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Antifungal Activities of *Chaetomium* spp. against Fusarium Wilt of Tea

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Abstract

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An isolate of *Fusarium* denominated as NHP-Fusa-2 from tea wilt and root-rot diseased sample collected in Vietnam was identified as *Fusarium oxysporum* based on molecular analysis of translation elongation factor-1α sequence. Interestingly, it is the first time *F. oxysporum* is reported as a causal pathogen of wilt and root-rot disease of tea in Vietnam. *Chaetomium* spp. were investigated to control *F. oxysporum* NHP-Fusa-2 in *in vitro* test. Three antagonists (*Ch. cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01) inhibited mycelial growth by 31.69–34.03% and reduced conidial production of the pathogen by 67.25–75.92% in the bi-culture antagonistic test after 30 days with non-significant difference in their effect. All the crude extracts of these antagonists significantly inhibited mycelial growth and conidial production of NHP-Fusa-2. MeOH extract of CC3003 was more effective in conidial inhibition of the pathogen than the others, the effective dose (ED_{50}) was 85.30 µg/ml of which, it was hexane (49.32 µg/ml) and EtOAc extract (62.17 µg/ml) in the case of CG05 and CL01, respectively.

Keywords: *Chaetomium* species; *Fusarium oxysporum*; growth; sporulation; inhibition

Chaetomium, a strictly saprophytic fungus that belongs to Ascomycota of the family Chaetomiaceae, was reported to be one of the largest genera of *Ascomycetes* with more than 300 species worldwide (von Arx *et al.* 1986). They are normally found in soil and organic compost, degrade cellulose and other organic materials relying on producing lytic enzymes (Soytong *et al.* 2001). Many species of *Chaetomium* with the potential as biological control agents suppress the growth of bacteria and fungi through competition, mycoparasitism, antibiosis, or their various combinations (Zhang & Yang 2007). Until now, more than 200 compounds with a wide range of bioactive effects have been isolated from *Chaetomium* spp., and many of them exhibited antifungal activity against plant pathogenic fungi (Zhang *et al.* 2012). Some species of *Chaetomium* have been reported to work as antagonists against several plant pathogens (Di Pietro *et al.* 1992; Park *et al.* 2005; Charoenporn *et al.* 2010; Sibounnavong *et al.* 2012).

Root-rot is found to be one of very dangerous diseases of tea in Vietnam. The disease is usually not detected until several neighbouring tea bushes have been seriously affected and died. With the progressing disease the tea leaves become yellow, wilted, and die, leading to final death of the whole bush (Nguyen 2001; Zeiss & Braber 2001). With a 1% loss of bushes which is owed to root disease, in the 10th years the loss of the potential crop would be 10% (Hainsworth 1952). In Kenya, the loss of tea bushes caused by root disease could reach up to 50% (Onsando *et al.* 1997).

This study aimed to isolate the pathogenic fungus causing wilt of root-rot disease of tea in Vietnam, and to evaluate antifungal potential of antagonistic fungi,

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namely *Chaetomium cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01, against the isolated pathogen in *in vitro* test.

MATERIAL AND METHODS

Isolation of the pathogen. Samples of tea wilted of root-rot disease were collected from tea plants showing the symptoms at Northern Mountainous Agricultural and Forestry Science Institute (Vietnam) in 2013. The pathogen was isolated by transferring surface sterilised diseased tissues to water agar (WA) containing streptomycin, then the growing mycelium tip of it was sub-cultured and purified in potato dextrose agar (PDA) to get the pure culture (NHP-Fusa-2).

DNA extraction and identification of the pathogen. DNA of NHP-Fusa-2 was extracted using the method described by Liu *et al.* (2000). A small lump of the fungal mycelia was added to an Eppendorf tube containing 500 µl of lysis buffer (400 mM of Tris-HCl (pH 8.0), 60 mM EDTA (pH 8.0), 150 mM NaCl, and 1% of sodium dodecyl sulphate), then left at room temperature (RT) for 10 min, then 150 µl of potassium acetate (60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic acid, and 28.5 ml of distilled water, pH 4.8) was added. Afterward, the tube was vortexed briefly and spun at 10 000 rpm for 1 min, the supernatant layer was transferred to a new tube and spun again under the same conditions. The new supernatant layer was transferred into another tube, and an equal volume of isopropyl alcohol was added. The contents of this tube was mixed briefly and spun at 10 000 rpm for 2 minutes. Subsequently, the supernatant layer was discarded to get DNA pellet, which was later washed in 300 µl of 70% ethanol and spun at 10 000 rpm for 1 min, ethanol was then poured off. The DNA pellet was air-dried and dissolved in 50 µl of 1X Tris-EDTA buffer (BioThema, Handen, Sweden).

