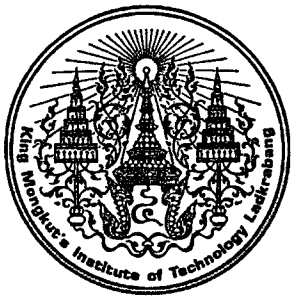


Graduate Studies

Faculty of Agricultural Technology, King Mongkut's Institute Technology Ladkrabang, Bangkok, Thailand

PROGRESS REPORT

The period of *Oct, 2014 – July, 2015*

The research project: Biological control tea pathogen by *Chaetomium* spp. *in vitro* test

Principal investigator: Assist. Prof. Dr. WATANACHAI PONGNAK

Research assistant: NGUYEN HUU PHONG

Department of Plant Production Technology – Faculty of Agricultural Technology – KMITL, Bangkok, Thailand

I. INTRODUCTION

The tea tree, *Camellia sinensis* (L.).O. Kuntze, is thought to have originated within the fan-shaped area extending from Assam/Burma border in the West to China in the East, and in the South from this line through Burma and Thailand to Vietnam (Kingdon-Ward, 1950). From the main center of cultivation in South East Asia, tea has been introduced into many other areas of the world and is now grown in condition which range from Mediterranean-type climate to the hot humid tropics (Carr, 1972). Nowadays, there are 46 countries planting tea tree all over the world. The main tea producing countries globally are: Burundi, Kenya, Malawi, Rwanda, Tanzania, Uganda, Zimbabwe...in Africa; Argentina, Brazil...in South America; Iran and Turkey in Near East; Bangladesh, China, India, Indonesia, Sri Lanka, Vietnam...in Asia (FAOSTAT data, 2011). Depending on the manufacturing technique, tea products may be described as many kind of tea such as green, black, oolong, white, yellow and even compressed tea (Alastair Hick, 2009).

Tea is one of the most popular and lowest cost beverages in the world (Majumder *et al.*, 2012), next only to water and, consumed by a wide range of age groups in all levels of society, with more than three billion cups of tea that are consumed daily worldwide (Alastair Hick, 2009). Tea is considered to be a part of the huge beverage market and be also of interest to functional foods markets (Alastair Hick, 2009), and has been one of the main agriculture export items for many developing countries. In addition to cultivation, tea picking and processing have provided job opportunities to millions of people in tea growing countries. For this reason, not only as an economic sector but also due to its social dimension, tea sector have been vital for these countries (Işil Alkan et al., 2009).

II. OBJECTIVES OF THE STUDY

1. To continue evaluating the antagonistic ability of *Chaetomium* spp. to control *Pestalotia* spp. and *Fusarium oxysporum* causing leaf spot disease and root rots disease, respectively, in tea

2. To continue testing bioactive substances from *Chaetomium* spp. to control *Pestalotia* spp. and *Fusarium oxysporum* causing leaf spot disease and root rots disease, respectively, in tea.

III. MATERIALS AND METHOD

During this period, all the work has been done as below:

- Two antagonists, viz. *Chaetomium globosum* and *Chaetomium lucknowense* were tested for their antagonistic ability against tea pathogen, *Fusarium oxysporum* in *vitro* test.

- The antibiotic substances that were extracted from three antagonist, viz. *Chaetomium cupreum, Chaetomium globosum* and *Chaetomium lucknowense* were tested for their antagonistic ability against tea pathogen, *Pestalotia* spp. in *vitro* test.

The materials and methods were applied as follow:

Part I: Bi-culture antagonistic test and Biological active substances test

The tea pathogens such as *Pestalotia* spp. that causes leaf spot disease and *Fusarium oxysporum* that causes root rot disease were isloated from susceptible tea variety of Vietnam and maintain in PDA and, confirmed by Assoc. Prof. Dr. Kasem Soytong, from Mycology and Bio-control Unit, Faculty of Agricultural Technology, King Mongkut's Institute of

Technology Ladkrabang (KMITL), Bangkok, Thailand. The isolates of *Pestalotia* spp. and *Fusarium oxysproum* had been proved for their pathogenic ability

The antagonists namely *Chaetomium cupreum* CC3003, *Chaetomium globosum* CG05, and *Chaetomium lucknowenese* CL01 were offered from Assoc. Prof. Dr. Kasem Soytong, Mycology and Biocontrol Unit, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Bi-culture antagonistic test was performed in Completely Randomized Design (CRD) with 4 replications. The test was conducted using the methods of Soytong (1992) and Charoenporn *et al.* (2010).

The antagonistic fungi and pathogen was separately cultured on PDA, then incubated at room temperature for seven days before used. A 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of cultures of the pathogenic and of antagonistic fungus. The agar plugs were then transferred onto the same sterilized 9 cm-diameter PDA plates, an agar plug of the pathogen was placed on one side of the plate which opposite an agar plug of the antagonistic fungus. Two separate PDA plates that were transferred with a single plug of the antagonistic fungus and single plug of the pathogen acted as the controls.

Abnormal spores and nornal spores of pathogen from each treatment were observed under compound microscope and take phoyograph for comparison.

Data were collected including colony diameter (cm) and the number of spore produced by the pathogen. The number of spore were counted using haemocytometer. Percentage inhibition of mycelial growth or spore production of pathogen were transformed according to the following formula:

% inhibition = [(colony diameter or spore production of pathogen in control plate - colony diameter or spore production of pathogen in bi-culture plate) x 100]/colony diameter or spore production of pathogen in control plate

Colony diameter and spore production were statistically computed analysis of variance, the treatment means will be compared using Duncan's Multiple Range Test (DMRT) at p = 0.05 and 0.01.

Part II: Biological active substances test

Method of crude extraction:

Crude extracts from *Chaetomium cupreum, Chaetomium globosum* and *Chaetomium lucknownese* were done by following the method of Kanokmedhakul *et al.* (2006). The fungi were cultivated in potato dextrose then stored at room temperature for 35 days before used, then fresh fungal biomass of antagonist were havested. The fresh fungal biomass were air dried for 3-5 days to get dry fungal biomass, then the dried fungal biomass were serial extracted by soaked in hexane, ethyl acetate and methanol respectively to get filtrates. The filtrates were then evaporated in a rotary vacuum evaporator to yield crude hexane, crude ethyl acetate (EtOAc), and crude methanol (MeOH) extracts, respectively.

