The new antagonistic fungus, *Emericella nidulans* strain EN against Fusarium Wilt of Tomato

P. Sibounnavong[1*], D.C. Cynthia. [1], S. Kanokmedhakul[2] and K. Soytong[3]

[1]Department of Biology, Central Luzon State University, Munoz, Philippines.
[2]Department of Chemistry. Faculty of Science, Khonkhan Unversity, Thailand.
[3]Department of Plant Pest Management, KMITL, Bangkok, Thailand

Sibounnavong, P., Cynthia, C.D., Kanokmedhakul, S. and Soytong, K. (2008). The new antagonistic fungus, *Emericella nidulans* strain EN against Fusarium Wilt of Tomato. Journal of Agricultural Technology 4(1): 89-99.

The research findings on the optimum growth of *Emericella nidulans* strain EN, a promising antagonist, which cultured on Coconut glucose medium at pH 7 gave the highest spore production of 87.81 x 10^6 spores and followed by Potato dextrose medium at pH 7 and Coconut glucose medium at pH 9 which the number of spores were 66.75 x 10^6 and 62.64 x 10^6 spores, respectively. The tested fungus growing on Coconut glucose medium at pH 3 gave dried mycelium weight of 0.625 g. Moreover, the SKP02-bioactive compound from *E. nidulans* strain EN for inhibition of sporulation of *F. oxysporum* f sp *lycopersici* showed that the concentration of 1000 ug/ml gave the highest inhibition of sporulation which the ED_{50} was 211 ug/ml. It was significantly inhibited at all tested concentrations when compared to the control (0 ug/ml). In this research finding of *E. nidulans* strain EN is reported for the first time as a new antagonistic fungus against *F. oxysporum* f. sp. *lycopersici.*

Key words: *Emericella nidulans*, Fusarium Wilt, Tomato

Introduction

Tomatoes have become one of the most popular and widely grown vegetables in the world and mainly reason of yield loss is the problems of pest and diseases. Fusarium wilt is one of the most prevalent and damaging tomatoes among several varieties in highland area wherever tomatoes are grown intensively, and its outbreaks occur wherever the tomatoes are grown, and the fungal pathogen can be survived in soils even if there is sterilization and crop rotation (Agrios, 1988). The use of chemical fungicides to control the disease is one of the practical methods for the growers, but there are many reports indicated that the fungus become resistant to fungicides and can be polluted to the surrounding environments. Biological control of plant pathogens is of increasing interested to plant pathologists and many researchers

* Coresponding author: Phutthasone Sibounnavong,e-mail: sone_lp@hotmail.com

(Soytong,1992). The growth of fungi require various media for their optimum growing and producing their structures for survival in the surrounding environment (Soytong, 1990). Research interest in biological control of plant pathogens has become extremely active. One of the main reasons for this burst of research activity is the hazardous impact of various fungicides and other agrochemical on ecosystem (Soytong, 1990). The search for promising microbial antagonists have been increasingly interested and used to control the diseases. There are numerous reports indicated that *Chaetomium globosum* could control seedling blight of wheat caused by *Helminthosporium victoriae* (Tveit and Moore, 1954) but it is needed to know how the antagonist grow well in various conditions. Spraying the spore suspension of *Ch. globosum* in the optimum conditions could significantly control apple scab caused by *Venturia inequalis* (Cullen *et al*., 1984). *Chaetomium cupreum* is also reported to be antagonistic to *Phomopsis sojae* which caused seed-borne pathogen of soybean (Manandhar, 1986). *Ch. globosum* is reported to be significantly suppressed tomato wilt caused by *F. oxysporum* f. sp. *lycopersici* (Soytong, 1991) and reported that *Ch.cupreum* could be controlled tomato wilt in the fields (Soytong, 1992).

