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Fungal Metabolites from *Chaetomium brasiliense* to Inhibit *Fusarium solani*

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Tomato (*Solanum lycopersicum* L.) is one of among important cash crop nowaday. Several countries have consumed a lot of tomato consumtion. Tomato plants are always been attacked by several soil borne fungal pathogens, which cause serious disease such as wilt caused by *Fusarium solani* and other species. Fusarium species are soil fungi and have a worldwide distribution. In this research finding, tomato dry root rot and wilt were isolated and tesed for their pathogenicity. In vitro, the experiment was done by using the bio active compounds of *chaetomium brasiliense* to inhibit *Fusarium solani*. The different concentrations of bio active compounds of *chaetomium brasiliense* was separately ameneded into potato dextrose agar in each treatment and transferred the tested pathogen onto middle plates and the result showed that bio active compounds gave a potent to inhibit the growth of *Fusarium solani*. The ED50 for spore inhibition were 66, 288, and 140 µg/ml, respectively. Further investigation will be conducted in pot experiment.

Keywords: *Fusarium solani*, *chaetomium brasilense*, bio active compound

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown and consumed vegetables all over the world. Tomatoes and tomato products are rich in health-related food components as they are good sources of carotenoids (in particular, lycopene), ascorbic acid (vitamin C), vitamin E, folate, flavonoids and potassium (Beecher, 1998; Leonardi *et al*., 2000). However, the tomato crop is usually attacked by many kinds of diseases such as Fusarium.

Fusarium solani is the most important soilborne fungal pathogens, which develop in both cultured and non-cultured soils, causing the symptoms of damping off and root rot diseases to wide range of vegetable and crop plants

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including tomato (Abu-Taleb *et al.*, 2011). *Fusarium solani* (Mart.) Appel and Wollenw is widely found in soil and constitutes one of the most important phytopathogens in agriculture. Symptoms include stunted growth with varying degrees of chlorosis, mottling and necrotic spotting on young foliage and cortical rot of tap root or discoloration of stele of the main lateral roots. In severely affected plants, the tap roots completely girdled and the crown was also rotted. Although plants are not often killed by the disease but it reduces the yield (Vawdrey and Petersone, 1988)

This resresch aimed to study morphological of *Chaetomium brasilense* and *Fusarium solani*, pathogenicity test of *Fusarium solani* and tested for the inhibition of pathogen using bioactive compound from *chaetomium brasilense*.

Materials and methods

Morphological study of Chaetomium brasilense

Chaetomium brasilense which offered from Assoc. Prof. Dr. Kasem Soytong, 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 pathogen, Fusarium solani

Fusarium solani causing wilt diseases were isolate form tomato root by tissue transplanting technique. Root of tomato were properly cleaned with running tap water and after air-dried for a few minutes and cut it in small pieces and soaked in sterilized water, and followed by 1% sodium hypochlorite (NaClO) for 3 min and then sterilized water again. All of the small pieces root 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

The pathogenicity test of fusarium wilt (*Fusarium solani*) was conducted in vivo to 15 day tomato seedlings and carried out using a rootdip inoculation method. Tomato seedlings were uprooted gently and roots were washed with tap water to remove all soil (Bao J.R. *et al.*, 2001). The concentration of conidia in the suspension was determined using Haemacytometer to adjust the number of spores to$1x10^6$ conidia/ml. The 3-4 root tips will be cut and soaked into

spore suspension for 30 seconds. Control plants were sown in soil and be treated with sterile distilled water. The disease severity was rated with follow the method of Sibounnavong (2012), as follows: 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21- 40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting 81-100% or die. The most virulent isolate was selected for further experiment.

Testing bio active compound of chaetomium brasilense to against Fusarium solani

The crude extracts of chaetomium brasilense were tested for inhibition of *Fusarium solani*. Experiment was designed by using two factors factorial experiment in CRD with four replications. Factor A represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100, 500, and 1,000 ppm. Each crude extract was dissolved in one drop 2% dimethyl sulphite (DMSO), mixed into 30 ml potato dextrose agar (PDA) before autoclaving at 121 ℃ , 15 psi for 30 minutes. The culture of *Fusarium solani* was cut at the edge of colony with sterilized cock borer (3mm). Agar plug of pathogen was transferred to the middle of PDA media in plate (5.0mm diameter) incorporated with each and incubated at room temperature (28 ℃-30 ℃) until the pathogen on the control plates growing full. Data were collected as colony diameter and the number of conidia. Percentage inhibition of pathogen colony growth and conidia production were calculated using the following formula:

$$\% \text{ inhibition} = (A-B) / A \times 100$$

Where, A is the diameter of colony or number of conidia produced by the pathogen in control plates and B is the diameter of colony or number of conidia produced by the pathogen in treatment plates.

