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Nano-particles derived from *Chaetomium elatum* against Phytophthora rot of durian

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Abstract Durian are the economically fruit trees in Thailand. The important problem of durian is root rot disease caused by *Phytophthora palmivora*. This study was used *Chaetomium elatum* to control the *P. palmivora* causing root rot disease of durian by dual culture method, crude extract test and nano particles test derived from *Ch. elatum*. Dual-culture test showed that *Ch. elatum* gave efficiency to inhibit of spore and colony growth of *P. palmivora* which were 46.13 and 38.89%, respectively. Testing efficacy of crude extract from *Ch. elatum* to control *P. palmivora* found that crude ethyl acetate from *Ch. elatum* gave significantly highest against pathogen of *P. palmivora* at the concentration of 1000 ppm which the ED_{50} of 175.31 ppm. Nano particles testing, nano particles of crude hexane, ethyl acetate and methanol from *Ch. elatum* showed the ED_{50} values of 3.49, 3.47 and 3.41 ppm.

Keywords: *Chaetomium elatum*, *Phytophthora palmivora*, durian

Introduction

Durian(Durio zibethinus Murr.) is king of tropical fruit refer to two facts of the fruit. Its superlative fresh, which is highly nutritional and its appearance, which resembles the thorny thrones of the Asian kings of old. Durian is one of the most famous fruit in South-East Asia. The fruit is very famous not only due to the taste richness but also the strong odour. Durian is an economically fruits in Thailand. The country is the world's largest producer and exporter of durian, followed by Malaysia and Indonesia (Somsri, 2014). In past, root rot has been reported to the serious rate of infection of durian because monoculture planting and high fertilizer applications could lead the increment of disease incidence caused by fungi such as *P. palmivora* and *Pythium* spp. Chemical compounds

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have been used to control plant diseases, but abuse in their employment has favored the development of pathogens resistant to fungicides. The objective was to use of Chaetomium elatum that antagonize plant pathogens is risk-free when it results in enhancement of resident antagonists. Moreover, biological control agents (BCAs) could reduce levels of fungicide.

Materials and methods

Morphological Studies

Soil samples were collected plant disease. Soil samples were isolated by using soil plate method on glucose-ammonium nitrate agar media(GANA) and incubated at 28-30℃ for 2 days, then the fungal growing mycelium tip of it was sub-cultured and purified in potato dextrose agar (PDA) until get the pure culture.

The macroscopic characteristics of colony appearance were determined including growth pattern and texture and growth rate onto PDA plates. For microscopic characteristics shapes of zoosporangia were observed by using a light microscope.

Pathogenicity Test

Pathogenicity test was done by agar plug method. The healthy durian detached leaves were sterilized by 10% sodium hypochlorite. The surface detached leaves were made wounds by sterilized needle. The agar plug of pathogen inoculated to wound on detached leaves. The controls were processed similarly but transferred an agar plug without the pathogen.

Bi-culture test

The experiment was conducted using a Completely Randomized Design (CRD) with 4 replications. The antagonistic fungi and pathogen were separately cultured on PDA at room temperature for 7 day. A 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of cultures of the pathogenic and antagonistic fungi and transferred onto 9 cm diameter PDA plates, an agar plug of the pathogen was placed on one side of the plate which opposited an agar plug of an antagonistic fungus. PDA plates were transferred with a single plug of an antagonistic fungus or of the pathogen acted as the controls. The bi-culture plates were incubated at room temperature for 30 days. Data were collected regarding colony diameter (cm) and the number of conidia reduced by the pathogen.

Crude extract test

The experiment was conducted by using factorials in Completely Randomized Design (CRD) with four replications. Each crude extract was dissolved in 2% dimethyl sulfoxide and added to PDA before autoclaving at 121 ℃ (15 psi) for 30 minutes. The agar plug of pathogen was transferred to the middle of PDA plates (amending with each crude extracts) in each concentration(0, 10, 50, 100, 500, 1000 ppm) and incubated at room temperature until the pathogen on the control plates growing full. Data were collected as colony diameter, Percentage inhibition of pathogen colony growth and conidia and The effective dose (ED_{50}). Data was statistically computed analysis of variance. Treatment means were compared with DMRT at P=0.05.

