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Evaluation of bio-formulation from *Chaetomium elatum* ChE01 to control banana anthracnose caused by *Colletotrichum musae*

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Abstract Anthracnose of banana is proved to cause by *Colletotrichum musae.* *Chaetomium elatum* ChE01 inhibited the growth of *C. musae* within 30 days in bi-culture plates over 60 % and inhibited the spore production of *C. musae* of 57 %. The metabolites from *C. elatum* ChE01 actively fungal activity against *C. musae* for spore inhibition which crude methanol, crude ethyl acetate and crude hexane extracts at the ED_{50} value was 5.43, 7.43 and 19.62 ppm., respectively. The new bio-formulation of *C. elatum* ChE01 reduced disease incidence of banana anthracnose of 13.34 % compared to benomyl that reduced the disease incidence of 13.05 %. Moreover, the crude extract mixture of *C. elatum* ChE01 and spore suspension of *C. elatum* ChE01 reduced the anthracnose incidence of 2.13 and 2.04 %, respectively. The research finding is reported for the first time that bioformulations of *C. elatum* ChE01 could control banana anthracnose caused by *C. musae.*

Keywords: *Chaetomium elatum*, bio-formulation, banana anthracnose

Introduction

Banana (*Musa sapientum* Linn.) is one of economic important exporting plant in Thailand (Center for Agriculture Information Office of Agricultural Economic, Ministry of Agriculture and Co-operative, Thailand, 2012). It increases the world's demand annually (Food and Agriculture Organization of the United Nation, 2006). The most important disease of export bananas is crown rot causing by *Colletotrichum musae* (Finlay and Brown, 1993). The most important problem of bananas is crown rot or anthracnose which may involve several species, the most commonly species is *C. musae* (Green and Goos, 1963; Griffee and Burden, 1976; Finlay and Brown, 1993). *C. musae* causes anthracnose which appear on the fruit peel (Griffee and Burden, 1974). The most commonly used fungicides to control the disease is

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benzimidazoles such as benomyl and thiabendazole (TBZ) , but commonly *C. musae* is developed to resist those fungicides (de Lapeye de Bellaire and Dubois, 1997). It is needed to investigate the alternative disease control. The studied isolate of *Chaetomium elatum* ChE01 was proved to antagonize *F. oxysporum* f.sp. *lycopersici* causing tomato wilt (Soytong, 2015). Morever, Sakunyarak and Satithorn (2014) stated that *Pantoea agglomerans* and *Enterobacter* sp.as the antagonistic bacteria had ability to inhibit the growth of *C. musae*, the causal agent of anthracnose don banana fruits. The objectives were to isolate, identify and prove for pathogenicity of the causal agent of banana anthracnose, to test the efficacy of *Chaetomium elatum* ChE01 against *C. musae* and to prove control mechanism through bioactive compound of *C. elatum* ChE01. Bio-formulation of *C. elatum* ChE01 was investigated to control banana anthracnose.

Materials and methods

Isolation and identificatioin of pathogen and prove for pathogenicity

Colletotrichum musae was isolated from disease plant parts by tissue transplanting method. The lesion was cut in advance margin, soaked in sodium hypochlorite 10% for 2-3 min, placed on Water Agar (WA). The hyphal mycelia were moved to Potato Dextrose Agar (PDA) until get pure culture. Pathogenicity test was performed by culturing *C. musae* on PDA for 15 days and the spore suspension at concentration of 1x 10^6 spore/ml, was inoculated into wounded on surface of banana fruits.

Antagonist: Chaetomium elatum ChE01

This strain of *C. elatum* ChE01 is isolated and reported to produce the secondary metabolites which reported by Thohinung *et al.* (2010) which is a new chaetoglobosin V, two new natural products, prochaetoglobosin III and prochaetoglobosin III$_{ed}$, six known chaetoglobosins B-D, F and G and isochaetoglobosin D (Figure 5). Morphology was reconfirmed species by culturing in PDA and incubated at 30 C for 30 days before observation under compound microscope and follow instruction of Domsch *et al*. (1980), Von Arx *et al. (*1986), Soytong (2001) and Pornsuriya *et al.* (2008).

