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Antifungal activities of *Chaetomium brasilense* CB01 and *Chaetomium cupreum* CC03 against *Fusarium oxysporum* f.sp. *lycopersici* race 2

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The antagonistic fungi of *Chaetomium brasilense* CB01 and *Chaetomium cupreum* CC03 were proved to antagonize *F. oxysporum* f.sp. *lycopersici* NKSC02 race 2 caused tomato wilt of sida abd cherry varieties. The bioactivities test demonstrated the antagonistic activity of *Ch. brasilense* CB01 and *Ch. cupreum* CC03 to inhibit the conidial production of *F. oxysporum* f. sp. *lycopersici* race 2. To elucidate the control mechanism involved in the inhibition of *F. oxysporum* f. sp. *lycopersici*, crude extracts of *Ch. brasilense* CB01 and *Ch. cupreum* CC03, were confirmed for antifungal activity against of *F. oxysporum* f. sp. *lycopersici* race 2. The other control mechanism involved in releasing antibiotic substances to inhibit *F. oxysporum* f. sp. *lycopersici* race 2. All tested crude extracts of *Ch. brasilense* CB01 and *Ch. cupreum* CC03, were significantly inhibited conidia production of *F. oxysporum* f. sp. *lycopersici* race 2. It is indicated that crude extracts from hexane, EtOAc and MeOH from *Ch. brasilense* CB01 inhibited *F. oxysporum* f.sp. *lycopersici* race 2 at the ED_{50} of 29.87, 38.99 and 2.99 µg/ml, respectively. Crude extracts from hexane, EtOAc and MeOH from *Ch. cupreum* CC03 inhibited *F. oxysporum* f.sp. *lycopersici* race 2 at the ED_{50} of 2.33, 2.38 and 2.65 µg/ml, respectively.

Key words: *Chaetomium brasilense*, *Chaetomium cupreum*, *F. oxysporum* f.sp. *lycopersici* race 2 , fungal metabolites

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Introduction

Researches on natural products for antimicrobial against plant pathogens have been reported from several recent works. There are many new species of promising antagonists that can be used to control Fusarium wilt of tomatoes. The biocontrol agents and their bioactive compounds extracted from different species of antagonistic fungi were reported to inhibit the growth of many plant pathogenic fungi, including Fusarium wilt of tomato (Kanokmedhakul *et al*., 2006 and 2003; Thongsri and Soytong, 2004; Srinon *et al*. 2004, Suwannapong and Soytong, 2002 and Sibounnavong *et al*. 2009ab). The bioactive compounds, Trichotoxin A50 extracted from *Trichoderma harzianum* PC01; and Chaetoglobosin C extracted from *Chaetomium globosum*. These compounds have also been reported to elicit resistance or immunity in plants by inducing oxidative burst in plant cells (Nuchdonrong *et al*. 2004; Soytong *et al*. 2001). The metabolites from fungi become one of potent antifungal against several plant pathogens. Crude extracts of *Trichoderma hamatum* WS01 and *Penicillium* sp.WS01 were reported to inhibit *Fusarium oxysporum* f.sp. *cucumerinum* and *F. oxysporum* f.sp. *lycopersici* isolated from wilt of cucumber and tomato (Srinon *et al*. 2006). Crude extracts from P. chrysogenum could protect cotton plants against wilt disease (*F. oxysporum* f. sp. *vasinfetum* and *Verticillium dahlidae*) and increases yield under field condition. (Dong *et al*. 2005, 2003; Dong and Cohen, 2001; Saidkarimov and Cohen, 2003) and *Colletotrichum gloeosporioides* (Soytong *et al*. 2005), *Phytophthora parasitica* (Meepeung and Soytong, 2004) and De Cal (2004) studied *P. oxalicum* to inhibit *F. oxysporum* f.sp. *lycopersici* and *Botrytis cinerea*. In addition, *Gliocladium virens* produced gliotoxin (Lumsden *et al*. 1992) and its properties against wood attacking fungi; *Postia placenta* and *Neolentinus lepideus* and *Trametes versicolor* and *Phlebia brevisspora* (Terry *et al*.,1996). Chulalak and Soytong (2006) reported that the bioactive compound extracted from *Chaetomium cochliodes* and *Ch. cupreum* inhibited plant pathogenic fungi, *Phytophthora palmivora* (root rot of pomelo) and *Fusarium oxysporum* f. sp. *lycopersici* (tomato wilt). Soytong *et al*. (2001) reported that the bioactive compound from *Ch. cupreum* inhibited the spore production of *F. oxysporum* which the ED_{50} was 113.43 µg/ml and inhibited the spore production of *P. palmivora* which the ED_{50} was 53.46 µg/ml. Moreover, the bioactive compounds revealed that *Ch. cupreum* could reduce the sporulation of *P. palmivora* which the ED_{50} was 279.67 µg/ml. With this, the ED_{50} of crude extracts from *Ch. cochliodes* was 323.01 µg/ml to inhibited *F. oxysporum* and the ED_{50} of crude henae and ethyl acetate from *Ch. cochliodes* inhibited *F. oxysporum* were 203.64 and 416.41 µg/ml, respectively. A mechanism of antibiosis can occur during interactions involving low-molecular-weight

