Effect of Chitosan (Unirradiated and Irradiated) Treatment on Anthracnose Disease and Its Potential to Increase the Shelf life of "Embul" Banana

Dinusha Jinasena, Pavithra Pathirathna, Suranga Wickramarachchi and Eshani Marasinghe

***Abstract*—The potential of chitosan (unirradiated and irradiated) to be used as an antifungal agent to reduce anthracnose disease on banana var. embul and thereby to improve its shelf life was investigated. Chitosan was irradiated using gamma radiation and a low dose of 5 kGy was used to determine the potential of using lower doses to irradiate chitosan in antifungal treatments. Minimum inhibitory concentration (MIC) of chitosan against *Colletotricum musae* was determined via a series of experiments on potato dextrose agar. A complete inhibition of *C. musae* was observed at chitosan concentrations ≥ 0.30% (irradiated) and chitosan concentrations ≥ 0.75% (unirradiated). In-vivo treatments showed 100% disease control when applied chitosan formulations ≥ 1.0% (irradiated) and chitosan formulations ≥ 1.5% (unirradiated) on fresh banana. Chitosan treatments had no adverse effects on the organoleptic and physiological properties of the fruit. Favorable changes were observed in chitosan treated banana with respect to firmness and weight loss. Overall acceptability of the chitosan treated banana was 70%. Irradiated chitosan is more effective than unirradiated chitosan in controlling the anthracnose pathogen and anthracnose disease. Irradiation has enhanced the antifungal activity of chitosan and a low dose such as 5 kGy was sufficient to increase the antifungal activity of chitosan against *C. musae*.**

***Index Terms*— Anthracnose, antifungal, banana, irradiated chitosan.**

I. Introduction

"Embul" (Musa accuminatea AAB) is one of the most popular banana varieties in Sri Lanka due to its sweet and sour taste. Because of its characteristic flavor and the small size it has a great potential for increased production to service the export market. The short storage life of banana is the major drawback in exporting this commodity. Postharvest diseases are the main cause for the short shelf life of banana. Anthracnose, caused by the fungus *Colletotricum musae*, is one of the major postharvest diseases in "embul" banana in Sri Lanka. Pale brown irregular shape spots at the early stage of anthracnose continuously develop into larger spots and coalesce as the ripening of the fruit progresses and eventually the center of the spot may burst open.

Manuscript received July 29, 2011, revised August 10, 2011. This work was supported by the Department of Chemistry under Grant RP/03/02/06/03/2007.

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Postharvest fungal diseases in banana are controlled by fungicides such as benlate (Benomyl), sorbic acid, thiabendazole, chlorothalonil, triazoles, strobilurin and imidazoles. The overuse of fungicides leaves chemical residues on the fruit surface and their environments creating hazardous impacts in the environment. The persistent use of fungicides also results in the emergence of resistant strains of *C. musae*. There is a great demand for alternative non-chemical methods for controlling this pathogen. Natural antifungal agents play a vital role in this regard.

A novel approach to extend the post harvest shelf life is the use of edible coatings of natural antimicrobial compounds. Chitosan, the deacetylated product of chitin, is one such natural coating derived from the outer shell of crustaceans. Due to its unique polycationic nature, chitosan has shown antimicrobial as well as inhibitory properties against a wide variety of bacteria and fungi [2]. Chitosan is considered as an ideal preservative coating for fresh fruits and vegetables due to its film-forming and biochemical properties. It acts in three ways to enhance the storage life of fresh fruits and vegetables; it acts as an antifungal agent to control the postharvest fungal disease, forms a semi-permeable coating around plant tissues which modifies the internal atmosphere of the fruit and decreases the loss of moisture due to transpiration and induces defense mechanisms which delay ripening and lower the rate of respiration [8]. Chitosan has been successful on controlling the decay and extending the storage life of apples [13], cucumbers [1], papaya [6], [22], strawberries [3], [17], [24], peaches, japanese pears, kiwi fruits [5] and litchis [7].

