Postharvest Control of Black Rot on African Star Apple (*Chrysophyllum albidum* L.) Fruits Following Treatment with Hot Water

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Abstract: Black rot is one of the most destructive diseases of African star apple fruits, caused by *Aspergillus niger*. This study aimed to explore the potential of using hot water (HW) in combating black rot on African star apple fruits. After harvest, the fruits were sorted and selected for maturity and uniform homogeneity and thereafter washed, disinfected in 10% NaOCl for 10 minutes and allowed to air dry at 26°C. All the fruits were inoculated with 1 ml of freshly prepared spore suspension (4.05×10^2 spores/ml) of the *Aspergillus niger*. After 12 hours of artificial inoculation, the fruits were later immersed separately in hot water bath at 50^0C for 5, 10, 15 and 20 minutes, while inoculated but untreated fruits served as control. Each treatment lot consisted of three fruits and both treated and control fruits were later stored in sterilized desiccators at 28 ± 2°C and 75 ± 5% relative humidity and observed daily for disease severity. Results obtained shows that both the control and all the HW treated fruits had same disease severity values (1.00 ± 0.00) for the first 10 days of storage, which implied non-diseased nature of the samples. However, as storage duration advanced to day 20, fruits treated at 50°C for 15 and 20 minutes still maintained their non-diseased status compared to the control fruits that already showed complete rottenness. Thus, HW treatments especially at 50^0C could probably be considered as effective non-chemical means that can be explored in fruits preservation.
Key word: Black rot, *Chrysophyllum albidum*, hot water, storage

INTRODUCTION

Chrysophyllum albidum L. is an indigenous fruit tree native to tropical Africa belonging to the Sapotaceae family (Akinmoladun *et al*., 2007). It is commonly referred to as the African star apple fruit and highly valued for its sweet taste and nutritional benefits. For instance, the fruit is very rich in vitamins, calcium and iron (Adisa, 2000). In fact, according to Amusa *et al*. (2003), African star apple fruit is one of the richest sources of vitamin C, about 100 times more than that of oranges and 10 times more than that of guava or cashew. Similarly, the fruit is known to be high in antioxidants, total phenols and flavonoids (Oloyede and Oloyede, 2014). Meanwhile, the presence of high antioxidant contents in the fruits have appreciably justified the increased demand for cherry-based products (Kelley *et al.,* 2018). Its rich sources of natural antioxidants have been established to promote health by acting against oxidative stress-related diseases such as diabetes, cancer, and coronary heart diseases (Adisa *et al.,* 2011).

Despite its benefits*, C. albidum* is vulnerable to diseases that can drastically affect fruit quality and yield. Black rot disease, caused by *Aspergillus* species, is a major concern and can result in severe fruit losses (Adebesin *et al.*, 2009; Adeniyi *et al.*, 2016). In fact, the infection can cause significant pre-harvest and post-harvest losses in the fruit harvest season. Unfortunately, the fruits are always abundant in large quantities on the tree during the harvest season, resulting into substantial postharvest losses because the fruits are mostly under-utilized in Nigeria except for consumption coupled with inadequate storage, pathogen attack and poor processing facilities. The disease manifests its symptomatic appearance as black necrotic lesions on the surface of the fruit, followed by the production of grayish-black spores which begins with small dark spots that rapidly expand, causing the fruit to shrivel (Rungjindamai *et al*., 2014).

Meanwhile, traditional management of black rot in *C. albidum* has relied on cultural practices and synthetic fungicides. However, concerns about the environmental and health impacts of chemical pesticides, along with the potential for pathogen resistance, have

driven the search for alternative, eco-friendly approach. Thus, with the increasing awareness of the adverse effects of chemical pesticides and the need for safer alternatives, physical methods like heat treatment is one of such substitutes that show promise in controlling post-harvest diseases. The common examples of such heat treatments include hot water, hot air and steam or vapour. Fortunately, immersing fruits in hot water is best preferred to either exposing fruits to hot air or spraying fruits with steam because heat transfer in water is more efficient and requires short temperature time durations when compared with others.