diffusible compounds or production of antibiotics by biological control agents (Benítez *et al*. 2004). With this, the effective biological control agents produce several types of antibiotics to play important role in disease control (Lewis *et al*. 1989; Handelsman and Stabb, 1996). Specific species of fungi can produce specific metabolite that either impede spore germination as fungistasis, or kill the cells as antibiosis (Benítez *et al*. 2004). *T. harzianum* PC01 reported to produce trichotoxin A50 that it would induce resistant to mamy crops like tomato and potato etc. (Suwan *et al*. 2000). *Ch. globosum* can produce Chaetoglobusin C (Soytong *et al*. 2001) and *Ch. cupream* can produce rotiorinol (Kanokmedhakul *et al*. 2006). Antibiotics chaetoglobosin C and rotiorinol were reported to inhibit several plant pathogen e.g. *F. oxysporum* f. sp. *lycopersici*, *C. gloeosporides* and *Phytopthora* spp. (Soytong *et al*. 2001). The objective was to evaluate the biological activities of antagonists against *F. oxysporum* f. sp. *lycopesici* race 2 caused tomato wilt of sida abd cherry varieties.

Materials and methods

Pathogen to be tested:- *Fusarium oxysporum* f.sp. *lycopersici* NKSC02 race 2 which pathogenic causing wilt to tomato var. Sida and Cherry from previous reports were used.

Effective antagonists to be tested:- *Chaetomium brasilense* CB01 and *Chaetomium cupreum* CC03 offered from Assoc. Prof. Dr. Kasem Soytong, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok, Thailand were used.

Crude extraction:- Crude extracts from each antagonistic fungus were done follwed the method of Kanokmedhakul *et al*. (2006) and Moosophon *et al*. (2009). The fungi were cultured in potato dextrose broth (PDB) at room temperature for 30 days. The dried fungal biomass of each antagonistic fungus was ground and sequentially extracted with hexane, ethyl acetate and methanol. The solvents were then evaporated in vacuo to yield crude hexane, crude ethyl acetate (EtOAc), and crude methanol (MeOH) extracts, respectively.

Bioassays:- Crude extracts were assayed for inhibition of the most virulent isolate of *F. oxysporum* f. sp. *lycopersici* NKSC02 race 2. The experiment was conducted by using a factorial experiment in Completely Randomized Design (CRD) with four replications. Factor A represented the different solvents: A1 = crude hexane, A2 = crude ethyl acetate and A3 = crude methanol. Factor B represented the different concentrations: B1 = 0 μg/ml (control), B2 = 50 μg/ml, B3 = 100 μg/ml, B4 = 500 μg/ml and B5 = 1,000 μg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxide and added to PDA before autoclaving at 121°C (15 psi) for 30 minutes. To perform the assay,

a sterilized 3-mm diameter cork borer was used to remove agar plugs from the actively growing edge of the pathogen culture. An agar plug was transferred to the center of 5 cm diameter Petri dishes of PDA containing crude extract at each concentration and incubated at room temperature until the pathogen on the control plates had grown over the plate. Data were collected regarding the number of conidia produced by the pathogen and used to calculate the percentage of conidia inhibition. The effective dose (ED_{50}) was calculated using Probit analysis. The experiment was repeated twice.

