

Control of *Rhizopus*: Induced decay in watermelon (*Citrullus lanatus* L.) fruits using heat treatment

Oluwole Olakunle Oladele

Department of Biology. Federal University of Technology. Akure. Ondo State. Nigeria. Email: prophetoladele2014@gmail.com.

Abstract. Control of *Rhizopus*, induced decay in watermelon (*Citrullus lanatus*) fruits using heat treatment was investigated. Watermelon fruits of uniform size and colour were selected, washed with clean water and disinfected after harvest. The fruits were then inoculated with spore suspension containing 3.02×10^3 spores mL⁻¹ of *Rhizopus* sp. inoculated fruits were immersed separately in a water bath at 50 °C for 2, 3 and 5 min and 53 °C for 2 and 3 min. Control fruits were inoculated but not heat treated. Each set up was in 3 replicates. Control and treated fruits were then stored in cleaned transparent plastic containers at 28 °C ± 2 °C and 75%-80% relative humidity and observed daily for disease severity. Result obtained on day 5 of storage showed there was no significance difference ($p \geq 0.05$) in the disease severities of control and all the treated fruits. The disease severity of both the control and all treated fruits was 1.00 ± 0.28 which implied that the fruits were healthy (disease free). However as storage period progressed to day 25, only fruits treated at 53 °C for 3 min maintained disease severity of 1.00 ± 0.00 , which implied that the fruits were still disease free, recording mean appearance and taste index of 3.50 and 2.00, respectively, which thus indicated good appearance and sweet taste of the fruits. Other treatments recorded various degrees of rottenness. Hence, treatment at 53 °C for 3 min could be considered as the most effective for extending the shelf life of water melon fruits among all temperatures and time combinations experimented in the study.

Keywords: Disease severity; Hot water; Time; Pathogen; Spore suspension; Storage.

Introduction

Watermelon (*Citrullus lanatus*) fruits belong to cucurbitaceae family, which consists of nearly 100 genera and over 750 species. In Nigeria, the largest production of watermelon fruits comes from the northern part of the country, where the suitable agro- ecology is found. They are widely distributed in the tropics and subtropics, and a few species

occur in temperate region. Economically, Watermelon is an important fruit crop and valuable alternative source of water in desert areas (Perkins-Veazie and Yamaguchi, 2001). It is a good source of lycopene, citrulline and important minerals and vitamins. It has the highest lycopene content among fresh fruits and vegetables (Lurie, 1998). Watermelon contains 60% more lycopene than tomato. Lycopene in the human diet is

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ORCID

0000-0001-9023-2646
Oladele Oluwole
Olakunle

associated with prevention of heart attacks and certain cancers. Rind of watermelon contains an important natural compound called citrulline, an amino acid that the human body makes from food.

However, watermelon fruits are mostly threatened by some pathogenic fungi which are seed-borne and also soil-borne such as *Mucor* spp and *Rhizopus* spp. Various post-harvest control measures of fruits are: use of low temperature, heat treatment, use of fungicides etc. Post-harvest disease of water melon fruits are primarily controlled by application of conventional fungicides. However, the development of resistance by pathogens to fungicides (Holmes and Eckert 1999) and the increased demand for fruit free of hazardous chemicals have revived interest in heat treatment (Smilanick et al., 2003). Heat is usually applied to a fruit commodity via air or water (Barkai-Golan and Philips 1991). Nevertheless, water is more efficient than air by virtue of its higher heat transfer coefficient (Jacobi and Giles, 1997).

The use of heat treatment is therefore investigated in this research work as alternative non chemical control measure against *Rhizopus* sp on watermelon fruits.

Materials and methods

Source of fruits and medium preparation

Mature, green healthy watermelon fruits were harvested from Ipinsa (7° 13' 26" N and 5° 39' 0" E) village located along Oke-Odu, in Akure, Ondo State, Nigeria, and brought to the research laboratory of Biology Department, FUTA. Fruits of uniform size and colour were selected and washed with clean water and immersed in 10% sodium hypochlorite solution for 5 min and allowed to air dry. Medium was prepared by dispensing 5.0 g of Potato Dextrose Agar into a sterile 250 mL conical flask. 100 mL of distilled water

was added to the agar and agitated carefully for homogeneity of the medium. The flask was corked and placed in the autoclave where it was sterilized for 15 min at 121 °C. Tetracycline was added to the sterilized medium and the medium after being allowed to cool was poured into sterile Petri dishes and left to solidify.