To identify the isolate, a polymerase chain reaction (PCR) was performed with the primer pair of ef1 (forward primer: 5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and ef2 (reverse primer: 5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') (Geiser *et al.* 2004). The PCR mixture with a total volume of 50 µl consisted of 1X PCR buffer, 3.5 mM $MgCl_2$, 0.16 mM each dNTP, 1.75 U Taq polymerase, 0.2 µM of each primer, 2 µl of DNA template, and deionised water (top up to 50 µl). Cycling conditions were: initial denaturation at 94°C for 1 min 25 s followed by 35 cycles of denaturation at 95°C for 35 s, annealing at 61°C for 55 s, and elongation at 72°C for 1 min 30 s; final extension was at 72°C for 10 minutes. The PCR product was sent to the 1st BASE DNA Sequencing Division (Singapore Science Park II, Singapore) for sequencing with the same primers. The partial DNA sequencing data was used as a blast query against the database, which is publicly accessible at Fusarium-ID (http://isolate.fusariumdb.org), for identifying the isolate to species included within the database. NHP-Fusa-2 was also identified via phylogenetic analysis by adding sequences of related taxa, which were downloaded from Fusarium-ID database, to the DNA sequence alignment using MEGA Version 6.06 software (Tamura *et al.* 2013).

Pathogenicity test. NHP-Fusa-2 was tested for its pathogenicity with tea cuttings using a method of Bhagat and Chakraborty (2010), which was modified. The inoculum (chaff-grains) of the pathogen was prepared following the method of Leslie and Summerell (2006). Chaff and grain were mixed together at the ratio of 5 : 1, respectively, the mixture was added into a 2-litre beaker up to a 0.5-litre level, and tap water was added up to a 1-litre level. The beaker was mixed thoroughly and placed at 5°C overnight. After that, the water was drained out, and the chaff-grain mixture was dried out. The drained mixture was inserted into an Erlenmeyer flask which was filled up to a height of approximately 5 cm, then autoclaved for 15 min on each two successive days before inoculating with the pathogen's conidial suspension (10^5 conidia/ml) at the rate of 2 ml of fungal suspension per 250 ml of chaff-grain mixture. The inoculum material was shaken daily for the 3–4 first days and incubated at 25°C until the material in the flask was completely colonised (14 days). Subsequently, the culture was removed from the flask and air-dried at RT overnight. The dried substrate of the inoculum was mixed with sterilised soil at the ratio of 1 : 8, respectively. One-year tea cuttings were separately planted in plastic pots containing 1 kg sterile soil each, and regularly watered for two weeks before inoculation. 100 g of the fungal soil mixture was added carefully into the rhizosphere area of the tea cutting. A pot planted with a tea cutting without inoculation by the pathogen served as a control. After inoculation the disease symptoms were observed daily. The pathogen was then re-isolated from feeder roots of the infected inoculated tea cuttings to confirm the pathogenic isolate.

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Bi-culture antagonistic test. The antagonistic fungi, namely *Chaetomium cupreum* strain CC3003, *Ch. globosum* strain CG05, and *Ch. lucknowense* strain CL01, were tested to inhibit NHP-Fusa-2 using bi-culture technique following the method of Charoenporn *et al.* (2010). A mycelial disc (5 mm in diameter) of NHP-Fusa-2 was placed singly (as controls) or oppositely to the mycelia disc of each of the above-mentioned antagonistic fungi in Petri dishes (9 cm in diameter) containing PDA. A mycelial disc of each antagonist was also placed singly on a separate 9-cm-diameter Petri dish for comparison. The experiment was conducted in a completely randomised design (CRD) with four replications, incubation took place at RT. After 30 days data on colony diameter and number of conidia produced by the pathogen were collected. The conidia were counted using a haemacytometer (Hausser Scientific, Horsham, USA). The inhibitory percentage of mycelial growth and conidial production of NHP-Fusa-2 was calculated by the formula:

