International Journal of Agricultural Technology 2015 Vol.11(4):975-996
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Testing Bioformulation of *Chaetomium elatum* Che01 to Control Fusarium Wilt of Tomato

Soytong, K.*

Department of Plant Production Technology, Faculty of Agricultural Technology, KMITL, Bangkok 10520, Thailand.

Soytong, K. (2015). Testing bioformulation of *Chaetomium elatum* ChE01 to control Fusarium wilt of tomato. International Journal of Agricultural Technology 11(4):975-996.

Abstract The research findings on tomato wilt collected from infested fields resulted to isolate and identify the causal agent as *Fusarium oxysporum* f. sp. *lycopersici* according to morphological and molecular phylogeny. The antagonistic fungus of *Chaetomium elatum* ChE01 was proved to antagonize *F. oxysporum* f.sp. *lycopersici* . The antagonism test demonstrated the antagonistic activity of Ch. *elatum* ChE01 to inhibit the conidial production of *F. oxysporum* f. sp. *lycopersici*. Bioactivities tests of crude extracts and pure compounds were proved as a control mechanism. All tested crude extracts of *Ch. elatum* ChE01 was significantly inhibited conidia production of *F. oxysporum* f. sp. *lycopersici.* With hexane crude extract at ED_{50} of 0.65 µg/ml and EtOAC crude extract at ED_{50} of 3.39 µg/ml. It is clearly demonstrated that chaetoglobosin-C, a pure compound produced by *Ch. elatum* ChE01 significantly inhibited conidial production of *F. oxysporum* f. sp. *Lycopersici* at ED50 of 5.94 µg/ml. Chaetoglobosin-C is expressed as a antibiotic substances to destroy the pathogen cells implies antibiosis. Inocula of *F. oxysporum* f. sp. *lycopersici* (1 × 107 spores/ml) were mixed with chaetoglobosin-C and inoculated to tomato seedlings caused no symptoms at day 21 while the treatment with pathogen alone showed significantly highest disease severity index. Withthis, no wilt incidences were appeared at all tested concentration of 10, 50 and 100 µg/ml of chaetoglobosin-C. It is stated that chaetoglobosin-C affected directly to the pathogen inocula implies antibiosis which the occurrences of ruptured cells and abnormal conidia of pathogen.
Ch. elatum ChE01 was formulated as powder bioformulations gave a good result to control wilt of tomato var Sida caused by *F. oxysporum* f.sp. *lycopersici*. The treated tomatoes showed the lowest wilt incidence in oil and powder bioformulations from *Ch. elatum* ChE01 which significantly differed from Prochoraz and inoculated control. Based on the result, oil bioformulation from *Ch. elatum* ChE01 gave significantly better plant parameters in terms of plant height, plant weight, root weight, number of fruits and fruit weight than powder bioformulation and Prochoraz when compared to the inoculated control with *F. oxysporum* f. sp *lycopersici*. It is suggested that this new reports of bioformulation of *Ch. elatum* ChE01 could be used as further biofungicide to control tomato wilt caused by *F. oxysporum* f.sp. *lycopersici.*

Keywords: tomato wilt, bioformulation, *Chaetomium elatum*

* **Corresponding author:** Soytong, K.**; Email:** ajkasem@gmail.com

Introduction

A tomato (*Lycopersicon esculentum* Mill.) is one of the most widely cultivated, popular and important vegetable crops in the world. There is increasing demand in developed countries for organic tomatoes, as well as heirloom tomatoes, to make up for flavor and texture in commercial tomatoes. Tomato crop is usually attacked by many kinds of diseases such as Fusarium wilt, bacterial wilt, and early blight (Agrios, 1997). Among these diseases, Fusarium wilt is one of the most serious that can cause serious economic losses. It is caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen. Management of this pathogen is very difficult due to their endophytic growth and persistence in soil (Alström, 2001). In general, this pathogenic fungus is a limiting factor in the production of many crops and accounts for 10 – 20 percent yield losses annually and can reach as high as 100 per cent (USDA, 2008). It has become one of the most prevalent and damaging diseases wherever tomatoes are grown intensively because the pathogen persists indefinitely in infested soils. The methods used to control vascular wilt are either not very efficient or are difficult to apply. The best way is recommended to control the disease would be selected resistant varieties of tomato (Silva and Bettiol, 2005). Tomatoes may develop resistance to their race from pathogenic fungus. In addition, there is a report from United State Department of Agriculture in 2008 that the pathogenic fungus is expected to increase when methyl bromide is no longer available. The pathogen has increased and become resistant to chemical fungicides (Silva and Bettiol, 2005). For this reason, alternative methods with emphasis on biological control using fungi or bacteria in controlling the disease have been studied by several researchers to reduce fungicide application and decrease cost of plant production. Recently, there have been many reports that some species of fungi can be used as source of biological fungicide to control the diseases (Soytong, 1992). The control of most plant diseases is dependent on the use of chemical fungicide because of its effectiveness, reliability, readily available and easy to apply. However, there are several disadvantages of using chemical fungicides. They are toxic not only to humans but also to other forms of life. The price of chemical fungicide is rapidly increasing which is beyonded the reach of ordinary farmers. There are also many reports of environmental pollution due to injudicious application of

