New Safe Methods for Controlling Anthracnose Disease of Mango (*Mangifera indica L.*) Fruits Caused by *Colletotrichum gloeosporioides* (Penz.)

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Abstract. Mango suffers from several diseases at all stages of its life. Anthracnose, caused by the fungus *Colletotrichum gleosporioides* is the most important post harvest disease of mango. The effect of various concentrations of chitosan solution on the mycelium growth and spore germination of *Colletotrichum gloeosporioides* (Penz.) the causal agent of anthracnose disease of mango fruits was studied under vitro conditions. Chitosan solution at 0.6mg/l obtained significantly reduction of *C. gloeosporioides* growth and inhibited spore germination, while, chitosan solution at 0.8mg/l resulted a complete reduction and inhibition of fungal mycelium growth and spore germination. Meanwhile, coating mango fruits with 0.2 and 0.4% (w/v) chitosan solution obtained a highly protective effect against anthracnose disease incidence of mango fruits, by 98.1% and 95.4% after 30 days of storage, respectively. At the same treatments were reducing the percentage of fruit rotted tissues by 89.3 and 95.0%, respectively. The results of this study showed that chitosan was a alternative safe coating method for prevent mango fruits against anthracnose disease which causes economic losses during transportation, marketing and storage. [Journal of American Science. 2011;7(1):80-86]. (ISSN: 1545-1003).

Key words: Chitosan – Mango fruits – Anthracnose disease –*Colletotrichum gloeosporioides.*

1-Introduction

Mango (*Mangifera indica* L.) is considered one of the most popular fruits among millions of people in the tropical area and increasingly in the developed countries (FAO STAT, 2005). Mango fruits are sensitivity to decay, low temperature and general fruit perish ability due to the rapid ripening and softening limits the storage, handling and transport potential.

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc., is the major postharvest disease of mango in all mango producing areas of the world (Dodd *et al*., 1997) and (Swart *et al*., 2002). The disease occurs as quiescent infections on immature fruit and the damage it incites is more important in the postharvest period (Muirhead and Gratitude, 1986; Dodd *et al*., 1997). Fungicides, either as preharvest or postharvest treatments, form the main approach to reduce losses from anthracnose. However, their use is increasingly restricted due to public concerns over toxic residues. Moreover, fungicides are unaffordable for many mango growers in developing countries (Dodd *et al*., 1989).

Figure 1, Anthracnose disease symptoms on mango fruits

Synthetic fungicides are the primary means to control postharvest diseases (Eckert,1990 and 1991). They are used alone, combined in mixtures, or applied separately in sequence (Ismail and Zhang,2004). However, several fungicides have been removed from the market due to possible toxicological risks. In addition, repeated use of certain systemic fungicides in packinghouses has led to the appearance of fungicide-resistant pathogens. Over recent decades there has been increasing public pressure to reduce the use of synthetic fungicides in agriculture products and their presence in the environment. Moreover, concerns have been raised

about the health risk involved in the use of synthetic fungicides on fresh fruits and vegetables shortly before consumption. Chitosan (poly-β-(1→4)*N*-acetyl-d-glucosamine) derived from the outer shell of crustaceans, has become a promising alternative treatment due to its natural character, antifungal activity, and elicitation of defense responses in plant tissue (Terry and Joyce, 2004). Some research results have indicated that chitosan can inhibit the growth of *Puccinia arachidis* Speg. (Sathiyabama and Balasubramanian, 1998), *Alternaria alternata* (Fr.) (Reddy *et al.*, 1998) and *Aspergillus niger* V. Tiegh. (Plascencia- Jatomea *et al.*, 2003). Coating citrus fruit with chitosan was effective in controlling fruit decay caused by *Penicillium digitatum* Sacc. And *Penicillium expansum* Link (Chien *et al.*, 2007) and rots including gray mould and blue mould caused by *B. cinerea* and *P. expansum* in sweet cherry fruit were reduced by preharvest spraying or postharvest dipping of chitosan (Romanazzi *et al.*, 2003). The objectives of this study were to investigate the effects of chitosan on the control of anthracnose disease caused by *Colletotrichum gloeosporioides* in mango fruits, as well as to evaluate the antifungal activity of chitosan against pathogen *in vitro*, and their effect of disease incidence on mango fruits.

2-Materials and methods

2.1. Fruits

Mango (*Mangifera indica* L.) fruits Sanara cv. were harvested at the mature stage, and sorted based on size and the absence of physical injuries or disease infection. Before treatments, fruit were surfaced disinfected with 2% sodium hypochlorite for 3 min, then rinsed with tap water, and air-dried.

