



## Management of postharvest anthracnose of banana using inorganic salts alone and in combination with hot water

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### Abstract

Experiments were conducted to evaluate selected inorganic salts alone and in combination with hot water for management of banana anthracnose disease. The efficacy of hot water treatment (HWT) and either sodium bicarbonate (SBC), sodium carbonate (SC) or sodium hypochlorite (SH) applied alone and in combination to suppress anthracnose disease was investigated in both naturally infected and artificially inoculated banana fruit cv. Kolikuttu during the storage. The *in vitro* efficacy of the selected salts on the inhibition of colony growth of the anthracnose fungal pathogen was also tested. Postharvest treatment involving fruit dipped for 30 min in 1% (w/v) SC following HWT (50°C, 3 min) reduced the severity of anthracnose, which was statistically similar ( $P > 0.05$ ) with that of homai fungicide treatment. Among the tested salts, SC recorded the significantly highest ( $P < 0.05$ ) *in vitro* inhibition of mycelial growth of anthracnose pathogen. Integration of SC with HWT could be a commercially acceptable and economically feasible non-fungicidal approach for the postharvest management of anthracnose during the storage of banana.

**Key words** – Eco-friendly disease management – Fruit disease – Generally regarded as safe salts – Postharvest disease

### Introduction

Banana (*Musa acuminata*) which belongs to the family Musaceae is one of the most ancient fruits in the world and has a high consumer demand throughout the world. The ripe fruit is a rich source of energy, vitamins, minerals and protein, and the fruit is prized for its excellent taste and medicinal value (Wasala et al. 2014). Under condition in Sri Lanka, more than 60% of the total land under fruit cultivation is with banana and it has been the most consumed fruit in the country (Anonymous 2011). Banana fruit is affected by a number of postharvest diseases such as anthracnose, crown rot and blossom end rot (Nikagolla et al. 2019). Although *Colletotrichum musae* is the most common species associated with anthracnose of banana, *C. gloeosporioides*, *C. siamense*, *C. theobromicola*, and *C. tropicale* have also been reported to cause banana anthracnose (Sakinah et al. 2014, Vieira et al. 2017). Black and sunken lesions with spore masses can be seen on the anthracnose lesions (Zakaria et al. 2009). Wounds on fruit due to handling and transportation make anthracnose disease to be severe (Sarkar 2016). It causes deterioration of quality of the fruit, making them unmarketable. Thus, anthracnose disease can cause heavy losses to farmers and sellers.

Benzimidazole fungicides such as benomyl were used on banana as a fruit dip for effective control of postharvest disease in several countries, including Sri Lanka (Perera & Karunaratne 2001). However, this systemic fungicide has been banned in Sri Lanka and many other countries due to its possible carcinogenicity and reproductive toxicity on human health and harmful effects on the environment. Long-term indiscriminate use can also lead to development of fungicide resistance (Lucas et al. 2015). Therefore, there is a high consumer-demand for non-chemically treated fruit in markets. The fruit sector urgently needs to develop eco-friendly postharvest treatments that could be alternative to the conventional fungicide treatment. Low-toxicity chemical alternatives which have acceptable antifungal activity, are known to have less toxicological impact on mammals and environment (Ranasinghe et al. 2002). In addition, they have been freed from residue tolerances on agricultural produce by the US Environmental Protection Agency and approved as food additives or preservatives by the US Food and Drug Administration (Karmaus et al. 2017). Among them, carbonates, benzoates, sorbates and paraben salts are the important GRAS (generally regarded as safe) salts (Hewajulige & Wijeratnam 2010, Palou et al. 2016).

Dipping the fruit in hot water is an effective non-chemical treatment to reduce the development of pathogens. Moreover, the advantages of hot water treatment (HWT) include its relatively easy application, short treatment time and its efficacy in killing pathogens that cause skin-borne diseases (Kechinski et al. 2012). HWT has been effective in reducing the viability of *C. gloeosporioides* in papaya (Li et al. 2013) and guava (Ferraz et al. 2013). Integration of HWT with either salt (Reyna et al. 2017) or bacterial antagonist (De Costa & Erabadupitiya 2005) was shown to reduce development of fungal pathogens in papaya and banana, respectively.

Banana fruit has been treated with some inorganic salts to suppress other postharvest diseases such as crown rot and blossom end rot, but a comprehensive study on the application of different salts with combination of HWT for the management of anthracnose remains unattended. Hence, the present study was conducted to assess the efficacy of selected inorganic salts alone and in combination with HWT as alternative sources replacing commercial fungicide to reduce anthracnose disease of banana.