The bioactive compounds from different fungi have been reported to inhibit many plant pathogenic fungi (Amemiya**,** 1994). The research on biological control agents against plant pathogens are increasingly interested among scientists in recent years. The reports on secondary metabolites from the antagonists to inhibit pathogens are also studied by several workers. *Emericella* is a fungus with a worldwide distribution. *Emericella* species are the perfect states of *Aspergillus*, a common hyphomycete (Domsch *et al*., 1993). *Emericella* sp. have been reported for the source of a variety of natural products. Kanokmedhaklul *et al*. (2007) reported that isolation of bioactive constituents from fungi *Chaetomium* sp., *Emericella* sp., afforded numerous type of compounds such as indol-3-yl-[13]cytochalasans, azaphilones, xanthones, xanthoquinodines, diterpenoids and diketopiperazine derivatives. Some of these compounds exhibited activity towards *Plasmodium falciparum* (cause of malaria), *Mycobacterium tuberculosis* (TB), *Candida albicans* and cancer cell lines (KB, BC and NCI-H187). Those compounds also showed activity against plant diseases such as *Phythophtora* sp. causing root rot of plants and *Colletotrichum gloeosporioides* causing anthracnose disease. In this research finding of *Emericella nidulans* strain EN is firstly reported to released some antibiotic substances against human pathogens. Despite description of *E. nidulans*, little information is available on its biology, including environmental requirement for growth. This information would valuable to further study on biological control using antagonistic fungi.

The objective of this study was to find out the optimum media for the growth of *E. nidulans* and to test the bioactive compound from *E. nidulans* strain EN to inhibit *F. oxysporum* f. sp. *lycopersici* causing tomato wilt.

Materials and Methods

Culture of the tested pathogen and antagonistic fungus

F. oxysporum f. sp. *lycopersici* was isolated tomato wilt from previous experiment and *E. nudulans* strain EN is an antagonistic fungus offered by Dr. Kasem Soytong (KMITL, Bangkok, Thailand) were transferred onto potato dextrose agar (PDA), incubated at room temperature (27-30 C) for 4 days before use in the experiment.

Effect on spore production and mycelia growth of Emericella nidulans strain EN on solid and broth media on various pH levels

The experiments were done by using two factors factorial (2x4) experiment in Completely Randomized Design (CRD) with four replications. There were two experiments which conducted both on agar and broth media. With this, factor A was media which A1 = Potato dextrose medium (PD) and A2 = coconut dextrose medium (CD) and factor B was pH level which B1 = pH 3, B2 = pH 5, B3 = pH 7 and B4 = pH 9. Media preparation:- Potato dextrose medium (PD) consisted of potato 200 g, dextrose 20 g, and water 1000 ml which either added agar of 20 g (PDA) or no agar (PDB). Coconut dextrose medium (CD) consisted of coconut water 1000 ml, dextrose 20 g which either added agar of 20 g (CDA) or no agar (CDB). Both media were separately prepared and adjusted pH level with either HCl or NaOH to get the required pH level in electrical pH meter before brought to sterilize in autoclave at 121 C, 15 lbs/inch^2 for 20 min. The tested fungus, *E. nidulans* was cultured on PDA and was cut at peripheral colony with sterilized cork borer (ca 0.3 cm) to get the agar plugs. The culture agar plugs were transferred into each media with different pH level as in treatment combination. The inoculated plates or flasks were kept at room temperature approximately 27-30 C for 7 days before collection of data. The experiment was repeated two times.

Data collection were spore production, fresh weight (g) and dry weight (g). Data were analyzed for analysis of variance (ANOVA) for two factors factorial experiment in CRD using SIRICHAI STATISTICAL PROGRAM 6. Treatment means were compared with Duncan Multiple Range Test at P=0.05 and P=0.01.

