Bioactivity test of *Chaetomium* isolate CNC1 to control *Pestalotia* spp causing leaf spot of Orchid

Yimcharoen, C.[1*], Tongon, R., Song, J. J.[1,2] and Soytong, K.[1,2]

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, CAS Bioengineering Co. Ltd., Wuxi, Jiangsu, China

Abstract The active strain of *Chaetomium* CNC1 isolated from forestry soil in China. It was confirmed to inhibit *Pestalotia* spp causing leaf spot of Orchid from Thailand by dual agar culture test. It revealed that the colonies of *Chaetomium* grew over the pathogren's colony within 30 days incubation. The tested *Chaetomium* proved to be inhibited the colony growth and spore of *Pestalotia* sp. Crude hexane, ethyl acetate and methanol extracts resulted to inhibit colony growth and spore of tested pathogen.

Keywords: biocontrol, antagonist, *Chaetomium*, Orchid, leaf spot

Introduction

Orchid is the most popular plant for decorate and expensive in many country (Pares and Whitecross, 1982) because there are beautiful plant many people always plant orchid to decorate garden or restaurant. But when orchid was inoculated by pathogen the price of orchid will decrease because when they got lesion on leaves that make them cant decorate anymore and the most important pathogen of orchid is *Pestalotia* spp. that will inoculate on leaves of orchid and make a leaves go brown so in this case called "Leaf spot" (Maharachchikumbura, 2014) to many prople always use chemical fungicide to control but in a long term that will made the pathogen mutation (Pasche, 2015) so bicontrol is a good choice to control *Pestalotia* spp. for avoid mutations of pathogen.

Materials and methods

Isolation of the pathogen from leaf spot of orchid

Disease samples were taken from orchid leaves showing leaf spot disease Symptom. The pathogen was isolated by tissue transplanting technique. The surface of the diseased orchid 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 orchid leaf samples were put into sterilized tissue papers until no more water drop on the surface of the orchid 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 (Chen *et al*., 2011) under a compound microscope 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 (27-30ºC) for 10 days before data collection. symptom was rate as Disease severity (DS) 0 = no disease, 1 = 1 – 10% leaf spot, 2 = >10 – 25% leaf spot , 3 = >25 – 50% leaf spot , 4 = >50 – 75% leaf spot , 5 = >75% leaf spot (Wheeler, 1969)

* **Corresponding Author:** Yimcharoen, C., **Email adress:** strugglegamer@gmail.com

Bi-culture test

A mycelial disc of *Pestalotia* spp. (5 mm diameter) was placed singly (as controls) or oppositely to a mycelial disc of each above antagonist on 9-cm-diameter Petri dishes, which contained PDA. After incubation at 25˚C for 30 days, data were collected as colony diameter and number of sporangia produced by *Pestalotia* spp. Numbers of sporangia were counted by using haemacytometer.

In vitro effects of antagonistic crude extracts on the growth of Pestalotia spp.

Chaetomium spp. were offered from Assoc. Prof. Dr. Kasem Soytong, from Faculty of Agricultural Technology, KMITL, Bangkok, Thailand. Crude extracts *Chaetomium* spp. 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), 866 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 121ºC (15 psi) for 20 minutes. A sterilized 5-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.

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 (ED50) was also calculated using the Probit analysis software.

Results

Isolation, Identification and Pathogenicity Test of pestalotia spp.

Pestalotia spp. were isolated and identified was confirmed is *Pestalotia* spp. from leaf spot of orchid with obvious pure culture and coloni indentified (Fig.1). The isolate was confirmed pathogenic isolate from pathogenecity test. The result showed that isolate could infected in the leaves of orchid (Table 1). *Pestalotia* spp. Was isolated from orchid leaves exhibiting leaf spot symptom the pathogenicity of isolate was confirmed. The isolate produced typical leaf spot on orchid leaves.

