CHAPTER 5

Effect of Chitosan on Carbendazim-resistant *Colletotrichum* spp. Causing Mango Anthracnose

5.1 Introduction

Mangoes are an important export commodity in Thailand. Anthracnose caused by *Colletotrichum gloeosporioides* is an important pre- and post-harvest diseases (Dinh *et al*., 2003), especially in the susceptible mango cv. Namdokmai (Sangchote, 1987), which leads to uncertain in Thai fruit market values. The export market is well developed, but fruit infection commonly occurs in the orchard, and usually results in serious decay during transit to market or after sales. Mango anthracnose, the fungus invades the skin of young fruit and remains in a "quiescent infection" state until ripening begins (Barkai-Golan, 2001). Susceptibility of fruits to post-harvest diseases results from physiological changes during ripening and increasing senescence that develops during storage (Prusky, 1996), necessitating treatment such as hot water, fungicide, or a combination of both to control the disease. Since the side effects of fungicides cause risks such as serious hazards to humans and the environment, induction of non-target pests and fungicide resistance (Bautista-Baños *et al*., 2006). Several studies have attempted to find alternative ways to solve this problem (Rabea *et al*., 2003; Bautista-Baños *et al*., 2006; Rahman *et al*., 2008; Aranaz *et al*., 2009; El Hadrami *et al*., 2010).

Chitosan is a safe material that acts as an antifungal activity against many plant pathogens. It is soluble in organic acids, and one in a range of natural

compounds that has been used successfully to maintain the quality of harvested fruits and vegetables (Rabea *et al*., 2003; Bautista-Baños *et al*., 2006; Aranaz *et al*., 2009; El Hadrami *et al*., 2010). It has been shown as effective in decreasing the incidence of diseases such as *Botrytis cinerea* in strawberries (El Ghaouth *et al*., 1991, 1992a, b; Han *et al*., 2004; Romanazzi, 2010), controlling anthracnose in artificially post-inoculated papaya fruit (Bautista-Baños *et al*., 2003; Rahman *et al*., 2008), and preventing the decay of longan fruit (Jiang and Li, 2001) and litchi (Jiang *et al*., 2005). Chitosan was also found to induce severe morphological changes in *Rhizopus stolonifer* (García-Rincón *et al*., 2010). One possible mechanism of chitosan appears through the cellular leakage induction of amino acids and to a lesser extent of proteins. Chitosan is reported as a partly effective in its potential to induce plant defense mechanisms, such as phenolic and lignin accumulation in chitosan-treated wheat seeds (Bhaskara *et al*., 1999), increased in peroxidase activity of chitosan-treated cucumber plants (Ben-Shalom *et al*., 2003). Pre-harvest treatment with chitosan can reduce apparent severity of the quiescent infected area and incidence of post-harvest decay, whereas post-harvest treatment inhibits development of quiescent infections (Prusky *et al*., 1983; Dodd *et al*., 1997).

The objective of this chapter was:

1. To evaluate the antifungal activity of chitosan against the carbendazim-resistant *Colletotrichum* spp. that causes mango anthracnose.

5.2 Materials and Methods

The chitosan used in this study was divided into 3 groups:-

- Group A: commercial chitosan solution (CC) product for plant production (2 samples; CC1 and CC2)
- Group B: polymer chitosan (PC) [2 samples; Poly-(1,4-β-D-glucopyranosamine) (PC1) and Poly (D-glucosamine) (PC2)]
- Group C: oligomer chitosan (OC) [2 samples; oligomer chitosan solution (OC1) and chitosan oligosaccharide lactate (OC2)].

Descriptions of chitosan samples are shown in Table 5.1.

Table 5.1 Description of chitosan samples used in this study

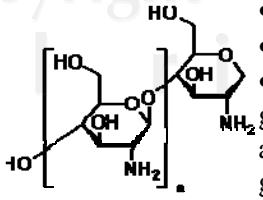
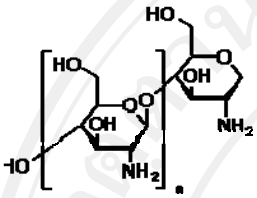
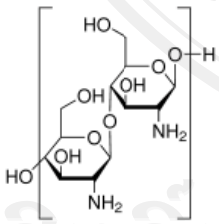
Chitosan samples	Descriptions	Source	Price (Baht)
Group A. Commercial chitosan solution (CC) product for plant production			
1. Sample code: CC1			0.38/ml
	• chitosan solution • from crab shells • 1 cc/l (recommended field-use concentration)	• General formula, Co. • Product of Thailand	
2. Sample code: CC2			0.45/ml
	• chitosan solution • from crab shells • 1 cc/l (recommended field-use concentration)	• Australia fertilize, Co. limited • Product of Australia	
Group B. Polymer chitosan (PC)			
3. Sample code: PC1			103.2/g
Synonym: 2-Amino-2-deoxy-(1→4)-β-D-glucopyranan, Poly-(1,4-β-D-glucopyranosamine)			
	• platelet form • from crab shells • 20% β1,4-linked N-acetyl-D-glucosamine (GlcNAc) and approximately 80% β1,4-linked D-glucosamine (GlcN) • total impurities ≤ 1% insoluble matter • viscosity 200-400 mPa.s., 1% in acetic acid (20°C)	• Fluka, BioChemika, Sigma-Aldrich, Co. • Product of Japan	

Table 5.1 Continued.

Chitosan samples	Descriptions	Source	Price (Baht)
4. Sample code: PC2 Synonym: : Deacetylated chitin, Poly (D-glucosamine) 	• platelet form • from shrimp shells • ≥ 75% deacetylated • >200 cP, 1% in 1% acetic acid	• Aldrich Chemistry, Sigma-Aldrich, Co. • Product of Iceland	86.9/g
Group C. Oligomer chitosan (OC)			
5. Sample code: OC1 Commercial name: Orchid-80	• oligomer chitosan solution • 1 cc/l (recommended field-use concentration) • from crab shell • MW 45,000 • >90% DD	• Center for chitin-chitosan Biomaterials, Chulalongkorn University • Product of Thailand	0.25/ml
6. Sample code: OC2 Synonym: chitosan oligosaccharide lactate $(C_{12}H_{24}N_2O_9)_n$ 	• powder form • average M_n 5,000 • 90% deacetylation	• (Aldrich Chemistry, Sigma-Aldrich, Co.) • Product of China	2,690/g

Preparation of chitosan solution

The CC1, CC2 and OC1 were prepared at 1cc/l in sterile distilled water as recommendation rate.

The PC1 and PC2 chitosan samples were prepared as described by El Ghaouth *et al.* (1991). One hundred milliliters of chitosan solutions were prepared with 0.5, 1.0 and 1.5 g of chitosan dissolved in 75 ml of sterilize distilled water, with 1% (v/v) of acetic acid. The mixture was heated at 35^{O}C, whilst continually stirring until properly dissolved. Tween 80 (0.1 ml (v/v)) was added, as a surfactant, to improve the wetting properties of the solution, and the volume was taken up to 100 ml with sterile distilled water. The final pH of the solution was adjusted to 5.5 with 1N sodium hydroxide (NaOH) (Jiang and Li, 2001).

The OC2 sample was prepared as a stock solution in sterilized distilled water.

Experiment 1: The effect of various chitosan on conidial inhibition

Seven isolates of carbendazim-resistant *C. gloeosporioides*, including 3 Car^{HR} isolates (CAN_F095, K_F103, NDM_F116), one isolate of Car^{MR} isolate (NDM_F063) and 3 isolates of Car^{S} isolates (FL_F003, NDM_ F006, PBL_F102) from genotypic detection by the second beta-tubulin sequence analysis were randomly selected for this experiment.

The tested chitosan of CC1, CC2 and OC1 at concentration of 1 cc/l, and PC1, PC2 and OC2 at the concentrations of 0.5, 1.0 and 1.5 % were tested for conidial inhibition to 7 isolates of carbendazim-resistant *C. gloeosporioides* by slide cultures.

Inoculum preparation of C. gloeosporioides

The conidia of each carbendazim-resistant *C. gloeosporioides* isolate were obtained from a 14 day old culture grown on PDA at room temperature. Conidia were subsequently harvested by flooding the medium surface with sterilized distilled water, containing Tween 80 (v/v), and gently agitating the plate with a bent sterilized glass rod to dislodge the conidia. The resulting suspension was filtered through a double layer of sterilized cheesecloth. The conidia concentration was adjusted to $1x10^6$ conidia/ml, with sterile distilled water, using a haemacytometer (Obagwa and Korsten, 2003).

