Control of Mango Anthracnose by Using Chinese Quince *(Pseudocydonia sinensis)* Seed Extract

Lia Angraeni [1], Yasunori Hamauzu [2], Wirongrong Thongdeesoontorn [1,4] and Matchima Naradisorn [1, 3,*]

Abstract

Chinese quince (*Pseudocydonia sinensis* Schneid.) has been known in its rich in phenolic compounds. However, Chinese quince seed is usually a waste which may also contain high phenolic compound. The objectives of this study were to investigate the potential *in vitro* and *in vivo* antimicrobial activity of Chinese quince seed extract against *Colletotrichum gloeosporioides*, the causal agent of anthracnose disease in mango cv. 'Nam Dok Mai Si Thong'. The seeds were extracted by using distilled water in ratio of seed powder to water at 1:10 (w/v) and tested at concentrations of 0.5%, 1% and 1.5% (w/v). *In vitro* antimicrobial activities were observed through mycelial radial growth and spore germination assays while *in vivo* assay was observed on mango fruits. Chinese quince seed extract at different concentrations was added into film-forming solution to prepare Chinese quince seed film for antifungal test. The total phenolic content in Chinese quince seed extract was 48.84 ± 1.71 mg GAE / 100 g wet sample. The 1.5% extract showed mycelial growth inhibition of *C. gloeosporioides* at 82.59% after 5 days of incubation, while 0.5% and 1% extract had lower inhibitory effect (78.85% and 78.41%, respectively). Spore germination in untreated control (23.68%) was significantly less ($p < 0.05$) than that in 0.5%, 1% and 1.5% extracts (61.31%, 52.86% and 55.75%, respectively). Inoculated mango fruit dipped in 1% extract solution exhibited smaller lesion diameter (1.36 cm) compared to those treated with 0.5% and 1.5% extract (1.52 cm and 1.43 cm, respectively) and control (2.17 cm) after 5 days of incubation. Antifungal test of Chinese quince seed film did not show the growth inhibition of *C. gloeosporioides*.

Keywords: Chinese quince, Seed extract, Mango, Anthracnose, *Colletotrichum gloeosporioides*

[1] School of Agro-Industry, Mae Fah Luang University, Chiang Rai, Thailand

[2] Graduate School of Agriculture, Shinshu University, Nagano, Japan

[3] Research Group of Postharvest Technology, Mae Fah Luang University, Chiang Rai, Thailand

[4] Unit of Innovative Food Packaging and Biomaterials, Mae Fah Luang University, Chiang Rai, Thailand

* Corresponding author(s), e-mail: matchima@mfu.ac.th

Received: 8 August 2018/ Revised: 11 October 2018 Accepted: 3 March 2019

1. Introduction

Mango (*Mangifera indica* L.) is one of the most common fruits grown throughout the tropical and subtropical climate of the world (Chacko, 1986). Postharvest disease contributes the major problem to commercialize high quality of mango fruit. It can reduce the quality of fruit and cause postharvest losses because the fruit does not meet the quality standard in the major import market (Cappellini *et al*., 1988). Anthracnose disease (caused by *Colletotrichum gloeosporioides* (Penz.) Sacc.) is the major postharvest disease of mango worldwide (Dodd *et al*., 1997). Initial infection starts from young leaves and flower panicles (causing blossom blight), forms quiescent infections on young immature fruit and causes the fruit damage in postharvest period (Muirhead and Gratitude, 1986; Dodd *et al*., 1989; Dodd *et al*., 1997). For many years, synthetic fungicides have been used to control anthracnose disease. However, the use of chemical is currently restricted because of public concerns of its toxicity and residue (Kefialew and Ayalew, 2008).

The plant extract is bio-efficacious, biodegradable, economical, and eco-friendly for use as agrochemicals (Macias *et al*., 1997). Natural compounds from plant extract and essential oil have a number of antimicrobial properties which could be applied to the perishable products as postharvest protection (Maqbool *et al*., 2011; Regnier *et al*., 2008). The secondary metabolites may express their effects at very low concentration and can be adsorbed easily; moreover, less harmful for human and animal and have a less chance of odors in the treated postharvest product (Tripathi and Dubey, 2004).

The use of natural extract or essential oil for controlling postharvest disease has been reported widely. Maqbool *et al*. (2011) found that Potato Dextrose Agar (PDA) medium amended with 10% Gum Arabic from acacia combined with 0.4% cinnamon oil showed the most promising results against *Colletotrichum musae* and *C. gloeosporioides* in suppressing the mycelial growth and inhibiting spore germination. Ethyl acetate extracts of *Lantana camara* was reported in inhibiting *C. gloeosporioides* (Ademe *et al*., 2013). Recently, aqueous extract of *Ruta chalepensis* was also reported in reducing the development of *C. gloeosporioides* to below 36% (Alemu *et al*., 2014).

Chinese quince fruits (*Pseudocydonia sinensis* Schneid.) cannot be consumed as fresh fruit because of its strong acidity, astringency and hard flesh; therefore, it is usually processed as a food in the form of syrup, jam, candy or jelly (Hamauzu *et al*., 2006). Phenolics are believed as the one of bioactive compounds for the medicinal effects of Chinese quince fruit and its product (Hamauzu *et al*., 2007). However, there have not been any data about antimicrobial activities of Chinese quince seed for reducing the fungal decay on postharvest perishable product. Preliminary study showed that aqueous extraction of Chinese quince seed

promotes antifungal activity against *C. gloeosporioides*, a causal agent of anthracnose disease on mango fruit, under *in vitro* (data not shown) compared to methanolic and ethanolic extraction. Chinese quince seed may contain phenolic compounds which could be further explored in the present study as alternative means for controlling anthracnose disease on mango.