fungicides. Applications of chemical fungicides affect the environmental condition and can be harmful to the ecosystem. Because of the problems associated with the use of chemicals there is needed to search for an alternative method of controlling the diseases which is not only effective and economical but also safe to the environment. Recently, the use of biological control of plant pathogens has been concerned to the most plant pathologists and many researchers. 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.,* 2003 and 2006, Thongsri and Soytong, 2004, Srinon *et al.*, 2004, Suwannapong and Soytong, 2002 and Sibounnavong *et al*, 2009ab). The bioactive compound Chaetoglobosin C extracted from *Chaetomium globosum* reported to elicit resistance or immunity in plants by inducing oxidative burst in plant cells (Soytong, *et al.,* 2001).

Materials and methods

Pathogenicity test of Fusarium wilt pathogen

Pure cultures of *F. oxysporum* spp was identified by morphological characteristics and molecular phylogeny from previous works with AFLP procedure.

Pathogenicity tests

F. oxysporum isolate was tested for pathogenicity to tomato seedlings var Sida using Koch's postulates to confirm pathogenic isolates. All isolates were sub-cultured and multiplied on PDA and incubated for 7–10 days at room temperature approximately (30–32 ℃). The inoculum of pathogen was adjusted to1×10^7 spores/ml before inoculating to 20–day–old tomato seedlings var. Sida. The roots of tomato seedlings were washed under running sterilized water and cut at five points on the root tips before dipping the roots into a 20 ml spore suspension for 15 min. A control was performed by dipping seedling roots into

sterile distilled water. The seedlings were then potted in sterilized soil. After 10 days, symptoms of disease were recorded using the Disease Severity Index (DSI) and rated according to Sibounnavong *et al.* (2009, 2010) as follows: 1 = no symptoms, 2 = 1–20% of leaves yellow and wilted, 3 = 21–40% of leaves yellow and wilted, 4 = 41–60 % leaves yellow and wilted, 5 = 61–80% of leaves yellow and wilted, and 6 = 81–100 % of leaves yellow and wilted. The experiment was conducted using a completely randomized design (CRD) with six replications of each treatment. The experiment was repeated twice. Virulence was categorized according to the DSI, following the method used by Charoenporn *et al.* (2010) as follows:- non-pathogenic (DSI =1), low virulence (DSI ≤ 3.50), moderate virulence (DSI > 3.50 – 4.50), and highly virulence (DSI > 4.50).

Bioactivities test against Fusarium oxysporum f sp lycopersici

Crude extracts were assayed for inhibition of *F. oxysporum* f. sp. *lycopersici.* The experiment was conducted by using a factorial experiment in 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℃ (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.

Pure compound bioassay against Fusarium oxysporum f sp. lycopersici

Pure compound, chaetoglobosin-C from *Ch. elatum* is offered from Prof. Dr. Somdej Kanokmedhakul, Khoan Khan Unversity, Thailand. It is tested for antifungal activity against *F. oxysporum* f. sp. *lycopersici*. 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 either pure compound Chaetoglobosin–C in each concentration and incubated at room temperature until the pathogen on the control plates grown over the plate. The experiment was performed using a CRD with four replications. Treatments comprised four different concentrations: 0, 10, 50 and 100 µg/ml. (Fig.3.3). The experiment was repeated twice. Data were collected regarding the number of conidia produced by the pathogen and calculated for percentage conidial inhibition. The ED_{50} was calculated using Probit analysis

Effect of chaetoglobosin-C from Ch. elatum to Fusarium oxysporum f. sp. lycopersici

The roots of 20– day– old tomato seedlings var. Sida were washed under running sterilized water and cut at five points on the root tips before dipping the roots into each treatment a 20 ml spore suspension of *F. oxysporum* f. sp. *lycopersici* at 1×107 spores/ml mixed with different concentration of pure compounds for 15 min. The experiment was conducted by using CRD with four replications. Treatments were chaetoglobosin-C from *Ch. elatum* in various concentrations as follows:- 0 (control), 10, 50, and 100 µg/ml. A

control was performed by dipping seedling roots into sterile distilled water. The seedlings were then planted in pots which contained sterilized soil. The experiment was repeated twice.

