SALINE HOT SPRING WATER TREATMENT TO CONTROL POST-HARVEST FUNGAL DISEASE ON MANGO CV. 'IRWIN'

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ABSTRACT

Japanese Mango cv. 'Irwin' is affected by two post-harvest diseases, anthracnose and stem-end rot, which are currently managed by chemical applications. Although effective, misuse of pesticides may lead to damage to the environment, human health, and potential resistant development by pathogens. Due to these, new strategies need to be developed for the effective management of these diseases. In this study, saline hot spring water (SHSW) was evaluated as a medium for hot water treatment to control post-harvest diseases in inoculated and non-inoculated mangoes while maintaining fruit quality in terms of weight loss, fruit hardness, peel color, and soluble sugar content. Non-inoculated mangoes harvested in Miyako Island, Japan, were dipped in SHSW at 60 °C for 1 min and sterilized distilled water (SDW) at the same conditions, cooled under running tap water for 10 min, and then stored at 25-27°C for 12 days in June 2019. Treated mangoes did not show symptoms of anthracnose at 6 days post-treatment but showed mild symptoms of anthracnose at 12 days post-treatment, while mild symptoms of stem-end rot appeared from 6 days post-treatment. The hot treatment with SHSW did not negatively affect the mango fruit quality compared to the control and SDW after 6 days. Furthermore, fungi causing anthracnose and stem-end rot were inhibited in inoculated mangoes followed by treatment at 60 °C for 1 min and stored at 25-27 °C. Hot water treatments with SHSW at 60 °C for 1 min effectively inhibited the quiescent (non-inoculated) and superficial (inoculated) infections of anthracnose without affecting fruit quality but these did not inhibit the quiescent infection of stem-end rot.

Key words: Anthracnose, natural resource, physical control, Okinawa, stem-end rot.

INTRODUCTION

Mango (*Mangifera indica* L.) is a tropical and subtropical tree whose climacteric fruit is appreciated worldwide. In 2019, Japan produced approximately 3,519 tons of mango fruit (Portal Site of Official Statistics of Japan 2022), mainly cv. 'Irwin' in greenhouse conditions in the southern prefectures of Kagoshima, Miyazaki, and Okinawa.

In Japan, as in other areas, mango fruit is affected by post-harvest diseases, principally anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletotrichum*

acutatum J.H. Simmonds (Taba et al. 2004; Takushi et al. 2013a; Takushi et al. 2013d), and stem-end rot (SER), caused *by Lasiodiplodia theobromae sensu stricto* (Pat.) Griffon & Maubl. (Takushi et al. 2013c), *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (Takushi et al. 2017), and *Diaporthe* sp. (Ajitomi et al. 2019).

The use of fungicides during the preharvest stage of cultivation is the primary method for controlling both diseases in Japan (Takushi et al. 2013b; Takushi et al. 2018), with six active ingredients (captan, iminoctadine, fludioxonil, azoxystrobin, kresoxim-methyl, and mancozeb) approved for the control of anthracnose, and three active ingredients (procymidone, copper sulfate, and *Bacillus subtilis* strain Y1336) approved for the control of SER (MAFF 2022). However, some of these fungicides may have side effects on the environment and consumers' safety (Brandhorst and Klein 2020; Brock et al. 2020; Ostby et al. 1999), including the potential development of resistance to these active ingredients by fungal causal agents (Al-Jabri et al. 2017; Chen et al. 2020; Dowling et al. 2020; Rehman et al. 2015; Schnabel et al. 2021).

As an alternative, physical control techniques such as hot water treatments have been developed as a postharvest treatment for fungal control and insect disinfestation (Asio and Cuaresma 2016), which varies in temperature and time of dipping depending on the mango variety and fruit size (Arauz 2000), showing an efficacy of 60-80% for control of anthracnose (McGuire 1991), while improving general appearance of fruits (Prusky et al. 1999). Immersion time can be 1h or more when the temperature is below 50 °C, while treatments can be less than 4 min at temperatures above 50°C (Lurie 1998). Along with the previous, protocols were developed for each case, for example, mango cv. ´Sindri´ had been treated at 45 °C for 75 min (Anwar and Malik 2007), cv. ´Keitt´ at 50 °C for 30 min (Djioua et al. 2008) and 52 °C for 5 min (Kumah et al. 2011), cv. ´Carabao´ at 47-48 °C for 15 min (Kitma and Esguerra 2009), 53 °C for 20 min (Alvindia and Acda 2015), 55°C for 10 min (Montecalvo et al. 2019), and 60 °C for 35 sec (Esguerra et al. 2004, Pasilan et al. 2020), cv. ´Kensington´ at 53 °C for 5 min followed by vapor heat at 47 °C for 15 min (Jacobi and Giles 1997), cv. ´Diab´ at 50 °C for 10 min (Yousef et al. 2019), cv. ´Tommy Atkins´ at 50 °C for 3 min (Gutiérrez-Alonso et al. 2004), and cv. ´Ataulfo´ at 46 °C for 70 min (Ochoa-Rosas et al. 2020).

In the case of Japanese mango, cv. ´Irwin´ was treated at 52°C for 60-90 min for inhibition of anthracnose on inoculated mangoes, without affecting the fruit quality (weight loss, moisture content and soluble solid content) for 14d when stored at 13°C and 90% relative humidity, showing tolerance to heat injury (Hasbullah et al. 2001); and at 60°C for 40 sec for inhibition of anthracnose on inoculated mangoes, being as effective as treatment at 52°C for 20 min followed by cooling for 10 min, showing mild symptoms after 4d (Teruya et al. 2012). While both studies clearly demonstrated the effectiveness of hot water treatments on mango anthracnose in Japan, neither have reported the effects of hot water treatments on quiescent infections of anthracnose, which occurs in Japanese mango during May and June (De la Cruz Padilla et al. 2020), nor on direct contact and quiescent infections of SER in Japanese mango cv. "Irwin".