2. Materials and Methods

2.1 Preparation of Seed Extract

Chinese quince seed was obtained from Nagano Prefecture, Japan. Seeds were dried at 50 °C until constant weight was obtained and were then ground into fine powder. Chinese quince seed powder sample was mixed with distilled water in ratio of sample to solvent at 1:10 (w/v). The mixture was shaken vigorously on shaker (Shel Lab, Shaking Incubators/ 3-2, Sheldon Manufacturing, Inc., Cornelius, OR, USA) maintained at 150 rpm and 40 °C for 24 h according to the method described in Alizadeh *et al*. (2013). The suspended solution was filtered through muslin cloth and then was concentrated by using rotary evaporator (Eyela Rotary Evaporator N-1000, Eyela Oil Bath OSB 2000, Tokyo Rikakikai Co., Ltd, Japan) at 40 °C for 3 h under vacuum condition (0.1 MPa). The extract was kept in an amber bottle and stored at 4 °C until further use.

2.2 Isolation of *Colletotrichum gloeosporioides*.

The fungi *C. gloeosporioides* was obtained from naturally infected mango fruit. The infected mango was cut into small pieces and surface sterilized by dipping in 2% sodium hypochlorite for 2 min. The tissues were washed 3 times using sterile distilled water, cultured on Potato Dextrose Agar (PDA) (Becton, Dickinson and Company, Sparks, MD, USA) and incubated at 25 °C. The causal pathogen was then confirmed through Koch's postulates.

2.3 Preparation of Spore Suspension

Spore suspension was prepared according to the method described by Tsortzakis and Economakis (2007) with slight modification. A two-week-old culture was used to prepare spore suspension. Culture surface was flooded with 3–4 mL of sterile water and then was scrapped off with a sterile glass rod to release the spores from the agar. The suspension was transferred to a sterile micro tube and centrifuged (Hettich Centrifuge/ 320R, Andreas Hettich GmbH and Co., Tuttlingen, Germany) at 10,000 rpm for 3 min at room temperature. The supernatant was discarded and the suspended spore was re-suspended in 1 mL sterile water. The number of spore was determined with a haemocytometer and was prepared at concentration of 10^5 spores/mL for further use.

2.4 Effect of Chinese Quince Seed Extract on Mycelial Growth

Mycelial growth inhibition assay was carried out according to the method described by Bautista-Banos *et al*. (2002) with some modifications. Extract at different concentrations (0.5%, 1% and 1.5%) were mixed with 15 mL PDA and poured into 9-mm Petri dish. Sterile distilled water was used as negative control, and Carbendazim at concentration of 10 µL / 10 mL (v/v, dissolved in sterile water) was used as positive control. Five mm fungal disc was taken from the edge of 7-day-old *C. gloeosporioides* pure culture using sterile cork borer and placed in the center of a Petri dish. Nine plates were prepared for each treatment. Plates were incubated at room temperature (25 ± 2 °C) and mycelium diameter for each plate was measured every 24 h. The measurement was carried out until the mycelium growth in any plate reached the edge of the plate. The ability of extract in reducing fungal mycelial growth was expressed as percentage of mycelia growth inhibition and calculated by using formula described by Pandey *et al*. (1982):

$$\text{Mycelial growth inhibition (\%)} = \frac{(dc - dt)}{dc} \times 100 \quad (1)$$

where dc is average diameter of fungal colony in control (cm)

dt is average diameter of fungal colony in treatment (cm)

2.5 Effect of Chinese Quince Seed Extract on Spore Germination

The *in vitro* spore germination test was carried out by cavity slide technique adopted from Cronin *et al*. (1996). An aliquot (40 µL) of each concentrated extract was pipetted on a cavity slide. A freshly harvested spore suspension (10 µL) of *C. gloeosporioides* (10^5 spores/mL) was placed into the cavity slide, covered with a cover slip and kept in the dark for 3 h at 25 °C. After incubation, the spores were killed by adding 10 µL of 2.0% sodium azide (Ajax Finechem Pty Ltd., NSW, Australia). Approximately 100 spores per slide were observed for germination under a light microscope (Motic BA 300, Meyer Instruments Inc., Houston, TX) at 40× magnification. A spore was considered germinated if its germ tube was longer than the spore itself. The percentage of spore germination was calculated by the following equation.

$$\text{Spore germination (\%)} = \frac{\text{Total germinated spores}}{\text{Total observed spores}} \times 100 \quad (2)$$

2.6 Effect of Chinese Quince Seed Extract on Anthracnose Disease in Mango

In vivo assay was conducted according to the method described by Abd-Alla and Haggag (2013) with some modifications. Fruits were surface sterilized in the solution of 2% sodium hypochlorite for 2 min, rinsed with reverse osmosis water, and air-dried before wounding. Fruits were wounded with a sterile needle to make 1 uniform 2-mm depth by 5-mm wide wound on their peel at the equatorial region. Five-mm fungal disc was placed into the wound and inoculated fruits were kept in plastic box at 25 °C for 15 h to allow the spores to colonize in the wound site. The humidity in each plastic box was maintained at 90 ± 5% relative humidity (RH) at all time.

Extract at different concentrations were prepared by dissolving extract in sterile distilled water containing 0.05% Tween 80 (Ajax Finechem Pty Ltd., NSW, Australia) followed by stirring for 10 min. After 15 h of inoculation, fruits were dipped in extract for 3 min. Dipping fruit in sterile water was used as negative control, and dipping in Carbendazim (1 mL/L water) was used as positive control. Fruits were then air-dried and kept into the plastic box. Each treatment was replicated 3 times with 3 fruits per replication. The lesion diameter was measured horizontally and vertically every 24 h (Regnier *et al*., 2008), and then average of lesion diameters were calculated.