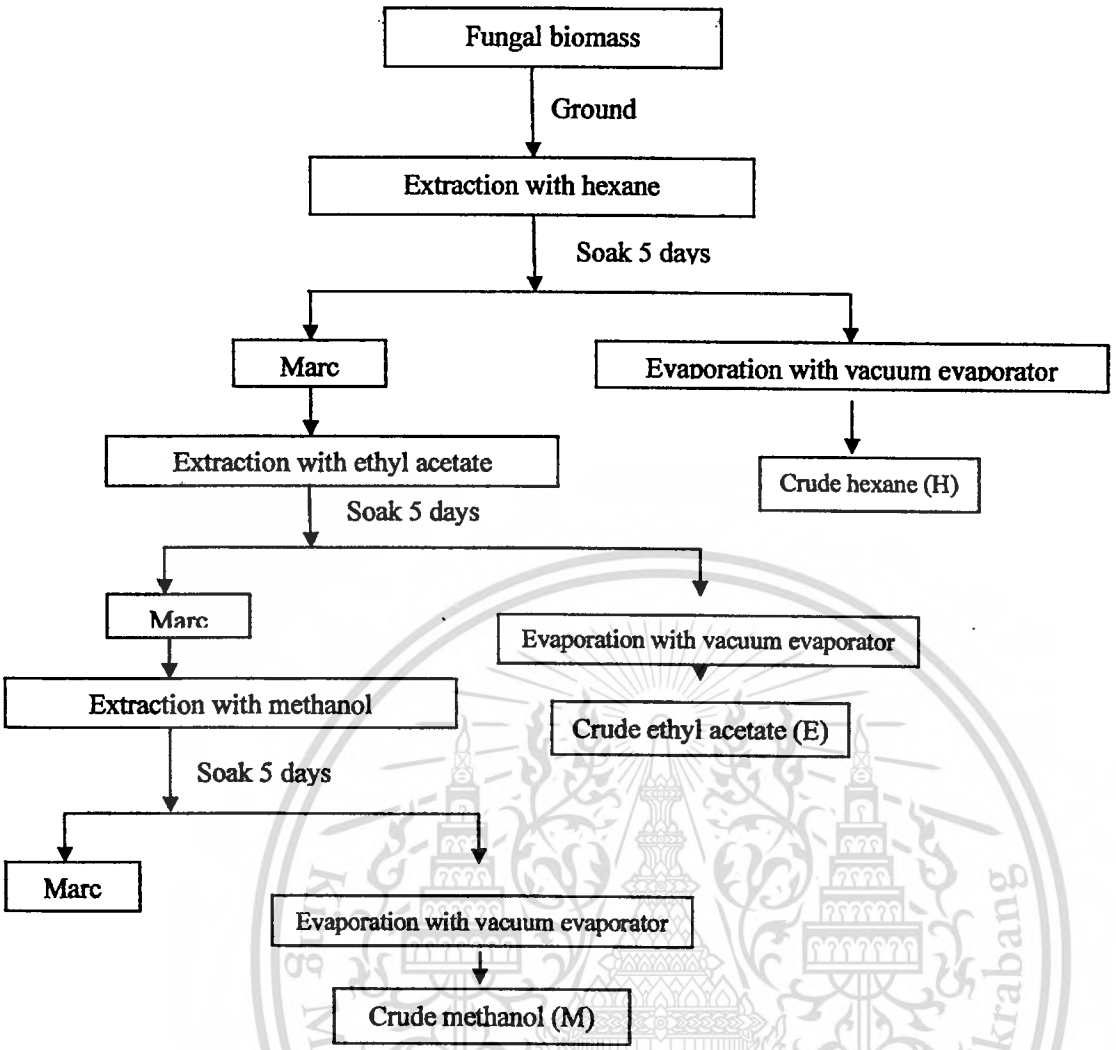


Fig.1. Chart of the extraction methods Crude extracts

Biological active substances from *Chaetomium* to control tea pathogen:

The crude extracts of antagonists were tested for inhibition of the pathogen. The experiment was conducted by using the two factorial experiment in CRD with four replications.

Factor A was represented the crude extracts:

A1 = crude hexane extract

A2 = crude ethyl acetate extract

A3 = crude methanol extract

Factor B was represented the different concentrations:

B1 = 0 µg/ml (control)

B2 = 50 µg/ml

B3 = 100 µg/ml

B4 = 500 µg/ml

B5 = 1,000 µg/ml

Each crude extract were dissolved in 2% dimethyl sulfoxide and were added to PDA before autoclaving at 121°C (15 psi) for 20 minutes. A sterilized 3-mm diameter cork borer was used to transfer 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 were incubated at room temperature until the colony of the pathogen on the control plates reach to the rim of the petri dish.

Abnormal spores and nornal spores of pathogen from each treatment were observed under compound microscope and take phoyograph for comparison.

Data were collected regarding the number of spore produced by the pathogen and used to calculate the percentage of conidia inhibition. The effective dose (ED_{50}) was calculated using probit analysis.

IV. RESULTS AND DISCUSSION

4.1. Bi-culture antagonistic test

The antagonist fungi including *Ch.globosum* and *Ch.lucknowense* were proved their abilities to inhibit the growth *Fusarium oxysporum*. Antagonistic test was conducted by using Biculture test. The colony diameter and spore production of pathogen were chosen to be parameters for evaluation.

Table 1. Effect of antagonists on mycelia growth of *Fusarium oxysporum*

Treatments	*Fusarium oxysporum*	
	Colony (cm)	% Inhibition of colony
Control	9.00[a1/]	
Ch.globosum vs *F.oxysporum*	5.94[b]	34.03[a]
Ch.lucnowens vs *F.oxysporum*	6.06[b]	32.64[a]

1/ Average of four replications, means followed by the same letter in a column were not significantly different by DMRT at P = 0.01 followed by P=0.05.

The Table 1 showed that *Ch.globosum* and *Ch.lucknowense* significantly inhibited colony growth of *F.oxysporum*. causing wilt of root-rot disease of tea which were 5.94 and 6.06 cm, respectively when compared to the control plate (9.00 cm). However, there were no significance differnece in the inhibitive percentage of the colony growth of the pathogen as compared between two antagonists, which was 34.03% in case of *Ch.globosum* and 32.64% in case of *Ch.lucknowense*. This result was in accordance with the study from Sibounnavong (2012) who reported that *Ch.lucknowense* CLT01 were tested for abilities to inhibit *Fusarium oxysporum* f.sp. *lycopersici* NKSC02 causing wilt disease of Tomato in bi-culture test, with the mycelia inhibitive percentage was 39.43%. This result was also in accordance with the study from Tathan *et al.* (2012) who reported that *Ch.globosum* were tested for abilities to inhibit *Drechslera oryzae* causing leaf spot of rice in bi-culture test, with the mycelia inhibitive percentage was 36.80%. It was also similar to the result of Charoenpoen *at al.* (2010) who reported that *Ch.lucknowense* significantly inhibited the mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici* as 88.89%.