## **Materials & Methods**

### **Banana fruit**

A highly susceptible banana cultivar to anthracnose, Kolikuttu (AAB type) was used for this study. Banana fruit at mature unripe stage was collected from a local farmer in Chenkalady, Sri Lanka, as hands from the same bunch of inflorescence, surface-sterilized by 10% Clorox (commercial bleach) solution for 3 min, washed with sterilized water three times and air-dried.

### **Fungal pathogen and culture**

*C. musae* was isolated from banana fruit cv. Kolikuttu showing typical anthracnose symptoms. A pure culture of *C. musae* was obtained and maintained in potato dextrose agar (PDA) at  $30 \pm 2^\circ\text{C}$  under 12 h/12 h light/dark conditions for 10 days. Based on the colony and spore morphology, the fungal culture was confirmed as *C. musae*.

### **Inorganic salts**

Sodium bicarbonate (SBC) as 1% (w/v), sodium carbonate (SC) as 1% (w/v) and sodium hypochlorite (SH) as 1% (v/v) were tested in the *in vivo* investigations. The concentration of salts and duration of salt treatment used in the study were based on the results of Alvindia (2013).

### **Determination of effective non-fungicidal methods to reduce anthracnose of naturally infected banana**

Nine different treatments including inorganic salts and HWT as single or in combination, were evaluated for the banana to reduce anthracnose development. Treatment groups included fruit dipped in SBC, SC or SH for 30 min; fruit subjected to HWT by submerging in a water bath at

50°C for 3 min; fruit subjected to HWT and one of the salt treatments; and fruit treated with homai fungicide dip at a concentration of 1.8 g L<sup>-1</sup>. The untreated controls were dipped in sterile distilled room temperature water (SDRTW). Each treatment had five replicate banana fingers. The temperature and duration of HWT used in the study was according to the findings of De Costa & Erabadupitiya (2005). After the dip treatments, fruit were air-dried and incubated in a glass chamber with a mean temperature of 30°C and RH of 80% until disease development. For quantification of anthracnose lesions, a method described by De Costa & Gunawardhana (2012) was followed. Briefly, the peels of each fruit were removed, and individual anthracnose lesions were traced on a transparent sheet and measured on 1 mm<sup>2</sup> graph paper. The total area of lesions per fruit was recorded and the severity of disease was calculated as a percentage of peel area containing lesions.

### **Determination of effective non-fungicidal methods to suppress anthracnose of artificially infected banana**

The most effective method of suppressing anthracnose pathogen was confirmed on artificially inoculated banana. A drop of 20 µL of *C. musae* spore suspension (10<sup>6</sup> spores mL<sup>-1</sup>) was injected on the fruit peel as a spot inoculation (3 spots per finger) using a syringe. Artificially inoculated fruit were placed in a glass chamber at a mean temperature of 30°C for 24 h. After the incubation, the nine treatments described previously were applied. For each treatment, five fingers were used as replicates. After the dip treatments, fruit were air-dried and kept in a glass chamber with a mean temperature of 30°C and RH of 80%. Days taken to develop initial anthracnose lesions were recorded and disease severity was quantified as percentage area of lesion developed from each point of inoculation in each finger on seventh day post inoculation. Total area of lesion development in a finger was measured by tracing the lesions (3 lesions per finger, i.e. three points of inoculations) on a transparent sheet and then estimating the area using a 1 mm<sup>2</sup> graph paper.

### **Evaluation of *in vitro* efficacy of inorganic salts for suppression of *C. musae***

Influence of inorganic salts on the inhibition of mycelial growth was determined by growing the pathogen in a PDA medium having final concentration of 0.3 M of the respective salt. The concentration of salts was based on the results of an experiment conducted by De Costa & Gunawardhana (2012). Treatment groups comprised PDA amended with SBC, SC or SH; and unamended PDA. A disc of 5 mm diameter from an actively growing margin of 10 day-old culture was placed in the centre of a PDA plate with a diameter of 90 mm. Each treatment with five replicate plates was kept for incubation at a mean temperature of 30°C. Colony diameter of each plate was taken by averaging the diameters measured at two perpendicular directions. Inhibition of mycelial growth (%) was calculated for each salt treatment using the colony growth data measured seven days after incubation on unamended and salt amended PDA plates (Reyna et al. 2017).