In fact, El-Ramady *et al*. (2015) remarked that dipping or fruit immersion in hot water, typically at temperatures between 40-60°C offers great advantage in inhibiting the growth of pathogenic micro-organisms. Additionally, hot water treatment is non-toxic, cheap with ease of application, and has great potential in retaining fruit quality during prolonged storage (Oladele and Jonathan, 2023). Hence, this study was carried out to evaluate the efficacy of hot water treatment (HWT) in controlling black rot pathogen of African star apple (*Chrysophyllum albidum*) fruits during ambient storage. Besides, there is scarcity of scientific literatures documented on the use of hot water for control of post-harvest decay of African star apple fruits at ambient temperature.

MATERIALS AND METHODS

Source of Fruits: Apparently healthy African star apple fruits were obtained from a commercial orchard in Akungba Akoko, Ondo State, Nigeria (7.4740° N, 5.7379° E). The sampled fruits were later sorted and selected for maturity and uniform homogeneity in terms of size (51.122 ± 53.672 g), appearance (skin clearly yellow in colour), free from bruises and diseases. The fruits were thereafter washed with soap and rinsed severally with water and disinfected in 10% sodium hypochlorite for 10 minutes and allowed to air dry at 26°C.

Isolations from Infected African Star Apple Fruits: The rind surface of decayed cherry fruit showing symptomatic appearance of *Aspergillus niger* (evident by formation of black spores on the fruit surface) was cut and a small piece without surface sterilization was placed on plates containing already prepared and solidified potato dextrose agar (PDA). The plates were incubated at 28 ± 2^0C for 72 hours. After incubation, sub culturing was carried out using agar cut of distinct mycelia of the isolates until pure cultures of *Aspergillus niger* were obtained.

Fruit Inoculation and Hot Water Treatment: All the fruits were inoculated with 1 ml of freshly prepared spore suspension (4.05×10^2 spores/ml) of *Aspergillus niger*. After 12 hours of artificial inoculation, the fruits were later placed or immersed separately inside hot water bath (CU – 420 model) at 50^0C for 5, 10, 15 and 20 minutes, while inoculated but not treated fruits served as control. Each treatment lot consisted of three fruits. Both treated and control fruits were later stored in sterilized desiccators at 28 ± 2°C and 75 ± 5% relative humidity and observed daily for disease severity.

Assessment of Disease Severity: Each fruit was visually evaluated for the presence and severity of decay (fruit surface with *Aspergillus* symptoms evident by formation of black spores) and rated on a scale of 1-4 according to Miller *et al.* (1991), but with slight modifications, where 1 = healthy fruit (100% disease free); 2 = slight rot (decay up to 10% of the fruit surface); 3 = moderate rot (decay up to 25% of the fruit surface) and 4 = severe rot (decay up to ≥35% of the fruit surface).

In Vitro Evaluation of Hot Water Treatment on Mycelia Growth of Aspergillus niger: Six millimeters of diameter of mycelium-agar discs plug from three-day old cultures of *Aspergillus niger* on PDA using sterile cork borer were transferred into sterilized test tubes. Then, each disc was separately heated inside water bath (CU-420 Model) with hot water at 50°C

for 5, 10, 15 and 20 minutes. Non-heated mycelium-agar discs plug served as control. Each disc was then placed at the centre of transects drawn on the reverse side of a 9-cm diameter Petri dish containing PDA medium. The plates were incubated at 28±2°C for 3 days in a Gallenkamp incubator after which mycelia growth was measured along transects in two directions at right angles to each other. The mean diameter of growth was obtained and the radius of growth was calculated as a mean of the measurements along the two lines. Three replicates of each treatment were set up.

In Vitro Evaluation of Hot Water Treatment on Spore Germination of Aspergillus niger: Sample aliquots of the spore suspension of *A. niger* containing $4.05x10^2$ spores per ml were transferred into sterilized test tubes and the tubes were heated separately in hot water at 50°C for 5, 10, 15 and 20 minutes each. Non-heated spores served as control. One drop of the heated spore suspension was then placed on a thin film of sterile PDA on microscope slide, placed in a Petri dish containing sterile filter paper moistened with sterile water and incubated for 10 hours at 28°C. Percentage of germinated spores was then determined in 50 microscope fields and the means calculated for each treatment.

Stastical Analysis: The data obtained from mycelial growth and percentage spore germination was subjected to one way ANOVA; and the means were compared at 95% confidence interval using Tukey's HSD Test (SPSS version 17).