Effect of metabolites for disease immunity of wilt incidence in tomato var Sida

The experiment was conducted by using a CRD with four replications. Treatments were conducted as follows: T1= control; non-inoculated with conidia of *F. oxysporum* f. sp. *lycopersici*; T2= control; inoculated with conidia of the *F. oxysporum* f. sp. lycopersici; T3= inoculated with *F. oxysporum* f. sp. *lycopersici* mixed with 500 µg/ml of the most effective crude extract and T4= inoculated with *F. oxysporum* f. sp. *lycopersici* mixed with 1000 µg/ml of the most effective crude extract. The roots of 20– day– old tomato seedlings var. Sida were washed under running sterilized water and cut at five points on the root tips before dipping the roots into each treatment. A 20 ml spore suspension of 1 ×107 spores/ml mixed with different concentrations of crude extract from Ch. *elatum* for 15 min. The seedlings were then planted in pots which contained sterilized soil. The experiment was repeated twice. DSI was scored as previous experiment and disease immunity (%) was computed as follows:- DSI in control – DSI in treatment/ DSI in control X 100.

Evaluation of Bio-agent formulations to control Fusarium wilt of tomato in vivo

Chaetomium elatum ChE01 is formulated as bioformulation and evaluate its efficacy to control Fusarium wilt of tomato var Sida in pot experiment. The biological fungicide or bioformulation were formulated as powder and oil formulations from *Ch. elatum* ChE01 which modified from the work of Soytong (2001). Soil preparation was prepared as soil mixture, sand :

compost at the ratio of 8:2:2 (vol/vol/vol) before autoclaving at121C, 15 lbs/inch2 for 2 hours then put into pot for each treatment before planting the inoculated tomato seedlings. Sida variety was used in this experiment.

Tomato seedlings at 20 days were used in the experiment. The root tip of tomato were cleaned by running off water then the roots were cut for 2 cm at 5 points and dipped in the pathogen inoculum suspension for 10 min before transplanting into sterilized soil in pot experiment. After transplanting the tomato seedlings were treated every 10 days for each treatment until harvesting. The treatments were done as follows:-T1 = Control, non-inoculated with pathogen and non-treated, T 2 = Control, inoculated with pathogen and non-treated with *Ch. elatum* ChE01, T 3 = Inoculated with pathogen and treated with powder formulation of *Ch. elatum ChE01* (A) at the rate of 20g/20L of water, T 4 = Inoculated with pathogen and treated with oil formulation of *Ch. elatum* ChE01(A) at the rate of 20 ml/20L of water, T 5 = Inoculated with pathogen and treated with powder formulation of *Ch. elatum* ChE01 (B) at the rate of 20 ml/20L of water, T6 = Inoculated with pathogen and treated with oil formulation of *Ch. elatum* ChE01 (B) at the rate of 20 ml/20L of water, T7 = Inoculated with pathogen and treated with powder formulation of *Ch. elatum* ChE01 (C) at the rate of 20 ml/20L of water and T8 = Inoculated with pathogen and treated with oil formulation of *Ch. elatum* ChE01 (C) at the rate of 20 ml/20L of water.

The experimental design was conducted by using Randomized Completely Block Design (RCBD) with four replications. The experiment was repeated two times. Data were collected as plant height (cm), plant weight (g), number of fruits/plant, day of flowering bloom, weight of fruit (g) were collected. Diseased Index (DI) was observed and rated every 30 days after inoculation based on a diseased rating scale. Disease index was scored by the

method of Sibounnavong et al (2010). The percent disease reduction (%DR) was determined using the formula as follows:- A-B/A X 100, where A = score of disease index rating from control treatment inoculated with pathogen and B = score of disease index rating from treatment applied with biofungicides

Data were statistically analyzed for significant differences using analysis of variance (ANOVA). Comparison among treatment mean were computed using Duncan Multiple Range Test at P=0.05 and P=0.01.The experiment was repeated two times.

Results

Disease sample was isolated to get pure culture of *Fusarium oxysporum* f. sp. *lycopersici* and proved for its pathogenicity (Fig.1).

Figure 1. *Fusarium oxysporum f. sp. lycopersici*

Bioactivities test against Fusarium oxysporum f sp lycopersici

Chaetomium elatum ChE01 at different concentrations of 0, 10, 50, 100, 500, and 1,000 g/ml were tested for inhibition of *F. oxysporum* f. sp. *lycopersici* which obtained from previous experiment. Hexane crude extract from *Ch. elatum* ChE01at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici*

which were 4.12, 4.02, 3.92, 3.27 and 3.12 cm, respectively when compared to the control (0 µg/ml) of 5.00 cm. EtOAc crude extract from *Ch. elatum* ChE01at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* which were 3.80, 3.35, 3.19, 2.55 and 2.44 cm, respectively when compared to the control (0 µg/ml) of 5.00 cm. MeOH crude extract from *Ch. elatum* ChE01at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* which were 4.75, 4.12, 4.04, 3.90 and 3.80 cm, respectively when compared to the control (0 µg/ml) of 5.00 cm (Table1). Crude extract at 1000 µg/ml from EtOAc of *Ch. elatum* ChE01was significantly better inhibited the colony growth of *Fusarium oxysporum* f.sp. *lycopersici* as 51.0 % better than crude extracts from hexane and MEOH which were 37.5 and 24.0 %, respectively (Table 2).