### **Experimental design and statistical analysis**

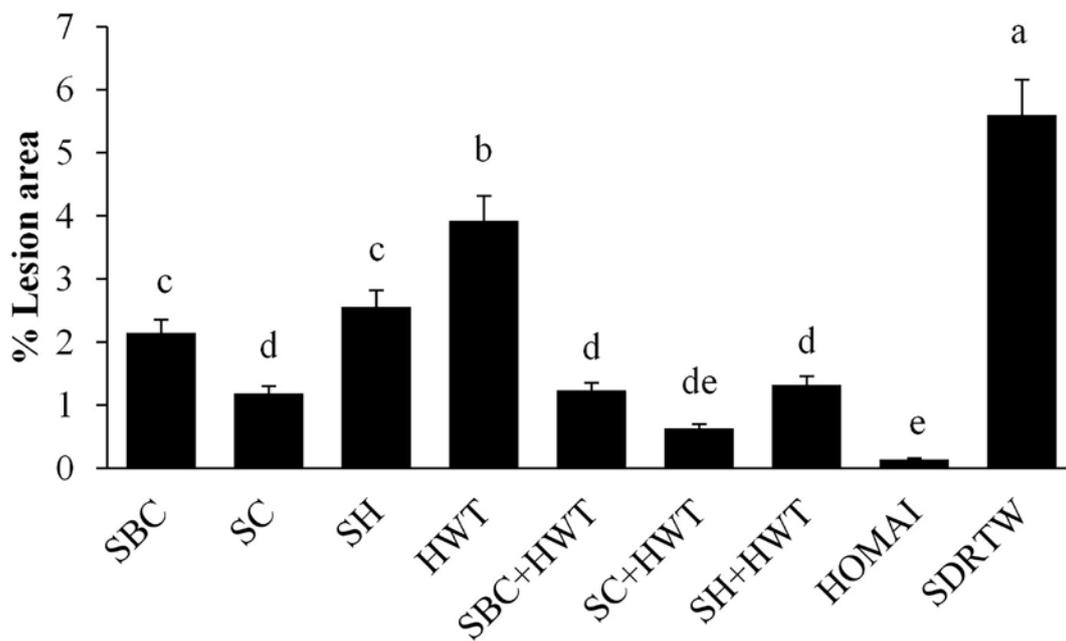
Completely randomized design was performed for both *in vitro* and *in vivo* experiments. All experiments were performed twice and the data from repeated independent trials were pooled after confirmation of homogeneity of variances. Significance of treatment effects on the measured variables was determined by analysis of variance and the treatment means were compared using Duncan's multiple range test at  $P = 0.05$ , using SAS 9.1 software.

## **Results**

### **Determination of effective non-fungicidal methods to reduce anthracnose of naturally infected banana**

The severity of anthracnose as percentage area of lesion development in naturally infected banana (cv. Kolikuttu) applied with different treatments is shown in Fig. 1. The various treatments showed significant difference ( $P < 0.05$ ) in their ability to suppress the disease. HWT significantly

reduced ( $P < 0.05$ ) symptoms, but treatment with salt alone or in combination with HWT significantly reduced ( $P < 0.05$ ) lesion development even more than HWT alone. SC treatment with or without HWT were statistically the same ( $P > 0.05$ ) in reducing symptoms, but the addition of HWT to SC treatment made it as effective as fungicide dipping. Integration of HWT with SBC and SH significantly reduced ( $P < 0.05$ ) the area of lesion in comparison to the corresponding individual salt treatment by 42.5% and 48.4%, respectively. Meanwhile, there was no significant difference ( $P > 0.05$ ) in anthracnose lesion development among the combined treatments, indicating that the inorganic salts with HWT contributed same degree in reducing the disease. Fruit dipped in salts after HWT showed 66.3-83.9% of reduction in disease severity than that of individual HWT. However, HWT alone had statistically lower ( $P < 0.05$ ) disease severity than the SDRTW dipping.