Results

Chaetomium brasilense CB01 and *Chaetomium cupreum* CC03 at different concentrations of 0, 10, 50, 100, 500, and 1,000 g/ml were tested for inhibition of *F. oxysporum* f. sp. *lycopersici* NKSC02 which obtained from previous experiment. Hexane crude extract from *Ch. brasilense* CB01 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 3.67, 3.19, 2.67, 2.37 and 1.94 cm, respectively when compared to the control (0 µg/ml) of 5 cm. EtOAc crude extract from *Ch. brasilense* CB01 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 3.05, 2.92, 2.64, 2.27 and 2.22 cm, respectively when compared to the control (0 µg/ml) of 5 cm. MeOH crude extract from *Ch. brasilense* CB01 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 3.67, 3.50, 2.97, 2.77 and 2.22 cm, respectively when compared to the control.

Hexane crude extract from *Ch.cupreum* CC03 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 5.00, 4.87, 4.47, 4.45 and 4.12 cm, respectively when compared to the control. EtOAc crude extract from *Ch. cupreum* CC03 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* NKSC02 which were 5.00, 3.92, 3.67, 3.54 and 3.40 cm, respectively when compared to the control. MeOH crude extract from *Ch. cupreum* CC03 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 4.47, 4.12, 3.74, 3.54 and 3.25 cm, respectively when compared to the control (Table 1 and 2).

Table 1. Effect of crude extracts from antagonistic fungi on mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici* NKSC02

Crude extracts	Colony diameter (cm) of *Fusarium oxysporum* f.sp. *lycopersici* at each concentration (µg/ml))					
	0	10	50	100	500	1000
C. brasilense						
Hexane	5a[1]	3.67b	3.19c	2.67f	2.37g	1.94h
EtOAc	5a	3.05cd	2.92de	2.64f	2.27g	2.22g
MeOH	5a	3.67b	3.50b	2.97de	2.77ef	2.22g
C. cupreum						
Hexane	5a	5.00a	4.87a	4.47b	4.45b	4.12b
EtOAc	5a	5.00a	3.92d	3.67ef	3.54f	3.40g
MeOH	5a	4.47b	4.12c	3.74e	3.54f	3.25 h

[1]Average of four replications. Means followed by the same letters in each antagonist were not significantly different by DMRT at P=0.01.

Table 2. Effect of crude extracts from antagonistic fungi for percentage of colony inhibition growth of *Fusarium oxysporum* f.sp. *lycopersici* NKSC02

Crude extracts of	Colony inhibition of *Fusarium oxysporum* f.sp. *lycopersici* (%)				
	10	50	100	500	1000
C. brasilense					
Hexane	26.5g[1]	36.0f	46.5c	52.5b	61.0a
EtOAc	39.0ef	46.0cd	47.0c	54.5b	55.5b
MeOH	26.5 g	30.0 g	40.5 def	44.5 cde	55.5b
C. cupreum					
Hexane	0.0h	2.5h	10.5g	11.0g	17.5f
EtOAc	0.0h	21.5 e	26.5 cd	29.0 bc	32.0 ab
MeOH	10.5 g	17.5 f	25.0 d	31.0 b	35.0 a

[1]Average of four replications. Means followed by the same letters in each antagonist were not significantly different by DMRT at P=0.01

Hexane crude extract from *Ch. brasilense* CB01 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in spore production of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 21.5 $x10^7$, 15.93 $x10^7$, 14.0 $x10^7$ and 2.16 x 10^7 spore/ml, respectively.when compared to the control (0 µg/ml) of 35.78 $x10^7$ spore/ml. EtOAc crude extract from *Ch. brasilense* CB01 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in spore production of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 26.71 $x10^7$, 19.61 $x10^7$, 11.48 $x10^7$, 5.35 $x10^7$ and 4.40 $x10^7$ spore/ml, respectively when compared to the control (0 µg/ml) of 36.24 $x10^7$ cm. MeOH crude extract from *Ch. brasilense* CB01 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in spore production of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 11.83 $x10^7$,

