EFFECTIVENESS OF THAI PLANT MEDICINAL EXTRACTS AGAINST THE FUNGI *COLLECTOTRICHUM* SPP., THE CAUSAL AGENTS OF CHILI ANTHRACNOSE

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ABSTRACT Anthracnose is a major disease effecting Chili production in many tropical and subtropical regions worldwide with significant economic consequences. The antifungal activity of eight Thai plant medicinal extracts obtained from *Zingiber montanum* (Koenig) Link ex Dietr, *Boesenbergia rotunda* (L.) Mansf., *Alpinia nigra* B.L. Burt, *Allium sativum* L., *Allium ascalonicum* L., *Curcuma longa* L., *Tinospora crispa* (L.) Miers ex Hook.f and Thomson, and *Cymbopogon citratus* was investigated for the ability to control the growth of *Collectotrichum capsici* and *Collectotrichum acutatum* in vitro. Following alcohol extraction using a poison food technique, three crude plant extracts of 20000 ppm concentration were obtained from *Z. montanum, B. rotunda,* and *A. nigra* that caused a higher reduction of the radial hyphal growth of *Collectotrichum* spp. (60-86%) on agar plates. The lowest reduction of mycelial growth of *Collectotrichum* spp. (33-61%) was obtained for the extract of *Tinospora crispa*. All of the plant extracts significantly decreased the growth of *Collectotrichum* spp. The results of this work suggest that the use of shoot underground extracts obtained from these plants is effective in reducing *Collectotrichum* spp. hyphal growth.

1. INTRODUCTION 943

Chili (*Capsicum annuum* L.) belongs to the family of Solanaceae and is an important spice not only in Thailand, but also in many countries of the world such as India, Japan Australia, Indonesia, New Zealand (Rashid et al., 2015; Gautam, 2014; Pakdeevaraporn, et al., 2005; Nireberg, et al., 2002; Johnston & Jones, 1997; Simmonds, 1965). The annual demand for this spice is very high. Several chili cultivars are grown throughout the year in our country.

Collectotrichum causing anthracnose diseases is a limiting factor in chili production. It leads to changes in developmental stages of chili fruit resulting in production yield losses of up to 50% (Pakdeevaraporn et al., 2005). In order to control this disease in Thailand, toxic chemicals in form of organophosphate are used by farmers. These agents may pollute terrestrial environments for long periods. Furthermore, the chili fruit becomes a reservoir of pesticides residues. The increasing use of chemical fungicides to control anthracnose diseases in chili crops leads to serious impacts on the health of farmers and consumers. Therefore, increasing concerns over residual toxicity resulting from the wide spread use of synthetic fungicide and proliferation of resistance in pathogen populations, attention has focused on natural substances. The exploration of new alternatives to increase storage life has to give priority to methods that reduce horticultural produce decay and concurrently avoid negative health or environmental effects (Das et al., 2010). In variously research, exploitation of natural biochemical agents controlled *Collectotrichum* growth (Shinde & Gawai, 2011; Mogle, 2011; Anaruma et al., 2010). Biologically active natural products have the potential to replace synthetic fungicides. They are in demand nowadays. The goal of this study was to evaluate the antifungal activity of some Thai medicinal plants for in vitro inhibitory activity against strains of *Collectotrichum capsici* and *Collectotrichum acutatum* isolated from chili farm land. The crude extracts of medicinal plants were evaluated and compared with Benomyl, a commercial chemical fungicide.

2. EXPERIMENT

2.1 Experimental Apparatus

2.1.1 Pathogen culture. The cultures of *Collectotrichum capsici* and *Collectotrichum acutatum* were obtained from the Department of Plant Pathology, Faculty of Agriculture, Khon Kaen University, Thailand. Pure cultures were maintained on Potato Dextrose Agar (PDA) slants and stored at 4°C.

2.1.2 Medical plant collection. On the basis of previous research on antifungal activity, eight medical plants with potential anti-*Collectotrichum* activity were collected from areas of farm houses and local markets in Amphur Naklang in Nong Bua Lum Poo Province, Thailand.

2.2 Technique

2.2.1 Preparation and extraction of plant material

A list of the studied plants, including botanical names, common names, and plant parts used is given in Table 1. The parts of collected plants were washed with distilled water and chopped to less than 5 mm in length. They were dried under shade at 60°C for 48 hours or until the weight was constant (Shinde & Gawai, 2011). Following the extraction procedures of Sutthisa et al. (2014), 50 grams of the plant sample was left to stand in 500 ml of 95% absolute ethanol at room temperature for 7 days. The suspension was collected. Residual solids were extracted a second and third time with the same ratio of solids and ethanol. All crude extract solutions were filtered through folded Whatman No.1 filter paper and reduced to dryness in rotary vacuum evaporator at 50°C. The extract was concentrated and stored at 4°C. The concentrated extract was diluted with distilled water before being tested against isolated *Collectotrichum* activities.

