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Control mechanism of *Chaetomium* spp. and its biological control of Citrus root rot in pot and field experiments in Vietnam

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Chaetoglobosin-C produced from *Chaetoimium* globosum gave significantly inhibited colony, sporangia, and oospores growth of *Phythothora parasitica* causing root rot of citrus which the ED_{50} values of 4.0, 35.4 and 125.7 ppm respectively. The research finding in pot experiment showed that Chaetomium treatment at soil pH 3,4,5,6 and 7 gave highly significant better reduction the disease incidence than the metalaxyl chemical fungicide and non-treated control. Result in the field trial revealed that the disease index of cturs root rot caused by *P. parasitica* in Chaetomium biofungicide treatment was not significantly differed from Metalaxyl chemical fungicide when compated to the non-treated control. Chaetomium biofungicide reduced disease of 64 % and Metalaxyly chemical fungicide reduced the disease of 61.3 %. However, Chaetomium biofungicide treatment was also not significantly differed from Metalaxyl chemical fungicide in term of yield.

Key words: *Chaetomium* spp.; Biological control; Citrus root rot

Introduction

Citrus is one of the major plants cultivated in North Vietnam. The citrus plantations have been applied chemical fungicides for disease control for years and now those pathogen are being resistant to chemical fungicides leading to low effective control, damaged citrus trees and low yield, especially root rot disease caused by *Phytophthira parasitica* (Soytong *et al*., 2001; Levy *et al*., 1983). Root rot pathogens are serious infectious disease causing basal stem rot, brown-rot, gummosis, root rot then yellowing leaves and die back (Ohazuruike and Obi (2000) which similar reports in many countries wherever citrus is grown (Timmer *et al*, 1989). Other diseases are helongjiang disease, greening

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and tristeza virus diseases which found to be associated with Phytophthora rot as a disease complex (Kean *et al*., 2010). It is now increasingly interested for alternative control of disease by using effective microorganism as biological control agents. Many reports stated that Chaetomium spp gave a good control of Phytophthora rot in Citrus (Soytong *et al*., 2001; Kean et al., 2010). Chaetomium spp are reported to be antaginized several pathogens (Soytong and Quimio, 1989). *Chaetomium globosum* and *Chaetomium cochlioides* reported as biocontrol agents against *Fusarium* spp. and *Helminthosporium* spp. (Tveit and Moore, 1954). *Ch. cupreum* and *Ch. globosum* are reported to reduce leaf spot disease of corn caused by *Curvularia lunata*, rice blast caused by *Magnaporthe grisea* (*Pyricularia oryzae*) and sheath blight of rice caused by *Rhizoctonia oryzae* (Soytong, 1989, 1992).

The research findlings were to find out control mechanism of *Chaetomium* sp in term of antibiosis and to develop and formulate as biological fungicide to control Citrus root rot caused by *Phytophthora parasitica* in pot and field experiments.

Materials and methods

Control mechanism of Chaetomium

Chaetoglobosin-C is reported to produce from Chaetoimium globosum (Kanokmedhakul et al, xxxx). Our Chaetomium isolates were molecular phylogeny compared to as Ch. globosum 0805 previous report. Pure compound of Chaetoglobosin-C was offered from Prof. Dr. Kanokmedhakul, Department of Chemistry, Faculty of Science, Khon Khan University, Thailand.

The experiment was conducted by using Completely Randomized Design (CRD) with four replications. Treatmente were set up different concentrations as follows:- 0, 10, 50, 100 and 500 ppm. Each treatment was dissolved in 2% dimethyl sulfoxide (DMSO), then mixed into potato dextrose agar (PDA) before autoclaving at 121°C, 15 lbs/inch^2 for 30 minutes. The tested pathogen, *Phytophthora parasitica* causing root rot of citrus was cultured on PDA and incubated at room temperature for 3 days, then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was moved to the middle of PDA plate (5 cm diameter) in each concentration and incubated at room temperature (28-30°C). Data were collected as colony diameter and number of sporangia and oospores. Percentage of inhibition was computed as seen in Table 1. Data was statistically computed analysis of variance. Treatment means were compared with DMRT at P = 0.05 and P = 0.01. The effective dose (ED_{50}) was computed by using probit analysis.

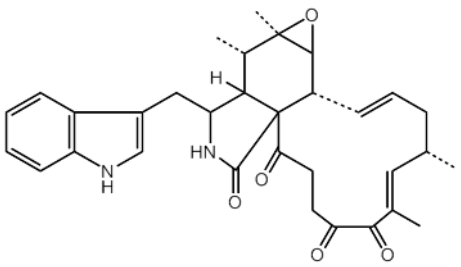

Fig. 1. Chaetoglobosin C produced from *Chaetomium globosum* KMITL N0802
Source: Kanokmedhakul et al. (2002)

Pot experiment

The experiment was conducted by using 3 x 5 factorial in Randomized Complete Block Design (RCBD) with four replications. Factor A represented chaetomium-biofugicide, metalaxyl and non-trated control and factor B represented soil pH levels of 3, 4, 5, 6 and 7.