Each chitosan solution and conidial suspension were dropped into each Eppendrof tube to give a final concentration. Each chitosan solution mixture of 10 µl was transferred onto 2 cm^2 PDA pieces and placed on a glass slide (two pieces per slide), in a Petri dish. Distilled water was used as a control. The slide cultures were maintained at room temperature, and evaluated by counting germinated conidia (Rappussi *et al*., 2009). 100 conidia was observed for germination and recorded using a compound light microscope. Conidia were considered to germinate when the germ tube length equaled or exceeded the length of the conidium (El Ghaouth *et al*., 1992a, b). Data on number of germinated conidia were recorded at 6, 12, 18 and 24 hr. The experiment was done by using 2 factors factorial in completely randomized design (CRD) with four replications.

Factor A represented chitosan application as follows:-

A1 = control (sterile distilled water)

A2 = 1% acetic acid (pH 5.5)

A3 = 0.1% Tween 80

A4 = 500 mg/l carbendazim

A5 = 1 cc/l CC1

A6 = 1 cc/l CC2

A7 = 0.5% PC1

A8 = 1.0% PC1

A9 = 1.5% PC1

A10 = 0.5% PC2

A11 = 1.0% PC2

A12 = 1.5% PC2

A13 = 1cc/l OC1

A14 = 0.5% OC2

A15 = 1.0% OC2

A16 = 1.5% OC2

Factor B represented 7 isolates of carbendazim-resistant *C. gloeosporioides* namely CAN_F095 (B1), K_F103 (B2), NDM_F116 (B3), NDM_F063 (B4), FL_F003 (B5), NDM_ F006 (B6) and PBL_F102 (B7)

Experiment 2: The effect of various chitosan on mycelial inhibition

Seven isolates of carbendazim-resistant *C. gloeosporioides*, including 3 Car^{HR} isolates (CAN_F095, K_F103, NDM_F116), one isolate of Car^{MR} isolate (NDM_F063) and 3 isolates of Car^{S} isolates (FL_F003, NDM_ F006, PBL_F102) were also used in this experiment.

The tested chitosan samples namely CC1, CC2 at concentration of 1 cc/l and PC1 and PC2 at concentration of 0.5, 1.0 and 1.5 % were used in this experiment.

Each chitosan solution of 1 ml was dropped onto each PDA plate, using a pipette, and spread over with the sterile bent glass rod. Mycelial discs (5 mm diameter) were taken from the peripheral region of a 5 day old *C. gloeosporioides* culture, which grown on PDA and transferred onto the amended chitosan plate (9 cm diameter). Sterilized distilled water was used as a control. The plates were then incubated at room temperature. Colony diameter was measured after incubation when the colony growing almost full plate in the control plates. The experiment was done by using 2 factors factorial in CRD with five replications.

Factor A represented chitosan application as follows:-

A1 = control (sterile distilled water)

A2 = 1% acetic acid (pH 5.5)

A3 = 500 mg/l carbendazim

A4 = 1 cc/l CC1

A5 = 1 cc/l CC2

A6 = 0.5% PC1

A7 = 1.0% PC1

A8 = 1.5% PC1

A9 = 0.5% PC2

A10 = 1.0% PC2

A11 = 1.5% PC2

Factor B represented 7 isolates of carbendazim-resistant *C. gloeosporioides* namely CAN_F095 (B1), K_F103 (B2), NDM_F116 (B3), NDM_F063 (B4), FL_F003 (B5), NDM_ F006 (B6), PBL_F102 (B7)

Experiment 3: The effect of chitosan to control anthracnose on mango fruits cv. 'Namdokmai'

Two isolates of highly carbendazim-resistant *C. gloeosporioides* (Car^{HR}) namely CAN_F095 and NDM_F116 were used in this experiment.

The tested chitosan samples namely CC1, CC2 at concentration of 1 cc/l and PC1 and PC2 at concentration of 0.5, 1.0 and 1.5 % and OC1 at concentration of 1 cc/l were used in this experiment.

Each Car^{HR} isolate of CAN_F095 and NDM_F116 was prepared spore suspension which adjusted to $1x10^6$ spores/ml using a haemacytometer. The harvested mango fruits cv. 'Namdokmai' were used in this experiment after disinfected thorough tap water and followed by surface sterilized using ethyl alcohol about 1 min and air-dried before application. All preparations were used in Experiment 3.1, and 3.2.

Experiment 3.1 Dropping chitosan solution to control mango anthracnose

Wound inoculation was done to test the chitosan efficacy on the harvested mango fruits cv. 'Namdokmai'. A circular inoculation site, with a 5 mm diameter, was marked on the surface of the fruit. The wound was created by a 2 mm deep puncture for 5 point using a sterile needle.

Before inoculation of pathogen in treatments, each tested chitosan was dropped onto the wounded areas of the mango fruits and allowed to dry for 15 min, and then 20 µl spore suspension ($1x10^6$ spores/ml) of each isolate was dropped onto wounded areas.

After inoculation of pathogen in treatments, 20 µl spore suspension ($1x10^6$ spores/ml) of each isolate was dropped onto the wounded areas of mango fruits and incubated for 24 hr, and then each tested chitosan was dropped onto the inoculated areas.

The treated fruits were incubated in the moist plastic box at room temperature. Lesion diameters were measured after 4 days. The percentage of disease decrease was calculated according to the formula below.

$$\text{percentage of disease decrease} = 100 - \left[\frac{100 \times C2}{C1}\right]$$

C1 = lesion diameter in pathogen without treated the sample (control)

C2 = lesion diameter in pathogen with treated the sample

The experiment was done by using 3 factors factorial in CRD with three replications.

Factor A represented chitosan application as follows:-

A1 = non-treated control (sterile distilled water)

A2 = non inoculated and wounded

A3 = inoculated and wounded

A4 = 1% acetic acid (pH 5.5)

A5 = 500 mg/l carbendazim

A6 = 1 cc/l CC1

A7 = 1 cc/l CC2

A8 = 1 cc/l OC1

A9 = 0.5% PC1

A10 = 1.0% PC1

A11 = 1.5% PC1

A12 = 0.5% PC2

A13 = 1.0% PC2

A14 = 1.5% PC2

Factor B represented before (B2) and after inoculation (B2)

Factor C represented 2 isolates namely CAN_F095 (C1) and NDM_F116 (C2).

Experiment 3.2 Spraying chitosan solution to control mango anthracnose

The harvested mango fruits cv. 'Namdokmai' were sterilized by surface disinfectant with ethyl alcohol and used to test chitosan efficacy.

Before inoculation of pathogen treatments, each tested chitosan was sprayed onto the surface of mango fruits and air dried for 15 min, and then 10 ml spore suspension ($1x10^6$ spores/ml) of each isolate was inoculated by spraying onto the surface of mango fruits.

After inoculation of pathogen treatments, 10 ml spore suspension ($1x10^6$ spores/ml) of each isolate was inoculated by spraying onto the surface fruits and incubated for 24 hr, and then each tested chitosan was sprayed onto surface of mango fruits.

The treated fruits were incubated in the moist chamber in plastic box at room temperature. The disease index was recorded 7 days after incubation by using disease index in Table 5.2. The experiment was done by using 3 factors factorial in CRD with three replications.

Factor A represented chitosan application as follows:-

A1 = non-inoculated control (sterile distilled water)

A2 = inoculated pathogen

A3 = 1% acetic acid (pH 5.5)

A4 = 500 mg/l carbendazim

A5 = 1 cc/l CC1

A6 = 1 cc/l CC2

A7 = 1 cc/l OC1

A8 = 0.5% PC1

A9 = 1.0% PC1

A10 = 1.5% PC1

A11 = 0.5% PC2

A12 = 1.0% PC2

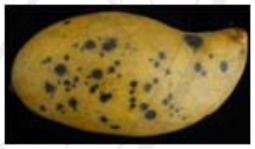
A13 = 1.5% PC2

Factor B represented before (B2) and after inoculation (B2)

Factor C represented 2 isolates namely CAN_F095 (C1) and NDM_F116 (C2).

Table 5.2 Disease index (DI) on mango anthracnose

Disease index was modified from Prabakar *et al*. (2008) as follows:-

Disease category	Description	
0	No disease symptom	
1	>0-25% disease symptoms on fruit surface	
2	>25-50% disease symptoms on fruit surface	
3	>50-75% disease symptoms on fruit surface	
4	>75% disease symptoms on fruit surface	

The data in all experiments were statistically analyzed by analysis of variance (ANOVA) and treatment means were compared by the Duncan's Multiple Range Test (DMRT).