Table 1. Effect of crude extracts from *Chaetomium elatum* ChE01 on mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici*

Crude extracts	**Colony diameter (cm) of *Fusarium oxysporum* f.sp. *lycopersici* at each concentration (µg/ml))**					
	0	10	50	100	500	1000
Hexane	5a	4.12b	4.02bc	3.92bc	3.27d	3.12d
EtOAc	5a	3.80c	3.35d	3.19d	2.55e	2.44e
MeOH	5a	4.75a	4.12b	4.04bc	3.90bc	3.80c

[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 *Chaetomium elatum* ChE01 for percentage of colony inhibition growth of *Fusarium oxysporum* f.sp. *lycopersici*

Crude extracts of	**Colony inhibition of *Fusarium oxysporum* f.sp. *lycopersici* (%)**				
	10	50	100	500	1000
Hexane	7.5d	19.5cd	21.5cd	34.5b	37.5b
EtOAc	24.0c	33.0b	37.0b	49.0a	51.0a
MeOH	5.0e	17.5d	19.0cd	22.0cd	24.0c

[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. elatum* ChE01at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* which were 13.79 $x10^7$, 11.83 $x10^7$, 8.75 $x10^7$, 7.76 $x10^7$and 5.90 $x10^7$, respectively when compared to the control (0 µg/ml) of 37.81 $x10^7$. EtOAc crude extract from *Ch. elatum* ChE01at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* which were 13.07 $x10^7$, 10.82 $x10^7$, 4.64 $x10^7$, 2.89 $x10^7$ and 1.65 $x10^7$, respectively when compared to the control (0 µg/ml) of 37.68 $x10^7$. MeOH crude extract from *Ch. elatum* ChE01 at the concentrations of 10, 50, 100, 500 and 1000 µg/ml gave significantly different in colony diameter of *F. oxysporum* f. sp. *lycopersici* which were 29.03 $x10^7$, 23.00 $x10^7$, 12.84 $x10^7$, 9.06 $x10^7$and 7.54 $x10^7$, respectively when compared to the control (0 µg/ml) of 38.62 $x10^7$ (Table 3). Crude extract at 1000 µg/ml from EtOAc of *Ch. elatum* ChE01gave significantly better inhibited the colony growth of *F. oxysporum* f.sp. *lycopersici* as 95.10 % better than crude extracts from hexane and MeOH which were 83.85 and 80.44 %, respectively (Table 4).

Table 3. Effect of crude extracts from *Chaetomium elatum* ChE01 against conidia production of *Fusarium oxysporum* f.sp. *lycopersici*

Crude extracts	**Number of conidia(x10^7) of *Fusarium oxysporum* f.sp. *lycopersici* at each concentration (µg/ml)**					
	0	10	50	100	500	1000
Hexane	37.81a	13.79d	11.83ef	8.75gh	7.76h	5.90i
EtOAc	37.68a	13.07d	10.82f	4.64j	2.89k	1.65l
MeOH	38.62a	29.03b	23.00c	12.84de	9.06g	7.54h

[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 4. Effect of crude extracts from *Chaetomium elatum* ChE01 for percentage of conidia inhibition of *Fusarium oxysporum* f.sp. *lycopersici*

Crude extracts	**Conidia inhibition of *Fusarium oxysporum* f.sp. *lycopersici* (%)**					
	10	50	100	500	1000	ED_{50}
Hexane	63.46j	68.67gh	76.35f	78.87ef	83.85d	0.65
EtOAc	65.30ij	71.26g	87.65c	92.29b	95.61a	3.39
MeOH	24.76l	40.43k	66.69hi	76.52f	80.44e	63.42

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

Pure compound bioassay against Fusarium oxysporum f sp lycopersici

Chaetoglobosin-C from *Ch. elatum* ChE01 was offered by Professor Dr. Somdej Kanokmedhakul, Department of Chemistry, Faculty of Sceince, Khon Khan University (Figure 2). Chaetoglobosin-C, a pure compound produced by *Ch. elatum* ChE01 inhibited conidia production of *F. oxysporum* f. sp. *lycopersici* with the ED_{50} value of 5.94 µg/ml (Table 5).

