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Chaetomium spp. as Biocontrol Potential to Control Tea and Coffee Pathogens in Vietnam

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Abstract The effective isolates of *Chaetomium cochliodes*, *Chaetomium bostrychodes* and *Chaetomium gracile* were isolated in Vietnam. Root pathogen of coffee causing wilt was isolated and identified as *Fusarium roseum* and leaf anthracnose of coffee was isolated and identified as *Colletotrichum gloeosporioides*. The witling tea was isolated the pathogen from roots and identified as *Fusariumroseum*. Result showed that in bi-culture antagonistic tests, *C. gracile*, *C. bostrychoides* and *C. cochliodes* could inhibit the colony growth and spore production of *F. roseum* causing wilt of coffee tree. With this, *C. cochliodes*could inhibit the spore production at 62.82 %. *C. gracile*, *C. bostrychoides* and *C. cochliodes* could inhibit the colony growth and spore production of *F. roseum* causing wilt of coffee tree. With this, *C. cochliodes* could inhibit the spore production at 50.49 %. *C gracile*, *C. bostrychoides* and *.C cochliodes*could inhibit the colony growth and spore production of *F. roseum* causing wilt of coffee tree. With this, *C. cochliodes*could inhibit the spore production at 81.26 %. Moreover, all tested crude extracts at 1,000 ppm gave the best inhibition of all tested pathogens. Crude extracts with hexane, ethyl acetate and methanol of *C. cochliodes* inhibited spore production of *F.roseum* causing wilt of coffee 60.87, 78.16 and 74.57 %, respectively. Crude extracts with hexane, ethyl acetate and methanol of *C. cochliodes*inhibited spore inhibition of *C. gloeosporiodes* causing anthracnose of coffee beans 74.76, 76.50 and 67.63 %, respectively. Crude extracts with hexane, ethyl acetate and methanol of *C. cochliodes*inhibited spore inhibition of *F. oxysporum* causing wilt of tea 71.32, 80.19 and 73.76 %, respectively. Further research findings would necessary do for optimum nutritional requirement, environmental impacts, toxicology, formulation development and extension to the growers in the field trials. However, *C. cochliodes*, *C. gracile* and *C. bostrychodes* are reported for the first time to inhibit *F. roseum* causing wilt of tea and coffee, *F. oxysporum* causing wilt of tea and *C. gloeosporiodes* causing coffee anthracnose.

Keywords: *Chaetomium cochliodes*, *Chaetomium bostrychodes, Chaetomium gracile*

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Introduction

Tea *(Camellia sinensis)* and coffee (*Coffea arabica*) is the common crops in Vietnam. Now, tea area in Vietnam is about 135 000 hectares, coffee area is approximately 500 000 ha. Tea and coffee products are exported to many countries around the world, providing important foreign currency for Vietnam and is the main income of whom plant tea and coffee. Area and production of tea and coffee in the Vietnam continues to increase. Along with the intensive cultivation of tea and coffee yield, the number of pests has also increased, especially pathogens and the amount of pesticides and polluting the environment, visual impact to worker's health and residues in products reduces the commercial value. Until now, the use of bio-pesticide for tea and coffee in Vietnam is still limited because the tea and coffee growers are not aware of the benefits of the use of biological drugs. On the other hand there'is almost no research and application of biological disease control products for tea and coffee (Phong *et al*., 2014). Soytong *et al.* (2001) satated that the research and development of biological control agents for use against plant diseases have been undertaken for several years in both government and private sectors, as natural agents are needed to take the place of chemical fungicides. The problem associated with the use of hazardous chemicals for disease control has received increasing attention worldwide, due to the fact that pathogens become resistant to chemical fungicides and the resulting environmental pollution and ecological imbalances. The implementation of practical integrated biological control technology to control plant diseases has been successfully introduced to growers in China, Philippines, Russia, Thailand and Vietnam by using new broad spectrum biological fungicides from *Chaetomium* (Thailand Patent No. 6266, International Code: AO 1N 25/12 and registered as Ketornium® mycofungicide). Since 1989, the biological product has been developed and improved from 22-strains of Chaetomium cupreum CCOI-CC I0 and *Chaetomium globosum* CGOI-CG 12 in the form of pellet and powder formulation. The formulation has successfully been applied to infested field-soils with integrated with cultural control measures and organic amendments for the long-term protection of Durian (*Durio zibethinus* L.) and Black Pepper (*Piper nigram* L.) caused by Phytophthora palmivora, Tangerine (*Citrus reticulata* Blanco) caused by Phytophthora parasitica and Strawberry (*Fragaria* spp.) caused by Phytophthora cactorum, Wilt of Tomato (*Lycopersicon esculentum* L.) caused by *Fusarium oxysporum* f. sp. *lycopersici* and Basal rot of Corn (*Zea mays* L.) caused by *Sclerotium rolfsii* against *Phytophthora* rot. Biological products are useful, not only for the protection against plant diseases, but can also be used for curative effects of plant diseases. Originating from that fact, with the help of biocontrol Research Unit and