Bioactive compounds from Emericella nidulans EN against Fusarium wilt of Tomato

The SKP02/E-bioactive compounds extracted from *E. nudulans* strain EN which offered from Assoc. Prof. Dr. Somdej Kanokmedhakul, Department of Chemistry , Khon Khan University, Thailand. The bioactive compound was tested to inhibit the growth of *F. oxysporum* f. sp. *lycopersici*. The experiment was done by using Completely Randomized Design (CRD) with 3 replications. Treatments were designed in different concentrations as follows:- 0, 10, 50, 100, 500 and 1,000 µg/ml. which mixed to potato dextrose agar (PDA). The bioactive compound in each concentration was dissolved by using 2 % methyl alcohol, then autoclaved at 121 C, 15 lbs/inch^2 for 20 min., and poured into sterilized Petri dishes. The tested pathogen was cultured on PDA and then incubated at room temperature for 5 days, then cut from actively growing colony margins by cork borer and placed either in the center of Petri dish or flask in each concentration and incubated at room temperature for 4 days.

Data were collected as colony diameter (mm) until the control plate growing almost full plates and spore production was counted by using Haemacytometer. Treatment means were computed analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and P=0.01. Effective Dose (ED_{50}) of compound was computed by probit analysis program. The experiment was repeated two times.

Results

Effect on spore production and mycelia growth of Emericella nidulans strain EN on solid and broth media on various pH level

Result showed that *E. nidulans* strain EN cultured on Coconut glucose medium (CD) at pH 7 gave the highest spore production and followed by Potato dextrose medium (PD) at pH 7 and Coconut glucose medium (CD) at pH 9 which the number of spores were 87.81 x 10^6 spores, 66.75 x 10^6 and 62.64 x 10^6 spores, respectively. It was showed that *E. nidulans* growing on the different broth media and pH levels which incubated on electrical shaker at room temperature (approx. 27-30 C) for 7 days revealed the tested fungus growing on Potato dextrose medium (PD) at pH 7 gave the highest fresh mycelium weight of 3.53 g which highly significant different when compared to the other treatments, and followed by the fungus growing on PD at pH 9, CD at pH5, 7 and 9 which the fresh mycelium weight were 3.11, 2.67, 3.18, 3.12 g, respectively. The lowest fresh mycelium weight was shown on growing fungus

on PD at pH 3 and CD at pH 3. It also revealed that the tested fungus growing on PD at pH 7 gave the highest dried mycelium weight of 0.445 g which highly significant different when compared to the other treatments, and followed by the fungus growing on PD at pH 9 which was 0.407 g and followed by growing the fungus in PD at pH 5 which the dried mycelium weight was 0.375 g.

There were not significant different when the fungus grew in CD at pH 5, 7 and 9. The lowest fresh mycelium weight was shown on growing fungus on PD and CD at pH 3 which were 0.005 g and 0.635 g, respectively. It was observed that dried mycelium weight in CD had less than dried mycelium weight in PD (Table 1).

Table 1. Growth of *Emericella nidulans* strain EN on different broth media and pH levels**.**

Media	pH	No of spore	Fresh weight(g)	Dry weight (g)
	3	1.37 d[1/]	0.05 c	0.005 d
PD	5	10.32 d	2.48 b	0.357 b
	7	66.75 b	3.53 a	0.445 a
	9	40.75 c	3.11 ab	0.407 ab
	3	0.87 d	0.23 c	0.625 a
CD	5	15.65 d	2.67 ab	0.164 c
	7	87.81 a	3.18 ab	0.197 c
	9	62.64 b	3.12 ab	0.170 c
CV (%)	-	24.98	19.24	16.56

[1/]Mean of four replications. Means followed by a common letter in each column is not significantly different by Duncan's Multiple Range Test (DMRT) at P=0.01.

Bioactive compounds from Emericella nidulans EN against Fusarium wilt of Tomato

Result showed that the SKP02-bioactive compound from *E. nidulans* strain EN gave significantly different to inhibit *F. oxysporum* f. sp. *lycopersici* at the concentrations of 50, 100, 500 and 1000 ug/ml which the colony diameter were 7.66, 6.00, 4.16 and 4.33 mm, respectively when compared to the control (0 ug/ml) and 10 ug/ml after incubation for 1 day as shown in Table 2 and Fig. 1. and 2. It was also gave significantly different to inhibit *F. oxysporum* f sp *lycopersici* at the concentrations of 100, 500 and 1000 ug/ml which the colony diameter were 17.33, 13.33 and 12.83 mm, respectively when compared to the control (0 ug/ml) and 10, 50 ug/ml after incubation for 2 days. But after incubation for 3 days, it was showed that all tested concentrations gave highly significantly different to inhibit the mycelia or colony growth at concentrations of 10, 50, 100, 500 and 1000 ug/ml which the colony diameter were 16.66, 23, 33, 21.33, 19.00 and 17.33 mm, respectively when compared to the control (33.33 mm).

