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Effect of Heat Treatment of Plantain (*Musa paradisiaca*) Fruits on Peel Characteristics and Control of Decay by *Fusarium verticillioides*

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Abstract: Mature green plantain fruits were inoculated with conidial suspension of *Fusarium verticillioides* and exposed to hot air at 38 and 44°C or dipped in hot water only at 50°C for 5 min, 53°C for 3 min, hot water at 50°C with 5 ppm Benlate (Benomyl) and 5 ppm Benlate only. The controls were neither heat treated nor dipped in chemical. Fruits were then stored at 28±2°C for 21 days. Results showed that heat treatment reduced severity of rot on plantain fruits. Prestorage hot air treatment at 38°C for 48 h and 44°C for 72 h resulted into total disease control and compared favourably with Benlate and hot Benlate treatments. Hot water only at the two time-temperature combinations tested also significantly reduced rot severity. *In vitro* studies also showed that the fungus was significantly inhibited at 44°C but not at 38°C. Heated fruits also had higher peel colour ratings except those dipped in hot water only at 50 and 53°C which remained green and more green than yellow, respectively. Hot benlate treated fruits had the highest colour rating among dipped fruits while hot air treatment at 44°C enhanced colour development more than 38°C.

Key words: Plantain, hot air, hot water, decay, colour

INTRODUCTION

Plantain (*Musa paradisiaca* Linn.) with world production index of 20.5 million tonnes per year is a very important fruit of the tropics and sub tropics where both ripe and unripe fruits are eaten (MacDonald and Low, 1990). Anonymous (2006) revealed that Nigeria produced 2.1 million metric tons in 2004. The major postharvest problems of plantains are mechanical injuries during handling, over ripening and pathological damage by microorganisms. The first two types predispose fruits to the last, accelerating and many times aggravating it. Fungal diseases were noted to cause the greatest pre and post harvest production losses and account for a large share of plantation management expenses (Nelson *et al*., 2006). Quimio (1986) and Nelson *et al*. (2006) reported several pre and post harvest fungal diseases of *Musa* sp. in the Philippines and Pacific Islands including finger-stalk rot or black decay of the finger stalk and adjacent parts of the finger caused by *Colletotrichum musae*, *Nigrospora sphaerica* or *Fusarium* sp. Alvindia *et al*. (2004) implicated *Fusarium verticillioides* with other fungi in crown rot of bananas. The fungus infects both ripe and unripe fruits and may progress into the fruit causing its total decay with time.

Post harvest methods of decay control include use of antagonistic organisms, chemicals, surface coatings, irradiation, atmosphere modification, low temperature application, protective packaging with polyethylene and prestorage heat treatment (Mari *et al*., 1996; Bhowmik and Pan, 1992). The use of chemicals although widespread because of the efficacy and convenience is becoming increasingly unpopular because of the problem of fungal resistance and toxic residues. The other methods are also sometimes injurious to personnel and consumers in addition to high cost and physiological damage to crops (Thomas *et al*., 1971; Olorunda and Aworh, 1984).

The benefits of prestorage heat treatment on various horticultural produce especially in controlling pathological problems have been reported (Fallik *et al*., 1993; Jacobi *et al*., 1996; Afek *et al*., 1999; Aborisade and Ojo, 2002). Heat treatment with hot water is usually for brief periods at higher temperatures because water is a more efficient heat transfer medium, while vapour heat treatment is usually done for longer periods at lower temperatures. Generally, the higher the temperature, the shorter the period of exposure required. The temperatures most commonly employed are 38 to 50°C. Fallik *et al*. (1993) described a process for vapour heat treatment at 38°C for 3 days which effectively controlled *Botrytis cinerea* decay of tomato fruits. Armstrong (1982) reported hot water treatment at 50°C for 45 and 15 min, respectively on mature green and ripe Hawaiian grown Brazillian bananas for quarantine purposes. Hassan *et al*. (2004) also reported extension of banana shelf life by hot water treatment at 50±2°C combined with fungicide application.

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For plantain however, there is paucity of information on postharvest heat treatment trials although there are reports of the application of other methods. These include use of permeable coatings (Olorunda and Aworh, 1984; Al- Zaemey *et al*., 1989) and ionizing radiations (Aina *et al*., 1999; Falana *et al*., 2000).

This study therefore seeks to assess the potentials of prestorage hot air and hot water treatment in controlling *Fusarium verticillioides* finger stalk rot of plantain fruits.