2.2. Pathogen culture

Colletotrichum gloeosporioides was cultured for 1–2 weeks on potato dextrose agar (PDA) at 25 ◦C. The isolate used was obtained from infected mango fruit in Egypt. Spores were harvested by adding 3–4 ml of sterile, de-ionized water (diH2O) to the Petri dish. The spores were then rubbed with a sterile glass rod to free them from the PDA medium, and the spore suspension was passed through two layers of cheese cloth. The suspension was diluted with water to obtain the spore concentrations (10^6 spores ml^{-1}) according to determination with a haemocytometer.

2.3. Chitosan solution preparation

Crab-shell chitosan, purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.), was ground to a fine powder (particle size smaller than 1 mm) by extensive grinding in a mortar, washed 3 times in distilled water (20 ml of water per g of chitosan), pelleted by low-speed centrifugation and air-dried at room temperature. The purified chitosan was prepared as described by Benhamou *et al.*, (1998). For experimental use the stock solution, (1%, w/v) of chitosan, was prepared by dissolving purified chitosan in 0.5% (v/v) glacial acetic acid (Du *et al.*,1998), under continuous stirring, and the pH was adjusted to 5.6 using 1 N NaOH.

2.4. Effect of different chitosan concentrations on linear growth of *C. gloeosporioides*

Different concentrations of chitosan solution prepared by the method described by El-Gaouth (1992). chitosan solution was added to conical flasks containing melted PDA medium to obtain final concentrations of 0.0, 0.2, 0.4, 0.6 and 0.8 mg/l and mixed gently and then dispensed in sterilized Petri plates (10 cm diameter).Plates were individually inoculated at the center with equal disks (10-mm-diameter) of the same physiological age of each *C. gloeosporioides***,** then incubated at 22-25°C. The average linear growth of fungi tested was calculated.

2.5.Effect of different chitosan concentrations on spore germination of pathogenic fungi

Conidia of 10 days old of *C. gloeosporioides* cultures were harvested in sterilized water containing 0.1% (Tween 80), aliquots of spore suspension (10^6 spore/ml) were inserted into plates containing different concentrations of chitosan. *i.e.*, 0.0, 0.2, 0.4, 0.6 and 0.8 mg/l and then PDA medium were poured into the plates before solidification. Four plates as replicates were used for each treatment. Inoculated plates were incubated at 22-25°C for 48hr. Spore germination was determined microscopically and the percent of germinated spores was calculated.

2.5.Effect of chitosan coating on anthracnose diseases incidence of mango fruits

Fresh mango fruits cv. Cenara apparently free from physical damage and diseases were used in this experiment. Fruits were surface disinfected with sodium hypocholorite (5%) and washed several times with sterilized water, fruits were gently injured with sterilized needle. Fruits were dipped in 0.05 , 0.1 ,0.2 and 0.4 % (w/v) chitosan solutions. Control fruits were dipped in sterilized water. The coated and control (uncoated) fruits were air dried for 2hr in laminar flow. Inoculation of fruits was carried out by spraying them individually with spore suspension (10^6 spore/ ml) of *C. gloeosporioides*.Coated-inoculated fruits were stored at 10°C for 30 days.

Mango fruits were examined daily for disease assessment. Each treatment was represented by 5 replicates with 10 fruits of each were used. Each experiment was repeated three times.

2.6. Disease assessment

Percentage of diseased fruits was recorded after3, 7,15 and 30 days of storage. Fresh weight of rotted tissue part and its percentage were recorded and calculated after 30 days of storage.

Statistical analysis

Tukey test for multiple comparisons among means was utilized (Neler *et al*.,1985)

3. Results

Results in Table (1) indicate that all chitosan concentrations reduced linear growth of tested fungus. Complete inhibition was obtained by 0.8m g/l and 76.6% linear growth reduction at 0.6mg/l and 54.1% fungal growth reduction at 0.4mg/l as compared with control treatment. Meanwhile, concentration of 0.2m g/l of chitosan was less effective.

Chitosan solutions in 4 concentrations *i.e*, 0.2, 0.4, 0.6 and 0.8 mg/l were tested for their inhibitory effect on *C. gloeosporioides* linear growth.

Chitosan solutions in 4 concentrations *i.e*. 0.2, 0.4, 0.6 and 0.8 mg/l were tested for their inhibitory effect on *C. gloeosporioides* spores germination.

Results in Table (2) indicate that spore germination of pathogenic fungus was inhibited by all tested chitosan concentrations. The inhibitor effect increasing with increased chitosan concentrations. The most effective concentration was 0.8mg/l, where a complete inhibition of *C. gloeosporioides* spore germination, meanwhile, it was 44.5% inhibition at 0.2mg/l of chitosan solution.