The incubation period of 4 days were also showed that the tested concentrations of 10, 50, 100 and 1000 ug/ml gave highly significantly different to inhibit the mycelia or colony growth which the colony diameter were 30.66, 27.33, 24.66 and 30.66 mm, respectively when compared to the control (37.50 mm), except for the concentration of 500 ug/ml that was not significant different to the control.

Lastly, the incubation period of 5 days, it was showed that all tested concentrations gave highly significantly different to inhibit the mycelia or colony growth at concentrations of 10, 50, 100, 500 and 1000 ug/ml which the colony diameter were 33.50, 31.66, 29.50, 25.00 and 22.66 mm, respectively when compared to the control (39.00 mm).

The SKP02-bioactive compound from *E. nidulans* strain EN for inhibition of sporulation of *F. oxysporum* f. sp. *lycopersici* gave the highest inhibition of sporulation which the ED_{50} was 211 ug/ml and gave 73.68 per cent inhibition (Table 2).

Table 2. Effect of SPK02-bioactive compound of *Emericella nidulans* strain EN for inhibition of mycelia growth (colony diameter, mm) of *Fusarium oxysporum* f. sp. *lycopersici*

Conc. (µg/ml)	1 day	2 days	3 days	4 days	5 days
0	9.33^{a1}	21.00^{a}	33.33^{a}	37.50^{a}	39.00^{a}
10	9.83^{a}	21.33^{a}	26.66^{b}	30.66^{ab}	33.50^{ab}
50	7.66^{ab}	20.83^{a}	23.33^{bc}	27.33^{bc}	31.66^{abc}
100	6.00^{bc}	17.33^{b}	21.33^{cd}	24.66^{bc}	29.50^{bcd}
500	4.16^{c}	13.33^{c}	19.00^{cd}	22.33^{bc}	25.00^{cd}
1000	4.33^{c}	12.83^{c}	17.33^{d}	20.16^{c}	22.66^{d}
C.V.(%)	16.40	6.35	10.98	11.63	9.66

[1]/Average of three replications. Means followed by a common letter in each column are not significantly different by Duncan Mulitiple Range Test (DMRT) at P=0.01.