RESULTS AND DISCUSSION

Effect of Hot Water (HW) Treatment on Disease Severity of African Star Apple Fruits Infected with Spores of *Aspergillus niger*

The effect of hot water (HW) treatment on disease severity of African star apple fruits infected with spores of *Aspergillus niger* during ambient storage for 5 days (Figure 1) shows that both the control and all the HW treated fruits had the same disease severity values (1.00 ± 0.00), which implied that the fruits were all non-diseased. Remarkably, the same trend of results was also observed as storage duration proceeded to day 10 (Figure 2) except for control fruits whose severity values had significantly increased to 2.00 ± 0.00, indicating slight rottenness. However, by day 15 of storage, only fruits treated at 50°C for 15 and 20 minutes still maintained their non-diseased statuses compared with other treated fruits. For instance, the severity values of fruits treated at 50°C for 5 and 10 minutes and the control had significantly increased to 2.00 ± 0.00 (slight rottenness) and 3.00 ± 0.00 (moderate rottenness) respectively (Figure 3). Meanwhile, as storage duration became extended to day 20 (Figure 4), same severity values of 1.00 ± 0.00 were observed for fruits treated at 50°C for 15 and 20 minutes. This consequently showed that such fruits still maintained their healthy status in comparison to other treated fruits at 50°C for 5 and 10 minutes that manifested slight rottenness as evident by their severity values of 2.00 ± 0.00 and the control fruits that showed complete rottenness because the severity values had significantly increased to 4.00 ± 0.00.

Findings in this present study reveals the efficacy of hot water treatment at 50°C for 5, 10, 15 and 20 minutes in controlling black rot in African star apple fruits during ambient storage. At least, all the HW treated fruits were healthy as observed during the first 10 days of storage, without actually showing any sign of decay / rottenness compared with the untreated fruits that had started manifesting slight rottenness. Even when the storage duration advanced to day 20, fruits treated at 50°C for 15 and 20 minutes still maintained their non-diseased status and manifesting no symptom of *Aspergillus* infection. This demonstrates the effectiveness of the HWT at 50°C in extending the shelf life of the African star apple fruits by completely inhibiting the black rot pathogen.

This is consistent with the works of Martins *et al*. (2010), who reported complete inhibition of *Collectotrichum*

gloeosporioides on papaya fruits when the fruits were immersed for 20 minutes at 50°C. Also, Essat (2002) buttressed the efficacy of hot water treatment in fruit preservation by dipping cantaloupe fruits in hot water at ≥ 50°C, but less than < 60°C. The author found out that the overall quality of the treated fruits in terms of decay, firmness and appearance was significantly better than the untreated fruits. Similarly, Arauz (2000) demonstrated that post-harvest HW dips at > 50°C for 1 - 5 minutes significantly reduced anthracnose incidence in mangoes.

Evaluation of Hot Water Treatment on Mycelia Growth and Spore Germination of *Aspergillus niger In Vitro*

The *in vitro* effect of HWT on mycelia growth of black rot pathogen (Table 1) shows that the mycelial growths of the black rot pathogen (*Aspergillus niger*) treated at 50°C for 5, 10, 15, 20 minutes and the control were not significantly different (P ≥ 0.050) from each other. In this study, the growth values ranged from 1.50 ± 0.14 cm of mycelia treated at 50°C for 20 minutes to 0.95 ± 0.03 cm for mycelia treated at 50°C for 5 minutes. However, the *in vitro* effect of HWT on spore germination of the black rot pathogen (Table 2) reveals that percentage spore germinations following the various HWT were significantly different (P ≤ 0.05) from the control which ranged from 38.00 ± 2.30 cm for unheated spores (control) to 6.25 ± 1.93 cm for spores heated at 50°C for 20 minutes.

Although, the mycelia of the black rot pathogen was not affected by HWT at 50°C for 5, 10, 15 and 20 minutes compared with the unheated mycelia, the same HW temperature–time combinations exhibited a signification reduction in spore germination of *A. niger* compared with the non-heated spores. This is in agreement with the works of Hali *et al.* (2020) who reported that germination of fungal spores are effectively reduced by heat treatments. In fact, the works of Oladele and Jonathan (2023) further proved that no fungal spores can survive heating temperatures ≥ 45°C. Therefore, the *in vitro* inhibition of germination of the *Aspegillus* spores by HWT as observed in this study suggests why treatments at 50°C for 5, 10, 15 and 20 minutes extended the shelf life of the African star apple fruits by delaying the manifestation of the *Aspergillus* symptoms for at least 10 days.