Although the hot water treatment's effectiveness, the treatment requires heating of the water, which could become prohibitive with the rising cost of oil and liquefied petroleum gas in international markets, as the local electricity generation in the Okinawa Prefecture relies in fossil fuels (Ministry of Economy, Trade and Industry-Agency for Natural Resources and Energy 2022). Due to this, the use of saline hot spring water (SHSW), a local natural resource, is proposed for the implementation of hot water treatments in Japanese mango, for which there is no previous study on either its effects on the fruit quality and the disease inhibition of postharvest diseases of mango.

In this study, the effect of hot water treatment (by dipping) using SHSW, a natural resource in Miyako Island, Okinawa Prefecture, was evaluated on the inhibition of non-inoculated and inoculated post-harvest fungal pathogens (anthracnose and SER) and mango fruit quality.

MATERIALS AND METHODS

Saline hot spring water. SHSW was collected from a pilot well in Bora, Gusukube in Miyako Island, Okinawa Prefecture, Japan in 2019, and stored in the lab for later use. According to the "Document of Mineral Spring Analysis No.2014-00145-C01", conducted following the "Standard Methods for Analysis for Mineral Springs" by the Ministry of Environment (2014) of Japan, the *in-situ* temperature of the HSW was 68.7 °C, with an *in-situ* pH of 7.3 and a laboratory pH of 7.2 at 21.6 °C. Based on the chemical composition of the collected HSW and following the criteria of the Ministry of Environment of Japan, the spring water was categorized as "Sodium chloride high salt hot spring", in which the quantity of dissolved substances (excluding gases) exceeds 1,000 mg in 1kg of spring water, and the main anion is chloride ion (Cl^-). The total cation content was 9,920 mg/kg, predominantly sodium ion (Na^+) with 9,270 mg/kg, and the total anion content was 15,855.7 mg/kg, primarily Cl^- with 15,300 mg/kg.

Mango fruit. For the experiments, mature and healthy-looking mangoes cv. 'Irwin' harvested in June 2019 on Miyako Island, Okinawa Prefecture, were used, 60 for hot water treatments in non-inoculated mangoes, and 27 for inoculation followed by treatments.

Pathogens. Four fungal isolates were selected for inoculation (Fig. 1): three *Colletotrichum* spp. isolates (CG1, CG2, and CG3) and one *Lasiodiplodia* spp. isolate (LT). *Colletotrichum* isolates were obtained from anthracnose lesions from 3 different mango fruits collected in Miyako Island, while *Lasiodiplodia* isolates were obtained from a mango fruit showing advanced symptoms of SER. Slices of lesions were put on 1.5% water agar plates and incubated for 1-2 days. Single hyphae growing from the slices were transferred to 2% malt extract agar medium and incubated for 7 days at 27 °C. For the inoculation assay, the fungal isolates were incubated at 27 °C in potato dextrose agar (PDA) for a week.

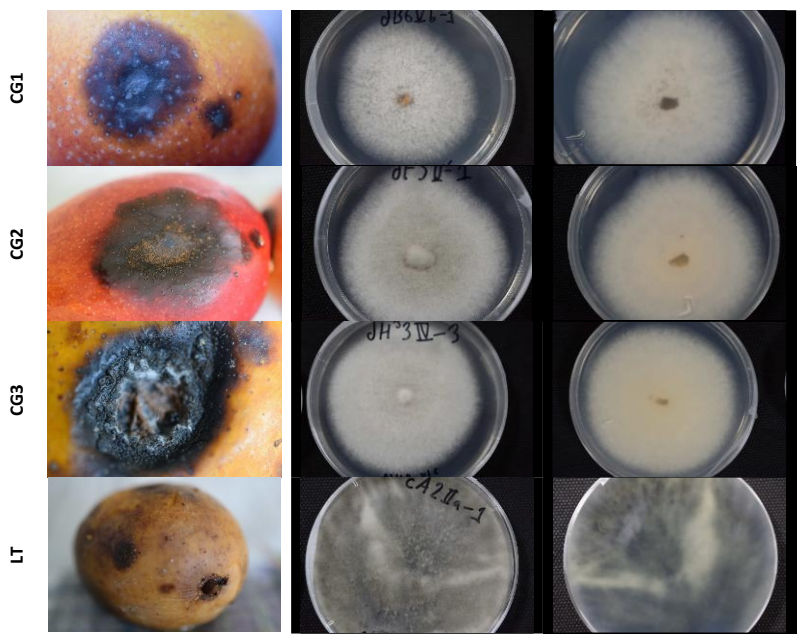

Figure 1. Fungal isolates from mangoes harvested in Miyako Island, Japan. At left, the sample materials from which the fungi were isolated. At center and right, the front and behind of each fungal isolate in PDA plates after 7d at 27°C. CG1, CG2, and CG3 correspond to three different isolates of *Colletotrichum* spp., while LT corresponds to an isolate of *Lasiodiplodia* spp.

Hot water treatment of non-inoculated mangoes for disease inhibition and fruit quality. The mangoes were divided into five groups (12 fruits/replicates per group): non-treated (control), SHSW at 60 °C for 60 sec followed by cooling under running tap water (wc = with cooling) for 10 min (SHSW 60 °C wc), sterile distilled water (SDW) at 60 °C for 60 sec followed by cooling for 10 min (SDW 60 °C wc), SHSW at room temperature (RT, 26~28 °C) for 10 min (SHSW RT) and SDW at room temperature for 10 min (SDW RT).

