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Antifungal activity of *Talaromyces muroii* against coffee anthracnose

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Colletotrichum coffeanum causing anthranose is proved for pathogenicity to coffee var. Arabica. *Talaromyces muroii* EU18 is taxonomic confirmed by morphological characters and molecular phylogeny based on *ß-tubulin* region. The crude ethyl acetate and crude methanol extracted from *T. muroii* EU18 showed significantly antifungal activity against *C. coffeanum* which the median effective dose (ED_{50}) values for colony growth inhibition of 580.00 and 420.00 ppm, respectively. Crude hexane, ethyl acetate and methanol extracts at 1,000 ppm suppressed the colony growth of 43.00, 60.75 and 76.25 %, respectively and sporulation of 61.76, 73.02 and 80.00 %, respectively. It is clearly demonstrated that metabolites from *T. muroii* EU18 acts as a new antagonist against *C. coffeanum* causing coffee anthracnose. The chemical elucidation of bioactive compound to prove control mechanism is being done and characterized.

Keyword: *Talaromyces muroii*, fungal metabolites, coffee anthracnose

Introduction

Coffee var. Arabica is distributed to highland in many places around the world (Clifford and Willson, 1985). It becomes export product value mostly producing in Latin America, Africa and Asia (Ridler, 1983). The main important factors for poor quality of coffee are disease and insect pests, especially coffee bean anthracnose caused by *Colletotrichum coffeanum* Noack. The coffee growers are usually applied chemical fungicides but later the pathogen become resistant to those fungicides (Soytong et al., 2001) leading to low quality of coffee beans. Biological control is increasingly interested by many researchers to investigate the new antagonists against plant pathogens. As reports in Ascomycetous fungi like *Chaetomium* spp. are reported to be antagonizing many plant pathogens (Soytong and Quimio, 1992). *Chaetoimum* as a board spectrum biofungicide is introduced to control several plant pathogen especially anthracnose caused by *Colletotrichum* spp. (Soytong *et al.*,

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2001). *Talaromyces* spp. is one of the Ascomycetous fungi which belongs to Eurotiales, Trichocomaceae which express both perfect and imperfect stages as *Penicillium* spp. (Houbraken and Samson, 2011; Yilmaz et al., 2014). With this, Madi et al. (1997) reported that metabolites from *Talaromyces flavus* could inhibit and decrease rotting disease of beans caused by *Sclerotium rolfsii*. Stosz et al. (1996) stated *T. flavus* could control wilt disease caused by *Verticillium dahlia* and tomato wilt caused by *Verticilium albo-atrum* (Naraghi et al., 2010). Moreover, cellulolytic enzymes from *T. flavus* expressed antifungal activity against *Botrytis fabae* causing grey mold on leaves of beans (Haggag et al., 2006). However, the bioactivity of *T. muroii* had not be found. The objective of this research was to investigate the promising antagonist, *Talaromyces muroii* EU18 to control coffee anthracnose caused by *Colletotrichum coffeanum*.

Materials and methods

Coffee Pathogen

Colletotrichum coffeanum was isolated from coffee anthracnose for Arabica in Paksong highland, Laos PDR and it is proved pathogenicity using Koch's Postulate Method by Vilavong and Soytong (2013).

Antagonist

Talaromyces spp. was isolated from soil in Chiang Mai, Thailand by using soil plate method. Pure culture was identification based on morphology and DNA sequencing (Soytong and Poeaim, 2014). To identify by DNA sequence was using the reference sequence of *ß-tubulin* region from Genbank with accession number: KJ865727, KM066155, KM066154 and KM066153 *T. muroii* and KC109774 *Chaetomium globosum. T. muroii* EU18 was selected by screening for antagonistic activity in vitro against coffee anthracnose causing by *Colletotrichum coffeanum.* (Soytong and Poeaim, 2015)

Biological activity of fungal metabolites from Talaromyces muroii against coffee anthracnose pathogen

Crude extracts were done by the extraction from antagonistic fungus, *T. muroii* by using the method of Kanokmedhakul et al. (2006). *T. muroii* EU18 was cultured in potato dextrose broth (PDB) at room temperature (30˚C) for 30 days. Fungal biomass was collected by filtration through cheesecloth and air-

dried until dried. Dried fungal biomass was ground in electrical blender, extracted with hexane and shaken for 24 hour at room temperature. The ground biomass was separated by filtration through Whatman filter paper No.1. The marc was then extracted with hexane as the same procedure described above. The filtrate was following evaporated to yield crude extract. The marc was then further extracted with ethyl acetate and methanol, respectively. Crude hexane, ethyl acetate and methanol were resulted for further experiment and kept in refrigerator at 4°C until use.