Testing nano-particles from Ch. elatum

Preparation of nano particles derived from *Ch.elatum* were used the method of Dar and Soytong (2014). Testing for inhibition of mycelial growth and sporangium formation of *P. palmivora* was done by using poison food method.The Experiment was conducted by using factorials in CRD with four replications. The concentration of nano particles; nano-CEH, nano-CEE, nano-CEM were as follows: 0, 3, 5, 10 and 15 ppm. Each concentration was dissolved in 2% dimethyl sulfoxide, then mixed into potato dextose agar(PDA) and added chitosan before autoclave at 121 ℃ for 30 minutes. The agar plug of pathogen was removed to PDA plates in each solvent and concentration. After incubated at room temperature until the pathogen on the control plates growing full collected data as colony diameter, number of sporangia, inhibition percentage and Effective dose ED_{50}. Data was statistically computed analysis of variance. Treatment means were compared with DMRT at P=0.05.

Results

Morphological Studies of P. palmivora

The fungal growth rapidly and colonized the plate within 4 days on PDA. Colony morphology on PDA is a chrysanthemum pattern with aerial mycelium. Sporangia are globose and ovoid shape, which was papillate. Zoospores were directly released from sporangia when flooded in water (Fig.1).

Pathogenicity test

Pathogenicity test on detached leaves after 3 days by the plug inoculation method. Leaves showed symptoms of brown hydrolysis expand around agar plug of pathogen. In control, Leaves remained healthy (Fig.2).

Figure 1. Morphological characteristics of *P. pamivora* (A); Colony appearance on PDA (B); Shape of sporangia (C); Zoospore release from sporangia (D); Oogonia

Figure 2. Pathogenicity test of *P. palmivora* on detached leaves. (A); The inoculated pathogen (B); The non - inoculated pathogen

Bi-culture test

Ch.elatum was proved its abilities to inhibit the growth of *P. palmivora* by using bi-culture test (Fig.3).The result showed that *Ch. elatum* inhibited colony growth and production of spore by *P. palmivora* of 38.89 and 46.13% inhibition, respectively (Table 1).

Table 1. Colony and spore inhibition of *P. palmivora*

Antagonist fungi	*P. palmivora*	
	Colony inhibition (%)	**spore inhibition (%)**[/2,3]
Ch. elatum	38.89	46.13
C.V. (%)	1.05	

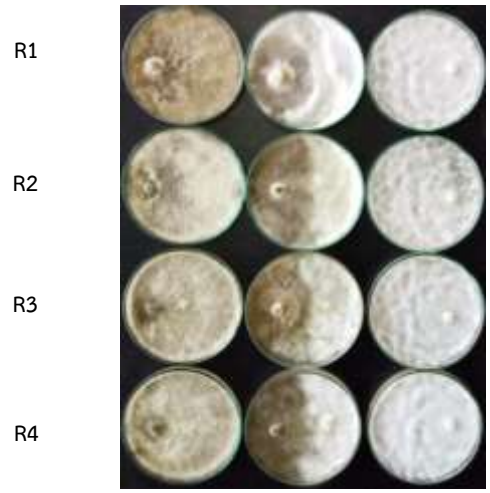

Figure 3. *Ch. elatum* inhibited colony growth of *P. palmivora* by using bi-culture test

Crude extract test

Crude-CEH at concentrations of 10, 50, 100, 500 and 1000 ppm were tested the colony growth inhibition of *P. palmivora* which were 19.25, 39.25, 46.5, 58.00 and 58.5% respectively (Fig 4). Test inhibition of sporangia information of *P. palmivora* which were 13.46, 25.88, 38.30, 53.86 and 62.73% respectively (Table 2) when compared to the control. Crude-CEE at concentrations of 10, 50, 100, 500 and 1000 ppm were tested the colony growth inhibition of *P. palmivora* which were 37.5, 39.75, 45.75, 60.5 and 76.5% respectively (Fig 4). Test inhibition of sporangia information of *P. palmivora* which were 20.04, 30.68, 39.87, 56.47 and 80.79% respectively (Table 2) when compared to the control. Crude-CEM at concentrations of 10, 50, 100, 500 and 1000 ppm were tested the colony growth inhibition of *P. palmivora* which were 12.25, 30.75, 45.25, 45.75 and 51.00% respectively (Fig 4). Test inhibition of sporangia information of *P. palmivora* which were 14.09, 20.45, 31.41, 43.73 and 54.69% respectively (Table 2) when compared to the control. Meanwhile

ED_{50} values of crude-CEH, CEE, CEM were 341.97, 175.31 and 58.96 µg/ml respectively.