For the hot water treatments, SHSW and SDW were heated in a water bath (IWAKI Thermo Bath THB-6D, 20cm diameter x 12cm depth) at 60±0.2 °C. The surface temperature of 5 hot-treated mangoes with HSW and SDW was measured at three sections (two lateral and the stem area) with a handheld infrared thermometer at four timepoints: before treatments, just after treatment, 10 min, and 60 min following the treatment. The mango fruits were dipped into hot water for 60 sec without water circulation. After treatments, the mangoes were stored at 26-28 °C in different boxes sorted by the treatment. Mangoes were evaluated for disease severity according to "Evaluation of disease severity", and for fruit quality according to "Evaluation of mango fruit quality".

Disease severity. Disease severity was evaluated using a 5-point scale, where: 0 = no symptoms, 1 = less than or equal to 10% of the diseased area, 2= 11 ~ 25% of the diseased area, 3 = 26 ~ 50% of the diseased area, and 4 = more than 50% of the diseased area. The mango fruit surface was divided into three sections for assessment: two longitudinal faces (X, Y), corresponding to the mango fruit cheeks, and one lateral face (Z), corresponding to the stalk or stem of the fruit. Six mangoes per treatment were selected for the evaluation, and the value was assessed at 6 days and 12 days after treatments.

Mango fruit quality.

Weight loss. The weight of six randomly selected mangoes of each group was measured at day 0 using a digital balance, with weight values expressed in grams (g). Later, 3 of those selected mangoes were measured 6 days after treatments, and the remaining 3 were measured at 12 days.

From the measurements, the percentage of weight loss (WL [%]) was computed using the following formula:

$$WL(\%) = \frac{(W_0(g) - W_d(g)) * 100}{W_0(g)},$$

where W_0 is the initial weight at day 0, and W_d is the weight at day 6 or 12.

Fruit hardness. Fruit hardness was measured using a hand-held fruit hardness tester (Fujiwara Scientific Fruit Hardness Tester KM-1, Tokyo, Japan) at 0, 6 and 12 days after treatments. Three fruits per treatment were selected and 4 measures (in kg/cm^2) were taken from the area near the equator of each fruit.

Peel color differences. Peel color was determined using a colorimeter (Konica Minolta Chroma Meter CR410, Tokyo, Japan) under the CIELAB color space configuration (where L represents the luminance, a* denotes the red/green hue, and b* is the yellow/blue hue). The values were measured just after the treatment and 6 days after treatment. Later, peel color differences were calculated using the following formulas: $\Delta L = L - L_0$, $\Delta a^* = a^* - a_0^*$ and $\Delta b^* = b^* - b_0^*$. In which L_0, a_0^*, and b_0^* are the values after treatments, and L, a^*, and b^* are the values at 6 days. The total color difference (ΔE*) was calculated using the following formula:

$$\Delta E^* = \sqrt{(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}.$$

The CIELAB values were then converted into LCh color space values for chroma (C^*) and hue (h) using the following formulas:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \text{ and}$$

$$h = \left(\arctan\left(\frac{b^*}{a^*}\right) \Rightarrow \arctan\left(\frac{b^*}{a^*}\right) \geq 0\right) \vee \left(arctan\left(\frac{b^*}{a^*}\right) + 360° \Rightarrow arctan\left(\frac{b^*}{a^*}\right) < 0\right).$$

The difference between each measure of C^* and h and the initial values were calculated as: $\Delta C^* = C^* - C_0^*$ and $\Delta h = h - h_0$.

Soluble sugar content. The soluble sugar content (°Bx) was measured from the mango fruit pulp juice with an analog handheld refractometer. Three fruits per treatment were assessed at 6 days after treatment, and the remaining were assessed at 12 days after treatment.

SHSW treatment of mango fruits inoculated with *Colletotrichum* spp. and *Lasiodiplodia* spp.. Twenty-seven mangoes were divided into 9 groups (3 fruits/replicates per group): one control consisting on mangoes treated by dip in hot water treatment (HWT) at 60 °C for 1 min with SHSW, 4 groups of mango fruits inoculated with post-harvest disease causal agent followed by HWT with SDSW (T1: CG1+HWT, T3: CG2+HWT, T5: CG3+HWT and T7: LT+HWT), and 4 groups of mango fruits with inoculation only (T2: CG1, T4: CG2, T6: CG3 and T8: LT).

The mangoes were inoculated by attaching two PDA fungal plugs of approximately 1 cm diameter for 24h on the mango fruit surface. In the case of the control group, non-inoculated PDA plugs were attached. Depending on the treatment group, the mangoes were soaked in SHSW at 60 °C for 1 min, without subsequent cooling. After the treatments, the mangoes were stored in boxes separated by treatment groups at 25~27 °C. The weight of the fruits of each mango fruit was assessed at 0 days, 8 days, and 16 days after treatments, from which weight loss (%) at 8 days and 16 days was calculated. Each disease spot in the inoculated areas was measured at 8 and 16 days after treatment. The fungi were reisolated from the diseased spots to fulfill Koch's postulates.

Statistical analysis. The data were analyzed using the Real Statistics Resource Pack (release 7.3) (Zaiont, 2020). The Shapiro-Wilk and D'Agostino-Pearson tests were used to determine whether the data were normally distributed. For the normally distributed datasets, parametric tests were applied: one-way ANOVA for single-factor comparisons and two-way ANOVA for two-factor comparisons, both followed by Tukey's HSD test. For the non-normally distributed datasets, non-parametric tests were applied: the Kruskal-Wallis' test and Dunn's test for single-factor comparison, and the Scheirer-Ray-Hare test and Tukey's HSD test for two-factor comparisons.