Table 1 Medicinal plant species and the parts used for extraction

Botanical names	Common names	Plant parts used
Zingiber montanum (Zm)	Koenig	Rhizome
Boesenbergia rotunda (Br)	Kaempfer	Rhizome
Alpinia nigra (An)	Galangal	Rhizome
Allium sativum (As)	Galic	Clove
Allium ascalonicum (Aa)	Shallot	Clove
Curcuma longa (Cl)	Turmeric	Rhizome
Tinospora crispa (Tc)	Borapet	Shoot
Cymbopogon citrus(Cc)	Lemongress	Shoot

2.2.2 Evaluation of plant extracts against *Collectotrichum* spp. The effect of crude plant extracts on the hyphal growth of isolated *Collectotrichum* strains was determined using the poison food technique of Tasiwal et al. (2009). The crude extract was dissolved in sterile distilled water to prepare a stock solution. This solution was serially diluted to prepare four different concentrations, 20,000 15,000 10,000 and 5,000 ppm, in warm PDA medium. They were mixed, and poured into the middle of sterile Petri dishes. After solidification, the plates were inoculated with young mycelia of the *Collectotrichum* spp. (5 mm in diameter), and incubated at 28ºC for 14 days. A Petri dish without the fungal crude extract was used as a negative control. The standard synthetic fungicide, Benomyl (methyl-1-(butylcarbamoyl)-2-benzi midazole carbamate) was a positive control for the study (Ogu & Owoeye, 2013). The inhibitory levels were determined using the formula: A-B/A×100, where A = colony radius of the plant pathogenic fungi in the control, and B = colony radius of plant pathogenic fungi in the presence of the tested crude extract (Boonsang et al., 2014). Each treatment was performed in triplicate with a completely randomized design.

2.2.3 Statistical analysis Data about the anti-mycelial effects of the treatments on growth were analyzed using ANOVA to determine the differences between treatments. Treatment means were separated with Duncan's Multiple Range Test (p=0.05), using SPSS (Khamna et al., 2009).

3. RESULTS AND DISCUSSIONS

To evaluate the antifungal activity against the tested *Collectotrichum* spp., eight Thai medicinal plants were selected from previously research. Four plants were prepared from dry rhizomes, two from dry cloves, and two from dry shoots. Results in Table 2 showed that the crude extracts of all plants expressed their ability to reduce the mycelial growth of *C. capsici* 10-2, *C. acutatum* 25-1, *C. acutatum* 29/2 and *C. acutatum* 2/4, especially at higher concentrations of the crude extracts. Similar effects of various other plant extracts effective against *Collectotrichum* spp. were reported by several authors (Sutthisa et al., 2014; Uddin et al., 2013; Nashwa & Abo-Elyousr, 2012).

In the current study, crude extracts of *Z. montanum* (Zm), *B. rotunda* (Br), and *A. nigra* (An) at 20,000 ppm concentration caused high inhibition of the mycelial growth of all *Collectotrichum* isolates (Figures 1 & 2), compared with other plant extracts. However, their fungicidal activities were less than those of Benomyl. Benomyl completely inhibited the growth of all *Collectotrichum* spp. at 5,000 ppm. It was observed that untreated plant extract could not suppress mycelial growth at all. The inhibition of mycelial growth of *Collectotrichum* spp. by the *A. nigra* crude extract at 20,000 ppm was similar to that reported by Sutthisa et al. (2014) who investigatged the role of plant extracts in *Collectotrichum* disease control for mango fruit. Furthermore, the results indicated that *Tinospora crispa* (Tc) caused the lowest inhibition of the mycelia growth of *Collectotrichum* (Figure 4). The other treatments with plant extracts were moderately effective. Amadioha (2000) recommented that the inhibitory effect of the plant extracts on mycelial growth may directly act on pathogenic fungi. Siva et al. (2008) also suggested that the differeces in the inhibitory effect of various plant extracts may be due to qualitative and quantitative differences in the antifungal compounds present in them.

CONCLUSIONS

This study showed that eight Thai medicinal plant extracts, i.e., Zingiber montanum, *Boesenbergia rotunda*, *Alpinia nigra, Allium sativum*, *Allium ascalonicum*, *Curcuma longa, Tinosporaispa*, and *Cymbopogon citrus*, could be utilized for biocontrol of *Collectotrichum*. These Thai medicinal plants significantly contribute to reducing the risks and hazards of toxic chemical fungicides for control the anthracnose disease on chili plants. Further research into these plant extracts will be need, as well as confirmation in field experiments for

their fungicidal activity. Additionally, the effect of plant extracts on fresh and dry parts of medicinal plants on antifungal activity should be studied.

Table 2 Effects of different crude extract concentrations and Benomyl on growth inhition of isolated *C. capsici* and *C. acutatum*.