Mycology Section, Department Plant Production Technology, Faculty of Agricultural Technology, King's Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. The aim of research project was to find out the original effective isolates of *Chaetomium* and screened to control some plant pathogens in Tea and Coffee in Vietnam.

Materials and methods

Isolation of pathogen from tea and coffee diseases

The pathogens fro tea and coffee diseases were isolated by tissue transplanting technique. The infected roots of tea and antharcnose of coffee beabs and leaves were cut into small pieces, then surface disinfested for 1 minute in 10 % clorox, and washed in sterile water then transferred to water agar (WA; consisted of 20 g agar and 1,000 ml distilled water) and incubated at room temperature (27-30oC). The mycelia growing out of the plant tissues were then transferred aseptically to potato dextrose agar (PDA; consisted of 200 g potato, 20 g dextrose, 20 g agar, and 1,000 ml distilled water), isolated to pure culture. The pure cultures were maintained in PDA slant at room temperature.

Isolation of Chaetomium spp

Baiting technique was used to isolate Chaetomium spp. Soil samples were placed in a sterile Petri dish and moistened with sterile water. The sterile pieces of rice straw and filter papers were placed on the top of soil. The Petri dish was incubated at the room temperature and periodically observed. The mycelium or the fruiting bodies of the fungus grown on the pieces of rice straw and filter papers were transferred to the isolating medium (water agar, WA). The hyphal tip was cut and transferred to PDA to get pure culture (Soytong, 1989).

All isolates obtained from baiting techniques were morphologically identified by observing the characteristic of the hypha, ascomata, asci, ascospores, and other specific structures, measured and taken photo under compound microscope. Those isolates were taken to screen for biological control activities against plant pathogens from tea and coffee.

Bi-culture antagonistic test

The experiments were conducted using the methods of Soytong (1992), Sibounavong (2009) and Charoenporn (2010). *Chaetomium* spp were tested for their antagonistic abilities to control tea and coffee pathogens. The experiment

was conducted by using bi-culture antagonistic test in Completely Randomized Design (CRD) with four replications. Means were compared by DMRT at P = 0.05 and P = 0.01. *Chaetomium* spp were separately grown on PDA for 10 days at room temperature. The agar plug was removed with a sterile cork borer (3 mm. diameter) from the leading edge of colony and placed on the middle of a half of Petri dishes containing PDA (9-cm diameter). The agar plug of pathogen at the age of 7 days at peripheral area was placed on the opposite side of Petri dish. The Petri dish contaning PDA was placed only the agar plug of Chaetomium or pathogen was served as control. All tested Petri dish were incubated at room temperature for 10 days.

Data were collected as colony diameter and number of spore of pathogenic fungus and computed to percent inhibition as follows:-

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100$$

A = colony diameter or number of conidia produced by the pathogen on the control petries.

B = colony diameter or number of conidia produced by the pathogen on the pathogen was cultured opposite an antaginistic fungus.

Data was statistically analyzed by Sirichai 6.0. Analysis of variance at p = 0.05 and p = 0.01. .\

