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Antimicrobial Substances from *Chaetomium* spp. against *Pestalotia* spp. causing grey Blight Disease of Tea

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Abstract This research was focused on grey blight disease of tea caused by *Pestalotia* spp. Hexane, EtOAc and MeOH crude extracts from *Chaetomium globosum* and *Chaetomium lucknowense* were proved as antifungal substances against *Pestalotia* spp. Result showed that ethyl acetate crude extract of *Ch. lucknowense* inhibited spore production of *Pestalotia* spp at ED_{50} of 86 µg/ml. The ED_{50} of crude ethyl acetate of *Ch.globosum* to inhibit spore production *Pestalotia* spp at 154 µg/ml. Hexane crude extract of *Ch.lucknowense* inhibited spore production of *Pestalotia* spp at ED50 of 200 µg/ml.

Keywords: tea, antifungal substance test, *Pestalotia* spp., *Chaetomium cupreum*, *Chaetomium globosum, Chaetomium lucknowense*

Introduction

The tea plant, *Camellia sinensis* (L.).O. Kuntze, is thought to have origin within the fan-shaped areas 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, 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 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, 2011). Tea is one of the most popular and lowest cost beverages in the world (Majumder *et al*., 2012), next only to

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water, and consumed by a wide range of age groups in all levels of society, with more than three billion cups that are consumed daily worldwide. 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, 2009). Tea is not only considered to be a part of the huge beverage market but also be of interest to functional foods markets (Alastair, 2009), and has been one of the main agriculture export items for many developing countries. In addition to tea cultivation, tea picking and tea 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 *et al*., 2009).

Chemical fungicides have been extensively used to control diseases of tea for a long time. Using chemical method to control plant pathogens leading to many risks such as rapid conversion of innocuous species into pests, development of resistance of pathogens, undesirable pesticide residues in made tea, and cause eco-environmental pollution. Biological control of plant pathogens is a recent successful strategy for disease control, and has been successfully integrated with other control measures. This method can reduce the heavy use of chemical fungicides, improving the agro-ecosystem and maintaining a natural balance (Soytong *et al*., 1999). There are several reports on the use of biological control agents against plant pathogens. *Chaetomium* species which belonging to Ascomycota of the Chaetomiaceae have been shown to be against several plant pathogens, e.g. *Botrytis cinerea* (Kohl *et al*., 1995), *Colletotrichum gloeosporioides* (Noiaium and Soytong, 1997), *Fusarium oxysporum* f.sp. *lycopersici* (Sibounavong, 2012), *Phytophthora palmivora* (Pechprome and Soytong, 1997), *Phytophthrola parasitica* (Usuwan and Soytong, 1998), *Venturia inegualis* (Heye and Andrews, 1983). To assessing the ability of *Chaetomium* spp. against tea pathogen, this research was concentrated on evaluating antifungal substances extracted from *Chaetomium* spp. against *Pestalotia* spp. causing grey blight disease of tea *in vitro* test.

Materials and methods

Isolation of the pathogen from grey blight disease of tea

Disease samples were taken from tea leaves showing grey blight disease symptom of ten tea varieties that collected from Vietnam and planted at the Bio-control Research Unit and Mycology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. The pathogen was isolated by tissue transplanting

technique. The surface of the diseased tea leaves were washed by sterile distilled water, then sterilized by rinsing them in alcohol 70% for 5 seconds, and then washed again by sterile distilled water. After that, the diseased tea leaf samples were put into sterilized tissue papers until no more water drop on the surface of the tea leaves. The advance margin of between healthy tissue and diseased tissue was then cut into small pieces of 2x2 mm in size, then transferred into water agar, then followed by potato dextrose agar (PDA) until get pure culture. The pure cultures of *Pestalotia* spp. were identified by morphological characteristic under a compound microscope, then maintained on PDA slant and deposited at the Bio-control Research Unit and Mycology Section, Faculty of Agricultural Technology, KMITL, Bangkok, Thailand.

Pathogenicity tests

The isolates were proved for their pathogenicity by using the detached leaf technique in the laboratory. A sterilized filter paper was placed in a sterilized 9 cm-diameter Petri dish. The leaves were wounded by a sterilized needle before placed on the filter paper in the Petri dish. A 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of the cultures of the *Pestalotia* spp. and placed onto the wounded position of the leaf surface. The filter paper in the Petri dish was moistened by sterilized distilled water. The non-inoculated leaves were treated with 0.5 cm sterilized agar plug served as control. All petri dishes were incubated at room temperature (27-30 ℃) for 10 days before data collection.

