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Effect of soap pod and tobacco on inhibition of *Colletotrichum capsici*.

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Abstract

The effect of types of solvent and time of exposure on two local plant extracts including soap pod *(Acacia concinna* (Willd.) DC.) and tobacco (*Nicotiana tabacum* L.) were studied individually by using 70% ethanol, 95% ethanol, and methanol for 1, 2 and 3 days. It was found that extraction with 70% ethanol for 3 days gave the highest yield of crude extract of 0.32 % (w/w) from soap pod and 0.11 % (w/w) from tobacco. The result from the plant extract analysis showed that crude extract of soap pod contains saponin as high as 60mg/g. These plant extracts were evaluated for growth inhibition of *Colletotrichum capsici* at 2,000, 10,000, 20,000, and 40,000 ppm. The result revealed that crude extracts from both soap pod and tobacco at 40,000 ppm was the most effective concentration for mycelium growth inhibition. The highest inhibition of 100% and 25.5% was obtained for crude extract of soap pod and tobacco, respectively. In addition, spore germination was inhibited to 100% by 20,000 ppm soap pod extract and 88.88% by 40,000 ppm tobacco extract.

Keywords: Chilli, Plant extract, *Collectotrichum capsici*, Saponin

Introduction

Chilli (*Capsicum annuam*) is a member of solanaceae family and one of important economic crops cultivated in Thailand. Most of chilli product is used for domestic consumption (Office of Agricultural Economics, 2007). The problems of chilli crop production are plant pathogen including insect, virus, fungi and other such as bacterial. Major problem for agriculturist cultivated chilli in Thailand is fungal pathogen. Anthracnose, caused by *Colletotrichum* sp., is a

common disease in chilli. It can damage the plant at all stage of growth (Agrios, 2005). The quick and effective management of anthracnose disease in chilli is generally achieved by the use of synthetic fungicide (Takeda and Sakuoka, 1997). However, chemical based fungicides are hazardous substances and create health hazards in human due to their toxicity residue. This is not only a problem for exported products, but also expensive and non-specific method. Therefore, biological material should be replaced for more acceptance by the consumer. Local plants in Thailand are natural resources, yielding valuable herbal product and often used in the treatment of various symptoms and diseases. Many reports concentrate on antifungal activity of saponin extract. (Grunweller *et al.* 1990; Phongpaichit *et al*., 1995; Chapagain *et al*., 2007) but the use of saponin extract to preserve chilli from *Collectotrichum* sp. has never been reported. In this study, tobacco and soap pod, which are local plants in the Northern of Thailand, have been selected to study saponin composition and antimicrobial activity.

Materials and Methods

Isolation of Colletotrichum sp. and *Test of pathogenicity on chilli*

Fresh chilli fruit was selected, soaked in the 5% Clorox solution for 1 min. and put on PDA medium plate. The plate was incubated at room temperature (28-32 ºc) for 3 days. The fungi grow on PDA medium plate were isolated and purified. Spore suspension of isolated pathogen was prepared (approximately 10^6 spores/ml) and spray onto the surface of fresh healthy chilli fruit, and incubated in moist chamber at room temperature for 7 days. Disease symptom was monitored and active isolated fungi was selected for re-isolation. Finally, the fungal morphology and characteristic was used for identification as described in Barnett and Barry (1987).

Preparation of plant extracts and HPLC analysis

Soap pod was purchased from local market in Chang Mai province. Dried Tobacco was obtained from Thailand Tobacco Monopoly, Chiang Mai. The extraction methods using methanol, 70% ethanol and 95% ethanol was modified from the procedure of Hsieh *et al*. (2001). Ten gram of dry plant was extracted with 100 ml solvent l in flask for 1,2 and 3 days. After that, the mixture was filtered by cotton cloth. Then, the solution was centrifuged at 5000xg for 10 min at room temperature. The supernatant was collected and concentrated on rotary evaporator at 45˚c. The crude extract was dry with hot air oven at 60˚c and kept at 4 ºc

The HPLC analysis was performed using ODS C18 column with HPLC instrument (Shimasu, Japan). The fractions were eluted isocratically with a binary mixture of acetonitrile and 0.10% phosphoric acid solution (42:58). The column temperature was set at 30°c. The total flow rate was at 1.0 ml/min, sample injection volume was 10 µl. and the detection wavelength was set at 203 nm on the diode array detector (Chen et al., 2007).