Table 2. Effect of antagonist fungi on conidial production of *Fusarium oxysporum*

Treatments	*Fusarium oxysporum*	
	Spores (x 10^7)	% inhibition
Control	108.18[a1/]	
Ch.globosum vs *F.oxysporum*	34.95[b]	67.25[a]
Ch.lucnowens vs *F.oxysporum*	27.95[b]	73.45[a]

1/ Average of four replications, means followed by the same letter in a column were not significantly different by DMRT at P = 0.01 followed by P=0.05.

The Table 2 showed that in reliability of 99 %, *Ch.globosum* and *Ch.lucknowense* significantly inhibited number of conidia of *F.oxysporum*, which were 34.95 x 10^7 and 27.95 x 10^7, respectively when compared to the control plate (108.18 x 10^7). However, similar to the results of colony diameter, the inhibition of conidial production of pathogen was not significant difference between *Ch.globosum* and *Ch.lucknowense*, which was 67.25 and 73.45% respectively. This result was similar to the study from Tathan *et al.* (2012) who reported that *Ch. globosum* were tested for abilities to inhibit *Drechslera oryzae* causing leaf spot of rice in bi-culture test, with the conidial inhibitive percentage were 87.94%. This result was also in accordance with the study from Charoenpoen *at al.* (2010) who reported that *Ch.lucknowense* significantly inhibited the conidial production of *Fusarium oxysporum* f.sp. *lycopersici* as 92.54 %.

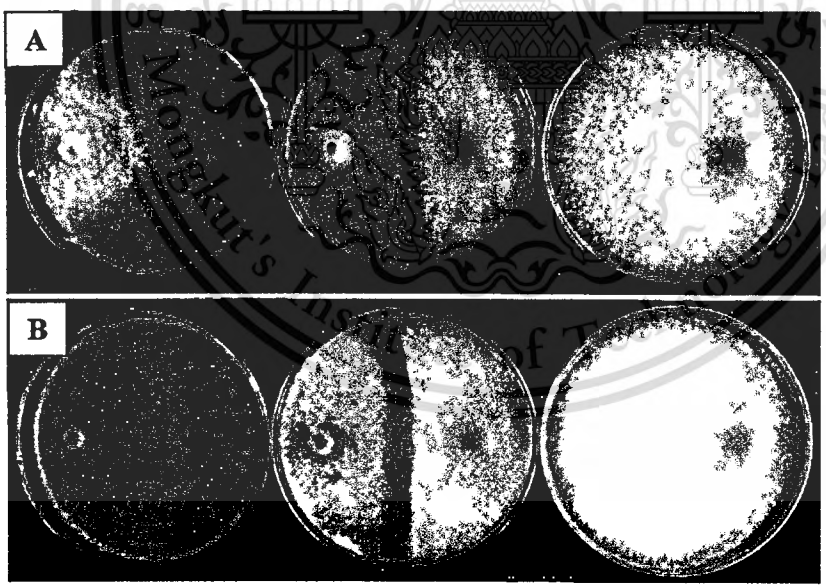
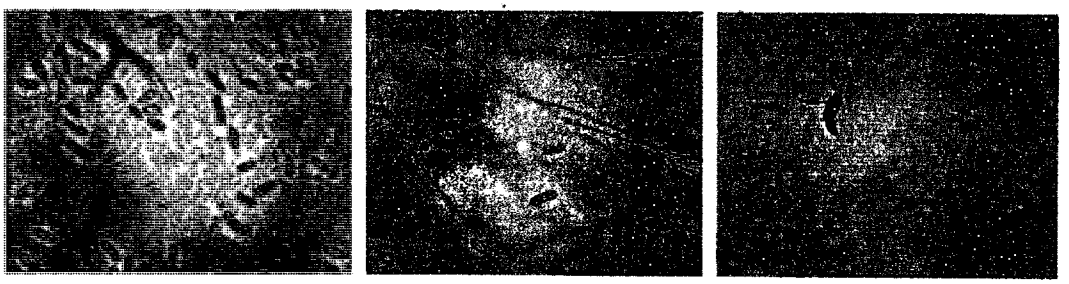


Fig.1. Colony growth of the fungi in the bi-culture antagonistic tests of *Ch.globosum* (A) and *Ch.lucknowenes* (B) against *F.oxysporum*



Control plate Bi-culture plate Abnormal spore

Fig.2. Numbers of conidia produced by *Fusarium oxysporum* in the bi-culture test

4.2. Effect of antifungal metabolites from *Chaetomium* spp. against *Pestalotia* spp. casing grey blight disease of tea in *vitro* test

The cultures of *Pestalotia* spp. were maintained on PDA that were already separately mixed with different concentrations of each crude extract of each *Chaetomium* spp., and incubated at room temperature (RT) for 6 days before colony diameter of the pathogen was measured (showed at Fig.3, Fig.4). The cultures were then continued to incubate at RT for next 9 days before number of spore counted. The results of colony diameters of *Pestalotia* spp. at each concentration are showed on the Table 3.

Table 3. Effect of crude extract from *Chaetomium* spp. on cmycelial growth of *Pestalotia* spp.

Crude extract	Colony diameter (cm) of *Pestalotia* spp. at each concentration (µg/ml)				
	0	50	100	500	1000
Ch.cuperum					
Hexane	5.00[a1/]	3.78[b]	3.43[bc]	2.18[fgh]	1.48[i]
Ethyl acetate	5.00[a]	3.06[cd]	2.60[def]	2.00[gh]	1.80[hi]
Methanol	5.00[a]	3.79[b]	3.41[bc]	2.94[cde]	2.49[efg]
Ch.globosum					
Hexane	5.00[a]	4.23[b]	4.09[bc]	3.46[e]	3.16[f]
Ethyl	5.00[a]	4.19[b]	3.83[d]	2.76[g]	2.56[g]
Methanol	5.00[a]	4.21[b]	3.94[cd]	3.51[e]	3.36[ef]
Ch.lucknowense					
Hexane	5.00[a]	3.76[b]	3.53[bc]	1.99[e]	1.70[e]
Ethyl acetate	5.00[a]	3.76[b]	3.13[cd]	2.93[cd]	2.01[e]
Methanol	5.00[a]	4.04[b]	3.07[cd]	3.11[cd]	2.74[d]

[1/] Average of four replications. Means followed by the same letter in columns and rows within an antagonist were not significantly different by DMRT at P=0.05.