Isolation from infected watermelon fruits

Isolation of associated fungi from spoilt watermelon fruit was made by cutting out the interface between the healthy and the disease tissue and placing pieces of the affected fruit and without surface sterilization on plates of solidified Potato Dextrose Agar (PDA). The plates were then incubated at for 3 days. Subcultures of the isolate were prepared by transferring agar cut with distinct mycelium to sterilized Petri dishes containing solidified PDA and then incubated at 28 °C ± 2 °C until pure cultures were obtained. The resulting pure culture was then used for morphological characterizations of the isolate.

After incubation, identification of the isolate was based mainly on the structural features as seen in the culture plate as well as microscopic characteristics. A drop of cotton in blue lactophenol solution was put on a slide. The isolate was placed on a slide. This was covered with a cover slip, excess liquid was drained with filter paper and the isolate was examined under microscope. Examination was done with x40 objective for the presence and type of hyphae, mycelium whether clear or dark and spore morphology. The isolate was then identified using the text of Alexopoulous et al. (1996).

Preparation of spore suspension and treatment of inoculated watermelon fruits with hot water

Sterile water was poured into a conical flask containing the pure culture

and shaken vigorously to dislodge the spores from the vegetative hyphae. The wash water was collected in a sterilized beaker. After serial dilution to $\times 10^3$, one milliliter of the suspension was injected using sterile syringe and needle into watermelon fruits and allowed to stay for 12 h before heat treatment. The suspension used to inoculate the watermelon fruits before treatment contained 3.02×10^3 spores/mL of the isolate. Inoculated watermelon fruits were immersed separately in a water bath at 50 °C for 2, 3 and 5 minutes, 53 °C for 2 and 3 min. Each set up was in three replicates. Control fruits were inoculated but not heat treated. All fruits were placed in cleaned transparent plastic containers and stored in 75%-80% relative humidity at $28 \text{ °C} \pm 2 \text{ °C}$ and observed daily for disease severity.

Both the control and all heat treated watermelon fruits stored in clean plastics containers at 75%-80% relative humidity at $28 \text{ °C} \pm 2 \text{ °C}$ were assessed daily for disease severity using the modified scale of Arekemase and Oyeyiola (2007) where: 1 = disease free; 2 = slight decay up to 10% of the fruit surface; 3 = moderate decay up to 35% of the fruit surface; 4 = severe decay $\geq 45\%$ of the fruit surface. Decay was recognized by dark coloration on the fruit or by appearance of whitish mycelium on the fruit surface. Sensory evaluation of fruits which remained healthy after 30 days of storage was done by an informal panel of 10 judges. Fruit appearance was observed. Fruit samples from each treatment were labelled and laid out for the panel of judges to avoid biased judgment. Appearance index was

determined by scoring graded fruit as very good/no sign of wilting on the entire fruit surface (4); good/no sign of wilting up to 90% of the fruit surface (3); showing sign of wilting up to 45% of the fruit surface (2) or wilted (1) (Babarinsa and Williams, 2002). Wittiness was evident by dark/brown colouration or appearance of mycelium on the fruit surface. Taste index was determined on hedonic scale of 5; where 1 = very sweet; 2 = sweet; 3 = not sweet; 4 = no taste and 5 = inedible (Lamond, 1997).

Statistical Analysis

The data obtained for disease severity were subjected to analysis of variance and where significant, the means were compared at 5% level of probability using New Duncan's Multiple Range Test (SPSS Version 20).

