International Journal of Agricultural Technology 2018 Vol. 14(7): 2181-2190
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

In vitro efficacy of *Chaetomium brasiliense* against *Pythium* spp. causing root rot disease of tangerine

Udompongsuk, M.[1] [*] , Soytong, K.[1], Kanokmedhakul, S.[2] and Kanokmedhakul, K.[2]

[1]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok, Thailand; [2]Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

Udompongsuk, M., Soytong, K., Kanokmedhakul, S. and Kanokmedhakul, K. (2018). *In vitro* efficacy of *Chaetomium brasiliense* against *Pythium* spp. causing root rot disease of tangerine. International Journal of Agricultural Technology 14(7): 2181-2190.

Abstract Root rot is one of the most serious disease in Tangerine which caused by *Pythium* spp. This study tested efficacy of *Ch. brasiliense* to control *Pythium* sp. by bi – culture, crude extracts and nano particles testing from *Ch. brasiliense* to control *Pythium* sp., in vitro. The results from the bi – culture testing, *Ch. brasiliense* inhibited mycelium growth and sporangia production by 42.50% and 48.41%, respectively. In crude extracts from *Ch. brasiliense* gave ED_{50} values of 30.15, 58.71 and 37.25 ppm for the hexane, EtOAc and MeOH, respectively. The efficacy of nano particles against *Pythium* sp. with the ED_{50} values of 2.69, 3.00 and 3.96 ppm for the hexane, EtOAc and MeOH, respectively.

Keywords: *Chaetomium brasiliense*, Root rot, Tangerine

Introduction

Thailand office of agricultural economics reported in 2007 – 2015 tangerine production tends to decrease from 757,000 to 141,000 ton due to yield loss and decline of tangerine tree. The major problem is root rot disease (Molina *et al*., 1998). Growers used fungicide for control this disease and were faced with high cost of production, poor yield and low prices. They quit cultivation of tangerines. In 2016, they turned to reactivate their orchards because the price hiked.

The symptoms after *Pythium* spp. infected, the leaves turn yellow and some drop, twinge and branch dieback. Roots turn soft and brown, sometimes bark cracks through which gum exudation. Plant will grow poorly, decline and die in the end (Vichitrananda, 1998). For controlling this disease has been applied chemical fungicides in orchards but application chemical fungicides have resulted in accumulation of toxics in environment and resistance of

[*] **Coressponding Author:** Udompongsuk, M.; **Email:** zw.mink@gmail.com

fungicides. To avoide them, there are many researches for alternative control by effective of biological control agents (BCAs) e.g. *Ch. globosum Ch. cupreum* and *Ch. lucknowense* were reported to control *Phytophthora palmivora and Phytophthora nicotianae* causing root rot in pomelo and citrus (Hung *et al.*, 2014; 2015). Chaetoglobosin – C that produce from *Ch.globosum* gave significantly inhibited colony and sporangia of *Phytophthora parasitica* (Quyet *et al*., 2016). The aim of this study was to evaluation of efficiency of *Ch. brasiliense* to control *Pythium* spp. causing root rot of tangerine, in vitro.

Materials and methods

Isolation and identification

Soil samples, at a depth of 15 – 30 cm, were collected from the tangerine orchards and isolated by baiting method. The soil samples were ground and placed into sterilized petri dish, added sterilized distilled water above the soil and floated leaf pieaces of tangerines (0.5 × 0.5 cm) on the water surface and incubated petri dish for 24-72 hours at room temperature. Place baits onto water agar (WA). When mycelia grown on WA agar were transferred to Potato Dextrose Agar (PDA) until get pure culture and incubated room temperature. For morphological studies, according to their cultural appearances and observation of sporangium and other structures of *Pythium* spp. Sporangia were produced by floating some mycelial discs in distilled water and sterilized grass blades.

Pathogenicity test

Pathogenicity was tested by detached leaf method from healthy leaves leaves of *Citrus reticulata.* The leaves were washed with distilled water, then surface sterilized with 70% ethyl alcohol and placed with the upper leaf surface in sterile petri dish moisture chambers. Wounding by sterile needle on the leaves for easy access of the fungus, then leaves were inoculated with mycelium discs of *Pythium* spp. on the wound. Non-inoculated controls were inoculated with an agar plug without the fungus. 4 replications of each treatment were used in the experiment. After inoculation petri dishes were incubated at room temperature and symptoms were observed after 3 days.