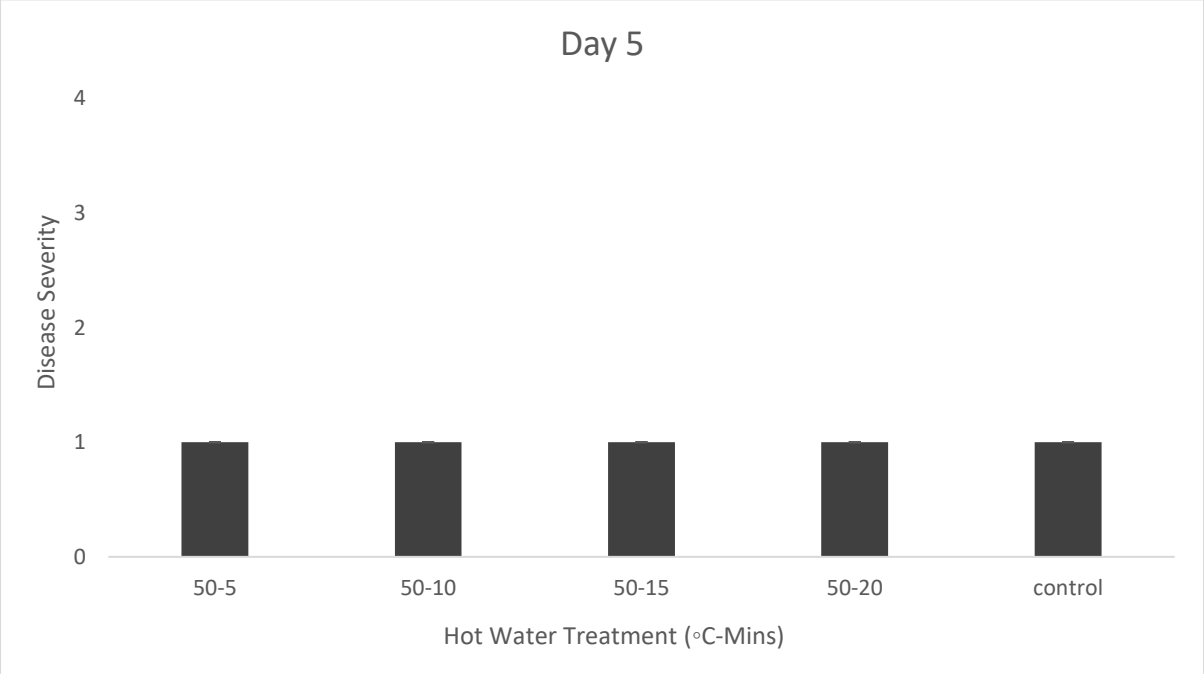

Figure 1: Effect of Hot Water Treatment on *C. albidum* Inoculated with Spores of *A. niger* and Stored for 5 Days at 28 ± 2°C