Results

Effect of hot water treatment on *Rhizopus*- induced decay of watermelon fruits during storage

Result obtained on day 5 of storage showed there was no significance difference ($p \geq 0.05$) in the disease severities of control and all the treated fruits. The disease severity of both the control and all treated fruits was 1.00 ± 0.28 (Figure 1). However, on day 10 of storage, there was a significant difference ($p \leq 0.05$) in the disease severity of control when compared with other treated fruits. The disease severity of fruits treated at 50 °C for 2, 3 and 5 min were 1.67 ± 0.14 , 1.33 ± 0.05 and 1.00 ± 0.56 , respectively (Figure 2).

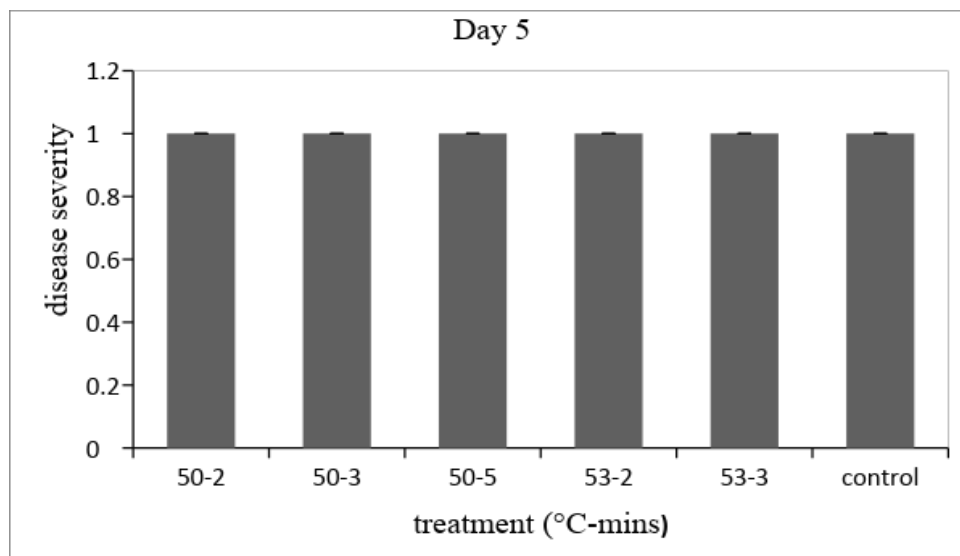


Figure 1. Effect of hot water treatment on disease severity of Watermelon fruits after 5 days of storage.

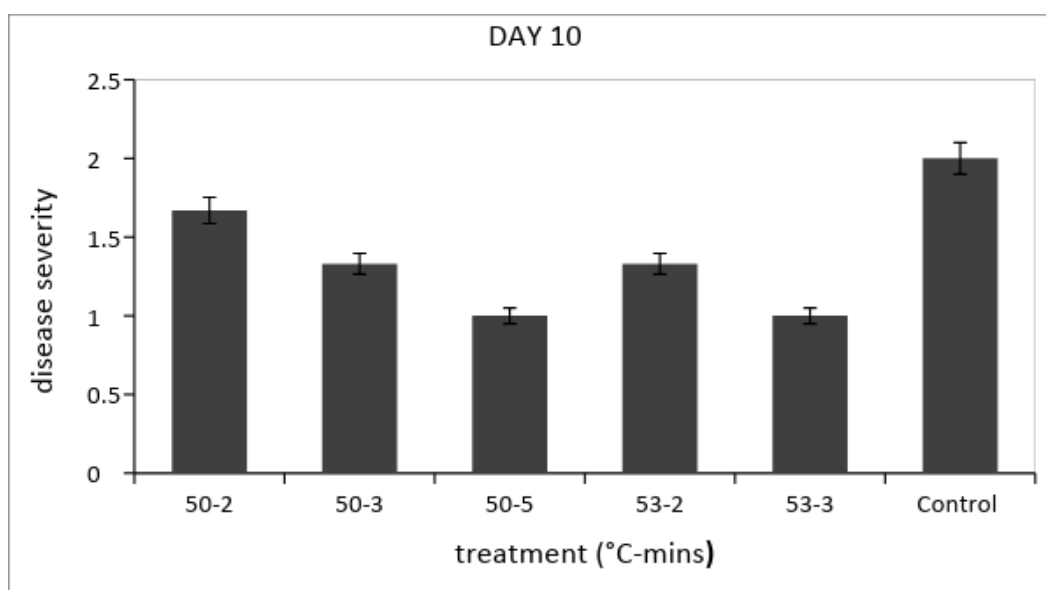


Figure 2. Effect of hot water treatment on disease severity of Watermelon fruits after 10 days of storage.