Bi-culture antagonistic test

Mycelial disc (0.5 m in diameter) of pathogen was placed on PDA at one side of petri dish and antagonist was placed at the opposite side of petri

dish and incubated at room temperature (28-30 ^{0}C). The agar discs of pathogen and antagonist was separately placed alone on PDA which served as controls. All bi-culture plates were incubated at room temperature until the pathogen in control growing full plate. Data were gathered as colony diameter and the number of spore. The inhibition of mycelial growth and spore of pathogen was calculated as a percentage according to the formula: Percent inhibition (I) = C – T/C ×100, where, C = number of spores in control plate, T = number of spores in bi-culture plate.

In vitro test of crude extract from C. elatum ChE01 ***against C. musae***

The antagonist was cultured in potato dextrose broth (PDB) for 30 days. The biomass was collected, air-dried, ground and extracted with hexane, ethyl acetate (EtOAc) and methanol (MeOH) to yield crude hexane, crude EtOAc and crude MeOH extracts, respectively. These crude extracts were tested to control the pathogen by poisoned food technique with different concentrations (0, 10, 50, 100, 500 and 1000 ppm). Each crude extract was dissolved in 2% dimethyl sulfoxide (DMSO), and poured into PDA before autoclave at 121℃, 15lbs/inch2 for 30 min. Mycelial disc of pathogen was placed on the middle of PDA plate (5 cm diameter) incorporated with each crude extract. The tested plates were incubated at room temperature until pathogen in control growing full plate. The experiment was done by using 2 factors factorial experiment in Completely Randomized Design (CRD) with 4 replications. Factor A represented crude extracts and factor B represented concentrations. Data were gathered as colony diameter and the number of spores. The inhibition of mycelial growth and spore of pathogen was calculated as percentage. The effective dose (ED_{50}) value was calculated using probit analysis. Data was statistically subjected to analysis of variance. Treatment means were compared with Duncan's multiple range test (DMRT).

Evaluation of bio-formulation from C. elatum ChE01 ***to control banana anthracnose***

The green banana fruits were used to inoculated by placing the culture agar block (0.5 cm) of the pathogen onto the wounded lesion on each fruit, then put in moist chamber plastic box. Thereafter inoculation, each treatment was done by spraying. Treatments were non-inoculated control, inoculated control, crude extract mixture at 0.1 mgl/L, spore suspension of antagonist at $1x10^6$ spores/ml, bio-formulation of antagonist 1g/100ml and benomyl chemical fungicide at recommendation rate. The experiment was done using CRD with

four replications. All treatments were incubated at room temperature to observe the lesion diameter. Data were subjected to ANOVA and DMRT treatment comparison.

Results

Isolation and identification of pathogen and prove for pathogenicity

It found that the causing agent of banana anthracnose was *Colletotrichum musae* which whitish colony when young and turn to yellowish orange when mature, acervuli orange in color and many long oval one cell spore, 4.5-5.5 x 10.0-17.50 um, sclerotia found, no satae (Figure 1). The tested pathogen was proved to be pathogenic isolate to cause anthracnose symptom on banana fruits (Figure 2). The inoculated areas were infected and appeared lesion within 3 days after incubation.

Antagonist: Chaetomium elatum ChE01

Colonies are rapidly growing, young colonies usually are white by aerial mycelium. Mature colonies become dark with ascomata. Ascomata are olivaceous, maturing within 20 days, dark to brown when old, ovate in shape. Ascomatal hairs usually straight and branches. Asci are clavate in shape with 8 ascospores per ascus. Ascospores are limoniform with an apical germ pore (Figure 1).

Bi-culture antagonistic test

Bi-culture antagonistic test found that *C. elatum* ChE01 can be suppressed the tested pathogen, *C. musae* within 30 days in bi-culture plates over 60 % (Figure 4). *C. elatum* ChE01 inhibited the spore production of *C. musae* of 57 % (Figure 3).

