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SCIENTIFIC ARTICLE

Antifungal activity of sodium bicarbonate alone or in combination with a low dose of thiabendazole against crown rot in banana cv. Enano Gigante

Actividad antifúngica del bicarbonato de sodio, solo o combinado con una dosis baja de tiabendazol contra la pudrición de la corona en banano cv. Enano Gigante

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ABSTRACT

Crown rot is the most important postharvest disease in banana worldwide. This study evaluated the antifungal efficacy and potential of sodium bicarbonate (SB) as an alternative for the control of crown rot (CR) in banana cv. Enano Gigante. The fruits were artificially inoculated with *Colletotrichum musae* and incubated at 26-27 °C for 7 d. Through various *in vivo* treatments, 500 mM SB was identified as the most effective concentration. In addition, the effect of dip temperatures on SB efficacy was analyzed. Results showed that 500 mM SB significantly reduced CR incidence and severity by 77.8 % and 92.9 %, respectively, at temperature of 40°C. The combination of SB with a low dose of thiabendazole (225 µL L^{-1}) achieved complete control of the disease. These findings contribute to postharvest disease management by proposing an effective and sustainable alternative to the use of conventional fungicides.

KEYWORDS

Colletotrichum musae, postharvest disease, severity, inoculation, fungus, antifungal treatment.

RESUMEN

La pudrición de la corona es la enfermedad postcosecha más importante del banano en todo el mundo. Este estudio evaluó la eficacia antifúngica y el potencial del bicarbonato de sodio (SB) como alternativa para el control de la pudrición de la corona (CR) en banano cv. Enano Gigante. Los frutos fueron inoculados artificialmente con *Colletotrichum musae* e incubados a 26-27 °C durante 7 d. Mediante diversos tratamientos *in vivo*, se identificó que 500 mM de SB fue la concentración más efectiva. Además, se analizó el efecto de la temperatura de inmersión sobre la efectividad del SB. Los resultados mostraron que 500 mM de SB redujo significativamente la incidencia y severidad de la CR en 77.8 % y 92.9 %, respectivamente, a una temperatura de 40 °C. La combinación de SB con una dosis baja de tiabendazol (225 µL L^{-1}) permitió el control total de la enfermedad. Estos hallazgos contribuyen al manejo de enfermedades postcosecha al proponer una alternativa efectiva y sostenible al uso de fungicidas convencionales.

PALABRAS CLAVE

Colletotrichum musae, enfermedad postcosecha, severidad, inoculación, hongo, tratamiento antifúngico.

Introduction

Bananas are cultivated in over 135 countries, with India, China, Indonesia, Brazil, Ecuador and the Philippines being the leading producers (Food and Agriculture Organization of the United Nations [FAO], 2022). Mexico ranks twelfth worldwide, with an annual production of 2.6 million tons (FAO, 2022). Despite their global importance, bananas are highly perishable, leading to significant postharvest losses (De Costa & Erabadupitiya, 2005).

Crown rot (CR) is the most prevalent postharvest disease affecting bananas, causing considerable economic losses during storage, transportation and marketing (Krauss & Johanson, 2000). The disease is primarily caused by *Colletotrichum musae*, a fungal pathogen that thrives under postharvest conditions (Krauss & Johanson, 2000; Lassois et al., 2010; Muirhead & Jones, 2000).

Current management strategies rely heavily on synthetic fungicides, such as thiabendazole (TBZ), prochloraz, and imazalil. Although effective, these chemicals raise several concerns, including environmental contamination, the accumulation of harmful residues in fruits, and the development of fungicide-resistant strains (Gatto et al. 2011; Jinasena et al. 2011; Mari et al. 2003). As the demand for more sustainable agricultural practices increases, there is a growing need to explore alternative methods for controlling CR.

This research addresses this need by investigating sodium bicarbonate (SB) as a low-toxicity alternative to traditional fungicides. SB is known for its antifungal properties, however, its potential for managing CR caused by *C. musae* in bananas remains largely unexplored.