9.84 x10^7, 8.52 x10^7, 4.28 x10^7 and 1.07 x10^7 spore/ml, respectively when compared to the control (0 µg/ml) of 35.72 x10^7 spore/ml. Hexane crude extract from *Ch. cupreum* CC03 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in spore production of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 15.93 x10^7, 8.64 x10^7, 6.82 x10^7, 5.94 x10^7 and 3.18 x10^7 spore/ml, respectively when compared to the control (0 µg/ml) of 39.50 x10^7 spore/ml. EtOAc crude extract from *Ch. cupreum* CC03 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in spore production of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 14.43 x10^7, 8.87 x10^7, 7.68 x10^7, 4.48 x$10^7$and 2.40 x10^7 spore/ml, respectively when compared to the control. MeOH crude extract from *Ch. cupreum* CC03 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in spore production of *F. oxysporum* f. sp. *lycopersici* NKSC02which were 13.93 x10^7, 8.43 x10^7, 6.16 x10^7, 2.86 x$10^7$and 1.07 x10^7 spore/ml, respectively when compared to the control (Table 3).

Table 3. Effect of crude extracts from antagonistic fungi against conidia production of *Fusarium oxysporum* f.sp. *lycopersici* NKSC02

Crude extracts of	Number of conidia(x10^7) of *Fusarium oxysporum* f.sp. *lycopersici* at each concentration (µg/ml)					
	0	10	50	100	500	1000
C. brasilense						
Hexane	35.78a[1]	21.51c	15.93d	14.50d	4.10gh	2.16hi
EtOAc	36.24a	26.71b	19.61c	11.48e	5.35g	4.40gh
MeOH	35.72a	11.83e	9.84ef	8.52f	4.28gh	1.07i
C. cupreum						
Hexane	39.50b	15.93d	8.64fg	6.82hi	5.94i	3.18k
EtOAc	38.47c	14.43e	8.87f	7.68gh	4.48j	2.40k
MeOH	41.00a	13.93e	8.43fg	6.16i	2.86k	1.07 l

[1]Average of four replications. Means followed by the same letters in each antagonist were not significantly different by DMRT at P=0.01.

It revealed that crude extract at 1000 µg/ml from MeOH of *Ch. brasilense* CB01 gave significantly better inhibited spore production of *F. oxysporum* f.sp. *lycopersici* as 96.98 % better than crude extracts from EtOAc and MeOH which were 55.50 %. Crude extract at 1000 µg/ml from MeOH of *Ch. cupreum* CC03gave significantly better inhibited spore production of *F. oxysporum* f.sp. *lycopersici* as 97.37 % better than crude extracts from hexane and EtOAc which were 93.75 and 91.92 %, respectively. It is indicated that crude extracts from hexane, EtOAc and MeOH from *Ch. brasilense* CB01 inhibited *Fusarium oxysporum* f.sp. *lycopersici* race 2 at the ED_{50} of 29.87, 38.99 and 2.99 µg/ml, respectively (Table 4). Crude extracts from hexane, EtOAc and MeOH from

Ch. cupreum CC03 inhibited *Fusarium oxysporum* f.sp. *lycopersici* race 2 at the ED_{50} of 2.33, 2.38 and 2.65 µg/ml, respectively (Table 4).

Table 4. Effect of crude extracts from antagonistic fungi for percentage of conidia inhibition of *Fusarium oxysporum* f.sp. *lycopersici* NKSC02

Crude extracts	Conidia inhibition of *Fusarium oxysporum* f.sp. *lycopersici* (%)					
	10	50	100	500	1000	ED_{50} µg/ml
C. brasilense						
Hexane	39.83k[1]	55.44i	59.47h	88.53c	93.95b	29.87
EtOAc	33.17l	45.88j	68.29g	85.22d	87.85c	38.99
MeOH	66.87g	72.38f	76.09e	87.98c	96.98a	2.99
C. cupreum						
Hexane	59.64j	78.23g	82.71de	84.38de	91.92b	2.33
EtOAc	62.46i	76.92g	82.17ef	88.34c	93.75b	2.38
MeOH	65.99h	79.41fg	85.49d	93.13b	97.37a	2.65

[1]Average of four replications. Means followed by the same letters in each antagonist were not significantly different by DMRT at P=0.01.