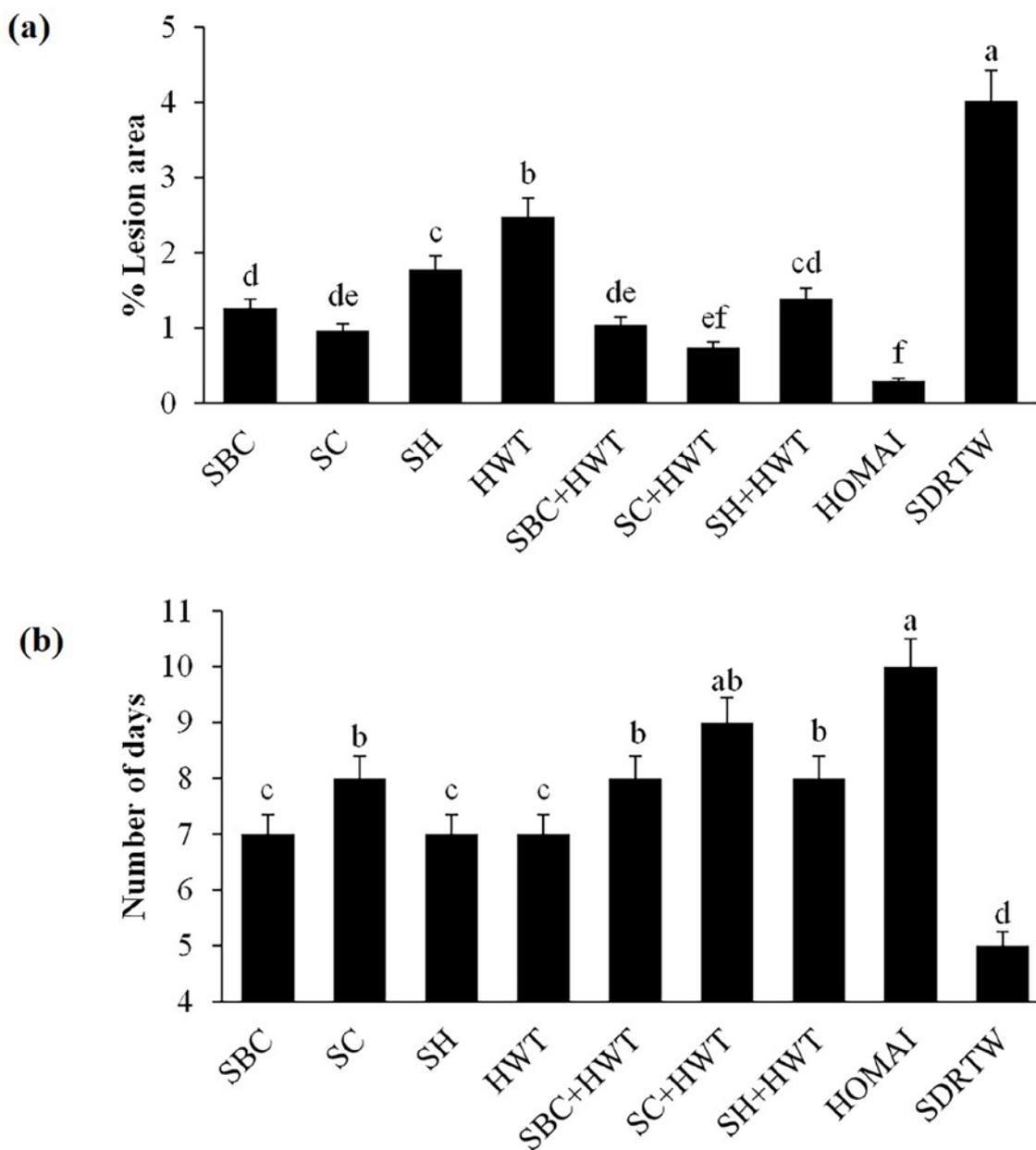
**Fig. 1** – Severity of anthracnose disease on naturally infected banana. Each bar is a mean of two independent trials with a total of ten replicate measurements per treatment. Error bar indicates standard error of mean. Means with the same letter are not significantly different at  $P = 0.05$ .

### Determination of effective non-fungicidal methods to suppress anthracnose of artificially infected banana

Fig. 2a shows the percentage lesion area developed seven days after inoculation of *C. musae* and treated with various treatments. The treatments had a significant influence ( $P < 0.05$ ) on the pathogenicity of *C. musae* and thereby on the degree of lesion development in banana cv. Kolikuttu. Treatment with fungicide or HWT + SC resulted in statistically the same ( $P > 0.05$ ) percentage lesion area of 0.3% and 0.74% respectively, indicating that HWT + SC was equally capable as homai fungicide in reducing anthracnose symptoms. Fruit dipped in a salt after HWT did not significantly differ ( $P > 0.05$ ) in lesion development compared to the corresponding individual salt treatment. Meanwhile, fruit subjected to single salt treatments had significantly lower ( $P < 0.05$ ) percentage lesion area than untreated fruit. Area of lesion development was reduced by 65.4-81.6% and 44-70.2% by the integrated methods compared to SDRTW treatment and HWT alone, respectively.