5.3 Results

Experiment 1: The effect of various chitosan on conidial inhibition

Result showed that after 6 hr of incubation, the number of germinated conidia in the tested isolates of carbendazim-resistant *C. gloeosporioid*es showed significant difference (*P*=0.01) between isolates and chitosan treatments when compared to the control. There was a significantly interaction between chitosan treatments and isolates. It revealed that carbendazim at 500 mg/l, CC1 and CC2 at 1 cc/l, PC1 and PC2 at 0.5%, 1.0% and 1.5% were completely reduced the numbers of germinated conidia in all tested isolates of CAN_F095, K_F103, NDM_F116, NDM_F063, FL_F003, NDM_ F006, and PBL_F102. Moreover, the tested OC2 at 1.5% in the isolates of NDM_F116 and NDM_F006 gave the lowest number of germinated conidia at 27.75 and 28.50, respectively. However, the number of germinated conidia in 1% acetic acid (pH 5.5) and 0.1% Tween 80 were not significantly difference with control in all tested isolates (Table 5.3).

In addition, the number of germinated conidia in all treatments were increased after incubation for 12 hr which showed significant difference (*P*=0.01) between isolates and chitosan treatments when compared to the control. There was gave a significantly interaction between chitosan treatments and isolates at 12 hr.

The result showed that the lowest number of germinated conidia was obtained in the treatment of 1.5 % PC1 for tested isolates of CAN_F095, NDM_F116, FL_F003, NDM_ F006, K_F103, PBL_F102 and NDM_F063 which the averaged numbers of germinated conidia were 1.50, 2.75, 3.75, 4.75, 5.75, 7.75 and 10.75 respectively. The 1.5% PC2 was tested to isolates of CAN_F095, NDM_F116, FL_F003, NDM_ F006, K_F103, PBL_F102 and NDM_F063 that the averaged

numbers of germinated conidia were 2.50, 2.50, 3.00, 4.25, 4.25, 7.25 and 10.00 respectively. The 1.0 % PC1 was tested to isolates of CAN_F095 and NDM_F116 that showed the average number of germinated conidia as 4.00 and 5.25, respectively. The 1.0% PC2 was tested to isolates of NDM_F116 and CAN_F095 showing average number of germinated conidia of 5.00 and 9.25, respectively. It revealed that 1 cc/l CC2 tested to isolates of NDM_F116, NDM_ F006, K_F103, NDM_F063, PBL_F102, CAN_F095 and FL_F003 showing average number of germinated conidia of 3.75, 6.00, 6.50, 6.75, 8.00 and 8.00, respectively.

Meanwhile, the tested carbendazim at 500 mg/l to 3 Car^{HR} isolates of CAN_F095, K_F103 and NDM_F116 was not inhibited conidial germination at 12 hour but Car^{MR} isolate of NDM_F063 and 3 Car^{S} isolates of FL_F003, NDM_F006 and PBL_F102 showed no germinated conidia.

However, number of germinated conidia in 1% acetic acid (pH 5.5), 0.1% Tween 80, 1 cc/l OC1 and 0.5%, 1.0% and 1.5% of OC2 were not significant difference when compared to the control in all isolates (Table 5.4).

Moreover, the number of germinated conidia after incubated at 18 hr showed significant difference (P=0.01) between isolates and chitosan treatments. There was gave a significantly interaction between chitosan treatments and isolates at 18 hr. The results showed that the lowest number of germinated conidia was obtained when treated 1cc/l CC2 to tested isolates of CAN_F095, NDM_ F006, K_F103, NDM_F116, NDM_F063 and PBL_F102 which the germinated conidia were 23.50, 24.50, 25.00, 25.50, 31.25 and 33.00 respectively.

The 1.5% PC1 was treated to isolates of CAN_F095, FL_F003, K_F103, PBL_F102, NDM_ F006, NDM_F063 and NDM_F116 showing the germinated conidia of 29.75, 32.50, 33.75, 33.75, 36.00, 36.75 and 37.75, respectively.

The 1.5% PC2 was treated to isolates of NDM_F116, FL_F003, NDM_ F006, K_F103, and CAN_F095 showing germinated conidia of 28.75, 31.75, 33.00, 34.75 and 36.75, respectively. Treatments of 1 % PC1 and 1 % PC2 tested to isolates of NDM_F116 revealed the number of germinated conidia of 36.25 and 37.25, respectively.

However, treatments of 1% acetic acid (pH 5.5), 0.1% Tween 80, 1 cc/l OC1 and 0.5%, 1.0% and 1.5% of OC2 to all tested isolates were completely germinated conidia (Table 5.5).

Moreover, after incubation for 24 hr, the number of germinated conidia showed significant difference (P=0.01) between isolates and chitosan treatments. There was shown a significantly interaction between chitosan treatments and isolates at 24 hr. The results showed that 1.5% PC2 tested to isolate K_F103 gave significantly lowest germination as 62 germinated conidia and followed by the isolate PBL_F102 which was 67.75 germinated conidia.

Result showed that 3 isolates of Car^{HR} maintained resistant to carbendazim at 500 mg/l as seen all germinated conidia. But 1 isolate of Car^{MR} and 3 isolates of Car^{S} showed sensitivity to carbendazim which no conidia germinated at 24 hours (Table 5.6).

It is demonstrated that all tested chitosan could inhibit conidial germination with time in term of delay germination.

Table 5.3 The effect of various chitosan on conidial inhibition of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose for 6 hr

Treatments	Number of germinated conidia for 6 hr						
	Car^{HR}[1/]			Car^{MR}	Car^{S}		
	CAN_F095	K_F103	NDM_F116	NDM_F063	FL_F003	NDM_F006	PBL_F102
control (sterile distilled water)	76.50f-j[2/]	86.00a	81.00a-f	83.75a-e	76.50f-j	85.00a-c	85.50ab
Acetic acid 1% (pH 5.5)	74.00i-l	84.50a-d	79.75d-h	82.75a-e	75.25h-k	82.50 a-e	84.25a-d
Tween 80 0.1% v/v	74.50i-k	84.75a-d	78.75e-i	82.50a-e	75.50g-k	81.25a-f	84.75a-d
Carbendazim 500 mg/l*	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s
CC1 1 cc/l*	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s
CC2 1cc/l*	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s
PC1							
0.5%	0.00s	0.75s	1.50s	0.25s	0.00s	1.50s	0.25s
1.0%	0.00s	0.25s	0.75s	0.00s	0.00s	0.75s	0.00s
1.5%	0.00s	0.00s	0.25s	0.25s	0.00s	0.25s	0.00s
PC2							
0.5%	0.00s	0.75s	0.50s	0.50s	0.00s	0.50s	0.00s
1.0%	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s
1.5%	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s
OC1 1 cc/l*	68.00mn	62.50o	64.25no	67.25m-o	70.75k-m	64.75no	67.25m-o
OC2							
0.5%	69.00l-m	79.75d-h	48.50p	79.75d-h	75.50g-k	47.50p	79.75d-h
1.0%	73.25j-l	82.50a-e	41.50q	80.75b-f	74.00j-l	41.00q	81.25a-f
1.5%	62.75o	80.00c-h	27.75r	80.50b-g	65.50no	28.50r	81.50a-f
A(chitosan application)				**			
B(isolates)				**			
A*B				**			
CV %				8.77			

[1/]Carbendazim-resistant phenotype: Car^{HR}, Car^{MR}, Car^{S} = highly resistant, moderately resistant, sensitive, respectively.
[2/]Means of number of germinated conidia followed by the same letter are not significantly different by Duncan's Multiple Range Test DMRT at 1% level.
Chitosan products: CC1, CC2 = Commercial chitosan sample 1, 2; PC1, PC2 = Polymer chitosan sample 1, 2; OC1, OC2= Oligomer chitosan sample 1, 2, respectively.
*Field-use recommended concentration.