Nano-particles test

Nano-CEH at concentrations of 3, 5, 10, 15 ppm were tested the colony growth inhibition of *P. palmivora* which were 31.25, 38.25, 38.75 and 58.5% respectively (Fig 5). Test inhibition of sporangia information of *P. palmivora* which were 26.15, 40.94, 51.60 and 76.65% respectively (Table 3) when compared to the control. Nano-CEE at concentrations of 3, 5, 10, 15 ppm were tested the colony growth inhibition of *P. palmivora* which were 2.5, 19.75, 28.5 and 37.75% respectively (Fig 5). Test inhibition of sporangia information of *P. palmivora* which were 20.22, 28.47, 42.55 and 62.77% respectively (Table 3) when compared to the control. Nano-CEM at concentrations of 3, 5, 10, 15 ppm were tested the colony growth inhibition of *P. palmivora* which were 14.00, 23.75, 36.5 and 40.75% respectively (Fig 5). Test inhibition of sporangia information of *P. palmivora* which were 12.77, 31.48, 52.21 and 61.46% respectively (Table 3) when compared to the control. Meanwhile ED_{50} values of nano-CEH, CEE, CEM were 3.49, 3.47 and 3.81 µg/ml respectively (Table 3).

Table 2. Effect of crude extracts from *Ch. elatum* to inhibit *P. palmivora*

Nano particles	Concentration (ppm)	Colony diameter (cm)	Inhibition of colony growth (%)	Number of sporangia ($\times 10^6$)	Inhibition of sporangia (%)	ED_{50} (µg/ml)
Crude CEH	0	5.00^{a}	0^{i}	59.87^{a}	0^{h}	341.97
	10	4.03^{c}	19.25^{g}	51.81^{b}	13.46^{g}	
	50	3.03^{e}	39.25^{e}	44.37^{cd}	25.88^{fe}	
	100	2.67^{g}	46.5^{d}	36.93^{e}	38.30^{d}	
	500	2.1^{h}	58.00^{b}	27.62^{f}	53.86^{c}	
	1000	2.02^{h}	58.5^{b}	22.31^{g}	62.73^{b}	
Crude CEE	0	5.00^{a}	0^{i}	59.87^{a}	0^{h}	175.31
	10	3.12^{e}	37.5^{e}	47.87^{c}	20.04^{f}	
	50	3.01^{e}	39.75^{e}	41.5^{d}	30.68^{e}	
	100	2.71^{f}	45.75^{d}	36.00^{e}	39.87^{d}	
	500	1.97^{h}	60.5^{b}	26.06^{f}	56.47^{c}	
	1000	1.17^{i}	76.5^{a}	11.5^{h}	80.79^{a}	
Crude CEM	0	5.00^{a}	0^{i}	59.87^{a}	0^{h}	58.96
	10	4.38^{b}	12.25^{h}	51.43^{b}	14.09^{g}	
	50	3.46^{d}	30.75^{f}	47.62^{c}	20.45^{f}	
	100	2.73^{f}	45.25^{d}	41.06^{d}	31.41^{e}	
	500	2.71^{f}	45.75^{d}	33.68^{f}	43.73^{d}	
	1000	2.45^{h}	51.00^{c}	27.12^{f}	54.69^{c}	
C.V.(%)		4.16		5.93		

