Antifungal activity of *Chaetomium* isolate CNC2 against *Alternaria* spp. causing leaf blight of kales

Chotikulapat, N. [1], Song, J. J. [1,2] and Soytong, K.[1*,2]

[1]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute vof Technologyb Lasdkrabang, Bangkok, Thailand, [2]CAS Bioengineering Co. Ltd., Wuxi, Jiangsu, China.

Abstract *Chaetomium* spp. isolate CNC2 isolated from forestry soil in China proved to be antagonized *Alternaria alternata* causing leaf blight of Kales in bi-culture antagonistic evaluation. The crude metabolites from Chaetomium sp CNC2 were extracted by hexane, ethyl acetate and methanol that expressed antifungal activity against *A. alternata*.

Keywords: biocontrol, antagonist, *Chaetomium*, kales, leaf blight

Introduction

Species of the genus *Brassica* are important vegetable crops grown in South east Asia, including Thailand, but their production is often limited by diseases (Tsou and Tsay, 1988). Diseases caused by the fungal genus *Alternaria* are among the most serious, and they have been reported from many countries including Australia (Sivapalan and Browning, 1992) and Taiwan (Wu, 1979) Spread of the disease during the growing season is by wind-blown or rain-splashed spores. Optimum conditions for sporulation are temperatures of 20 –30˚C and a minimum wet period of 13 h, epidemics occurring when rainfall is frequent (Humpherson-Jones and Phelps, 1989; Fontem *et al.,* 1991). These conditions correspond to the climate in some tropical countries such as Thailand, where the average annual temperature is 26–28˚C and there is daily rainfall during the growing season for vegetables. Nevertheless, the disease appeared to cause considerable damage to the crop in the country (Visethsung and Saranak, 1988). This experiment uses by *chaetomium* isolate CNC2 to biocontrol methods to inhibit the growth of fungal pathogens.

Materials and methods

Isolation and identification

Kale pathogens were isolated CAN-1 from leaf using moist chamber at room temperature, the signs of pathogens were transferred to water agar (WA) and then subcultured to potato dextrose agar (PDA) until get pure culture. The most frequency found pathogen would then prove for pathogenicity test followed the Koch's postulate method.

Pathogenicity test

The kale leaves were detached from healthy plant, and then surface sterilized with 70% ethyl alcohol and placed with the upper leaf surface in a sterile petri dish containing filter paper moist with distilled water to maintain high humidity. Wounding by sterile needle on the leaves for easy access of the fungus, then leaves were inoculated with mycelium discs of *Alternaria* spp. isolate CAN-1 on the wound. Non-inoculated controls were inoculated with an agar plug without the fungus. The petri dishes were incubated at room temperature for 7 days. Four replications of each treatment were used in the experiment. The result showed that isolate could infected in the leaves of kale and caused symptom. Disease severity (DS) scored as level 0 was no symptoms, level 1 was 1-10% leaf blight level 2 was 11-25 % leaf blight, level 3 was 26-50 % leaf blight, level 4 was 51-75 % leaf blight and level 5 was over 75 % leaf blight. (Wheeler, 1969).

Bi-culture test

A mycelial disc of *Alternaria* 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

*Coressponding Author: Chotikulapat, N.; **E-mail address:** nitipat0123@gmail.com

15 days, data were collected as colony diameter and number of sporangia produced by *Alternaria* spp. in both bi-culture and control plates. Numbers of sporangia were counted by using haemacytometer. Data were computed in a form of inhibition percentage of mycelial growth and sporangial production of the pathogen by using the formula below:

$$\% \text{ Inhibition} = 100 \times (A - B)/A$$

where A = colony diameter or numbers of sporangia of *Alternaria spp.* in control plates; B = colony diameter or numbers of sporangia of *Alternaria spp.* in bi-culture plates. Finally, variance and the treatment means were analyzed and compared by using Duncan's multiple range tests at 0.05.

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

The antagonistic substances were extracted from *Chaetomium* spp isolate CNC2 as crude extracts. These antagonistic substances were tested for their abilities to inhibit the growth of *Alternaria* spp. The crude extraction from antagonistic fungi was performed using the method of Kanokmedhakul *et al*., (2006). *Chaetomium* spp. was separately cultured in PDB at room temperature (28-30˚C) for 30 days. Fungal biomass were removed from PDB, filtered through cheesecloth and air-dried overnight. Fresh weight and dry weight of fungal biomass were weighed. Dried fungal biomass were ground with electrical blender, extracted with 200 ml hexane (H) and shaken for 24 hour at room temperature. The ground biomass was separated by filtration through Whatman No. 4 filter paper. The marc was extracted again with hexane using method described above. The filtrates were evaporated in vacuo to yield crude extract. The marc was further extracted with ethyl acetate (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. CNC2 cultured in PDB for 30 days. It exposed in air dry room temperature 27-30˚C for 5 days. Fungal biomass was the soaked in hexane for 3 days filtered to separate marc, the filtrate was performed in rotary vacuum evaporator yielded crude Hexane. The marc was then soaked in ethyl acetate for 3 days and filtered to get filtrate and passed through rotary vacuum evaporator yielded crude ethyl acetate. Marc was further then soaked in methanol for 3 days, then filtered and filtrate and passed through rotary vacuum evaporator yielded crude methanol.