% inhibition = [(colony diameter or conidial number of the pathogen in the control plate – colony diameter or conidial number of the pathogen in the bi-culture plate) × 100]/colony diameter or conidial number of the pathogen in the control plate

Antifungal crude extract test. The three above-mentioned antagonists were separately cultured in potato dextrose broth (500 plates per each) and incubated at RT for 35 days before collecting fresh fungal biomass, which was then dried out to get dried fungal biomass. Crude extraction of the dried fungal biomass of each antagonist was done separately using the method of Kanokmedhakul *et al.* (2006). Initially, the dried biomass was ground and extracted with hexane (1 : 1 v/v), and incubated by shaking for 72 h at RT. Afterwards, the filtrate was collected and the marc was separated out by filtration through a filter paper (Whatman No. 4), then it was subjected to a rotary vacuum evaporator to yield hexane crude extract. The marc from hexane extraction was further extracted by ethyl acetate (EtOAc) and subsequently by methanol (MeOH) using the same procedure as described above to yield EtOAc and MeOH crude extract, respectively. The experiment was set up at two-factor factorial design in the CRD with four replications. Factor A was represented by the three above-given crude extracts. Factor B was represented by the following concentrations: 0 (control), 50, 100, 500, and 1000 µg/ml. To obtain the desired concentrations of the crude extracts, stock crude extracts of each concentration were weighed, then dissolved in 2% dimethyl sulfoxide, and added to molten PDA before autoclaved at 121°C (15 psi) for 20 minutes. To perform the assay, a mycelia disc (3 mm in diameter) of the pathogen at the actively growing edge of the culture was placed in the centre of a 5-cm-diameter Petri dish containing PDA with the predetermined crude extract at each concentration. The experiment was incubated at RT until the pathogen on the control plates had grown over. Data collection was the same as in the case of the bi-culture antagonistic test. The dose effective on conidial inhibition of the pathogen (ED_{50}) was also calculated by probit analysis using IBM SPSS Statistics v. 19.0 (Armonk, USA).

RESULTS

Isolation and identification of the pathogen. After two days of the diseased tissue transplanting in WA, a single mycelium tip of the pathogen was isolated and transferred to PDA to get the pure culture, whose DNA was extracted and used for a PCR to amplify the translation elongation factor-1α (TEF-1α) gene region using the ef1 and ef2 primer. The PCR product was purified before sequenced. BLAST search for similarities using Fusarium-ID indicated that NHP-Fusa-2 showed 100% (629/629) of similarity level to *Fusarium oxysporum*. Alternatively, the phylogenetic tree (Figure 1), which illustrated

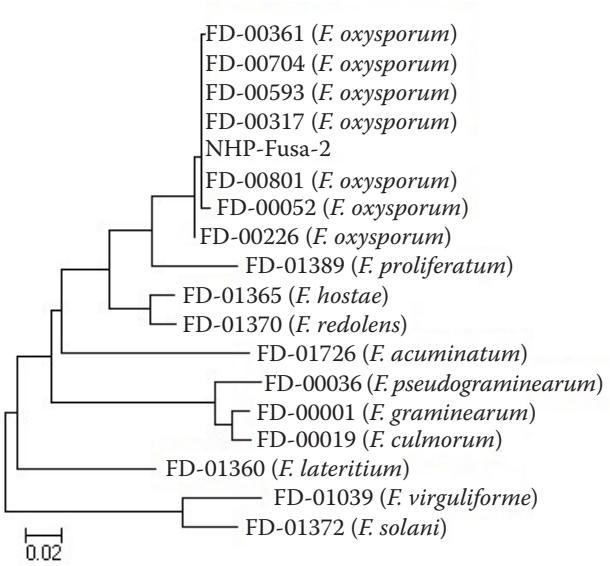

Figure 1. Phylogenetic tree reveals the relationships among NHP-Fusa-2 and the related taxa

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Figure 2. Pathogenicity test of *F. oxysporum* NHP-Fusa-2 with PH8 tea variety: (**a**) after 3 days of inoculation; (**b**) after 10 days of inoculation; (**c**) after 10 days of inoculation in bare soil; inoculated: in the left side, control: in the right side

relationships between NHP-Fusa-2 and related taxa, whose TEF-1α sequences were stored in Fusarium-ID database, also denoted that NHP-Fusa-2 belongs to the *F. oxysporum* species complex.

