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Application of Bio-Formulation of *Chaetomium* for Biocontrol of *Colletotrichum Gloeosporioides* Causing Coffee Anthracnose in Arabica Variety in Laos

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Coffee anthracnose pathogen was isolated from coffee leaves and coffee. Morphological data confirmed the species as *Colletotrichum gloeosporioides*.The pathogenicity of the isolate was also confirmed. Bioactive substances from *Chaetomium cupreum* CC3003 including crude hexane, ethyl acetate and methanolextracts showed good inhibition efficacy against *C.gloeosporioides*. A powder bio-formulation of *C. cupreum* gave the highest disease reduction when compared with the inoculated control.

Keywords: coffee anthracnose, Arabica variety

Introduction

Arabica was introduced into Laos in 1920 and distributed to the growers in the uplands between 800 and 1,200 m above sea level. Coffee culture spread to the plateau and down to an elevation of 600 m. Then commercial coffee production was distributed to many places around the world (Clifford and Willson, 1985). Coffee has become one of the primary sources of income and most important export product for approximately 50 coffee producing countries in Latin America, Africa and Asia (Ridler, 1983).Coffee production is the most important perennial crop in the Lao People's Democratic Republic (PDR), mainly grown in the Phouphieng Paksong area. There are 41,000ha of coffee plantations, especially in the Saravan, Sekong, Champasak provinces in the Lao PDR, because of their excellent agronomic characteristics for production of the crop. The government has promoted the production of this crop to support local

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and international needs (MAF, 1997). However, the export price of Phouphiang Paksong coffee is 10% lower than the international market price due to its lower quality. The lower quality is mainly caused by poor agronomic practices, early harvesting, manual harvest processing from screening to drying, insects and disease problems. One of the important reasons for poor quality is that most of farmers have limited knowledge regarding quality improvement including control of insects and diseases especially anthracnose on coffee beans. The coffee growers routinely apply many chemical fungicides for disease control but the anthracnose pathogen has become resistant to chemical fungicides (Soytong *et al.*, 2001) leading to an increase in anthracnose incidence on coffee beans.

Bio-control has proven effective in reducing the pathogen inoculums and disease incidence in a number of economically important plants. *Chaetomium* is a board spectrum biological fungicide used to control several plant pathogens, especially anthracnose caused by *Colletotrichum* spp. (Soytong *et al*., 2001).About 26 million cases of poisoning from toxic chemical pesticides have been reported and 220,000 people die each year from pesticide exposure (WHO and UNEP as cited by Richter, 2002). The need to find alternative effective methods of disease and pest control to safe guard human health and protect the environment is evident (Li and Wu, 2011). Precision farming allows farmers to maximize crop production while utilizing minimum farm inputs especially chemical pesticides and fertilizers (Ditta, 2012). Many research reports have indicated that fungal metabolites from *Chaetomium* spp. gave good control of plant pathogens (Soytong *et al.*, 2001). Metabolites from *Chaetomium* sp. at a concentration of 10 ppm reduced tomato wilt caused by *Fusariumoxysporum* f. sp. *lycopersici* race 2 after incubation for 7 d.

The research aimed to isolate the causal agent of coffee anthracnose and evaluate bio-formulations to control anthracnose of coffee var. Arabica.

Materials and methods

Isolation of coffee anthracnose pathogen

The pathogen was isolated by the tissue transplanting technique from symptomatic leaves and beans of coffee var. Arabica. The diseased plant parts were cut at the advanced margin of lesions into small pieces (5 mm × 5 mm) and surface disinfected with 10% sodium hypochlorite for 1 min, followed by rinsing with sterile distilled water two times, and transferred to water agar (WA). The mycelia growing out of the plant tissue were sub-cultured to potato dextrose agar (PDA), and incubated at room temperature (approximately 28-30 ℃) for 7-10 d. Single spore isolation was done to obtain a pure culture. The

isolate was morphologically identified to species under a compound micros cope.