The Table 3 showed that the colony diameter of *Pestalotia* spp. gradually reduced following by the decrease of the concentration of the crude extracts in all the antagonists. In the reliability of 95%, the crude extracts from all the *Chaetomium* spp. at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in colony diameter of *Pestalotia* spp. when compared to the control (0 µg/ml).

The result of the antimicrobial test from *Ch.cupreum* showed that Hexane crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp., which were 3.78, 3.43, 2.18 and 1.48 cm, respectively when compared to the

control (0 µg/ml, 5.00 cm). EtOAc crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp., which were 3.06, 2.60, 2.00 and 1.80 cm, respectively when compared to the control (0 µg/ml, 5.00 cm). MeOH crude extract at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp., which were 3.97, 3.41, 2.94 and 2.49 cm, respectively when compared to the control (0 µg/ml, 5.00 cm).

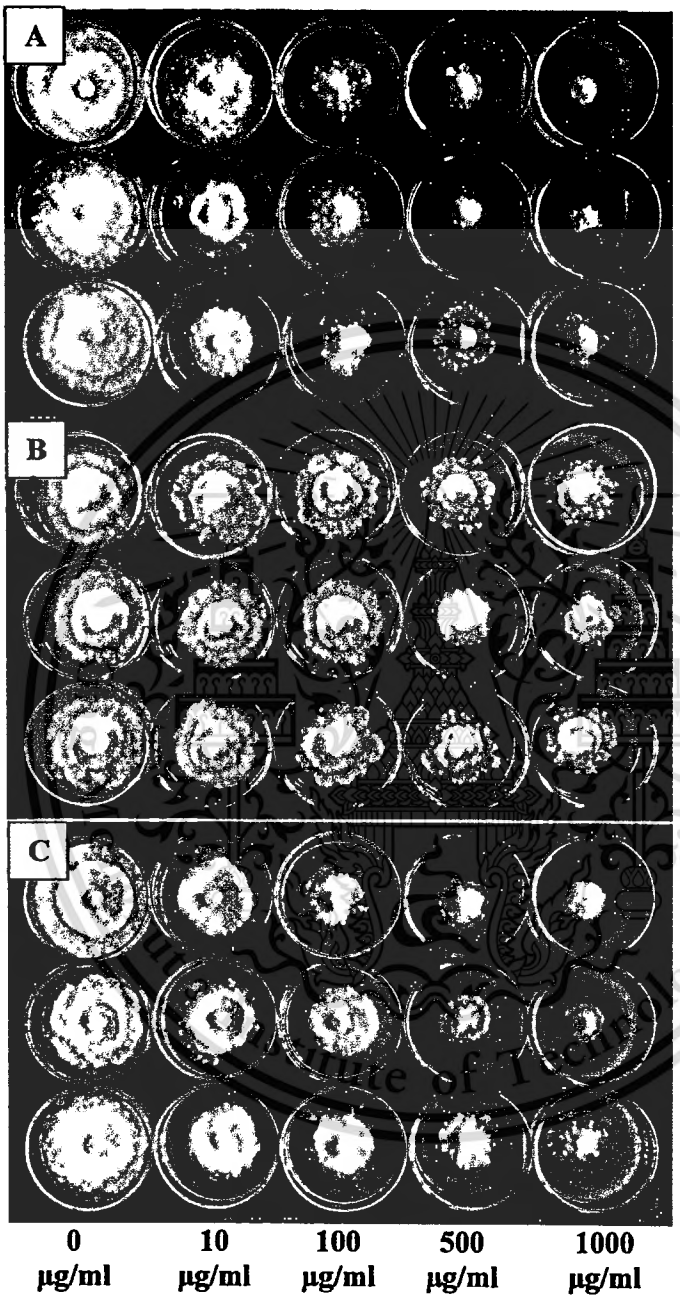


Fig.3. Crude extract test of *Ch.cuperum* (A), *Ch.globosum* (B) and *Ch.lucknowense* (C) against *Pestalotia* spp; from the top to the bottom of each test: hexane, EtOAc and MeOH extract, respectively

The result of the antimicrobial test from *Ch.globosum* showed that Hexane crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp. that were 4.23, 4.09, 3.46 and 3.16 cm, respectively when compared to the control (0 µg/ml, 5.00 cm). EtOAc crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp. that were 4.19, 3.83, 2.76 and 2.56 cm, respectively when compared to the control (0 µg/ml, 5.00 cm). MeOH crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp. that were 4.21, 3.94, 3.51 and 3.36 cm, respectively when compared to the control (0 µg/ml, 5.00 cm).

The result of the antimicrobial test from *Ch.globosum* showed that Hexane crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp. that were 3.76, 3.53, 1.99 and 1.70 cm, respectively when compared to the control (0 µg/ml, 5.00 cm). EtOAc crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp. that were 3.76, 3.13, 2.93 and 2.01 cm, respectively when compared to the control (0 µg/ml, 5.00 cm). MeOH crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp. that were 4.04, 3.07, 3.11 and 2.74 cm, respectively when compared to the control (0 µg/ml, 5.00 cm).

The parameter of percentage of colony growth inhibition was calculated to evaluate the colony growth inhibition ability of antagonist against the pathogen. The results are showed on the Table 4.

Table 4. Effect of crude extract from *Chaetomium* spp. on percentage of mycelial growth inhibition of *Pestalotia* spp.