Table 5.4 The effect of various chitosan on conidial inhibition of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose for 12 hr

Treatments	Number of germinated conidia for 12 hr						
	Car^{HR}[1/]			Car^{MR}	Car^{S}		
	CAN_F095	K_F103	NDM_F116	NDM_F063	FL_F003	NDM_F006	PBL_F102
Control (sterile distilled water)	100.00a[2/]	98.75a	100.00a	100.00a	100.00a	100.00a	100.00a
Acetic acid 1% (pH 5.5)	100.00a	96.25a	100.00a	97.75a	100.00a	100.00a	99.00a
Tween 80 0.1% v/v	100.00a	94.25a	100.00a	99.00a	100.00a	100.00a	98.00a
Carbendazim 500 mg/l*	71.50b	70.70b	71.50b	0.00z	0.00z	0.00z	0.00z
CC1 1 cc/l*	10.00p-y	20.00l-o	16.75l-q	11.75o-v	13.25n-u	18.00l-p	11.25p-w
CC2 1cc/l*	8.00r-z	6.50t-z	3.75v-z	6.75t-z	8.00r-z	6.00t-z	7.00t-z
PC1							
0.5%	22.00j-m	43.00e-g	21.50j-n	56.25cd	29.00ij	21.50j-n	46.75ef
1.0%	4.00v-z	16.00l-r	5.25u-z	39.50f-h	9.75p-y	22.00j-m	30.00ij
1.5%	2.50x-z	4.25v-z	2.50x-z	10.00p-y	3.00w-z	4.25v-z	7.25s-z
PC2							
0.5%	15.75l-s	37.25g-i	20.25k-o	58.50c	34.00hi	28.75i-k	48.75de
1.0%	9.25q-y	23.00j-l	5.00u-z	33.50hi	10.75p-x	14.00m-t	34.25hi
1.5%	1.50yz	5.75t-z	2.75w-z	10.75p-x	3.75v-z	4.75u-z	7.75r-z
OC1 1 cc/l*	100.00a	98.75a	100.00a	93.00a	100.00a	100.00a	93.50a
OC2							
0.5%	100.00a	98.75a	100.00a	97.00a	100.00a	100.00a	97.00a
1.0%	100.00a	98.75a	100.00)a	100.00a	100.00a	100.00a	95.50a
1.5%	100.00a	98.75a	100.00a	96.75a	100.00a	100.00a	95.75a
A(chitosan application)				**			
B(isolates)				**			
A*B				**			
CV %				8.86			

[1/]Carbendazim-resistant phenotype: Car^{HR}, Car^{MR}, Car^{S} = highly resistant, moderately resistant, sensitive, respectively.
[2/]Means of number of germinated conidia followed by the same letter are not significantly different by Duncan's Multiple Range Test DMRT at 1% level.
Chitosan products: CC1, CC2 = Commercial chitosan sample 1, 2; PC1, PC2 = Polymer chitosan sample 1, 2; OC1, OC2= Oligomer chitosan sample 1, 2, respectively.
*Field-use recommended concentration.

Table 5.5 The effect of various chitosan on conidial inhibition of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose for 18 hr

Treatments	Number of germinated conidia for 18 hr						
	Car^{HR} 1/			Car^{MR}	Car^{S}		
	CAN_F095	K_F103	NDM_F116	NDM_F063	FL_F003	NDM_F006	PBL_F102
Control (sterile distilled water)	100.00a 2/	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Acetic acid 1% (pH 5.5)	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Tween 80 0.1% v/v	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Carbendazim 500 mg/l*	100.00a	100.00a	100.00a	0.00F	0.00F	0.00F	0.00F
CC1 1 cc/l*	54.50j-p	48.25o-s	47.25p-s	57.50h-m	54.75j-p	54.75j-p	58.00g-l
CC2 1cc/l*	23.50E	25.00C-E	25.50B-E	31.25z-E	45.25r-u	24.50DE	33.00y-C
PC1 0.5%	67.50d-f	84.50b	44.50r-w	81.50b	66.25d-g	56.00j-o	84.50b
1.0%	43.00r-x	45.00r-v	36.25w-A	49.50m-s	44.75r-v	45.75q-t	45.00r-v
1.5%	29.75z-E	33.75y-B	37.75t-z	36.75v-A	32.50z-D	36.00x-A	33.75y-B
PC2 0.5%	68.50c-e	64.50d-i	57.25i-m	76.25bc	60.00f-k	65.75d-h	72.75cd
1.0%	54.00k-q	50.75l-r	37.25u-z	62.75e-j	41.25s-y	45.50r-u	56.75i-n
1.5%	36.75v-A	34.75x-A	28.75A-E	50.00l-r	31.75z-E	33.00y-C	48.50n-s
OC1 1 cc/l*	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
OC2 0.5%	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
1.0%	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
1.5%	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
A(chitosan application)				**			
B(isolates)				**			
A*B				**			
CV %				6.51			

1/Carbendazim-resistant phenotype: Car^{HR}, Car^{MR}, Car^{S} = highly resistant, moderately resistant, sensitive, respectively.
2/Means of number of germinated conidia followed by the same letter are not significantly different by Duncan's Multiple Range Test DMRT at 1% level.
Chitosan products: CC1, CC2 = Commercial chitosan sample 1, 2; PC1, PC2 = Polymer chitosan sample 1, 2; OC1, OC2= Oligomer chitosan sample 1, 2, respectively.
*Field-use recommended concentration.

Table 5.6 The effect of various chitosan on conidial inhibition of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose for 24 hr

Treatments	Number of germinated conidia for 24 hr						
	Car^{HR}[1/]			Car^{MR}	Car^{S}		
	CAN_F095	K_F103	NDM_F116	NDM_F063	FL_F003	NDM_F006	PBL_F102
Control (sterile distilled water)	100.00a[2/]	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Acetic acid 1% (pH 5.5)	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Tween 80 0.1% v/v	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Carbendazim 500 mg/l*	100.00a	100.00a	100.00a	0.00t	0.00t	0.00t	0.00t
CC1 1 cc/l*	84.00f-l	89.75c-e	94.00bc	82.75g-l	82.25c-f	82.75g-l	80.50k-n
CC2 1cc/l*	82.25h-m	80.00k-n	84.50e-l	75.25n-q	84.50e-l	79.25l-n	70.50p-r
PC1 0.5%	99.50ab	100.00a	99.25ab	100.00a	100.00a	99.50ab	100.00a
1.0%	88.00d-g	79.25l-n	81.50i-m	90.75cd	82.25h-m	83.00g-l	84.50e-l
1.5%	80.75j-n	71.25o-r	76.75m-o	80.25k-n	75.75n-p	75.25n-q	69.75q-r
PC2 0.5%	99.25ab	100.00a	98.00ab	100.00a	100.00a	99.25ab	100.00a
1.0%	85.50d-k	80.00k-n	86.50d-I	87.25d-h	86.25d-j	83.00g-l	80.50k-n
1.5%	85.25d-k	67.75r	85.25d-k	72.50o-r	73.50o-q	79.25l-n	62.00s
OC1 1 cc/l*	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
OC2 0.5%	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
1.0%	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
1.5%	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
A(chitosan application)				**			
B(isolates)				**			
A*B				**			
CV %				3.38			

[1/]Carbendazim-resistant phenotype: Car^{HR}, Car^{MR}, Car^{S} = highly resistant, moderately resistant, sensitive, respectively.
[2/]Means of number of germinated conidia followed by the same letter are not significantly different by Duncan's Multiple Range Test DMRT at 1% level.
Chitosan products: CC1, CC2 = Commercial chitosan sample 1, 2; PC1, PC2 = Polymer chitosan sample 1, 2; OC1, OC2= Oligomer chitosan sample 1, 2, respectively.
*Field-use recommended concentration.

Experiment 2: The effect of various chitosan on mycelial inhibition

Seven isolates of carbendazim-resistant *C. gloeosporioides*, including 3 Car^{HR} isolates (CAN_F095, K_F103, NDM_F116), one isolate of Car^{MR} isolate (NDM_F063) and 3 isolates of Car^{S} isolates (FL_F003, NDM_ F006, PBL_F102) were tested with chitosan samples namely CC1, CC2 at concentration of 1 cc/l and PC1 and PC2 at concentration of 0.5, 1.0 and 1.5 % were tested the mycelia inhibition. It revealed that CC2 at 1 cc/l gave significantly highest in reduction of colony growth of isolate NDM_F063 which the colony diameter was 6.60 cm and followed by CC1 and CC2 at 1 cc/l treated to isolate NDM_F063 and isolate NDM_F116 which colony diameter were 7.22 and 7.40 cm., respectively when compared to carbendazim treatment. It observed that chitosan samples of PC1 and PC 2 at 0.5 %, 1.0 % did not significantly different when compared the acetic acid treatment and non-treated control.

With comparison to the tested carbendazim at 500 mg/l, it was completely reduced the colony growth in isolates of Car^{S} group (FL_F003, NDM_F006 and PBL_F102), and followed by the isolates of Car^{MR} (NDM_F063) as seen in Table 5.9 and Figure 5.1.