This study aims to: 1) evaluate the primary *in vivo* effect of SB on CR, 2) assess the influence of dip temperature on the effectiveness of SB, and 3) determine the antifungal efficacy of SB applied alone or in combination with low doses of TBZ against CR. By identifying effective, and environmentally friendly treatments, this research advances postharvest disease management and contributes to the development of sustainable practices in banana production.

Materials and methods

Fungal pathogen

Colletotrichum musae strain PAC-1 used in this study was isolated from diseased banana fruits showing typical symptoms of crown rot at the Laboratorio de Fitopatologia del Colegio de Postgraduados, Campus Tabasco (Cardenas, Tabasco, México). The strain was identified through molecular analysis of the internal transcribed spacer (ITS) region, showing 99.101 % sequence similarity to *C. musae*, with the following nucleotide sequence:

(GACCTGCGGAGGGATCATTACTGAGTTTACGCTCTGCAACC
CTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGGGTC
CCCGTGACCCTCCCGGCCCCCCGCCCCCGGGCGGGTCGGCGC
CCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTC
TGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT
AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTC
GAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTA
CAGCAGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGC
CTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGG
ACTCTTGCCGTAAAACCCCCCAATTTTCCAAAGGTTGACCTCGGA
TCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATA).

The fungus was cultured on potato dextrose agar (PDA) medium (BD BIOXON, Estado de Mexico, Mexico) in Petri dishes. Cultures were incubated at 25 °C for 7-14 d to ensure optimal growth.

Fruit

The experiments were performed using bananas (*Musa paradisiaca*), specifically the Enano Gigante cultivar. Banana bunches were harvested from commercial fields in Cunduacan, Tabasco, Mexico. Prior to the experiments, the bunches were dehanded and thoroughly washed with tap water to remove dust, floral remnants and latex. The bananas were randomized and allowed to air-dry at ambient temperature before further processing.

Fruit disinfection and ripening

The bananas were disinfected by immersion in an 1 % aqueous chlorine solution (sodium hypochlorite, 5 % v/v, Hycel Reactivos Quimicos, Jalisco, Mexico) for 1 min. They were then rinsed with water and air-dried at room temperature. Following disinfection, the fruits were immersed in 2,000 ppm ethephon (ETHREL 21.7 % LS Bayer, Mexico) for one min to induce ripening. Prior to inoculation, the cut surfaces of the crown were disinfected again by immersion in 70 % ethanol for 30 s.

Inoculum preparation of *Colletotrichum musae*

Conidia from a 14-day-old *Colletotrichum musae* strain PAC-1 grown on PDA medium were harvested and suspended in a 0.05 % (w/v) aqueous Tween© 80 solution. The conidial suspension was filtered through sterile cloth to remove debris. The conidial concentration was then adjusted to 1×10^6 conidia mL^{-1} using a hemocytometer.

Fungal inoculation

A 100 µL aliquot of the *C. musae* conidial suspension (1×10^6 conidia mL^{-1}) was applied to the cut surface of the crown of each banana hand. The conidia were then evenly distributed with a small brush to ensure uniform coverage.

Antifungal activity of sodium bicarbonate against crown rot in primary in vivo screenings

The most effective SB concentration for reducing mycelial growth and inhibiting conidial germination in *in vitro* experiments (data not shown) was selected to determine the concentration range for the *in vivo* experiments. Fruit disinfection and forced ripening were performed as described in the "Fruit disinfection and ripening" section. The following preventive and curative SB treatments were evaluated: 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 475 mM, and 500 mM. Treatments were applied using a manual atomizer to ensure complete coverage of the crown cut surface.

Inoculation of the crown cut surface was carried out either 1 h before or 1 h after SB application, following the procedure described in the "Fungal inoculation" section. Each treatment consisted of three replicates, with three banana hands arranged in a completely randomized design. Each hand contained three or four fruits. Inoculated banana hands were placed in 10 kg plastic boxes and incubated at 24-26 °C and 80-90 % relative humidity for 7 d.