However, drawbacks of the application of chitosan are its low solubility in aqueous diluted acids and high viscosity of the solution even at low concentrations [25]. Insolubility is mainly due to its high molecular weight. Molecular weight of the polysaccharide can be suitably modified by radiation degradation. Irradiation of chitosan results in oligochitosan (depends on the dose rate: high dose rate favors cross linking reactions and low dose rates favor chain scission). Low molecular weight chitosan has more applications compared to high molecular weight chitosan as a fungicide. Irradiation has shown an increase in antimicrobial properties of chitosan [12], [15]. A few studies on the use of irradiated chitosan as an antimicrobial agent are reported. In most of these studies chitosan in the powder form has been irradiated and very high doses of gamma ray have been used for the irradiation of the chitosan powder [10], [18]. Degradation of chitosan by radiation is more efficient in aqueous medium due to the generation of hydroxyl radicals which in turn attack chitosan molecules by H-abstraction, thus forming radicals at carbon atoms [20].

However, not many studies have been reported on the usage of chitosan to improve the storage life of banana. The objectives of the present investigation are to determine the antifungal activity of both unirradiated and irradiated chitosan against the anthracnose disease pathogen, *Colletotrichum musae*, under *in-vitro* conditions and the effect of these treatments on anthracnose disease and the postharvest storage life of embul banana. Further, during this study an attempt was made to use a lower dose of gamma radiation to degrade chitosan in order to cut down irradiation costs in view of developing an economically viable postharvest treatment.

II. Materials and Methods

Chitosan: Chitosan (Degree of deacetylation, 80%) was purchased from the Nuclear Research Institute, Vietnam. A chitosan stock solution (2.5% w/v) in acetic acid (1% w/v) was prepared. Half of it was used to prepare a concentration series of unirradiated chitosan solutions while the other half was irradiated with a dose of 5 kGy by ^{60}Co gamma cell (GAMMA CHAMBER 5000 at Atomic Energy Authority, Sri Lanka, Dose rate 5 kGy/h). The irradiated 2.5% chitosan stock solution was used to prepare the irradiated series of the chitosan solutions.

Isolation of fungi: Ripe fruits of 'embul' banana having the symptoms of anthracnose were collected from local markets in Sri Lanka. *Colletotricum musae* was isolated from anthracnose infected "embul" banana tissues using the method described by Anthony *et al* (2004*)* and pure cultures were maintained on PDA plates.

Antifungal activity against *Colletotricum musae*: Mycelial discs (1.0 cm, diameter) were cut from the periphery of a 7 day old viable culture of *Colletotricum musae,* grown on PDA and transferred onto the centre of a 9 cm diameter PDA plates, which had been amended by incorporating irradiated and unirradiated chitosan aqueous solutions at concentrations ranged from 0.1 to 2.0% (w/v). Plates were incubated for 7 days at room temperature (28±2 ^{0}C). Plates incorporated with sterile distilled water, and 1% acetic acid served as controls. The colony diameter was measured after 7 days. The inhibition (%) of the pathogen was calculated using the following equation;

$$\boldsymbol{Inhibition} = \frac{(C-I)-(T-I)}{(C-I)}\boldsymbol{X100}$$

where,

T = Mean radial diameter of mycelium of test plate,

C = Mean radial diameter of mycelium of control plate,

I = Mean radial diameter of initial inoculum.

In vitro studies were conducted with five replicates of each treatment and control and arranged according to a completely randomized design (CRD). The experiment was repeated twice. The data were compared using Analysis of Variance and Tukey's pair wise comparison test.

Effect of chitosan on anthracnose disease of fresh 'embul' banana: A few bunches of "embul" banana at colour index 2 (10% yellow stage) were bought from a retail shop and banana fingers were detached from the crown. Ten banana hands were dipped in unirradiated chitosan solutions ≥ MIC for 5 min. Another set of ten banana hands were dipped in irradiated (5 kGy) chitosan solutions ≥ MIC for 5 min. Then fruits were air dried for 5 min. at 28±2 ^{0}C. Banana fingers treated with sterile distilled water and 1% acetic acid served as controls. Each set of banana was placed separately in cardboard cartons (18''×18"×6") with air holes. The cartons were stored at 13.5 ^{0}C and 95% RH for 14 days. Fruits were removed from cold storage after 14 days and kept at room temperature for 2 more days. The experiment was repeated twice. Disease severity was assessed by measuring the anthracnose lesion diameter. Anthracnose incidence was assessed by the ratio of fruits showing disease symptoms to the total number of fruits in each treatment.