Pathogenicity test

Pathogenicity was done by the plug inoculation method using a modified protocol from Rattanacherdchai (Ratanachredchai *et al*., 2010). Coffee leaves were surface disinfected with 10 % sodium hypochlorite and left to air-dry in the laboratory. Leaves were wounded by gently pricking with a sterilized needle; two 0.5 cm-diameter wounds were made per leaf, 0.5 cm diameter. Inoculum was prepared by culture on potato dextrose agar (PDA) at room temperature (approximately 28-30 ℃) for 15 d. Agar plugs of the pathogen (0.3 cm-diameter) were cut from actively growing areas of the colony by sterilized cork borer, and transferred to the wound sites on the leaves. The control was wounded and inoculated with a PDA plug without the pathogen. The inoculated leaves were maintained in a moist chamber at room temperature. The experiment was done by Completely Randomized Design (CRD) with four replications. Lesion diameter (mm) was recorded and analysis of variance (ANOVA) was computed. Treatment means were statistically compared by Duncan's Multiple Range Test (DMRT) at P =0.05 and 0.01.

Evaluation of bioactive substances of Chaetomium sp. Against anthracnose pathogen of coffee var. Arabica

The crude extraction from antagonistic fungi was performed using the method of Kanokmedhakul (Kanokmedhakul *et al*., 2006). *Chaetomium cupreum* CC3003 was cultured in PDB at room temperature for 30 d. Fungal biomass was moved from PDB, filtered through cheesecloth and air-dried overnight. Fresh and dried fungal biomass were recorded. Dried fungal biomass was ground with an electrical blender, extracted with 200 ml hexane, and shaken for 24 h at room temperature. The ground fungal biomass was separated by filtration through What man No. 4 filter paper. The marc was extracted again with hexane using the method described above. The filtrates were evaporated *in vacuo* to yield crude extracts. The marc was further extracted with ethyl acetate and methanol using the same procedure as hexane. Each crude extract was weighed, and then kept at 5 ℃ until use. The crude extracts of *C.cupreum* CC3003 were tested for inhibition of the anthracnose pathogen. The experiment was done by using a 3x6 factorial Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts which consisted of crude hexane, ethyl acetate and methanol extracts and factor B

represented extract concentrations of 0, 10, 50, 100, 500, and 1,000 ppm. Each crude extract was dissolved in 2% dime thylsulfoxide, and mixed into PDA before autoclaving at 121℃, 15 lbs/in.2for 30 min. The tested pathogen was cultured on PDA and incubated at room temperature for 5 d and 3 mm-diameter agar plugs were removed from the colony margin using as terilized cork borer. The agar plug of pathogen was transferred to the middle of a 5.0 cm-diameter PDA plate containing each extract concentration and incubated at room temperature (28-30℃) for 4 d. Colony diameter and number of conidia were recorded.

Testing bio-formulations to control coffee anthracnose in a pot experiment

One-year-old coffee var. Arabica plants were inoculated with a $1x10^6$ spores/ml suspension of the anthracnose pathogen. Ten wounds on leaves/seedling with the fifth leaf from the top; a wound was puncture with sterilized needles 10 times. Thereafter, the following treatments were appliedat 15-day intervals:- T1 was inoculation with anthracnose pathogen, T2 was a spore suspension of *C.cupreum* CC3003 at a concentration of $1x10^6$ spore/ml, T3 was a bio-formulation in powder of *C.cupreum* CC3003 at a concentration of 10 g/20 L of water, at the Bio-control Research Laboratory, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Data were collected using the following disease severity index: level 1 was no symptoms, level 21-25 % symptoms, level 3 was 26-50%, level 4 was 51-75% and level 5 was over 75%. Disease incidence was assessed at 90 d after treatment based on a disease incidence scale (Vilavong and Kasem, 2013) as follows:-1=no infected leaves, 2=1–25% infected leave per plant, 3=26–50%, 4=51–75% and 5=76–100%. Therefore, disease reduction (%) = disease rating in inoculated control – disease rating in treatment/ disease rating in inoculated control × 100. The experiment was arranged in Randomized Complete Block Design (RCBD) with four replications.