Pathogenicity test. To prove the pathogenic ability of the isolate, *F. oxysporum* NHP-Fusa-2 was tested for pathogenicity with young tea cuttings. Observation of the symptoms after 3 days of inoculation showed that leaf colour of the inoculated tea plants turned from green to yellowish-green, and wilting symptom of the tea leaves occurred. After 10 days of inoculation, whole tea leaves of the inoculated plants became completely wilted; most of the tea feeder roots became rotten with black wood discoloration (Figure 2). The pathogen was then re-isolated from the infected roots of the inoculated tea plant, and its morphological characteristics (Figure 3) were compared with those of NHP-Fusa-2.

Table 1. Inhibition of mycelial growth and conidial production of *Fusarium oxysporum* NHP-Fusa-2 in the bi-culture antagonistic test

Treatments	Inhibition (%)	
	colony	conidial
CC3003 vs NHP-Fusa-2	31.69 ± 1.32[a]	75.92 ± 4.44[a]
CG05 vs NHP-Fusa-2	34.03 ± 0.97[a]	67.25 ± 3.25[a]
CL01 vs NHP-Fusa-2	32.64 ± 0.73[a]	73.45 ± 3.57[a]

Values are means of four replications ± SE; values within a column followed by the same letter are not significantly different by Duncan's Multiple Range Test at $P = 0.05$

Bi-culture antagonistic test. *Ch. cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01 significantly inhibited mycelial growth and conidial production of *F. oxysporum* NHP-Fusa-2. The inhibitory percentage of the pathogen's mycelial growth given by the three antagonists ranged from 31.69% to 34.03%, with just insignificant difference (Figure 4A and Table 1). However, CC3003 gave a significantly lower number of conidia of NHP-Fusa-2 than those of CG05 and CL01, while there was non-significant difference between CG05 and CL01 (data not shown). Though, there was insignificant difference in the conidial inhibitory percentage of the pathogen among the three antagonistic fungi, which ranged from 67.25% to 75.92% (Table 1). To assess the antifungal efficiency of the tested antagonists on the conidial production of the pathogen, the production of conidia of NHP-Fusa-2 both in the bi-culture and

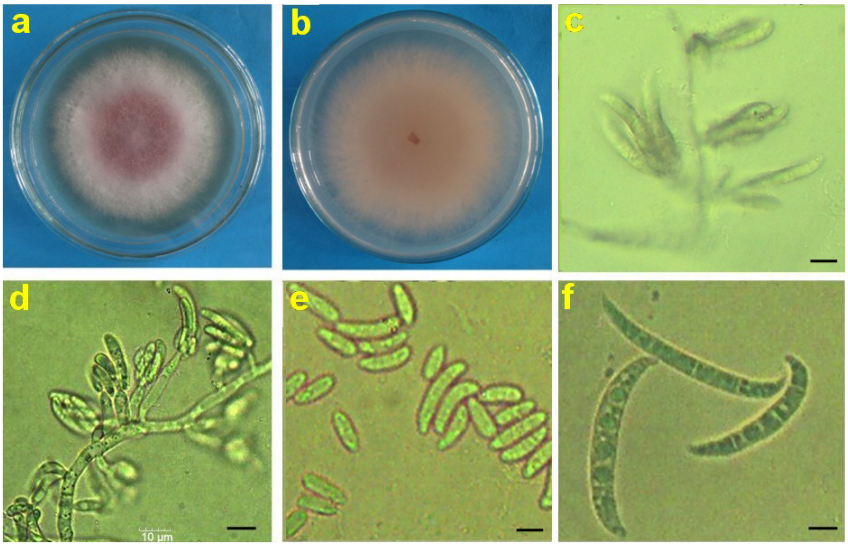

Figure 3. Morphological characteristics of *F. oxysporum* NHP-Fusa-2 at a 7-day-old culture on potato dextrose agar (colony) and water agar (conidia) (scale bar 10 µm): (**a**) front surfaced colony; (**b**) back surfaced colony; (**c**) macroconidia *in situ* on monophialides; (**d**) microconidia *in situ* on sporodochia; (**e**) microconidia; (**f**) macroconidia