MATERIALS AND METHODS

Plant material: Mature green healthy plantain fruits of the short horn variety were harvested from a commercial farm in Ilara-Mokin, Ondo State, Nigeria. Fruits of similar size, colour and maturity were selected. The fruits were treated within 24 h of harvest. The experiments were carried out from December 2004 to September 2005.

Preparation of inoculum: Ten-day old agar slant cultures of *F. verticillioides* were used for inoculum. The slants were washed repeatedly several times by pouring sterile water into them and shaking to dislodge conidia into the water. The wash water was collected in a sterilized beaker. One milliliter of the suspension was spread on an area of 1 cm^2 and allowed to dry on clean microscope slide before counting using the high dry (×40) objective. The conidia concentration of the suspension was determined by using the following formula (Breed Direct Counting Technique).

Number of conidia mL^{-1} = Average number of conidia (about 50 fields) ×MCF

$$\text{MCF} = \text{Microscope Correction Factor} = \frac{\text{Area of smear}}{\text{Area of microscope field}} \times \text{dilution factor}$$

Area of microscope field = A = πr^2

The suspension was used to inoculate the point of excision from hands on fruits stalks. The suspension used to inoculate hot air treated fruits contained 9.87×10^3 conidia mL^{-1} while that for hot water dips contained 7.48×10^5 conidia mL^{-1}. The higher inoculum used for dip treatments was to compensate for the conidia lost when inoculated fruits were dipped in water.

Inoculation, heat treatment and assessment of rot severity: Individual plantain fingers were separated from the bunch and surface disinfected in 1.05% sodium hypochlorite solution. The stalk end was then dipped into the conidial suspension for 5 sec with constant agitation of the suspension. Inoculated fruits were then separately exposed to hot air at 38°C or 44°C for 3, 6, 9, 12, 15, 18, 21, 24, 48, 72 h and hot water at 50°C for 5 min, 53°C for 3 min, 5 ppm Benlate with and without heating at 50°C for 5 min. The fruits dipped in water were first incubated for 12 h at 28°C after inoculation before the heat treatment. The control fruits were not exposed to heat after inoculation. All fruits were afterward stored in an atmosphere of 100% relative humidity at 28±2°C in sterilized desiccators (hot air treated fruits) and sterile polyethylene wrappers of 2.5 microns thickness (dipped fruits) and rated subjectively for disease severity on a scale of 1-10 twenty-one days in storage at 28±2°C after treatment.

By this rating 1 = No mould growth/no decay; 2 = Appearance of mycelia/discoloration at the point of inoculation; 3 = Dark brown/black colouration of fruit stalk; 4 = mould growth up to 2 cm on fruit with white mycelium; 5 = mould growth up to 2 cm on fruit with pinkish mycelium; 6 = decay up to 2.5 cm but not > 5.0 cm; 7 = decay up to 5.0 cm but not > 7.0 cm; 8 = decay up to 7.0 cm but not > $^1/_3$ fruit length; 9 = fruit decayed more than $^1/_3$ length but not total; 10 = total decay.

Assessment of peel colour change during storage: Fruits exposed to hot air and dipped in hot water were assessed for peel colour change on the twenty-first day after treatment. Colour rating was done on a scale of 1 to 7 using the modified banana ripening guide (Anonymous, 1972). By this guide 1 = green; 2 = green with traces of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = full yellow; 6 = yellow lightly flecked with dark brown; 7 = yellow with more dark brown. Fruits with full yellow peel colour are more preferred for eating, while green peel colour is preferred for storage.

Effect of heat treatment on conidia germination and linear growth of fungus on agar: Ten-day old cultures of the mould on Lab M™ potato dextrose agar (PDA) medium were exposed to hot air at the same time-temperature combinations as for fruits. Six millimetre diameter of mycelium-agar discs were then cut from the cultures using sterile cork borer. One disc each was placed at the center of transacts drawn on the reverse side of Petri dishes containing PDA. The plates were incubated at 28°C for 4 days in a Gallenkamp incubator after which mycelial extension was measured along the lines as the diameter of growth. Two drops of conidial suspensions prepared from the discs were placed on a thin film of sterile PDA on microscope slide, placed on a support in a Petri dish containing filter paper moistened with sterile water and incubated for 12 h at 28°C.

Percentage of germinated conidia was then determined in about 10 different microscope fields and the means calculated for each treatment.