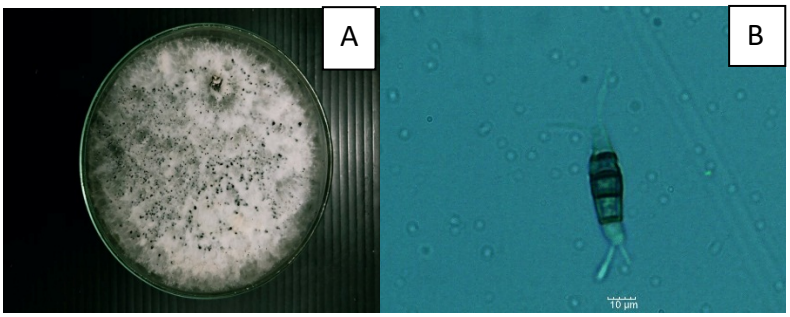

Figure 1. *Pestalotia* spp.A: Pure culture in PDA ; B: Conidia

Table 1 : Pathogenicity test on orchid leaves

Treatmants	*Disease severity (DS)*
Control	0
Pestalotia spp.	3

Table 1. Pathogenic test of leaf spot caused *Pestalotia* spp. 0 = no disease, 1 = 1 – 10% leaf spot, 2 = >10 – 25% leaf spot , 3 = >25 – 50% leaf spot , 4 = >50 – 75% leaf spot , 5 = >75% leaf spot (Wheeler, 1969)

Bi-culture antagonistic tests

Chaetomium spp. isolate CNC-1 was proved its abilities to inhibit the growth of *Pestalotia* spp. by using bi-culture tests. The result showed that CNC-1 gave significantly colony inhibition of *Pestalotia* spp. when compared to the control. The number of spores that producing by the pathogen *Pestalotia* spp. was counted by using Hemacytometer. The results showed that CNC-1 significantly inhibited pathogen spores when compared to the control plate. The results showed that CNC - 1 significantly inhibited number of pathogen spores of 40 % when compared to the control

Bioactivity test of crude extracts from Chaetomium* spp.** ***isolate CNC-1 against Pestalotia* spp.** ***causing leaf spot of orchid

Antagonistic *Chaetomium* spp. isolate CNC-1 was yielded metabolites as crude extracts and examined to control the growth of plant pathogen *Pestalotia* spp. The results showed that crude ethyl acetate from CNC-1 gave significantly highest inhibition of the colony growth of *Pestalotia* spp. when compared to the control, followed by crude methanol. All tested crude extracts, ethyl acetate, hexane and methanol crude extracts from CNC-1 gave significantly inhibition of the colony growth and spore production of *Pestalotia* spp. It showed that antagonistic fungus *Chaetomium* isolate CNC-1

The result from *Chaetomium* spp. Isolate CNC1 that hexane crude extract at the concentrations of 50, 100, 500 and 1000 µg/ml gave significant difference in the percentage of sporangium inhibition of *Pestalotia* spp. Which were 52.61 , 57.98 , 74.10 , 79.97 and 89.06 respectively. Followed growth inhibiton which were 10.50 , 17.50 , 17.00 , 21.00 and 30.00 respectively. EtOAc crude extract at concentrations of 50, 100, 500 and 1000 µg/ml significant difference in the percentage of sporangium inhibition of *Pestalotia* spp. Which were 59.20 , 66.29 , 77.52 , 87.78 and 91.81 respectively. Followed growth inhibition which were 10.00 , 13.00 , 18.50, 19.00 and 31.00 respectively. MeOH crude extract at concentrations of 50, 100, 500 and 1000 µg/ml significant difference in the percentage of sporangium inhibition of *Pestalotia* spp. Which were 32.46 , 43.20 , 49.43 , 65.00 and 71.42 respectively. Followed growth inhibition which as 8.00 , 9.50 , 10.50 , 11.50 , 14.00 respectively. (Table.2) In this case *Chaetomium* spp. isolate CNC1 with EtOAc significant inhibition to *Pestalotia* spp.

Table 2. Effect of crude extracts from *Chaetomium* spp. Isolated CNC1 on colony growth of *Pestalotia* spp.