RESULTS AND DISCUSSION

Fungal pathogens in inoculated mango fruits. The inoculated and treated mangoes (T1, T3, T5, T7) were less affected by the pathogens than the inoculation-only mangoes (Table 1, Fig. 2 and Fig. 3), where T4 (CG2) and T8 (LT) had the largest disease spots after 8 days. T8 exhibited the highest weight loss and was significantly different from the other samples after 16 days. Measurements of the disease spots were not taken on T8 fruits at 16 days due to decay. In the CG-inoculated groups, the disease spot size (Table 1 and Fig. 3) was larger in the T4 (CG2) and T6 (CG3) fruits than in the T2 (CG1) fruits 16 days after treatments. The disease spot left by the pathogens in the hot water treated mangoes did not grow any further, suggesting that the inoculated pathogens were inhibited by the hot water treatment (Fig. 3).

Figure 2. Inoculated mangoes followed by hot water treatments (HWT) with SHSW. Control (HWT), inoculation followed by HWT (T1: CG1+HWT, T3: CG2+HWT, T5: CG3+HWT, and T7: LT+HWT), and inoculation-only (T2: CG1, T4: CG2, T6: CG3, and T8: LT) mangoes after 7 and 14 days.

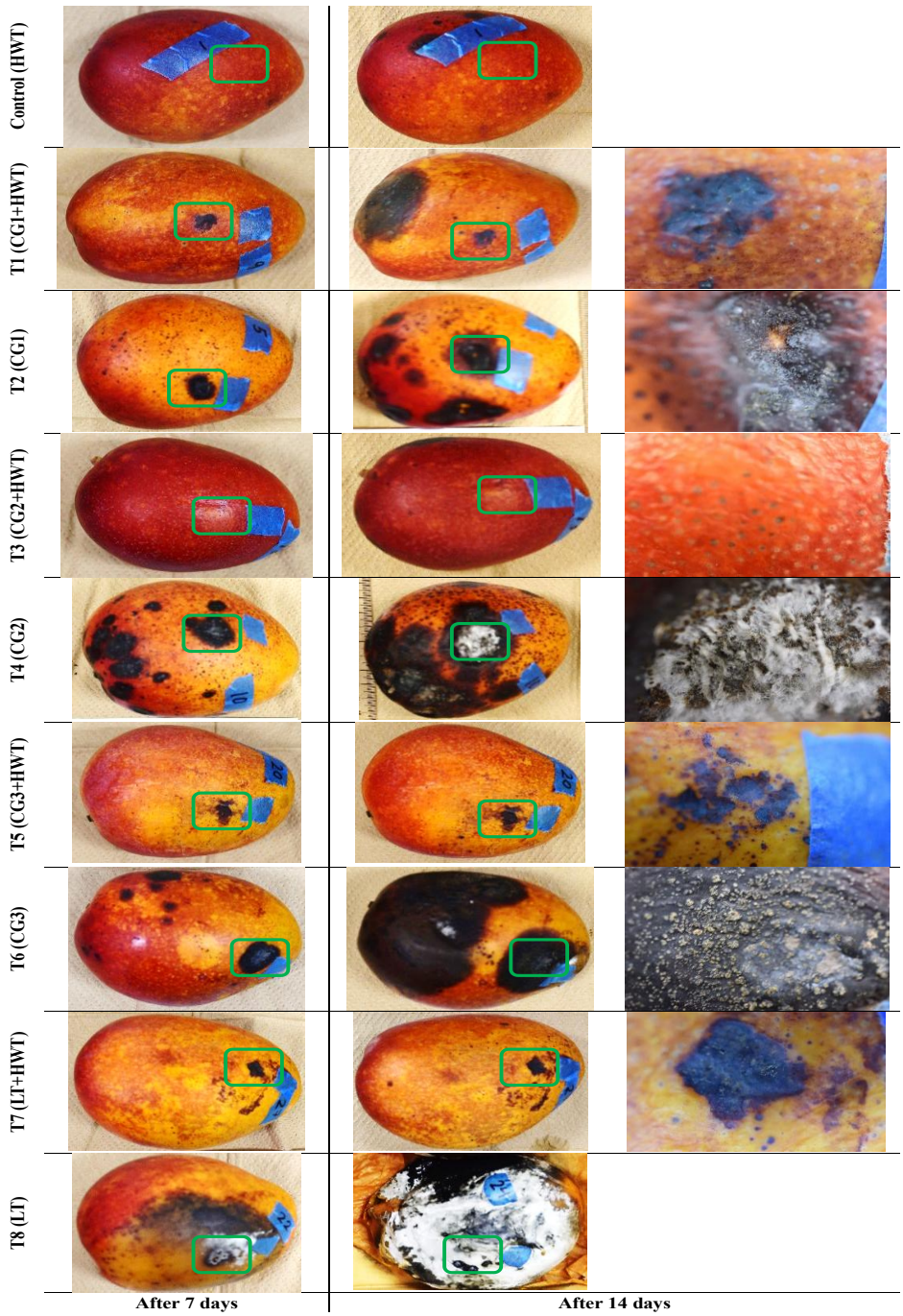

Figure 3. Disease spot on control, inoculation-only mangoes with *Colletotrichum* spp. (CG1, CG2 and CG3) and *Lasiodiplodia* spp. (LT), and inoculation followed by hot water treatment (HWT) with SHSW. At left, mangoes from 7d after treatments; at center, mangoes from 14d after treatments; and at right, closeup of the inoculated areas after 14 days (photos of control and T8 were not taken). Green boxes indicate the inoculated area.