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Table 2. Effect of the crude extracts on mycelial growth of *Fusarium oxysporum* NHP-Fusa-2

Treatments	Colony diameter (cm) of NHP-Fusa-2 at each concentration (µg/ml)				
	0	50	100	500	1000
CC3003					
Hexan	5.00 ± 0.00^{a}	4.82 ± 0.12^{a}	3.85 ± 0.17^{d}	2.58 ± 0.06^{g}	1.99 ± 0.04^{h}
EtOAc	5.00 ± 0.00^{a}	4.92 ± 0.02^{a}	4.21 ± 0.10^{c}	3.49 ± 0.04^{e}	2.96 ± 0.04^{f}
MeOH	5.00 ± 0.00^{a}	4.50 ± 0.11^{b}	3.85 ± 0.17^{d}	3.25 ± 0.07^{e}	2.63 ± 0.07^{g}
CG05					
Hexan	5.00 ± 0.00^{a}	4.13 ± 0.03^{b}	3.96 ± 0.04^{b}	3.66 ± 0.10^{cd}	3.23 ± 0.01^{e}
EtOAc	5.00 ± 0.00^{a}	3.75 ± 0.05^{c}	3.58 ± 0.07^{d}	3.08 ± 0.06^{e}	2.53 ± 0.04^{f}
MeOH	5.00 ± 0.00^{a}	3.99 ± 0.02^{b}	3.98 ± 0.03^{b}	3.53 ± 0.02^{d}	3.08 ± 0.04^{e}
CL01					
Hexan	5.00 ± 0.00^{a}	4.15 ± 0.08^{bc}	4.15 ± 0.02^{bc}	3.10 ± 0.16^{g}	2.64 ± 0.03^{h}
EtOAc	5.00 ± 0.00^{a}	4.04 ± 0.04^{c}	3.66 ± 0.04^{ef}	3.49 ± 0.12^{f}	3.48 ± 0.05^{f}
MeOH	5.00 ± 0.00^{a}	4.28 ± 0.03^{b}	4.03 ± 0.06^{c}	3.95 ± 0.07^{cd}	3.80 ± 0.06^{de}

Values are means of four replications ± SE; values in the columns and the rows of three crude extracts within an antagonist followed by the same letters are not significantly different by Duncan's Multiple Range Test at $P = 0.05$

control plate was observed for comparison also under a compound microscope (×40) (Figure 5A).

Antifungal crude extract test. Crude extracts of *Ch. cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01 were tested for their ability to inhibit *F. oxysporum* NHP-Fusa-2 in *in vitro* test. Data were collected after five days of experiment. The inhibitive efficiency of the crude extracts on mycelial growth and conidial production of NHP-Fusa-2 gradually increased following the rise of the concentrations (Figures 4B and 5B). All the crude extracts at the concentrations of 50–1000 µg/ml significantly reduced the number of conidia of NHP-Fusa-2 if compared to the control (0 µg/ml) (Table 3). Meanwhile, only hexane and EtOAc extract of CC3003 inhibited significantly the mycelial growth of NHP-Fusa-2 at the concentration of 100–1000 µg/ml, which the other crude extracts of the antagonists inhibited significantly at the concentration of 50–1000 µg/ml with the same comparison (Table 2).