The experiment was conducted in Completely Randomized Design (CRD) with four replications.

Antifungal substance tests

Chaetomium globosum (*Ch.globosum)* and *Chaetomium lucknowense (Ch.lucknowense)* were offered from Assoc. Prof. Dr. Kasem Soytong, from Faculty of Agricultural Technology, KMITL, Bangkok, Thailand. Crude extracts from *Ch.globosum* and *Ch.lucknownese* were done by following the method of Kanokmedhakul *et al.* (2006). The antagonists were cultured in PDA and incubated at room temperature for 35 days before fresh fungal biomass were collected. The dried fungal biomass of the antagonists were serial extracted by soaked in hexane, ethyl acetate (EtOAc) and methanol (MeOH), respectively to get filtrates before subjected to a rotary vacuum evaporator then yielded hexane, EtOAc and MeOH crude extract.

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

Factor A represented crude extracts:

A1 = crude hexane extract

A2 = crude ethyl acetate extract

A3 = crude methanol extract

Factor B represented the concentrations:

B1 = 0 µg/ml (control)

B2 = 50 µg/ml

B3 = 100 µg/ml

B4 = 500 µg/ml

B5 = 1,000 µg/ml

The crude extracts at different concentration were separately dissolved in 2% dimethyl sulfoxide and added to separate PDA, then autoclaved 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 culture of *Pestalotia* spp. An agar plug of the pathogen was transferred to the center of a 5 cm-diameter Petri dish containing the prepared media, then incubated at room temperature until seeing colony of the pathogen on the control plates reach to the rim of the Petri dish.

Abnormal spores and nornal spores of *Pestalotia* spp. from each treatment were observed under a compound microscope and taken photograph for comparison.

Data were collected regarding colony diameter and number of spores produced by *Pestalotia* spp. The number of observed spores and the colony diameter was then used to calculate percentage of spore producing inhibition and percentage of colony growth inhibition. The effective dose (ED_{50}) was also calculated using the Probit analysis software.

Results

The result from *Ch.globosum* showed that hexane crude extract at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp., which was 4.23, 4.09, 3.46 and 3.16 cm respectively when compared to the control (5.00 cm). EtOAc crude extract at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp., which was 4.19, 3.83, 2.76 and 2.56 cm respectively when compared to the control (5.00 cm). MeOH crude extract at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant

difference in the colony diameter of *Pestalotia* spp., which was 4.21, 3.94, 3.51 and 3.36 cm respectively when compared to the control (5.00 cm). The result from *Ch.lucknowense* showed that hexane crude at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp., which was 3.76, 3.53, 1.99 and 1.70 cm respectively when compared to the control (5.00 cm). EtOAc crude at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp., which was 3.76, 3.13, 2.93 and 2.01 cm respectively when compared to the control (5.00 cm). MeOH crude at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the colony diameter of *Pestalotia* spp., which was 4.04, 3.07, 3.11 and 2.74 cm respectively when compared to the control (5.00 cm) as seen in Table 1 and Fig1, 2.

Table 1. Effect of crude extracts from *Chaetomium* spp. on colony growth of *Pestalotia* spp**.**

Crude extract	Colony diameter (cm) of *Pestalotia* spp. at each concentration (µg/ml)				
	0	50	100	500	1000
Ch.globosum					
Hexane	5.00^{a} [1/]	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.

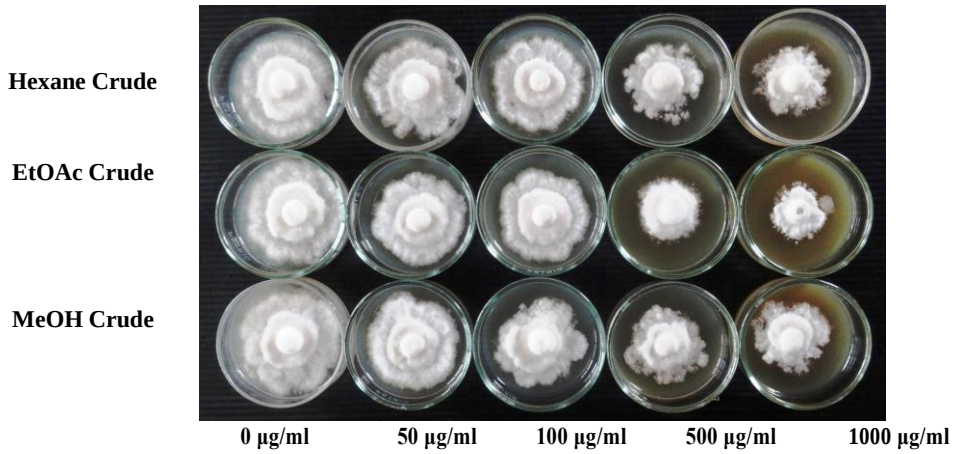

Fig. 1. Crude extract test of *Ch.globosum* against *Pestalotia* spp.

