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Antimicrobial Activity of Metabolites from *Chaetomium* to Control Root Rot (*Phytophthora parasitica*) and *Colletotrichum gloeosporioides* (anthracnose) in Citrus

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ABSTRACT

Citrus diseases were studied which found anthracnose caused by *Colletotrichum gloeosporioides* and root rot caused by *Phytophthora parasitica* which seriously invaded in Thailand where citrus are grown. The chemical fungicides have been traditionally using by the growers until those chemicals are non-effective control. *Chaetomium* species are proved to be antagonistic fungi against phytopathogens by using their biopreparation or active metabolites to control those diseases. The purpose of investigation was evaluated active metabolites derived from *Chaetomium cupreum* and *Chaetomium cochliodes* to control those pathogens. The phytopathogens were isolated, identified and proved to be pathogenic isolates. The factorial experiments in Completely Randomized Design were conducted to gathered data for statistical analysis. *Chaetomium*'s metabolites were crude hexane, ethyl acetate and hexane extracts that tested to 0, 10, 50, 100, 500 and 1000 ppm. Results showed that crude EtoAC extract of *C. cupreum* showed the best anifungal activity against *C. gloeosporioides* with the ED_{50} of 275 ppm. Moreover, crude MeOH extract derived from *C. cochliodes* inbibited the sporulation *C. gloeosporioides* with the ED_{50} of 532 ppm. However, crude EtoAC extract derived *C. cupreum* suppressed the production of sporangia of *P. parasitica* at ED_{50} of 98 ppm., and crude MeOH extract from *C. cochliodes* showed the highest sporangia supression of *P. parasitica* at the ED_{50} of 44 ppm. Further investigation is being conducted to control those citrus diseases in pot and field trials.

Keywords: *Citrus, Root rot, Anthracnose, Chaetomium*

1. INTRODUCTION

Citrus rot caused by *Phytophthora parasitica* or Pythium spp and anthracnose caused by *Colletotrichum gloeosporioides* occurs on the same citrus trees namely disease complex or disease interaction leadsing to loss of yield or even plants died. The growers have been controlled using chemical fungicides that known to be causing resistant by mutate pathogens [1]. *Phytophthora* spp. is reported to be seriously disease which appeared the symptoms of basal stem and brown rot, gummosis, fruit drop and rotting roots, and followed by yellowing leaves and die back [2] in many countries [3]. The other disease interaction may be involved are greening and tristeza virus diseases which can be possible found in the infected trees with Phytophthora rot and anthracnose pathogens [4]. The antagonistic fungi against phytopathogens have been investigated my many researchers in recent years. There are reported that application biological fungicides from *Chaetomium* and *Trichoderma* can be recovered citrus root rot disease in 3-4 months. Surprisingly, the biological products of *Chaetomium* and *Trichoderma* can be controlled the disease at the same level of metalyxyl fungicide [4] *Chaetomium* spp have been reported to inhibit many phytopathogens and Chaetomium formulation gave a good control in several diseases [5]. The control mechanism of *Chaetomium* is found to be released metablite activity going into destroy the pathogen cells and lost of pathogenicity. *Chaetomium globosum* is reported to produce a pure compound, Chaetoglobosin-C which eliminate the colony, sporangia, and oospores growth of *P. parasitica* at the ED_{50} values of 4, 35 and 125 , respectively. Chaetomium biofungicide produced from *Ch. globosum* was not significantly differed to reduce disease of 64 % when compared to metalaxyly fungicide that reduced the disease of 61.3 % in the fields [6]. Our previous research investigation of *Chaetomium cupreum* and *Chaetomium cochliodes* found that the secondary metabolites of *Ch. cochliodes* , compounds chaetoviridines F, chaetochalasin A and 24(R)-5a,8a-epidioxyergosta-6-22-diene-3b-ol