There was a significant difference in mycelial growth inhibition of NHP-Fusa-2 among the concentrations of 100, 500, and 1000 µg/ml in all the crude extracts of CC3003 and CG05, and EtOAc extract of CL01;

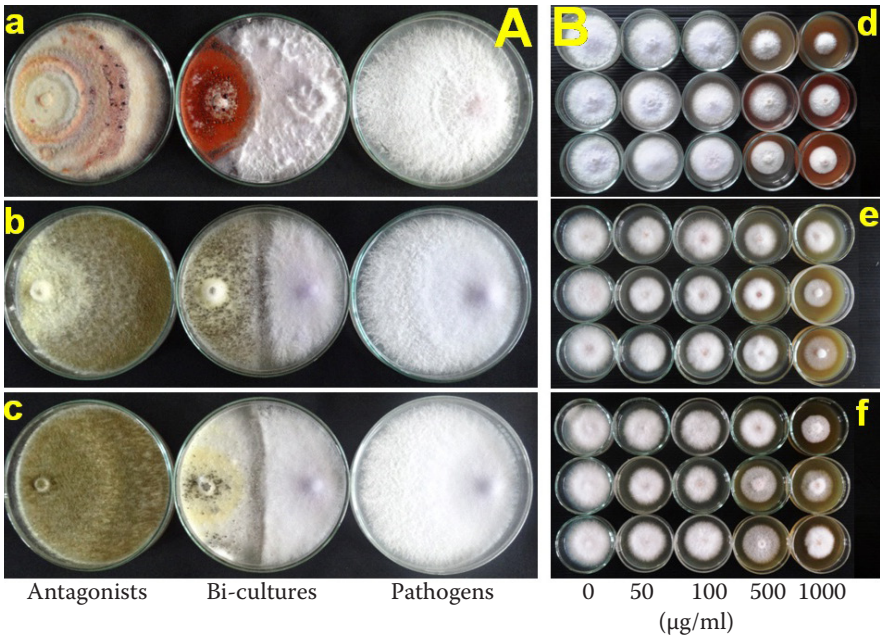

Figure 4. Colony growth of the pathogen observed from the antifungal activities test of *Chaetomium* spp. against *F. oxysporum* NHP-Fusa-2. (**A**) bi-culture test of CC3003 (**a**), CG05 (**b**), and CL01 (**c**) against NHP-Fusa-2; (**B**) crude extract test of CC3003 (**d**), CG05 (**e**), and CL01 (**f**) against NHP-Fusa-2; from the top to the bottom of each test: hexane, EtOAc, and MeOH extract, respectively

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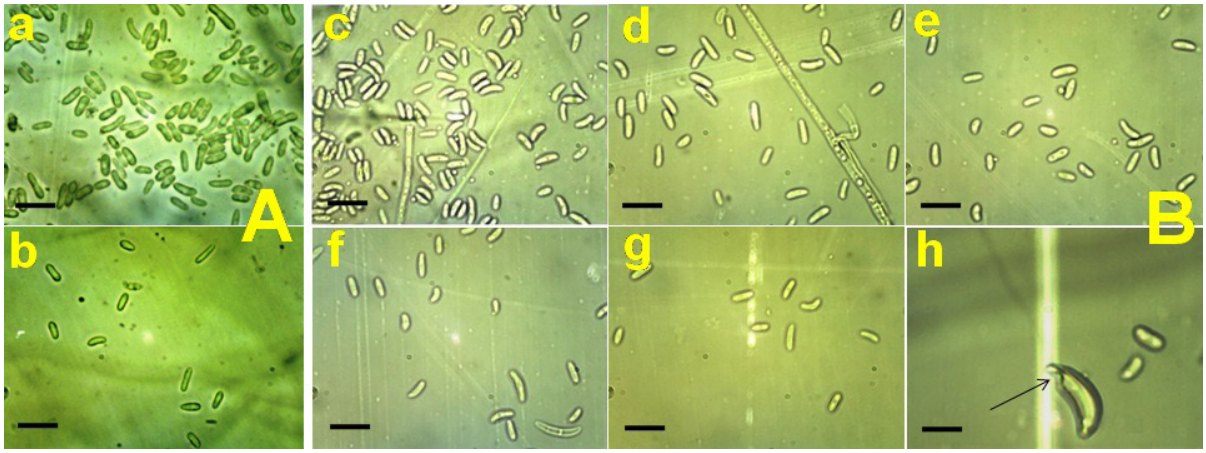