Effect of chitosan on organoleptic properties of fresh 'embul' banana: Flavor, taste, odor, peel colour and overall acceptability of chitosan treated bananas were tested by a taste panel consisted of ten taste panelist along with a questionnaire. Each quality parameter was rated as 0-25%: poor, 25-50%: fair, 50-75%: good, 75-100%: excellent. Overall acceptability was assessed according to the score based on the above properties. The percentage overall acceptability was calculated as a ratio of the number of bananas with overall acceptability of 50-75% or 75-100% against the total number of fruits used per treatment. Fresh banans at the same yellow stage as treatments but without extended storage life were selected for the control sample.

Effect of chitosan on physicochemical properties of fresh 'embul' banana: Water loss from the fruits was calculated as a percentage of initial weight. Fruit firmness was measured with a fruit pressure tester (FT 011). Total soluble solids (^{0}Brix) content was measured using a hand held refractometer (ATAGO, ATC-1E, Japan). pH was measured using a pH meter (Orion, 410A, USA). Titrable acidity (TA) was determined by titrating a homogenate prepared from the middle part of the finger with NaOH. TA was expressed as grams of malic acid per 100g of fresh weight of the pulp.

III. Results and Discussion

Effect of chitosan on radial mycelial growth of *Colletotricum musae:*

Both unirradiated and irradiated chitosan were effective against the anthracnose pathogen *C. Musae.* Chitosan treatments showed significant inhibition of radial mycelial growth of *C. musae* compared to controls; sterile distilled water and acetic acid. The inhibition increased with increasing concentration of chitosan (Table 1, Table 2). There is no significant difference in radial diameter and growth inhibition in 1.0% acetic acid treated samples compared with sterile distilled water samples indicating that there is no growth control effect of acetic acid on *C. musae*.

Minimum inhibitory concentration (MIC) of unirradiated chitosan against *C. musae* was 0.75%. whereas that of irradiated chitosan was 0.3%. Irradiated chitosan showed a higher antifungal activity against *C. musae* compared to unirradiated chitosan.

TABLE 1: MEAN RADIAL GROWTH AND GROWTH INHIBITION OF *COLLETOTRICUM MUSAE* AS AFFECTED BY DIFFERENT CONCENTRATIONS OF UNIRRADIATED CHITOSAN FORMULATIONS

Concentration of chitosan % (w/v)	Unirradiated chitosan	
	Mean radial diameter (cm)	Growth Inhibition, %
0.10	6.0(±0.2)[a]	25.00(±1.25)[a]
0.20	5.7(±0.1)[ab]	28.75(±1.30)[b]
0.30	5.4(±0.1)[b]	32.50(±1.25)[c]
0.40	4.9(±0.2)[c]	38.75(±1.20)[d]
0.50	3.3(±0.1)[d]	58.75(±1.52)[e]
0.75	0.0[e]	100.0[f]
1.00	0.0[e]	100.0[f]
1.50	0.0[e]	100.00[f]
2.00	0.0[e]	100.00[f]
Sterile distilled water	8.0(±0.2) [f]	0.00[g]
Acetic acid (1.0%, v/v)	8.0(±0.2) [f]	0.00[g]

Note: The results were analyzed using One-way ANOVA and Tukey's pair-wise comparison test (P<0.05). Means with the same letters do not differ significantly.

TABLE 2: MEAN RADIAL GROWTH AND GROWTH INHIBITION OF *COLLETOTRICUM MUSAE* AS AFFECTED BY DIFFERENT CONCENTRATIONS OF IRRADIATED CHITOSAN FORMULATIONS

Concentration of chitosan % (w/v)	Irradiated chitosan (5 kGy)	
	Mean radial diameter (cm)	Growth Inhibition, %
0.10	3.2(±0.1)[a]	68.75(±1.70)[a]
0.20	3.0(±0.1)[a]	81.25(±1.25)[b]
0.30	0.0[b]	100.0[c]
0.40	0.0[b]	100.0[c]
0.50	0.0[b]	100.0[c]
0.75	0.0[b]	100.0[c]
1.00	0.0[b]	100.0[c]
1.50	0.0[b]	100.00[c]
2.00	0.0[b]	100.00[c]
Sterile distilled water	8.0(±0.2)[c]	0.00[d]
Acetic acid (1.0%, v/v)	8.0(±0.2)[c]	0.00[d]

Note: The results were analyzed using One-way ANOVA and Tukey's pair-wise comparison test (P<0.05). Means with the same letters do not differ significantly.