Disease incidence [(number of diseased crowns/ total number of crowns) × 100] and severity were assessed at 7 d post-inoculation. Disease incidence was expressed as the percentage reduction relative to the control treatment. Disease severity was evaluated using the scale proposed by Alvindia et al. (2004), defined as follows:

0	No discoloration or mycelial growth on the crown.
1	Discoloration or mycelial growth limited to the crown cut surface.
2	Discoloration or mycelial growth covering less than 10 % of the crown area.
3	Discoloration or mycelial growth affecting 11-40 % of the crown area.
4	Discoloration or mycelial growth affecting 41-70 % of the crown area.
5	Discoloration or mycelial growth affecting 71-100 % of the crown area.
6	Discoloration or mycelial growth spreading to the finger stalks.
7	Discoloration or mycelial growth reaching the pulp of the fingers.

Disease severity was expressed as the percentage reduction in CR severity and calculated using the following formula:

$$Percentage\ reduction\ of\ crown\ rot\ severity = \left(\frac{CRIC - CRIT}{CRIC} \right) \times 100$$

Where CRIC is the crown rot index of the control group and CRIT is the crown rot index in the treatment group.

All assays were performed in duplicate to ensure the reliability of the results.

Influence of dip temperature on sodium bicarbonate effectiveness

To evaluate the effect of dip temperature on the efficacy of SB for controlling CR, a series of experiments

was conducted using Enano Gigante banana fruits. Fruit disinfection and ripening were performed as described in the "Fruit disinfection and ripening" section. For these assays, an SB concentration of 500 mM and a dip duration of 20 min were used.

The following treatments were evaluated: 1) non-inoculated treatment (WI), 2) control (water at ambient temperature), 3) hot water at 30 °C (HW 30 °C), 4) hot water at 40 °C (HW 40 °C), 5) hot water at 45 °C (HW 45 °C), 6) 500 mM sodium bicarbonate at 30 °C (SB 30 °C), 7) 500 mM sodium bicarbonate at 40 °C (SB 40 °C), and 8) 500 mM sodium bicarbonate at 45 °C (SB 45 °C).

The crown cut surface was inoculated either 1 h before or 1 h after the dip treatments, following the procedure described in the "Fungal inoculation" section. A total of 18 L of a 500 mM aqueous SB solution was used for the dip treatments, either heated or unheated. For heated treatments, the solution was warmed using a manual heater (Silverline, DC-CA, 1000 W, Cardenas, Tabasco, Mexico). Banana hands were placed in a 15 L multi-perforated plastic container and immersed in the treatment solution.

Each treatment consisted of three replicates, with four banana hands per replicate (each hand containing 3-4 fingers). After treatment, the banana hands with treated and inoculated crowns were placed in 10 kg plastic boxes and incubated at 24-26 °C and 80-90 % relative humidity for 7 d.

Crown rot incidence [(number of diseased crowns/ total number of crowns) × 100] and disease severity were assessed after 7 d using the scale proposed by Alvindia et al. (2004). Disease incidence and severity were expressed as percentage reductions relative to the control treatment. All experiments were performed in duplicate to ensure the reliability of the results.

Effect of sodium bicarbonate alone or in combination with thiabendazole

Dip assays were conducted on Enano Gigante bananas to evaluate the effect of SB applied alone or in combination with thiabendazole (TBZ). Treatments were applied for 20 min at either ambient temperatures or 40 °C. Fruit disinfection and forced ripening were performed as described in the "Fruit disinfection and ripening" section.

The following treatments were tested: *1*) control (water), *2*) non-inoculated treatment (WI), *3*) 500 mM SB applied alone (SB), *4*) 500 mM SB + 225 μL L^{-1} TBZ (SB+TBZ), *5*) 225 μL L^{-1} TBZ (TBZ 225), *6*) 450 μL L^{-1} TBZ (TBZ 450), and *7*) 600 μL L^{-1} TBZ (TBZ 600).