Bi-culture test

Mycelial disc (0.5 m in diameter) of *Pythium* spp. was placed on PDA at one side of petri dish and *Ch. brasiliense* was placed at the opposite side of

petri dish with 4 replications and incubated at room temperature. As control an agar disc of *Pythium* spp. and *Ch. brasiliense* was placed alone on PDA. All petri dishes were incubated at room temperature until the pathogen in control growing full. The data were collected as colony diameter and the number of sporangium. The inhibition of mycelial growth and sporangium formation of pathogen was calculated as a percentage according to the formula:

Percent inhibition (I) = C – T/C × 100

Where, C = colony diameter / number of sporangium of the control

T = colony diameter / number of sporangium of the in bi-culture test

In vitro test of crude extract from Ch. brasiliense to control Pythium spp.

Ch. brasiliense was cultured in potato dextrose broth (PDB) for 30 days. The fungal biomass was collected, air-dried, ground and extracted with hexane, ethyl acetate (EtOAc) and methanol (MeOH) to produce crude hexane, crude EtOAc and crude MeOH extract, respectively. The crude exract from *Ch. brasiliense* were tested to control *Pythium* spp. by poisioned food technique with different concentrations (0, 10, 50, 100, 500 and 1000 ppm). Each crude exract was dissolved in 2% dimethyl sulfoxide (DMSO), and mixed into PDA before autoclaving at 121˚C, 15lbs/inch2 for 20 min. Mycelial disc of *Pythium* spp. was placed on the center of PDA in plate (5 cm diameter) incorporated with each crude exract. All petri dishes were incubated at room temperature until *Pythium* spp. in control growing full. Experiment was designed by using 2 factors factorial experiment in Completely Randomized Design (CRD) with 4 replications. Factor A represented solvents and factor B represented concentrations

The data were collected as colony diameter and the number of sporangium. The inhibition of mycelial growth and sporangium formation of pathogen was calculated as a percentage and the effective dose (ED_{50}) value was then calculated using probit analysis. Data was statistically computed and analysis of variance. Treatment means were compared with Duncan's multiple range test (DMRT) (p=0.05)

In vitro test of nano particles from Ch. brasiliense to control Pythium spp.

Nano particles were done using the method of Dar and Soytong (2014) to get Nano-CBH, Nano-CBE and Nano-CBM and were tested to control *Pythium* spp. by poisioned food technique. Experiment was designed by using 2 factors factorial experiment in CRD with 4 replications. Factor A represented nano particles and factor B represented concentrations at 0, 3, 5, 10 and 15 ppm.

Each nano particle was dissolved in 2% dimethyl sulfoxide (DMSO), and then mixed into PDA and added chitosan before autoclaving at 121˚C, 15lbs/inch2 for 20 min. Mycelial disc of *Pythium* spp. (7mm) was placed on the center of PDA in plate incorporated with each nano particles. All petri dishes incubated at room temperature until the pathogen in control plates growing full.

The data were collected as colony diameter and the number of sporangium. The inhibition of mycelial growth and sporangium formation of pathogen was calculated as a percentage and the effective dose (ED_{50}) value was then calculated using probit analysis. Data was statistically computed and analysis of variance. Treatment means were compare with Duncan's multiple range test (DMRT) (p=0.05).

Results

Pythium spp. was isolated from soil samples by baiting method and identified based on morphological characteristic. The cultural appearances were observed on PDA. Colony has a cottony aerial mycelium. The fungus grows fast, mycelium hyaline (Fig. 1, A). Sporangia formed on sterile grass blades in water cultures. Sporangia are of filamentous inflated (Fig. 1, B). Oogonia are smooth-walled, spherical, terminal, intercalary (Fig. 1, C-D). Oospores are aplerotic (Fig. 1, C-D).

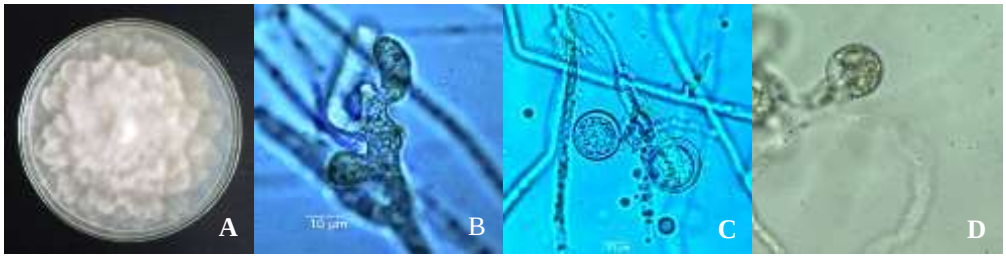

Figure 1. Colony patterns and morphology of *Pythium* spp. A; Colony patterns on PDA, B; Filamentous inflated sporangium, C; Oogonium with monoclinous antheridium, D; Oogonium with aplerotic oospores