Results

Isolation, identification and pathogenicity test of the coffee anthracnose agent *5*

Colletotrichum sp. was isolated from coffee leaves of var. Arabica exhibiting anthracnose symptom. The pathogenicity of the isolate was confirmed; the isolate produced typical anthracnose symptoms on coffee leaves

20 d after inoculation. The non-inoculated leaves remained anthracnose-free. *C.gloeosporioides*.

Bioactivity of Chaetomiumsp. substances against Colletotrichum Gloeosporioides causing anthracnose of coffee var. Arabica

The results showed that the crude ethyl acetate extract of *C. cupreum* CC3003 showed the highest inhibition of *C. coffeanum,* followed by the crude hexane and methanol extracts. Colony growth and conidial production were inversely related to bioactive substance concentration. At 1000 ppm, the crude ethyl acetate extract showed the significantly highest inhibition of spore production of *C. gloeosporioides*, followed by the crude methanol hexane extracts.The crude ethyl acetate extract significantly inhibited colony growth, while the crude hexane and methanol extracts produced a reduction.

Testing bio-formulations for control of coffee anthracnose in a pot experiment

The results showed that the powder bio-formulation of *C.cupreum* CC3003 at a concentration of 10 g/20 L of water gave the significantly highest control of coffee anthracnose *gloeosporioides*, followed by spore suspension of *C.cupreum* when compared with the inoculated control that the powder bio-formulation of *C. cupreum* CC3003 produced the highest disease reduction, followed by spore suspension of *C. cupreum* CC3003 when compared with the inoculated control.

Discussions

Colletotrichum gloeosporioides was isolated from coffee var Arabica leaves and beans symptomatic of coffee anthracnose, identified morphologically and by molecular phylogeny, its pathogenicity was confirmed on that host. We previously reported and confirmed through molecular and morphological tests that anthracnose on coffee var. Arabica in Laos is caused by *C.gloeosporioides* (Bailey and Jeger, 1992; Sutton, 1980; Vilavong and Soytong, 2013). Previous research indicated that crude extracts of *C.cupreum* CC3003 significantly inhibited *C. gloeosporioides* causing anthracnose of mango (Noiaium and Soytong, 1999) chili (Soytong *et al*., 2001), and grape (Soytong *et al*., 2005). *C. cupreum* is reported to produce antagonistic substances that breakdown the pathogen cells resulting in loss of viability and pathogenicity (Soytong, 1992a; Soytong, 1992b). Moreover, The mechanism of plant disease control by *C. cupreum* CC3003 involves the production of

antibiotics including three new azaphilones named, rotiorinol A, B, and C, and two new stereo isomers, rotiorinol and epi-isochromophilone II that exhibited antifungal activity against *Candida albicans* with IC50 values of 10.5, 16.7, 24.3 and 0.6 ppm, respectively (Kanokmedhakul, 2006; Soytong *et al*., 2001). Moreover, *C. cupreum* CC3003 could inhibit several plant pathogens, eg. *Pyricularia oryzae*, *C. gloeosporioides*, *Colletotrichum dematium*, *Fusarium oxysporum*, *Phytophthora parasitica*, *P. palmivora*, *P. cactorum* (Pechprome and Soytong, 1997; Soytong, 1992a; Soytong, 1992b; Soytong *et al*., 2001; Soytong *et al*., 2013). In the current research bio-formulations *C. cupreum* CC3003 showed effective reduction of coffee anthracnose. In addition a microbial elicitor from *Chaetomium* was shown to induce immunity against anthracnose caused by *Colletotrichum capsici*. In chili by production of the phytoalexincapsidiol (Soytong *et al*., 2014).

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