Data were statistically computed analysis of variance and treatment means were compared using Duncan Multiple's Range Test (DMRT) at P = 0.05 and 0.01. The effective dose (ED50) will be calculated using probit analysis.

Results and Discussion

Morphological study of Chaetomium brasilense

Chaetomium brasiliense was cultured and morphological observation. Ascocarp, asci and ascospores were taken photograph under compound

microscope. Culture is dark gray color, perithecia globose, subglobose or broadly ovoid, cylindrical asci. This result is similar to report of Soytong and Quimio (1989) and Bell (2005). who stated that *C. brasiliense* is characterised by subglobose to ovate ascomata, undulate or spirally coiled terminal hairs, cylindrical asci, uniseriate, broadly ovoid, bilaterally flattened, comparatively small ascospores with an apical germ pore. It is reported that this species frequently grows on a variety of herbivore dungs (Ames, (1963) and Bell (2005) as seen in Fig.1.

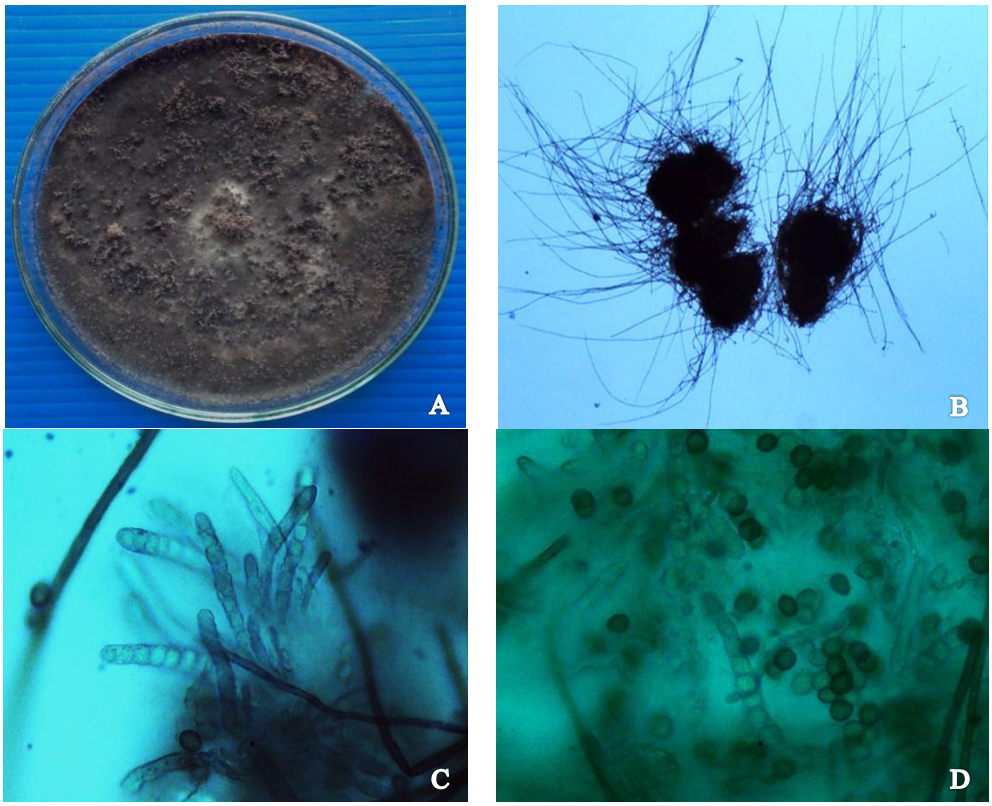


Figure1. *Chaetomium brasilense*, A= colony, B= ascocarps, C= asci and D= ascospores

Isolation of pathogen

Fusarium solani is isolated from disease plant parts, especially from roots of tomato var Sida. The characteristics are fast growing with discrete sporodochia and white-ochraceous colour, aerial mycelium floccose. Macro-conidia found abundant and more-celled, curved or bent at the pointed ends. It

is morphological identification as *Fusarium solani* as also reported by Grunwald *et al*. (2003) and Hafizi *et al*. (2013) as seen in Fig. 2.