In vitro test of crude extract from C. elatum. ChE01 *against C. musae*

Result showed that crude methanol of *C. elatum* ChE01 gave significantly highest spore inhibition of *C. musae* which the ED_{50} value was 5.43 ppm., and followed by crude ethyl acetate and crude hexane which the ED50 values were 7.43 and 19.62 ppm., respectively (Figure 4, Table 1). The higher concentration gave higher inhibition of pathogen than the lower concentration.

Evaluation of bio-formulation from C. elatum ChE01 ***to control banana anthracnose***

Result showed that Bio-formulation of *C. elatum* ChE01 and benomyl chemical fungicide were significantly higher disease reduction which were 13.34 and 13.05 %, respectively than the crude extract mixture of *C. elatum* ChE01 and spore suspension of *C. elatum* ChE01 which the disease reduction were 2.13 and 2.04 %, respectively (Table 2).

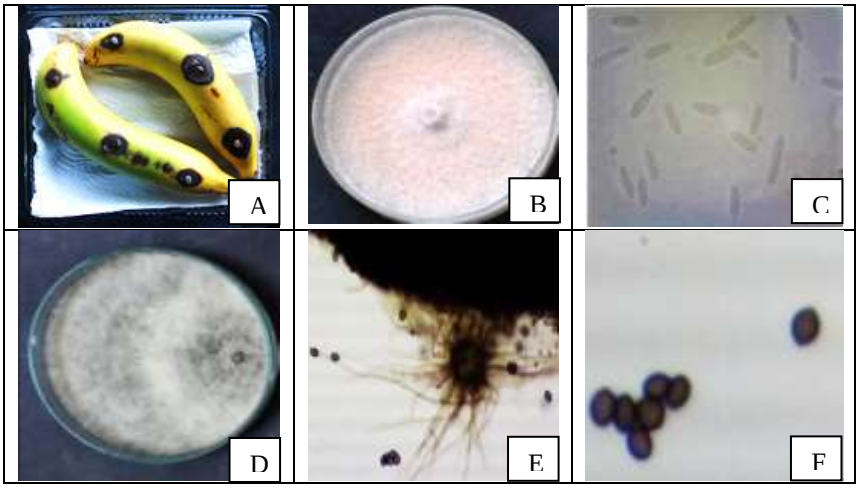

Figure 1. *Colletotrichum musae* A = symptoms , B = colony on PDA at 14 days, C = spores (400X) and *Chaetomium elatum* ChE01 D= colony on PDA at 20 days, E = Ascomatum (100X) F = ascospores (400X)