Table 5. Assay of bioactive compound against *Fusarium oxysporum* f. sp. *lycopersici*

Pure compound	Inhibition of conidia production (%)[1]	ED_{50} µg/ml
Chaetoglobosin-C	89.00	5.94

[1]Inhibition (%) = average number of conidia in control plate – average number of conidia in treated plate/ average number of conidia in control plate X 100.

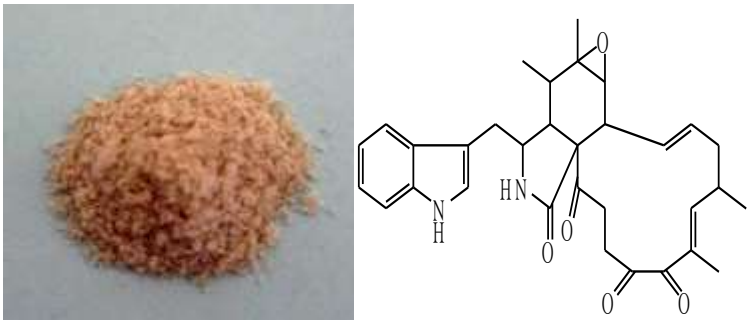

Figure 2. A pure compound of chaetoglobosin-C

Effect of fungal metabolites to Fusarium oxysporum f. sp. lycopersici

Inoculum of *F. oxysporum* f. sp. *lycopersici* (1×10^7 spores/ml) treated with pure compounds of chaetoglobosin-C inoculating to tomato seedlings caused no symptoms at 21 days while the treatment with pathogen alone showed significantly highest disease severity index. No disease incidences were appeared at all tested concentration of 10, 50 and 100 µg/ml of chaetoglobosin-C which significantly differed from the control. It revealed that the antibiotic substances of chaetoglobosin-C affected directly to the pathogen conidial inocula which implies antibiosis mechanism of control. Moreover, the occurrences of ruptured cells and abnormal conidia thereafter mixing with each

pure compound of chaetoglobosin-C is observed under the microscope (Fig. 3; Table 6).

Chaetoglobosin-C Normal conidia Abnormal conidia

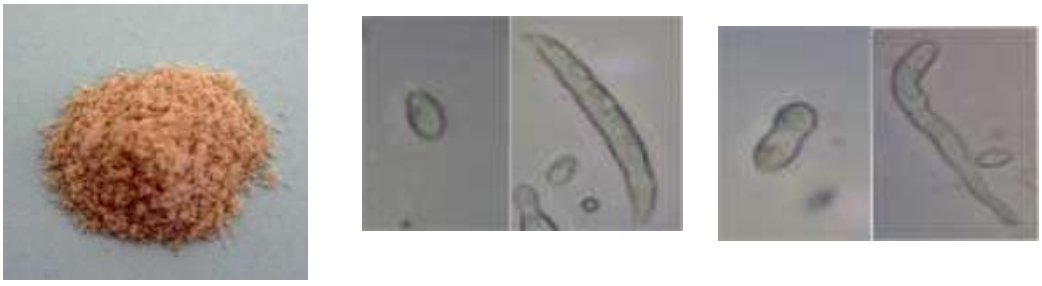

Figure 3. Abnormal conidial lysis of *F. oxysporum* f. sp. *lycopersici* owing to chaetoglobosin-C

Table 6. Effect of fungal metabolites to *Fusarium oxysporum* f. sp. *lycopersici* and its pathogenicity loss

Pure compounds	**Concentrations µg ml^{-1}**	**DSI[1]**
	0	6.00a[2]
	10	1.00b
Chaetoglobosin-C	50	1.00b
	100	1.00b

[1]Tomato plants were assessed for disease symptoms 21 days after inoculation using theDisease Severity Index (DSI): 1 = no symptoms; 2 = plant showed 1–20% yellowing leaves and wilting, 3 = plant showed 21–40% yellowing leaves and wilting, 4 = plant showed 41–60% yellowing leaves and wilting, 5 = plant showed 61–80% yellowing leaves and wilting, and 6 = plant showed 81–100% yellowing leaves and wilting or death.

[2]Average of four replications. Means with the same common letters in each column were not significantly different according to Duncan's multiple range test at $p = 0.01$.