Table 5.7 The effect of various chitosan on colony diameter of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose

Treatments	colony diameter (cm)						
	Car^{HR}[1/]			Car^{MR}	Car^{S}		
	CAN_F095	K_F103	NDM_F116	NDM_F063	FL_F003	NDM_F006	PBL_F102
Control (sterile distilled water)	9.00a[2/]	9.00a	9.00a	9.00a	9.00a	9.00a	9.00a
Acetic acid 1% (pH 5.5)	9.00a	9.00a	9.00a	9.00a	9.00a	9.00a	9.00a
Carbendazim 500 mg/l*	8.20p-r	7.84u	7.84u	3.04y	0.50z	0.50z	0.50z
CC1 1 cc/l*	8.36m-o	8.70c-e	7.92tu	7.22w	8.68c-f	8.88ab	8.88ab
CC2 1cc/l*	8.10rs	8.28op	7.40v	6.60x	8.10rs	8.00st	8.08rs
PC1 0.5%	9.00a	9.00a	9.00a	8.80bc	9.00a	9.00a	9.00a
PC1 1.0%	8.72cd	9.00a	8.90ab	8.62d-h	8.66d-g	8.90ab	9.00a
PC1 1.5%	8.46j-n	8.54g-l	8.60d-i	8.36m-o	8.44k-n	8.48i-m	8.58e-j
PC2 0.5%	9.00a	9.00a	9.00a	8.86b	9.00a	9.00a	9.00a
PC2 1.0%	9.00a	9.00a	9.00a	8.60d-i	8.62d-h	8.58e-j	9.00a
PC2 1.5%	8.12q-s	8.42l-n	8.24o-q	8.14qr	8.34no	8.56f-k	8.52h-l
A(chitosan application)				**			
B(isolates)				**			
A*B				**			
CV %				0.91			

[1/]Carbendazim-resistant phenotype: Car^{HR}, Car^{MR}, Car^{S} = highly resistant, moderately resistant, sensitive, respectively.
[2/]Means of number of germinated conidia followed by the same letter are not significantly different by Duncan's Multiple Range Test DMRT at 1% level.
Chitosan products: CC1, CC2 = Commercial chitosan sample 1, 2; PC1, PC2 = Polymer chitosan sample 1, 2, respectively.
*Field-use recommended concentration.

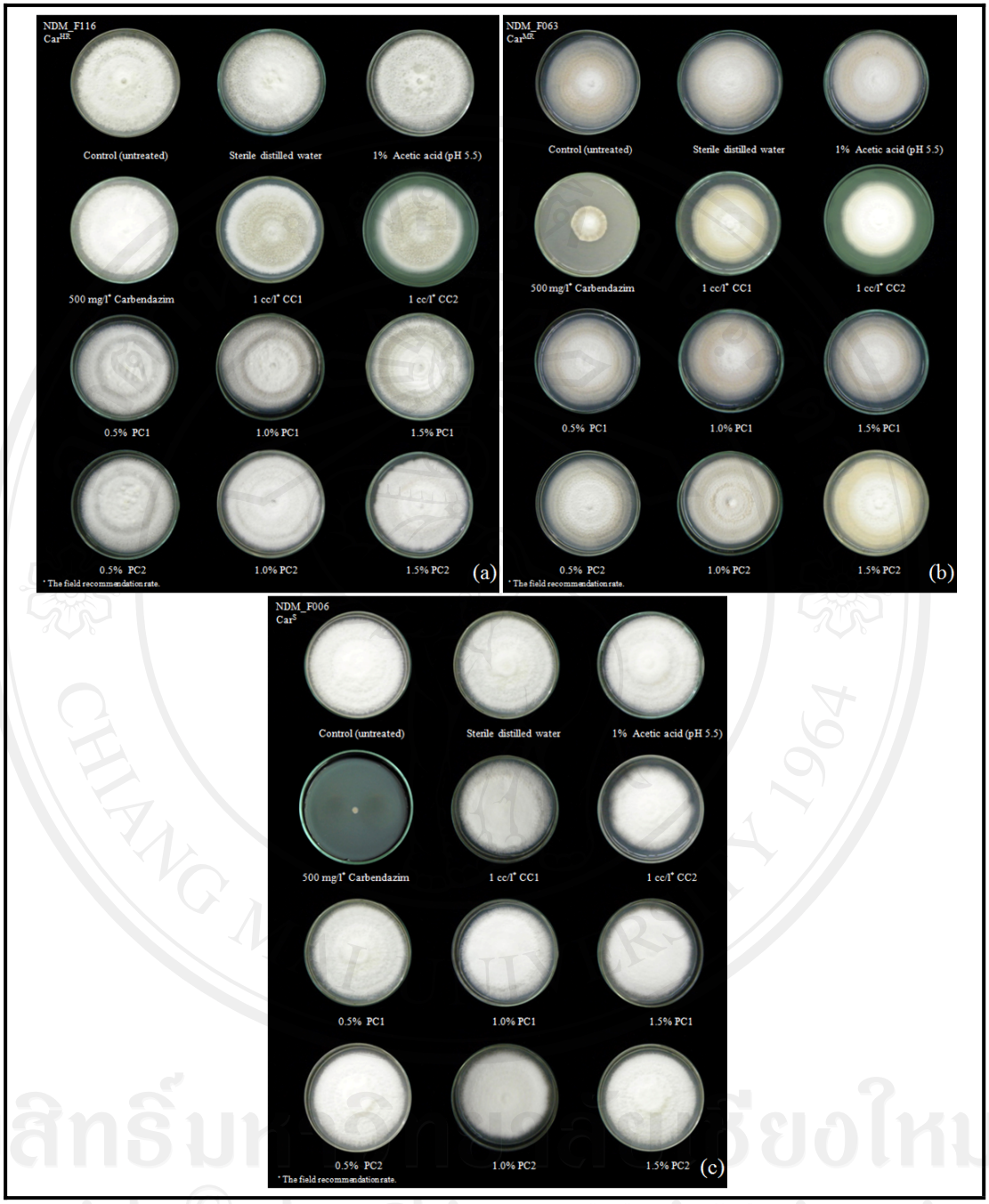


Figure 5.1 The effect of chitosan on the mycelial growth of carbendazim-resistant *Colletotrichum gloeosporioides* causing mango anthracnose. Carbendazim-resistant phenotype; highly resistant (Car^{HR}) (a), moderately resistant (Car^{MR}) (b), sensitive (Car^{S}) (c).

Experiment 3: The effect of chitosan to control anthracnose on mango fruits cv. 'Namdokmai'

Experiment 3.1 Dropping chitosan solution to control mango anthracnose

Two isolates of highly carbendazim-resistant *C. gloeosporioides* (Car^{HR}) namely CAN_F095 and NDM_F116 were tested chitosan samples namely CC1, CC2 at concentration of 1 cc/l and PC1 and PC2 at concentrations of 0.5, 1.0 and 1.5 % and OC1 at concentration of 1 cc/l to control post harvest mango cv. 'Namdokmai'.

Result showed that lesion diameter on fruits showed significantly differences (P=0.01) between chitosan treatments. There was shown a significantly interaction among treatment combination of chitosan, inoculations, and isolates.

It revealed that CC2 at 1 cc/l and PC2 at 1.5 % were treated before inoculation with isolate of CAN_F095 showing the lesion diameter of 1.30 cm which decreased disease incidence of 68.81%, and 1.36 cm which decreased disease incidence of 66.09%, respectively.

The tested PC1 and PC2 at 1.5% in the isolate of NDM_F116 gave the lesion diameter of 1.46 cm which decreased disease incidence of 62.58% and 1.50 cm which decreased disease incidence of 61.40%, respectively.

Furthermore, lesion diameter in treated 1% acetic acid (pH 5.5) was not significantly difference when compared to the control in both isolates.

The carbendazim at 500 mg/l were treated before and after inoculation with isolates of CAN_F095 and NDM_F116 that was not decreased disease incidence on fruits (Table 5.8 and Figure 5.2).

