Natural products of fine particles derived from *Chaetomium globosum* to inhibit Fusarium wilt of tomato

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Abstract *Fusarium solani* was proved to cause wilt of tomato var Sida. It is confirmed by morphology and molecular phylogenic identification on the basis of ITS1-5.8S-ITS2 ribosomal gene sequence acquisition and analyses. It is pathogenic confirmed by pathogenicity test. Thease antagonists expressed ability to inhibit the growth and spore production of *F. solani* causing wilt of tomato var sida. The Hexane crude extract of *Ch. globosum* inhibited the spore production of *F. solani* at the ED_{50} of 200 ppm, and followed by crude EtOAc crude extract and methanol extract which the ED_{50} values of 314 and 378 ppm, respectively. The nautral products of fine particles constructed from the tested *Chaetomium* gave significantly inhibited *F. solani* at all tested crude extracts. Fine particle CGM actively expressed antifungal activity of *F. solani* which the ED_{50} of 1.48 ppm, and followed by fine particle -CGH and fine particle-CGE which the ED_{50} values were 3.41 and 3.48 ppm, respectively.

Keywords: *Fusarium solani*, *Chaetomium globosum*, Fine particle

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important crop in the world due to the high value of its fruits for fresh market consumption (Arici *et.al*, 2013). Tomato is a products 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). Regular consumption of tomatoes has been correlated with a reduced risk of various types of cancer (Gerster, 1997) and heart diseases (Lavelli *et al*., 2000; Pandey *et al*., 1995). Tomato is an economically important vegetable crop, suffering from many fungal diseases (Ketelaar and Kumar, 2002). Fusarium wilt is one of the most serious disease in tomato throughout the world, especially in upland. This disease is caused by *Fusarium solani* leading to serious economic losses (Snyder and Hansen, 1940). *Fusarium solani* is an abundant saprophyte in soil and organic matter and occurs worldwide in the rhizosphere of many plant species. Plants infected by this soil-dwelling fungus show leaf yellowing and wilting that progress upward from the base of the stem. Initially, only one side of a leaf midrib, one branch, or one side of a plant would be affected. The symptoms soon spread to the remainder of the plant. Wilted leaves usually drop prematurely to minimize losses from *Fusarium* wilt, it is advisable to plant resistant varieties, and many resistant varieties are available. It is reported tomato wilt and pathogenic to other economic plants (Ajilogba *et al*., 2013)

Recently, there have been many reports that antagonistic fungi can be used to control Fusarium wilt in tomato plant such as *Trichoderma asperellum, Chaetomium elatum* ChE0, *Chaetomium globosum* N0802, *Chaetomium lucknowense* CLT, *Trichoderma harzianum* PC01, *Emericella rugulosa* ER01, *Chaetomium cupreum* (Mahmoud *et.al*., 2015; Soytong,2015; Sibounnavong *et.al*., 2011). Moreover, the use of bioactive compound extracted from difference species of antagonistic fungi were reported to inhibit the growth of the Fusarium wilt disease such as Trichotoxin A50 extracted from *Trichoderma harzianum* PC01 and Chaetoglobosin C extracted from *Chaetomium globosum*. These compounds have been reported to elicit resistance or immunity in plants by inducing oxidative burst in plant cells for plant immunity (Soytong *et al*., 2001).

Material and Methods

Isolation, identification and pathogenicity test

Fusarium solani causing wilt disease was isolated form tomato root by tissue transplanting technique. Roots 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, followed by 1% sodium hypochlorite for 3 min and then sterilized water again. All of the small piece roots were transferred onto water agar (WA) medium for firstly observation of

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appearing colonies and sub-cultured to PDA until get pure culture. Morphological identification was done by observation fungal characteristic under binocular compound microscope.

Morphology study of the *Fusarium* spp.

Isolate of *Fusarium* spp was morphological identified by culturing in potato dextrose agar (PDA) and incubated at room temperature for 14 day observation. The characters of *Fusarium* spp were determined under binocular compound microscope and the details of fungal morphology were recorded as mycelia structure, shape and size of macroconidia, microconidia, conidiophores and chamydospores.

Pathogenicity test

The experiment was designed by using Completely Randomized Design (CRD) with four replications. The mycelia of *F. solani* were removed into sterilized distilled water and conidia suspension which adjust to 1×10^6 conidia/ml by using haemacytometer. Seedling of tomato var. sida was grown in mix soil for 15 days. The root dip method was used to inoculate which followed the method of Bao *et al*. (2002), gently removed dirt and excess soil from roots of tomato var. sida seeding by using tap water. Root tips of seedlings were cut by sterilized scissors for 5 mm and dipped into conidia suspension for 30 seconds. Seedling roots in control were cut root tips and dipped into sterilized distilled water without inoculum. Before transfer to plastic pots that contained the sterilized soil which autoclaved at 121°C, 15lbs/inch^2 for 1 hour. Disease severity index (DSI) was scored by followed the modified method of Sibounnavong *et al.* (2012), as follows: 1= no symptom; 2= yellowing leaves and root rot 1-20%, 3= yellowing leaves and root rot 21- 40%, 4= yellowing leaves and root rot 41-60%, 5= yellowing leaves and root rot 61-80%, and 6= yellowing leaves and root rot 81-100% or die. Disease severity index (DSI) was analysed by using analysis of variance (ANOVA) and mean comparison was computed Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05.