TABLE 3: ANTHRACNOSE INCIDENCE AND DISEASE SEVERITY OF UNIRRADIATED CHITOSAN TREATED BANANA SUBSEQUENTLY STORED AT LOW TEMPERATURE FOR 14 DAYS.

Concentration of chitosan % (w/v)	Unirradiated chitosan	
	Anthracnose incidence, %	Disease severity-lesion diameter (cm)
0.3	-	-
0.50	71(±2)[a]	0.90(±0.2)[a]
0.75	51(±2)[b]	0.70(±0.1)[a]
1.00	10(±1)[c]	0.20(±0.05)[b]
1.50	0(±0.0)[d]	0(±0.0)[c]
2.00	0(±0.0)[d]	0(±0.0)[c]
Sterile distilled water	100(±0)[e]	1.10(±0.3)[a]
Acetic acid (1.0%, v/v)	90(±1)[f]	0.80(±0.1)[a]

Note: The results were analyzed using One-way ANOVA and Tukey's pair-wise comparison test (P<0.05). Means with the same letters do not differ significantly.

TABLE 4: ANTHRACNOSE INCIDENCE AND DISEASE SEVERITY OF IRRADIATED CHITOSAN TREATED BANANA SUBSEQUENTLY STORED AT LOW TEMPERATURE FOR 14 DAYS.

Concentration of chitosan % (w/v)	Irradiated chitosan (5 kGy)	
	Anthracnose incidence, %	Disease severity-lesion diameter (cm)
0.3	53(±1)[a]	0.73(±0.1)[a]
0.50	40(±2)[b]	0.50(±0.1)b
0.75	10(±1)[c]	0.20(±0.05)[c]
1.00	0(±0)[d]	0(±0.0)[d]
1.50	0(±0.0)[d]	0(±0.0)[d]
2.00	0(±0.0)[d]	0(±0.0)[d]
Sterile distilled water	100(±o)[e]	1.10(±0.2)[g]
Acetic acid (1.0%, v/v)	90(±1)[f]	0.80(±0.1)[a]

Note: The results were analyzed using One-way ANOVA and Tukey's pair-wise comparison test (P<0.05). Means with the same letters do not differ significantly.

Effect of chitosan on anthracnose disease of fresh 'embul' banana

Application of chitosan formulations 0.75% (irradiated) and 1.0% (unirradiated) resulted in only ~ 10% disease incident after 14 days at low temperature storage while it was 80% in acetic acid and 100% in sterile distilled water. Chitosan formulations ≥ 1.0% (irradiated) and ≥ 1.5% (unirradiated) were capable of controlling the disease completely. There is an enhancement in the antifungal effect of chitosan with increasing concentration and irradiation.

Effect of chitosan on physicochemical properties of fresh 'embul' banana

As anthracnose disease in 1% (irradiated) and 1.5% (unirradiated) chitosan treated bananas was completely controlled by chitosan treatments these banans were selected for determining the organoleptic and physicochemical parameters of the treatments. TSS or ^{0}Brix of banana ranged from 4% to 5%. No significant changes were observed in pH and TA (% Malic acid) of the chitosan treated fruits compared to the control (Table 5). Firmness of the fruits treated with chitosan formulations was significantly higher than the control. Percentage weight loss in chitosan treated bananas was significantly lower than the controls. There is no significant difference in physicochemical properties between irradiated and unirradiated chitosan treated bananas except in fruit firmness. Fruit firmness of the irradiated chitosan treated banana was significantly higher than that of the unirradiated treatment.

