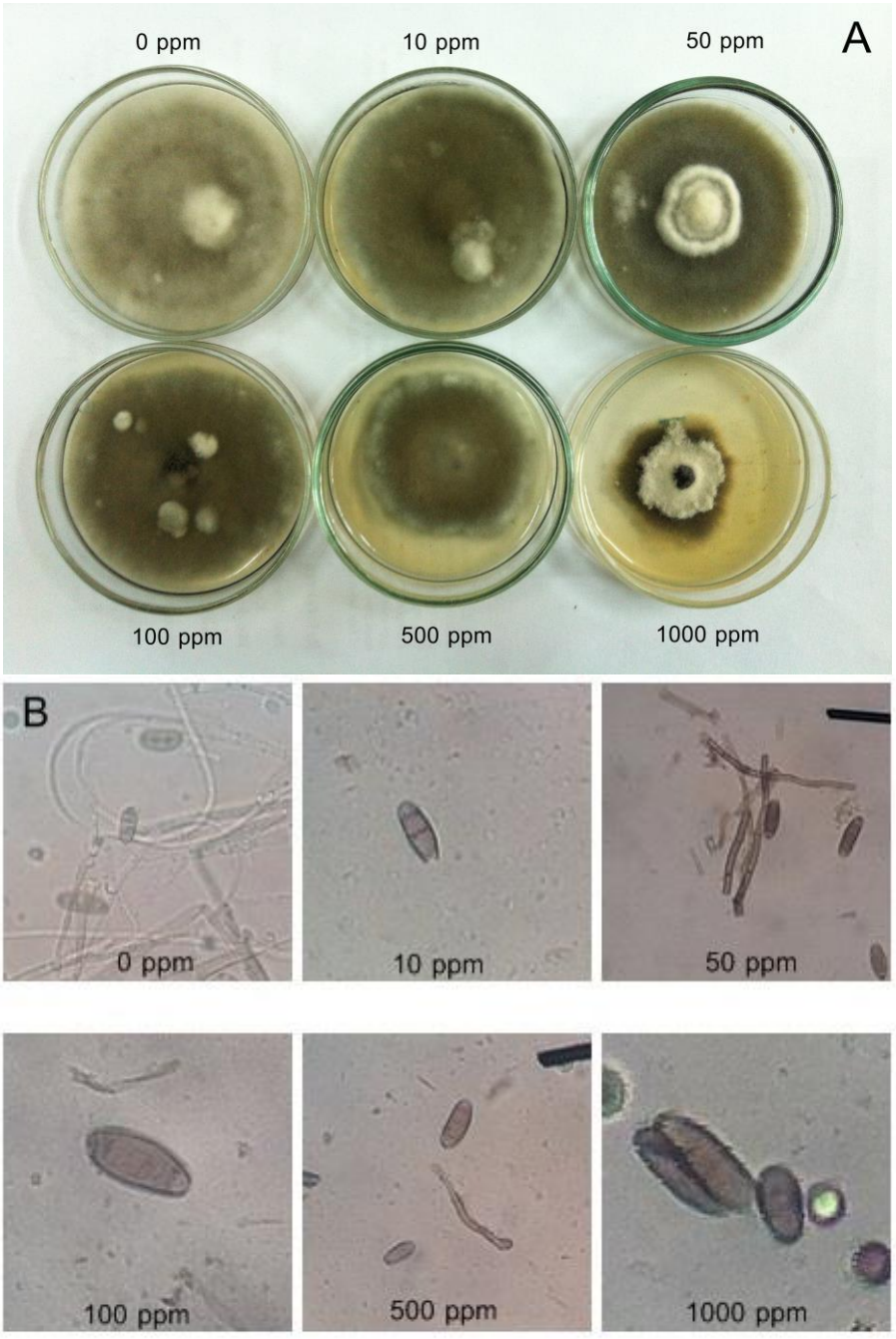

Fig. 7. Crude Hexane from *Chaetomium cochliodes* at various concentrations (A) and abnormal spores of *Drechslera oryzae* ; (B)