2.7 Antifungal Test of Chinese Quince Seed Film

Chinese quince seed film was prepared according to the method described by Jouki *et al*. (2014a) with slight modification. Film-forming solution was prepared by slowly dissolving 0.5%, 1% and 1.5% mucilage in water (w/v) and 30% glycerol (w/w, based on Chinese quince seed mucilage weight) as a plasticizer was then added into the solution, under constant stirring at 750 rpm and 45 ± 2 °C for 30 min. The film-forming solution (25 mL) was poured onto the plastic Petri dishes (130 mm diameter) and dried at room temperature (25 ± 2°C) with 37 ± 2% RH for 24 h. After completely dry, the films were peeled off and conditioned at 25 ± 2°C in desiccators containing saturated solutions of $Mg(NO_3)_2$, $6H_2O$ (50 ± 2% RH) for at least 48 h prior tests.

Antifungal test of Chinese quince seed film was carried out under *in vitro* condition. Twenty µL of spore suspension (concentration of 10^5 spores/mL) were spread over the PDA surface with sterile glass rod. Discs of film at concentrations of 0.5%, 1% and 1.5% were placed on the PDA. Sterile filter paper discs were also prepared, impregnated with a drop of sterile water (for negative control) and a drop of 0.1% Carbendazim (for positive control). The fungal growth inhibition was measured in the presence of clear zone surrounding the film disc containing the antimicrobial agent when putting in direct contact with fungal culture (Weerakkody *et al*., 2010).

2.8 Total Phenolic Compound Analysis of Chinese Quince Seed Extract

Total phenolic content was determined using Folin-Ciocalteu method described by the ISO 14502-1 (International Organization for Standardization, 2005) with slight modification. Gallic acid (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard. Five g of Chinese quince seed extract was homogenized in 20 mL of methanol (80% v/v) then centrifuged at 9000 rpm for 10 min. One mL of diluted sample was added into test tube and mixed thoroughly with 5 mL of Folin-Ciocalteu reagent (previously pre-diluted 10 times with distilled water). After 5 min, 4 mL of 7.5% sodium carbonate (Na_2CO_3) was added, followed by brief vortexing to mix and allowed to stand for 60 min at room temperature. The absorbance was measured using spectrophotometer (Thermo Scientific Genesys 20, Becthai Bangkok Equipment and Chemical Co., Ltd., Phayathai, Bangkok, Thailand) at 765 nm and performed in ten replications. The results were expressed as Gallic Acid Equivalent (GAE) in mg/ 100 g of wet sample.

$$\text{TPC} = \frac{(\text{Concentration from STD curve} \times \text{vol. extract} \times \text{dilution factor} \times 100}{\text{Sample weight} \times 1000} \quad (3)$$

2.9 Statistical Analysis

Data were arranged in Completely Randomized Design test and expressed as mean ± SD. Analysis of variance (ANOVA) was conducted and compared significance of difference within sample using Duncan's multiple range test. Difference at $p < 0.05$ was considered to be significant (SPSS 16.0 for Windows, SPSS Inc., IL, USA).

3. Results and Discussion

3.1 Effect of Chinese Quince Seed Extract on Mycelial Growth Inhibition

The mycelial growth of *C. gloeosporioides in vitro* was completely inhibited by Chinese quince seed extract at all concentrations tested during storage for 6 days at 25 ± 2 °C compared to control. The mycelial growth inhibition among the concentrations of Chinese quince seed extracts clearly increased from day 2 until day 5 of incubation and significantly higher ($p < 0.05$) than control. Chinese quince seed extract at concentration of 1.5% showed a strong mycelial growth inhibition at 82.59% after 5 days of incubation, slightly lower than mycelial growth inhibition rate of fungicide 0.1% Carbendazim (100%). On the other hand, Chinese quince seed extract at concentrations of 0.5% and 1% promoted mycelial growth inhibition at 78.85% and 78.41%, respectively. There was no mycelial growth inhibition observed in untreated control; however, at day 6 of incubation, the mycelial radial growth of fungi in control had reached the edge of the plate (Table 1 and Figure 1).

Table 1. Effect of Chinese quince seed extract at different concentrations on mycelial growth inhibition of *Colletotrichum gloeosporioides* during incubation at 25 ± 2°C.

Treatments	Mycelial Growth Inhibition (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}
0.5% Extract	17.47 ± 1.65^{b}	65.28 ± 0.60^{c}	73.66 ± 0.28^{c}	78.11 ± 0.61 ^{c}	78.85 ± 0.97 ^{c}	77.69 ± 1.01 ^{c}
1% Extract	14.52 ± 2.34^{b}	64.72 ± 1.21^{c}	73.29 ± 0.90^{c}	77.89 ± 0.39 ^{c}	78.41 ± 0.80 ^{c}	76.69 ± 0.52 ^{c}
1.5% Extract	16.50 ± 0.27^{b}	68.58 ± 0.68^{b}	77.35 ± 0.80^{b}	82.08 ± 0.55 ^{b}	82.59 ± 0.70 ^{b}	81.40 ± 0.34 ^{b}
0.1% Carbendazim	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00 ^{a}	100.00 ± 0.00 ^{a}	100.00 ± 0.00 ^{a}

Note: Values are mean ± SD. Values with different letters represent significant differences between treatments separated using Duncan's Multiple Range Test (DMRT) ($p < 0.05$).