inhibited *Plasmodium falciparum* (malaria disease of human being). Moreover, chaetoviridines E and F and 24(R)-5a,8a-epidioxyergosta-6-22-diene-3b-ol inhibited *Mycobacterium tuberculosis* (tubercurosis) We found that chaetoviridines E and F inhibited the KB, BC1, and NCI-H187 cell lines [7]. Moreover, *Chaetomium cupreum* found to release rotiorinols A-C, (–)-rotiorin, and rubrorotiorin which inhibited *Candida albicans* [8]. *Ch. cupreum* and *Ch. cochliodes* reported to be inhibit phytopathogens, *Pyricularia oryzae* (rice blast) and *Fusarium exospore* f sp *lycopersici* (wilt of tomato). The fungal metabolites from *Ch. cupreum*, crude hexane, ethyl acetate and methanol extracts inhibited *Phytophthora parasitica* causing citrus root rot in Vietnam at the ED_{50} of 88, 97, 165 ppm., respectively [9]. The objectives were evaluated the active microbial metabolites derived from *Chaetomium cupreum* and *Chaetomium cochliodes* to control *P. parasitica* causing root rot and *C. gloeosporioides* causing anthracnose of citrus.

2. MATERIALS AND METHODS

2.1. Isolation of causal agents

The collected root rot and anthracnose samples were done isolation using tissue transplanting and baiting techniques. The samples of infected plants were separately kept in moisten plastic bags until reached laboratory, then cleaned with sterilize distilled water, then cut the lesions in advanced margin (in between healthly and diseased parts) into small pieces, placed to water agar (WA) and kept at room temperature (37 C). The hyphal tips growing out from advanced margin were sub-cultured to potato dextrose agar (PDA) until gathered pure cultures. Pure cultures were morphologically identified according the works of Waterhouse [10], Trow [11], Johnson [12] and van der Plaats- Niterink [13].

2.2. Pathogenicity tests

The pathogen isolates were proved to be pathogenic isolates by Kock's postulate. Experiment was set up in Completely Randomized Design (CRD) with four repeated experiments. The fungal plugs (5 mm diameter) of expected *Phytophthora* sp. and *Colletotrichum* sp. were separately inoculated to the wounded leaves. The detached leaf method was used by inoculation the target pathogen onto wounded and placed in moist chambers, then incubated at room temperature (28-30C) for 7 days. The non-inoculated control were placed the sterile agar discs onto leaves which served as controls. The lesions on leaves were indexed as of 1 to 4, where 1=green is normal and non-aggressive, 2 = pale green is low aggressive, 3=pale brown is medium aggressive and 4 = dark brown is high aggressive, 7 days after inoculation. The lesions on leaves were periodically observed and separately re-isolated the pathogens to confirm the pathogenic isolates.

2.3. Testing metabolites from Chaetomium spp. against citrus root rot and anthracnose

The effective of *Ch. cupreum* and *Ch. cochliodes* were derived from Biocontrol Research Unit, Agricultural Technology Faculty, KMITL, Thailand, They were sub-cultured on potato dextrose agar medium and incubated at room temperature (28-30 C) for 3 weeks. The fungal structures were morphologically observed under compound microscope. The metabolites from *Ch. cupreum* and *Ch. cochliodes* were extracted by culturing in potato dextrose broth (PDB) for 30 days to get the dried biomass. The crude extractions were done using the method of Kanokmedhakul et al. [8]. Research design was performed using 2x6 factorial in CRD, and four replications. Crude extracts derived from *Ch. cochliodes* namely Chc / EtoAC, and *Ch. cupreum* namely Chc MeOH. These crude extracts were evaluated through the concentrations of 0, 10, 50, 100, 500, and 1,000 µg/ml. 2% dimethyl sulfoxide (DMSO) was used to dissolve the crude extracts before mixed to PDA, then autoclaved at 121°C, 15 lbs/inch2 for 30 minutes. The tested pathogenic isolates, *P. parasitica* and *C. gloeosporioides* were separately transferred onto PDA, and maintained at room temperature for 5 days. The advanced margin of colony was cut using 3 mm diameter sterilized cork borer, and those agar plugs of each pathogenic isolate was separately moved to the middle of amended metabolite-PDA plate (5.0 cm diameter) in each concentration of both experiments. were incubated at room temperature (27°C) for 5 days. Data were gathered as diameter of colony and sporangia and/or conidia number. Inhibition was calculated as percentage. Data were statistically analyzed the variance of data. Duncan's Multiple Range Test (DMRT) at P= 0.05 and P=0.01 was used to compare the means of treatment combination. Probit program was used for analysed the effective dose (ED50).