Crude extract	Colony inhibition percentage (%) of *Pestalotia* spp. at each concentration (µg/ml)					Average of four treatments (%)
	0	50	100	500	1000	
Ch.cuperum						
Hexane	0.00^{i}[1/]	24.50^{h}	31.50^{gh}	56.50^{bcd}	70.50^{a}	45.75^{b}[2/]
Ethyl acetate	0.00^{i}	38.75^{fg}	48.00^{def}	60.00^{bc}	64.00^{ab}	52.69^{a}
Methanol	0.00^{i}	24.25^{h}	31.75^{gh}	41.25^{efg}	50.25^{cde}	36.88^{c}
Average of three crudes	0.00^{e}[3/]	29.17^{d}	37.08^{c}	52.58^{b}	61.58^{a}	
Ch.globosum						
Hexane	0.00^{g}	15.50^{f}	18.25^{ef}	30.75^{c}	36.75^{b}	25.31^{b}
Ethyl acetate	0.00^{g}	16.25^{f}	23.50^{d}	44.75^{a}	48.75^{a}	33.31^{a}
Methanol	0.00^{g}	15.75^{f}	21.25^{de}	29.75^{c}	32.75^{bc}	24.88^{b}
Average of three crudes	0.00^{e}	15.83^{d}	21.00^{c}	35.08^{b}	39.42^{a}	
Ch.lucknowense						
Hexane	0.00^{e}	24.75^{d}	29.50^{cd}	60.25^{a}	66.00^{a}	45.13^{a}
Ethyl acetate	0.00^{e}	24.75^{d}	37.50^{bc}	41.50^{bc}	59.75^{a}	40.88^{ab}
Methanol	0.00^{e}	19.25^{d}	38.63^{bc}	37.75^{bc}	45.25^{b}	35.22^{b}
Average of three crudes	0.00^{e}	22.92^{d}	35.21^{c}	46.50^{b}	57.00^{a}	

[1/] The average of four replications. Means followed by the same letter in the columns and the rows of three crude extracts within each antagonist were not significantly different by DMRT at P=0.05.

[2/] The average of the concentrations of 50, 100, 500, 1000 µg/ml of each the crude extract. Means followed by the same letter in the row of each crude extract within each antagonist were not significantly different by DMRT at P=0.05.

[3/] The average of three crude extract of each concentration. Means followed by the same letter in the column of each concentration within each antagonist were not significantly different by DMRT at P=0.05.

A comparison in the percentage of colony growth inhibition of the pathogen among three crude extracts showed that in the reliability of 95%, there was significant difference in the percentage of colony inhibition from the crude extracts of *Ch.cuperum* and *Ch.globosum* against *Pestalotia* spp. EtOAc crude gave the highest average of colony inhibition percentage that were 52.69% on the average of five treatments in case of *Ch.cupreum* and 33.31% in case of *Ch.globosum*, followed by Hexane crude which were 45.75%, 25.31% respectively and MeOH crude which were 36.86%, 24.88% respectively. The percentage of colony inhibition of *Pestalotia* spp. given by EtOAc crude of *Ch.lucknowense* was not significant different from that given by Hexane and MeOH crude of the same antagonist. The percentage of colony inhibition of *Pestalotia* spp. given by EtOAc, Hexane and MeOH crude of *Ch.lucknowense* were 40.88%, 45.13% and 35.22% respectively on the average of five treatments. However, there was significant difference in the percentage of colony inhibition between Hexane and MeOH crude. Among three crude extracts of *Ch.lucknowense,* Hexane crude gave higher percentage average of colony growth inhibition of *Pestalotia* spp. than the others.

A comparison in the percentage of colony growth inhibition of the pathogen among the treatments showed that in the reliability of 95%, there was significant difference in the average of colony inhibition percentage of *Pestalotia* spp. among five treatments in all the antagonists. The average of colony inhibition percentage of *Pestalotia* spp. significantly increased following by the gradual increase of the concentrations (50, 100, 500, 1000 µg/ml), which were 29.17, 37.08, 52.58 and 61.58%, respectively in case of *Ch.cuperum*; 15.83, 21.00, 35.08 and 39.42% respectively in case of *Ch.globosum*; 22.92, 35.21, 46.50 and 57.00%, respectively in case of *Ch.lucknowense.*

The result of the antimicrobial test from *Ch.cupreum* showed that Hexane crude at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the percentage of colony inhibition of *Pestalotia* spp., which were 24.50, 31.50, 56.50 and 70.50% respectively when compared to the control (0 µg/ml). It showed similar results to EtOAc and MeOH crude extract, which were 38.75, 48.00, 60.00 and 64.00 % respectively in case of EtOAc crude, and 24.25, 31.75, 41.25 and 50.25% respectively in case of MeOH crude.

The result of the antimicrobial test from *Ch.globosum* showed that Hexane crude at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the percentage of colony inhibition of *Pestalotia* spp., which were 15.50, 18.25, 30.75 and 36.75%, respectively when compared to the control (0 µg/ml). It also showed similar results to EtOAc and MeOH crude which were 16.25, 23.50, 44.75 and 48.75%, respectively in case of EtOAc crude, and 15.75, 21.25, 29.75 and 32.75%, respectively in case of MeOH crude.

The result of the antimicrobial test from *Ch.lucknowense* showed that Hexane crude at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the percentage of colony inhibition of *Pestalotia* spp., which were 24.75, 29.50, 60.25 and 66.00% respectively when compared to the control (0 µg/ml). Again, it showed similar results to EtOAc and MeOH crude that were 24.75, 37.50, 41.50 and 59.75% respectively in case of EtOAc crude, and 19.25, 38.63, 37.75 and 45.25% respectively in case of MeOH crude.

To evaluate the ability of spore producing inhibition of antagonist against the pathogen, the number of spore produced were counted by using a Haemacytometer under a compound microscope. The result is showed on the Table 5.

The Table 5 showed that the number of spore of *Pestalotia* spp. gradually reduced following by the decrease of the concentrations of almost of the crude extracts in all the antagonists excepting EtOAc crude of *Ch.globosum* which showed the concentration of 100 µg/ml gave insignificant higher number of spore than the concentration of 500 µg/ml. However, whether significant difference or not in number of spore among five treatments of each crude extract from all the antagonists were not the same.

The result of the antimicrobial test from *Ch.cupreum* showed that Hexane, EtOAC and MeOH crude at the concentrations of 50 - 1000 µg/ml gave significant difference in the spore numbers of *Pestalotia* spp. that were 8.41 x 10^7, 5.19 x 10^7, 2.09 x 10^7 and 2.06 x 10^7 spores, respectively when compared to the control (0 µg/ml, 18.48 x 10^7 spores) in case of Hexane crude; 12.42 x 10^7, 9.77 x 10^7, 5.15 x 10^7 and 0.93 x 10^7 spores, respectively when compared to the control (0 µg/ml, 16.74 x10^7 spores) in case of EtOAc crude; 7.17 x 10^7, 3.86 x 10^7, 2.56 x 10^7 and 2.07 x 10^7 spores, respectively when compared to the control (0 µg/ml, 10.71 x 10^7 spores) in case of MeOH crude.

Table 5. Effect of crude extract for *Chaetomium* spp. on spore production of *Pestalotia* spp.