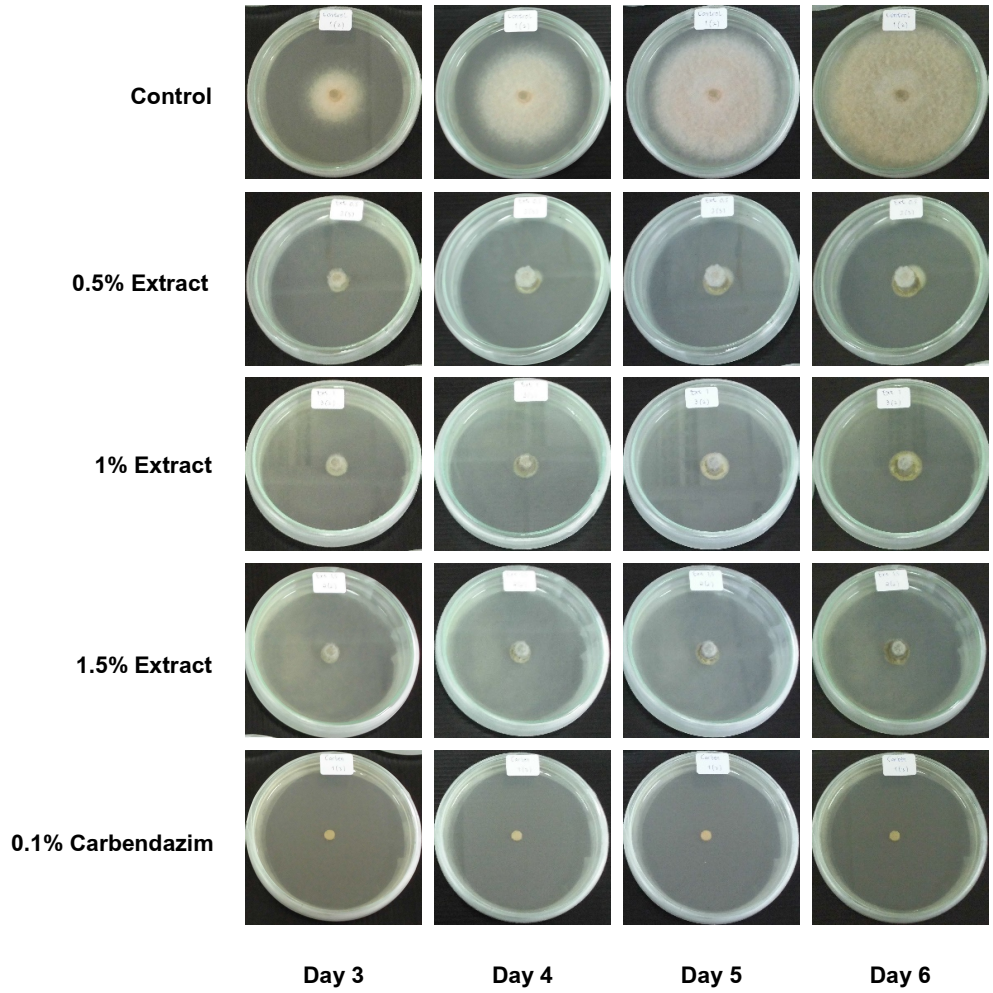

Figure 1 Effect of Chinese quince seed extract at different concentrations on mycelial growth of *Colletotrichum gloeosporioides in vitro*, during 6 days of incubation at 25 ± 2 °C.

3.2 Effect of Chinese Quince Seed Extract on Spore Germination

Cavity slide technique showed that the percentage of *C. gloeosporioides* spore germination was significantly different ($p < 0.05$) between the extract concentrations and the untreated control after incubation at 25 ± 2°C for 3h. Spore germination rate in untreated control (23.68%) was significantly less ($p < 0.05$) than that in all extract concentrations. Spore germination in 1% and 1.5% extract treatments (52.86% and 55.75%, respectively) were significantly lower ($p < 0.05$) than that in 0.5% extract treatment (61.31%). The efficacy of extract in reducing spore germination was affected by the extract concentration which was found to be more effective in the higher extract concentration. There was no spore germination observed in fungicide Carbendazim as positive control.

Table 2. Effect of Chinese quince seed extract at different concentrations on spore germination of *Colletotrichum gloeosporioides* after incubation at 25 ± 2 °C for 3 h.

Treatment	Spore Germination (%)
Control	23.68 ± 2.22 [c]
0.5% Extract	61.31 ± 2.51 [a]
1% Extract	52.86 ± 0.57 [b]
1.5% Extract	55.75 ± 2.63 [b]
0.1% Carbendazim	0.00 ± 0.00 [d]

Note: Values are mean ± SD. Values with different letters represent significant differences between treatments separated using Duncan's Multiple Range Test (DMRT) ($p < 0.05$).

3.3 Effect of Chinese Quince Seed Extract on Anthracnose in Mango

There was slight significant difference of Chinese quince seed extract in inhibiting disease development in inoculated mango fruit (Figure 2). Table 3 shows that fruit treated with extract at all concentrations showed significantly smaller lesion diameter ($p < 0.05$) compared to fruit in untreated control during storage at 25 ± 2 °C. Fruit treated with extract at concentrations of 1 and 1.5% showed the smaller lesion diameter (0.43 and 0.44 cm, respectively), followed by 0.5% extract (0.53 cm) compared to the control (0.61 cm) after 2 days of storage. Mango fruit treated with extract at concentration of 1% exhibited the smaller lesion diameter (1.36 cm) compared to 0.5% and 1.5% extract (1.52 and 1.43 cm, respectively) after 5 days of storage, but there was no significant difference between them.

Figure 2 Lesion development on mango cv. 'Nam Dok Mai Si Thong' inoculated with *Colletotrichum gloeosporioides* 6 days after inoculation.

Table 3. Effect of Chinese quince seed extract at different concentrations on the lesion diameter of *Colletotrichum gloeosporioides* on treated mango fruit during storage at 25 ± 2 °C.