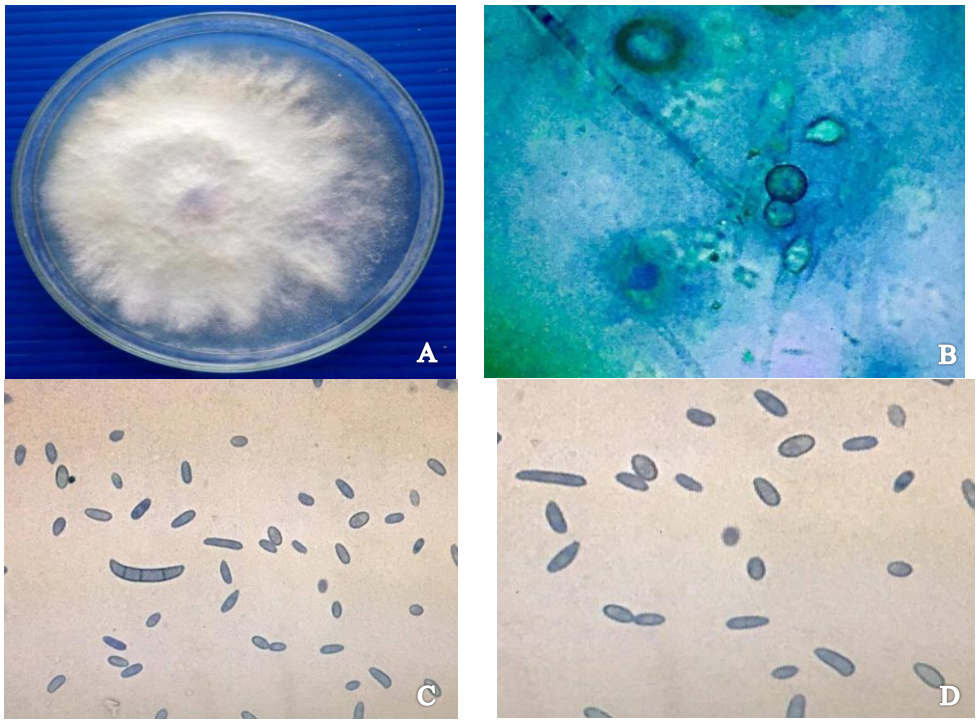


Figure2. *Fusarium solani*, A= colony, B= clamydospore, C= macrospore, D= microspore

Pathogenicity test

Pathogenicity test was conducted by dipping cut root into sopore suspension of *Fusarium solani* at the concentration of 1 x10^6 conodia/ml for 30 seconds which resulted tomato seedligs showed yellowing leaves and wilting 41-60 %. It was significantly differed when compared to the non-inoculated control (Fig.3, Table 1). As a result, this procedure for pathogenicity test is successfully done by Sibounnavong et al. (2012).

Table 1. Percent disease index of *Fusarium solani* in tomato.

Treatments	Disease index
T1=Control	1
T2= *Fusarium solani*	4

Disease index are as follows: 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting 81-100% or die.

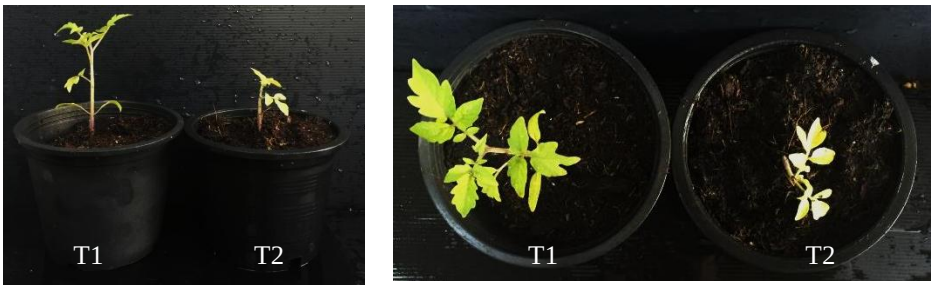


Figure3. Pathogenicity test of *Fusarium solani* caused tomato wilt, T1= dip root with sterile water (control), T2=dip root with spore suspension of *Fusarium solani*