Testing bioformulation of Chaetomium elatum ChE01to control Fusarium wilt of tomato

The disease severity index (DSI) of Fusarium wilt was lowest wilt incidence in oil and powder bioformulations of *Ch. elatum* ChE01 (DSI 1.75 and 2.00) and followed by culture filtrate (DSI 2.5) which significantly differed from Prochoraz (DSI 3.25) and inoculated control(DSI 4.75). The non inoculated control was no wilt incidence. With this, application of oil bioformulation leaded to reduce wilt incidence and followed by application of powder bioformulation, culture filtrate and Prochoraz which also reduced wilt incidence. Based on the result, oil bioformulation gave significantly highest in plant height (125cm) and followed by powder bioformulation, culture filtrate and Prochoraz which were 105.75, 100.50 and 87.50 cm, respectively when compared to the inoculated control (75.75 cm). Plant weight showed the highest after apply oil bioformulation (184.25 g), and followed by powder formulation, culture filtrate and Prochoraz which were 169.25,151.00 and 134.25 g, respectively when compared to the inoculated control (73.25 g). With this regards, the root weights of oil and powder bioformulations gave significantly better than culture filtrate and Prochoraz treatments. Oil bioformulation gave significantly highest in fruit weight (327.5 g) and followed by powder bioformulation (279 g), culture filtrate (217.5 g) and Prochoraz(172 g) which significanyly differed from the inoculated control (185 g). The number of fruits in oil bioformulation application was 21.5 fruits/plant which gave significantly higher than powder bioformulation (17.25 fruits/plant) , culture filtrate (16 fruits/plant) and Prochoraz (11.75 fruits/plant) treatments which significantly differed from the inoculated control(11.75 fruits/plant) as seen in Tables 7, 8 anf Fig.4).

Table 7. Testing bio-agent formulation of *Chaetomium elatum* ChE01 to control Fusarium wilt of tomato var Sida in pot experiment

Treatments	**Plant height (cm)**	**Plant weight (g)**	**Root Weight (g)**	**Fruit weight (g)**	**fruits/plant**	**DSI[1]**
non inoculated control	100.50bc[1]	166.25b	10.25b	229.00c	15.00b	1.00d
inoculated control	75.75d	73.25e	5.37c	185.00cd	11.75c	4.75a
powder bioformulation	105.75b	169.25b	13.75b	279.00b	17.25b	2.25c
Oil bioformulation	125.00a	184.25a	24.25a	327.50a	21.50a	2.25c
culture filtrate	100.50bc	151.00c	10.75b	217.50cd	16.00b	3.25bc
Prochoraz	87.50cd	134.25d	10.25b	172.00d	11.75c	3.50b
CV(%)						

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

Table 8. Percent increased in plant growth and disease reduction after apply bio-agent formulation of *Chaetomium elatum* ChE01

Treatments	**Plant height**	**Plant weight**	**Root weight**	**Fruit weight**	**Numbers of fruit/plant**	**DR[2]**
powder bioformulation	28.83	56.72	60.94	33.69	31.88	46.31
Oil bioformulation	39.40	60.24	77.85	43.51	45.34	46.31
culture filtrate	24.62	51.49	50.04	14.94	26.75	31.57
Prochoraz	13.42	45.43	47.60	00.00	0.00	26.31

[1]Increased in plant growth parameters = treatment– inoculated control /treatment X 100.

[2]Disease reduction (DR) = disease index of inoculated control - disease index of treatment/disease index of inoculated control X 100.

Figure 4. Testing bioformulation of *Chaetomium elatum* ChE01to control Fusarium wilt of tomato

Discussion

The pathogenicity tests performed on tomato seedlings in this study showed that the *F. oxysporum* f. sp. *lycopersici* was aggressive islate as similar work reported by Charoenporn *et al*. (2010). This observation is supported by *in vitro* studies of virulence by Soytong *et al*. (2001), Sibounnavong *et al*. (2009. *F. oxysporum* f. sp. *lycopersici* was confirmed morphologically and based on molecular phylogeny. Charoenporn *et al*. (2010) reported that some isolates were low virulent to cause wilt of tomato var. Sida. It can explain that the

different varieties of tomatoes may affect to pathogenicity level of wilt disease infected by same isolate of *F. oxysporum* f. sp. *lycopersici* (Cai 2003).

Ch. elatum ChE01 was proved to antagonize *F. oxysporum* f.sp. *lycopersici*. The antagonism test demonstrated the antagonistic activity of *Chaetomium elatum* ChE01 to inhibit the conidial production of *F. oxysporum* f. sp. *lycopersici*. The result was in accordance with Charoenpoen *et al* (2010) reported that *Ch lucknowense* CLT significantly inhibited the mycelia growth and conidial production of *F. oxysporum* f. sp. *lycopersici* as 88.89 and 92.54 %, respectively.

Bioactivities tests of crude extracts and pure compounds from antagonistic fungi were also proved as a control mechanism. To elucidate the control mechanism involved in the inhibition of *F. oxysporum* f. sp. *lycopersici* , crude extracts of *Chaetomium elatum* ChE01, were confirm for antifungal activity against of *F. oxysporum* f. sp. *lycopersici*. The other control mechanism of *Chaetomium elatum* ChE01 involved in releasing antibiotic substances to inhibit *F. oxysporum* f. sp. *lycopersici*.