For treatment at 53 °C for 2 and 3 min, the disease severities were 1.33 ± 0.14 and 1.00 ± 0.57 (Figure 2) while the disease severity of control was 2.00 ± 0.79 (Figure 2). On day 15 of storage, there was a significant difference ($p \leq 0.05$) in the disease severity of control when compared with other treated fruits. The disease severity of

fruits treated at 50°C for 2, 3 and 5 min were 2.33 ± 0.34 , 2.33 ± 0.05 and 1.00 ± 0.56 , respectively (Figure 3). For treatment at 53 °C for 2 and 3 min, the disease severities were 1.33 ± 0.14 and 1.00 ± 0.57 (Figure 3) while the disease severity of control was 3.00 ± 0.79 (Figure 3).

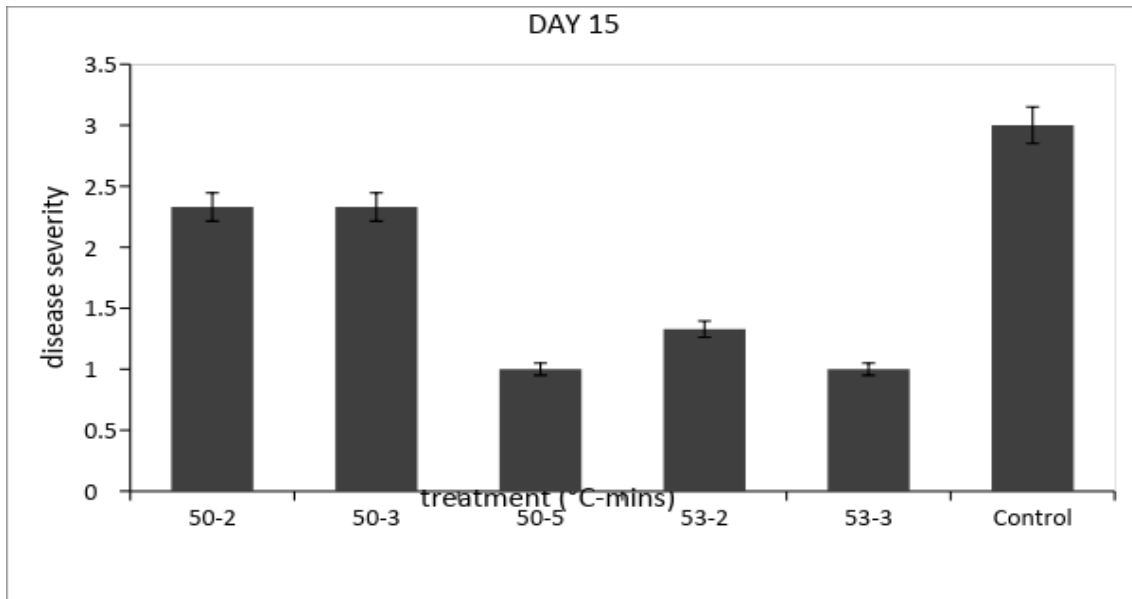


Figure 3. Effect of hot water treatment on disease severity of Watermelon fruits after 15 days of storage.

On day 20 of storage, there was a significant difference ($p \leq 0.05$) in the disease severity of control when compared with other treated fruits. The disease severity of fruits treated at 50 °C for 2, 3 and 5 min were 3.67 ± 0.34 , 3.67 ± 0.05 and 2.00 ± 0.56 , respectively (Figure 4). For treatment at 53 °C for 2

and 3 min, the disease severities were 1.33 ± 0.64 and 1.00 ± 0.97 (Figure 4) while the disease severity of control was 4.00 ± 0.39 (Figure 4). On day 25 of storage, there was also a significant difference ($p \leq 0.05$) in the disease severity of control when compared with other treated fruits.