Determination of minimal inhibitory concentration (MIC)

A. Mycelium inhibition

The fungal culture was prepared in PDA and incubate at room temperature for 5 days. Plant extracts was diluted in sterile distilled water to produce serial dilutions ranging from 2,000 to 40,000 ppm. One ml of each concentration were mix with PDA medium. After that, the mycelium was placed on the PDA mixing with plant extract. The plate was incubated at room temperature for 7 days. Antifungal activity was observed at 1, 3, 5 and 7 days by diameter measuring for of inhibition mycelium growth. The value was recorded as mean diameter of two replication. The percentage of inhibition for radial growth (PIRG) was calculate as the following:

$$PIRG = \frac{(R1 - R2)}{R1} \times 100$$

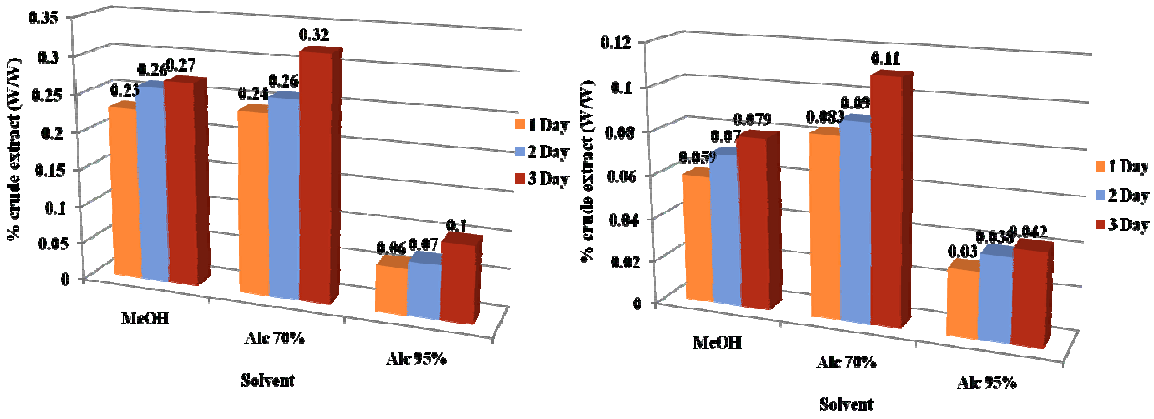
When; R1= Radial of colony in control plate
R2= Radial of colony in sample plate

B. Spore germination inhibition

Spore suspension was prepared by cultured *C. capsici* on PDA plate and incubated at room temperature for 3-5 days until sporulation. After that, the fungal spores were suspended in 100 ml sterile distilled water. PDA plate was spread with 100 µl spore suspension and incubated at room temperature for 30 min. After that, the PDA medium was cut to produce agar wells. The plant extracts (100 µl) after diluted in sterile distilled water to produce serial dilutions ranging 2,000 to 40,000 ppm, were added into the wells. The plates were incubated at room temperature for 2 days. The inhibition zone was recorded as the mean diameter of 3 replication.

Results and Discussion

Seven fungal strains were isolates from fresh chilli fruit and designated as CMBT1, CMBT2, CMBT3, CMBT4, CMBT5, CMBT6 , and CMBT7. After identification, it was found that four isolates (CMBT1, CMBT2, CMBT3 and CMBT5) were *C. gloeosporioides*, CMBT6 was *C. capsici*, CMBT4 was *Fusarium oxysporum* and CMBT7 was *Aspergillus niger*. All five isolates of *Colletotrichum* sp. were infected on fresh healthy chilli fruit for pathogenicity test. The 5 isolated showed symptom on fruit and the symptom was similar to that was reported by Melanie and Miller (2004). The *Colletotrichum capsici* isolated was selected and used in this experiment. For the result of plant extraction, it was found that extraction with 70% ethanol for 3 days gave highest yield of crude extract of 0.32 %(w/w) from soap pod and 0.11 % (w/w) from tobacco (Fig. 1). The result from the plant extract analysis showed that crude extract of soap pod and tobacco contain saponin at 60 mg/g and 30 mg/g (Fig. 2).



