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 extract 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, Colletotrichum capsici, saponin

Introduction

Chilli (Capsicum annuum) is a member of solanaceae family and one of important economic crops cultivated in Thailand. The major problem for agriculturist cultivated chilli in Thailand is fungal pathogen. Anthracnose, caused by Colletotrichum sp., is a common disease in chilli (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, some fungicides are hazardous substances and create health hazards in human due to their residue toxicity. Therefore, biological material should be used instead of chemical substance 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. (Chapagain et al., 2007) but the use of saponin extract to preserve chilli from *Colletotrichum* sp. has never been reported. In this study, tobacco and soap pod which are local plants have been selected to study saponin composition and antimicrobial activity.

**Materials and Methods**

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

The fresh chilli fruit was selected and soaked in the 5% Clorox solution for 1 min. and put on PDA medium plate. The plate was incubated at room temperature for 3 days. The fungi grow on PDA medium plate were isolated and purified. Spore suspension of isolated pathogen was prepared and spray onto the surface of fresh healthy chilli fruit and incubated in moist chamber at room temperature for 7 days. Selected the active isolated fungi from the plant for re-isolation. Finally, The fungal morphology and characteristic was used for identification (Barnett and Barry, 1987).

**Preparation of plant extracts**

Ten gram of dry soap pod and tobacco was extracted with 100 ml methanol, 70% ethanol and 95% ethanol in flask for 1, 2 and 3 days. After that the mixture was filtered by a cotton cloth. The supernatant was collected and concentrated on rotary evaporator at 45 °C for methanol, 70% and 90% ethanol. After that the crude extract was dry with hot air oven at 60 °C and kept at 4 ºC.

**HPLC analysis**

The HPLC analysis was performed with a HPLC instrument (Shimazu, Japan) with ODS C18 column. Then fraction was 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 and sample injection volume was 10 µl. The detection wavelength was set at 203 nm on the diode array detector (Chen et al., 2007).

**Determination of minimal inhibitory concentration (MIC)**

**Mycelium inhibition**

Plant extracts was diluted in sterile distilled water to produce serial dilutions ranging 2,000 to 40,000 ppm and 1ml of each concentration were mix to PDA medium. Then, the mycelium was put on PDA mixing of each concentration plant extract medium plate. The plate was incubated at room temperature for 7 days. Antifungal activity was observed 1, 3, 5 and 7 days by measuring diameter of inhibition mycelium growth.

**Spore germination inhibition**

Prepared spore suspension. PDA plate was spread with 100 µl spore suspension and Incubate at room temperature for 30 min. After that, cut the PDA medium to produce agar wells. 100 µl of plant extracts, diluted in sterile distilled water to produce serial dilutions ranging 2,000 to 40,000 ppm, were add into the well. The plate was incubated at room temperature for 2 days. The inhibition zone was recorded as the mean diameter of triplicate.

**Results and Discussion**

Seven fungal strain were isolates from fresh chilli fruit. After identification, It was found that four isolates were *C. gloeosporioides*, one isolate was *C. capsici*, one isolate was *Fusarium oxysporum* and one isolate was *Aspergillus niger*. All five isolates of *Colletotrichum* sp. were infected on fresh healthy chilli fruit for pathogenicity. The symptoms were similar to that reported by Melanie and Miller (2004). *C. capsici* was selected and used in this experiment. 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 contains saponin as high as 60 mg/g (Fig. 2). Fig. 3 is the result of antifungal inhibition soap pod and tobacco extracts with 70% ethanol at 40,000 ppm was the most effective to inhibited mycelium. Maximal
inhibition for soap pod and tobacco extract were 100% and 25.5%, respectively. Table 1 shows percentage of spore germination inhibition varied according to the kinds and concentration was greater than 20,000 ppm. Some of the result in this experimental were similar to that reported by Vudhivanich (2003) but the difference in the extraction methods and solvents caused some variation in experimental result. Therefore, further investigation on suitable extraction and determination of minimal inhibitory concentration (MIC) are necessary. The latter aspect application of effective Plant extract on control anthracnose of chilli should also be further study.

Table 1 Percentage of spore germination inhibition.

<table>
<thead>
<tr>
<th>Test Plant</th>
<th>Concentration of plant extract (ppm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Soap pod</td>
<td>-</td>
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<tr>
<td>Tobacco</td>
<td>-</td>
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Figure 1 Yield of crude extract (A): Soap pod and (B): Tobacco.

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

Figure 3 Inhibitory effect of soap pod and tobacco extracts on mycelium growth of *C. capsici*. 
Summary

From 7 isolates, 4 isolate were identified as *C. gloeosporioides*, 1 isolate was *C. capsici*, 1 isolated was *F. oxysporum*, and 1 isolated was *A. niger*. Five isolates of *Coletotrichum* sp. present pathogenicity on chilli fruit. Soap pod and tobacco extracted by 70% ethanol for three day gave highest crude extract at 0.32 % (w/w) and 0.11 % (w/w), respectively. Crude extract of soap pod and tobacco contain saponin at 60 mg/g and 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. This study indicates that some of Thai plant extracts had high potential for the inhibition of *C. capsici* growth and might be applicable for anthracnose control.

Literature cited