All crude extracts from *T. muroii* EU18 were evaluated to inhibit *Colletotrichum coffeanum* causing anthracnose of coffee var. Arabica. The experiment was performed using 3x6 factorial experiments in Completely Randomized Design (CRD) with four replications. Factor A was crude extracts of hexane crude (A1), ethyl acetate crude (A2) and methanol crude (A3). Factor B was various concentrations of 0 (B1), 10 (B2), 50 (B3), 100 (B4), 500 (B5), and 1,000 ppm (B6). Each crude extract was separately dissolved in 2% dimethyl sulfoxide (DMSO), then mixed into potato dextrose agar (PDA), then autoclaved at 121°C, 15 lbs/inch^2 for 30 minutes. The coffee pathogen was cultured on PDA and incubated at room temperature for 5 days; colony peripheral was cut by 3 millimeters diameter sterilized cock borer. The agar plug of pathogen was transferred to middle of PDA plates incorporated with each concentration of each crude extract (5.0 centimeters diameter), then incubated at room temperature for gathering data. Data were collected as colony diameter and number of spores. Number of pathogen spores in each treatment was counted by using haemocytometer under light microscope. Percentage of inhibition was calculated. Data was statistically computed analysis of variance. Treatment means were compared with Duncan Multiple's Range Test (DMRT) at P= 0.05. The median effective dose (ED_{50}) was computed by using probit analysis.

Results and discussion

Description of Talaromyces muroii EU18

Colony character is pale yellow to deep yellow, yellowish ascocarps, 220.78-420.59 x 244.4-482.06 micrometers, subglobose. Asci are globose, 8.51-9.95 x 8.69-11.69 micrometers, ascospores are broadly ellipsoidal shape 2.91-4.42 x 4.27-5.46 micrometers, thick wall with small spiny, without ridge. It is morphological confirmed and similar to reports by Domsch et al. (1993) and Yilmaz et al. (2014) as seen in Figure 1A-D. Their morphology seems to be *Talaromyces flavus*. However, Phylogeny tree of EU18 is reconfirmed species

level based on *ß-tubulin* region using Neighbor-joining, bootstrap = 1000 (Figure 2). *Talaromyces muroii* EU18 is confirmed identification by morphological characters and molecular technique.

