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Use of Chitosan to Control Anthracnose On "Embul" Banana

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Abstract. The demand for the pesticide free products prompted investigating the potential of chitosan to be used as an antifungal agent to reduce anthracnose disease on banana var. embul. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of chitosan against Colletotricum musae were determined via a series of experiments using Poisoned food bioassay. A complete inhibition of C. musae was observed at concentration of 0.75% (MIC) and above. Fungicidal effect was observed with no mycelial growth in the growth revival test at concentration of 1.0% (MLC) and above. "Embul" banana at colour index 2 (10% yellow stage) were subjected to in-vivo treatments. In-vivo study using 1.0% chitosan significantly reduced both anthracnose incidence and severity on banana. Significant changes were observed in chitosan treated banana in firmness, and percentage weight loss with respect to controls. These results show that chitosan can be used to control Anthracnose disease in "embul" banana.

Keywords: chitosan, antifungal, anthracnose, shelf life

1. 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.

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 (Sandford and Hutchings, 1987; Sanford 1989). Due to its unique polycationic nature, chitosan has shown antimicrobial as well as inhibitory properties against a wide variety of bacteria and fungi (Hirano and Nagae, 1989). 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

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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 (Zhang and Peter, 1997).

Chitosan has been successful on controlling the decay and extending the storage life of apples (Due *et al,* 1998), cucumbers (E1-Ghaouth *et al.,* 1994), papaya (Bautista – Banos *et al.,* 2003; Shivakumar *et al.,* 2005), strawberries (Park et al., 2005; Reddy et al., 2000; Vargas et al., 2006), peaches, japanese pears, kiwi fruits (Jianming et al., 1997) and litchis (Zhang and Quantick, 1997). 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 chitosan against the anthracnose pathogen, *Colletotrichum musae* and its potential as an antifungal agent to control the anthracnose disease in 'embul' banana during postharvest storage.

2. Materials and Method

2.1 Isolation of fungi

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.

2.2. Effect of chitosan on radial mycelia growth of *Colletotricum musae*

A mycelial disc (1.0 cm, diameter) was 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 chitosan aqueous solutions at concentrations ranged from 0.1 to 1.0% (w/v) introduced into the medium at 50^0 C before plating. 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. Five replicates of each treatment and controls were arranged according to a completely randomized design (CRD). The colony diameter was measured after 7 days. Minimum inhibitory concentration (MIC) of chitosan was determined. Where the growth was completely inhibited by chitosan treatments, fungal discs were transferred onto fresh PDA plates without chitosan. After 7 days survival of the pathogen was observed. MLC was assessed by noting the minimum concentration which shows no signs of the survival of the pathogen**.**

2.3. Effect of chitosan on banana inoculated with *Colletotricum musae*

Banana fingers at colour index 2 (10% yellow stage) were surface sterilized with 70% ethanol and wounded the surface with a No 05 cork borer (1 cm) to a depth of 3mm. The wound was inoculated with 0.2 mL of conidial suspension (10^3 conidia/ mL) of the fungi and incubated for 3 hours at 28±2 ^{0}C and 95% relative humidity (RH). At the end of the incubation period, 10 banana fingers were dipped in 1% chitosan formulation for five minutes. Fruits (10 each) dipped in 1% acetic acid and sterile distilled water served as controls. Then fruits were air dried and kept 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. At the end of the storage period, disease severity was assessed by measuring the anthracnose lesion diameter.

2.4. Effect of chitosan on banana fruit

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. 10 Bananas were dipped in 1% chitosan solution for 5 minutes and 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 as mentioned in section 2.3. Anthracnose incidence was assessed by the ratio of fruits showing disease symptoms to the total number of fruits in each treatment.

2.4.1. Assessment of physico-chemical properties:

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.

The results were compared using Analysis of Variance and Tukey's pair wise comparison test.

3. Results and Discussion

3.1. Effect of chitosan on radial mycelial growth of *Colletotricum musae*

Chitosan treatment 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). The results of mean radial diameter and percent inhibition in sterile distilled water and 1.0% acetic acid were not significantly different indicating that there is no effect of acetic acid on the radial mycelial growth of *C. musae*.