Citrus seedlings were grown to mixed-soil in pots for 30 days before inoculation with pathogen. The root-dipped method was used for inoculation followed the method of Marlatt et al. (1996). Dirt and excess soil was removed from the roots of seedlings and washed with tab water. Root tips of seedlings were cut with sterilized scissors of 5 mm and then dipped into inoculum suspension of *Phytophthora parasitica* at a concentration of $1x10^6$ sporangia/ml for 15 minutes before transplanting into pots containing a sterilized soil (soil mixture consists of loam soil: fine coconut shield: sand = 2:1:1) which autoclaved at 121°C, 15 lbs/inch^2 for 1 hour. Seedling roots in control were cut and dipped into sterilized distilled water without inoculum.

Data were collected as disease index (DI) which rated as follows:- level 1 was no symptom, level 2 was shown symptom 1-25 %, level 3 was 26-50, level 4 was 51-75 and level 5 was over 75% (Sandler et al., 19089). Percent disease reduction was calculated based on the formula:- disease reduction (DR) was computed as disease rating in inoculated control – disease rating in treatment/ disease rating in inoculated control × 100. Data were statistically computed analysis of variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test (DMRT) at P = 0.05 and P=0.01.

Field experiment

The experimental site was set up in the infested field wirh roor rot disease planted to citrus approximately 0.75 hectares which was covered about 100 trees of 4-5 year-old citrus trees. The experiment was designed by using a Randomized Complete Block Design (RCBD) with four replications. Treatments were set up as follows:- T1 was Chaetomium-biofungicide at 40 g/20 L of water around the rhizosphere soil and above plants, T2 was metalyxyl-chemical fungicide at 20g/20 L of water around the rhizosphere soil and above plants and T3 was non-treated control. Each treatment consisted of 20 citrus trees.Then, all tested citrus trees were 60 trees. Symptom of yellow leaves, die back and root rot was scored as disease index (DI) and evaluated monthly during the experiment. Disease Index was recorded and classified into 5 levels as follows:- Level 1=0%, no symptom (healthy plant), Level 2=1–25% of yellow lesions on leaves, Level 3=26–50% of yellow lesions on leaves, Level 4=51-75% of yellow lesions on leaves and Level 5=76–100% of yellow lesions on leaves. Data was statistical calculated analysis of variance (ANOV), treatment means were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and P=0.01. Disease Reduction was computed as disease index in control – disease index in treatment / disease index in control × 100.

Results and discussions

Control mechanism of Chaetomium

Chaetoglobosin-C is reported to produce from *Chaetoimium globosum* (Kanokmedhakul *et al*, 2002). Chaetomium isolates were confirmed by molecular phylogeny compared to as *Ch. globosum* 0805 previous report (Nguyen The Quyet *et al*, 2014). As a result, Chaetoglobosin-C gave significantly inhibited colony, sporangia, and oospores growth of P. parasitica causing root rot of citrus which the ED_{50} values of 4.0, 35.4 and 125.7 ppm respectively. It was highly significant inbited the colony sporangia and oospore growth of P. parasitica at 500 ppm which were 92, 76.1 and 71 % respectively (Table1). Chaetoglobosin-C at 500 ppm gave highest significantly inhibite colony (0.4 cm) when compared to the control (5.5 cm). Chaetoglobosin-C at 500 ppm was also given highest inhibition of sporangia production which was 1.8 X105 sporangia when the control was 7.8 X105 sporangia and also gave highest inhibition of oospore production which was 0.6 oospore while the control was 2.3 oospores. Similar reports was comfirmed by Pechprome and Soytong (1997) that *Ch. globosum* significantly inhibited *Phytophthora*

palmivora causing root rot of durian. Ch. globosum reported to be inhibited *P. parasitica* causing root rot of citrus in Cambodia (Kean et al, 2010). Control mechanism could involve in the production of anyibiotic substances against the pathogen as Kanokmedhakul *et al.* (2002) reported that *Ch. globosum* produces new compounds, chatomanone, chaetoglobosin-c and echinuli which expressed inthibition to *Mycobacterium tuberculosis* causing human disease. Kanokmedhakul *et al* (2006) reported that antifungal azaphilones eg chaetomanon produced from *Ch. cupreum* CC3003 expresed actively against some humam pathogen as well. It is concluded that control mechanism of Ch. globosum is proved as antibiosis which it could produce antibiotic substance, Chaetoglobosin C to supress *P. parasitica* causing root rot of Citrus.