Table 1. Mango fruit weight loss and disease spot size (mean ± SEM) treated with hot water treatment (HWT) with SHSW on inoculated mangoes.

(unit: %, cm)

Treatments	**Weight loss (n=3)** [1,2]			**Disease spot size (n=6)** [2]	
	Day 8	**Day 16**		**Day 8**	**Day 16**
Control	7.22±0.19 [a]	14.2±0.31 [b]	B	-	-
T1 (CG1+HWT)	4.2±2.4 [a]	11.39±1.52 [b]	B	0.35±0.17 x 0.31±0.14 [D]	0.35±0.17 x 0.31±0.14 [D]
T2 (CG1)	5.78±0.41 [a]	14.68±1.22 [b]	B	1.78±0.17 x 1.31±0.1 [B]	2.3±0.08 x 2.06±0.07 [B]
T3 (CG2+HWT)	6.68±0.96 [a]	13.75±1.81 [b]	B	0.48±0.22 x 0.48±0.22 [D]	0.48±0.22 x 0.48±0.22 [D]
T4 (CG2)	6.59±0.65 [a]	18.88±4.76 [ab]	B	2.65±0.23 x 2.11±0.12 [A]	3.5±0.24 x 3.06±0.23 [A]
T5 (CG3+HWT)	8.05±0.59 [a]	15.83±0.7 [b]	B	0.8±0.33 x 0.6±0.24 [CD]	0.8±0.33 x 0.6±0.24 [CD]
T6 (CG3)	6.74±0.18 [a]	15.34±1.41 [b]	B	1.71±0.26 x 1.43±0.26 [B]	3.4±0.15 x 2.88±0.27 [A]
T7 (LT+HWT)	7.49±1.5 [a]	15.44±2.37 [b]	B	1.03±0.18 x 1.26±0.22 [BC]	1.03±0.18 x 1.26±0.22 [C]
T8 (LT)	7.57±0.63 [a]	32.98±6.01 [a]	A	8.08±1.61 x 7.08±1.33 [A]	- [3]

[1] In the same column, different letter by row means significant difference ($p < 0.05$) under an one-way ANOVA test followed by Tukey's HSD test.
[2] Different capital letters by row means significant difference ($p<0.05$) under a two-way ANOVA test followed by Tukey's HSD test.
[3] Samples completely covered by the pathogen and with advanced decay.

Disease incidence of non-inoculated mangoes. The symptoms of SER and some mild symptoms of anthracnose were observed in the control mango fruits at 6 days after treatment. Symptoms of SER were distinguished from anthracnose by their size and watery appearance as streaking in the conductive tissues is a characteristic sign of SER (Fig. 4).

Hot water treated mangoes (SHSW 60°C wc and SDW 60 °C wc) showed significant suppression when compared with control (Table 2 and Fig. 4), with mild symptoms of SER, after 6 days. Furthermore, the hot water treated mangoes (HSSW 60°C wc and SDW 60 °C wc) showed significant suppression compared with control and non-heated treatments (Table 2 and Fig. 4), with symptoms of SER and mild symptoms of anthracnose after 12 days.

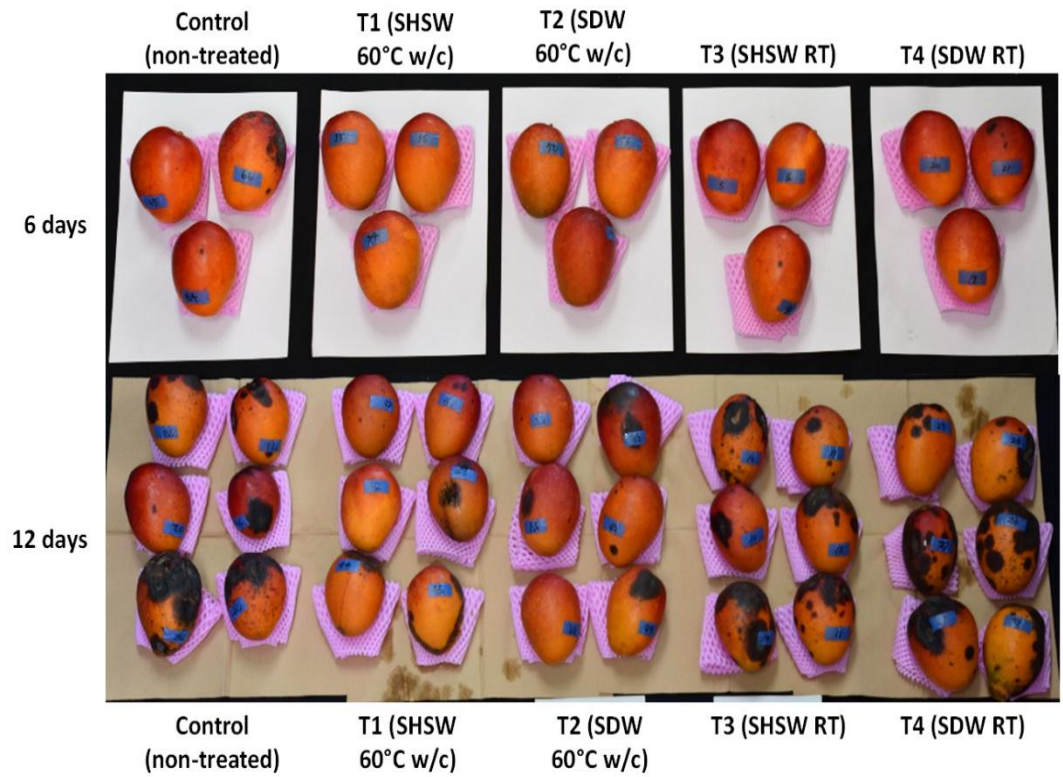

Figure 4. Non-inoculated mangoes after hot water treatments. Control, hot water treated mangoes with SHSW and SDW followed by cooling for 10 min (T1=SHSW 60°C w/c and T2=SDW 60°C w/c), and SHSW and SDW at room temperature (T3=SHSW RT and T4= SDW RT) soaked mangoes after 6d (at up) and 12d (at down). Symptoms of stem-end rot from quiescent infections were detected in all the treatments after 12d.