Effect of chitosan on organoleptic properties of fresh 'embul' banana

The values obtained for the peel colour, odor, flavor and the taste of chitosan treated bananas were slightly lower than those obtained for the control sample. There was no significant difference between irradiated and unirradiated chitosan treated bananas in above properties. The overall acceptability of the bananas was estimated by considering the

TABLE 5: PHYSICOCHEMICAL PARAMETERS OF CHITOSAN TREATED BANANA AFTER LOW TEMPERATURE STORAGE FOR 14 DAYS

Treatment	TSS (^{0}Brix)	Fruit firmness (Kg/cm^2)	TA (% Malic Acid)	pH	% Weight loss
Unirradiated Chitosan, 1.5% (w/v)	4.50 (±0.1)^a	1.73 (±0.03)^a	0.35 (±0.03)^a	5.2 (±0.1)^a	8.6 (±0.1)^a
Irradiatd chitosan, 1% (w/v)	4.3 (±0.1)^a	1.82 (±0.03)^b	0.45 (±0.05)^a	4.9 (±0.1)^{ab}	8.5 (±0.1)^a
Control	4.00 (±0.1)^b	1.29 (±0.01)^c	0.40 (±0.02)^a	5.1 (±0.1)^a	12.9 (±0.2)^b

Note: The results were analyzed using One-way ANOVA and Tukey's pair-wise comparison test ($P<0.05$). Means with the same letters do not differ significantly.

above organoleptic parameters. There is no significant difference in overall acceptability between the two chitosan treatments while the control sample showed a significantly higher value compared with the treatments. However, a good overall acceptability of 70% was obtained for both treatments.

Chitosan is already known to interfere with the growth of several pathogenic fungi including *Colletotricum gloeosporioides* [9], *Bortrytis cinerea* [13]. The mechanism by which chitosan affects the growth of pathogen is still controversial. The most feasible hypothesis is a change in cell permeability due to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membranes. This interaction leads to the leakage of proteinaceous and other intracellular constituents [3], [11], [19].

Irradiation of the polymeric chitosan results in low molecular weight oligochitosan. Gamma irradiation is known as a useful tool for degradation of chitosan polymer [14] thus reducing the molecular weight [20]. Antifungal activity of chitosan powder irradiated at higher doses on fruit spoiling fungi strains has been demonstrated prviously [10], [18]. Radiation treatment of chitosan powder at doses higher than 20 kGy has incresed the anifungla activity of chitosan [18]. However, a recent study on *Colletotricum gloeosporioides* has shown that irradiated chitosan powder is less effective in controlling the pathogen irrespective of the radiation dose applied than chitosan irradiated in aqueous medium [10]. It has been further observed that the viscosity decrease rate is higher from 0 – 5 kGy and it slows down at higher doses [10].

The present study showed that both chitosan formulations ≥ 0.3% (irradiated at 5 kGy) and ≥ 1.0% (unirradated) has an ability to inhibit the radial mycelial growth of *Colletotricum musae* significantly. Chitosan formulations ≥ 1.0% (irradiated) and ≥ 1.5% (unirradated) could successfully control the anthracnose disesase thus extending the storage life of the fruit. It was found that the chitosan treatments have no adverse impact on the physiological or oraganoleptic properties of the fruit.

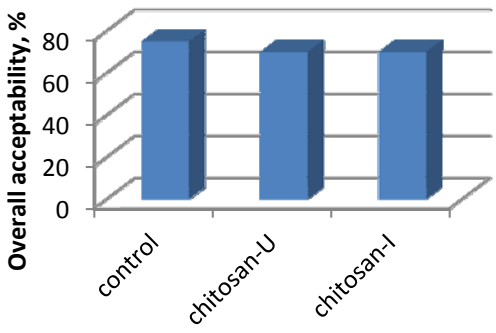

Fig 1. Overall acceptibility of banana after chitosan treatments followed by two week storage period; chitosan-U: unirradiated chitosan treatment, chitosan-I: irradiated chitosan treatment, Control-fresh banans at the same yellow stage as treatments without extended storage life.

Irradiation of chitosan clearly showed an enhanced effect on the anthracnose pathogen and in controling the disease. A low dose such as 5 kGy is sufficient to increase the antifungal activity of chitosan. Since chitosan is natural and biodegradable, it will be a biologically sound alternative to fungicides.

ACKNOWLEDGMENT

We carried out this work in collaboration with the Atomic Energy Authority, Sri Lanka and we thank the Atomic Energy Authority for providing the irradiation facility.

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