Extract	Lesion Diameter (cm)				
	Day 2	Day 3	Day 4	Day 5	Day 6
Control	0.61 ± 0.09^{a}	0.84 ± 0.18^{a}	1.27 ± 0.34^{a}	2.17 ± 0.53^{a}	2.88 ± 0.69^{a}
0.5% Extract	0.53 ± 0.11^{a}	0.71 ± 0.21^{a}	0.98 ± 0.35^{a}	1.52 ± 0.53^{ab}	2.25 ± 0.96^{ab}
1% Extract	0.43 ± 0.10^{ab}	0.60 ± 0.11^{ab}	0.75 ± 0.15^{ab}	1.36 ± 0.39^{ab}	1.94 ± 0.54^{ab}
1.5% Extract	0.44 ± 0.10^{ab}	0.68 ± 0.10^{a}	0.89 ± 0.19^{ab}	1.43 ± 0.37^{ab}	2.05 ± 0.47^{ab}
0.1% Carbendazim	0.30 ± 0.00^{b}	0.30 ± 0.00^{b}	0.32 ± 0.03^{b}	0.61 ± 0.44^{b}	0.96 ± 0.93^{b}

Note: Values are means ± SD. Values with different letters represent significant differences between treatments separated using Duncan's Multiple Range Test (DMRT) ($p < 0.05$).

The lesion diameter of fruits in all treatments increased with the storage period. During the storage period (day 2 to day 6), control fruit showed the biggest lesion diameter compared to fruits treated with extracts, while the smallest lesion diameter was observed in Carbendazim treatment. However, the lesion diameter in mango fruit treated with Chinese quince seed extract was significantly lower ($p < 0.05$) than that in untreated control, the inhibition by the extracts was not as dramatic as that in *in vitro* study.

3.4 Antifungal test of Chinese Quince Seed Film

Film incorporated with Chinese quince seed extract at concentrations of 0.5, 1 and 1.5% did not inhibit the growth of *C. gloeosporioides*, similar to the result in the control. There was no clear inhibitory zone formed around the film disc on the PDA (Figure 3). The fungus could even grow over the film disc on the PDA. The clear zone only observed surrounding the disc containing Carbendazim as positive control.

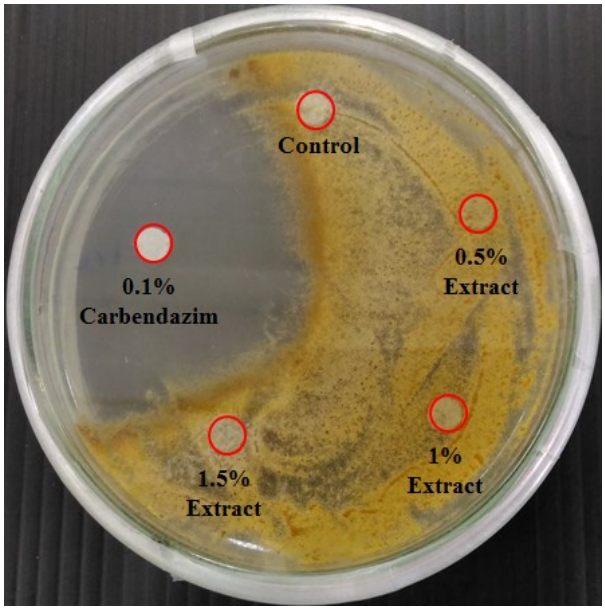

Figure 3 Antifungal test of Chinese quince seed extract film against *Colletotrichum gloeosporioides* after 7 days of inoculation.

3.5 Total Phenolic Content Analysis

The total phenolic content in Chinese quince seed extract measured by the Folin-Ciocalteu method was 48.84 ± 1.71 mg GAE / 100 g wet sample. The total phenolic content of the seed extract in this experiment was higher than previously published data in quince and Chinese quince seed.

3.6 Discussions

Chinese quince seed extract at different concentrations had high effect on reducing growth of *C. gloeosporioides* under *in vitro* rather than on *in vivo* test. Mycelial growth of *C. gloeosporioides* was inhibited by Chinese quince seed extract at all concentrations. Antifungal activity exhibited by Chinese quince seed extract might due to the presence of secondary metabolites, such as phenolic compound, in the extract which could inhibit the mycelial growth of *C. gloeosporioides* under *in vitro*. These compounds can combat with pathogens by different mode of action. Cell wall of pathogens is the main target of phenolic compounds to disrupt the permeability barrier of cell membrane and inhibit respiration (Abdl-Alla and Haggag, 2013). Nychas (1995) found that phenolic compounds in low concentration disrupted proteins and in high concentrations damaged the enzymes outbreak in production of energy. The phenolic substance may inhibit the growth of fungi either temporarily (fungistatic) or permanently (fungicidal) (Feng and Zheng, 2007; Abdl-Alla and Haggag, 2013). However, the mechanism of Chinese quince seed extract by which it affects the growth of the fungi is still unclear.

A lot of plant extracts had been reported in its antimicrobial activity against *C. gloeosporioides* under *in vitro*. Abd-Alla and Haggag (2013) reported that orange oil at concentration of 150 µg/ mL caused a complete reduction in mycelium linear growth of *C. gloeosporioides*. Another study stated that the leaf extract of *Citrus limon* and *Persea americana* completely inhibited the radial growth of *C. gloeosporioides* (Bautista-Banos *et al*., 2002). It seems like the seed extract of Chinese quince showed antimicrobial activity that can reduce the mycelial growth of *C. gloeosporioides*.

Chinese quince seed extract had no effect on spore germination of *C. gloeosporioides*. The pathogen might be more sensitive to Chinese quince seed extract at the mycelial stage rather than the conidial stage. The extract at all concentrations showed inhibition effect on the spore germination of *C. gloeosporioides* for only less than 50%. Chemical substances extracted from plant contain several chemicals such as sugar, amino acid, mineral salt, phenols and alkaloids which may either stimulate or inhibit spore germination (Narayanasamy, 2002; Mishra *et al*., 2005). It may be also caused by the dormancy of the spores. The spore dormancy can be broken by physical or chemical treatments, which may change permeability of cell wall (Mishra *et al*., 2005) to germinate. Pizana *et al*. (2011) reported that spore germination of *Fusarium oxysporum* f. sp. *gladioli* was less affected by plant extracts compared with mycelial growth.