3. RESULTS AND DISCUSSION

3.1. Isolation of causal agents

Phytophthora parasitica and *Colletotrichum gloeosporioides* were isolated and identified to cause root rot and anthracnose of citrus. The identification was morphologically based on the reviewed references of Waterhouse [10], Trow [11], Johnson [12] and van der Plaats- Niterink [13].

P. parasitica produces mobile zoospores in sporangia and releasing zoospores from papulae pore. It has one antheridium per one oogonium. Oospore is double thick

walls. It belongs to Oomycota, Peronosporales, Peronosporaceae. [14]. *C. gloeosporioides* produces many conidia in pycnidia without setae, one-cell conidium, hylaline, and oblong shape. It is an imperfect stage and it is a asexual stage of *Glomerella cingulate* but most of the this pathogen in tropical zone refers to *C. gloeosporioides* which is a significant problem worldwide causing anthracnose and fruit rotting diseases on hundreds of economically important plants. It belongs to Ascomycota, Sodariomycetes, Glomerellales, Glomereallaceae [15].

3.2. Pathogenicity tests

It is proved that the isolated *Phytophthora parasitica* caused rot disease and *Colletotrichum gloeosporioides* caused anthracnose diseases of citrus which were pathogenic isolates. Citrus root rot caused by *P. parasitica* is confirmed by Daniel et. al [16] and Quyet et al. [9]. But there were reported that root rot of *Phytophthora citrophthora* is mostly found in winter and summer. *P. citrophthora* is most damaging when citrus roots are inactive and their resistance to infection is low and *P. parasitica* is active during warm weather especially in tropical zone [14]. Withthis, citrus rot is also reported to caused by *P. parasitica* in Vietnam and the pathogen is proved to be aggressive isolate causing root rot of citrus [9, 6].

3.3. Testing metabolites from Chaetomium spp against Colletotrichum gloeosporioides causing citrus anthracnose

Result showed that *Ch. cupreum* that extracted to be crude Chc/EtoAC significantly inhibited the spore production of *C. gloeosporioides* of 68 % at concentration of 1,000 ppm. But it inhibited the colony growth only 16 % at concentration of 1,000 ppm (Table 1). Crude Chc/EtoAc extract from *Ch. cupreum* against *P. parasitica* causing root rot of durian resulted to inhibit the colony growth of 16 %, while inhibited the oospore production of 41 % and inhibited sporangia production of 94 % (Table 2). Similar research reported that Chaetoglobosin-C released from *Ch. globosum* significantly inhibited colony, sporangia, and oospores of *P. parasitica* which the ED_{50} of 4.0, 35.4 and 125.7 ppm., respectively [9, 14].

Crude Chg/MeOH extract from *Chaetomium cochliodes* resulted to inhibit *Colletotrichum gloeosporioides* causing citrus anthracnose. The colony growth was inhibited 16 % by crude Chg/MeOH extract from *Ch. cochliodes* at concentration of 1,000 ppm. Crude Chg/MeOH extract from *Chaetomium cochliodes* inhibited spore production of 54 % at concentration 1,000 ppm (Table 3). The crude Chg/MeOH extract from *Ch. cochliodes* showed antifungal activity against *P. parasitica* which inhibited the colony growth by 16 %, oospore production by 60 % and sporangia by 93 % (Table 4). *Chaetomium* spp gave a good control citrus root rot in Cambodia [4]. The fungal metabolites from *Ch. cupreum* (Chc/EtoAC) and *Ch. cochliodes* (MeOH) expressed antifungal activities against citrus anthracnose (*C. gloeosporioides*) which the ED_{50} values of 275 and 532 ppm, respectively (Table 5).