Inoculated leaves under moist chamber condition showed water-soaked brownish lesions expand around agar plug of pathogen size 1.5 × 3.2 cm. Non-inoculated leaves showed no symptoms, leaves remained healthy.

In bi – culture test, result showed that *Ch. brasiliense* grew over and degraded *Pythium* spp. after inoculation 30 days. *Pythium* spp. was inhibited 42.5% growth colony and 48.1% sporangia production when compare to control.

Figure 2. Pathogenicity test of *Pythium* spp. on detached tangerine leaves. A; Inoculated control. B; Non-inoculated control

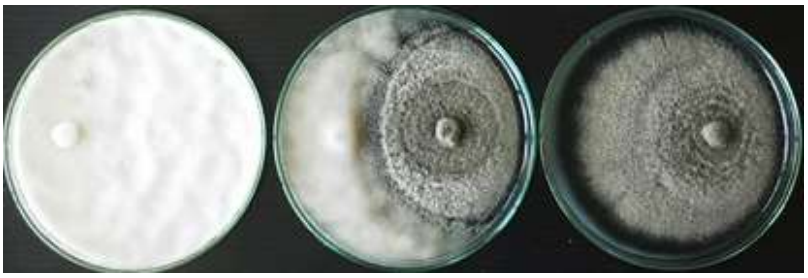

Figure 3. Growth of *Pythium* spp. in bi–culture test of *Pythium* spp. (at 30 days)

Efficacy of crude extract and nano particles from *Ch. brasiliense* to control *Pythium* spp. were tested by poisioned food technique with different concentrations when *Pythium* spp. in control petri dishes grew fully (2 days).

All of concentrations gave significantly different when compare to the control (0 ppm) and at 1000 ppm gave the best growth inhibition and sporangia inhibition and follow by 500, 100, 50 and 10 ppm. All of crude extracts at the concentrations of 1000 ppm, *Pythium* spp. did not grow from mycelial discs. The hexane extract of *Ch. brasiliense* at the concentrations of 500, 100, 50 and 10 ppm inhibited the colony growth of 88, 76.5, 58.25 and 34.25%, respectively when compared to the control. The EtOAc extract of *Ch. brasiliense* at the concentrations of 500, 100, 50 and 10 ppm inhibited the colony growth of 88, 69.25, 42.75 and 27.5%, respectively when compare to the control. The MeOH extract of *Ch. brasiliense* at the concentrations of 500, 100, 50 and 10 ppm inhibited the colony growth of 86, 71, 51.5 and 44 %, respectively when compared to the control (Table 1).

There is sporangia production 46.81×10^6 spore/ml in control. When calculated the sporangia inhibition at the concentrations of 1000, 500, 100, 50 and 10 ppm, the hexane extract of *Ch. brasiliense* inhibited the sporangia production of 95.59, 88.12, 73.56, 55.14 and 32.18%, respectively when compare to the control. The EtOAc extract of *Ch. brasiliense* inhibited the sporangia production of 95.33, 87.72, 59.41, 35.38 and 25.23%, respectively when compare to the control. The MeOH extract of *Ch. brasiliense* inhibited the sporangia production of 95.46, 86.78, 63.68, 43.92 and 36.98%, respectively when compare to the control (Table 1).

The crude extracts gave ED_{50} values of 30.15, 58.71 and 37.25 ppm for the hexane, EtOAc and MeOH, respectively (Table 1).

Table 1. Effect of crude extracts from *Ch. brasiliense* to inhibit *Pythium* spp.