However, some plant extracts had been reported in their abilities on reducing fungal spore germination. Mustard oil and basil oil at concentration of 50 µg/ mL significantly reduced the spore germination of *C. gloeosporioides* for up to 70.8% and 64.7%, respectively (Abd-Alla and Haggag, 2013). Bussaman *et al*. (2012) reported that the maximum inhibition of *C. gloeosporioides* spore germination could be obtained after application with crude methanolic extract of *Piper sarmentosum* leaves at 1.25%. The present results suggested that Chinese quince seed extracts did not suppress fungal spore germination; however, they showed an apparent potential in inhibiting mycelial radial growth of *C. gloeosporioides* under *in vitro*.

In vivo assay of the efficacy of Chinese quince seed extract against *C. gloeosporioides* showed that the lesion diameter on the mango fruit treated with 0.5, 1 and 1.5% extract were smaller than that in the control. The use of plant extract to inhibit the growth of anthracnose disease on its host fruits had been previously reported in many researches. The use of mustard oil and basil oil at concentration of 250 ppm highly reduced the anthracnose incidence in mango fruits for more than 60% (Abd-Alla and Haggag, 2013). Other research reported that aqueous extract of *Echinops* sp. (25%) was found to be effective in the reduction of *C. gloeosporioides* on papaya fruit (Ademe *et al*., 2013). Cruz *et al*. (2013) also reported the efficacy of 4% citric extract on suppressing disease incidence at 19.44% and disease severity at 9.34% caused by *C. musae* on banana fruit.

Quince seed mucilage was composed of cellulose, D-xylose, aldobiouronic acids and small amounts of L-arabinose, and the major water-soluble polysaccharide was a partially *O*-acetylated (4-*O*-methyl-D-glucurono)-D-xylan with an exceptionally high proportion of glycuronic acid residues (Lindberg *et al*., 1990; Vignon and Gey, 1998). Jouki *et al*. (2014a) reported that quince seed films alone did not inhibit the growth of the ten pathogenic bacteria tested. The combination of film with natural compound contained antimicrobial activity may possess the synergistic effect and greatest potential in controlling postharvest disease. Quince seed film incorporated with 1% oregano essential oil exhibited strong inhibitory effect against *Staphylococcus aureus*, *Shewanella putrefaciens*, *Escherichia coli*, and *Yersinia enterocolitica* (Jouki *et al*., 2014b). In another study, Jouki *et al*. (2014a) reported quince seed mucilage film containing 1% thyme essential oil showed antibacterial effect and significantly more effective against Gram-positive bacteria (*S. aureus* and *Listeria monocytogenes*) than against Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*).

Colletotrichum gloeosporioides in papaya fruit was reported to be controlled by coating treatments of 10% gum Arabic combined with 0.4% cinnamon oil (Maqbool *et al*., 2011) and mesquite gum-based coating incorporated with thyme and Mexican lime essential oils (Bosquez-Molina *et al*., 2010). The combination of chitosan film and thyme oil was also known

to significantly reduce the disease severity of anthracnose in avocado fruit during postharvest storage (Bill *et al*., 2014).

Silva *et al*. (2004) reported that the quince seed total phenolic content was 27.36 mg/100 g of methanolic extract dry matter. In another study, Mahalgaes *et al*. (2009) reported that the highest total phenolic content of quince was found in peel (630 mg/100 g of methanolic extract), followed by pulp and seed (250 and 40 mg/100 g of methanolic extract, respectively). Similar result was also reported by Carvalho *et al*. (2010) who found that total phenolic content of quince decreased in the following order: leaf >peel >pulp >seed (2796 mg/100 g, 741 mg/100 g, 117 mg/100 g and 52 mg/100 g of methanolic extract, respectively). The total phenolic content of *Chaenomeles sinensis* fruit was about 28 mg GAE/g fresh weight, lower than that reported in *Chaenomeles speciosa* (46.92 ± 2.76 mg GAE/g fresh weight) and *Chaenomeles thibetica* (46.28 ± 0.59 mg GAE/ g fresh weight), but higher than total phenolic content in *Chaenomeles japonica* (19.35 ± 0.59 mg GAE/g fresh weight) (Du *et al*., 2013).

This different result might be due to the extraction procedure and solvent used to extract the seed. The phenolic content of hot-water extract of Chinese quince pomace (13.3 ± 0.3 mg ChAE/g) was much higher than that of the ethanol extract (7.3 ± 0.1 mg ChAE/g) (Kabir *et al*., 2015). Water is a universal solvent. Nearly all of the identified antimicrobial compounds from plants are aromatic or saturated organic compounds, thus water is among other solvents that are most commonly used for preliminary investigation of antimicrobial activity in plants (Gurjar *et al*., 2012). Mierina *et al*. (2011) reported that Japanese quince seeds extracted with water, mixture of water and ethanol or ethanol showed total phenolic content between 1 to 7 mg GAE/100 g Japanese quince seed, while the ground seeds extracted at room temperature had total amount of polyphenol varied from 20 to 50 mg GAE/100 g Japanese quince seed. The total amount of polyphenols strongly depends on solvent, temperature and the seed particles (degree of grounding) (Mierina *et al*. 2011).

Hamauzu *et al*. (2005) reported that Chinese quince pulp extracted by aqueous acetone had total phenolic content about 1280 mg/100 g of fresh weight, four times higher than that in quince fruit (302.7 mg/100 g of fresh weight). Chinese quince had the largest amount of phenolics consisting mainly of (+)catechin and (-)-epicatechin (high polymeric procyanidins) as a major component and a small amount of 3-caffeoylquinic acid (Hamauzu *et al*., 2005), with high proportion of flavan-3-ols to its total phenolics (Hamauzu *et al*., 2006). The use of aqueous acetone as extraction solvent permitted the proportion of polymeric procyanidins, which may not be extracted by pressing the fruits or when only methanol or ethanol was used as extraction solvent (Hamauzu and Hanakawa, 2003).