However, crude Chc/EtoAC and Chg/MeOH extract gave a good result to inhibit root rot pathogen (*P. parasitica*) which the ED_{50} values of 98 and 44 ppm, respectively. However, the reviewed literature found that ED_{50} values of CG- methanol derived from *Ch. globosum* inhibited *P. parasitica* at 16 ppm, and followed by CC-hexane (Ch. cupreum) at 88 ppm, CC-ethyl acetate (*Ch. cupreum*) 97 ppm, CC-methanol (*Ch. cupreum*) 165 ppm, CG-hexane (Ch. globosum) 185 ppm and CG-ethyl acetate (*Ch. globosum*) 4487 ppm [9] It is proved the control mechanism as antigiosis. The antagonistic substances were extracted from *Chaetomium* spp as crude extracts showed their abilities to inhibit *P. parasitica* and *C. gloeosporioides.*

Table 1. Chc/EtoAC *Chaetomium cupreum* against *Colletotrichum gloeosporioides* causing citrus anthracnose

	Concentrations (ppm)					
	0	10	50	100	500	1000
Colony (cm)	4.97	4.97	4.86	4.81	4.41	4.14
% inhibition	-	0.50	2.26	3.26	11.29	16.81
Conidia ($x10^6$)	17.80	13.83	8.80	7.18	5.63	5.35
% inhibition	-	18.87	47.45	54.77	64.92	68.46

Table 2. Chc/EtoAc *Chaetomium cupreum* against *Phytophthora parasitica*

	Concentrations (ppm)					
	0	10	50	100	500	1000
Colony (cm)	4.19	4.98	4.86	4.40	4.25	4.20
% inhibition	-	0.50	0.98	10.44	13.39	16.51
Oospores (x106)	5.22	3.90	4.05	3.60	3.30	3.05
% inhibition	-	24.42	21.98	30.68	36.49	41.37
Sporangia (x106)	1.75	0.85	0.70	0.55	0.15	0.10
% inhibition	-	50.00	59.03	69.38	91.25	94.72

Table 3. Chg / MeOH *Chaetomium cochliodes* against *Colletotrichum gloeosporioides* causing citrus anthracnose

	Concentrations (ppm)					
	0	10	50	100	500	1000
Colony (cm)	4.97	4.97	4.86	4.81	4.41	4.14
% inhibition	-	0.50	2.26	3.26	11.29	16.81
Conidia ($x10^6$)	36.44	33.06	22.00	18.88	17.13	16.50
% inhibition	-	8.99	39.56	48.08	52.90	54.55

Table 4. Chg / MeOH *Chaetomium cochliodes* against *Phytophthora parasitica*

	Concentrations (ppm)					
	0	10	50	100	500	1000
Colony (cm)	4.98	4.90	4.95	4.77	4.60	4.15
% inhibition	-	1.52	0.50	4.02	7.55	16.59
Oospores (x106)	5.15	3.95	3.05	2.75	2.30	2.00
% inhibition	-	22.63	40.74	46.38	54.88	60.56
Sporangia (x106)	1.70	1.60	0.70	0.50	0.45	0.00
% inhibition	-	3.13	48.86	81.25	93.56	92.73

Table 5. ED_{50} of Chc/EtoAC and Chg/MeOH against *Colletotrichum gloeosporioides* and *Phytophthora parasitica*

pathogens	extracts	ED_{50} (ppm)
C. gloeosporioides	Chc / EtoAC	98
	Chg / MeOH	44
P. parasitica	Chc / EtoAC	98
	Chg / MeOH	44

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4. CONCLUSION

The natural product fungal metabolites derived from *C. cupreum* and *C. cochliodes* expressed antimicrobial activity against anthracnose pathogen (*C. gloeosporioides*) at the ED_{50} of 275 and 532 ppm, respectively. Moreover, the metabolites crude EtoAC derived from *C. cupreum* and crude MeOH from *C. cochliodes* gave a good suppression to inhibit sporangia proliferation at ED_{50} of 98 and 44 ppm. It has further conducted to develop these metabolites to be the nanoparticles and testing to control citrus diseases in pot and field experiments.

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