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Efficacy of Nano Particles from *Chaetomium cochliodes* to Control *Pythium* spp. causing Root Rot of Tangerine (*Citrus reticulata*)

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Tangerine (*Citrus reticulata*) is one of the most popular fruit in Asia. The seriously disease of tangerine is found to be root rot which caused by *Pythium* spp. and proved for pathologenicity. The tested nano particles derived from *Chaetomium cochliodes* to control the *Pythium* spp. *in vitro* was evaluated. The result showed that the tested nano particles exhibited antifungal activities against mycelial growth and sporangia formation of *Pythium* spp. with effective doses (ED_{50}) of 2.0~3.8 µg/mL and 1.2~3.7 µg/mL respectively. It is the first report of nano particles derived from *Ch. cochliodes* to control *Pythium* spp. causing root rot of *Citrus reticulata*.

Keywords: Root rot, *Pythium* spp., Tangerine, *Citrus reticulate*

Introduction

Tangerines (*Citrus reticulata*) are important fruit crops, being widely and commercially grown in Southeast Asia. In Thailand, tangerine trees mostly grow in the Northern provinces. The major causes of yield loss and decline of tangerine tree is root rot disease (Molina *et al*., 1998). *Pythium* spp. is reported that caused root rot disease of citrus (Maseko and Coutinho, 2001 and Kean *et al*., 2010). In some poor orchard sites, root rot is often presented with high population densities of *Pythium* spp. in the apparent absence of *Phytophthora* spp. and nematodes (Tsao *et al*., 1978, Thompson *et al*., 1995). When the pathogen infected near the ground level, infected bark cracks through which gum exudation. Roots turn soft and brown, the leaves turn yellow and may drop. The tree will grow poorly, stored energy reserves will be depleted, and production will decline (Vichitrananda, 1998). Chemical fungicides used to control root rot as reported by Davis (1982), Matheron and Matejka (1991)

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and Graham (2011) but they usually reduced soil quality, thus the biological control is an alternative practice to control these disease. The objective of the present study was to evaluation of efficiency of nano particles from *Ch. cochliodes* to control *Pythium* spp. causing root rot of tangerine.

Materials and methods

Isolation and identification

Soil and disease samples were collected from the tangerine orchards in Nan province, at a depth of 15 – 30 cm and stored at room temperature. Soil samples were isolated by baiting method. The soil samples were ground and placed into sterilized petri dish, then added sterilized distilled water and leaf pieaces of tangerines (15 × 15 mm) and incubated at room temperature for 1 – 2 days. After the pathogen grew out from the leaf pieaces of tangerines, the leaf pieaces were placed on water agar (WA). When mycelium grown from the tissue sections were transferred to Potato Dextrose Agar (PDA) until get pure culture and incubated at 25 °C. 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 10 ml of distilled water and sterilized grass blades.

Pathogenicity test

The tangerine 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 *Pythium* spp. on the wound. Non-inoculated controls were inoculated with an agar plug without the fungus. The petri dishes were incubated at room temperature for 3 days. Four replications of each treatment were used in the experiment.

In vitro test of nano particles from Chaetomium cochliodes to control Pythium spp. Causing root rot of Citrus reticulate

Ch. cochliodes 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. Nano particles were done using the method of Dar and Soytong (2014) to get Nano-CCOH, Nano-CCOE and

Nano-CCOM. The nano particles were tested to control *Pythium* spp. causing root rot of Citrus reticulate. Experiment was designed by using two factors factorial experiment in Completely Randomized Design (CRD) with four replications. Factor A represented Nano-CCOH, Nano-CCOE and Nano-CCOM 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 (5 cm diameter) incorporated with each nano particles. All plates 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 (ED50) 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 and discussion

The cultural appearances were observed on PDA. Colonies have a cottony aerial mycelium with chrysanthemum pattern. 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).

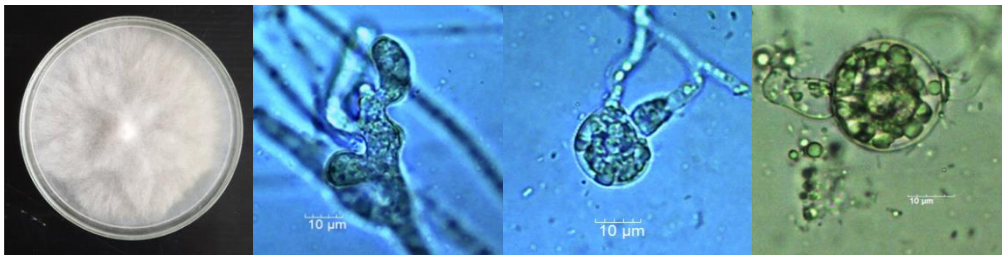

Fig. 1. Colony patterns and morphology of *Pythium* spp. A; Colony patterns on PDA, B; Filamentous inflated sporangium, C; Oogonium with monoclinous antheridium, D; Oogonium with aplerotic oospores.

Pathogenicity test on detached leaves after 3 days, infected leaves showed water-soaked grayish brown lesion expand around agar plug of pathogen. Non-inoculated leaves showed no symptoms, leaves remained healthy. The result

showed the fungus caused disease symptoms on tangerine leaves that were similar to the report of Mida *et al*. (2015) which reported pathogenicity test on detached leaves of *Citrus jambhiri*.