Table 5.8 Lesion diameter and decreased disease incidence of harvested mango fruits before and after inoculation with carbendazim-highly resistant *Colletotrichum gloeosporioides* for 4 day

Treatments	Lesion diameter (cm) (Decreased disease percentage)			
	CAN_F095		NDM_F116	
	Before	After	Before	After
Control (non-treated control)	0.00s	0.00s	0.00s	0.00s
Control (non inoculated and wounded)	0.50r	0.50r	0.50r	0.50r
Control (inoculated and wounded)	3.03ab[1/] (0.00)	3.10a (0.00)	3.10a (0.00)	3.16a (0.00)
1% Acetic acid (pH 5.5)	2.93ab (3.80)[2/]	3.10a (0.00)	2.90a-c (7.55)	3.00ab (2.56)
500 mg/l* Carbendazim	2.73b-d (11.62)	2.50d-f (22.91)	2.76a-d (12.73)	2.76a-d (11.74)
1 cc/l* CC1[2/]	2.03h-m (39.26)	1.96i-n (43.34)	2.10g-l (38.35)	2.06h-m (38.92)
1 cc/l* CC2	1.30q (68.81)	1.93j-n (44.72)	1.66n-p (54.99)	1.73m-o (52.05)
1 cc/l* OC 1	2.06h-m (38.18)	2.26e-j (31.94)	2.00h-n (42.15)	2.03h-m (40.21)
PC1				
0.5%	2.43d-g (23.23)	2.56c-e (20.24)	2.46d-f (24.43)	2.50d-f (21.95)
1.0%	2.46d-f (22.45)	2.33e-h (29.22)	2.30e-i (30.65)	2.20f-k (33.69)
1.5%	1.53o-q (59.45)	1.90k-n (45.95)	1.46o-q (62.58)	1.80l-o (49.28)
PC2				
0.5%	3.00ab (1.39)	2.26e-j (31.88)	3.00ab (3.70)	2.90a-c (6.51)
1.0%	2.23e-k (31.91)	2.06h-m (39.30)	2.16f-k (35.64)	2.06h-m (38.87)
1.5%	1.36pq (66.09)	2.00h-n (42.20)	1.50o-q (61.40)	1.53o-q (59.64)
A(chitosan application)	**			
B(before and after inoculation)	ns			
C(isolates)	ns			
A*B	**			
A*C	ns			
B*C	ns			
A*B*C	*			
CV (%)	8.43			

[1/]Means of lesion diameter followed by the same letter are not significantly different by Duncan's Multiple Range Test DMRT at 1% level.
[2/]Values in parentheses are means of percentage of disease decrease over control.
Chitosan products: CC1, CC2 = Commercial chitosan sample 1, 2; PC1, PC2 = Polymer chitosan sample 1, 2; OC1 = Oligomer chitosan sample 1, respectively.
*Field-use recommended concentration.

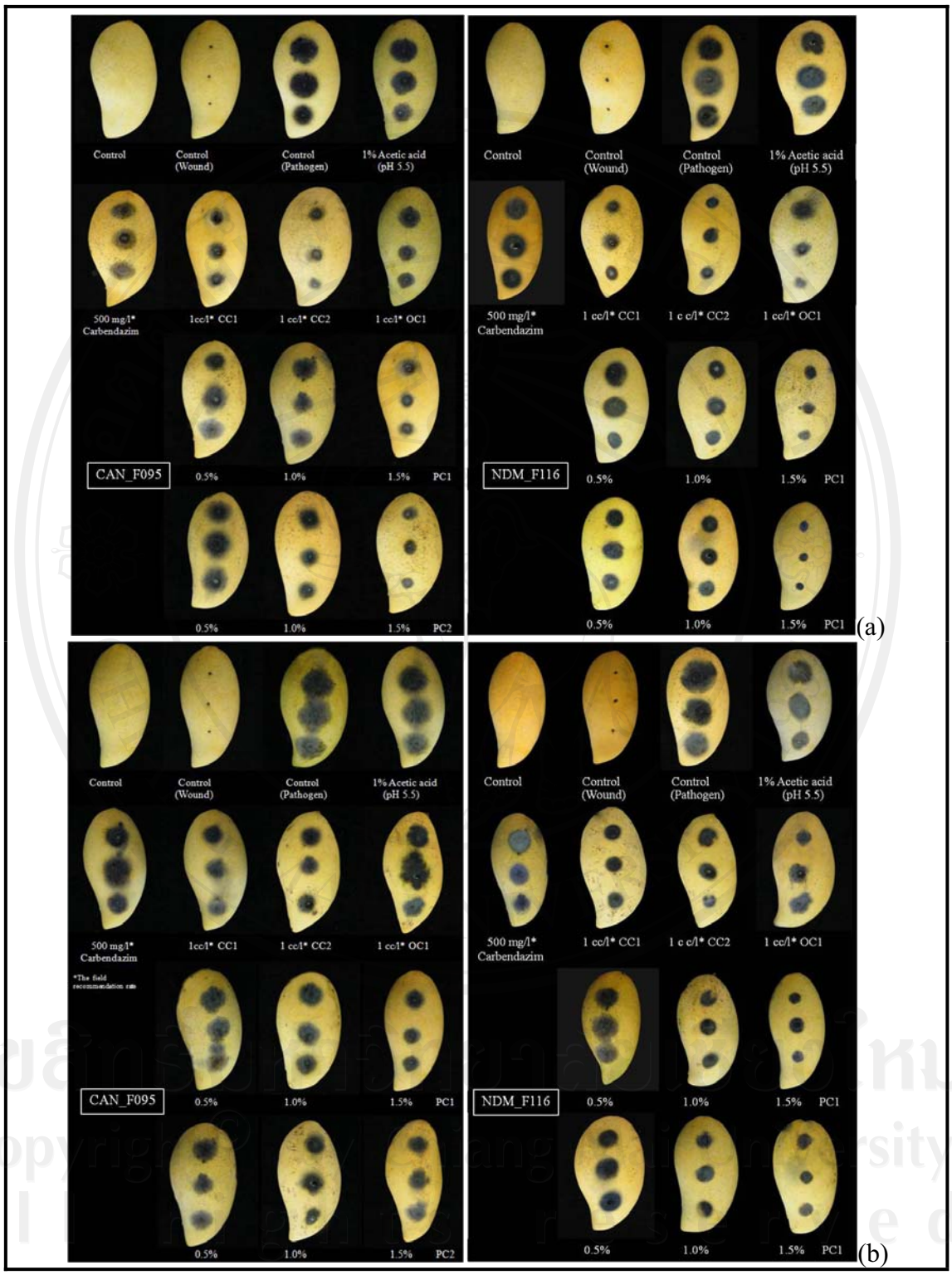


Figure 5.2 The effect of chitosan on decreasing anthracnose on mango fruits that treated; before (a) and after (b) inoculation of highly carbendazim-resistant *Colletotrichum gloeosporioides* (CAN_F095, NDM_F116).

Experiment 3.2 Spraying chitosan solution to control mango anthracnose

Two isolates of highly carbendazim-resistant *C. gloeosporioides* (Car^{HR}) namely CAN_F095 and NDM_F116 were tested chitosan samples namely CC1, CC2 at concentration of 1 cc/l and PC1 and PC2 at concentration of 0.5, 1.0 and 1.5 % and OC1 at concentration of 1 cc/l to control post harvest mango cv. 'Namdokmai'.

The result showed that disease index on mango anthracnose showed significantly differences (P=0.01) between chitosan treatments, before and after inoculations, and isolates There was shown a significantly interaction among treatment combination of chitosan, inoculations, and isolates.

Result showed that treated chitosan PC1 and PC2 at concentrations of 1.0% and 1.5% before and after inoculation with isolates of CAN_F095 and NDM_F116 and treated chitosan CC2 at concentration of 1cc/l after inoculation with isolate of CAN_F095 that expressed the lowest disease index and highly significant different when compared to inoculated control, acetic acid and carbendazim treatments (Table 5.9 and Figure 5.3).

It revealed that treated chitosan PC1 and PC2 at concentration of 1.5% before and after inoculation with isolates of CAN_F095 and NDM_F116 could reduce disease incidence of harvested mango anthracnose at 75 %. The disease reduction of harvested mango anthracnose was also shown when treated 1.0 % PC1 at before and after inoculation with isolates of CAN_F095, treated 1.0 % PC1 after inoculation with isolates of NDM_F116, treated 1.0 % PC2 after inoculation with isolates of CAN_F095, treated 1.0 % PC2 before and after inoculation with isolates of

NDM_F116, and treated chitosan 1 cc/l CC2 after inoculation with isolate of CAN_F095 could also reduce disease incidence of 75%.

The chitosan 1.0 % PC1 treatment before inoculation with isolates of NDM_F116 and 1.0 % PC2 treatment before inoculation with isolates of CAN_F095 reduced disease incidence of 66.75%.