Crude extract	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	Number of sporangia ($\times 10^6$)	Sporangia inhibition (%)	ED_{50} (ppm)
hexane	0	5.00a	-	46.81a	-	30.15
	10	3.29c	34.25h	31.75c	32.18i	
	50	2.09f	58.25e	21.00f	55.14f	
	100	1.18h	76.50c	12.38i	73.56c	
	500	0.60ij	88.00ab	5.56j	88.12b	
	1000	0.50j	90.00a	2.06k	95.59a	
ethyl acetate	0	5.00a	-	46.81a	-	58.71
	10	3.63b	27.5i	35.00b	25.23j	
	50	2.86d	42.75g	30.25d	35.38h	
	100	1.54g	69.25d	19.00g	59.41e	
	500	0.60ij	88.00ab	5.75j	87.72b	
	1000	0.50j	90.00a	2.19k	95.33a	
methanol	0	5.00a	-	46.81a	-	37.25
	10	2.80d	44.00g	29.50d	36.98h	
	50	2.43e	51.50f	26.25e	43.92g	
	100	1.45g	71.00d	17.00h	63.68d	
	500	0.70i	86.00b	6.19j	86.78b	
	1000	0.50j	90.00a	2.13k	95.46a	
C.V. (%)		4.28		3.98		

[1]Average of 4 replications. Means followed by the same letters in each antagonist were not significantly different by DMRT at P=0.05.

The nano particles were done from all crude extracts of *Ch. brasiliense* according to the method of Dar and Soytong (2014). All of concentrations gave significantly different when compare to the control and at 15 ppm gave the best growth inhibition and sporangia inhibition and follow by 10, 5 and 3 ppm.

All of nano particles at the concentrations of 15 ppm, *Pythium* spp. did not grow from mycelial discs. Nano-CBH at the concentrations of 10, 5 and 3

ppm inhibited the colony growth of 77.75, 55.5 and 41.75 %, respectively when compare to the control. Nano-CBE at the concentrations of 10, 5 and 3 ppm inhibited the colony growth of 67.5, 44 and 22.4 %, respectively when compare to the control. Nano-CBM at the concentrations of 10, 5 and 3 ppm inhibited the colony growth of 78.25, 57 and 47.25 %, respectively when compare to the control (Table 2).

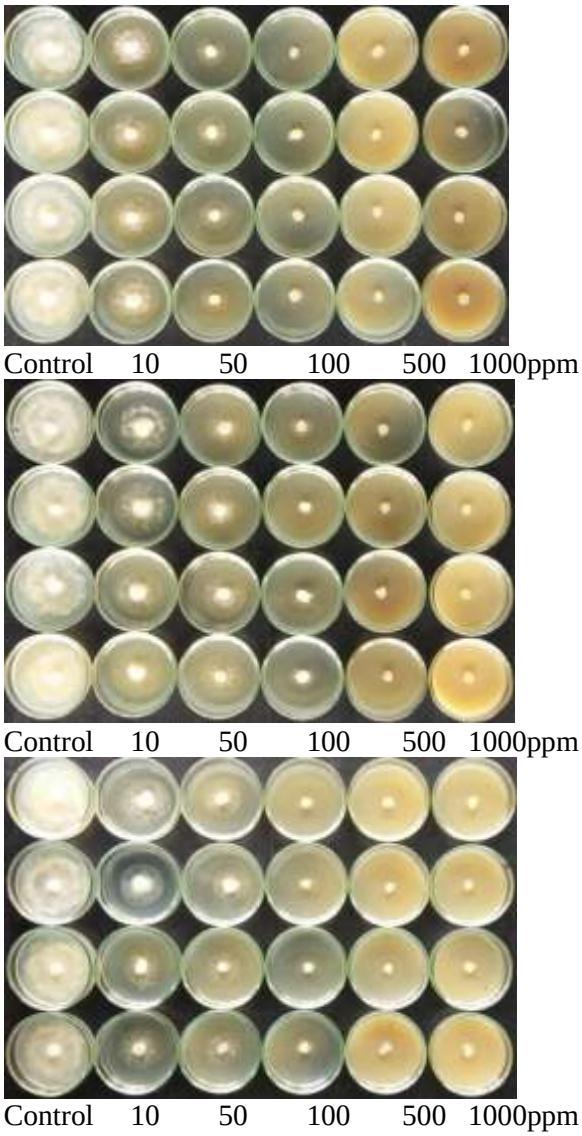

Figure 4. Testing crude extracts from *Ch. brasiliense* to inhibit *Pythium* spp.