Crude extract	Number of spore (x 10^7) of *Pestalotia* spp. at each concentration (µg/ml)				
	0	50	100	500	1000
Ch.cuperum					
Hexane	18.48^{a}	8.41^{cde}	5.19^{efg}	2.09^{gh}	2.06^{gh}
Ethyl acetate	16.74^{a}	12.42^{b}	9.77^{bcd}	5.15^{efg}	0.93^{h}
Methanol	10.71^{bc}	7.17^{def}	3.86^{fgh}	2.56^{gh}	2.07^{gh}
Ch.globosum					
Hexane	6.23^{a}	5.39^{ab}	5.43^{ab}	3.14^{cd}	1.84^{def}
Ethyl acetate	5.01^{ab}	4.93^{ab}	1.89^{def}	2.11^{de}	0.18^{f}
Methanol	5.85^{a}	4.59^{abc}	3.99^{bc}	1.77^{def}	1.21^{ef}
Ch.lucknowense					
Hexane	11.50^{a}	10.17^{ab}	6.08^{cd}	3.07^{de}	3.02^{de}
Ethyl acetate	11.31^{a}	6.98^{c}	4.67^{cde}	3.91^{cde}	2.01^{e}
Methanol	7.23^{bc}	5.61^{cd}	5.27^{cde}	2.85^{de}	2.84^{de}

[1/] Average of four replications. Means followed by the same letter in columns and rows within an antagonist were not significantly different by DMRT at P=0.05.

The result of the antimicrobial test from *Ch.globosum* showed that only the concentrations of 500 and 1000 µg/ml of Hexane and MeOH crude gave significant difference in the spore production of *Pestalotia* spp. that were 3.14 x 10^7 and 1.84 x 10^7 spores respectively when compared to the control (0 µg/ml) of 6.23 x 10^7 spores in case of Hexane crude; 1.77 x 10^7 and 1.21 x 10^7 spores respectively when compared to the control (0µg/ml) of 5.85 x 10^7 spores in case of MeOH crude. However, there was no significant difference in the spore production of *Pestalotia* spp. between the concentrations of 500 and 1000 µg/ml in both Hexane crude and MeOH crude. The concentrations of 50 and 100 µg/ml of Hexane and MeOH crude did not give significant difference in spore production of *Pestalotia* spp. when compared to the control (0 µg/ml). Furthermore, EtOAc crude showed the level of concentration that gave significant difference in the spore production the pathogen was lower than that of Hexane and MeOH crude, which started giving significant difference in the spore production at the level of 100 µg/ml.

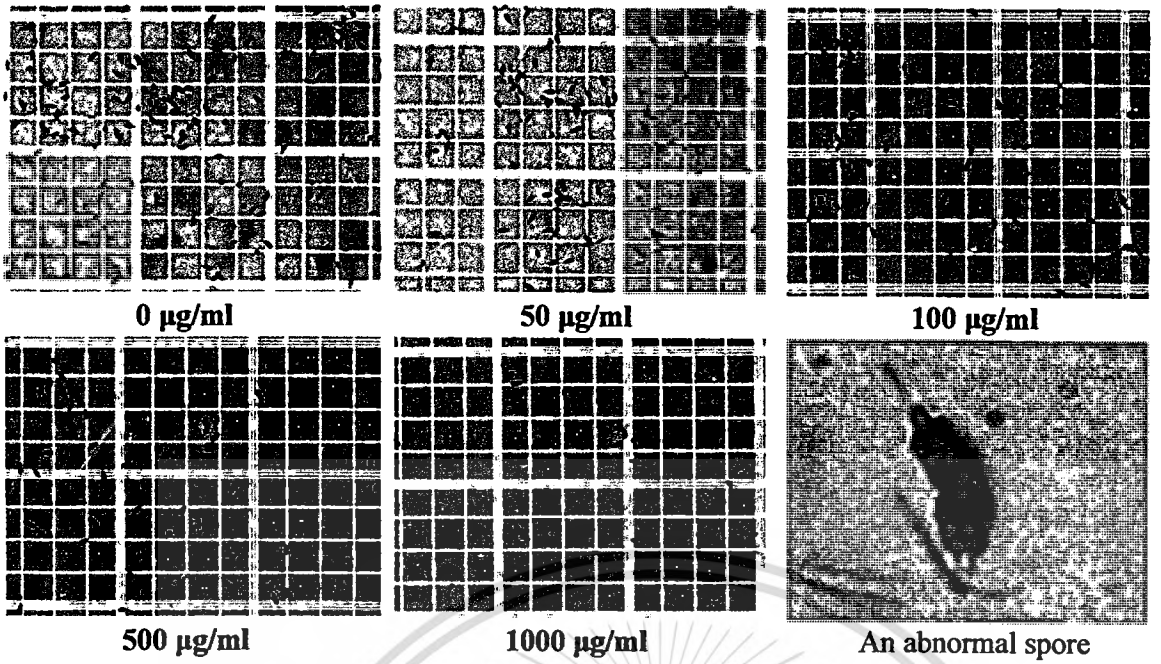


Fig 4. Spore production of *Pestalotia* spp. at different concentrations

The result of the antimicrobial test from *Ch.lucknowense* showed that Hexane crude at the concentrations of 100, 500 and 1000 µg/ml gave significant difference in the spore production of *Pestalotia* spp., which were 6.08 x 10^7, 3.07 x 10^7 and 3.02 x 10^7 spores, respectively when compared to the control (0 µg/ml) of 11.50 x 10^7 spores. EtOAc crude at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the spore production of *Pestalotia* spp., which were 6.98 x 10^7 and 4.67 x 10^7, 3.91 x 10^7 and 2.01 x 10^7 spores respectively when compared to the control (0 µg/ml) of 11.31 x 10^7 spores. MeoH crude at the concentrations of 500 and 1000 µg/ml gave significant difference in the spore production of *Pestalotia* spp., which were 2.85 x 10^7 and 2.84 x 10^7 spores respectively when compared to the control (0 µg/ml) of 7.23 x 10^7 spores. It can say that only EtOAc crude started giving significant difference in the spore production of *Pestalotia* spp. at the level of the concentration of 50 µg/ml. Meanwhile Hexane crude started giving significant difference in the spore production of *Pestalotia* spp. at the concentration level of 100 µg/ml and of which was 500 µg/ml for MeOH crude. This result explained why the ED50 value of EtAOc was lower than that of Hexane and MeOH crude (will be showed as the Table 6).