Fungal inoculation was performed as described in the "Fungal inoculation" section. Dip treatments were carried out as previously described, with SB and TBZ dissolved in water at the desired concentrations and mixed thoroughly using a plastic rod to ensure homogeneity.

Each treatment consisted of three replicates, with four banana hands per replicate (each hand containing 3-4 fingers). After treatment, banana hands with inoculated and treated crowns were placed in 10 kg plastic boxes and incubated at 24-26 °C and 80-90 % of relative humidity for 7 d.

Disease incidence [(number of diseased crowns/ total number of crown) × 100] and disease severity were assessed 7 d after inoculation using the scale proposed by Alvindia et al. (2004). Both variables were expressed as percentage reductions relative to control treatment.

Statistical analysis

Data on CR incidence and severity reduction were analyzed using analysis of variance (ANOVA) under a completely randomized design using Statgraphics Plus version 5.1 software. Percentage data were transformed using the arcsine square root transformation of the proportion of diseased crowns to meet ANOVA assumptions. Mean comparisons were performed using Fisher's protected least significant difference (LSD) test at a significance level of $p \leq 0.05$. Results are presented as non-transformed values for clarity.

Results

Antifungal activity of sodium bicarbonate against crown rot in primary *in vivo* screenings

Curative effect. Among all SB treatments evaluated in the primary *in vivo* curative assays against banana CR, only the 500 mM and 475 mM concentrations significantly reduced disease incidence, achieving reductions of 77.8 % and 33.4 %, respectively. The remaining treatments did not significantly affect disease incidence compared with the control. Similarly, these two treatments were the most effective in reducing CR severity,

with reductions of 92.9 % and 78.6 %, respectively. Treatments with 450 mM and 400 mM SB showed moderate effects, reducing disease severity by 58.7 % and 11.1 %, respectively. The remaining treatments did not significantly reduce disease severity (Figure 1).

Preventive effect. Overall, no significant reduction in CR incidence was observed in bananas treated with SB, inoculated with *Colletotrichum musae*, and incubated under the specified conditions during the primary *in vivo* experiments. However, SB significantly reduced CR severity. The 500 mM SB treatment was the most effective, achieving a 65.5 % reduction in severity, followed by the 475 mM SB treatment, with resulted in a 45.2 % reduction. The remaining SB treatments had little effect on CR severity