The antagonistic fungi, *Chaetomium globosum* were tested by using method of Soytong (1992). The experiment was designed as Completely Randomized Design (CRD) with four replications. *Fusarium solani* and *Chaetomium* sp were cut with 0.5 mm sterilized cork borer and one agar plug of each fungus were transferred to PDA plate at one side 4 cm from center of plate. For control treatment either agar plug of *F. solani* or *Ch. globosum* was placed on PDA plate at 4 cm from center of the medium. The tested plates were incubated at room temperature. The data were collected as colony diameter, number of conidia of pathogenic fungus. Percentage of growth and conidia inhibition of pathogen was calculated using formula below:

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

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 for analysis of variance (ANOVA) and mean comparison was computed by using Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05. The effective dose (ED_{50}) was computed by using probit analysis

Extraction antagonistic crude extracts and preparation of fine particle from *Ch. globosum*

The fungal antagonistic *Ch. globosum* were separately cultured in potato dextrose broth (PDB) and incubated at room temperature for 30 days. Biomass of each antagonistic fungus was removed from PDB by filtering thorough cheesecloth and air-dried at room temperature. Biomass was collected and weighted as fresh and dried. Dried biomass were ground with electric blender. Dried biomass of each antagonist was extracted by the method described by Kanokmedhakul *et.al* (2003) Each dried biomass was extracted with hexane (1:1 v/v) in 1000 ml of flask and incubation at room temperature. The ground marc was separated from the solvent by filtering through filter paper (Whatman No.4). The hexane filtrate was evaporated thought rotary vacuum evaporator to yield crude hexane extract. Further, the marc was then extracted with ethyl acetate (EtOAc) and methanol (MeOH), respectively using the same method as hexane. Finally, it yielded crude hexane, crude ethyl acetate and crude methanol of each antagonist. Preparation of nano particles was done using the method of Dar and Soytong (2013) to get fine particles of each *Chaetomium* sp.

Bioactivity test of crude extracts from *Chaetomium* sp against *F. solani*

The crude extracts of *Ch. globosum* were tested ability to inhibit the growth of mycelia and spore production of *F. solani.* The experiments were designed as 2 factors factorial in Completely Randomized Design (CRD) with four replications. Factors A represented crude extracts which included of crude extracts from hexane, ethyl acetate (EtOAc) and methanol (MeOH). Factors B represented different concentrations of crude extracts as follows 0, 10, 50, 100, 500, 1,000 µg/ml. Each crude extracts were dissolved by 2% dimethyl sulfoxide (DMSO) and mixed with PDA before autoclaved at 121°C, 15lbs/inch^2 for 30 min. *F. solani* was cultured on PDA and incubated at room temperature for 5 days, then colony margin was cut by 0.5 mm sterilized cork borer. The agar plugs of *F. solani* was transferred into the middle of 5 cm of petri dish in different concentrations and incubated at room temperature for 5-7 days. The normal and abnormal spores were observed and compared under compound microscope.

Testing fine particles form *Chaetomium* sp against *F. solani*

The experiments were designed as 2 factors factorial in Completely Randomized Design (CRD) with four replications. Factors A represented different kinds of nano particles. Nano particles derived *Ch. globosum* namely fine particles- CGH, fine particles- CGE and fine particles- CGM.

Factor B represented concentrations of 0, 3, 5, 10, 15 µg/ml. Each nano particle was dissolved by 2% dimethyl sulfoxide and mixed with PDA before autoclaved at 121°C, 15lbs/inch^2 for 30 min. The pathogen was cultured on PDA and incubated at room temperature for 5 days, then colony margin was cut by 0.5 mm sterilized cork borer. The agar plugs of *F. solani* was transferred into the middle of 5 cm of petri dishes in deferent concentrations and incubated at room temperature for 5-7 days. Data were collected as colony diameter, number of conidia. Percentage of inhibition of mycelial growth and number of conidia was calculated using formula (1) above and data were statistically computed for analysis of variance (ANOVA) and mean comparison was calculated by Duncan's Multiple Range Test (DMRT) at P=0.01 and P=0.05. The effective dose (ED_{50}) was computed by using probit analysis. The comparison of normal and abnormal spores was observed under compound microscope.