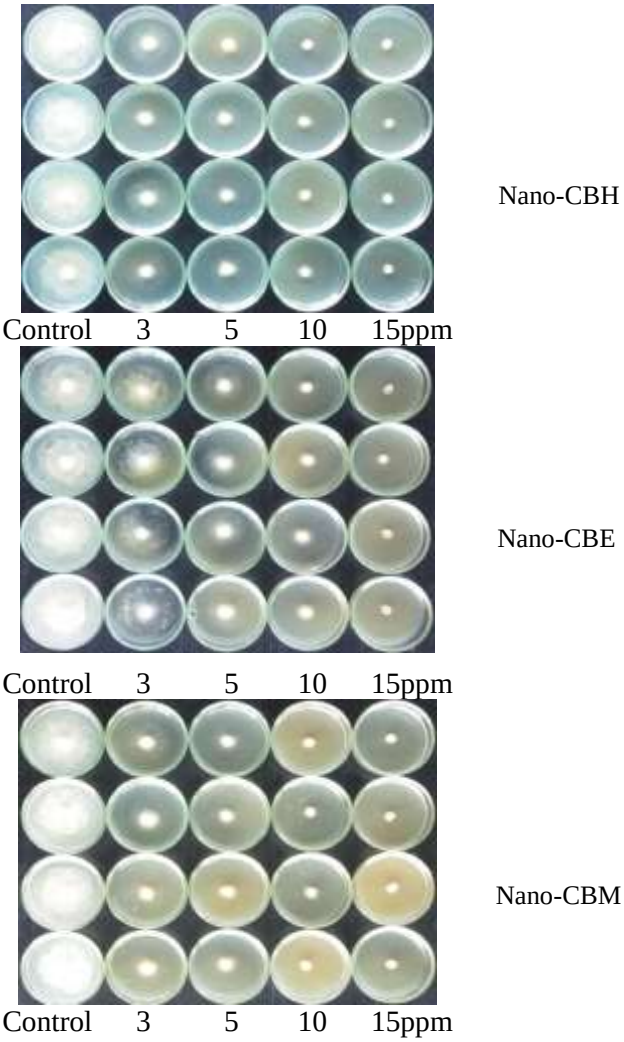

Figure 5. Testing the nano particles from *Ch. brasiliense* to inhibit *Pythium* spp.

In control, the number of sporangia was 42.38×10^6 spore/ml. When compare to the control, Nano-CBH at the concentrations of 15, 10, 5 and 3 ppm inhibited the sporangia production of 95.13, 86.43, 54.42 and 41.45 %, respectively. Nano-CBE at the concentrations of 15, 10, 5 and 3 ppm inhibited the sporangia production of 94.99, 86.43, 54.42 and 41.45 %, respectively when compare to the control. Nano-CBM at the concentrations of 15, 10, 5 and 3 ppm inhibited the sporangia production of 95.43, 89.38, 70.21 and 55.6 %, respectively when compare to the control (Table 2).

The nano particles of *Ch. brasiliense* gave ED_{50} values of 3, 3.96 and 2.69 ppm for the hexane, EtOAc and MeOH, respectively (Table 2).

Table 2. Effect of the nano particles from *Ch. brasiliense* to inhibit *Pythium* spp.

Nano product	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	Number of sporangia ($\times 10^6$)	Sporangia inhibition (%)	ED_{50} (µg/ml)
Nano-CBH	0	5.00a	-	42.38a	-	3.00
	3	2.91c	41.75f	20.81c	50.88e	
	5	2.23e	55.50d	13.44e	68.29c	
	10	1.11g	77.75b	5.00f	88.20b	
	15	0.50h	90.00a	2.06g	95.13a	
Nano-CBE	0	5.00a	-	42.38a	-	3.96
	3	3.88b	22.40g	24.81b	41.45f	
	5	2.80cd	44.00ef	19.31cd	54.42de	
	10	1.63f	67.50c	5.75f	86.43b	
	15	0.50h	90.00a	2.13g	94.99a	
Nano-CBM	0	5.00a	-	42.38a	-	2.69
	3	2.64d	47.25e	18.81d	55.60d	
	5	2.15e	57.00d	12.63e	70.21c	
	10	1.09g	78.25b	4.50f	89.38b	
	15	0.50h	90.00a	1.94g	95.43a	
C.V.(%)		5.01		6.52		

[1]Average of 4 replications. Means followed by the same letters in each antagonist were not significantly different by DMRT at P=0.05.

Discussion

The most serious root rot disease of citrus in Thailand is caused by *Phytophthora parasitica* but *Pythium* spp. is reported that caused root rot disease of citrus (Maseko and Coutinho, 2001 and Kean *et al*., 2010).