Crude extract	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	Number of sporangium ($\times 10^6$)	Sporangium inhibition (%)	ED_{50} (µg/ml)
	0	5.00a	-	20.47a	-	10.53
	10	4.47bc	10.50l	9.69e	52.61e	
Hexane	50	4.14def	17.50k	8.60f	57.98e	
	100	4.12def	17.00k	5.29h	74.10b	
	500	3.94f	21.00j	4.09j	79.97bc	
	1000	3.5g	30.00i	2.23jk	89.06c	
	0	5.00a	-	20.47a	-	4.43
	10	4.50bc	10.00l	8.35f	59.20de	
EtOAc	50	4.34cd	13.0l	6.86g	66.29d	
	100	4.07ef	18.50k	4.60i	77.52c	
	500	4.05f	19.00k	2.50j	87.78b	
	1000	3.44g	31.00i	1.67k	91.81a	
	0	5.00a	-	20.47a	-	91.29
	10	4.60bc	8.00l	13.82b	32.46g	
MeOH	50	4.52b	9.50l	11.62c	43.20f	
	100	4.47bc	10.50l	10.34d	49.43ef	
	500	4.42bc	11.50k	7.16g	65.00d	
	1000	4.30cde	14.00k	5.84h	71.42c	
C.V.(%)		3.48			4.78	

Discussion

As the results, *Chaetomium* isolate CNC-1 is proved to act as biological activity against *Pestalotia* spp. causing leaf spot of orchid from crude extracts of CNC-1 was successful to control *Pestalotia* spp. (Tathan *et al.*, 2012) . at low concentration. It is useful resource as nature product to inhibit the pathogen which causing leaf spot of orchid. It is not only reduced the production loss of growers but also reduced to pollute the environment as compared the traditional method. Some report showed in the same result that *Pestalotia* spp. can control by biocontrol. For instance grey blight of tea caused *Pestalotia* spp. (Phong *et al*., 2017) *Trichoderma viride* can control *Pestalotia* spp. in post – harvest (Bhuvaneswari and Subba, 2001) and *Trichoderma harzianum* control *Pestalotia* spp on lettuce (Hutchinson, 1972). *Chaetomium* also control many disease such as *Chaetomium globosum* in biocontrol of *Pythium ultimum* (Di Pietro, 1992) , *Chaetomium cupreum* for biocontrol of *Colletotrichum gloeosporioides* Causing Coffee Anthracnose on Arabica (Vilavong and Soytong, 2017).

Acknowledgement

I would like to express my sincere thanks to my advisors: Prof. Dr. Kasem Soytong and Jiao jiao song for their support and valuable advices. Thanks to Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand for supporting my PhD research.

References

Bhuvaneswari, V., and M. Subba Rao. "Evaluation of Trichoderma viride antagonistic to post-harvest pathogens on mango." *Indian Phytopathology* 54.4 (2001): 493-494.

Chen, C., Zhang, B., Yang, L., & Gao, J. (2011). Identification and biological characteristics of round leaf spot on blueberry caused by Pestalotiopsis photiniae. *Journal of Northeast Forestry University*, *39*(1), 95-98.

Di Pietro, A., Gut-Rella, M., Pachlatko, J. P., & Schwinn, F. J. (1992). Role of antibiotics produced by Chaetomium globosum in biocontrol of Pythium ultimum, a causal agent of damping-off. *Phytopathology*, *82*(2), 131-135.

Hutchinson, S. A., and M. E. Cowan. "Identification and biological effects of volatile metabolites from cultures of Trichoderma harzianum." *Transactions of the British mycological Society* 59.1 (1972): 71-77.

Kanokmedhakul, Somdej. (2006). Antifungal Azaphilones from the Fungus Chaetomium cupreum CC3003.*Journal of natural products* 69.6: 891-895.

Maharachchikumbura, S. S., Hyde, K. D., Groenewald, J. Z., Xu, J., & Crous, P. W. (2014). Pestalotiopsis revisited. *Studies in Mycology*, *79*, 121-186.

Phong, N. H., Wattanachai, P., Kasem, S., & Luu, N. T. (2014). Antimicrobial substances from Chaetomium spp. against Pestalotia spp. causing grey blight disease of tea. *Journal of Agricultural Technology*, *10*(4), 863-874.

Soytong, Kasem (2015) Testing bioformulation of Chaetomium elatum ChE01to control Fusarium wilt of tomato. Journal of Agricultural Technology 11(4):975-996.

Vilavong, S., & Soytong, K. (2017). Application of A New Bio-Formulation of Chaetomium cupreum For Biocontrol of Colletotrichum gloeosporioides Causing Coffee Anthracnose on Arabica Variety in Laos. *AGRIVITA, Journal of Agricultural Science*, *39*(3), 303-310.

Wheeler, B.E.J. 1969. An Introduction of Plant Disease. John Wiley & Sons Ltd., London, 301p.