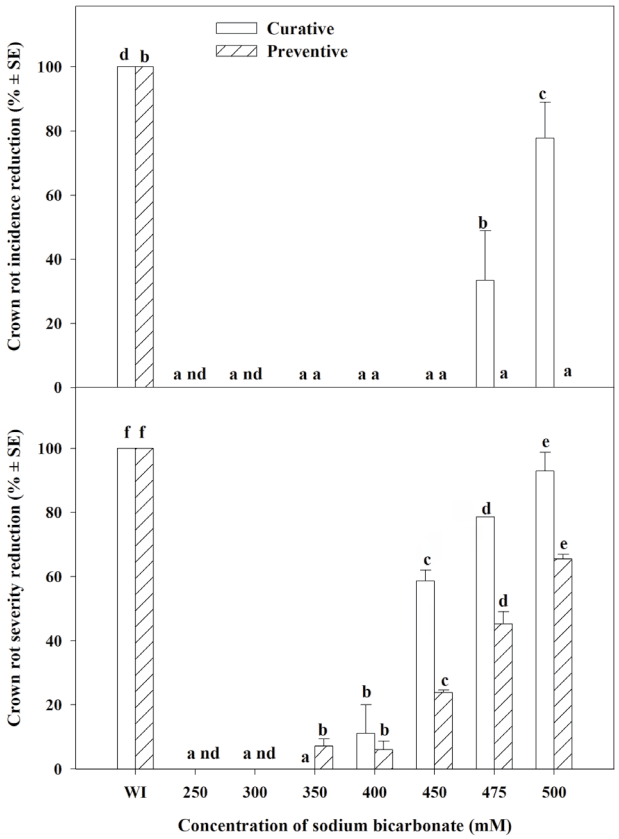

Figure 1. Curative and preventive effect of sodium bicarbonate at different concentrations on crown rot (CR) in primary *in vivo* experiments using artificially inoculated banana CV. Enano Gigante with *Colletotrichum musae*. Treatments were applied 1 h before or 1 h after inoculation and incubated for 7 d at 24-26 °C and 80-90 % RH. Reductions in disease incidence and severity were determined relative to the control treatment (water), which showed 100 % disease incidence and disease severity values ranging from 95.2 % to 100 % in curative assays and 100 % for both variables in preventive assays. Values for CR incidence and severity reduction represent means of two independent experiments. Within each treatment type, columns followed by different letters indicate significant differences according to Fisher's protected LSD test ($p \leq 0.05$) following ANOVA. Incidence and severity reduction data were arcsine-transformed prior to analysis; however, non-transformed means are presented. Each treatment consisted of three replicates. nd = not determined, WI = treatment without inoculation.

(Figure 1). Based on these results, the 500 mM SB concentration was selected for further experiments.

Effect of the dip temperature on sodium bicarbonate effectiveness

Curative effect. Among all treatments evaluated to determine the influence of dip temperature on the efficacy of 500 mM SB applied for 20 min, only the SB 40 °C treatment significantly reduced CR incidence, achieving a reduction of 83.3 %. The SB 45 °C treatment followed, with a 41.6 % reduction in CR incidence, whereas the remaining treatments had no significant effect. Similarly, the SB 40 °C and SB 45 °C treatments were the most effective in reducing CR severity, achieving reductions of 90.5 % and 91.7 %, respectively. The remaining treatments showed moderate reductions in CR severity, ranging from 41.7 % to 44.0 %, except for the HW 30 °C treatment, which showed no reduction (Figure 2).

Preventive effect. Overall, SB treatments evaluated to assess the influence of dip temperature in preventive applications did not significantly affect CR incidence in Enano Gigante bananas inoculated with *Colletotrichum musae* and incubated under the specified conditions for 7 d. However, these treatments significantly reduced CR severity, except for the HW 30 °C treatment. The SB 40 °C and SB 45 °C treatments were the most effective, reducing CR severity by 61.9 % and 63.1 %, respectively. The HW 40 °C, HW 45 °C, and SB 30 °C treatments followed, with reductions in disease severity of 50.0 %, 50.6 %, and 36.9 %, respectively (Figure 2). Based on these results, the SB 40 °C treatment, with a 20 min immersion, was selected for subsequent curative assays.

Curative activity of sodium bicarbonate alone or in combination with thiabendazole. The 500 mM SB treatment applied alone significantly reduced CR incidence, achieving an 83.3 % reduction. Its curative effect was statistically similar to that of the 600 µL L^{-1} TBZ treatment (TBZ 600), which resulted in a 95.8 % reduction in CR incidence. The combined treatment of SB with 225 µL L^{-1} TBZ (TBZ 225) significantly improved curative control compared with SB alone, achieving complete CR control (100 % reduction in disease incidence). The effectiveness of this combined treatment was statistically equivalent to that of the 600 µL L^{-1}