Results

Isolation, Identification and Pathogenicity test of Alternaria spp.

CAN-1 were isolated and identified was confirmed is *Alternaria alternata* from leaf blight of kale with obvious symptom and get pure culture. The isolate CAN-1 was confirmed pathogenic isolate from pathogenicity test. The result showed that isolate CAN-1 could infected in the leaf of kale as seen in Table 1.

Table 1 : Pathogenicity test on kale leaf

Treatmants	*DS*
Control	1
Alternaria alternata	3

Disease severity (DS) scored as level 0 was no symptoms, level 1 was 1-10% leaf blight level 2 was 11-25 % leaf blight, level 3 was 26-50 % leaf blight, level 4 was 51-75 % leaf blight and level 5 was over 75 % leaf blight.

Bi-culture antagonistic tests

Chaetomium spp. isolate CNC-2 was proved its abilities to inhibit the growth of *A. alternata* by using bi-culture tests. The result showed that CNC-2 gave significantly colony inhibition of *A. alternata* when compared to the control. The number of spores that producing by the pathogen *A. alternate* was counted by using Hemacytometer. The results showed that CNC-2 significantly inhibited number of pathogen spores of 55% when compared to the control.

Bioactivity test of crude extracts from Chaetomium spp. isolate CNC-2 against Alternaria spp. isolate CAN-1 causing laft blast of kales.

The crude extracts in CNC2 from hexane at concentrations of 10, 50, 100, 500, 1,000 ppm were tested inhibition of sporangia information of *A. alternata* which were 58.57 and 61.61 % in concentrations of 500 and 1000 ppm respectively (Table 2) (when compared to the control.) EtAOc at concentrations of 10, 50, 100, 500, 1,000 ppm were tested inhibition of sporangia information of *A. alternata* which were 58.73, 61.22, 75.15, 83.62 and 95.94 % in concentrations of 10, 50, 100, 500 and 1,000 ppm respectively Table 2 (when compared to the control.) MeOH at concentrations of 10, 50, 100, 500, 1,000 ppm were tested inhibition of sporangia information of *A. alternata* which were 56.39 and 67.30 % in concertrations of 500 and 1,000 ppm respectively Table 2 (when compared to the control.) Meanwhile ED50 values of CNC2-H, CNC2-Et, CNC2-M were 308.91, 7.92 and 272.85 µg/ml respectively (Table 2).

Table 2 : Effect of erude extracts from CNC2 to inhibit *A. alternate*

Crude extracts	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	Number of sporangium ($\times 10^6$)	Sporangium inhibition (%)	ED_{50} (µg/ml)
Hexane	0	5.00^{a}	0^{a}	48.150^{a}	0^{a}	
	10	4.60^{b}	8.00^{l}	44.325^{ab}	7.84^{m}	
	50	4.42^{cd}	11.50^{j}	43.225^{ab}	10.13^{l}	
	100	4.27^{f}	14.50^{g}	27.425^{c}	42.98^{h}	308.91
	500	4.25^{fg}	15.00^{g}	19.925^{d}	58.57^{f}	
	1000	4.12^{h}	17.50^{e}	15.575^{de}	67.61^{d}	
EtAOc	0	5.00^{a}	0^{a}	48.150^{a}	0^{a}	
	10	4.37^{de}	12.50^{i}	19.85^{d}	58.73^{f}	
	50	4.17^{gh}	16.50^{f}	18.65^{d}	61.22^{e}	
	100	3.92^{i}	21.50^{d}	11.95^{ef}	75.15^{c}	7.92
	500	3.82^{j}	23.50^{b}	7.875^{f}	83.62^{b}	
	1000	3.57^{k}	28.50^{ab}	1.95^{g}	95.94^{ab}	
MeOH	0	5.00^{a}	0^{a}	48.150^{a}	0^{a}	
	10	4.95^{ab}	1.00^{m}	37.575^{b}	21.88^{k}	
	50	4.50^{c}	10.00^{k}	35.975^{b}	25.20^{j}	
	100	4.30^{ef}	14.00^{h}	29.85^{c}	37.94^{i}	272.85
	500	4.10^{h}	18.00^{e}	20.975^{d}	56.39^{g}	
	1000	3.87^{ij}	22.50^{c}	15.725^{de}	67.30^{d}	
C.V.(%)		1.61		14.01		

Rage of four replications. Means followed by a common letter are not significantly different by DMRT at P = 0.05

