Effect of Chitosan Coating on Postharvest Life of Papaya (*Carica papaya* L.) var. Rathna Grown in Sri Lanka

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ABSTRACT. *The potential use of chitosan, as an antifungal agent to reduce anthracnose disease on papaya var. Rathna during storage was investigated. Chitin extracted from locally available prawn waste was used to prepare N,O- Carboxymethyl chitosan. Effective concentration of chitosan formulation to inhibit the radial mycelial growth and spore germination was selected via a series of experiments on potato dextrose agar. Finally the selected chitosan concentration was tested on papaya var. Rathna. In-vitro studies revealed complete inhibition on radial mycelial growth and spore germination of the pathogen at a treatment of 1.0% chitosan and above. In-vivo study using 1.0% chitosan significantly reduced both disease incidence and severity on papaya fruit. Significant changes were observed in chitosan treated fruits with respect to fruit firmness, rate of respiration and CO_2 concentration in the internal cavity of the fruits while other physicochemical and organoleptic characters of the fruits were not affected. Chitosan at a concentration of 1.0% showed improved fruit firmness after ripening, and improved the keeping quality reaching 70-80% marketability throughout the storage period of 14 days at 13.5^{0}C and 95% relative humidity followed by two days at ambient temperature (28^{0}C±2).*

INTRODUCTION

Papaya (*Carica papaya* L.) is amongst the four most popular fruit crops in Sri Lanka with a potential for increased production to service lucrative export markets. Postharvest diseases are major cause for postharvest losses of this commodity during storage and transportation. The causal organism of papaya anthracnose is *Colletotrichum gloeosporioides* (Bolkan *et al*., 1976). Symptoms of the disease are known to be particularly destructive once ripening has been initiated. Anthracnose in papaya can be controlled by fungicide application, hot-water-dip treatment at 43-49^{0}C for 20 min (Couey *et al*., 1984) and hot-water-dip treatment in combination with fungicides (Couey and Farias, 1979). However, hot-water-dip treatment affects the ripening process in papaya (Paull, 1990). Use of fungicides for extended periods may lead to the emergence of fungicide-resistant strains and the hazard due to fungicide residues left on fruit surface. Hence, there is an urgent need for developing an effective alternative method of controlling this pathogen.

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Chitosan, which is a natural coating, derived from crab or prawn shell after deacetylation of chitin. It is the second most abundant naturally available, easily degradable biopolymer. Chitosan is known to form a semi permeable film, which modifies the internal atmosphere of the fruit and decreases loss of moisture due to transpiration. It is also known to induce defense mechanisms, which delay the ripening -process and lower respiration rates. It also acts as an anti-fungal agent and helps to retain the quality of fruit during storage (Zhang and Peter, 1997). In fact, chitosan is an ideal preservative coating for fresh fruits and vegetables because of its film forming and biochemical properties. Its ability to prolong the storage life and control decay in temperate fruits such as strawberries (El-Ghaouth *et al*., 1991), litchi (Zhang and Peter, 1997) and apples (Du *et al*., 1998) has been observed. The objective of this study was to examine the possibility of using chitosan, as an antifungal agent to reduce the incidence and severity of anthracnose during storage and transportation of papaya.

MATERIALS AND METHODS

Synthesis of chitosan

Chitin from locally available head and shell waste of farm shrimp (*Penaeus monodon)* and lagoon shrimp (*Penaeus indicus*) was extracted by deproteinization with 5.0% NaOH and demineralization with 2.0% HCl. Extractions were done in triplicate. After extraction the chitin was deacetylated with 50% NaOH to form chitosan and purified chitosan was prepared as described by Benhamou *et al*. (1994). Chitosan was ground into a fine powder (particle size - 0.5 mm) and 0.1% to 3.0% chitosan solutions were prepared by dispersing 0.1, 0.3, 0.5, 1.0, 2.0 g and 3.0 g of the powder separately in 100 ml of 10% acetic acid. The pH of the solution was adjusted to 5.6 with 50% NaOH and homogenized at 6000 rpm for 5 min.

Effect of chitosan on radial mycelial growth of *Colletotrichum gloeosporioides*

The fungus *C. gloeosporioides* was isolated from diseased papaya fruit. Pure cultures were maintained on Potato Dextrose Agar (PDA) at 25^0C. A mycelial disc (0.9 cm, diameter) was cut from the peripheral region of a 5 days old culture of *C. gloeosporioides* grown on PDA and transferred to the centre of a 9 cm diameter, PDA plate, which had been amended by incorporating the chitosan aqueous solution at concentrations ranging from 0.1-3.0% separately into the medium at 50^0C before plating. Growth was assessed after 10 days by measuring the colony diameter. This study was conducted in Completely Randomised Design (CRD) with ten replicates and repeated twice to confirm the results. Data were analyzed by analysis of variance and mean separation was done by Duncan's Multiple Range Test (DMRT) at $p<0.05$. Plates incorporated with sterile distilled water served as control. Radial growth reduction was calculated in relation to growth of the control.