Data analysis: The data obtained were subjected to analysis of variance and the means were compared by New Duncan's multiple range test where significant differences occurred.

RESULTS

There was significant decay control on fruits exposed to hot air. Hot air treatment for 48 h at 38°C and 72 h at 44°C before storage did not show any sign of decay or mould growth 21 days post treatment. Those exposed for 48 h at 44°C showed slight mould growth at the point of inoculation on the stalk. All other hot air treated and untreated fruits had varying degrees of symptoms of decay (Table 1).

Full yellow peel colour was observed on fruits exposed to hot air for 12, 15 and 48 h with means of 5.0 for the first period and 4.50 for the latter two periods. None of the fruits exposed to 38°C developed colour to stage 6.0 but fruits treated at 44°C for 9, 48 and 72 h showed browning of their peels. There was more yellowing of peel among fruits exposed to 44°C before storage than among those exposed to 38°C. All exposure to hot air at 44°C resulted into peel colour change to some extent but no colour change was observed on peels of fruits exposed to 38°C for 3, 6 and 9 h as they all remained green (Table 1).

There was also significant reduction in disease severity on fruits dipped in hot water. Mean disease ratings were 1.20 and 1.60 for 50°C and 53°C, respectively compared with 2.40 for control. Fruits on which Benlate was applied with and without heating did not show any sign of decay or mould growth (Table 2). There was also no significant difference in disease severity in fruits dipped in hot water only at 50°C and the two Benlate treatments. Hot water treatment at 50°C before storage kept the fruits green twenty-one days after treatment while those dipped in hot water at 53°C had developed traces of yellow with mean of 2.60 for the same period. Hot Benlate treated fruits had ripened more with more yellow than green at mean rating of 4.0 while fruits with Benlate treatment alone and the controls were at the same rating of 3.40 (Table 2).

Reduction in mycelial growth and percentage spore germination of *F. verticillioides* was observed with treatment at 44°C but this was not so definite at 38°C (Table 3 and 4).

Table 1: Mean finger stalk rot severity and peel colour ratings of plantain fruits exposed to hot air and subsequently stored at 28±2°C for 21 days

	Temperature of treatment			
	38°C		44°C	
Time (h)	Disease rating	Colour rating	Disease rating	Colour rating
0	2.75±0.25^{c}	1.00±0.00^{a}	2.75±0.25^{b}	1.00±0.00^{a}
3	2.50±0.29^{bc}	1.00±0.00^{a}	3.00±0.00^{b}	2.50±0.29^{bc}
6	2.75±0.25^{c}	1.00±0.00^{a}	2.75±0.25^{b}	1.75±0.25^{ab}
9	2.75±0.25^{c}	1.00±0.00^{a}	6.00±0.00^{c}	7.00±0.00^{d}
12	3.00±0.00^{c}	5.00±0.00^{d}	3.00±0.00^{b}	3.50±0.50^{c}
15	3.00±0.00^{c}	4.50±0.29^{d}	3.00±0.00^{b}	3.00±0.41^{c}
18	3.00±0.00^{c}	2.75±0.63^{c}	3.00±0.00^{b}	1.50±0.29^{ab}
21	2.75±0.25^{c}	1.25±0.25^{a}	3.00±0.00^{b}	3.25±0.25^{c}
24	3.00±0.00^{c}	2.00±0.00^{b}	3.00±0.00^{b}	3.50±0.29^{c}
48	1.00±0.00^{a}	4.50±0.29^{d}	1.25±0.25^{a}	6.25±0.75^{d}
72	2.00±0.00^{b}	------	1.00±0.00^{a}	6.00±0.00^{d}

----- = abnormal ripening, Each value is a mean of four replicates±standard error of the mean. Means followed by the same letter in the same column are not significantly different by New Duncan's multiple range test (p>0.05)

Table 2: Mean disease severity and peel colour ratings 21 days in storage at 28°C after plantain fruits were dipped into hot water and Benlate

Treatment	Disease rating	Peel colour rating
Control	2.40±0.51^{b}	3.40±0.24^{c}
HW 50°C	1.20±0.20^{a}	1.00±0.00^{a}
HW 53°C	1.60±0.40^{ab}	2.60±0.24^{b}
Benlate	1.00±0.00^{a}	3.40±0.24^{c}
HW+Benlate 50°C	1.00±0.00^{a}	4.00±0.00^{d}