Figure 5. Conidial production of the pathogen observed from the antifungal activities test of *Chaetomium* spp. (an example for CL01) against *Fusarium oxysporum* NHP-Fusa-2 (scale bar 10 µm; observed under a 40× magnification optical microscopy). (**A**) **a** – control; **b** – bi-culture; (**B**) concentrations from **c** to **g** – 0, 50, 100, 500, and 1000 µg/ml, respectively; **h** – an abnormal conidium affected by antifungal activities of the antagonists

the higher concentrations exhibited a significantly higher inhibitive efficiency. Whereas hexane and MeOH extract of CL01 exhibited non-significant difference in mycelial growth inhibition of NHP-Fusa-2 as compared between the concentrations of 500 and 1000 µg/ml; in which MeOH extract of CL01 also did not exhibit significant difference as compared between the concentrations of 100 and 500 µg/ml (Table 2). It could be inferred that within the concentrations of 100–1000 µg/ml in the case of hexane and MeOH extract of CL01, the inhibitive efficiency on mycelia growth of the pathogen did not significantly increase following the rise of the concentrations.

Unlike the inhibition of mycelial growth (Table 2), EtOAc and MeOH extract of CC3003, and hexane and EtOAc extract of CG05 exhibited non-significant inhibition of conidial production of NHP-Fusa-2 as compared among the concentrations of 100, 500, and 1000 µg/ml, and also amongn the concentrations of 50, 100, and 500 µg/ml. While on which hexane extract of CC3003, MeOH extract of CG05, and MeOH extract of CL01 gave non-significant inhibition as compared among the concentrations of 50, 100, and 500 µg/ml, and also between the concentrations of 500 and 1000 µg/ml, except for MeOH extract of CL01. Hexane and EtOAc extract of CL01 at the con-

Table 3. Effect of the crude extracts on conidial production and effective dose (ED_{50}) of *Fusarium oxysporum* NHP-Fusa-2

Treatments	Number of conidia* (× 10^7) of NHP-Fusa-2 at each concentration (µg/ml)					ED_{50} (µg/ml)
	0	50	100	500	1000	
CC3003						
Hexan	8.02 ± 1.04^{a}	5.03 ± 0.19^{b}	4.41 ± 0.20^{bc}	2.65 ± 0.54^{bcd}	1.48 ± 0.19^{d}	133.60
EtOAc	8.88 ± 2.00^{a}	5.14 ± 0.13^{b}	3.88 ± 0.32^{bcd}	3.07 ± 0.18^{bcd}	1.99 ± 0.15^{cd}	88.39
MeOH	8.68 ± 1.53^{a}	4.84 ± 0.4^{b}	3.79 ± 0.41^{bcd}	3.01 ± 0.33^{bcd}	1.75 ± 0.26^{d}	85.30
CG05						
Hexan	28.60 ± 5.30^{a}	14.78 ± 1.94^{cd}	11.44 ± 1.98^{de}	9.52 ± 1.15^{defg}	5.78 ± 1.05^{efg}	49.32
EtOAc	20.64 ± 4.08^{bc}	12.28 ± 1.72^{de}	9.99 ± 0.19^{def}	5.81 ± 0.83^{efg}	3.28 ± 0.72^{fg}	92.11
MeOH	26.89 ± 4.49^{ab}	14.44 ± 1.64^{cd}	10.11 ± 1.28^{def}	7.74 ± 0.97^{defg}	2.12 ± 0.19^{g}	59.92
CL01						
Hexan	20.61 ± 1.46^{ab}	15.28 ± 0.06^{bcd}	11.27 ± 0.82^{cde}	7.21 ± 0.62^{efg}	1.78 ± 0.34^{g}	151.78
EtOAc	24.89 ± 5.87^{a}	13.58 ± 1.13^{cde}	10.09 ± 1.27^{def}	4.32 ± 1.18^{fg}	2.46 ± 0.49^{g}	62.17
MeOH	24.43 ± 3.81^{a}	17.82 ± 2.86^{bc}	13.11 ± 1.23^{cde}	12.09 ± 1.26^{cde}	1.92 ± 0.62^{g}	174.57

*values are means of four replications ± SE; values in the columns and the rows of three crude extracts within an antagonist followed by the same letters are not significantly different by Duncan's Multiple Range Test at $P = 0.05$

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centration of 50 µg/ml showed a significantly higher number of conidia of NHP-Fusa-2 as compared to that at the concentrations of 500 and 1000 µg/ml, but not to that at the concentration of 100 µg/ml. There was non-significant difference between the concentrations of 100 and 500 µg/ml, and between the concentrations of 500 and 1000 µg/ml (Table 3). This implies that at the concentrations ranging from 50 to 1000 µg/ml, the higher concentrations of the crude extracts were not imperative to express the significantly higher inhibitive efficiency on conidial production of the pathogen.