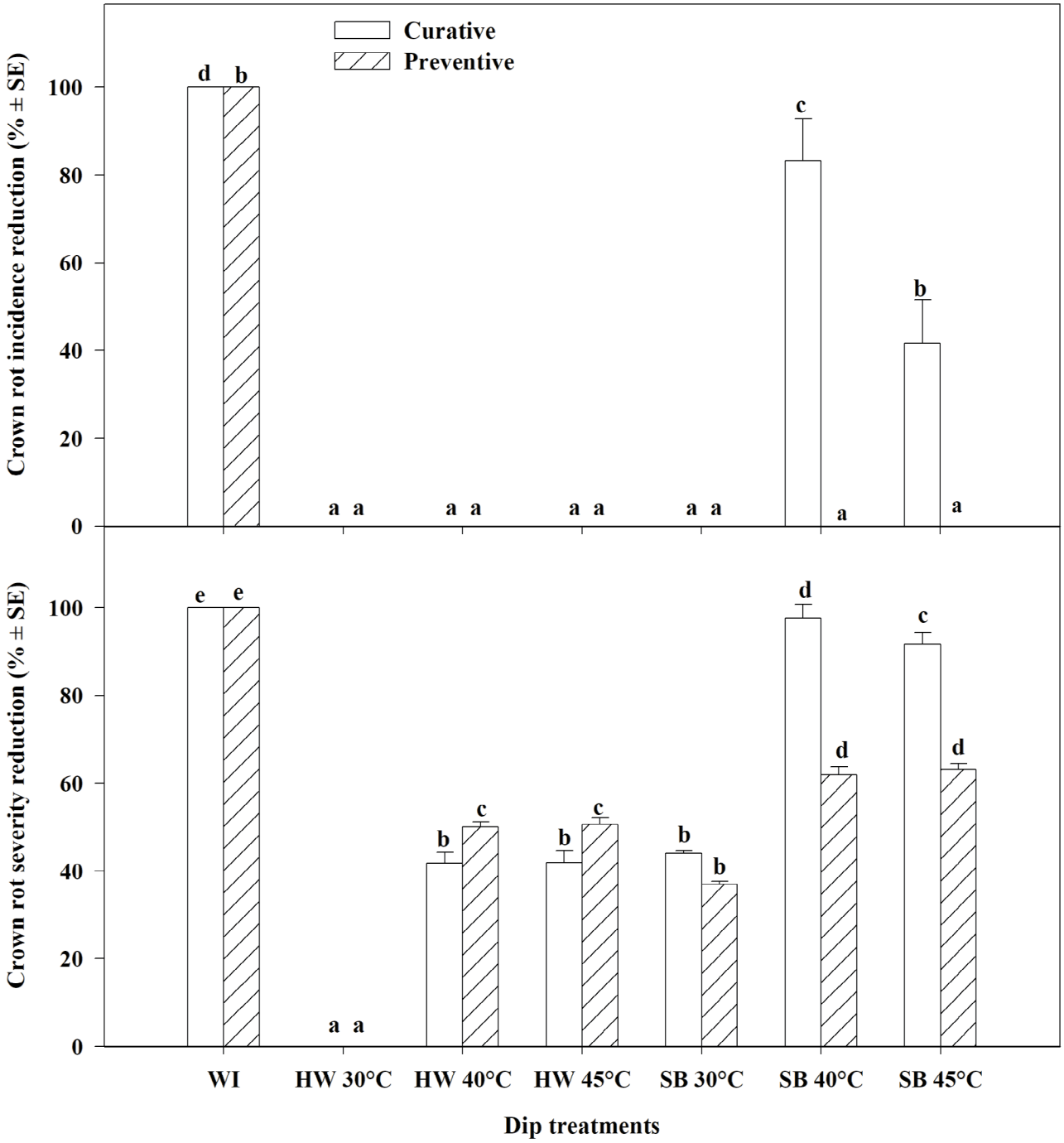

Figure 2. Effect of dip temperature on the efficacy of water alone or 500 mM sodium bicarbonate (SB) applied for 20 min to control crown rot (CR) in artificially inoculated banana cv. Enano Gigante with *Colletotrichum musae*. Treatments were applied 1 h before or 1 h after inoculation and incubated for 7 d at 24-26 °C and 80-90 % relative humidity. Reductions in disease incidence and severity were calculated relative to the control treatment (water), which showed 100 % disease incidence and severity across all treatment types and temperatures. Values for CR incidence and severity reduction represent means of two independent experiments. Within each treatment type, columns followed by different letters indicate significant differences according to Fisher's protected LSD test ($p \leq 0.05$) following ANOVA. Incidence and severity reduction data were arcsine-transformed prior to analysis, non-transformed means are presented. Each treatment consisted of three replicates. WI = non-inoculated treatment.