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

Nano particles of *Ch. cochliodes* were tested with different concentrations for colony growth inhibition and sporangium inhibition of *Pythium* spp. The nano particles from crude extracts of *Ch. Cochliodes* were done according to the method of Dar and Soytong (2014). Data were collected after 24 hours of experiment. Nano-CCOH at the concentrations of 3, 5, 10 and 15 ppm inhibited the colony growth of 47.75, 53.50, 63.25 and 80%, respectively when compare to the control (0 ppm). Nano-CCOE at the concentrations of 3, 5, 10 and 15 ppm inhibited the colony growth of 54.25, 74.75, 80.75 and 86%, respectively when compare to the control. Nano-CCOM at the concentrations of 3, 5, 10 and 15 ppm inhibited the colony growth of 56.75, 65.75, 75.50 and 81%, respectively when compare to the control. Nano-CCOH, Nano-CCOE and Nano-CCOM gave ED_{50} values of 3.83, 2.62 and 2.01 µg/ml, respectively. Nano-CCOH at the concentrations of 3, 5, 10 and 15 ppm inhibited sporangium formation of 46.58, 7.14, 67.24 and 83.23%, respectively when compare to the control. Nano-CCOE at the concentrations of 3, 5, 10 and 15 ppm inhibited sporangium formation of 58.70, 75.16, 79.04 and 95.03%, respectively when compare to the control. Nano-CCOM at the concentrations of 3, 5, 10 and 15 ppm inhibited sporangium formation of 62.42, 68.94, 71.43 and 83.54%, respectively when compare to the control. Nano-CCOH, Nano-CCOE and Nano-CCOM gave ED_{50} values of 3.68, 2.17 and 3.80 µg/ml, respectively. (Table 1, Fig.3). It is similar to the report of Soytong (2014) which studied Bio-formulation of *Ch. cochliodes* for controlling *Drechslera oryzae* gave good

result to inhibit *D. oryzae* and increased in plant growth. 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.

Table 1. Effect of nano particles from *Chaetomium cochliodes* to inhibit *Pythium* spp.

Nano product	Concentration (ppm)	Colony diameter (cm)	Growth inhibition (%)	ED_{50} (µg/ml)	Number of sporangia ($\times 10^6$)	Sporangia inhibition (%)	ED_{50} (µg/ml)
Nano-CCOH	0	5.00a[1]	-	3.83	40.25a	-	3.68
	3	2.61b	47.75f		21.50b	46.58h	
	5	2.33c	53.50e		17.25c	57.14g	
	10	1.84d	63.25d		13.19e	67.24e	
	15	1.00f	80.00b		6.75h	83.23b	
Nano-CCOE	0	5.00a	-	2.62	40.25a	-	2.17
	3	2.29c	54.25e		16.63cd	58.70fg	
	5	1.26e	74.75c		10.00fg	75.16cd	
	10	0.97f	80.75b		8.44g	79.04c	
	15	0.70g	86.00a		2.00i	95.03a	
Nano-CCOM	0	5.00a	-	2.01	40.25a	-	3.80
	3	2.16c	56.75e		15.13d	62.42f	
	5	1.71d	65.75d		12.50e	68.94e	
	10	1.22e	75.50c		11.50ef	71.43de	
	15	0.95f	81.00b		6.63h	83.54b	
C.V.(%)		6.03			6.53		

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

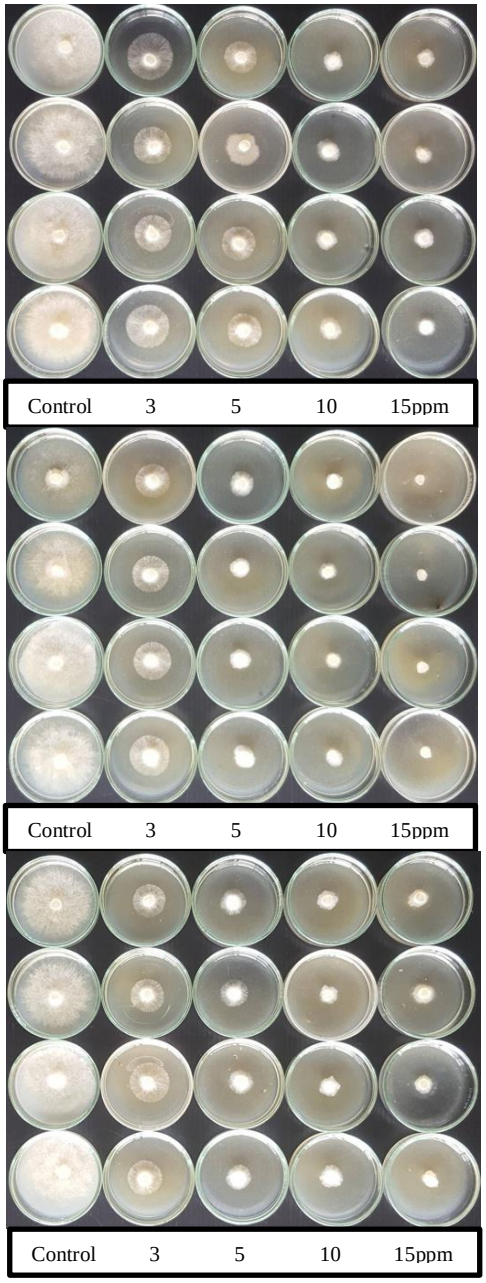

Fig. 3. Testing the nano particles from *Chaetomium cochliodes* to inhibit *Pythium* spp.

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