Each value is a mean of five replicates±standard error of the mean. Means followed by the same letter in the same column are not significantly different (p>0.05) by New Duncan's Multiple range test. HW = Hot Water

Table 3: Effect of hot air treatment of *Fusarium verticillioides* on mycelial extension at 28°C on potato dextrose agar four days after heat treatment

	Mean mycelial diameter (cm)	
Exposure period (h)	38°C	44°C
0 (Control)	5.72±0.24	5.72±0.24
3	5.42±0.04	4.40±0.20
6	5.62±0.11	5.10±0.04
9	5.75±0.21	4.57±0.11
12	5.42±0.27	3.00±0.34
15	5.42±0.24	4.62±0.11
18	5.72±0.25	4.80±0.76
21	5.92±0.30	5.05±0.06
24	5.57±0.11	2.95±0.78
48	5.67±0.21	4.02±0.34
72	5.47±0.16	0.75±0.05

Each value is a mean of four replicates±standard error of the mean

Table 4: Effect of exposure to hot air on germination of spores of *Fusarium verticillioides* on potato dextrose agar at 28°C

	Percentage spore germination	
Exposure period (h)	38°C	44°C
0 (Control)	91±1.45	91±1.45
3	98±0.68	95±0.93
6	100±0.41	83±3.54
9	90±2.59	91±3.80
12	74±10.23	75±1.50
15	97±1.25	61±7.01
18	90±4.74	23±6.39
21	85±2.82	3±0.78
24	95±2.80	0±0.00
48	89±2.63	0±0.00
72	95±1.11	0±0.00

Each value is a mean of ten replicates±standard error of the mean

It was observed that normal ripening related colour changes on the peel coincided with some stages of disease symptoms thereby interfering with observations of rot symptoms on the fruits and this slightly affected disease rating.

DISCUSSION

In this study, heat treatment of inoculated mature green plantain fruits with hot air and hot water resulted into reduction of the severity of finger stalk rot caused by *F. verticillioides*. Total disease control was observed with hot air treatment at 38°C for 48 h and 44°C for 72 h. Similar level of control was observed for hot water alone at 50°C and Benlate treatment. Hot air at these two time-temperature combinations therefore compared favourably with Benlate and hot Benlate treatments. In vitro studies showed that the fungus was more effectively inhibited at 44°C than at 38°C. Disease development was however more severe at 44°C than at 38°C. This greater severity in rot may have been aided by the higher level of ripening indicated in the yellowing and browning of peel observed with treatment at 44°C. Disease development is usually more severe in ripe than unripe fruits. Arpaia (1994) also observed a fairly high level of avocado fruit decay after ripening. The same reason may be advanced for the successful reduction of decay in hot water treated fruits especially those treated at 50°C whose peels remained green 21 days after treatment in which there was only a slight indication of infection. The reduction in disease severity observed with this treatment supports earlier reports by Hassan *et al*. (2004) on banana a similar crop to plantain. The generally lower disease severity in wet heat treatments compared with dry heat treatment despite the higher inoculum received by the former, suggest that hot water may be more effective for controlling this fungus on plantain.

Same peel colour rating on controls and Benlate treatment alone suggest that the chemical had no effect on peel colour at ambient temperature even though it resulted into disease control. With heat however, the increase in chemical solubility and fruit peel permeability, probably allowed greater penetration of the peel and possibly resulted into more chlorophyll degradation and increased carotenoids contents (Gaffney *et al*., 1998; Garcia *et al*., 2002) than what occurred at ambient temperature. Also, the higher colour rating in the treatment which combined heat with Benlate compared to other dip treatments, suggest that the chemical in hot water enhanced colour development while hot water only suppressed it as evidenced by the lower ratings at both water temperatures. This latter observation thereby confirms some earlier reports that heat treatment retarded ripening related colour changes (Paull, 1990). Hot air therefore seemed to enhance colour development to yellow while hot water dip retarded it. Considering the fact that hot water at 50°C reduced disease very significantly and the fruits remained green at the same time, this treatment therefore seem to be more promising in developing methods of extending the storage life of short horn plantain fruits. Lunardi *et al*. (2002) suggested that the best heat treatment for Fuji apples was 5 m hot water dip at 52°C. However, normal ripening related processes were noticed to have interfered with observations on disease development in this study especially with respect to colour changes of the peel which were similar for early disease symptoms, ripening and overripening. Future studies should attempt to separate plantain fruit physiology from disease development in order to assess the efficacy of heat treatment in decay control more accurately.

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