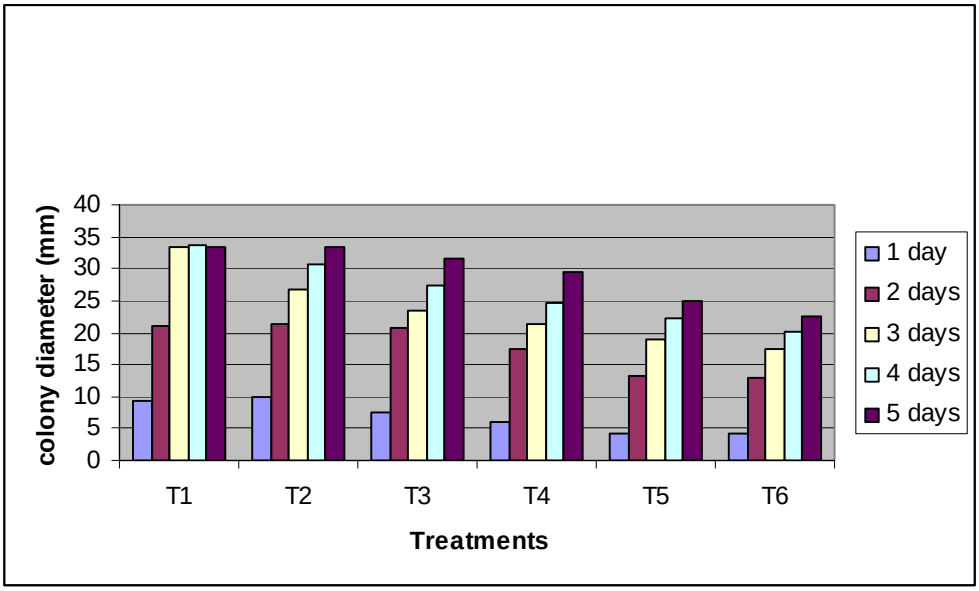

Fig. 1. Effect of SPK02-bioactive compound from *Emericella nidulans* for inhibition of *Fusarium oxysporum* f. sp. *Lycopersici.*

Table 3. Effect of SPK02-bioactive compound of *Emericella nidulans* strain EN for inhibition of sporulation of *Fusarium oxysporum* f. sp. *lycopersici*

Concentrations (ug/ml)	Average	Inhibition (%)
0	42.41 a[1]	----------
10	40.00 a	5.68[2]
50	33.25 ab	21.59
100	24.58 abc	42.04
500	16.83 bc	60.03
1000	11.16 c	73.68

[1]/Average of three replications. Means followed by a common letter are not significantly different by Duncan Mulitiple Range Test (DMRT) at P=0.01. C.V. = 27.33 %

[2]/Per cent Inhibition of sporulation = control (0 ppm) – treatment / control x 100. Effective Dose (ED_{50}) = 211 µg/ml.

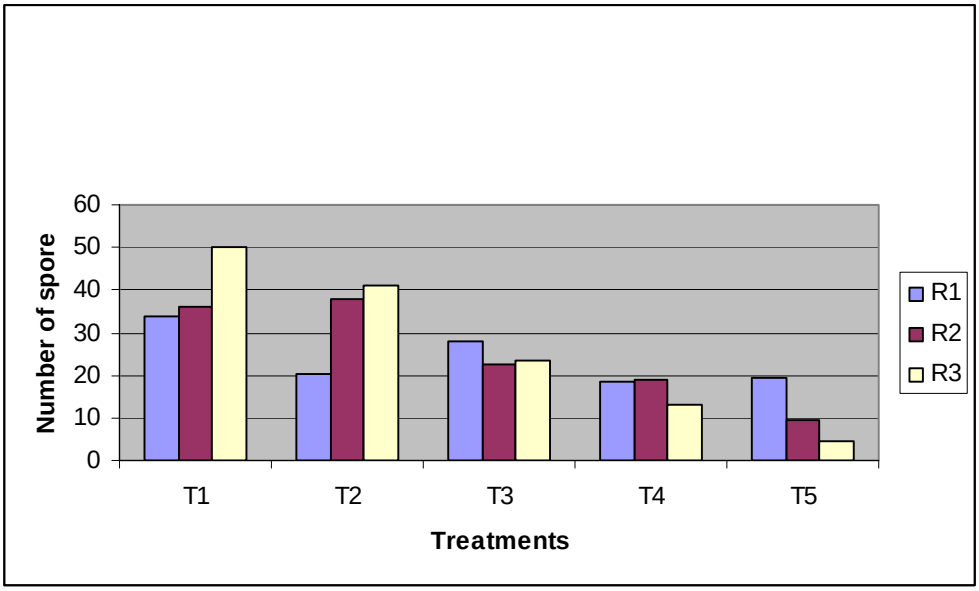

Fig. 2. Effect of SPK02-bioactive compound of *Emericella nidulans* on sporulatuion of *Fusarium oxysporum* f. sp. *lycopersici*

Discussion

It is clearly demonstrated that coconut water gave the best for spore production growing of the tested fungus, *E. nidulans*. This experiment showed that *E. nidulans* cultured on Coconut glucose (CD) at pH 7 was 87.81 x 10^6 spores that could produce the most sporulation. This may be concerned on the major chemical constituents of coconut water which are sugars and minerals and minor ones are fat and nitrogenous substances that serve as base food of the fungus (Fasola *et al*., 2007). However, Smith & Onions (1994) stated that many fungi grow well on PDA that encouraging the growth of mycelia and sporulation. Our result showed that *E. nidulans* grew on PDA at pH 7 gave more sporulation than the other pH levels.