Effect of chitosan on spore germination of *Colletotrichum gloeosporioides*

Cellulose membranes comprising 2 cm^2 pieces were cut from dialysis tubing (Medicell International Ltd., UK.) The pieces were boiled in distilled water for 5 min to remove the surface coating. The pieces were subsequently washed with distilled water and placed on a glass slide in a petri dish (one piece per dish), lined with a tissue paper. This

setup was sterilized by autoclaving for 15 min at 121^{0}C and 15 psi after which the setup was allowed to reach room temperature (28^{0}C). The tissue paper line was moistened with 4 ml of sterile distilled water. A 10^{3}spores/ml spore suspension was made in 3.0% glucose as described by Sivakumar *et al.* (2002). The conidia suspension (0.1 ml) was pipetted onto each membrane and samples were air dried in the laboratory at 28^{0}C for 30-60 min. The tested chitosan (0.1 ml) was introduced into the inoculated membranes at the required concentration and incubated at 28^{0}C for 24 h. Inoculated membranes treated with sterile distilled water (0.1 ml) served as the control. The cellulose membranes were removed from the Petri dishes at the end of the incubation period and observed for conidial germination, using an Olympus CH2 light microscope at x100 magnification. Three hundred spores were randomly counted and the number that had germinated was recorded when the germ tube had exceeded half the length of the spore. The experiment was conduced in CRD with three replicates and repeated twice. Data were analyzed by analysis of variance and mean separation was done by DMRT at $p<0.05$.

Effect of chitosan on papaya inoculated with *Colletotrichum gloeosporioides*

Papaya fruits (var. Rathna) were picked from a commercial cultivation in Puttalam and transported to Industrial Technology Institute (ITI) laboratory on the same day. A set of 40 fruits at color index 2 (10% yellow stage) were surface sterilized with 70% ethanol and wounded with a No 05 cork borer (0.9 cm) to a depth of 3 mm on the fruit surface. The wound was inoculated with 0.2 ml of conidial suspension (10^3 conidia/ml) of the pathogen and incubated for 3 h at 28^{0}C and 95% relative humidity (RH). At the end of the incubation period, 20 of the inoculated fruit were then dipped in 4 l of 1.0% chitosan formulation for 5 min. A set of 20 inoculated fruit dipped in sterile distilled water for 5 min served as control. Fruits were allowed to air-dry and held at 13.5^{0}C and 95% RH for 10 days. At the end of the storage period, disease severity was assessed by measuring the lesion diameter in centimeters. The inoculated lesions were then cut and removed from the healthy margin of the fruit and homogenized in sterile distilled water. The preparation was serially diluted and 0.1 ml of each dilution was plated on PDA by the spread plate method. The plates were incubated at 28^{0}C for 8-9 days and observed for growth of *C. gloeosporioides*. This experiment was repeated twice for the reproducibility of results. CRD was approved and data were analyzed by DMRT, at $p<0.05$.

Effect of chitosan on papaya fruit

A set of papaya fruits (var Rathna) was harvested at color index 2 (10% yellow stage) from commercial cultivation in Puttalam, Sri Lanka and transported to the ITI laboratory on the same day. Twenty fruits were then dipped in 4 l of 1.0% chitosan formulation for 5 min, and fruits were allowed to air-dry for 5 min at 28^{0}C. Fruits treated with sterile distilled water served as the control. The treated and untreated fruits were then lined with Styrofoam netting and placed vertically (stem end facing down) in corrugated fiberboard carton separately. Each carton consisted of 10 fruits. The packed fruits were stored at 13.5^{0}C and 95% RH for 14 days. The experiment was repeated twice with 20 replicates. Fruits were removed from cold storage at the completion of the storage period, and held at 25^{0}C for 48 h. Then the anthracnose incidences were assessed by the ratio of fruit showing disease symptoms on the surface to the total number of fruits stored in the commercial packaging. Disease severity was evaluated by measuring the lesion diameter (Sivakumar *et al*., 2002). Water loss from the fruit was calculated as percentage of initial