(A) **(B)**

Figure 1: Crude extract by different methods (A): Soap pod and (B): Tobacco

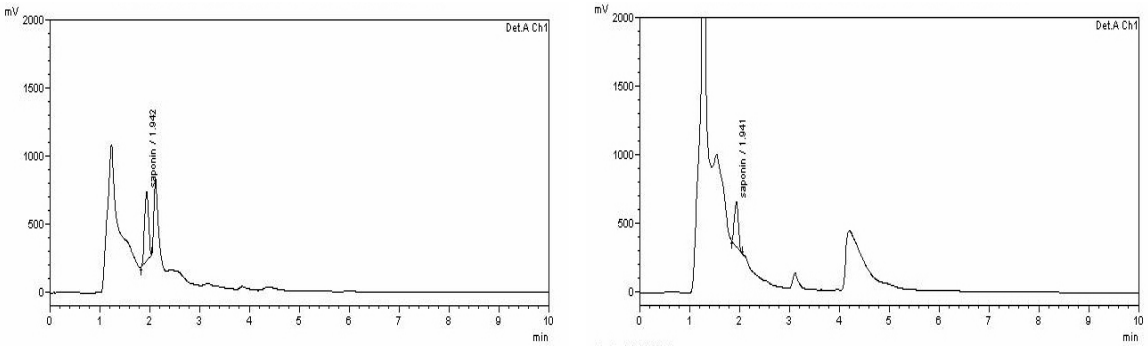


Figure 2: Chromatograms of plant extract analysis (A): soap pod and (B): tobacco

The result of antifungal inhibition was shown in Figure 3. Soap pod and tobacco extracted with 70% ethanol was the most effective to inhibited *C. capsici*, mycelium at 40,000 ppm. Maximal inhibition for soap pod and tobacco extracts was 100% and 25.5%, respectively.

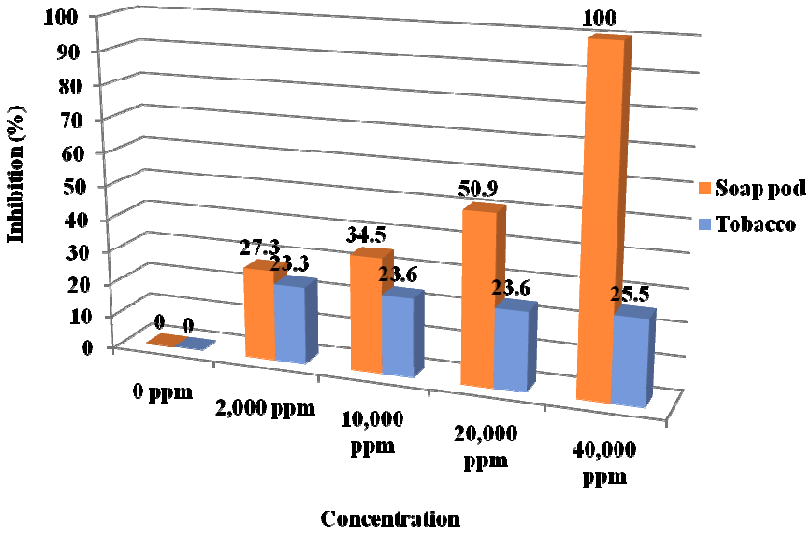


Figure 3: Inhibitory effect of plant extracts on mycelium growth of *C. capsici*

Table 1 shows percentage of spore germination inhibition of the plant extracts. The activity varied according to both the types and concentration. However, spore inhibition present at concentration higher than 20,000 ppm. The result in this experimental was similar to that was reported by Vudhivanich (2003) and Wongkaew *et al.* (1997). Difference extraction methods as well as types of solvents gave variation in activity of the plant extract. Application of this effective plant extract to control anthracnose in chilli in each production cycle should be further studied.

Table 1: Spore germination inhibition (%) of the plant extract on *C. capsici*

	Concentration of plant extract (ppm)				
Test Plant	0	2,000	10,000	20,000	40,000
Soap pod	-	-	-	100	100
Tobacco	-	-	-	53.26	88.88

Conclusions

From 7 isolates, 5 isolates of *Coletotrichum* sp. present pathogenicity on chilli fruit. The *C. capsici* isolated, CMBT6, was used in this experiment as fungal pathogen for chilli fruit. Soap pod and tobacco extracted by 70% ethanol for three day gave highest yield of crude extract at 0.32 % (w/w) and 0.11 % (w/w), respectively. Crude extract of soap pod contains saponin at 60 mg/g and tobacco contain saponin at 30 mg/g. Soap pod and tobacco extracts with 70% ethanol at 40,000 ppm was the most effective to inhibited mycelium and spore germination of *C. capsici*. This study indicates potential of crude extracts from local plants for inhibition of *C. capcisi* growth and the result can be applied for anthracnose control in chilli production.

Acknowledgement

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