Discussion

The antagonistic fungi *Ch. brasilense* CB01 and *Ch. cupreum* CC03, were proved to antagonize *F. oxysporum* f.sp. *lycopersici* NKSC02 race 2 causing wilt to tomato var. Sida and Cherry. The antagonism test demonstrated the antagonistic activity of *Ch. brasilense* CB01 and *Ch. cupreum* CC03, to inhibit the conidial production of *F. oxysporum* f. sp. *lycopersici* NKSC02 between 63 – 77 %. Similar result was in accordance with the study from Charoenpoen *et al.* (2010) reported that *Chaetomium lucknowense* CLT significantly inhibited the mycelia growth and conidial production of *F. oxysporum* f. sp. *lycopersici* as 88.89 and 92.54 %, respectively. Furthermore, Sibounnavong *et al*. (2009) reported crude extacrts of *Emericella nidulans* strongly inhibited colonial growth and sporulation of *F. oxysporum* f. sp. *lycopersici*. Crude extracts of *Ch. Brasilense* CB01 and *Ch. cupreum* CC03 were confirmed for antifungal activity against of *F. oxysporum* f. sp. *lycopersici* NKSC02 race 2. The other control mechanism of *Ch. brasilense* CB01 and *Ch. cupreum* CC03 involved in releasing antibiotic substances to inhibit *F. oxysporum* f. sp. *lycopersici*. All tested crude extracts of *Ch. brasilense* CB01 and *Ch. cupreum* CC03 were significantly inhibited conidia production of *F. oxysporum* f. sp. *lycopersici*. This result was similar to the report of Charoenpoen *et al*. (2010) who stated that crude hexane, crude ethyl acetate and crude methanol from *Ch. lucknowense* CLT inhibited *F. oxysporum* f. sp. *lycopersici* NKSC01 with the ED_{50} of 188, 209 and 212 µg/ml while in this study, crude extracts from methanol, ethyl acetate and hexane *Ch. brasilense*

CB01 inhibited the conidial production of different isolate of *F. oxysporum* f. sp. *lycopersici* NKSC02 race 2 with the ED_{50} of 29.87, 38.99 and 2.99 µg/ml, respectively and crude extracts from methanol, ethyl acetate and hexane *Ch. cupreum* CC03 inhibited the conidial production of different isolate of *F. oxysporum* f. sp. *lycopersici* NKSC02 race2 with the ED_{50} of 2.33, 2.38 and 2.65 µg/ml. Similar results were also reported by Srinon *et al*. (2006) and Sibounnavong *et al*. (2009) who stated that crude hexane, ethyl acetate and methanol extracts from *E. nidulans* inhibited the colony and sporulation of *F. oxysporum* f. sp. *lycopersici*. Moreover, Soytong *et al*. (2005) reported that crude ethyl acetate extract of *Ch. globosum* CG at 1000 µg/ml inhibited conidia production of this pathogen. As a result, Sibounnavong *et al*. (2009) reported that methanol crude extract from E. nidulans gave the highest inhibition of *F. oxysporum* f. sp. *lycopersici.* It is explained that ethyl acetate crude extract from *E. rugulosa* might have different fungal mrtabolites from methanol crude extract of *E. nidulans* as reported by Moosophon *et al*. (2006).

It concluded that *Ch. cupreum* CC03 can be produced some metabolites to inhibit *F. oxysporum* f. sp. *lycopersici* race 2 which Kanokmedhakul *et al*. (2006) found antifungal azaphilones from *Ch. cupreum* CC3003 effectively inhibited some human pathogens. Moreover, in this study *Ch. brasilense* CB01 proved to produce antifungal metabolites against *F. oxysporum* f. sp. *lycopersici* race 2 cause tomato wilt which it is the same isolate of reported *Ch. brasilense* CB01 by Khumkomkhet *et al*. (2009) found four new depsidones, mollicellins K-N which exhibited antimalarial activity against Plasmodium falcipurum and mollicellin K exhibited antmycobacterial activity against *Mycobacterium tuberculosis* and antifungal activity against *Candida albicans* and some cancer cell lines. The result of research finding would extend for testing to cotrol tomato wilt in the fields and further study would convey to apply these bioactive compounds as microbial elicitors to induce plant immunity.

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