These results are different from those of mango cv. 'Carabao' at 59- 60 °C for 25 sec, showing symptoms of anthracnose and SER at 5 and 4-6 days after treatments respectively, although the incidence of both diseases were lower than the control (Pasilan et al. 2020). At 12 days, all the sides of the fruits in treatments and control showed disease incidence, being the stem area (side 'Z') of the fruit the most affected, as confirmed by Scheirer-Ray-Hare test and Tukey's HSD test ($p<0.05$), with symptoms of quiescent infection of SER detected in this area (Table 2), consistent with the characteristics of quiescent infections of SER, in which the pathogens colonize the conductive tissues of stem of the fruit (Galsuker et al. 2018).

Table 2. Effect of SHSW and SDW treatments on disease severity of non-inoculated mango fruits (mean ± SEM)

Treatment	Disease severity (n=6) [3]							
	Day 6				Day 12			
	$X^{A\,1}$	$Y^{A\,1}$	$Z^{A\,1}$		$X^{A\,2}$	$Y^{A\,2}$	$Z^{B\,1}$	
Control	1±0.57 [a]	1.3±0.88 [a]	2±1 [a]	A	2.4±0.42 [a]	2.3±0.39 [ab]	3.6±0.22 [a]	A
SHSW 60°C wc	0 [a]	0 [a]	0 [b]	B	1.44±0.29 [b]	1.66±0.37 [bc]	2.22±0.54 [b]	B
SDW 60°C wc	0 [a]	0.66±0.66 [a]	0 [b]	B	1.33±0.37 [b]	0.77±0.36 [c]	1.33±0.47 [b]	B
SHSW RT	0.33±0.33 [a]	0 [a]	0.66±0.33 [ab]	AB	2.88±0.26 [a]	3±0.33 [ab]	3.77±0.22 [a]	A
SDW RT	0.33±0.33 [a]	0 [a]	1 [a]	AB	3±0.28 [a]	3.2±0.27 [a]	4 [a]	A

[1] In the same column, different letter by rows means significant difference ($p<0.05$) under a Kruskal-Wallis's test followed by Dunn's test.

[2] In the same column, different letter by rows means significant difference ($p<0.05$) under an one-way ANOVA test followed by Tukey's HSD test.

[3] Different capital letters by rows/columns mean significant difference ($p<0.05$) under a Scheirer-Ray-Hare test followed by Tukey's HSD test.

Mango fruit surface temperature during treatments. The surface temperature of the fruits before treatment (23.5~25.5°C) was close to room temperature (24~25°C) and rose to 43~49°C after being dipped at 60°C (Table 3). After 60 min, none of the mango fruits from the SHSW and SDW comparison groups (27~28°C) reached room temperature, which was later confirmed by Scheirer-Ray-Hare test. The temperature values were not significantly different between treatments but were significantly different when considering the time. The temperature values between the mango sides were not significantly different.

As such, the surface temperature of hot-treated mangoes in both SHSW and SDW was not different between these two treatments, changing along with the time of application.

SHSW, SDW and temperature. As was observed in non-inoculated mangoes, both SHSW and SDW at room temperature had little to no effect on disease severity as it did not differ from the disease severity values of the control and showed signs of advanced decay like control 12 days after treatment (Table 2 and Fig. 4).Therefore, SHSW and SDW were effective at reducing the disease incidence when heated to 60°C and not effective at room temperature, the effectiveness of the treatment on the inhibition of the fungal pathogens relies on the temperature rather than the water sample used for the treatment. Plus, SHSW and SDW had similar effects on fruit when used for hot water treatments, as was observed in temperature surface, fruit quality and disease severity.

However, because the *in-situ* temperature of the SHSW used in this study was 68.7°C, heating is not necessary for the application of this treatment if performed near the spring source. The application of hot water treatments by Philippine exporters requires water tanks powered by electricity, liquefied petroleum gas, or kerosene for heating, and the water should be changed every three days (Aveno and Orden 2004). The use of SHSW might reduce the cost of heating, thereby making the application of hot water treatments for mango fruits even more cost-effective.

Inhibition of Anthracnose The results of both experiments suggest that hot water treatment at 60°C for 1 min is effective in inhibiting quiescent and direct contact infections of anthracnose.

In the case of inoculated mangoes, the disease spot sizes of the three *Colletotrichum* spp. (Fig. 4) followed by treatment did not show any growth after treatment. By contrast, inoculated mangoes cv. `Irwin` treated at 60°C for 40 sec showed mild symptoms of anthracnose after 4-5 days when stored at RT (Teruya et al. 2012), while inoculated mangoes cv. 'Carabao' treated at 53 °C for 20 min reduced symptoms of anthracnose in 48.71-52.53% after 14 days due to possible quiescent infections (Alvindia and Acda 2015). Although in this study, inoculation was done using fungal plugs instead of conidia spraying, those showed that the conidia of *C. gloeosporioides* can be completely inhibited when treated at 51 °C in a chamber (Teruya et al. 2012), while both conidia and mycelium can be inhibited *in-vitro* over 70% at 53 for 20 min (Alvindia and Acda 2015), or over 90% at 55°C for 15 sec (Nascimiento et al. 2014).