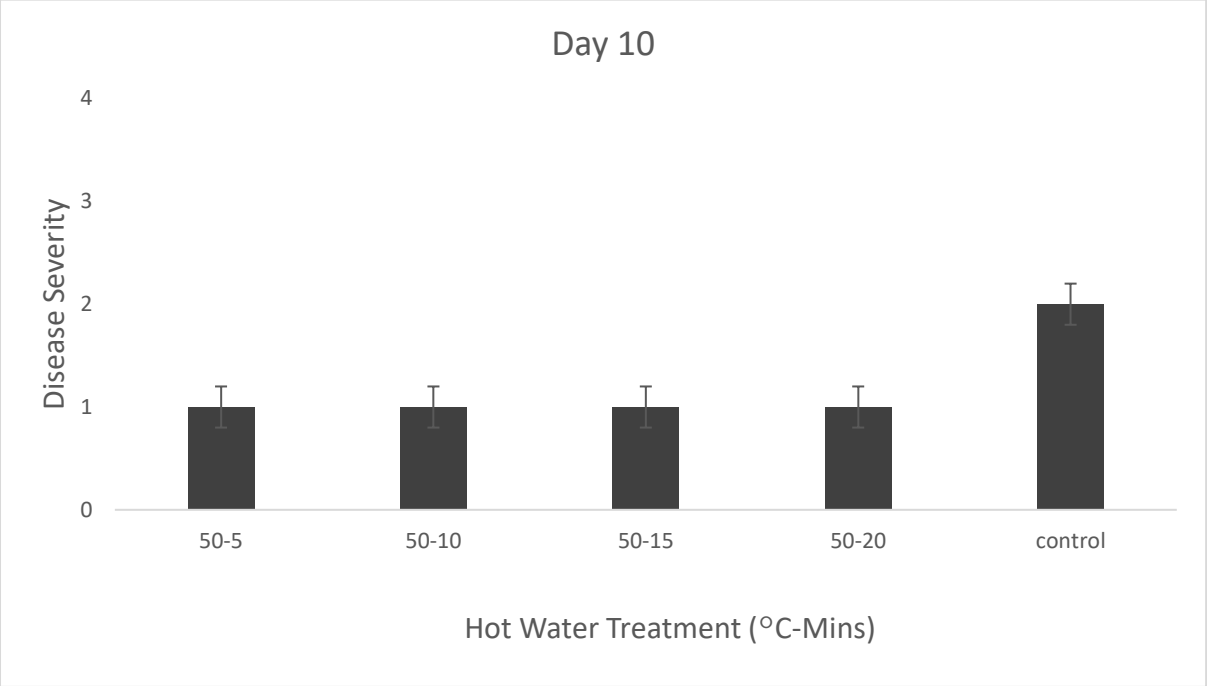

Figure 2: Effect of Hot Water Treatment on *C. albidum* Inoculated with Spores of *A. niger* and Stored for 10 Days at 28 ± 2°C

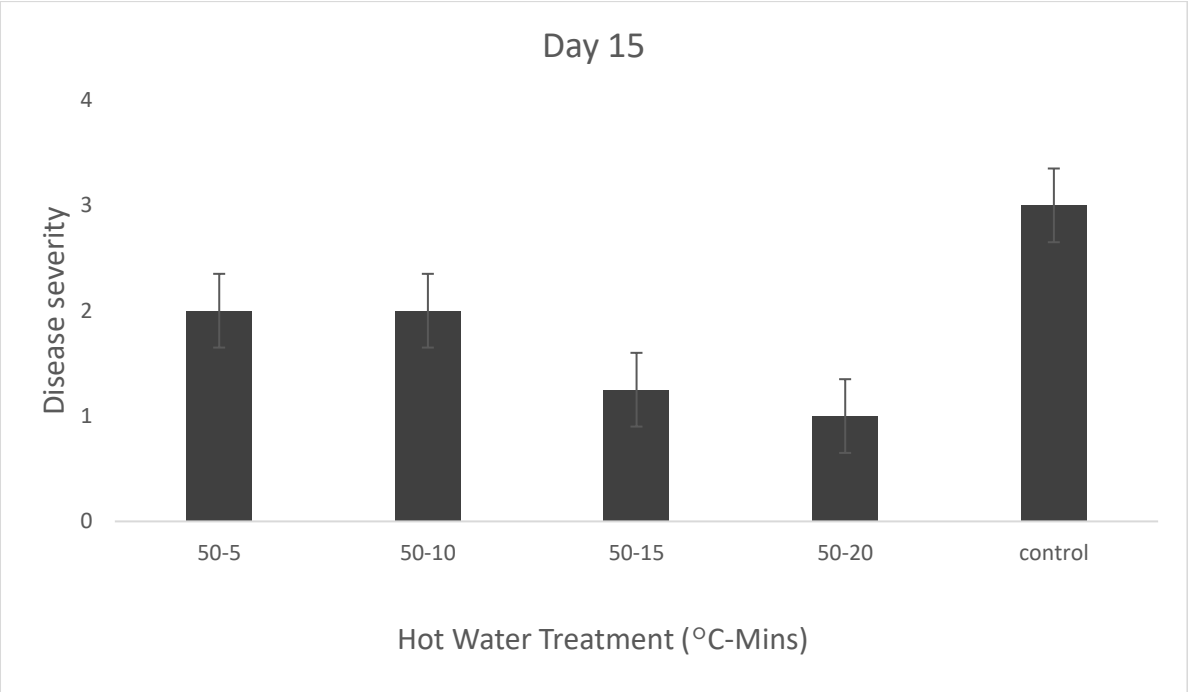

Figure 3: Effect of Hot Water Treatment on *C. albidum* Inoculated with Spores of *A. niger* and Stored for 15 Days at 28 ± 2°C