Bioactivitiy tests

Chaetomium spp were separately cultured on PDA for 10 days and transferred to Petri dish containing potato dextrose broth (PDB). The culture was incubated at room temperature for 30 days, thereafter filtered to yield fungal biomass. The fungal biomass were air dried, ground and weighted. The ground fungal biomass was extracted successively with hexane, ethyl acetate and methanol. Hexane was added into the flask containing ground mycelium and incubated for 5 days, then filtrated with filter paper. The filtrate was further extract through rotary vacuum evaporator to yield crude extract. The ethyl acetate was added into the marc and incubated for 5 days then filtered and evaporated the solvent to get crude extract. The methanol crude extract was as described above (Suwannapong, 2004). Crude extracts were kept in refrigerator until used. The experiment was conducted using 3x6 factorials in CRD with 4 replications. Factor A was crude extracts of Chaetomium sp which extracted with hexane, ethyl acetate, and methanol. Factor B was the concentration of crude extract at 0 (control), 10, 50, 100, 500, and 1,000 µg/ml. Each crude extract in each concentration was dissolved in Dimethylsulfoxide (DMSO), mixed with PDA before autoclaving at 121oC, 15 lbs/inch2 for 15 min. The

most virulent isolate of Rigidoporus microporus was cultured on PDA and incubated at room temperature for 6 days. The agar plug from actively growing colony was transferred and placed in the middle of PDA plate (5-mm diameter) incorporating with each concentration of crude extract, then incubated at room temperature for 5 days. The culture in PDA which mixed with DMSO was served as control. Data were collected as colony diameter (cm). Treatment means were compared using DMRT at P = 0.05 and P = 0.01. Effective dose (ED_{50}) of each crude extract was computed by probit analysis.

Results

Isolation of Chaetomium spp

It is found many isloates of Chaetomium spp and morphologicaly identied into species level as follows:- *Chaetomium bostrychodes*, *Chaetomium cochliodes* and *Chaetomium gracile*. With this, *C. cochliodes* belongs to Ascomycotina, Pyrenomycetes, Chaetomiales, Chaetomiaceae which showing perfect stage. It produces perithecia, cylindricall asci and 8-ascospore per ascus which reported by Soytong (1992), Soytong *et al*. (2001), Tveit and Moore (1954) and Johnston and Booth (1983).

Isolation of pathogen from tea and coffee diseases

The pathogen from root coffee was isolated and identified as *Fusarium roseum* and coffee leaf anthracnose was identified as *Colletotrichumgloeosporioides*. The witling tea was isolated the pathogen and identified as *Fusarium roseum.*

Bi-culture antagonistic test

C. gracile could inhibit the colony growth and spore production of *F. roseum* causing wilt of coffee tree at 32.96 and 52.97 %, respectively. *C. bostrychoides*could inhibit the colony growth and spore production of *F. roseum* causing wilt of coffee tree at 28.51 and 76.35 %, respectively.*C. cochliodes* could inhibit the colony growth and spore production of *F. roseum* causing wilt of coffee tree at 31.11 and 62.82 %, respectively (Table 1). *C. gracile* could inhibit the colony growth and spore production of *C. gloeosporiodes* causing anthracnose of coffee beans at 40.00 and 45.96 %, respectively. *C. bostrychoides* could inhibit the colony growth and spore production of *C. gloeosporiodes* causing anthracnose of coffee beans at 45.37 and 57.91 %, respectively. *C. cochliodes*could inhibit the colony growth and

spore production of *C. gloeosporiodes* causing anthracnose of coffee beans at 41.85 and 50.49 %, respectively (Table 2). *C. gracile* could inhibit the colony growth and spore production of *F. oxsporum*causing wilt of teas at 30.92 and 72.11 %, respectively. *C. bostrychoides* could inhibit the colony growth and spore production of *F. oxsporum* causing wilt of teas at 36.66 and 75.69 %, respectively. *C. cochliodes*could inhibit the colony growth and spore production of *F. oxsporum* causing wilt of teas at 30.92 and 81.26 %, respectively (Table 3). Soytong (2014) reported *C. cochliodes* proved to be antagonized *D. oryzae* causing leaf spot of rice varPitsanulok 2. It could inhibit colony growth and spore production of *D. oryzae* as 71.55 % in bi-culture antagonistic test which similar report of Soytong (1992). Bi-culture test showed lysis of pathogen spore which served as control mechanism (Soytong, 2005).