Table 1, Effect of different concentrations of chitosan on linear growth (mm) of *C. gloeosporioides.*

Chitosan concentration (mg/l)	*C. gloeosporioides.*	
	linear growth (mm)	Reduction (%)
0.2	56.5 b	37.2
0.4	41.3 c	54.1
0.6	21.0 d	76.6
0.8	0.0 e	100.0
Control (0.0)	90.0 a	----

Table 2, Effect of different concentrations of chitosan on percentage of conidia germination of *C. gloeosporioides.*

Chitosan concentration (mg/l)	*C. gloeosporioides.*	
	Conidia Germination (%)	Reduction (%)
0.2	51.3 b	44.5
0.4	31.4 c	66.0
0.6	17.8 d	80.7
0.8	0.0 e	100.0
Control (0.0)	92.5 a	----

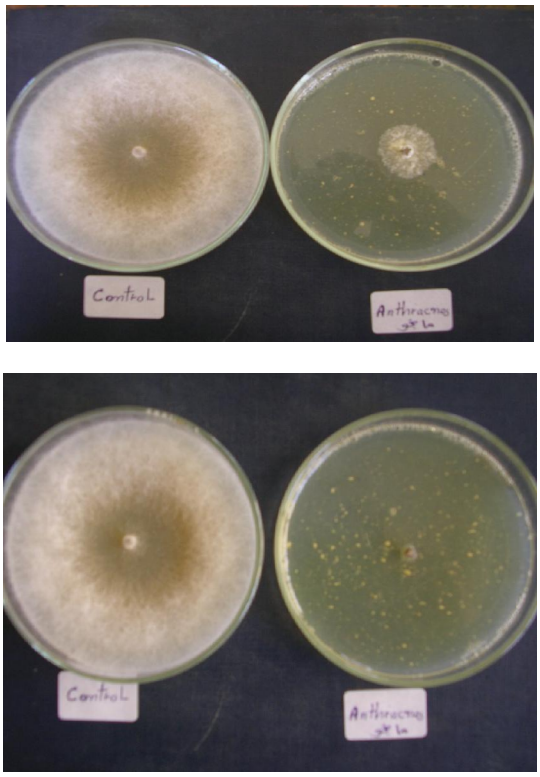

Figure 2, Effect of different concentrations of chitosan on linear growth (mm) and spore germination of *C. gloeosporioides* : 0.6mg/l (up) and 0.8mg/l (down).
Figures with the same letter in the same column are not significantly different (P=0.05). Figures with the same letter in the same column are not significantly different (P=0.05).

Results in Table (3) indicate that all concentrations tested of chitosan significantly reduced the of anthracnose diseases incidence. The percentage of decayed fruits increased by prolonging the storage period, reaching its maximum after 30 days. Chitosan concentrations of 0.05 and 0.1%

resulted in the highest reduction in disease incidence of mango fruits. At2.0% chitosan solution showed a complete protective effect of mango fruits against anthracnose incidence during 30day of storage, and after 30days of storage gave 85% reduction of disease incidence. Meanwhile, at4.0% chitosan solution showed a highly protective effect after 30days by 95.4% reduction of disease incidence, and 92.1% at the end of storage.

Results in Table (4) indicate that all concentrations tested of chitosan significantly reduced the percentage of mango fruits rotted tissue.

On the other hand, Fruits coated with chitosan solution at0.2 % and 0.4 % gave a strongly reduction of the percentage of mango fruit rotted tissue by 89.3 % and 95.0% , respectively, followed by fruit treated with chitosan solution at1.0% by 50.5 % reduction of fruit rotted tissue . At low concentration 0.5 % resulting less effect for reducing the percentage of fruit rotted tissue.

Table 3: Effect of chitosan coating on anthracnose disease incidence of mango fruits during storage period.

Chitosan Concentration (%)	Fruit rotted tissue (%)	
	Fresh weight of rotted tissue (%)	Reduction (%)
0.05	33.3 b	34.1
0.1	25.0 b	50.5
0.2	5.4 c	89.3
0.4	2.5 d	95.0
Control (0.0)	50.6 a	-----

Figures with the same letter are not significantly different (P=0.05).

Table 4, Effect of chitosan on Fruit rotted tissue of diseased mango caused by *C. gloeosporioides* after 30 days of storage.

Chitosan Concentration (w/v) %	Anthracnose disease incidence %			
	Storage period (days)			
	3	7	15	30
0.05	10.0 b	20.4 b	25.3 b	44.5 b
0.1	6.4 c	6.0 c	8.1 c	14.4 c
0.2	0.0 d	0.0 d	0.0 d	13.5c
0.4	0.0 d	0.0 d	4.1 d	7.1 d
Control (0.0)	15.8a	51.2a	60.0a	90.5a

Figures with the same letter are not significantly different (P=0.05).

Figure 3, Effect of chitosan coating on anthracnose disease incidence of mango fruits cv.canara during storage period.