TBZ treatment. In contrast, the 450 µL L^{-1} TBZ treatment (commercial dose) had not significantly affect CR incidence. Similarly, the combined SB + TBZ 225 treatment improved CR severity control, achieving a 100 % reduction, whereas SB applied alone reduced disease severity by 97.6 % (Figure 3).

Discussion

This study evaluated the *in vivo* antifungal activity of sodium bicarbonate (SB) against postharvest crown rot (CR) in banana cv. Enano Gigante. Bananas were artificially inoculated with *Colletotrichum musae* on the crown of banana hands, and treatments were applied before and after storage for 7 d at 24-26 °C and 80-90 % relative humidity.

Regarding the primary *in vivo* antifungal activity of SB, the 500 mM concentration was the most effective treatment for reducing CR incidence under curative conditions, achieving a reduction of 77.8 %. In contrast, the same treatment applied preventively did no reduce CR incidence, suggesting that SB exhibits curative activity but limited preventive efficacy under conditions evaluated. Additionally, SB at 475 mM and 500 mM effectively reduced CR incidence. The reductions achieved with 500 mM SB in both incidence and severity (77.8 % and 92.9 %, respectively) exceeded those reported for other salts, such as sodium silicate at 130 mM and potassium carbonate at 175 mM (González-Jiménez et al., 2023; 2024), suggesting a potentially stronger antifungal effect under the conditions tested.

Similarly, curative dip treatments with 500 mM SB at 40 °C for 20 min effectively controlled CR, reducing incidence and severity by 83.3 % and 97.6 %, respectively. These reductions exceeded those reported by De Costa & Gunawardhana (2012), who observed a 62 % reduction with 300 mM SB. This difference may be associated with the higher SB concentration and longer immersion period used in the present study. Compared