Bioactivitiy tests

Crude extracts with hexane of *C cochliodes* at concentrations of 10, 50, 100, 500, and 1,000 g/ml were tested for spore inhibition of *F. roseum* causing wilt of coffee which were 46.47, 49.55, 53.75, 57.30 and 60.87 %, respectively. Crude extracts with ethyl acetate of *C cochliodes*at concentrations of 10, 50, 100, 500, and 1,000 g/ml inhibited spore production of *F. roseum* causing wilt of coffee which were 30.67, 43.49, 49.94, 72.82 and 78.16 %, respectively. Crude extracts with methanol of *C cochliodes*at concentrations of 10, 50, 100, 500, and 1,000 g/ml inhibited spore production of *F. roseum* causing wilt of coffee which were 48.03, 61.76, 64.78, 68.65 and 74.57 %, respectively (Table 5). Crude extracts with hexane of *C cochliodes* at concentrations of 10, 50, 100, 500, and 1,000 g/ml inhibited spore production of *C. gloeosporiodes* causing anthracnose of coffee beans which were 29.60, 39.13, 43.54, 57.45 and 74.76 %, respectively. Crude extracts with ethyl acetate of *C cochliodes* at concentrations of 10, 50, 100, 500, and 1,000 g/ml inhibited spore production of *C. gloeosporiodes* causing anthracnose of coffee beans which were 44.92, 54.63, 64.20, 69.55 and 76.50 %, respectively. Crude extracts with methanol of *C cochliodes*at concentrations of 10, 50, 100, 500, and 1,000 g/ml inhibited spore production of *C. gloeosporiodes* causing anthracnose of coffee beans which were 27.59, 32.67, 42.10, 54.62 and 67.63 %, respectively (Table 6). Crude extracts with hexane of *C cochliodes*at concentrations of 10, 50, 100, 500, and 1,000 g/ml inhibited spore production of *F.oxysporum* causing wilt of tea which were 38.05, 49.68, 57.46, 62.53 and 71.32 %, respectively. Crude extracts with ethyl acetate of *C. cochliodes* at concentrations of 10, 50, 100, 500, and 1,000 g/ml inhibited spore production of *F. oxysporum* causing wilt of tea which were 35.83, 51.51, 66.76, 73.13 and 80.19 %, respectively. Crude extracts with methanol of *C. cochliodes* at concentrations of 10, 50, 100, 500, and 1,000 g/ml

inhibited spore production of *F. oxysporum* causing wilt of tea which were 38.60, 51.43, 63.47, 68.01 and 73.76 %, respectively (Table 7).

Phong *et al.* (2014) reported on grey blight disease of tea caused by *Pestalotia* spp. Hexane, EtOAc and MeOH crude extracts from *Ch. globosum* and *C. lucknowense* were proved as antifungal substances against *Pestalotia* spp. Result showed that ethyl acetate crude extract of *C. lucknowense* inhibited spore production of *Pestalotia* spp at ED_{50} of 86 µg/ml. The ED50 of crude ethyl acetate of *C. globosum* to inhibit spore production *Pestalotia* spp at 154 µg/ml. Hexane crude extract of Ch.lucknowense inhibited spore production of *Pestalotia* spp at ED_{50} of 200 µg/ml.

It may possible to prove for control mechanism as antibiosis, due to the tested *C. cochliodes*is used as same isolate reported by Nutchanat *et al* (2009) who demonstrated that could produce four new depsidones, mollicellins K-N (1-4), and six known depsidones, mollicellins B (5), C (6), E (7), F (8), H (9), and J (10). Among these isolates,1-3, 5-7, and 10 exhibited antimalarial activity against *Plasmodium falciparum*. Only 1 exhibited antimycobacterial activity against *Mycobacterium tuberculosis* and antifungal activity against *Candida albicans* using in vitro assays for human pathogens. In addition, 1-10 showed cytotoxicity against the KB, BC1, NCI-H187, and five cholangiocarcinoma cell lines.The research finding found that *C. gracile* could inhibit *F. roseum* and *F. oxysporum* causing wilt of tea and coffee which similar report with Soytong et al (2001) who stated that *C. gracile* could inhibit *F. oxysporum* f sp *lycopersici* causing tomato wilt. However, *C. gracile*, *C. bostrychodes*, *C. cochliodes* that reported for the first time to inhibit *F. roseum* causing wilt of tea and coffee, *F. oxysporum* causing wilt of tea and *C. gloeosporiodes* causing coffee anthracnose.