4. Conclusion

Chinese quince seed extract is a safe and applicable means, a natural product for controlling anthracnose disease in mango. It may have a promising role for future postharvest disease management during storage and long distance transportation. Nevertheless, because *C. gloeosporioides* typically showed quiescent infection, further work is needed to evaluate the field application of Chinese quince seed extract and its potential for using in combination with other postharvest treatment.

Acknowledgements

The authors would like to thank Mae Fah Luang University and Shinshu University for the support.

References

Abd-Alla, M.A. and Haggag, W.M. 2013. Use of some plant essential oils as post-harvest botanical fungicides in the management of anthracnose disease of mango fruits (*Mangifera indica L.*) caused by *Colletotrichum gloeosporioides* (Penz). International Journal of Agriculture and Forestry. 3(1): 1–6.

Ademe, A., Ayalew, A. and Woldetsadik, K. 2013. Evaluation of antifungal activity of plant extracts against papaya anthracnose (*Colletotrichum gloeosporioides*). Journal Plant Pathology and Microbiology. 4(10): 1–4.

Alemu, K., Ayalew, A. and Woldetsadik, K. (2014). Antifungal activity of plant extracts and their applicability in extending the shelf life of mango fruits. Food Science and Quality Management. 33: 47–53.

Alizadeh, H., Rahnema, M., Semnani, S.N. and Hajizadeh, N. 2013. Detection of compounds and antibacterial effect of quince (*Cydonia oblonga* Miller) extracts *in vitro* and *in vivo*. Journal of Biologically Active Products from Nature. 3(5–6): 303–309.

Bautista-Banos, S.B., Barrera-Necha, L.L., Bravo-Luna, L. and Bermudez-Torres, K. 2002. Antifungal activity of leaf and stem extracts from various plant species on the incidence of *Colletotrichum gloeosporioides* of papaya and mango fruit after storage. Mexical Journal of Phytopathology. 20(1): 8–12.

Bill, M., Sivakumar, D., Korsten, L. and Thompson, A.K. 2014. The efficacy of combined application of edible coatings and thyme oil in inducing resistance components in avocado (*Persea americana* Mill.) against anthracnose during post-harvest storage. Crop Protection. 64: 159–167.

Bussaman, P., Namsena, P., Rattanasena, P. and Chandrapatya, A. 2012. Effect of crude leaf extracts on *Colletotrichum gloeosporioides* (Penz.) Sacc. Psyche. 1–6. doi:10.1155/2012/ 309046.

Cappellini, R.A., Ceponis, M.J. and Lightner, G.W. 1988. Disorders in avocado, mango, and pineapple shipments to the New York market 1972–1985. Plant Disease. 72:270–273.

Carvalho, M., Silva, B.M., Silva, R., Valentao, P., Andrade, P.B. and Bastos, M.L. 2010. First report on *Cydonia oblonga* Miller anticancer potential: Differential anti proliferative effect against human kidney and colon cancer cells. Journal of Agricultural and Food Chemistry. 58(6): 3366–3370.

Chacko, E.K. 1986. Physiology of vegetative and reproductive growth in mango (*Mangifera indica* L.) trees. Proceedings of the 1st Australian Mango Research Workshop, Queensland. CSIRO, Melbourne. 54–70.

Cronin, M.J., Yohalem, D.S., Harris, R.F. and Andrews, J.H. 1996. Putative mechanism and dynamics of inhibition of apple scab pathogen *Venturia inaequalis* by compost extracts. Soil Biology and Biochemistry. 28: 1241–1249.

Cruz, M.E.S., Schwan-Estrada, K.R.F., Clemente, E., Itako, A.T., Stangarlin, J.R. and Cruz, M.J.S. 2013. Plant extracts for controlling the post-harvest anthracnose of banana fruit. Revista Brasileira de Plantas Medicinais. 15(4): 727–733.

Dodd, J.C., Prusky, D. and Jeffries, P. 1997. Fruit diseases. In Litz, R. E. (Ed.), The mango: Botany, production and uses (pp. 257–280). UK: CAB International.

Dodd, J.C., Jeffries, P. and Jeger, M.J. 1989. Management strategies to control latent infection in tropical fruits. Aspect of Applied Biology. 20: 49–56.

Du, H., Wu, J., Li, H., Zhong, P.X., Xu, Y.J., Li, C.H., Ji, K.X. and Wang, L.S. 2013. Polyphenols and triterpenes from Chaenomeles fruits: Chemical analysis and antioxidant activities assessment. Food chemistry. 141(4): 4260–4268.

Feng, W. and Zheng, X. 2007. Essential oils to control *Alternaria alternata in vitro* and *in vivo*. Food Control. 18: 1126–1130.

Gurjar, M.S., Shahid, A., Masood, A. and Kangabam, S.S. 2012. Efficacy of plant extracts in plant disease management. Agricultural Sciences. 3: 425–433.

Hamauzu, Y. and Hanakawa, T. 2003. Relation of highly polymerized procyanidin to the potential browning susceptibility in pear fruits. Journal of the Japanese Society for Horticultural Science. 72: 415–421.

Hamauzu, Y., Yasui, H., Inno, T., Kume, C., and Omanyuda, M. 2005. Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.) and apple (*Malus domestica* Mill.) fruits. Journal of Agricultural and Food Chemistry. 53(4): 928–934.

Hamauzu, Y., Takaroni, I., Kume, C., Irie, M. and Hiramatsu, K. 2006. Antioxidant and antiulcerative properties of phenolics from Chinese quince, quince, and apple fruits. Journal of Agricultural and Food Chemistry. 54: 765–772.