Results

Morphological identification

Fusarium solani was isolated from diseased plant parts, especially from roots of tomato var sida. The characteristics of colony were fast growing with aerial mycelium floccose, white or cream colour, reaching 9 cm diameter in 5-7 day at 29-32 °C. Conidiophores formed singly, slender and cylindrical shape. Macroconidia found abundant, moderately curved, blunt apical and pedicellate basal cell. Microconidia usually were abundant with 1 septate. Chlamydospores were singly or in paired in terminal, lateral or more rarely intercalary positions, smooth wall.

Pathogenicity test

Pathogenicity test was conducted by dipping cut root into spore suspension of *F. solani* at the concentration of 1 x10^6 conodia/ml for 30 seconds which resulted tomato seedlings showed yellowing leaves and root rot 41-60 %. It was significantly differed when compared to the non-inoculated control.

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

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

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

Morphological of antagonistic fungi

The culture of *Ch. globosum* was slow growing with olivaceous colour. Ascospore dark brown colour when mature with an apical germ pore. Colonies of *Ch. globosum* was slow growing with little superficial mycelium and a dense olivaceous layer on ascomata. Ascomata was dark brown or black colors, globose to subglobose. lateral hairs dark brown with paler tips, minutely roughened, terminal hairs dark olive brown with paler tips, wavy or loosely coiled and interwined. Ascopores was pale greenish to dark olive-brown, flattened lemon-shaped.

Dual-culture test

Ch. globosum were proved their abilities to inhibit plant pathogen *F. solani* causing disease of tomato by using dual-culture tests. The results showed that *Ch. globosum* gave significantly inhibition of *F. solani* which were 5.00 cm in colony diameter when compared to the control plate. After 3 month *Ch. globosum* showed ability to grow over the colony of *F. solani*

Table 2. Colony growth on dual-culture antagonistic tests.

Antagonist fungi	*Fusarium solani*	
	Colony(cm)	% inhibition of colony
Control	9.00 [a 1/]	-
Chaetomium globosum	5.00[b]	44.35
CV%	1.02	1.37

1/: Average of four replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.05

Bioactivity test of crude extracts from *Ch. globosum* against *F. solani*

Crude hexane extract from *Ch. globosum* gave significantly highest inhibition of 64.75% for the colony growth of *F. solani*, followed by crude methanol extract which inhibited 52.00% (Table 3). Crude hexane extract from *Ch. globosum* gave significantly highest inhibition for the spore production of *F. solani* as 93.29% with ED_{50} of 200.05 ppm. Crude methanol extract gave 79.75% inhibition with the ED_{50} was 378.47 ppm. Crude ethyl acetate extract showed 72.08% inhibition with ED_{50} was 314.16 ppm

Table 3 Crude extracts of *Ch. globosum* testing for growth and spore inhibition of *F. solani* at 7 days

Crude extracts	Concen-tration (ppm)	Colony diameter (cm)/1	Growth inhibition (%)/2,3	Number of spores /1 (10^6)	Inhibition (%)/2,3	ED_{50} (ppm)
Hexane	0	5[a]	-	6.52[a]	-	200.05
	10	5[a]	-	5.81[bc]	11.01[ij]	
	50	5[a]	-	4.88[ef]	25.13[fg]	
	100	5[a]	-	4.75[f]	27.23[f]	
	500	3.82[c]	23.50[e]	2.52[h]	61.27[d]	
	1,000	1.76[g]	64.75[a]	0.44[k]	93.29[a]	
EtOAc	0	5[a]	-	6.52[a]	-	314.16
	10	5[a]	-	5.58[bc]	12.85[ij]	
	50	5[a]	-	5.10[def]	21.84[fgh]	
	100	5[a]	-	4.74[f]	27.34[f]	
	500	3.72[d]	25.50[d]	2.91[gh]	55.40[de]	
	1,000	3.40[e]	32.00[c]	1.82[i]	72.08[c]	
MeOH	0	5[a]	-	6.52[a]	-	378.47
	10	5[a]	-	6.04[b]	7.28[j]	
	50	5[a]	-	5.43[cd]	16.59[hi]	
	100	5[a]	-	5.23[de]	19.83[gh]	
	500	3.92[b]	21.00[f]	3.08[g]	52.65[e]	
	1,000	2.39[f]	52.00[b]	1.32[j]	79.75[b]	
C.V. (%)		4.39	11.46	4.41	11.66	

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.