Bio-formulation of Chaetomium cochliodes testing to control brown leaf spot

Bio-formulation of *Ch cochliodes* can be significantly reduced leaf spot of rice var Pitsanulok 2 caused by *D. oryzae*. Result showed that biopowder of *Ch cochliodes*, Bio-crude extract and benlate treated to rice at 40 days showing plant height of 13.41, 13.16 and 12.00 cm, respectively when compared to the control (8 cm). At 70 days, bio-powder, crude extract of *Ch cochliodes* and benlate showed plant height of 50.83, 50.33 and 48.91 cm, respectively when compared to the control (21 cm). At 85 days, bio-powder, crude extract of *Ch cochliodes* and benlate showed plant height of 30.91, 30.58 and 29.91 cm, respectively when compared to the control (33.91 cm). At 100 days, bio-powder, crude extract of *Ch cochliodes* and benlate showed plant height of 70.83, 68.25 and 59.91 cm, respectively when compared to the control (47.66 cm). For number of tillers at 40 days, spore suspension of *Ch cochliodes*, benlate, bio-powder and crude extract of *Ch cochliodes* showed number of tillers of 3.58, 3.23, 3.16 and 3.16 tillers, respectively when compared to the control (3.0 tillers). At 55 days, benlate, crude extract and bio-powder of *Ch cochliodes* showed number of tillers of 13.00, 12.83 and 12.16 tillers, respectively when compared to the control (6.5 tillers). For number of tillers at 70 days, bio-powder, crude extract of *Ch cochliodes* and benlate showed number of tillers of 18.66, 18.58 and 18.00 tillers, respectively when compared to the control (7.33 tillers). For number of tillers at 85 days, bio-powder, crude extract of *Ch cochliodes* and benlate showed number of tillers of 19.08, 18.83 and 18.25 tillers, respectively when compared to the control (7.50 tillers). For number of tillers at 100 days, bio-powder, crude extract of *Ch cochliodes* and benlate showed number of tillers of 19.16, 18.91 and 18.50 tillers, respectively when compared to the control (10.58 tillers). Fresh weight of plant, bio-powder, spore suspension of *Ch cochliodes* and benlate and crude extract of *Ch cochliodes* gave fresh weight of 583.75, 572.50, 560.00 and 555.00 g, respectively when compared to the control (83.75 g). Rice panicle weight showed that spore suspension, benlate, bio-powder of *Ch cochliodes* gave fresh panicle weight of 48.75, 46.25 and 35.00 g respectively when compared to the control (28.75 g). Spore suspension of *Ch cochliodes*, benlate and crude extract of *Ch cochliodes* gave panicle dried weight of 85.75, 85.00 and 71.75 g when compared to the control (31.25 g). Root dried weight, It showed that spore suspention, crude extract and bio-powder of *Ch cochliodes* showed root dried weight of 52.50, 32.00 and 31.25 g respectively when compared to the control (9.50 g). Bio-poweder, spore suspension and crude extract of *Ch cochliodes* gave significantly controlled leaf spot of rice cause by *D oryzae* when compared to inoculated with pathogen and benlate chemical fungicide was

significantly better disease control than those all bio-formualtion of *Ch cochliodes* (Table 6).

Discussion

Drechslera oryzae was isolated from leaf spot of rice from different varieties. It belongs to Deuteromycotina, Hyphalaes, Dematiaceae which showing Imperfect stage. The fungus morphology is similar to report of Breda de Haan (1900) which four cell conidia, 63-153 X 14-22 µm, colony deep brown to blackish brown as similar report by Ellis (1971) and Ou (1984) *Chaetomium cochliodes* belongs to Ascomycotina, Pyrenomycetes, Chaetomiales, Chaetomiaceae which showing perfect stage. It produces perithecia, cylindricall asci and 8-ascospore per ascus which reported by Soytong) 2535), Soytong *et al*. (2001), Treit and Moore (1954) and Johnston and Booth (1983). As result, *Ch. cochliodes* proved to be antagonized *D. oryzae* causing leaf spot of rice var Pitsanulok 2. *Ch. cochliodes* could inhibit colony growth and spore production of *D. oryzae* as 71.55 % in bi-culture antagonistic test which similar report of Soytong (1992). Bi-culture test showed lysis of pathogen spore which served as control mechanism aseport by Soytong (2548).

Crude hexane of *Ch cochliodes* inhibited spore production of pathogen at concentrsation of 1,000 µg/ml (93.85 %) which ED_{50} was 66.45 ppm. This similar report by Biswas *et al.* (2002) showed that crude extract of *Chaetomium* sp could *Drechslera sorokiniana* causing spot blotch of wheat (Biswas *et al*., 2002).

As a result, bio-powder, crude extract and spore suspension of *Ch cochliodes* which similar to Soytong *et al*. (2001) who applied biopowder formulated from *Chaetomium globosum* and *Chaetomium cupreum* can be inhibited several plant pathogens especially *D. oryzae* causing brown spot of rice and Soytong (2535) reported that rice seed coated with *Ch globosum, Ch cupreum* and *Ch cochliodes* could inhibit rice blast caused by *P. oryzae.*

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