It is clearly demonstrated that chaetoglobosin-C, a pure compound produced by *Ch. elatum* ChE01 compounds significantly inhibited conidial production of *F. oxysporum* f. sp. *lycopersici* with the ED_{50} of 5.94 µg/ml. It is suggested that chaetoglobosin-C expressed as a antibiotic substances to destroy the pathogen cells implies antibiosis. As previously reported by Soytong *et al*. (2001), chaetoglobosin-C from *Ch. globosum* inhibited several plant pathogens including *F. oxysporum* f. sp. *lycopersici*. Thohinung et al. (2010) also reported that *Ch. elatum* ChE01 produce chaetoglobosin-C that showed cytotoxicity against the human breast cancer and cholangiocarcinoma cell lines. Inocula of *F. oxysporum* f. sp. *lycopersici* (1×10^7 spores/ml) were treated with pure compounds of chaetoglobosin-C and inoculated to tomato seedlings caused no

symptoms at day 21 while the treatment with pathogen alone showed significantly highest disease severity index . With this, no wilt incidences were appeared at all tested concentration of 10, 50 and 100 µg/ml of either chaetoglobosin-C. It is stated that chaetoglobosin-C affected directly to the pathogen inocula implies antibiosis which the occurrences of ruptured cells and abnormal conidia of pathogen. It is concluded that *Ch. elatum* ChE01, are confirmed to produce chaetoglobosin-C. In this study, these compounds exhibited antifungal activity against *F. oxysporum* f. sp. *lycopersici* at low concentration. In addition, Park *et al.* (2005) reported thatchaetoviridin-A purified from *Ch. globosum* F0142 exhibited moderate control of tomato late blight at 125 µg/ml. Chaetoglobosin-C, which produced by *Ch. elatum* ChE01, was not only shown to exhibit cytotoxicity against the human pathogens (Thohinung *et al.,* 2010) but also inhibited the tomato wilt pathogen; *F. oxysporum* f. sp. *lycopersici* in this study. Moreover, this study demonstrated that chaetoglobosin-C mixed in a solution with pathogen cells of *F. oxysporum* f. sp. *lycopersici* caused cells ruptured and abnormal conidia. It is suggested that these pure compounds can lyse the cell wall of the pathogen and the protoplast becomes a plug inside the cells. These observations were similar to those reported by Sibounnavong *et al*. (2009) and Soytong (1992) who showed that the crude extracts of these antagonists ruptured the cells of the *F. oxysporum* f. sp. *lycopersici* inoculums. In this study, the abnormal conidia of pathogen cells affected by chaetoglobosin-C leading to loss of its pathogenicity when inoculated to tomato seedlings var Sida and no symptoms were observed.

The biological fungicides has been released and distributed to the growers over a decade. Kaewchai *et al.* (2009) stated that mycofungicides have been promoted for agricultural use because of their ability to control plant diseases and to increase crop production in an environmental friendly manner.

The registered biological fungicide formulated from *C. cupreum* in Thailand could decrease disease incidence of tomato wilt and also increased in yield (Soytong, 1992). In this study, Fusarium wilt was lowest wilt incidence in oil and powder bioformulations from *Chaetomium elatum* ChE01, which significantly differed from Prochoraz and inoculated control. As comparison to the work of Charoenporn *et al.* (2010) reported that oil bio-agent formulation from the other antagonistic fungi of *Chaetomium globosum* and *Ch. lucknowense* also showed their biological ability to control tomato wilt.. However, bioformulation from *Ch. elatum* ChE01, this research finding revealed a good result to control wilt incidence of tomato caused by *F. oxysporum* f. sp *lycopersici.* This result is similar to the report of Charoenporn *et al.* (2010) stated that all tested bio-agent formulations of antagonistic fungi; *Ch. globosum* and *Ch. lucknowense* could significantly reduce tomato wilt caused by *F. oxysporum* f. sp *lycopersici* and increase in yield of tomato when compared to prochoraz and inoculated control. Soytong et al. (2001) showed that the biological products consist of *Chaetomium* sp. (22 strains of *C. cupreum* and *C. globosum*) in biopellet and biopowder formulations which when applied to the soil could suppress the growth of *F. oxysporum* f.sp. *lycopersici* and reduce infection rate in tomato and those bioproducts has been released to the market. It is suggested that this new reports of bioformulation of *Ch. elatum* ChE01, could be used for further biofungicide to control tomato wilt caused by *F. oxysporum* f.sp. *lycopersici*.

References

Agrios, G. N. (1997). Plant Pathology 4th edition. San Diego: Academic Press.

Charoenporn, C., Kanokmedhakul, S., Lin, F. C., Poeaim, S. and Soytong, K. (2010). Evaluation of bio-agent formulations to control *Fusarium* wilt of tomato. African Journal of Biotechnology 9:5836-5844.