Nevertheless, even when the surface temperature of the mangoes, just after treatments, was lower (45.6±0.36 ~ 46.04±0.68°C in SHSW 60 °C wc, and 44.04±4.03 ~ 49.08±0.4 °C in SDW 60 °C wc) than the previously suggested temperatures, symptoms of anthracnose appeared at 12 days after hot water treatment in this study. This suggests that direct contact between the fruit surface and the heated medium is critical in inhibiting anthracnose. The heat transfer to the mango surface was uniform from both SHSW and SDW, as the statistical analysis on the temperature of the mango surface after treatments in our study suggests (Table 3).

Table 3. Temperature of the mango fruit surface before and after hot water treatments (mean ± SEM, n=5).

(unit: °C)

Treatment	Before [d]			After [a]			10min [b]			60min [c]		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
SHSW	23.66	24.14	24.28	46.04	45.6	42.9	29.22	29.38	29.56	27.74	27.78	28.04
60°C wc	±0.09	±0.42	±0.39	±0.68	±0.36	±0.63	±0.15	±0.07	±0.19	±0.16	±0.15	± 0.34
SDW	24.86	25.02	25.34	49.08	44.04	46.14	29.54	29.82	30.1	27.5	28.3	28.32
60°C wc	±0.08	±0.11	±0.2	±0.4	±4.03	±0.73	±0.15	±0.2	±0.2	±0.17	± 0.21	± 0.08

Different letter by columns indicates significant difference (<0.05) under a Scheirer-Ray-Hare test followed by Tukey's HSD test.

Inhibition of SER Hot water treatment at 60 °C for 1 min with SHSW was effective in inhibiting direct contact SER infections, as shown in the results of the inoculation with *Lasiodiplodia* spp. followed by hot water treatment (T8: LT+HWT) (Table 1 and Fig. 3). However, it is ineffective against quiescent infections of stem-end rot as observed in the treatments in non-inoculated mangoes (Fig. 4).

Treated mangoes with SDW showed symptoms of SER at 6d after treatment and in SHSW and SDW at 12 days, with special presence in the stem area (side Z), while in inoculated mangoes followed by treatments, symptoms of stem-end rot appeared in non-inoculated areas, even in the mangoes inoculated with *Lasiodiplodia* spp. followed by hot water treatment.

Quiescent infections of SER occur during the inflorescence and flowering stages in mango trees, in which *Lasiodiplodia theobromae*, as an endophytic fungus, colonize the mango stem area, turning pathogenic when fruit ripening due to biochemical and physiological changes in the mango fruit. (Galsuker et al. 2018). In previous studies, *in-vivo* assays inhibited conidia and mycelium of *L. theobromae* over 90% after treatment at 55 °C for 15 sec (Nascimento et al. 2014) and at 53 °C for 50 min (Alvindia and Acda 2015), although treatments at 50 °C for 15 sec inhibited *L. theobromae* conidia by around 60% and mycelium by 5.5% (Nascimiento et al. 2014).

In *in-vivo* treatments, 53 °C for 20 min reduced SER incidence by 48-60.68% after 14 days (Alvindia and Acda 2015), 55 °C for 10 min reduced SER incidence by 70% after 21 days at 13 °C (Montecalvo et al. 2019), and 59-60 °C for 35 sec reduced SER incidence by 20-25% in cv. 'Carabao' (Esguerra et al. 2004; Pasilan et al. 2020). The previous, combined with our findings, might suggest that the heat of the water during the treatments is effective in inhibiting surface infections of stem-end rot, due to the direct contact between the mango skin and the medium (SHSW and SDW), even when mango skin reached a temperature of 43~49 °C just after treatments. However, this heat is ineffective on quiescent infections of stem-end rot, as the surface temperatures of the treated mangoes just after treatments in our study were lower (max. 46.04 °C in SHSW 60 °C wc and 49.08 °C in SDW 60 °C wc) than the necessary for inhibiting the mycelium of the stem-end rot fungus and even lower than the fruit surface temperature of 55 °C reached by Teruya et al. (2012). The difference in the surface temperature obtained by Teruya et al. (2012) and this study might be due to the absence of water circulation in this study, reducing the heat transfer to the fruits.

In future studies, it is suggested that the application of temperatures higher than 60 °C for 1 min or shorter with water circulation, which is expected to increase the chances of the fruit surface reaching an adequate temperature for the control of quiescent infections of stem-end rot. Moreover, as the mango fruits in this study were stored post-treatment at room temperature, the use of refrigeration after treatments should be evaluated to extend the shelf life of mango and the inhibition of SER, as was previously reported in Montecarlo et al. (2019) in cv. 'Carabao', while trying to prevent chilling injury (Rodeo and Esguerra 2013).

Mango fruit quality. Values of weight loss showed that treated mangoes with SDW 60 ºC w/c had the highest weight loss at 6 days (16.74±10.24) and 12 days (27.05±13.97). However, there was no significant difference between the treatments, including SDW 60 ºC w/c, and the control in weight loss at 6 days and 12 days (Table 4). Mangoes treated with SHSW and SDW at 60 °C for 1 min showed no significant difference in fruit quality (weight, peel color, firmness, and soluble sugar content) compared to the control after 6 days at room temperature. In the case of firmness, the values of all the treatments were similar and there was no significant difference between them. Soluble sugar content at 6 d and color differences in all the treatments and the control were also similar (Table 4 and 5).