Figure 2. Pathogenicity test

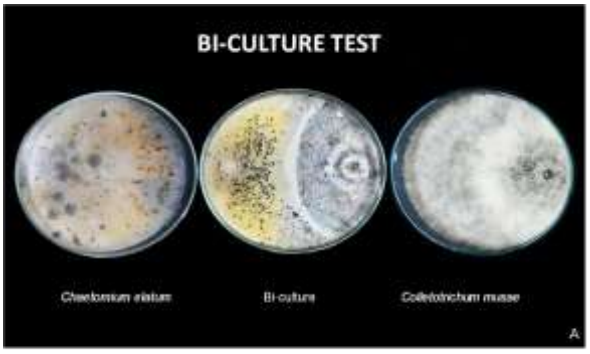

Figure 3. Bi-culture antagonistic test between *C. elatum* ChE01 and *C. musae*

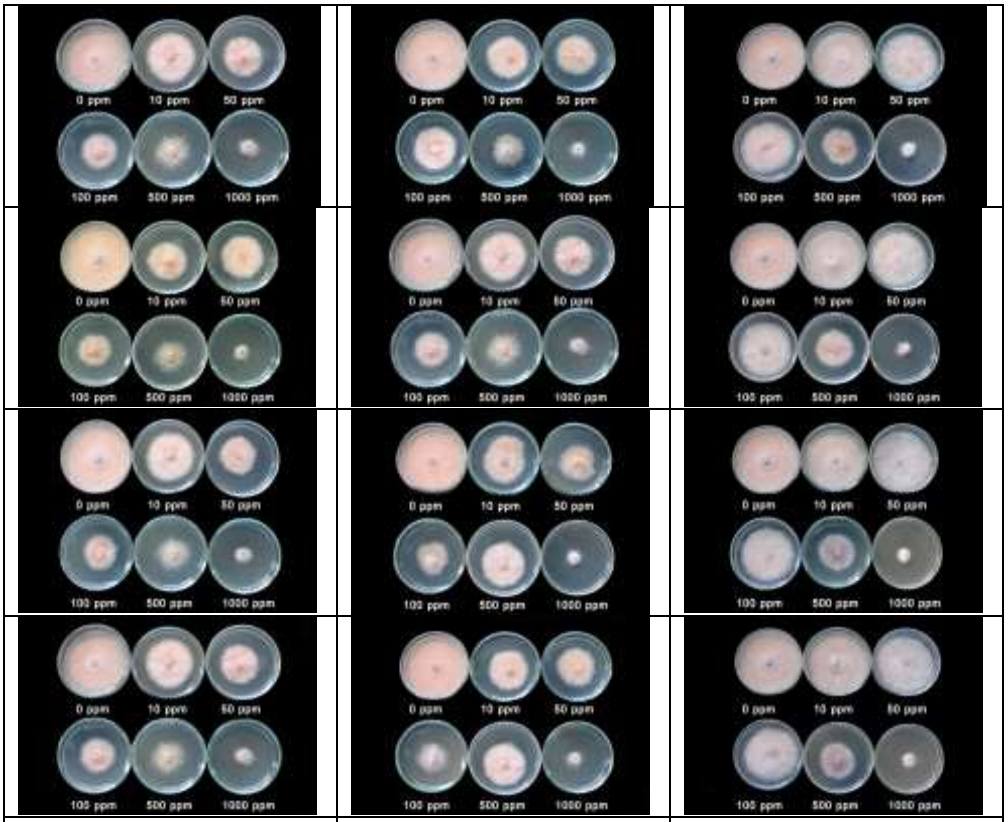

Figure 4. Crude extracts of *C. elatum* ChE01 testing to inhibit spore production of *C. musae*

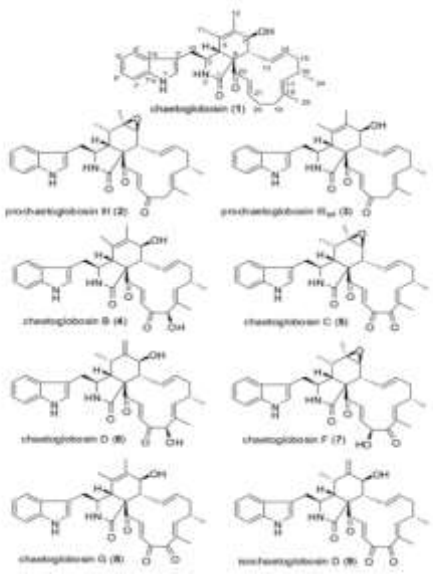

Figure 5. *Chaetomium elatum* ChE01, a new chaetoglobosin V, two new natural products, prochaetoglobosin III and prochaetoglobosin IIIed, six known chaetoglobosins B-D, F and G and isochaetoglobosin D (from Thohinung *et al*., 2010)

Table 1. Crude extracts of *C. elatum* ChE01 against *C. musae* causing banana anthracnose in 5 days

Extracts	Conc. (ppm)	Colony dia. (cm.)[2]	spores	growth inhibition	spore inhibition	ED_{50} (ppm)
CEH	0	5.00a	94.95a	0.00e	0.00g	
	10	3.37b	66.70b	32.50d	28.96f	
	50	2.85cd	59.50bc	43.00bc	36.39ef	
	100	2.67cd	13.59def	46.50bc	85.61abc	19.62
	500	2.62d	6.19f	47.50b	93.32ab	
	1000	1.67e	4.65f	66.50a	94.95a	
CEE	0	5.00a	82.29b	0.00e	0.00g	
	10	3.02c	39.00cde	39.50c	47.31ef	
	50	2.82cd	22.34def	43.50bc	68.13cd	7.43
	100	2.77cd	17.40def	44.50bc	74.32bcd	
	500	2.72cd	12.95ef	45.50bc	81.80abc	
	1000	1.47e	3.60f	70.50a	96.41a	
CEM	0	5.00a	95.25a	0.00e	0.00g	
	10	5.00a	40.25cd	0.00e	56.18de	
	50	4.82a	20.64def	3.50e	78.04abc	5.43
	100	4.96a	16.00def	6.00e	83.23abc	
	500	2.67cd	6.75f	46.50bc	92.54ab	
	1000	1.52e	3.44f	69.50a	96.72a	
	C.V. (%)	5.37	37.55	10.61	16.53	-