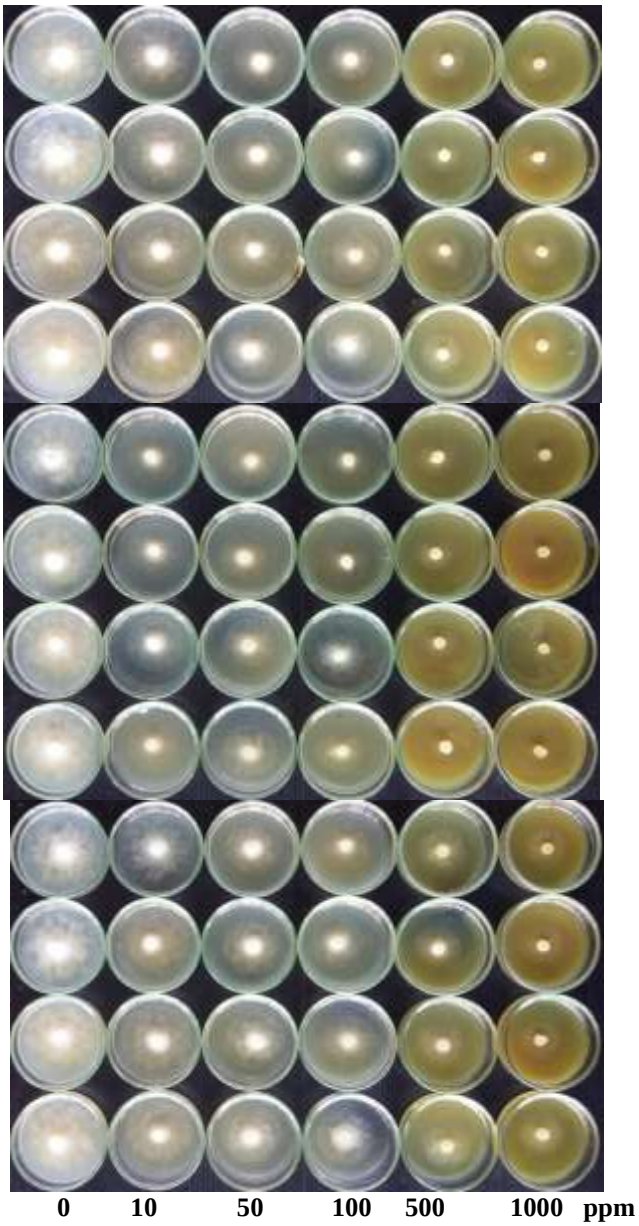

Figure 4. Testing crude extracts from *Ch. elatum* against *P. palmivora*

Figure 5. Testing nano particles from *Ch. elatum* against *P. palmivora*

Table 3. Effect of nano particles from *Ch. elatum* to inhibit *P. palmivora*

Nano particle	Concentration (ppm)	Colony diameter (cm)	Inhibition of colony growth (%)	Number of sporangia ($\times 10^6$)	Inhibition of sporangia (%)	ED_{50} (µg/ml)
Nano CEH	0	5.00[a]	0[i]	62.12[a]	0[h]	3.49
	3	3.43[f]	31.25[d]	45.87[d]	26.15[e]	
	5	3.08[g]	38.25[c]	36.68[e]	40.94[d]	
	10	3.06[gh]	38.75[cb]	30.06[f]	51.60[c]	
	15	2.07[i]	58.5[a]	14.5[h]	76.65[a]	
Nano CEE	0	5.00[a]	0[i]	62.12[a]	0[h]	3.47
	3	4.87[a]	2.5[i]	49.56[c]	20.22[f]	
	5	4.01[c]	19.75[g]	44.43[d]	28.47[e]	
	10	3.57[e]	28.5[e]	35.68[e]	42.55[d]	
	15	3.11[g]	37.75[c]	23.12[g]	62.77[b]	
Nano CEM	0	5.00[a]	0[i]	62.12[a]	0[h]	3.81
	3	4.3[b]	14.00[h]	54.18[b]	12.77[g]	
	5	3.81[d]	23.75[f]	42.56[d]	31.48[e]	
	10	3.17[g]	36.5[c]	29.68[f]	52.21[c]	
	15	2.96[h]	40.75[b]	23.93[g]	61.46[b]	
C.V.(%)		4.79		6.68		

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

Discusion

The bi-culture tests *Ch. elatum* gave significantly inhibition colony growth of *P.palmivora* and production of spore by P. palmivora of 38.89 and 46.13%, respectively. Similar reported by Tathan (2012) *Ch. elatum* gave significantly inhibition colony growth of *P.palmivora* and production of spore by *P. palmivora* of 32.49 and 26.23%, respectively. The crude extracts of *Ch. elatum* gave significantly highest inhibited sporangia production of *P. palmivora* at concentration of 1,000 ppm. Meanwhile ED_{50} values of Crude-CEE was 175.31 µg/ml. Similar reported by Soythong (2015) that crude extract of *Ch. elatum* gave significantly highest inhibited *Fusarium oxysporum* f.sp. *lycopersici* causing wilt of tomato at concentration of 1,000 ppm with the ED_{50} value of 5.94 µg/ml. The nano particle of *Ch. elatum* gave significantly highest inhibited sporangia production of *P. palmivora* at concentration of 15 ppm. Meanwhile ED_{50} values of Crude-CEH was 3.49 µg/ml. Similar reported by Song and Soytong (2016) that nano particle of *Ch. elatum* gave significantly highest inhibited *Pyricularia oryzae* causing blast of rice at concentration of 15 ppm.

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