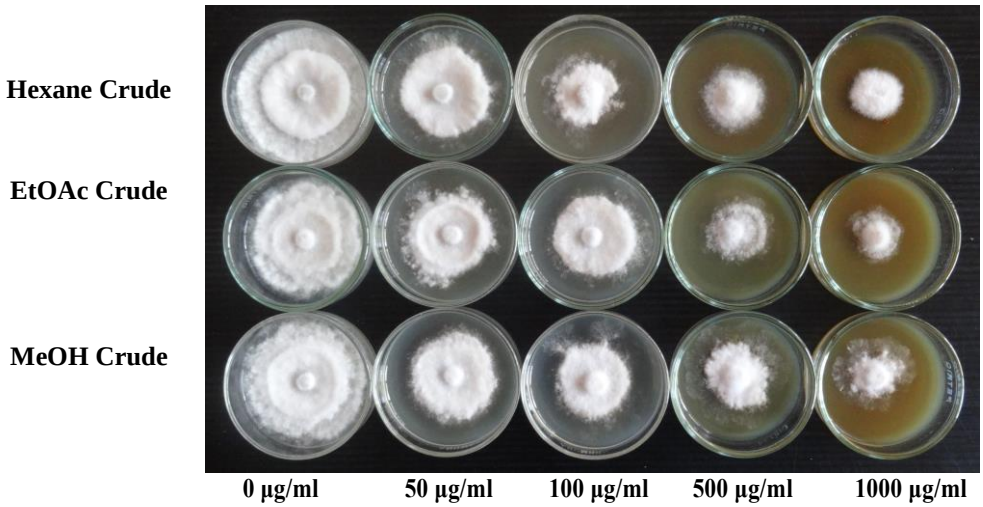

Fig. 2. Crude extract test of *Ch.lucknowense* against *Pestalotia* spp.

A comparison in the percentage of colony growth inhibition of the pathogen among three crude extracts showed that there was significant difference in the percentage of colony inhibition from the crude extracts of *Ch.globosum* against *Pestalotia* spp. EtOAc crude extract gave the highest average of colony inhibition percentage that was 33.31% on the average of five treatments, followed by hexane crude which was 25.31% and MeOH crude which was 24.88%. 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 extract 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 (Table 2).

A comparison in the percentage of colony growth inhibition of the pathogen among the treatments showed that 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 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* (Table 2).

Table 2. Effect of crude extract from *Chaetomium* spp. on percentage of colony 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.globosum						
Hexane	0.00$^{g1/}$	15.50^{f}	18.25^{ef}	30.75^{c}	36.75^{b}	25.31$^{b2/}$
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$^{e3/}$	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.

The result from *Ch.globosum* showed that hexane crude extract at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the percentage of colony inhibition of *Pestalotia* spp., which was 15.50, 18.25, 30.75 and 36.75% respectively when compared to the control (0 µg/ml). It also showed similar result 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 from *Ch.lucknowense* showed that hexane crude extract 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). It showed similar result to EtOAc crude which was 24.75, 37.50, 41.50 and 59.75% respectively, and MeOH crude which was 19.25, 38.63, 37.75 and 45.25% respectively (Table 2).

It observed 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.

Table 3. 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.globosum					
Hexane	6.23^{a}[1/]	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.