Table 1. Effect of *Chaetomium* isolates on the growth of *Fusarium roseum* causing wilt in coffee trees

Treatments	Colony diameter (cm)	Inhibition of colony growth (%)	Number of spore (x 10^7)	Inhibition of spore (%)
Fusariumroseum (Control)	9.00 a	---	47.46 a	
*C. gracile*vs*F. roseum*	6.03 b	32.96 a	22.33 b	52.97 c
*C. bostrychoides*vs*F. roseum*	6.43 b	28.51 a	11.20 d	76.35 a
*C. cochliodes*vs*F. roseum*	6.20 b	31.11 a	16.66 c	64.82 b
CV (%)	4.03	11.60	11.03	4.83

Table 2. Effect of *Chaetomium* isolates on the growth of *Colletotrichum gloeosporiodes* causing anthracnose of coffee beans

Treatments	Colony diameter (cm)	Inhibition of colony growth (%)	Number of spore (x 10^7)	Inhibition of spore (%)
C gloeosporiodes (Control)	9.00 a		80.16 a	
C. gracile vs *C. gloeosporiodes*	5.40 b	40.00 b	43.40 b	45.96 a
C. bostrychodes vs *C. gloeosporiodes*	4.91 c	45.37 a	39.30 b	50.91 a
C. cochliodes vs *C. gloeosporiodes*	5.23 bc	41.85 ab	39.66 b	50.49 a
CV (%)	2.06	3.83		

Table 3. Effect of *Chaetomium* isolates on colony growth of *Fusarium oxysporum* causing wilt of tea

Treatments	Colony diameter (cm)	Inhibition of colony growth (%)	Number of spore (x 10^7)	Inhibition of spore (%)
Fusarium oxysporum (Control)	9 a		6.56 a	
C. gracile vs *F. oxysporum*	6.21 b	30.92 a	1.83 b	72.11 b
C. bostrychodes vs *F. oxysporum*	5.70 b	36.66 a	1.60 bc	75.69 b
C. cochliodes vs *F. oxysporum*	6.21 b	30.92 a	1.23 c	81.26 a
CV (%)	6.86	18.18	9.58	2.62

Table 4. Effect of *Chaetomium* isolates on colony growth of *Fusarium roseum causing wilt of tea*

Treatments	Colony diameter (cm)	Inhibition of colony growth (%)	Number of spore (x 10^7)	Inhibition of spore (%)
F. roseum (Control)	9.00 a		13.43 a	
C. gracile vs *F . roseum*	5.56 b	38.14 a	6.00 b	55.41 b
C. bostrychodes vs *F .roseum*	5.40 b	40.00 a	3.36 c	74.97 a
C. cochliodes vs *F . roseum*	5.25 b	41.66 a	5.50 b	59.04 b
CV (%)	4.07	8.24	9.50	3.99

Table 5. Effect of crude extracts from *Chaetomium cochliodes* for spore production of *Fusarium roseum* causing wilt of coffee

Crude extracts	Concentrations (µg/ml)	Number of spores (X10^6)	spore inhibition (%)
Crude hexane	0	7.55 e	---
	10	4.05 gh	46.47 gh
	50	3.81 gh	49.55 gh
	100	3.48 gh	53.75 fg
	500	3.22 gh	57.3 ef
	1000	2.96 h	60.87 def
Crude ethyl acetate	0	17.16 a	---
	10	11.85 b	30.67 i
	50	9.65 cd	43.49 h
	100	8.56 de	49.94 gh
	500	4.75 fg	72.82 ab
	1000	3.71 gh	78.16 a
Crude methanol	0	10.85 bc	---
	10	5.53 f	48.03 gh
	50	4.12 fgh	61.76 cde
	100	3.78 gh	64.78 cd
	500	3.32 gh	68.65 bc
	1000	2.68 h	74.57 ab
CV (%)		15.42%	8.37%

Table 6. Effect of crude extracts from *Chaetomium cochliodes* for spore production *Colletotrichum gloeosporiodes* causing anthracnose of coffee

Crude extracts	Concentrations (µg/ml)	Number of spores (X10^6)	spore inhibition (%)
Crude hexane	0	22.36 b	---
	10	15.78 cd	29.60 f
	50	13.65 de	39.13 e
	100	12.63 ef	43.54 e
	500	9.47 gh	57.45 d
	1000	5.63 i	74.76 ab
Crude ethyl acetate	0	26.55 a	---
	10	14.50 de	44.92 e
	50	12.03 efg	54.63 d
	100	9.30 gh	64.20 c
	500	7.78 hi	69.55 bc
	1000	6.00 i	76.50 a
Crude methanol	0	17.56 c	---
	10	12.70 ef	27.59 f
	50	11.80 efg	32.67 f
	100	10.15 efh	42.10 e
	500	7.96 hi	54.62 d
	1000	5.67 i	67.63 c
CV (%)		15.94 %	8.02%