The Table 6 showed that three crude extracts of *Ch.cuperum* significantly inhibited spore production of *Pestalotia* spp. at the concentrations of 50, 100, 500 and 1000 µg/ml that were 51.34, 70.00, 88.56 and 88.49% respectively when compared to the control (0 µg/ml) in case of Hexane crude; 25.84, 41.13, 68.54 and 93.92% respectively when compared to the control (0 µg/ml) in case of EtOAc crude; 31.05, 65.40, 75.94 and 80.66% respectively when compared to the control (0 µg/ml) in case of MeOH crude.

EtOAc and MeOH crude of *Ch.lucknowense*, and MeOH crude of *Ch.globosum* also significantly inhibited spore production of *Pestalotia* spp. at the concentrations of 50, 100, 500 and 1000 µg/ml, which were 37.02, 54.47, 64.08 and 81.08% respectively when compared to the control (0 µg/ml) in case of EtOAc crude of *Ch.lucknowense*; 21.80, 26.75, 54.62, and 54.65% respectively when compared to the control (0 µg/ml) in case of MeOH crude of *Ch.lucknowense*; 21.40, 31.07, 67.12 and 76.34% respectively when compared to the control (0 µg/ml) in case of MeOH crude of *Ch.globosum*. Meanwhile, Hexane crude of *Ch.globosum* significantly inhibited spore production of *Pestalotia* spp. at the concentrations

of 500 and 1000 µg/ml, which were 48.73 and 74.36% respectively when compared to the control (0 µg/ml). EtOAc crude of *Ch.globosum* and Hexane crude of *Ch.lucknowense* significantly inhibited spore production of *Pestalotia* spp. at the concentrations of 100, 500 and 1000 µg/ml, which were 63.40, 58.11 and 95.71 % respectively when compared to the control (0 µg/ml) in case of EtOAc crude of *Ch.globosum*, and 46.52, 72.41 and 72.49% respectively when compared to the control (0 µg/ml) in case of Hexane crude of *Ch.globosum*.

Table 6. Effect of crude extract for *Chaetomium* spp. on percentage of spore producing inhibition of *Pestalotia* spp.

Crude extract	Spore inhibition percentage (%) of *Pestalotia* spp. at each concentration (µg/ml)					Average of four treatments (%)	ED50 (µg/ml)
	0	50	100	500	1000		
Ch.cuperum							
Hexane	$0.00^{h1/}$	51.34^{e}	70.00^{cd}	88.56^{ab}	88.49^{ab}	$74.60^{a2/}$	28.40
Ethyl acetate	0.00^{h}	25.84^{g}	41.13^{ef}	68.54^{cd}	93.92^{a}	57.36^{b}	154.12
Methanol	0.00^{h}	31.05^{fg}	65.40^{d}	75.94^{bcd}	80.66^{bc}	63.26^{b}	78.16
Average of three crudes	$0.00^{e3/}$	36.08^{d}	58.84^{c}	77.68^{b}	87.69^{a}		
Ch.globosum							
Hexane	0.00^{g}	12.93^{efg}	12.34^{efg}	48.73^{cd}	74.36^{b}	37.09^{b}	452.70
Ethyl acetate	0.00^{g}	1.79^{fg}	63.40^{bc}	58.11^{bc}	95.71^{a}	54.75^{a}	154.67
Methanol	0.00^{g}	21.40^{ef}	31.07^{de}	67.12^{bc}	76.34^{b}	48.98^{a}	224.50
Average of three crudes	0.00^{e}	12.04^{d}	35.60^{c}	57.99^{b}	82.14^{a}		
Ch.lucknowense							
Hexane	0.00^{h}	11.46^{gh}	46.52^{cde}	72.41^{ab}	72.49^{ab}	50.72^{a}	200.12
Ethyl acetate	0.00^{h}	37.02^{def}	57.47^{bcd}	64.08^{abc}	81.08^{a}	50.92^{a}	86.99
Methanol	0.00^{h}	21.80^{fg}	26.75^{efg}	54.62^{bcd}	54.65^{bcd}	39.45^{b}	359.21
Average of three crudes	0.00^{d}	23.43^{c}	43.58^{b}	63.71^{a}	69.41^{a}		

1/ Average of four replications. Means followed by the same letter in the columns and the rows of three crude extracts within each antagonist were not significantly different by DMRT at P=0.05.

2/ Average of the concentrations of 50, 100, 500, 1000 µg/ml of each the crude extract. Means followed by the same letter in the row of each crude extract within each antagonist were not significantly different by DMRT at P=0.05.

3/ Average of three crude extract of each concentration. Means followed by the same letter in the column of each concentration within each antagonist were not significantly different by DMRT at P=0.05.

A comparison in the percentage of the spore inhibition of the pathogen among three crude extracts showed that in the reliability of 95%, Hexane crude of *Ch.cupreum* gave the highest percentage of spore production of *Pestalotia* spp. among three crude extracts, which was 74.6% on the average of five treatments, followed by EtOAc and MeOH crude which were 62.26 and 57.36% respectively. It may explain why Hexane crude gave the lowest value of ED50 which was 28.40 µg/ml in compare with the ED50 value of EtOAc and MeOH crude, which were 154.20 µg/ml and 78.16 µg/ml respectively. EtOAc and MeOH crude of *Ch.globosum* gave significant higher of *Pestalotia* spp. that were 54.75 and 48.98% respectively when compared to the percentage of spore production of Hexane crude, which was 37.09% on the average of five treatments. However, there was no significant difference

in the percentage of spore inhibition between EtOAc and MeOH crude extracts. Among three crude extracts of *Ch.globosum,* EtOAc crude gave much lower value of ED50 that was 154.67 µg/ml than the ED50 value of Hexane crude that were 452.70 µg/ml. Nevertheless, the ED50 value of EtOAc crude was not so different from that of MeOH crude, which was 224.50 µg/ml.

EtOAc and Hexane crude of *Ch.lucknowense* gave significant higher percentage of spore production of *Pestalotia* spp. that were 50.92 and 50.72% respectively on the average of five treatments when compared to MeOH crude which was 39.45%. However, there was no significant difference in the percentage of spore inhibition between EtOAc and Hexane crude. Among three crudes of *Ch.globosum,* EtOAc gave much lower value of ED50 which was 86.99 µg/ml than MeOH crude which were 359.21 µg/ml. The value of ED50 of EtOAc crude was not so different from that of Hexane crude, which was 200.12 µg/ml.