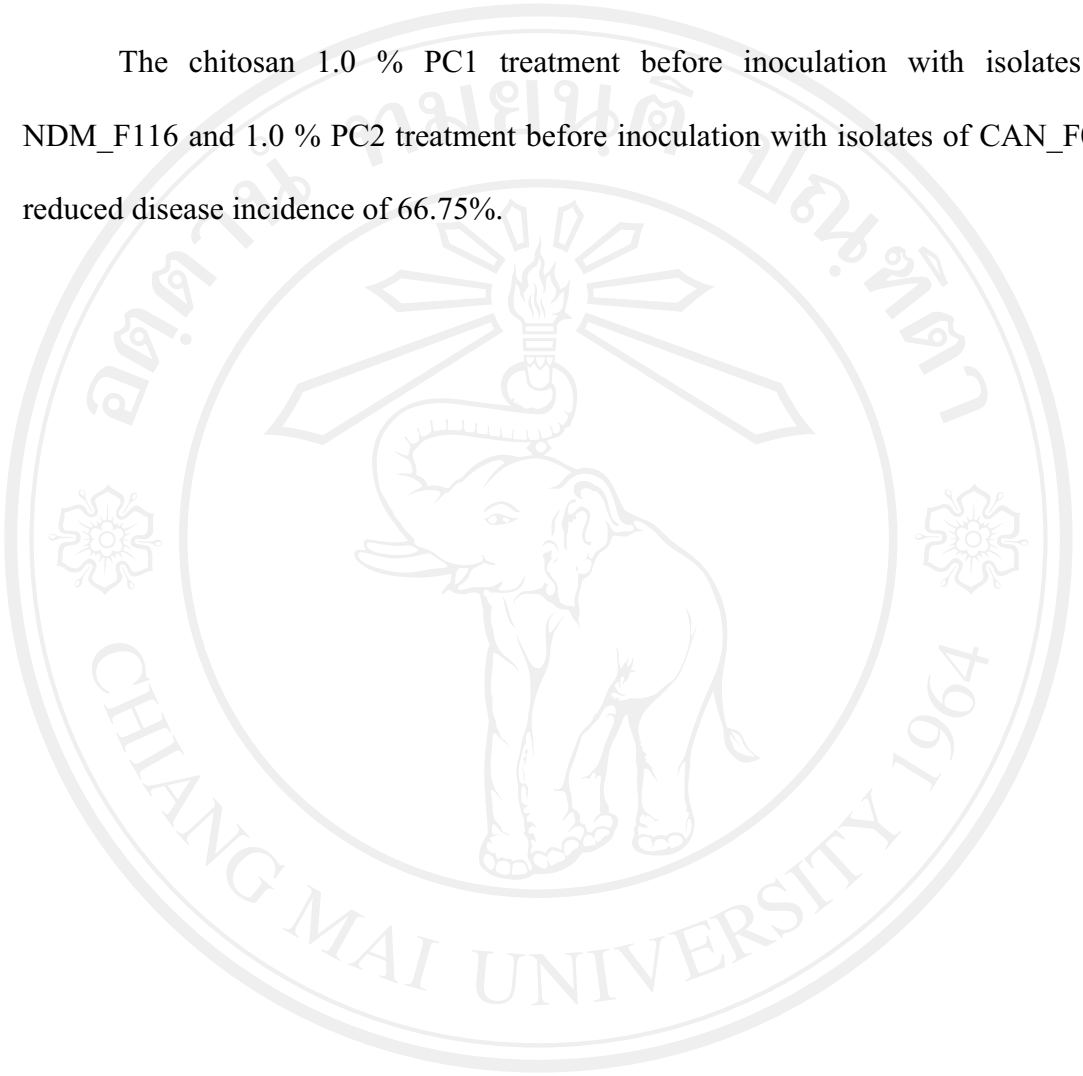


Table 5.9 Disease index and disease reduction of chitosan treated to control harvested mango anthracnose caused by *Colletotrichum gloeosporioides* for 7 days

Treatments	Disease index[1/] (Percentage of disease reduction)			
	CAN_F095		NDM_F116	
	Before	After	Before	After
Control (non-treated control)	0.00f[2/]	0.00f	0.00f	0.00f
Control (inoculated pathogen)	4.00a (0.00)	4.00a (0.00)	4.00a (0.00)	4.00a (0.00)
1% Acetic acid (pH 5.5)	4.00a (0.00)	4.00a (0.00)	4.00a (0.00)	4.00a (0.00)
500 mg/l* Carbendazim	4.00a (0.00)	3.00c (25.00)	4.00a (0.00)	4.00a (0.00)
1 cc/l* CC1[2/]	3.00c (25.00)	2.33d (41.75)	3.00c (25.00)	3.00c (25.00)
1 cc/l* CC2	2.00d (50.00)	1.00e (75.00)	2.00d (50.00)	2.00d (50.00)
1 cc/l* OC 1	3.66ab (8.50)	3.33k (16.75)	3.00c (25.00)	3.00c (25.00)
PC1				
0.5%	2.33d (41.75)	2.00d (50.00)	2.33d (41.75)	2.33d (41.75)
1.0%	1.00e (75.00)	1.00e (75.00)	1.33e (66.75)	1.00e (75.00)
1.5%	1.00e (75.00)	1.00e (75.00)	1.00e (75.00)	1.00e (75.00)
PC2				
0.5%	3.00c (25.00)	2.00d (50.00)	3.00c (25.00)	3.00c (25.00)
1.0%	1.33e (66.75)	1.00e (75.00)	1.00e (75.00)	1.00e (75.00)
1.5%	1.00e (75.00)	1.00e (75.00)	1.00e (75.00)	1.00e (75.00)
A(chitosan application)	**			
B(before and after inoculation)	**			
C(isolates)	**			
A*B	**			
A*C	**			
B*C	**			
A*B*C	**			
CV (%)	10.24			

[1/] Disease index; 0 = No disease symptom, 1>0-25% disease symptoms on fruit surface, 2>25-50% disease symptoms on fruit surface, 3>50-75% disease symptoms on fruit surface and 4>75% disease symptoms on fruit surface.
[2/] Means of disease index followed by the same letter are not significantly different by Duncan's Multiple Range Test DMRT at 1% level.
*Field-use recommended concentration.
Chitosan products: CC1, CC2 = Commercial chitosan sample 1, 2; PC1, PC2 = Polymer chitosan sample 1, 2; OC1 = Oligomer chitosan sample 1, respectively.
Disease reduction = 100-[(100* inoculated pathogen)/treated the sample]