Complete inhibition of the pathogen was observed at chitosan concentrations of 0.75% and above. This concentration also appeared to have a fungi-static effect on the pathogen as mycelial growth occurred when the mycelia disks were transferred to fresh un-amended PDA and incubated for 7 days. Minimum inhibitory concentration (MIC) of *C. musae* was considered as 0.75%. Fungicidal effect was observed with no mycelial growth in the growth revival test at concentration of 1.0% and above. Minimum lethal concentration (MLC) of test pathogen was taken as 1.0% and it was selected to be used in subsequent *in vivo* tests.

Table 1: Mean radial growth and percent inhibition of *Colletotricum musae* as affected by different concentrations of chitosan formulations

Concentration of chitosan (% w/v)	Mean radial diameter	Percent inhibition
0.10	6.0^{a}	25.00^{a}
0.20	5.7^{a}	28.75^{b}
0.30	5.4^{a}	32.50^{c}
0.40	4.9^{a}	38.75^{d}
0.50	3.3^{b}	58.75^{e}
0.75	0.0^{c}	100.0^{f}
1.00	0.0^{c}	100.0^{f}
2.00	0.0^{c}	100.00^{f}
3.00	0.0^{c}	100.00^{f}
Sterile distilled water	8.0^{d}	0.00^{g}
Acetic acid (1.0%, v/v)	8.0^{d}	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.

3.2. Effect of chitosan on banana inoculated with *Colletotricum musae*

Anthracnose severity in inoculated banana was significantly reduced when treated with 1.0% chitosan formulation, when compared with the controls (Table2). Disease incidence of inoculated banana treated with 1.0% chitosan was 40% and it was significantly lower than the controls.

Table 2: Effect of chitosan on anthracnose incidence and disease severity in banana

Treatment	Anthracnose incidence- the ratio of fruit showing disease (%)	Disease severity-the lesion diameter (cm)
1.0% Chitosan	40^{a}	1.73^{a}
Sterile distilled water	100^{b}	2.67^{b}
Acetic acid (1.0%, v/v)	80^{c}	1.90^{c}

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.

3.3. Effect of chitosan on 'embul' banana

Disease incidence and disease severity on banana were significantly reduced when treated with 1.0% chitosan formulation compared to banana dipped in sterile distilled water and acetic acid (Table 3). Application of 1.0% chitosan formulation resulted in only ~ 10% disease incident after 14 days in low temperature storage while it was 80% in acetic acid and 100% in sterile distilled water. Banana treated with 1.0% chitosan formulation significantly reduced the average lesion diameter when compared with controls.

Table 3: Effect of chitosan on anthracnose incidence and disease severity in banana

Treatment	Anthracnose incidence- the ratio of fruit showing disease (%)	Disease severity-the lesion diameter (cm)
1.0% Chitosan	10.4^{a}	0.2^{a}
Sterile distilled water	100^{b}	1.1^{b}
Acetic acid (1.0%, v/v)	80^{c}	0.8^{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.

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 4). Fruit firmness of the fruits treated with chitosan was higher than the control. Percentage weight loss in 1.0% chitosan treated banana was less than the controls.

Table 4: Effect of chitosan on physicochemical parameters of banana after 14 days at low temperature storage

Treatment	TSS (^{0}Brix)	Fruit firmness (Kg/cm^{2})	TA (% Malic Acid)	pH	% Weight loss
1.0% Chitosan	4.50^{a}	1.73^{a}	0.35^{a}	5.2^{a}	$8.63(\pm0.09)^{a}$
Control	4.00^{a}	1.29^{b}	0.40^{a}	5.1^{a}	$12.96(\pm0.51)^{b}$

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.

The present study showed that 1.0% and above concentrations of chitosan has an ability to inhibit the radial mycelial growth of *Colletotricum musae* significantly. Authors believe the anti-fungal effect of chitosan against *C. musae* was both fungicidal and fungistatic. Chitosan is already known to interfere with the growth of several pathogenic fungi including *Colletotricum gloeosporioides* (Hewajulige et al., 2006), *Bortrytis cinerea* (Du et al., 1998). Chitosan is known to form a semi-permeable coating around plant tissues and thereby inhibits the activity of plant pathogenic fungi. 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 (Young *et al* 1982; Leuba and Stössel 1986; Papineau *et al* 1991; Sudarshan *et al* 1992; Fang *et al* 1994). The above results show that chitosan has a great potential to control anthracnose in 'embul' banana. Since chitosan is natural and biodegradable, it will be a biologically sound alternative to fungicides.

4. References

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