In this study, the bioactive compound extracted from *E. nidulans* coded SKP02 gave the gave the highest inhibition of *F. oxysporum* f. sp. *lycopersici* which the ED_{50} value was 211 µg/ml. It was expressed a very good potent to inhibit the tesed pathogen at low concentration. Similarly result of bioactive compound against human was reported by Kanokmedhakul *et al.* (2001) which stated that antimycobacterial anthraquinone-chromanone compound and diketopiperazine alkaloid from the fungus *Ch. globosum* KMITL-N0802 could

inhibit *Mycobacterium tuberculosis*, a human pathogen and also reported that a bioactive triterpenoid and vulpinic acid derivatives from *Scleroderma citrinum* gave a good control to *M. tuberculosis* and the antifungal Azaphilones from the fungus, *Ch. cupreum* CC3003 gave a good control to *Candida albican*. Kanokmedhakul *et al*. (2003, 2006). Moreover, there are several reports indicated that the bioactive compounds could inhibit plant pathogenic fungi (Amemiya,1994oytong,2001).

This experiment, the tested bioactive compound from *E. nidulans* strain EN against *F. oxysporum* f. sp. *lycopersici* gave a positive effect to inhibit the spore production. The higher concentration gave better inhibition of spore production than the lower concentrations. Our research finding is also similar to Taechowisan et al (2005) which reported the application of secondary metabolites from endophytic *Streptomyces aureofaciens* CMUAc130 isolated from the root tissue of *Zingiber officinale* Rosc.. could inhibiy *Colletotrichum musae* and *Fusarium oxysporum*, the causative agents of anthracnose of banana and wilt of wheat, respectively. The other fungal metabolites, Hans *et al*. (2005) reported that the antifungal activity of chitinase from *Trichoderma harzianum* can be effectively lyses the hypha wall of the phytopathogenic fungus like *Colletotrichum gloeosporioides.*

The further research interesting is also to identify the active pure compounds to be tested the plant pathogenic fungi and formulated to be used by the farmers which may help to reduce the application of toxic chemical fungicides. However, Kanokmedhaklul *et al*. (2007) reported that isolation of bioactive constituents from *Emericella* sp., afforded numerous type of compounds such as indol-3-yl-[13]cytochalasans, azaphilones, xanthones, xanthoquinodines, diterpenoids and diketopiperazine derivatives. Some of these compounds exhibited activity towards *Plasmodium falciparum* (cause of malaria), *Mycobacterium tuberculosis* (TB), *Candida albicans* and cancer cell lines (KB, BC and NCI-H187). Those compounds also showed activity against plant diseases such as *Phythophtora* sp. causing root rot of plants and *C. gloeosporioides* causing anthracnose disease (Soytong, K; personal communication).

In this study, the tested bioactive compound from *E. nidulans* strain EN against *F. oxysporum* f. sp. *lycopersici* gave a positive effect to inhibit the spore production of tested plant pathogen. The higher concentration gave better inhibition of spore production than the lower ones. It is indicated that the SKP02-bioactive compound from *E. nidulans* strain EN could inhibit the spore production of the pathogen. The tested compound may possible has a potent of reduction of sporulatiuon or reduction the pathogen's inocula in term of reduce disease infection to the tomato plants.

References

Agrios, G.N. (1998). Plant Pathology, Third edition, Academic Press, Inc., London.p.408-415.

Amemiya, Y., Kondo, A., Hirukawa, T. and kato, T. (1994). Antifungal substances produced by *Chaetomium globosum*. Technical Bulletin of Faculty of Horticulture Chiba University 48(2): 13-18.