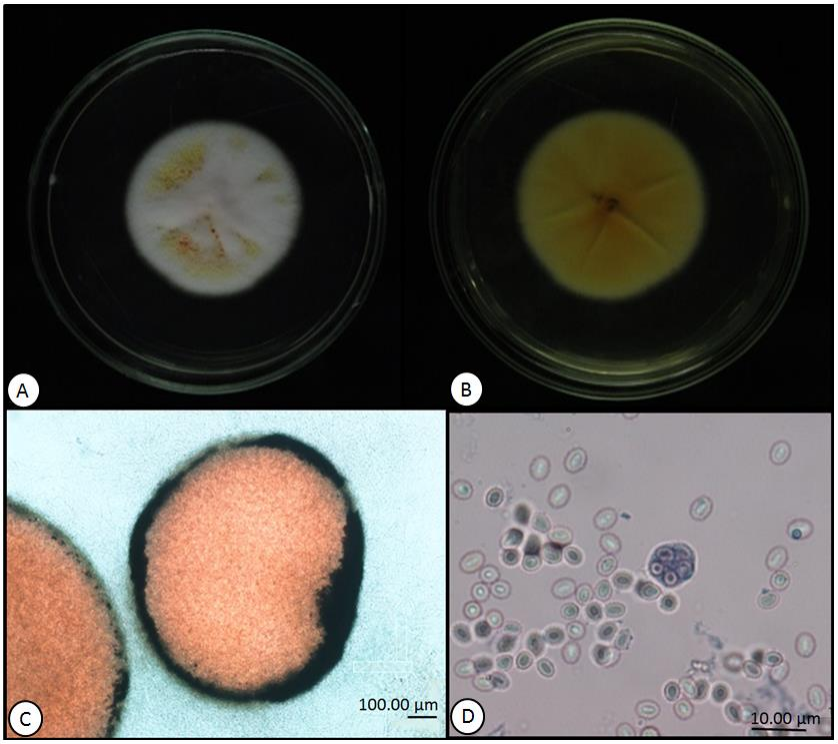

Figure 1 Characteristics of *Talaromyces muroii* EU18, A = upper surface of colony in pure culture on PDA, B = lower surface of colony in pure culture on PDA, C = ascocarps and D = ascus and ascospores

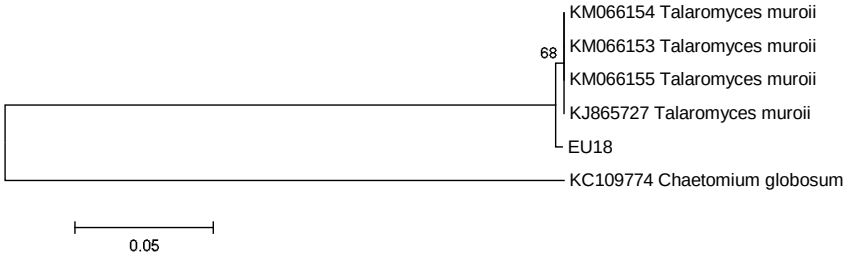

Figure 2 Phylogeny tree of *Talaromyces muroii* EU18 based on *ß-tubulin* region using Neighbor-joining, bootstrap = 1000.

Anthracnose of coffee var. Arabica caused by Colletotrichum coffeanum

Colletotrichum coffeanum is proved to be pathogenicity to coffee var. Arabica which shown symptoms on leaves and coffee beans (Figure 3A). Pure culture is pale greyish brown (Figure 3B), conidia or spores are produced on acervuli which are asexual fruiting body (Figure 3C).

Figure 3 Characteristics of *Colletotrichum coffeanum* causing anthracnose of coffee var. Arabica, A = coffee anthracnose, B = pure culture at 15 days and C = conidia

Biological activity of fungal metabolites from Talaromyces muroii against Coffee anthracnose pathogen

As a result, crude hexane, crude ethyl acetate and crude methanol extracted from *T. muroii* EU18 expressed antifungal activity against *C. coffeanum* causing anthracnose of coffee var. Arabica which the ED_{50} values for growth inhibition of >1000.00, 580.00 and 420.00 ppm, respectively.

Crude hexane, ethyl acetate and methanol extracts from *T. muroii* EU18 at 1,000 ppm significantly inhibited the colony growth of 43.00, 60.75 and 76.25 %, respectively and inhibited spore production of 61.76, 73.02 and 80.00 %, respectively (Table 1 and Figure 4). Similar results from previous reports confirmed the metabolites from *T. flavus* inhibited *S. rolfsii* causing bean rot (Madi et al., 1997), *V. dahlia* causing wilt disease (Stosz et al., 1996), *V. albo-atrum* causing tomato wilt (Naraghi et al, 2010) and *B. fabae* causing grey mold of beans (Haggag et al., 2006). It is clearly demonstrated that *T. muroii* EU18 acts as a new antagonist against *C. coffeanum* causing coffee anthracnose. Moreover, the pathogen cells were broken to be abnormal spores and possible due to fungal metabolites released from *T. muroii* EU18 (Figure 5). This phenomenon is also appeared in similar work of *Chaetomium* spp., antagonistic Ascomycetous fungi which released antibiotic substances eg. chaetoglobosin-c to broken the pathogen cells of *Fusarium oxysporum* f sp

lycopersici (Soytong et al., 2001). It is stated that *T. muroii* antagonized *C. coffeanum* causing coffee anthracnose as reported for the first time.