Treatment	%growth inhibition of mycelia after 14 days of inoculation			
	C. capsici	*C. acutatum*		
	khon 10-2	khon 25-1	khon 29/2	khon 2/4
PDA+sterile water	0.0	0.0	0.0	0.0
PDA+bonomyl 5,000ppm	100.0	100.0	100.0	100.0
PDA+Aa 5,000ppm	26.1	42.1	43.3	49.0
PDA+Aa 10,000ppm	28.8	52.1	50.0	54.5
PDA+Aa 15,000ppm	33.8	54.7	55.9	59.2
PDA+Aa 20,000ppm	39.2	62.9	62.6	66.7
PDA+Zm 5,000ppm	50.0	57.5	64.8	61.3
PDA+Zm 10,000ppm	51.7	56.7	65.6	69.4
PDA+Zm 15,000ppm	62.1	60.8	65.2	79.2
PDA+Zm 20,000ppm	68.3	82.9	77.0	86.0
PDA+Cl 5,000ppm	39.6	58.8	60.4	65.5
PDA+Cl 10,000ppm	45.0	61.7	63.7	67.4
PDA+Cl 15,000ppm	48.8	69.2	68.1	59.7
PDA+Cl 20,000ppm	54.2	73.8	73.2	64.7
PDA+Br 5,000ppm	51.1	42.1	51.1	50.6
PDA+Br 10,000ppm	59.7	64.2	58.9	54.9
PDA+Br 15,000ppm	67.3	73.2	64.1	64.7
PDA+Br 20,000ppm	74.2	78.6	73.0	74.9
PDA+As 5,000ppm	23.8	24.6	47.4	43.9
PDA+As 10,000ppm	28.8	33.3	55.6	55.3
PDA+As 15,000ppm	35.3	43.3	62.2	58.4
PDA+As 20,000ppm	41.2	56.3	73.1	61.7
PDA+Cc 5,000ppm	30.4	51.3	53.3	58.8
PDA+Cc 10,000ppm	37.1	55.8	54.4	52.9
PDA+Cc 15,000ppm	46.3	58.8	55.6	64.7
PDA+Cc 20,000ppm	50.0	61.3	71.5	68.6
PDA+Tc 5,000ppm	16.6	33.8	43.3	29.8
PDA+Tc 10,000ppm	24.5	36.3	46.3	31.8
PDA+Tc 15,000ppm	32.2	40.1	51.1	35.7
PDA+Tc 20,000ppm	33.0	55.8	61.1	38.8
PDA+An 5,000ppm	41.3	57.6	47.8	45.9
PDA+An 10,000ppm	59.6	59.6	56.7	52.9
PDA+An 15,000ppm	64.6	70.0	62.4	53.7
PDA+An 20,000ppm	68.8	74.2	71.6	60.0

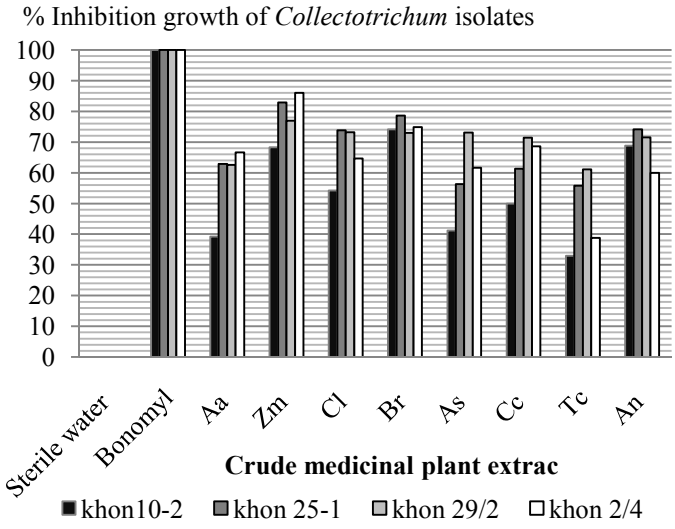

Fig. 1 The effect of crude Thai medicinal plant extracts at 20,000 ppm on the growth inhibition of *Collectotrichum* isolates at 14 days after inoculation, comparing negative (sterile water) and positive controls (Bonomyl).

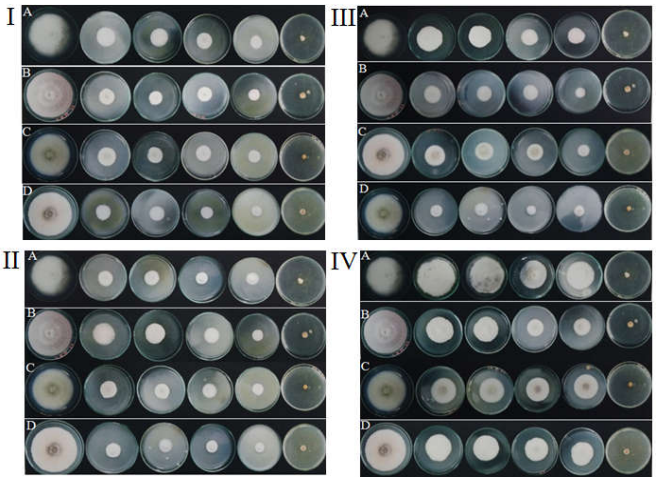

Fig. 2 The effect of concentration of crude *Z. montanum* (I), *A. nigra* (II), *B. rotunda* (III), and *T. crispa* (IV) plant extracts at (left) 0, 5,000, 15,000, 20,000 and Benomyl 5,000 ppm (right) on the growth inhibition of *Collectotrichum* spp. at 14 days after inoculation: A, khon 10-2; B, khon 25-1; C, khon 29/3; D, khon 2/4

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