Meepeung, W. and Soytong, K. (2004). Testing crude extract mixture form microbial antagonists to control plant pathogens of lime. Proceeding of The 1st KMILT International Conference on Integration of Science and Technology for Sustainable Development, KMITL, Bangkok, Thailand, 25-26 August 2004. 2:229-231.

Moosophon, P., Kanokmedhakul, S., Kanokmedhakul, K. and Soytong, K. (2009). Prenylxanthones and a bicycle (3.3.1) nona-2,6-diene derive from the fungus *Emericella rugulosa*. Journal of Natural Products 72:1442-1446.

Sibounnavong, P., Soytong, K., Divina, C. C. and Kalaw, S. (2009). *In vitro* biological activities of *Emericella nidulans,* a new fungal antagonist, against *Fusairium oxysporum* f. sp. *lycopersici.* International Journal of Agricultural Technology 5:75-84.

Sibounnavong, P., Keoudone, C., Soytong, K., Divina, C. C. and Kalaw. S. P. (2010). A new mycofungicide *Emericella nidulans* against tomato wilt caused by *Fusarium oxysporum* f sp *lycopersici*. International Journal of Agricultural Technology 6:19-30.

Sibounnavong, P., Chaoenporn, C., Kanokmedhakul, S., and Soytong, K. (2012). Antifungal metabolites from antagonistic fungi used to control tomato wilt fungus, *Fusarium oxysporum* f sp *lycopersici*. African Journal of Biotechnology 10:19714-19722.

Sibounnavong, P., Soytong, K., Davina, C. C. and Kalaw, S. P. (2009). *In vitro* biological activities of *Emericella nidulans* a new fungal antagonist, against *Fusarium oxysporum* f. sp. *lycopersici.* Journal of Agricultural Technology 5:75-84.

Soytong, K. and Ratanacherdchai, K. (2005). Application of mycofungicide to control late blight of potato. Journal Agricultural Technology 1:19-32.

Soytong, K., Srinon, W., Ratanacherdchai, K., Kanokmedhakul, S. and Kanokmedhakul, K. (2005). Application of antagonistic fungi to control anthracnose disease of grape. Journal Agricultural Technology 1:33-42.

Soytong, K., Kanokmedhakul, S., Kanokmedhakul, K. and Suksamrarn, A. (2007). Bioactive compounds from *Chaetomium cupreum*, *C. globosum* and *Trichoderma harzianum* for plant disease control and plant growth stimulant. International conference on Engineering, Applied Sciences and Technology, Bangkok, Thailand, 21-23 November 2007. 46 pp.

Soytong, K., Kanokmadhakul, S., Kukongviriyapa, V. and Isobe, M. (2001). Application of *Chaetomium* species (Ketomium®) as a new broad spectrum biological fungicide for plant disease control: A review article. Fungal Diversity 7:1-15.

Srinon, W., Chuncheen, K., Jirattiwarutkul, K., Soytong, K. and Kanokmedhakul, S. (2006). Efficacies of antagonistic fungi against *Fusarium* wilt disease of cucumber and tomato and the assay of its enzyme activity. Journal of Agricultural Technology 2:191-201.

Suwan, S., Isobe, M., Kanokmedhakul, S., Lourit, N., Kanokmedhakul, K., Soytong, K. and Koga, K. (2000). Elucidation of high micro-heterogencity of an acidic-neutral trichotoxin mixture from *Trichoderma harzianum* PC01 by electrospray ionization quadrupole time-of-flight mass spectrometry. Journal of Mass Spectrometry 35:1438-1451.

Silva, J. C. and Bettiol, W. (2005). Potential of non-pathogenic Fusarium oxysporum isolates for control of Fusarium wilt of tomato, Fitopatologia Braileira 30:409-412.

Talubnak, C. and Soytong, K. (2010). Biological control of Vanilla anthracnose using *Emericella nidulans*. Journal of Agricultural Technology 6:47-55.

Thongsri, V. and Soytong, K. (2004). A study on Nigrospora sp. strain L-03, a new potential antagonist to plant pathogenic fungi. Proceeding of The 1st KMILT International Conference on Integration of Science and Technology for Sustainable Development, KMITL, Bangkok, Thailand. 25-26 August 2004. 2:25-29.

Thohinung, S., Kanokmedhakul, S., Kanokmedhakul, K., Kukongviriyapan, V., Tusskorn, O. and Soytong, K. (2010). Cytotoxic 10-(indol-3-yl)-[13]cytochalasans from the fungus *Chaetomium elatum* ChE01. Archives of Pharmacal Research 33:1135-1141.

(Received: 30 March 2015, accepted: 25 April 2015)