Cullen, D., Berbeem, F.M. and Andrews, J.H. (1984). *Chaetomium globosum*, antagonizes the apple scab pathogen, *Venturia inequalis* under field conditions. Can. J. Bot. 62,1814-1818.

Domsch, K.H., Gams, W. and T.H. Anderson. (1993). Compendium of Soil Fungi. Vol I.IHW-Verlag .459 p.

Fasola, T.R., J.S. Gbolagade and I.O. Fasidi. (2007). Nutritional requirements of *Volvariella speciosa* (Fr.Ex.Fr.) Singer, a Nigerian edible mushroom. Food Chemistry 100: 904-908.

Han, JS., Cheng J.H., Yoon T.M., Song J., Rajkarnikar, A., Kim, W.G., Yoo, I.D., Yang, Y.Y., and Suh, J.W. (2005). Biological control agent of common scab disease by antagonistic strain *Bacillus* sp. sunhua. Journal of Applied Microbiology 99(1): 213-221.

Kanokmedhakul, S., Kanokmedhakul, K., Phonkerd, N., Soytong, K., Kongsaree, P. and A. Suksamrarn. (2001). Antimycobacterial anthraquinone-chromanone compound and diketopiperazine alkaloid from the fungus *Chaetomium globosum* KMITL-N0802. Planta Medica 68: 834-836.

Kanokmedhakul, S., Kanokmedhakul, K., Prajuabsuk, T., Soytong, K., Kongsaeree, P. and A. Suksamran. (2003). A bioactive triterpenoid and vulpinic acid derivatives from *Scleroderma citrinum.* Planta Medica 69: 566-568.

Kanokmedhakul, S., Kanokmedhakul, K., Nasomjai, P., Loungsysouphanh, S., Soytong, K., Isobe, M., Kongsaeree, K., Prabpai, S. and Suksamran, A. (2006). Antifungal Azaphilones from the fungus, Chaetomium cupreum CC3003. J. Natural Products (69): 891-895.

Kanokmedhakul, S., Kanokmedhakul, K., Phonkerd, N., Thohinung, S., Moosophon, P., Lekphrom, R.,Buayairaksa, M., Soytong, K. and P. Kongsaeree. (2007). Bioactive compounds from fungi to control human and plant pathogens. Proc of the Conference on Mycology in Thailand. 23 June 2007, Chiang Mai University, Thailand.

Manandhar, P.N., Thapliyal, P.N. and J.B. Sinclair. (1986). Potential biocontrol fungi for selected soybean fungal pathogen. Biological Control and Cultural Test.1: 36.

Soytong, K. (1990). Biological control of tomato wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* using a new biofungicide. Proc of the International Conference on Biotechnology and Environmental Science, August, 21-24,1990. Chulabhorn Research Institute.

Soytong, K. (1991). Species of *Chaetomium* in Thailand and screening for their biocontrol properties against plant pathogens. Proc of the XII International Plant Protection Congress. Rio de Janeiro, Brazil, 11-16 August, 1991.

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. (1993). Biological control of plant pathogens using *Chaetomium* spp. Proc of the 6th International Congress of Plant Pathology. Canada. July, 28-August, 6, 1993.

Soytong, K. 1996. Chaetomium as a new broad spectrum mycofungicide. Proc. of the first International Symposium on Biopesticides, October 27-31, 1996, Thailand. 124-132 pp.

Soytong, K., Kanokmedhakul, S., Kukongviriyapan, 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.

Taechowisan, T., Lu, C., Shen, Y., and Lumyong, S. (2005). *Secondary metabolites from endophytic Streptomyces aureofaciens* CMUAc130 and their antifungal activity. Microbiology 151(3): 1691-1695.

Tveit, M. and Moore, M.B. (1954). Isolates of *Chaetomium* that protect oats from Helminthosporium victoriae.Phytopathology 44: 686-689.

.

(received 10 April 2008; accepted 26 May 2008)