Fig. 2b gives the number of days taken to develop initial anthracnose symptoms in fruit treated with different treatments. Number of days for symptoms development differed significantly ( $P < 0.05$ ) between the treatments. Treatment with fungicide or HWT + SC resulted in a statistically similar ( $P > 0.05$ ) time for symptoms development of 10 and 9 days, respectively.

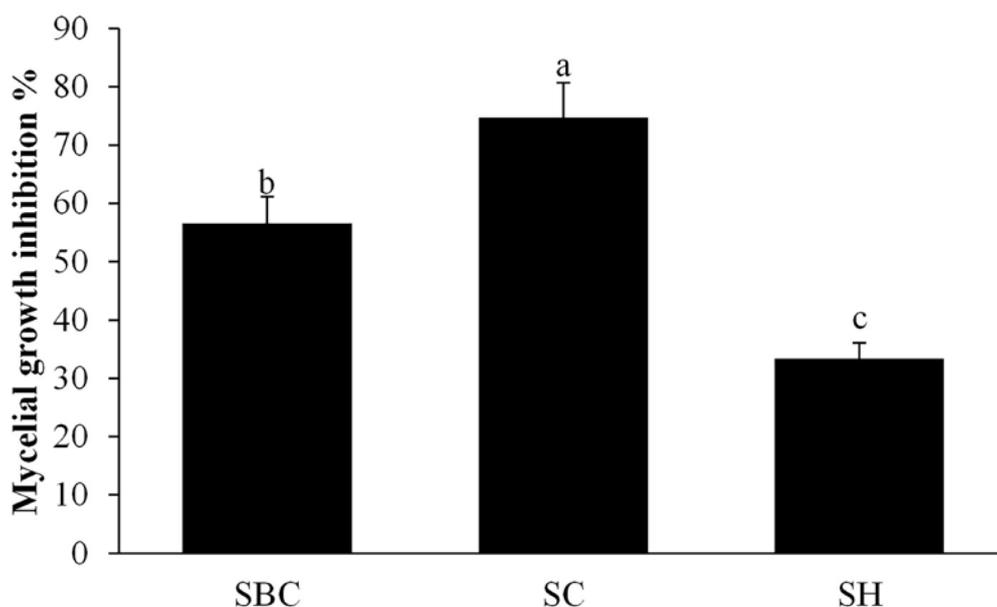
Among the integrated treatments, there was no significant variation ( $P > 0.05$ ) in the number of days taken to develop symptoms, indicating the equal efficacy in suppressing symptoms development. On the other hand, disease developed significantly later ( $P < 0.05$ ) in fruit treated with individual salt or HWT than untreated fruit.



**Fig. 2** – Severity of anthracnose on artificially inoculated banana. a percentage area of lesion. b number of days for anthracnose symptoms to appear. Each bar is a mean of two independent trials with a total of ten replicate measurements per treatment. Error bar indicates standard error of mean. Means with the same letter are not significantly different at  $P = 0.05$ .

#### Evaluation of *in vitro* efficacy of inorganic salts for suppression of *C. musae*

Fig. 3 indicates that different inorganic salts showed significant variation ( $P < 0.05$ ) in their ability to inhibit colony growth of *C. musae*. SC reduced the mycelial growth of the pathogen by 74.7%, SBC by 56.6% and SH by 33.4% compared to colony growth in unamended PDA. Among the salts tested, SC which recorded more than 70% reduction of mycelial growth, was significantly greater ( $P < 0.05$ ) than the inhibition efficacy of SBC and SH.



**Fig. 3** – Reduction of colony growth of anthracnose pathogen in salts amended PDA. Each bar is a mean of two independent trials with a total of ten replicate measurements per treatment. Error bar indicates standard error of mean. Means with the different letters are significantly different at  $P = 0.05$ .