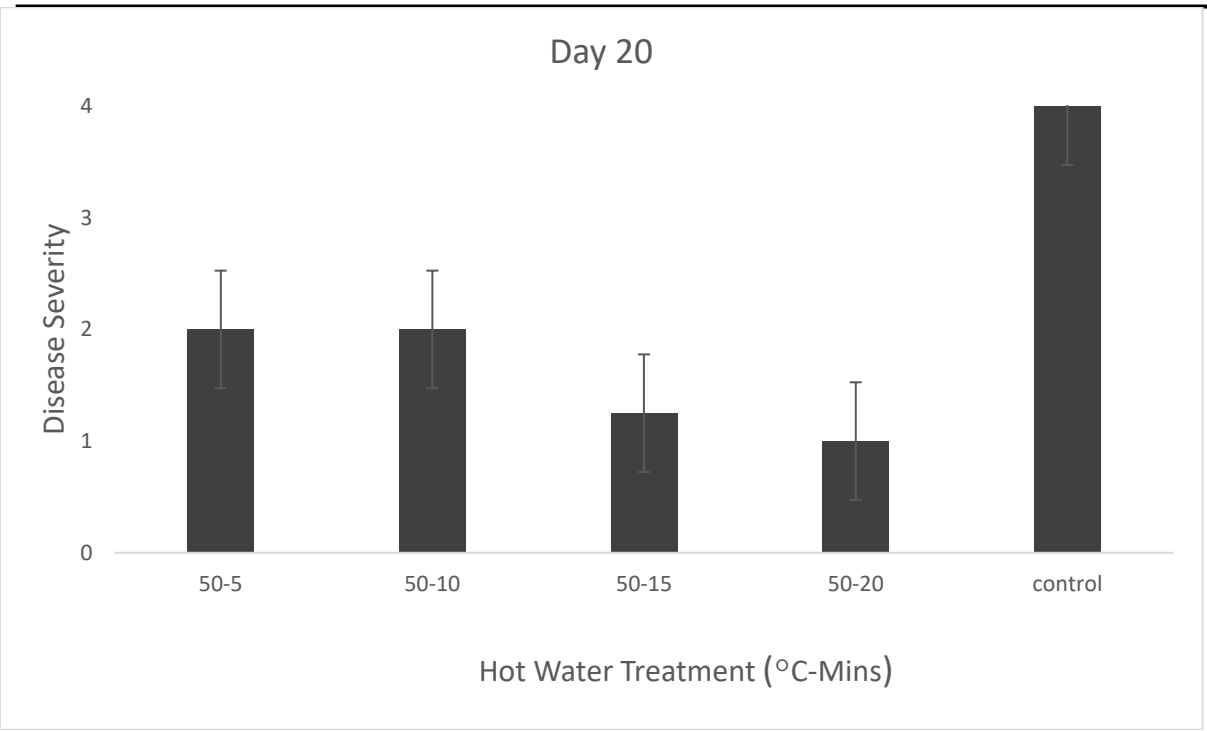

Figure 4: Effect of Hot Water Treatment on *C. albidum* Inoculated with Spores of *A. niger* and Stored for 20 Days at 28 ± 2°C

Table 1: Effect of Hot Water Treatment on Mycelial Growth of *Aspergillus niger In vitro*

Hot Water Treatment (^{0}C- mins)	Mean Mycelia Growth (cm)
50 – 5	0.95 ± 0.03^a
50 – 10	1.00 ± 0.00^a
50 – 15	1.00 ± 0.00^a
50 – 20	1.50 ± 0.14^a
Control	1.20 ± 0.30^a

Means followed by same letters along the column are not significantly different (P ≥ 0.05). Values are expressed as means ± SE (Standard error of means).

Table 2: The Effect of Hot Water Treatment on Spore Germination of *Aspergillus niger In vitro*

Hot Water Treatment (^{0}C- mins)	Germinated Spores (%)
50 – 5	14.33 ± 3.52^a
50 – 10	11.500 ±1.50^a
50 – 15	9.67 ± 4.06^a
50 – 20	6.25 ± 1.93^a
Control	38.00 ± 2.30^b

Means followed by same letters along the column are not significantly different (P ≥ 0.05). Values are expressed as means ± SE (Standard error of means).

CONCLUSION

In this study, HWT at 50°C for 5, 10, 15 and 20 minutes was found to keep the fruits healthy, causing no decay for at least 10 days and such could be exploited as a biopesticide, and non-toxic. This could serve as safer substitute to synthetic fungicides in fruit preservation particularly African star apple fruits.

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