A comparison in the percentage of spore inhibition of the pathogen among the treatments showed that in the reliability of 95%, there was significant difference in the average of spore inhibition percentage of *Pestalotia* spp. among five treatments in all the antagonists. The result from *Ch.cupreum* and *Ch.globosum* showed that the average of spore inhibition percentage of *Pestalotia* spp. significantly increased following by the gradual increase of the concentrations (50, 100, 500, 1000 µg/ml), which were 36.08, 58.84, 77.68 and 87.69% respectively on the average of three crude extracts in case of *Ch.cuperum*; 12.04, 35.60, 57.99 and 82.14% respectively on the average of three crude extracts in case of *Ch.globosum.* This result was showed the same to *Ch.lucknownese,* but there is only a difference that the average of spore inhibition percentage of *Pestalotia* spp. given by the concentration of 500 and 1000 µg/ml, which were 63.71 and 69.41% respectively on the average of three crude extracts were not different. It means that there was no significant difference in spore inhibition efficiency between the concentration of 500 µg/ml and 1000 µg/ml for MeOH crude of *Ch.lucknowense*.

All tested crude extracts of *Ch.cupreum*, *Ch.globosum* and *Ch.lucknowense* significantly inhibited colony growth of *Pestalotia* spp. This result was similar to the report of Sibounnavong (2012) who stated that *Ch.cupreum*, *Ch.lucknowense* significantly inhibited colony growth of *Fusarium oxysporum f.sp. lycopersici* causing wilt disease in tomato which were 10.50-35.00 % and 5.5-28.5% respectively. It also demonstrated that all tested crude extracts of *Ch.cupreum*, *Ch.globosum* and *Ch.lucknowense* significantly inhibited spore production of *Pestalotia* spp. This result was similar to the report of Tathan *et al.* (2012) who stated that Hexane, EtOAc and MeOH crude from *Ch.cupreum* and *Ch.globosum* inhibited *Drechslera oryzae* causing leaf spot of rice with the ED50 of 0.58, 8.72 and 14.92 µg/ml respectively in case of *Ch.cupreum*, 10.15, 49.74 and 63.01 µg/ml respectively in case of *Ch.globosum*. Another report from Sibounnavong (2012) also showed that Hexane, EtOAc and MeOH from *Ch.cupreum* and *Ch.lucknowense* inhibited *F. oxysporum f.sp. lycopersici* NKSC02 with the ED50 of 2.33, 2.38, and 2.65 µg/ml respectively in case of *Ch.cupreum*; of which were 921, 393 and 53 µg/ml respectively in case of *Ch.lucknowense*. This result was also similar to the report of Charoenpoen *et al.* (2010) who said that Hexane and MeOH crude from *Ch.lucknowense* significantly inhibited *Fusarium oxysporum* f.sp. *lycopersici* causing wilt disease in tomato with the ED50 of 188 and 212 µg/ml, respectively.

The result of the study revealed that *Chaetomium* spp. could produce some metabolites to inhibit *Pestalotia* spp. This statement was proved by some previous researches. Kanokmedhakul *et al.* (2002) reported that *Ch.globosum* (KMITL 0802) produces chaetoglobosin C which inhibits some pathogens. Later, Park *et al.* (2005) proved that *Ch.globosum* (F0142) produces chaetoviridin A to control rice blast, wheat leaf rust and tomato late blight. Soytong *et al.* (2001) showed that a specific isolate of *Ch.cupreum*

produced rotiorinols A-C and rotiorin that significantly suppressed *F. oxysporum* f.sp. *lycopersici* causing wilt disease in the tomato.

REFERENCE

Alastair, Hick. (2009). Current status and future development of global tea production and tea product. AU J.T 12(4): p.251-264.

Carr, M.K.V. (1972). Climate Requirements of the Tea plant. Expl. 39 Agric. 8, pp.1-14.

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 Vol. 9(36), pp. 5836-5844

Işil Alkan, Onur Köprürü and Bora Alkan (2009). Latest advance in world tea production and trade, Turkey's aspect World Journal of Agriculture Sicence 5 (3): p.345-349.

Kanokmedhakul, S., Kanokmedhakul, K., Phonkerd, N., Soytong, K., Kongsaeree, P. and Suksamrarn, A. (2002). Antimycobacterial antraquinone-chromanone compound and diketopiperazine alkaloid from the fungus *Chaetomium globosum* KMITL-N0802. Planta Medica 68: 834-836.

Kanokmedhakul, S., Kanokmedhakul, K., Nasomjai, P., Loungsysouphah, S., Soytong, K., Isobe, M., Kongsaeree, K., Prabpai, S. and Suksamran, A. (2006). Antifungal azaphilone from the fungus: *Chaetomium cupreum* CC3003. J. Nat. Prod. 69: 891-895.

Kingdon-Ward, F. (1950). Does wild tea exist ? Nature, 165, 297-99.

Majumder, A.B., Bera, B. and Rajan, A. (2012). Tea Statistics: Global Scenario. SJTS January 2012.

Soytong, K. (1992). Biological control of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* using *Chaetomium cupreum*. Kasetsart J. Nat. Sci. 26: 310-313.

Soytong K, Kanokmedhakul S, Kukongviriyapan V and Isobe M. Application of *Chaetomium* species (Ketomium) as a new broad spectrum biological fungicide for plant disease control. Fungal Div 2001;7:1-15.

Soytong, K., Srinon, W., Rattanacherdchai, K., Kanokmedhakul, S. and Kanokmedhakul, K. (2005). Application of antagonistic fungi to control anthracnose disease of grape. Journal of Agricultural Biotechnology 1: 33-41.

Sibounnavong, P. (2012). Bioilogical activities of antagonistic fungi to control Fusarium Wilt of Tomato. A Thesis submitted in partial fulfillment of the requirement for the degree of doctor of philosophy in biotechnology in plant pathology. International College-King Mongkut's Institute of Technology Lakrabang-2012.

Park, J.H., Choi, G.J., Jang, K.S., Lim, H.K., Kim, H.T., Cho, K.Y. and Kim, J.V. (2005). Antifungal activity against plant pathogenic fungi of *chaetoviridins* isolated from *Chaetomium globosum*. FEMS Microbiol. Letters 252: 309-313.

Tathan, S., Sibounnavong, P., Sibounnavong, P.S., Soytong, K. and To-anun, C. (2012). Biological metabolites from Chaetomium spp to inhibit Drechslera oryzae causing leaf spot of rice. Jornal of Agricultural Technology 8(5): 1691-1701.

http://faostat.fao.org/site/613/