4. Discussion

Among natural elicitor compounds, chitosan offers a great potential as a biodegradable substance that has antimicrobial and eliciting activities (Benhamou , 1996). Previous studies have shown that chitosan reduces decay incidence, mainly caused by *B.cinerea* in tomato fruits. (El-Ghaouth *et al*., 1992).The results of the present experiments showed that, all tested chitosan concentrations effective to reduced the linear growth and inhibited spore germination of *C. gloeosporioides* , but complete inhibition was obtained by used 0.8mg/l chitosan under vitro experiments. Meanwhile, at 0.4 and 0.6 mg/l , shown medium effect for reducing fungal mycelium growth and spore germination if compared with control treatments under the same conditions, These results were confirmed with Pongphen *et al*., 2007 , found that, the effects of chitosan on mycelial growth and spore germination of *Colletotrichum gloeosporioides* were investigated on potato dextrose agar (PDA) containing 0%, 0.5%, 1.0%, 1.5%, and 2.0% (w/v) chitosan dissolved in 0.5% acetic acid, the highest concentrations of chitosan, at 1.5% and 2.0%, were more inhibitory effect of fungal mycelia growth and spore germination than the lower concentrations (0.5% and 1.0%). Hewajulige *et al*.,2006 and 2009, reported that , chitosan solution at 0.1 % and above was inhibited effect to the radial mycelium growth and spore germination for *C. gloeosporioides* the causal agent of anthracnose disease on papaya var.Rathna during storage , at 0.1% chitosan under vitro conditions, a complete inhibition of radial mycelium growth and spore germination of the pathogen. Recently, two models have been proposed to explain the antifungal activity of chitosan, according to Leuba and Stossel ,1986, the activity of chitosan is related to its ability to interfere with the plasma membrane function. In the model of Hadwiger and Loschke,1981, the interaction of chitosan with fungal DNA and mRNA is the basis of its antifungal effect. In this study, mango fruits were coated with chitosan solution at 0.2% and 0.4% resulted in the highest reduction in anthracnose disease incidence of mango fruits and also the above treatments showed reducing the percentage of fruit rotted tissues during all storage periods. Complete protective effect was obtained when mango fruits were coated with 0.2% chitosan during 30 days of storage, while fruits coated with 0.4% chitosan showed a highly protective effect and reduction of disease incidence by 92.1% and 95.0% and reducing the percentage of fruit rotted tissues during all storage periods, these results agreement with, Zhu *et al*., 2008, reported that, disease progress in the mango fruits inoculated with *Colletotrichum gloeosporioides* was significantly inhibited by the treatment with chitosan coating. The disease incidence and lesion diameter in the fruits treated with 2.0% chitosan were 71.3 and 49.8% lower than that in the control fruits after 4 and 16 days of inoculation, respectively. Pongphen *et al*., 2007 reported that,chitosan treatment (0.5%, 1.0%, 1.5%, and 2.0%) of mangoes (*Mangifera indica*) previously inoculated with *C. gloeosporioides* resulted in a lower rate of disease progression compared with the controls and added that chitosan concentrations of 0.5% and 1.0% had stimulatory effects on chitinase and β-1,3-glucanase activities. Nadeem *et al*., 2009, found that, the decay controls of irradiated chitosan on mango fruits was better as compared with uncoated fruits. Chitosan treated fruit inhibited the growth of a wide variety of bacteria and fungi as compared to the control treatments. Various defense responses in several fruit have been induced, including the elicitation of phenylalanine ammonia lyase (PAL) activity in grape berries (Romanazzi *et al*., 2002), and chitinase and β_-1,3- glucanase in oranges, strawberries and raspberries (Fajardo *et al*., 1998; Zhang and Quantick, 1998). Jiang *et al.* (2005) observed an increase in anthocyanin levels in chitosan-coated fruit already after 6 hours of storage. Jiang and Li (2001) who investigated that, 1%, 2% and 3% chitosan coating on the activity of polyphenol oxidase on the third and sixth day of

storage of lychee fruit, Jiang *et al*. (2005) confirmed chitosan's inhibitory effect when analyzing the influence of 2% chitosan coating on the activity of polyphenol oxidase in lychee fruit stored at 25 °C. Pen and Jiang (2003) also noted that chitosan's inhibitory effect on polyphenol oxidase increases at higher concentrations of the chitosan solution which is applied to coat food products. The present study shows that chitosan, as a natural substance, could directly inhibit the growth of *Colletotrichum gloeosporioides in vitro* and potently induce defense reactions in mango fruits. This suggests that chitosan improves resistance of mango fruits against anthracnose disease and suggests that chitosan is promising as a natural fungicide to partially substitute for the utilization of synthetic fungicides in fruit and vegetables.

Acknowledgments
This manuscript funded from the project "New applied approaches to promote productivity and Quality of some fruit crops (Mango)" National Research Centre, 2007 to 2010.

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7/5/2010