Testing fine particles form *Ch. globosum* against *F. solani*

The fine particle from *Ch. globosum* was showed that fine particles-CGH inhibited colony growth of *F. solani* as 90.00%. fine particles -CGE inhibited colony growth of *F. solani* as 90.00% and nano-CGM inhibited colony growth of *F. solani* as 90.00% (Table 4). The resulted showed that fine-CGH, fine particles -CGM and fine particles -CGM gave the highest ability to inhibit spore production of *F. solani* as 99.39%, 99.54% and 99.69% respectively and ED_{50} were 3.41, 3.48 and 1.48 ppm respectively

Table 4 Growth inhibition and ED_{50} of fine-particles against *F. solani* at 7 days

Fine particles	Concen-tration (ppm)	Colony diameter (cm)[1/]	Growth inhibition (%)[2,3]	Number of spores[1] (10^6)	Inhibition (%)[2,3]	ED_{50} (ppm)
fine	0	5[a]	-	82.37[a]	-	
particles-	3	4.71[b]	5.75[h]	50.87[b]	38.24[g]	
CGH*	5	3.56[c]	28.75[g]	17.75[d]	78.48[e]	3.41
	10	2.02[f]	59.50[d]	6.12[e]	92.56[c]	
	15	0.50[i]	90.00[a]	0.50[g]	99.39[a]	
fine	0	5[a]	-	82.37[a]	-	
particles-	3	4.69[b]	6.00[h]	52.62[b]	36.13[h]	
CGE	5	3.67[c]	26.50[g]	17.25[d]	79.07[e]	3.48
	10	2.73[e]	45.25[e]	7.50[e]	90.91[d]	
	15	0.50[i]	90.00[a]	0.37[g]	99.54[a]	
fine	0	5[a]	-	82.37[a]	-	
particles-	3	3.23[d]	35.25[f]	19.87[c]	75.88[f]	
CGM	5	1.73[g]	65.25[c]	7.50[e]	90.90[d]	1.48
	10	0.77[h]	84.50[b]	3.12[f]	96.21[b]	
	15	0.50[i]	90.00[a]	0.25[g]	99.69[a]	
C.V. (%)		3.08	4.29	4.51	1.61	

[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.

Discussion

Fusarium solani was isolated from diseased plant tissues of tomato and ensured by pathogenicity test which also reported by Sibounnavong *et al*. (2011). The morphological identification of pathogen was done by culture into potato dextrose agar (PDA) and observed under microscope. The colony of *Fusarium solani* are fast growing, white or cream colour, reaching 9 cm diameter in 5-7 day at 29-32 °C. Macroconidia found abundant, moderately curved, blunt apical and pedicellate basal cells 3-5. Microconidia usually are abundant with 1 septate. Chlamydospores are singly or in paired in terminal, lateral or more rarely intercalary positions, smooth wall. This is similar to the reported of Domsch and Gams (1993) who stated that colony of *F. solani* are fast growing, cream colour. Macroconidia produced in variable quantities on shorter, branched conidiophores which soon form sporodochia, usually moderately curved, blunt apical and indistinctly pedicellate basal cells, mostly indistinctly 3 septate. Microconidia usually abundant, produced on elongate, sometime verticillate. Chlamydospores are produced singly or in pairs, in terminal, lateral, or more rarely positions, hyaline, smooth or rough walled.

Dual culture test exhibited that *Ch. globosum* could control *F. solani* causing tomato crown and root rot. Based on the result *Ch. globosum* had ability to inhibit the growth of *F. solani* and the result was similar to the report of Tongon and Soytong (2015). Tongon and Soytong (2016) stated that *Ch. globosum* showed efficacies to inhibit colony growth of *F. solani* and *Curvularia lunata* causing leaf spot disease in rice and also *Ch. globosum* showed efficacies to inhibit conidial production of *F. solani* over 50% and this resulted similar to the report of Moya *et al.* (2016) who stated that *Chaetomium* spp showed high potential to inhibit *Drechslera teres* and *Bipolaris sorokiniana* causing foliar diseases of barley.

The result showed that crude hexane from *Ch. globosum* gave significant highest inhibit colony of *F. solani*. *Ch. globosum* were shown highest inhibition for the spore production of *F. solani* by treated with crude ethyl acetate, crude ethyl acetate and crude hexane. *Ch. globosum* also gave highest significantly inhibition for the spore production of *F. solani*. Soytong (2014) reported that crude hexane of *Ch. cochliodes* inhibited spore production of *Drechslera sorokiniana* causing spot blotch of wheat at concentration of 1,000 µg/ml (93.85 %) which ED_{50} was 66.45 ppm (Biswas *et al.* 2002) and similar to Sibounnavong (2012) who reported that *Ch. brasilense* CB01 and *Ch. cupreum* CC03 inhibited the spore production of *F. oxysporum* f.sp. *lycopersici* NKSC02 between 63-77%. The fine particles from *Ch. globosum* were used to test their abilities to inhibit the growth of *F. solani* the result similar with Hung *et al.* (2015) stated that methanol extract of *Ch. globosum* CG05 expressed strongest inhibitory effects on mycelial growth and sporangium formation of *P. palmivora* PHY02 with effective dose ED_{50} values of 26.5 µg/mL and 2.3 µg/mL, respectively.

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