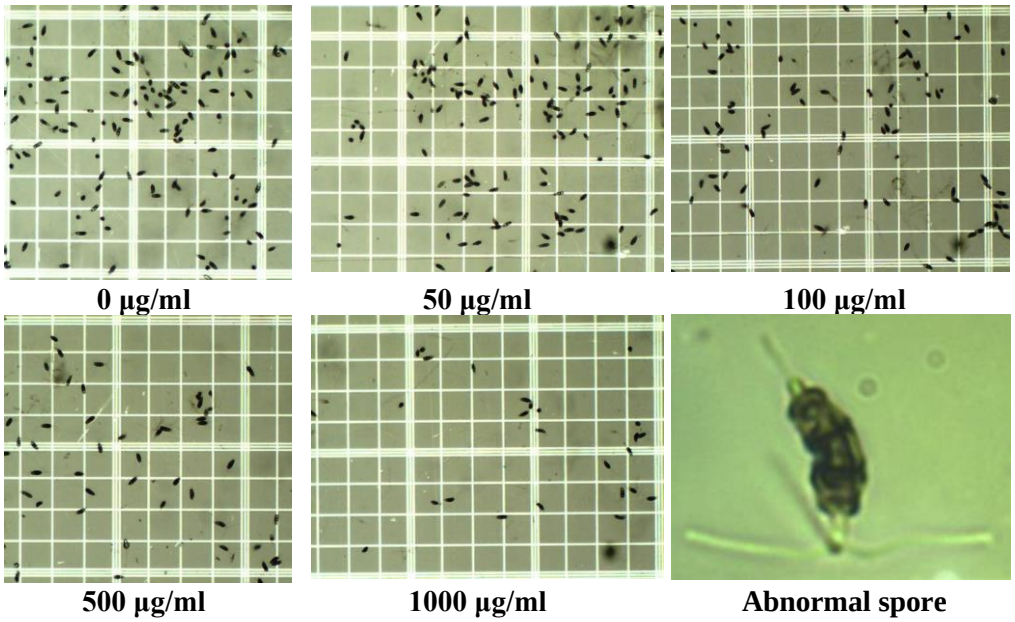

Fig. 3. Spore production of *Pestalotia* spp. at different concentrations

The result 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., which were 3.14 x 10^7 and 1.84 x 10^7 spores respectively when compared to the control (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 (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 crude 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 extract gave significant difference in the spore production of the pathogen that 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 (Table 3).

The result from *Ch.lucknowense* showed that hexane crude extract at the concentrations of 100, 500 and 1000 µg/ml gave significant difference in the spore production of *Pestalotia* spp., which was 6.08 x 10^7, 3.07 x 10^7 and 3.02 x 10^7 spores respectively when compared to the control (11.50 x 10^7 spores). EtOAc crude extract at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the spore production of *Pestalotia* spp., which was 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 (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 was 2.85 x 10^7 and 2.84 x 10^7 spores respectively when compared to the control (7.23 x 10^7 spores). It could say that only EtOAc crude extract 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 (Table 3).

Table 4. 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.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 3 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 3 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.

The result of spore inhibition showed that EtOAc and MeOH crude extract of *Ch.lucknowense*, and MeOH crude extract of *Ch.globosum* 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 in case of EtOAc crude of *Ch.lucknowense*; 21.80, 26.75, 54.62, and 54.65% respectively in case of MeOH crude of *Ch.lucknowense*; 21.40, 31.07, 67.12 and 76.34% respectively in case of MeOH crude of *Ch.globosum*. Meanwhile, hexane crude extract of *Ch.globosum* significantly inhibited spore production of *Pestalotia* spp. at the concentrations of 500 and 1000 µg/ml, which was 48.73 and 74.36% respectively when compared to the control (0 µg/ml). EtOAc crude extract 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 in case of EtOAc crude of *Ch.globosum*, and 46.52, 72.41 and 72.49% respectively in case of hexane crude of *Ch.globosum* (Table 4).

A comparison in the percentage of the spore inhibition of the pathogen among three crude extracts showed that EtOAc and MeOH crude of *Ch.globosum* gave significant higher percentage of the spore inhibition of *Pestalotia* spp., which were 54.75 and 48.98% respectively when compared to that 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 was 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 extract of *Ch.lucknowense* gave significant higher percentage of spore production of *Pestalotia* spp., which 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 was 359.21 µg/ml. The ED50 value of EtOAc crude was not so different from that of hexane crude, which was 200.12 µg/ml (Table 4).

A comparison in the percentage of spore inhibition of the pathogen among the treatments showed that 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.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 was 12.04, 35.60, 57.99 and 82.14% respectively on the average of three crude extracts. 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* (Table 4).

Discussions

All tested crude extracts of *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.lucknowense* significantly inhibited colony growth of *Fusarium oxysporum* f.sp. *lycopersici* causing wilt disease in tomato which were 5.5-28.5%. It also demonstrated that all tested crude extracts of *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.globosum* inhibited *Drechslera oryzae* causing leaf spot of rice with the ED50 of 10.15, 49.74 and 63.01 µg/ml. Another report from Sibounnavong (2012) also showed that Hexane, EtOAc and MeOH from *Ch.lucknowense* inhibited *F. oxysporum* f.sp. *lycopersici* NKSC02 with the ED50 of 921, 393 and 53 µg/ml respectively. 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* produces chaetoglobosin C which inhibits some pathogens. Later, Park *et al*. (2005) proved that *Ch.globosum* produces chaetoviridin A to control rice blast, wheat leaf rust and tomato late blight.

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