## Discussion

The study was undertaken in order to find alternative options to commercial fungicides to manage postharvest anthracnose of banana. The findings of the present study elucidate the potential of inorganic salts and HWT on suppression of anthracnose disease of banana when used as a single control method or their combination. They also highlight the efficacy of salts on inhibiting the colony growth of anthracnose pathogen. The present investigation reveals that salt or HWT used as individual postharvest treatment had a significant influence on reduction of the disease in comparison to SDRTW treatment. Even though application of either salt or hot water significantly lessened the disease development, these single treatments were not effective as fungicide dip to suppress anthracnose development. In general, integrated treatment of a salt with HWT did not significantly reduce disease severity than the individual treatment of the salt. Nevertheless, combination of HWT and SC resulted in prominent improvement in suppression of the pathogen *C. musae*. This treatment was statistically similar to homai fungicide in the efficacy of reducing anthracnose disease in banana.

HWT is effective for control of fungal pathogen, because latent infections and spores of fungi are either on the surface or in the first few cell layers under the peel of the fruit (Lurie 1998). The consequence of HWT on fungal pathogen is principally the reduction in the viability of fungal spores. HWT is ineffective in killing dormant spores of fungal pathogens but does cause a significant delay in conidial germination and mycelial growth (Alvindhia 2012). Heat can also retard fungal growth by inducing resistance mechanisms in the outer layers of fruit epicarp (Fallik 2004). Moreover, HWT could change the chemical environment of the fruit peel by stimulating the antimicrobial compounds present on it (Janisiewicz & Korsten 2002). De Costa & Chandima (2014) showed that host plant exudates present on the banana fruit peel positively influence the spore germination and appressoria formation of *C. musae*. Washing with hot water could eliminate these plant exudates and thereby lessen pathogen development.

Salts inhibit *in vitro* mycelial growth, spore germination and germ tube elongation of fungi (Boumaaza et al. 2015). In addition, the antifungal activities of salt against fungal pathogens could be because of its ability to increase the pH of its exogenous environment, inactivation of extracellular enzymes of fungi and direct interaction with cell membranes (Palou et al. 2001). Salt

treatment could increase pH of banana fruit peel and thereby reduce postharvest anthracnose development. Furthermore, bicarbonate salts increase osmotic stress which reduces the fungal cell turgor pressure and leads to collapse and shrinkage of hyphae and spores (Gamagae et al. 2003). In the present investigation, significantly highest *in vitro* inhibition of mycelial growth of *C. musae* was achieved by SC treatment. It is in agreement with Zoeir et al. (2017) showing that SC largely inhibited the linear growth of banana postharvest pathogen *C. musae*. Ability to grow on host tissues for rapid colonization, sporulation, conidial germination and appressoria formation are crucial steps of a successful infection process of *C. musae* (De Costa & Gunawardhana 2012). Having negative effects on any of the above steps would retard infection of *C. musae* and following disease development. Considering the detrimental effects on the pathogenicity of *C. musae*, combined HWT + SC could be used as a non-fungicidal strategy for management of banana anthracnose disease.

Although the present study showed an improved performance of salt used after HWT against anthracnose disease, considerations should be required before the postharvest application. For instance, salt solution at more than 1% causes discoloration to banana fruit due to its phytotoxicity (Alvindhia & Natsuaki 2007). A remarkable performance of HWT + SC in anthracnose reduction was achieved in the present investigation. Our results are supported by Alvindhia (2013) who reported that incidence of banana crown rot disease caused by *C. musae* was reduced by combined HWT + SC treatment as equally as mancozeb fungicide. It is worth discovering a method that would improve the retention of salts on the fruit surfaces after being treated with hot water. Extending the dipping time, repeating the dipping process and adding a surfactant would enhance the salt retention (Alvindhia 2013). Surfactants would increase the adherence of salts and distribute them uniformly on the fruit surface (Alvindhia & Natsuaki 2007). Further, surfactants would facilitate salts to easily reach natural openings of fruit to compete with the pathogen (Alvindhia 2013).

Undoubtedly, complete control of anthracnose is difficult to achieve by non-fungicidal approach. However, increasing consumers' consistent preference for non-fungicidal banana of excellent quality is driving our future work to investigate on the effects of salt and HWT treatments on fruit quality. The findings demonstrated the potential for using SC in combination with HWT in suppressing natural infections of anthracnose pathogen. This approach could be an applicable alternative to fungicide treatment of postharvest banana anthracnose disease. The alternative method can be of benefit and accessibility to banana growers in low socio-economic environments.

### **Acknowledgement**

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### **Conflict of interest**

The authors report no conflict of interest.

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