Testing bioactive compound of chaetomium brasilense against Fusarium solani

Result showed high efficacy antimicrobial activity of active compound from *Chaetomium brasilense* against *Fusarium solani*. Hexane, ethylacetate and methanol cude extracts from *C. brasilense* gave significantly highest inhibition of *Fusarium solani* for colony growth which was 52.00, 73.00 and 71.75 per cent at the concentration of 1,000 ppm, when compared to the control (Table 2). All crude extracts from *C. brasilense* showed significantly highest inhibition for the spore production of *Fusarium solani* at the concentration of 1,000 ppm. The ED_{50} for spore inhibition were 66.66, 288.94, 140.88 µg/ml, respectively (Table 3, Fig. 4). This study was similar to the study of Sibounnavong et al.(2012) who reported that *Chaetomium brasilense* CB01 and *Chaetomium cupreum* CC03 gave effectively inhibition of *F. oxysporum* f. sp. *lycopersici* race 2 cuased wilt disease in tomato.

Table 2 Crude extracts of *Chaetomium brasilense* testing for growth inhibition of *Fusarium solani* at 7 days

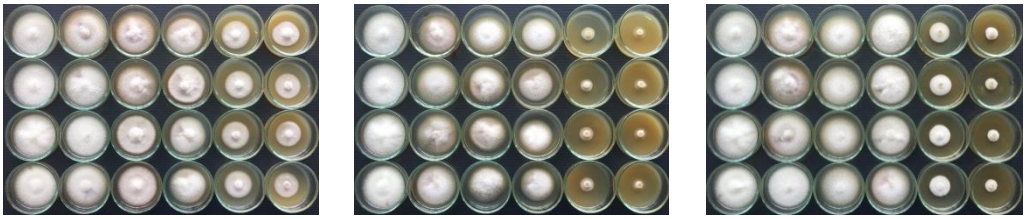
Crude extracts	Concentration (ppm)	Colony diameter(cm)[/1]	Growth inhibition(%)[/2,3]
Hexane	0	5[a]	-
	10	5[a]	0[h]
	50	4.98[ab]	0.50[h]
	100	4.98[ab]	0.50[h]
	500	3.43[e]	31.25[d]
	1,000	2.37[f]	52.00[c]
EtOAc	0	5[a]	-
	10	4.61[b]	7.75[g]
	50	4.41[c]	11.75[f]
	100	3.73[d]	25.25[e]
	500	1.62[g]	67.50[b]
	1,000	1.34[h]	73.00[a]
MeOH	0	5[a]	-
	10	5[a]	0[h]
	50	5[a]	0[h]
	100	4.95[ab]	10.00[h]
	500	2.37[f]	52.50[c]
	1,000	1.41[h]	71.75[a]
C.V. (%)		1.87	6.95

[1/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. , [2/]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05., [3/]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 3 Spore production inhibition of crude extracts from *Chaetomium brasilense* to *Fusarium solani* at 7 days and effective dose (ED_{50}) values

Crude extracts	Concentration (ppm)	Number of spores [/1] (10^6)	Inhibition(%) [/2,3]	ED_{50}
Hexane	0	6.60[a]	-	66.66
	10	5.73[ab]	13.23[h]	
	50	5.52[b]	16.53[h]	
	100	3.55[de]	45.45[ef]	
	500	2.22[g]	65.78[cd]	
	1,000	1.91[gh]	70.63[c]	
EtOAc	0	6.60[a]	-	288.94
	10	5.23[b]	20.33[h]	
	50	4.18[cd]	36.11[fg]	
	100	3.26[ef]	50.22[e]	
	500	1.06[h]	83.75[b]	
	1,000	0.23[i]	97.06[a]	
MeOH	0	6.60[a]	-	140.88
	10	5.59[ab]	15.25[h]	
	50	4.45[cd]	31.83[g]	
	100	4.35[cd]	33.67[g]	
	500	2.62[fg]	59.95[d]	
	1,000	0.18[i]	96.43[a]	
C.V. (%)		12.82		

[1 /]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [2 /]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.05. [3 /]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .



Hexane Ethylactate Methanol

Figure 4 Testing bio active compound of *chaetomium brasilense* to against *Fusarium solani*

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