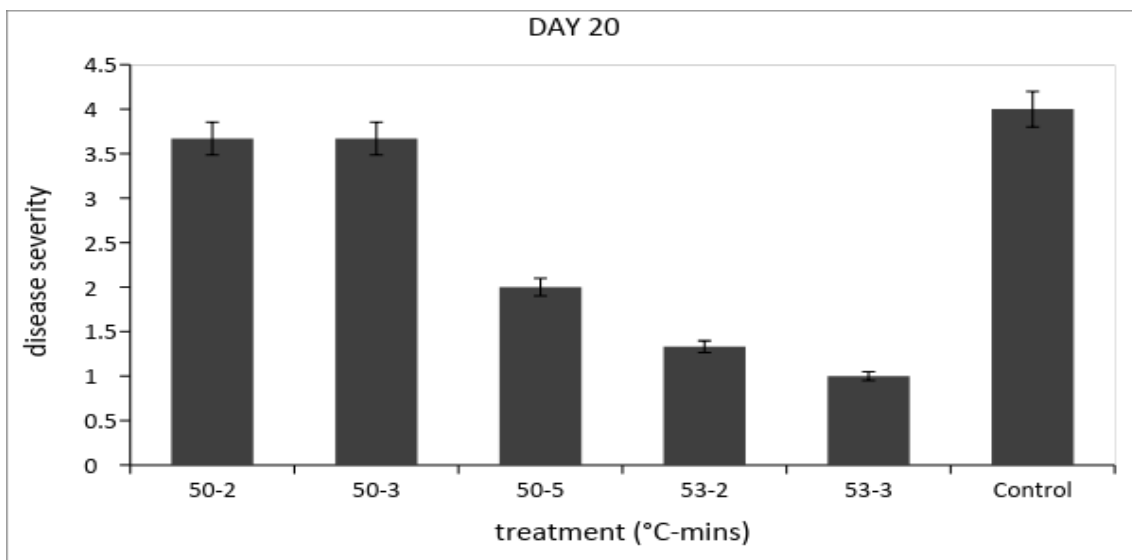


Figure 4. Effect of hot water treatment on disease severity of Watermelon fruits after 20 days of storage.

The disease severity of fruits treated at 50 °C for 2, 3 and 5 min were 4.00 ± 0.44 , 4.00 ± 0.15 and 3.00 ± 0.76 , respectively (Figure 5). For treatment at 53 °C for 2 and 3 min, the disease severities were 3.33 ± 0.24 and 1.00 ± 0.00 (Figure 5) while the disease severity of control was 4.00 ± 0.69 (Figure 5). Hot

watermelon treatment at 53 °C for 3 min was judged the best among all the treatments investigated. By day 25 in storage, the mean appearance and taste of the treated fruits were 3.50 and 2.00, respectively (Table 1), indicating good appearance and sweet taste of the treated fruits.