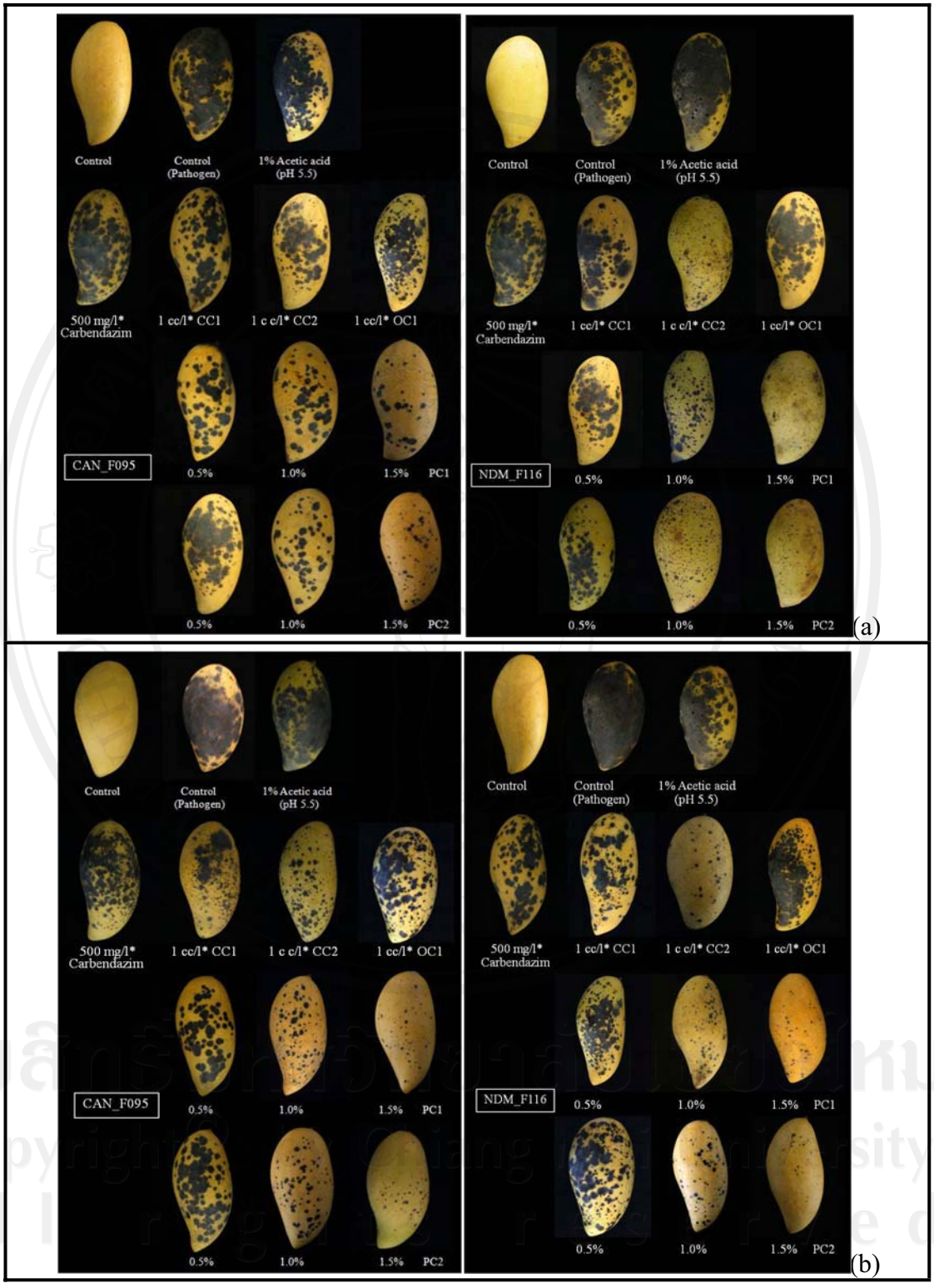


Figure 5.3 The effect of chitosan on reducing anthracnose on mango fruit when sprayed chitosan before (a) and after (b) inoculation of highly carbendazim-resistant *Colletotrichum gloeosporioides* (CAN_F095 and NDM_F116).

5.4 Discussion

Trade in mangoes makes a significant contribution to Thailand's economy, and the fruit itself is important for its nutritional and sensory qualities. Mangoes are popular in domestic and export markets because they possess excellent qualities such as attractive colour, fibre-free flesh and good taste. Mangoes are climacteric fruits and therefore, high post-harvest losses are associated ripening. Currently, the method used for reducing disease in mangoes is primarily through the application of fungicides. This chapter was investigated the possibility of alternative preservation technique by using chitosan, which is natural, non-toxic product and may become a potential product to control mango anthracnose in Thailand.

The results in this study tested three chitosan groups, including group A was commercial chitosan, CC1 and CC2 at concentration of 1 cc/l. Group B was polymer chitosan, PC1 [poly-(1,4-β-D-glucopyranosamine)] and PC2 [poly (D-glucosamine)] at concentrations of 0.5%, 1.0% and 1.5%. Group C was oligomer chitosan, OC1 (oligomer chitosan solution) at concentration of 1 cc/l and OC2 (chitosan oligosaccharide lactate) at concentrations of 0.5%, 1.0% and 1.5%. It was observed that all tested those chitosan could significantly delay in conidia germination and mycelia growth of 7 isolates of carbendazim-resistant *C. gloeosporioides* (CAN_F095, K_F103, NDM_F116, NDM_F063, FL_F003, NDM_ F006 and PBL_F102) causing mango anthracnose. This result was contradicted to the work of Sasananun *et al*., (2002) which reported that chitosan at 0.25 to 1.00% could not inhibit the spore germination of *C. gloeosporioid*es. But similar to the work of Tamthong *et al*., (2006) which reported that 0.8% chitosan showed the best in delay

the germination of *Fusarium solani*, *Sclerothium rolfsii*, *Pythium aphanidermatum* and *Macrophomena phaseolina*.

Moreover, all tested chitosan in this study could also delay mycelia growth of 7 isolates of carbendazim-resistant *C. gloeosporioide*s (CAN_F095, K_F103, NDM_F116, NDM_F063, FL_F003, NDM_ F006 and PBL_F102). This result was contradicted to the work of Luimnark (1998) which stated that chitosan at 0.50% in PDA that unable to inhibit the mycelial growth of *C. gloeosporioides* causing mango anthracnose. It is recommended that it might be different in chitosan type or tested isolates. In this regards, Abd-AllA and Haggag (2010) stated that chitosan at 0.8 mg/l could inhibit the mycelial growth of *C. gloeosporioides* by 100%, as the same result in this study. However, it is tended that the higher concentration of chitosan would possible to be higher inhibition of fungi as reports by Roller and Covill (1999), Bautista Baños *et al.* (2006) and Muñoz *et al*. (2009) who reported that in different types of chitosan but in higher concentration up to 2.5% could completely inhibit the mycelial growth of some fungi. It is suggested that the higher concentration of chitosan would be considered for further study for these tested carbendazim resistant isolates.

Moreover, the different types of chitosan application to control harvested mango anthracnose in this study showed that poly-(1, 4-β-D-glucopyranosamine) and poly (D-glucosamine) at 1.0 and 1.5% applied to harvested mango fruits cv. Namdokmai could decrease the anthracnose incidence before and after inoculation. With this, Sasananun *et al*., (2002) reported that 1.00% chitosan coated to mango fruit cv. 'Mahajanaka' could also reduce anthracnose incidence. In addition, Zhu *et al*.,

(2008) reported that 2.0% chitosan could reduce the disease incidence on mango fruits cv. 'Tainong', which was higher concentration of chitosan application in this study.

In this study, it can be explained that chitosan might act as a physical barrior against the pathogen as it coated to the harvested mango fruits in this experiment. However, Du *et al*., (1997) reported that it would be possible that treating mango fruits with polymer chitosan may form a layer of film on the outer skin surface that acts as a physical barrier, which limiting penetration of the germ tube of *C. gloeosporioides* and responded to the modification of internal CO_2, O_2 and ethylene levels of fruit.

The result in this experiment showed delay germination of conidia and hyphal growth that could delay in onset of mango anthracnose as described by Romanazzi (2010) which stated that the ability of chitosan treatment to delay fruit ripening could also enhance its resistance to pathogen infection during storage.

5.5 Conclusion

Three chitosan groups, including group A (commercial chitosan), CC1 and CC2 applied at concentration of 1 cc/l, group B (polymer chitosan), PC1 [poly-(1,4-β-D-glucopyranosamine)] and PC2 [poly (D-glucosamine)] at concentrations of 0.5%, 1.0% and 1.5% and group C (oligomer chitosan), OC1 (oligomer chitosan) applied at concentration of 1 cc/l and OC2 (chitosan oligosaccharide lactate) applied at concentration of 0.5%, 1.0% and 1.5% could significantly delay in conidial germination of 7 carbendazim resistant isolates of *C. gloeosporioides* (CAN_F095, K_F103, NDM_F116, NDM_F063, FL_F003, NDM_ F006 and PBL_F102) when compared to the non-treated one, acetic acid, tween 80 and carbendazim.

Moreover, CC1 and CC2 at concentrations of 1 cc/l, and PC1 and PC2 at 0.5%, 1.0% and 1.5% could also delay mycelia growth of 7 carbendazim resistant isolates of *C. gloeosporioides* (CAN_F095, K_F103, NDM_F116, NDM_F063, FL_F003, NDM_ F006 and PBL_F102).

The application of chitosan CC2 at concentration of 1 cc/l onto wounded mango fruits 15 min before inoculation of isolate CAN_F095 at $1x10^6$ spore/ml decreased anthracnose incidence of 68.81%, PC2 at concentration of 1.5 % with isolates CAN_F095 and NDM_116 decreased anthracnose incidence of 66.09 % and 61.40 %, respectively, PC1 at concentration of 1.5 % with isolate NDM_116 decreased anthracnose incidence of 62.58 %.

Spraying chitosan to un-wounded mango fruits at 15 min before inoculation with carbendazim resistant isolates at $1x10^6$ spore/ml, it concluded that chitosan 1.5 % PC1 and PC2 could protect the inoculated isolates of CAN_F095 and NDM_F116 as shown disease reduction of 75 %. It was also shown disease reduction of 75 % when sprayed with 1.0 % PC1 against the inoculated isolate of CAN_F095 and sprayed 1.0 % PC2 against the inoculated isolate of NDM_F116. Moreover, chitosan 1.0 % PC1 treatment inhibited the isolate of NDM_F116 for infection to mango fruits and the treated 1.0 % PC2 also inhibited the isolate of CAN_F095 to infect on mango fruits as the disease reduction of 66.75%.

Morover, after inoculation of carbendazim resistant isolates at $1x10^6$ spore/ml for 24 hr and then sprayed chitosan to un-wounded mango fruits, it revealed that 1.5 % PC1 and PC2 treatments could inhibit the isolates of CAN_F095 and NDM_F116 to infect mango fruits as the disease reduction of 75 %. The disease reduction was also shown when sprayed 1.0 % PC1 and 1 cc/l CC2 against inoculated isolates of

CAN_F095 and sprayed 1.0 % PC2 against the inoculated isolates of NDM_F116 as 75 %. The research findings suggested that chitosan may become a potent natural substance to protect post-harvest anthracnose of mango and it is further possible to apply in the field.