weight. Overall quality was assessed according to the following score based on disease severity and color; 1-2 = fruits not marketable; 3 = poor quality, limited marketability; 4-5 = fair quality, and marketable; 6-7 = good quality, marketable; 8-9 = excellent quality (Sivakumar *et al*., 2002). Fruit firmness was measured with a Chatillon Penetrometer. The flesh color of the fruit was determined using a Minolta chromameter on color space L* a* b* and hue angle (Hº) was calculated with a* and b* using the formula, Hº = arctan b*/a*. Total soluble solid (ºBrix) content was measured using a hand held refractometer (Erma, Japan; Scale 0-25%). Organoleptic characters were evaluated by a trained tasting panel, based on the following score 1-3 = Poor; 4-5 = Fair; 6-8 = Good; 9-10 = Excellent. Treatments were arranged in a CRD in low temperature storage with 20 replicates per treatment. CO_2 concentration in the internal cavity of ten randomly selected fruits from each treatment was determined by gas chromatography (Shimadzu, GC-9A). Respiration study of papaya was conducted using a closed system at 13.5^0C. Papaya treated with 1.0% chitosan (10 fruit per treatment) and untreated control fruit were placed separately in respiration chambers (volume of jar-30 l) made from Perspex glass with a sealable lid. Chambers were kept closed for 2 h and gas samples were drawn using airtight 5 ml glass syringe. Gas samples were analyzed for CO_2 concentration via Gas Chromatography (GC-9A, Shimadzu, Japan) equipped with Porapack Q-180/100 column and thermal conductivity detector. The respiration rate was expressed as ml CO_2/kg (fresh weight)/h.

RESULTS AND DISCUSSION

Effect of chitosan on radial mycelial growth of *Colletotrichum gloeosporioides*

Chitosan treatment showed significant inhibition of radial mycelial growth of *C. gloeosporioides* compared to control. The inhibition increased with increasing concentrations of the chitosan as shown in Table 1.

Complete inhibition of the pathogen was observed at concentration of 1.0% and above. These concentrations also appeared to have a fungi-static effect on the pathogen as mycelial growth occurred when the mycelial disks were transferred to fresh un-amended PDA and incubated for 10 days. Concentration of 1.0% chitosan was selected to use in subsequent *in vivo* trials.

Effect of chitosan on spore germination of *Colletotrichum gloeosporioides*

Chitosan treatments showed significant retardation of germination of *C. gloeosporioides* spores when compared to control (Table 2). Effect of 0.1% and 0.3% chitosan on spore germination of *C. gloeosporioides* was not significantly different from each other. Chitosan at 1.0% and above showed complete inhibition in germination of *C. gloeosporioides* spores. Observations revealed that complete inhibition of spore/conidia germination occurred in the presence of 1.0% Chitosan after 74 h. Germination of conidia was not observed when the conidia were transferred to a potato dextrose broth in the absence of chitosan solution. Concentration of 1.0% chitosan was selected to be used in subsequent *in vivo* trials.

Table 1. Mean radial growth of *Colletotrichum gloeosporioides* as affected by different concentrations of chitosan formulations.

Chitosan concentration (% w/v)	Radial mycelial inhibition (%)	Mean radial diameter (cm)
0.1	10.94	8.01[b]
0.3	42.66	5.16[c]
0.5	67.10	2.99[d]
1.0	100.00	0.00[e]
2.0	100.00	0.00[e]
3.0	100.00	0.00[e]
Control	0.00	9.00[a]

Note: Mean values denoted by the same superscripts within columns are not significantly different by DMRT, P=0.05 (P<0.0001), N=20.

Table 2. Mean number of spores germinated within 24 h on each of chitosan concentrations.

Chitosan concentration (% w/v)	Spore germination (%)	Number of germinated spores*
0.1	7.05	21[b]
0.3	6.10	18[b]
0.5	2.44	7[c]
1.0	0.00	0[d]
2.0	0.00	0[d]
3.0	0.00	0[d]
Control (sterile distilled water)	-	290[a]

Note: * Number of germinated spores out of randomly counted 300; Mean values denoted by the same superscripts within columns are not significantly different by DMRT, P=0.05 (p<0.0001), N=6.

Effect of chitosan on papaya inoculated with *Colletotrichum gloeosporioides*

Anthracnose severity in inoculated fruit was significantly (P<0.05) reduced when treated with 1.0% chitosan formulation, compared with the control (Table 3). The recovery of *C. gloeosporioides* from fruits treated with 1.0% chitosan was significantly lower than the control fruit (Table 3). Fruits treated with sterile distilled water (control) showed the highest recovery rate.

Table 3. Effect of chitosan on disease severity in inoculated papaya and recovery of *Colletrotrichum gloeosporioides* from the fruits.

Treatment	Disease severity-lesion diameter (cm)	Recovery of *C. gloeosporioides* (Arcsine transformed data)
1.0% Chitosan	1.30[b]	19.82[b]
Control (sterile distilled water)	4.64[a]	69.90[a]

Note: Mean values denoted by the same superscripts within columns are not significantly different by DMRT, P=0.05 (p<0.0001), N=20.