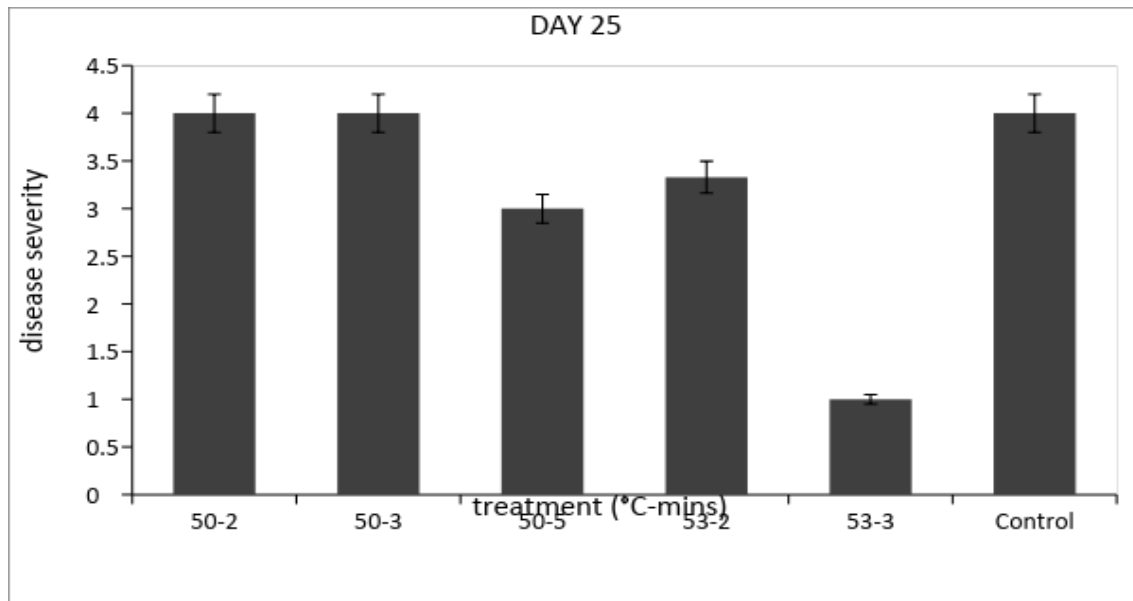


Figure 5. Effect of hot water treatment on disease severity of Watermelon fruits after 25 days of storage.

Table 1. Sensory attributes of the infected watermelon fruits on day 25 of storage after hot water treatment at 53 °C - 3min (most effective treatment).

Sensory attributes	Sensory values	Remarks
Fruit appearance	3.50 ± 0.20	Good appearance
Fruit taste	2.00 ± 0.00	Sweet taste

Each value is a mean of ten replicates.

Discussion

Results obtained on day 25 of storage showed that only fruits treated at 53 °C for 3 min were disease free. Other treatment temperatures and times recorded various degrees of rottenness. Hence, treatment at 53 °C for 3 min can be considered as the most effective treatment among all the treating

temperature-time combinations. The efficacy of this effective treatment cannot be connected with the physiological condition of the fruit which clearly influences the susceptibility of the fruit to decay. More so, part of the efficacy of hot water is due to its potential to remove spores from the wounds and also the direct effect of heat on the pathogenic agent.

This was also the similar observation of Lurie (1998) who reported that immersing orange fruits in hot water for 3 minutes at 53 °C significantly controlled *Penicillium digitatum*. Also, Llus et al. (2001) reported that hot water controlled blue mould in oranges at 50 °C to 55 °C for 3 min each for 14 days of storage.

Similarly, observation from this study showed that treatment at 53 °C for 3 min successfully controlled *Rhizopus* sp for 25 days of storage, although efficacy of hot water treatment depends on the product and is restricted to a narrow range of temperatures and exposure time. Moreover, variety of crop and pre harvest agronomic practices in the field and climatic regions of crops grown could vary with hot water efficiently (De Costa and Erabadupitya, 2005).

Lurie (1998) reported that heat treatment has a beneficial method for control of postharvest problems especially diseases on various horticultural produce. Heat treatments of fruit have been demonstrated to protect against postharvest decay. Heat treatments have been reported to inhibit postharvest fungal germination and growth (Fallik et al., 2003). The benefits of pre storage heat treatment on various horticultural produce especially in controlling pathological problems have been reported (Aborisade and Ojo, 2002; Aborisade and Akomolafe, 2007). Therefore, Use of heat treatment for postharvest control of pathogen on fruits due to ease of preparation, application and being free of risks due to fungicide residues is special importance and regarding the efficacy of 53 °C-3 min for effective decay control as demonstrated in this study, use of 53 °C-3 min is hereby recommended for control of pathogens on water melon fruits.

Conclusion

The results of this research work revealed that all the selected hot water (HW) treatments at 50 °C-2 min, 50 °C-3

min, 50 °C-5 min, 53 °C-2 min and 53 °C-3 min delayed the growth of *Rhizopus* sp for the first 5 days of storage. Interestingly, treatment at 53 °C-3 min showed 100% effectiveness for the control of *Rhizopus* sp on watermelon by 25th day of storage.

Conflicts of interest

The author declares that have no conflict of interests.

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