This is consistent with an earlier study wherein peel color was not affected by hot water treatments at 60 °C (Teruya et al. 2012). Likewise, mango cv. 'Carabao' treated at 59-60 °C for 35 sec showed no difference in peel color and visual quality at 6 days after treatment (Pasilan et al. 2020).

Table 4. Effect of SHSW and SDW treatments on fruit quality of non-inoculated mango fruits (mean ± SEM).

Treatment	Weight Loss (n=3), %		Hardness (n=3) kg/cm^2			Soluble sugar content, ^{O}Brix	
	Day 6 [1]	Day 12 [1]	Day 0 [2]	Day 6 [1]	Day 12 [2]	Day 6 (n=3) [2]	Day 12 (n=9) [2]
Control	4.87± 22^a	12.8± 1.22^a	0.92^a	0.78± 0.01^a	- [3]	11.43± 0.69^a	- [3]
SHSW 60°C wc	6.17± 0.31^a	11.18± 0.88^a	0.93^a	0.79^a	0.73± 0.01^i	12.33± 0.33^a	10.79 ±0.41^a
SDW 60°C wc	16.74± 10.24^a	27.05± 13.97^a	0.92^a	0.8^a	0.77± 0.01^i	12.83± 0.92^a	11.12±0.3^a
SHSW RT	4.87± 0.22^a	12.8± 1.22^a	0.92^a	0.8± 0.01^a	- [3]	10.66± 1.2^a	- [3]
SDW RT	14.64± 8.81^a	13.21± 1.45^a	0.9^a	0.8± 0.01^a	- [3]	11.33± 0.88^a	- [3]

[1] In the same column, different letter by rows indicates a significant difference ($p<0.05$) under a Kruskal-Wallis's test, followed by Dunn's test.

[2] In the same column, different letter by rows indicates significant difference ($p<0.05$) under a one-way ANOVA test followed by Tukey's HSD test.

[3] Samples discarded due to fruit decay

The effect of both SHSW 60 ºC wc and SDW 60 ºC wc on mango fruit quality (weight loss, firmness, soluble sugar content, color difference) was not different at 12 days. The values of hardness and soluble sugar content were not taken at 12 days for Control, SHSW RT, and SDW RT mangoes, as the fruits had to be discarded due to decay. In a previous study, treated mangoes cv. 'Carabao' had better peel color and suffered less deterioration of visual quality than the control at 12 days (Pasilan et al. 2020).

In general, hot water treatments on mango tend to increase the fruit quality in terms of peel color on cv. 'Kensington' and cv. 'Sindh' (Anwar and Malik 2007; Djioua et al. 2009; Jacobi and Giles 1997), while color, fruit hardness, pH, total soluble solids and titratable acids are either increased or not compared to the control in cv. 'Carabao' and 'Keitt' (Alvindia and Acda 2015; Kitma and Esguerra 2009; Kumah et al. 2011; Montecalvo et al. 2019), although some protocols might provoke lenticel spotting in cv. 'Carabao' (Esguerra et al. 2004). Due to this, the design of treatment protocols should be carried out taking into consideration the mango fruit variety and the desired result: improving storage time, fruit quality, and/or reducing the incidence of pests or diseases.

Some of the samples were destroyed during the measurement of quality values; thus, it is suggested that non-destructive measurements be used for the assessment of fruit quality when the main objective is the evaluation of disease inhibition on the fruit, as maintaining the number of samples during the entire experiment is critical for the evaluation.

Table 5. Effect of SHSW and SDW treatments on peel color of non-inoculated mango fruits (mean ± SEM).

Treatment	Peel color differences (n = 3) [1]					
	ΔL	**Δa**	**Δb**	**ΔC***	**Δh**	**ΔE***
Control	-0.5 ± 1.83[a]	-1.92 ± 0.74[a]	12.65 ± 2.84[a]	6.22 ± 1.87[a]	13.63 ± 2.85[a]	13.2 ± 2.6[a]
SHSW 60°C wc	-2.8 ± 1.09[a]	-2.97 ± 1.71[a]	2.95 ± 1.71[a]	-0.29 ± 2.34[a]	5.29 ± 1.56[a]	6.38 ± 0.78[a]
SDW 60°C wc	0.37 ± 2.26[a]	-4.54 ± 0.87[a]	8.79 ± 2.52[a]	2.14 ± 1.48[a]	12.45 ± 2.4[a]	10.68 ± 2.08[a]
SHSW RT	-4.01 ± 0.75[a]	-1.24 ± 2.19[a]	7.63 ± 3.23[a]	3.37 ± 0.4[a]	8.25 ± 4.4[a]	9.38 ± 3.11[a]
SDW RT	-4.46 ± 0.84[a]	-2.21 ± 0.16[a]	6.83 ± 5.39[a]	1.99 ± 3.01[a]	7.87 ± 5.2[a]	10.32 ± 3.51[a]

[1] In the same column, different letter by rows indicates significant difference ($p<0.05$) under a one-way ANOVA test followed by Tukey's HSD test.

CONCLUSION

This is a pioneering study that used saline hot spring water for hot water treatments of mango fruits and reported the effect of hot water treatments at 60 °C for 1 min on stem-end rot. Hot water treatment with saline hot spring water at 60 °C for 1 min, followed by cooling for 10 min, is effective in reducing the appearance of anthracnose from recent direct contact and quiescent infections, while maintaining mango fruit quality. The treatment is only effective against recent direct contact infections, but not against quiescent infections of stem-end rot.

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