Effect of chitosan on papaya fruit

Disease incidence and severity on fruits were significantly ($P<0.05$) reduced when treated with 1.0% chitosan formulation compared to fruits dipped in sterile distilled water. Application of 1.0% chitosan formulation resulted in only ~20% disease incident after 14 days in low temperature storage while it was nearly 90% in the control. Fruits treated with 1.0% chitosan formulation significantly reduced the average lesion diameter (0.72cm) when compared with the control (6.12 cm).

TSS or ºBrix of the fruit ranged from 10% to 11%. Color of the flesh varied from 55º to 57º (Hº). The overall quality (score based on disease severity and shell color) of the fruits treated with 1.0% chitosan formulation was significantly ($P<0.01$) higher than the control. The total marketability of 1.0% chitosan treated fruit after ripening was 80% and fruits were not marketable at the end of the storage period. No significant changes were observed in any of the physicochemical characteristics of the chitosan treated fruits compared to the control except in firmness and CO_2 concentration in the internal cavity of the fruit (Table 4). Significantly higher values (2.93 N) of fruit firmness were observed in fruit treated with chitosan than those in the control. The highest percentage of CO_2 concentration was recorded in fruits treated with 1.0% chitosan as shown in Table 4.

Table 4. Effect of chitosan on physicochemical parameters and overall quality of papaya var. Rathna after 14 days at low temperature storage.

Treatment	ºBrix	Firmness (N)	Flesh color (H^0)	pH	CO_2 (%)	water loss (%)	Overall quality (average score)
1.0% Chitosan	10.0^a	2.93^a	56.32^a	5.3^a	7.15^a	1.80^a	6.92
Control	10.3^a	0.85^b	55.76^a	5.3^a	3.12^b	2.35^b	2.07

Note: Mean values denoted by the same superscripts within columns are not significantly different by DMRT, P = 0.05, N = 6).

Variation in the rates of respiration (ml CO_2 kg/h) of papaya during storage clearly indicated that chitosan treated papaya has lower rate of respiration than that of control fruit. Fruit in the control showed the highest rate of respiration (Fig. 1). This could be due to the formation of a chitosan film on the fruit, which can act as a barrier for O_2 uptake thereby slowing the metabolic activity and consequently the ripening process. Chitosan coating is likely to modify the internal atmosphere without causing anaerobic respiration, since chitosan films are selectively permeable to O_2 than CO_2 (Bai *et al.*, 1988). The suppressive effect of chitosan on decay due to *C. gloeosporioides* can be attributed to delaying the ripening and senescence process.

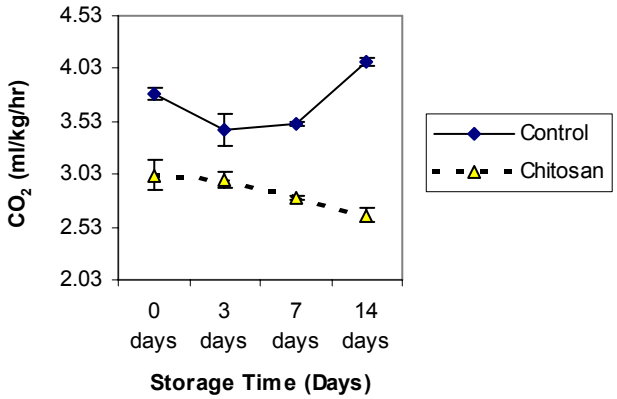

Fig. 1. Respiration behavior of papaya (treated with chitosan and untreated control) during low temperature storage.

The use of natural compounds to control plant pathogen may lead to reduction in use of fungicides. The present study showed that 1.0% and above concentrations of chitosan has an ability to inhibit the radial mycelial growth and spore germination of *C. gloeosporioides* significantly. The anti-fungal effect of chitosan against *C. gloeosporioides* was fungi-static. Chitosan is already known to interfere with the growth of several phytopathogenic fungi including *Botrytis cinerea* (Du *et al*., 1998), but the mechanism by which it affects the growth of the pathogen is still unclear. The inhibitory effect of chitosan on anthracnose in papaya seems to originate from the combination of its antifungal property and stimulation of defensive response in the host. The chitosan film formed around the fruit can act as a barrier to the outward flux of nutrients and consequently affects the establishment of nutritional relationship between host and pathogen (El-Ghaouth *et al*., 1994). The observations of this study revealed that flesh firmness of the chitosan treated fruits were significantly higher than the control.

CONCLUSIONS

The results showed that papaya fruit treated with chitosan had low disease severity and maintained 70-80% of marketability after 14 days of cold storage followed by 2 days at ambient temperature (28^0C±2). Since chitosan is natural and biodegradable, it will be a biologically sound alternative for exporters.

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