The obvious mechanisms of action of *Ch. brasiliense* in bi – culture test is competition because at first, *Pythium* spp. grew more than 50% of petri dish and faster than *Ch. brasiliense.* After 30 days *Ch. brasiliense* can grow over *Pythium* spp. moreover, *Pythium* spp. were degraded, mycelial deflated. There is probably antibiosis mechanism, therefore studying antifungal metabolite in terms of crude extract and nano particles from *Ch. brasiliense* to control *Pythium* spp. They gave high inhibition with low ED_{50} values. The ED_{50} values of crude extracts were 30.15 – 58.71 ppm and nano particles were 2.69 – 3.96 ppm. It was similar to the study of Tongon and Soytong (2016) studied using crude extracts of *Ch. brasiliense* to inhibit *Fusarium solani* and got effectively inhibition of *F. solani* with ED_{50} were 66.66 – 288.94 ppm. Khumkomkhet *et al.* (2009) found many depsidones from *Ch. brasiliense* and tested to control *Plasmodium falciparum, Mycobacterium tuberculosis* and *Candida albicans*.

Nano particles have one dimension less than 100 nm at least and ability to adsorb and carry compounds. The nano particles gave stronger inhibition than

crude extracts with lower ED_{50} values. It was similar to the report of Dar and Soytong (2014) tested nanomaterials derived *Ch. globosum* and *Ch. cupreum.* Moreover, Tongon and Soytong (2015) reported nano particles from *Ch. globosum* showed highly inhibitory effects on *Curvularia lunata* causing leaf spots of rice with low ED_{50} values.

Acknowledgement

I would like to acknowledge the King Mongkut's Institute of Technology Ladkrabang (KMITL) to offer a research fund (2562-02-04-018) supported by Faculty of Agricultural Technology, KMITL, Bangkok, Thailand. The financial support from Thailand Research Fund (Grant No RTA5980002) is also gratefully acknowledged.

References

Dar, J. and Soytong, K. (2014). Construction and characterization of copolymer nanomaterials loaded with bioactive compounds from *Chaetomium* species. Journal of Agricultural Technology. 10:823-831.

Hung, P. M., Wattanachai, P. and Soytong, K. (2014). Biological control of pomelo diseases using *Chaetomium* spp. Journal of Agricultural Technology. 10:833-844.

Hung P. M., Wattanachai, P., Soytong K. and Poeaim, S. (2015). Biological control of *Phytophthora palmivora* causing root rot of Pomelo using *Chaetomium* spp. Mycobiology. 43:63-70.

Kean, S., Soytong, K. and To-anun, C. (2010). Application of biological fungicides to control citrus root rot in Cambodia. Journal of Agricultural Technology. 6:219-230.

Khumkomkhet, P., Kanokmedhakul, S., Kanokmedhakul, K., Hahnvajanawong, C., and Soytong, K. (2009). Antimalarial and cytotoxic depsidones from the fungus *Chaetomium brasiliense*. Journal of natural products. 72:1487-1491.

Maseko, B. O. Z. and Coutinho, T. A. (2001). Pathogenicity of *Phytophthora* and *Pythium* species associated with citrus root rot in South Africa. South African Journal of Botany. 68:327-332.

Molina, A. B., Roa, V. N., Bay-Petersen, J., Carpio, A. T. and Joven, J. E. A. (1998). Regional Workshop on Disease Management of banana and citrus through the use of disease-free planting materials held in Davao city, Philippines. pp. 14-16.

Tongon, R. and Soytong, K. (2015). Application of nano-particles from *Chaetomium globosum* to control leaf spot of rice. Journal of Agricultural Technology. 11:1919-1926.

Tongon, R. and Soytong, K. (2016). Fungal Metabolites from chaetomium brasilense to Inhibit Fusarium solani. International Journal of Agricultural Technology. 12:1463-1472.

Quyet, N. T., Cuong, H. V., Hong, L. T. and Soytong, K. (2016). Control mechanism of *Chaetomium* spp. and its biological control of Citrus root rot in pot and field experiments in Vietnam. International Journal of Agricultural Technology. 12:329-336.

Vichitrananda, S. (1998). Disease Management of Citrus Orchards Planted with Disease-free Seedlings in Thailand. pp. 49-56. In: Proceedings of a reglonal workshop on disease management of banana and citrus throught the use of disease free planting materials helo in Davao City Philippines. October 14-16, 1998. Davao City, Philippines.

(Received: 25 August 2018, accepted: 7 October 2018)