Table 2. Evaluation of bio-formulation from *C. elatum* ChE01 to control banana anthracnose

Treatments		lesion (mm)[1/]	Disease reduction(%)[2/]
1	Inoculated control	5.1a	-
2	Crude extract mixture	3.1b	2.13
3	Spore suspension	3.1b	2.04
4	Bio-formulation	2.7c	13.34
5	benomyl	2.7c	13.05
C.V. (%)		9.7	-

Discussion

Colletotrichum musae found to cause anthracnose disease of banana. The research finding is similar resulted to Su *et al.* (2011) who stated that *C. musae* is whitish colony when young and turns to pale brown and orange conidial mass, conidium is cylindrical shape, single cell, hyaline. *Chaetomium elatum* belongs to Ascomycotina, Pyrenomycetes, Chaetomiales, Chaetomiaceae. It produces ascomata, clavate asci, 8 ascospores/ascus which reported by Domsch *et al*. (1980) and Von Arx *et al*. (1986). *C. elatum* ChE01 is proved to antagonize *C. musae* causing banana anthracnose. Withthis, Soytong (2015) reported that *C. elatum* ChE01 could inhibit *Fusarium oxysporum* f sp *lycopersici*. Result found that crude methanol, crude ethyl acetate and crude hexane extracts expressed antifungal activity against *C. musae* at the ED_{50} values of 5.43, 7.43 andc 19.62 ppm., respectively. Song and Soytong (2016) also reported that crude hexane, ethyl acetate and methanol extracts of *C. elatum* ChE01 were actively against *Pyricularia oryzae* (rice blast pathogen). Our reserch findings, the isolates *C. elatum* ChE01 proved to be antagonized *C. musae* (banana anthracnose) which this isolate was reported by Thohinung *et al.* (2010) that produces chaetoglobosin V, prochaetoglobosin III and prochaetoglobosin III$_{ed}$, chaetoglobosins B-D, F and G and isochaetoglobosin D. These compounds showed cytotoxicity against the human breast cancer (IC_{50} 2.54-21.29 ppm) and cholangiocarcinoma cell lines (IC_{50} 3.41-86.95 ppm). Similar result was shown in previous report that crude extracts of *C. elatum* ChE01 inhibited spore production of *F. oxysporum* f. sp. *lycopersici* causing tomato wilt which hexane crude extract at ED_{50} of 0.65 ppm and EtOAC crude extract at ED_{50} of 3.39 ppm (Soytong, 2015). It may involve the mechanism of control in term of antibiosis. As results, the bio-formulation of *C. elatum* ChE01 reduced tha disease incidence of 13.34 % which not significantly differed from the application of benomyl which reduced disease incidence of 13.05%. The crude extract mixture of *C. elatum* ChE01 and spore suspension of *C. elatum* reduced the disease incidence of 2.13

and 2.04 %, respectively. The research finding was similar result as previous report that *C. elatum* ChE01 formulated as powder bioformulation could control wilt of tomato var Sida caused by *F. oxysporum* f.sp. *lycopersici*. The lowest wilt incidence of tomato after apply oil and powder bioformulations of *C. elatum* ChE01 that differed from Prochoraz chemical fungicide Further research finding, Zhimo *et al.* (2016) found that *Candida tropicalis* YZ1, *C. tropicalis* YZ27 and *Saccharomyces cerevisiae* YZ7 as biocontrol agents against *Colletotrichum musae* were significantly reduced anthracnose of banana fruits. It concluded *C. elatum* ChE01 could be developed to be biological fungicide to control banana anthracnose caused by *C. musae.*

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