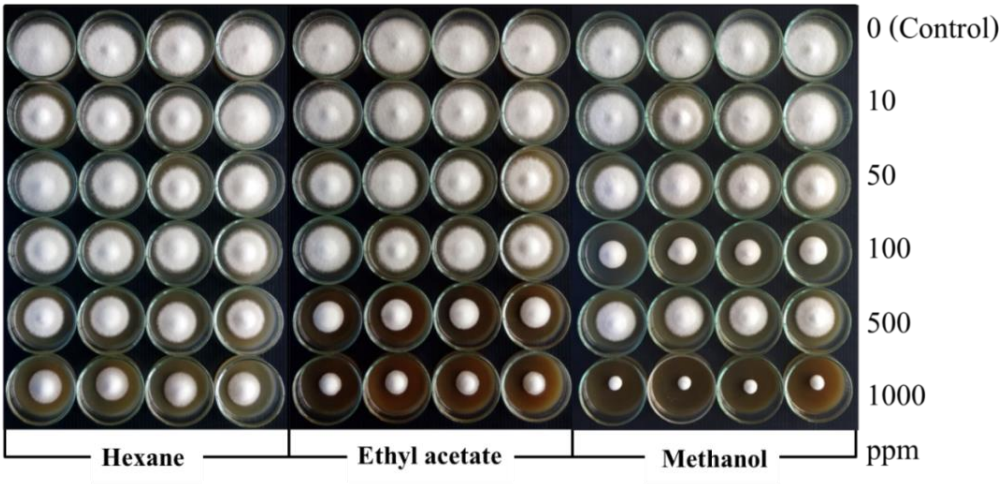

Figure 4 Fungal metabolites from *Talaromyces muroii* EU18 against *Colletotrichum coffeanum* causing anthracnose of coffee

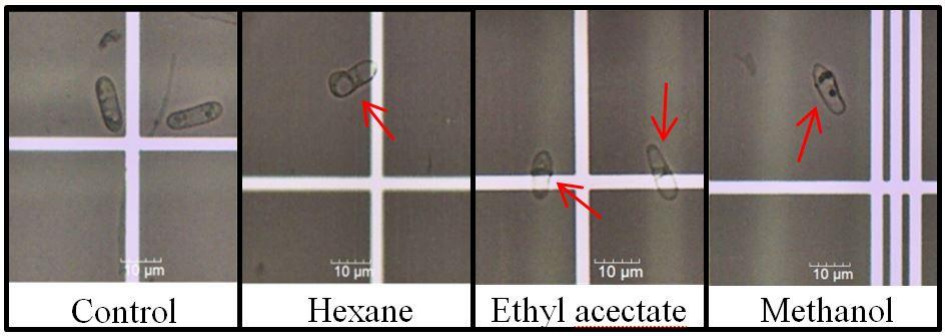

Figure 5 Effect of fungal metabolites from *Talaromyces muroii* to the pathogen cells of *Colletotrichum coffeanum* causing anthracnose of coffee

Table 1. Fungal metabolites from *Talaromyces muroii* EU18 against *Colettotrichum coffeanum*

crude extracts	concentration (ppm)	[1]% inhibition of spore production (25 days)	[1]% inhibition of colony growth (4 days)	[2]ED_{50} (ppm)
Hexane	0	0.00^{h}	0.00^{k}	>1000.00
	10	41.18^{g}	6.50^{j}	
	50	45.59^{fg}	7.50^{j}	
	100	50.00^{efg}	16.00^{hi}	
	500	52.94^{defg}	30.75^{f}	
	1000	61.76^{bcde}	43.00^{e}	
Ethyl acetate	0	0.00^{h}	0.00^{k}	580.00
	10	55.56^{def}	1.25^{k}	
	50	61.90^{bcde}	14.00^{i}	
	100	68.25^{abc}	19.25^{g}	
	500	71.43^{ab}	46.75^{d}	
	1000	73.02^{ab}	60.75^{b}	
Methanol	0	0.00^{h}	0.00^{k}	420.00
	10	42.86^{g}	1.25^{k}	
	50	57.14^{cdef}	17.75^{gh}	
	100	64.29^{bcd}	31.75^{f}	
	500	74.29^{ab}	53.75^{c}	
	1000	80.00^{a}	76.25^{a}	

[1] % inhibition of spore production or colony growth = R1-R2/R1 x 100; R1 = number of spore or colony diameter at 0 ppm and R2 = number of spores or colony diameter in each concentration. Means followed by a common letter are not significantly differed by Duncan's Multiple Range Test (DMRT) at P =0.05.

[2] the median effective dose (ED_{50}) values for colony growth inhibition

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