Hamauzu, Y., Kume, C., Yasui, H. and Fujita, T. 2007. Reddish coloration of Chinese quince (*Pseudocydonia sinensis*) procyanidins during heat treatment and effect on antioxidant and anti-influenza viral activities. Journal of Agricultural and Food Chemistry. 55(4): 1221–1226.

International Organization of Standardization. 2005. Determination of substance characteristic of green tea and black tea. Part 1: Content of total polyphenols in tea – calorimetric method using Folin–Ciocalteu reagent. ISO 14502-1: 2005. Retrieved March 2014, from http://www.iso.org/iso/catalogue_detail.htm?csnumber=31356.

Jouki, M., Mortazavi, S.A., Yazdi, F.T. and Koocheki, A. 2014a. Characterization of antioxidant–antibacterial quince seed mucilage films containing thyme essential oil. Carbohydrate Polymers. 99: 537–546.

Jouki, M., Yazdi, F.T., Mortazavi, S.A. and Koocheki, A. 2014b. Quince seed mucilage films incorporated with oregano essential oil: Physical, thermal, barrier, antioxidant and antibacterial properties. Food Hydrocolloids. 36: 9–19.

Kabir, F., Tow, W.W., Hamauzu, Y., Katayama, S., Tanaka, S. and Nakamura, S. 2015. Antioxidant and cytoprotective activities of extracts prepared from fruit and vegetable wastes and by-products. Food Chemistry. 167: 358–362.

Kefialew, Y. and Ayalew, A. 2008. Postharvest biological control of anthracnose (*Colletotrichum gloeosporioides*) on mango (*Mangifera indica*). Postharvest Biology and Technology. 50: 8–11.

Lindberg, B., Mosihuzzaman, M., Nahar, N., Abeysekera, R. M., Brown, R.G. and Willison, J. M. 1990. An unusual (4-O-methyl-D-glucurono)-D-xylan isolated from the mucilage of seeds of the quince tree (*Cydonia oblonga*). Carbohydrate research. 207(2): 307–310.

Macias, F.A., Castellano, D., Oliva, R.M., Cross, P. and Torres, A. 1997. Potential use of allelopathic agents as natural agrochemicals. Proceedings of Brighton Crop Protection Conference—Weeds Brighton, UK. 33–38.

Mahalgaes, A.S., Silva, B.M., Pereira, J.A., Andrade, P.B., Valentao, P. and Carvalho, M. 2009. Protective effect of quince (*Cydonia oblonga* Miller) fruit against oxidative hemolysis of human erythrocytes. Food and Chemical Toxicology. 47: 1372–1377.

Maqbool, M., Ali, A., Alderson, P.G., Mohamed, M.T.M., Siddiqui, Y. and Zahid, N. 2011. Postharvest application of gum Arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. Postharvest Biology and Technology. 62: 71–76.

Mierina, I., Serzane, R., Strele, M., Moskaluka, J., Seglina, D. and Jure, M. 2011. Extract of Japanese quince seeds - potential source of antioxidants. Conference Proceedings of 6th Baltic Conference on Food Science and Technology: Innovations for Food Science and Production. 98–103.

Mishra, A.K., Bohra, A. and Mishra, A. 2005. Plant pathology: Disease and management. Jodhpur, India: Agrobios.

Muirhead, I.F. and Gratitude, R. 1986. Mango diseases. Proceedings of the First Australian Mango Research Workshop, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Cairns. 248–252.

Narayanasamy, P. 2002. Microbial plant pathogens and crop disease management. Enfield, New Hampshire, USA: Science Publishers Inc.

Nychas, G.J.E. 1995. Natural antimicrobials from plants. In Gould, G.W. (Ed.), New methods of food preservation (pp. 58–89). London: Blackie Academic and Professional.

Pandey, D.K., Tripathi, N.N., Tripathi, R.D. and Dixit, S.N. 1982. Fungitoxic and phytotoxic properties of the essential oil of *Hyptis suaveolens*/ Fungitoxische und phytotoxische Eigenschaften des ätherischen Öis von *Hyptis suaveolens*. Journal of Plant Diseases and Protection/ Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. 89: 344–349.

Pizana, C.G., Necha, L.L.B. and Gomez, M.Y.R. 2011. Evaluation of the fungicidal activity of leaf powders and extracts of fifteen Mexican plants against *Fusarium oxysporum* f.sp. *gladioli* (Massey) Snyder and Hansen. Plant Pathology Journal. 9: 103–111.

Regnier, T., duPlooy, W., Combrinck, S. and Botha, B. 2008. Fungitoxicity of *Lippia scaberrima* essential oil and selected terpenoid components on two mango postharvest spoilage pathogens. Postharvest Biology and Technology. 48: 254–258.

Silva, B.M., Ferreres, F., Andrade, P.B., Seabra, R.M., Valenta, P. and Ferreira, M.A. 2004. Quince (*Cydonia oblonga* Miller) fruit (pulp, peel, and seed) and jam: Antioxidant activity. Journal of Agricultural and Food Chemistry. 52: 4705–4712.

Tripathi, P. and Dubey, N.K. 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biology and Technology. 32(3): 235–245.

Tsortzakis, N.G. and Economakis, C.D. 2007. Antifungal activity of lemongrass (*Cympopogon citratus* L.) essential oil against key postharvest pathogens. Innovative Food Science and Emerging Technologies. 8: 253–258.

Vignon, M.R. and Gey, C. 1998. Isolation, 1H and 13C NMR studies of (4-O-methyl-d-glucurono)-d-xylans from luffa fruit fibres, jute bast fibres and mucilage of quince tree seeds. Carbohydrate Research. 307(1–2): 107–111.

Weerakkody, N.S., Caffin, N., Turner, M.S. and Dykes, G.A. 2010. *In vitro* antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. Food Control. 21(10): 1408–1414.