Table 7. Effect of crude extracts from *Chaetomium cochliodes* for spore production *Fusarium oxysporum* causing wilt of tea

Crude extracts	Concentrations (µg/ml)	Number of spores ($X10^6$)	spore inhibition (%)
Crude hexane	0	0.93 c	---
	10	0.58 de	38.05 h
	50	0.47 efg	49.68 g
	100	0.39 fgh	57.46 f
	500	0.35 gh	62.53 f
	1000	0.26 h	71.32 bc
Crude ethyl acetate	0	1.41 a	---
	10	0.90 c	35.83 h
	50	0.68 d	51.51 g
	100	0.47 efg	66.76 cde
	500	0.38 gh	73.13 b
	1000	0.28 h	80.19 a
Crude methanol	0	1.05 b	---
	10	0.65 d	38.60 h
	50	0.51 ef	51.43 g
	100	0.38 gh	63.47 de
	500	0.33 h	68.01 cd
	1000	0.27 h	73.76 b
CV (%)		14.06 %	5.33 %

Discussion

Crude hexane of Chcochliodes inhibited spore production of pathogen at concentrsationof 1,000 µg/ml (93.85%) which ED_{50} was 66.45 ppm. This similar report showed that crude extract of *Chaetomium* sp could inhibit *Drechslera sorokiniana* causing spot blotch of wheat (Biswas *et al.,* 2002). Soytong (2014) reported that *C. cochliodes* proved to be a new antagonistic fungus against brown leaf spot of rice var Pittsanulok 2 caused by *Drechslera oryzae*and inhibited inoculum production of 71.55 per cent. Crude extracts from *C. cochliodes* using hexane, ethyl acetate and methanol at 1,000 ppm could significantly inhibited the inoculum production of rice pathogen 93.85 per cent which ED_{50} was 66.45 ppm. Bioformulation of *C. cochliodes* showed that bio-powder form gave significantly highest to control leaf spot and highest plant growth when compared to the non-treat control, followed by applying crude extract of *C. cochliodes*, benlate and spore suspension of *C. cochliodes*. Moreover, bio-powder form gave significantly increased in plant growth over 44 % and followed by crude extract of *C. cochliodes*, spore suspension of *C.cochliodes* and benlate.

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References

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 Microbiology Research. 9:5836-5844.

Phonkerd, N., Kanokmedhakul, S., Kanokmedhakul, K., Soytong, K., Prabpai, S. and Kongsearee, P. (2008). Bis-spiro-azaphilones and azaphilones from the fungi *Chaetomium cochliodes* vth01 and *C. cochliodes* Cth05. Tetrahedron 64:9636-9645.

Phong, N. H., Wattanachai, P., Soytong, K. and ThiLuu, N. (2014). Antimicrobial substances from *chaetomium* spp. Against pestalotia spp. Causing grey blight disease of tea. Journal of Agricultural Technology 10:863-874.

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.* Journal of Agricultural Technology 5:75-84.

Soytong, K. and Quimio, T. H. (1989). A taxonomic study on the philippines species of *chaetomium*". The Philippine Agriculturist 72:59–72

Soytong, K., Kanokmedhakul, S., Kulongviyapa, 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.

Soytong, K. (1992). Antagonism of *Chaetomium cupreum* to *Pyricularia oryzae*. Journal of Plant protection in the tropics 9:17-24.

Soytong, K., Srinon, W., Rattanacherdchai, K., Kanokmedhakul, S. and Kanokmedhakul, K. (2005). Application of Antagonistic Fungi to Control Antracnose Disease of Grape. Journal of Agricultural Technology 1:33–41.

Soytong, K. (2014). Bio-formulation of *chaetomium cochliodes* for controlling brown leaf spot of rice. Journal of Agricultural Technology 10:321-337.

Tviet, M. and Moor, M. B. (1954). Isolates of chaetomium that protect oats from *helminthosporiumvictoriae*. Phytopathology 44:686–689.

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