Table 1. Inhibition of *Phytophthora parasitica* by Chaetoglobosin-C

ppm					Inhibition(%)[1]	
	Colony dia	Sporangia($X10^5$)	oospores	Colony(mm)	sporangia	oospores
0	5.5 a[2]	7.8 a	2.3 a	---	---	---
10	2.0 b	4.2 ab	1.7 b	63.6	46.0	26.7
50	2.2 b	2.6 b	1.6 b	59.2	66.6	29.2
100	1.1 c	5.7 ab	1.4 b	79.8	27.0	39.3
500	0.4 d	1.8 b	0.6 c	92.0	76.1	71.1
ED_{50}	---	---	---	4.0	35.4	125.7

[1/]Inhibition (%) = number of spore in control – number of spore in treatment/number of spore in control

[2/]Average of four replications. Means followed by a common letter are not significantly different by DMRT at P=0.01.

Pot experiment

The research finding showed that Chaetomium treatment at soil pH 3,4,5,6 and 7 gave highly significant better reduction the disease incidence of cturs root rot caused by *P. parasitica*; which showing the disease index of 1.0, 1.6, 2.0, 1.6 and 1.6 respectively; than Metalaxyl chemical fungicide; which showing the disease index of 5.0, 4.8, 4.5, 4.4 and 4.2 respectively; when compated to the controls the disease incidence were 5.0, 4.9, 4.6, 4.5 and 4.1 respectively as seen in Table 2. Chaetomium biofungicide gave higher disease reduction at all tested with soil pH level of 3, 4, 5, 6 and 7 which were 80.0, 67.3, 56.5, 64.4 and 60.9 % respectively than Metalaxyl chemical fungicide which the disease reduction were 0.0, 2.04, 1.17, 4.34 and 0.00 % respectively. It is indicated that Chaetomium can grow well to supress the pathogen in various pH levels from pH 3-7. The tested Metalaxyl chemical fungicide gave lowest disease reduction

at all tested soil pH levels. It is indicated that the pathogen become resistant to chemical fungicide (Kean *et al*, 2010).

Table 2. Testing of Chaetomium biofungicide to control citrus root rot caused by *P. parasitica* in pot experiment for 4 months

Methods	Soil pH	Disease index	Disease reduction (%)
Chaetomium	3	1.0 c[1]	80.0
	4	1.6 bc	67.3
	5	2.0 b	56.5
	6	1.6 bc	64.4
	7	1.6 bc	60.9
Metalaxyl	3	5.0a	0.00
	4	4.8ab	2.04
	5	4.5cde	1.17
	6	4.4e	4.34
	7	4.2f	0.00
control	3	5.0a	-
	4	4.9a	-
	5	4.6bcd	-
	6	4.5cde	-
	7	4.1f	-

[1]Disease reduction = disease index of control – disease index of treatment/disease index of control X 100.

[2]Average of four replications. Means followed by a common letter are not significantly different by DMRT at P=0.01.

Field experiment

Result in the fiend trial showed that the disease index (DI) of cturs root rot caused by *P. parasitica* in Chaetomium biofungicide treatment (DI =1.17) was not significantly differed from Metalaxyl chemical fungicide (DI = 1.09) when compated to the non-treated control (DI = 3.00) as see in Table 3. Chaetomium biofungicide reduced disease of 64 % and Metalaxyly chemical fungicide reduced the disease of 61.3 %. Moreover, Chaetomium biofungicide treatment was also not significantly differed from Metalaxyl chemical fungicide in term of yield. Chaetomium biofungicide and Metalaxyl chemical fungicide treatments yielded 52.3 and 45.2 kg/tree which significantly differed from the

non-treated control (38.0 kg/tree). The result was similar to the result of Soytong *et al (*1999) and Usuwan and Soytong (2000) who reported that application of Ketomium products to control citrus root rot that was not significant different from chemical treatment (metalaxyl) in the fields and the control was steadily declined from month to month by showing the symptom of yellow leave, die back and root rot. It is noticed that Kean *et al* (2010) reported that *Pythium ultimum* caused citrus root rot in Cambodia and stated that In field trials, the chemical and biological fungicides namely Chaetomium product and metalaxyl were compared and applied to four year old citrus trees in one year showing that the biological product of Chaetomium gave significantly disease control as equal as the chemical fungicide (metalyxyl) when compared to the non-treated control.

Table 3. Field experiment

Treatments	DI	DR(%)	Yield/tree/kg
Chaetomium	1.17 b	64.0	52.3 a
Metalaxyl	1.09 b	61.3	45.2 a
Control	3.00 a	----	38.0 b

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