In the aspect of conidial inhibition of NHP-Fusa-2, there was a non-significant difference in the conidial inhibitory percentage among the three crude extracts at any concentration in the case of all the tested antagonistic fungi (data not shown). However, in comparison of ED_{50}, which could kill 50% conidia of the pathogen, MeOH extract of CC3003 expressed a better level of efficiency than its other crude extracts (ED_{50} value was 85.50 µg/ml). Of which, it was hexane extract with the ED_{50} of 49.32 µg/ml, and EtOAc extract with the ED_{50} of 62.17 µg/ml in the case of CG05 and CL01, respectively (Table 3).

DISCUSSION

The pathogenic fungus causing wilt and root-rot disease of tea was isolated and identified as *Fusarium oxysporum.* The observed symptoms of the infected tea cuttings in the pathogenicity test are similar to those described by Nguyen (2001) and Zeiss and Braber (2001). There were several pathogenic fungi reported as causal agents of root-rot and wilt disease of tea, e.g. *Poria hypolaterite* Berk, *Phellinus* sp., *Sphaerostilbe repens*, *Ganoderma pseudoferreum, Ganoderma philippi, Rosellinia* spp., and *Armillariella mellea* (Chen & Chen 1982; Lehmann-Danzinger 2000; Nguyen 2001; Zeiss & Braber 2001). This is the first report on *F. oxysporum* causing wilt and root-rot disease of tea in Vietnam.

The antifungal activities of *Ch. cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01 against NHP-Fusa-2 were investigated using bi-culture antagonistic test and antifungal crude extract test. The results showed that *Ch. cupreum, Ch. Globosum,* and *Ch. lucknowense* significantly inhibited both mycelial growth and conidial production of NHP-Fusa-2. These results are in accordance with some previous studies which reported that *Ch. cupreum* CC03, *Ch. globosum* N0802, and *Ch. lucknowense* CLT significantly inhibited *F. oxysporum* causing wilt disease of tomato (Charoenporn *et al*. 2010; Sibounnavong *et al*. 2012). The result from the crude extract test suggested that the disease control mechanism of CC3003, CG05, and CL01 against NHP-Fusa-2 involves antibiosis, which was already reported. *Ch. cupreum* CC3003 was stated to produce rotiorinols A–C and rotiorin, which exhibited antifungal activity against *Candida albicans* (Kanokmedhakul *et al.* 2006), *F. oxysporum* f.sp. *lycopersici* (Sibounnavong *et al*. 2012). *Ch. globosum* was shown to produce Chaetomin (Di Pietro *et al*. 1992), Chaetoviridin A, B (Park *et al*. 2005), Chaetoglobosin C (Soytong *et al.* 2001) against various pathogens and in different plant species. *Ch. lucknowense* CLT was reported to produce Chaetoglobosin C against *F. oxysporum* f.sp. *lycopersici* causing wilt of tomato (Charoenporn *et al*. 2010).

Hexane, EtOAc, and MeOH extract in the case of all the tested antagonists did not show significant difference in the conidial inhibitory percentage of the pathogen. It demonstrated that the conidial inhibitory efficiency of these crude extracts against NHP-Fusa-2 is comparable. However, according to the ED_{50} value, in the case of CC3003, MeOH extract was more effective on conidial inhibition of NHP-Fusa-2 than the others; of which it was hexane and EtOAc extract in the case of CG05 and CL01, respectively.

In conclusion, *Fusarium oxysporum* is reported for the first time as Fusarium wilt of tea in Vietnam. *Ch. cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01 could be used as biological agents to control this fungal pathogen. MeOH, hexane, and EtOAc could be used as a solvent to extract antibiotic substances from CC3003, CG05, and CL01, respectively. However, the results proved just by *in vitro* tests should be followed by *in vivo* testing.

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