Discussion

The results, *Chaetomium* isolate CNC-2 is proved to act as biological activity against Alternaria spp. causing leaf blight of kales from crude extracts of CNC-2. The result showed that crude extracts from EtAOc, able to inhibit sporerangium the most and still have a low ED_{50} until satisfactory. Was successful to control *Alternaria* spp. It is useful resource as nature product to inhibit the pathogen which causing leaf blight of kales. It is not only reduced the production loss of growers but also reduced to pollute the environment as compared the traditional method. Previous research indicated that crude extracts of other species of *Chaetomium* spp. are also reported to believe antagonize to many plant pathogens. Sibounnavong *et al*. (2011). reported that *Chaetomium lucknowense* showed greater antifungal activity against *F. oxysporum f. sp. lycopersici* NKSC02. The crude extract of endophyte *Chaetomium globosum* No.04 which isolated from the medicinal plant Ginkgo biloba showed significant growth inhibitory activity against the phytopathogenic fungi *Rhizopus stolonifer* and *Coniothyrium diplodiella*. (Guizhen Zhang *et al*. 2013). Charoenporn *et al*. (2010). reported that oil bio-agent formulation from the antagonistic fungi of *Chaetomium globosum* and *Ch. lucknowense* showed their biological ability to control tomato wilt. The efficacy of *Chaetomium globosum* as a biocontrol agent against the late blight pathogen *Phytophthora infestans* was evaluated in potato plants by Shanthiyaa *et al*. (2013). Among eight *Chaetomium* isolates evaluated *C. globosum* isolate Cg-6 showed greater inhibition to mycelial growth of *P. infestans* in vitro. Phung et al. (2015). examined the in vitro and in vivo effects of *Chaetomium globosum*, *Chaetomium lucknowense*, *Chaetomium cupreum* and

their crude extracts as biological control agents in controlling *Phytophthora nicotianae* causing root rot in citrus, and the result showed that *Chaetomium* species and their crude extracts strongly inhibited the growth of *Phytophthora. nicotianae* KA1.

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 research.

References

Charoenporn C, Kanokmedhakul S, Lin FC, Poeaim S, Soytong K (2010). Evaluation of bio-agent formulations to control Fusarium wilt of tomato. Afr. J. Biotech., 9: 5836– 5844.

Guizhen Zhang, Yanhua Zhang, Jianchun Qin, Xiaoyan Qu, Jinliang Liu, Xiang Li, and Hongyu Pan (2013). Antifungal Metabolites Produced by *Chaetomium globosum* No.04, an Endophytic Fungus Isolated from Ginkgo biloba.Indian J Microbiol.; 53(2): 175–180.

Humpherson-Jones FM, Phelps K, (1989). Climatic factors influencing spore production in *Alternaria brassicae* and *Alternaria brassicicola*. Annals of Applied Biology 114, 449–58.

Kanokmedhakul, S., Kanokmedhakul, K., Nasomjai, P., Louangsysouphanh, S., Soytong, K., Isobe, M., ... and Suksamrarn, A. (2006). Antifungal Azaphilones from the Fungus *Chaetomium cupreum* CC3003. Journal of natural products, 69(6), 891-895.

Phung Manh Hung, Pongnak Wattanachai, Soytong Kasem, and Supattra Poeaim. (2015). Efficacy of Chaetomium Species as Biological Control Agents against *Phytophthora nicotianae* Root Rot in Citrus. Mycobiology. 43(3): 288–296.

Shanthiyaa V., D. Saravanakumar, , L. Rajendran, G. Karthikeyan, K. Prabakar, T. Raguchander (2013). Use of *Chaetomium globosum* for biocontrol of potato late blight disease. Crop Protection, V(52), pp.33–38

Sibounnavong P., Charoenporn, C. , S. Kanokmedhakul and K. Soytong (2011). Antifungal metabolites from antagonistic fungi used to control tomato wilt fungus *Fusarium oxysporum f. sp. lycopersici*. African Journal of Biotechnology Vol. 10(85), pp. 19714-19722.

Sivapalan A, Browning JW, (1992). Incidence of Alternaria brassicicola (Schw.) Wiltsh. on *Brassica oleracea* seeds. Australian Journal of Experimental Agriculture 32, 535–7.

Tsou SCS, Tsay JTS, (1988). Vegetable research in Southeast Asia – an overview. In: McLean BT, ed. Vegetable Research in Southeast Asia. Taipei, Taiwan: Asian Vegetable Research and Development Center, AVRDC publication no. 88–303, pp. 119–32.

Visethsung O, Saranak J, (1988). Leaf spot disease of crucifer crops and their control. 1st conference on Integrated pest and disease control, 1–3 March 1988. Petchaburi, Thailand, pp. 171–81 (in Thai).

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

Wu WS, (1979). Survey on seed-borne fungi of vegetable. Plant Protection Bulletin (Taiwan) 21, 206–19.