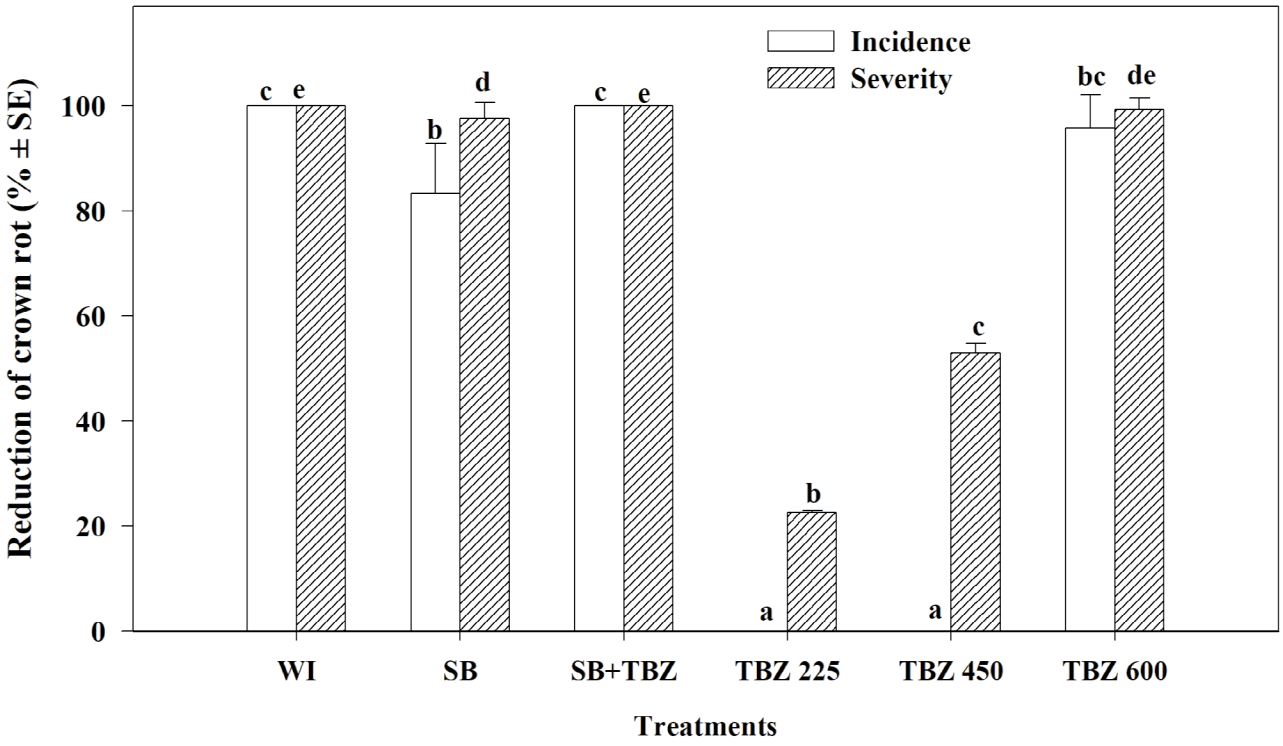

Figure 3. Efficacy of 500 mM sodium bicarbonate (SB) applied alone, 225 µL L-1 thiabendazole (TBZ 225), 450 µL L-1 thiabendazole (TBZ 450), 600 µL L-1 thiabendazole (TBZ 600), and the combination of 500 mM SB and 225 µL L-1 TBZ (SB + TBZ) for controlling crown rot (CR) in artificially inoculated banana cv. Enano Gigante with *Colletotrichum musae*. Treatments were applied for 20 min at 40 °C, 1 h after inoculation, and incubated for 7 d at 24-26 °C and 80-90 % relative humidity. Reductions in disease incidence and severity were determined relative to the control treatment (water), which showed 100 % incidence and severity. For each variable, columns followed by different letters indicate significant differences according to Fisher's protected LSD test ($p \leq 0.05$) following ANOVA. Incidence and severity reduction data were arcsine-transformed prior to analysis; however, non-transformed means are presented. Each treatment consisted of three replicates. WI = non-inoculated treatment.

with the results of Alvindia (2013), who applied SB at 50 °C for 30 min, a slightly greater reduction in CR incidence was observed in the present study (83.3 % vs. 77.6 %), despite differences in treatment conditions.

Chi & Anh (2019) reported that SB concentrations between 250 mM and 350 mM applied for 25 min had no significant effect on CR incidence, which is consistent with our findings. Alvindia et al. (2004) observed an 86 % reduction in CR severity when 6 g L^{-1} SB was applied as a curative treatment, whereas Qiao et al. (2022) reported a 72.47 % reduction using 100 mg L^{-1} hinokitiol. Although these reductions were substantial, they were lower than those achieved in the present study, possibly due to differences in treatment conditions and application parameters.

In preventive treatments, SB did not significantly reduce CR incidence, suggesting that *Colletotrichum musae* conidia remained viable after exposure, in agreement with the findings of Alvindia & Natsuaki (2007). However, SB significantly reduced CR severity, indicating a partial inhibitory effect on disease progression. Longer immersion periods may improve preventive efficacy, potentially by increasing the exposure of infected tissues to SB. The 61.9 % reduction in CR severity observed with 500 mM SB was comparable to the 65 % reduction reported by Alvindia & Natsuaki (2007) using 5 g L^{-1} SB combined with 2.5 mL L^{-1} surfactant.

The application of SB at 40 °C enhanced its curative effectiveness, achieving an 83.3 % reduction in CR incidence, compared to the 0 % reduction observed in preventive dips treatments under the same conditions. In curative assays, hot water treatments at 40 °C and 45 °C reduced CR severity by up to 41 % relative to the control. Thus, heated SB provided greater fruit protection that non-heated SB. The enhanced effectiveness of heated SB may be attributed with additive or complementary effects between thermal treatment and salt activity. Hot water treatments alone were more effective in reducing CR severity and CR incidence. Previous studies have shown that hot water enhances the efficacy of SB against *Penicillium italicum* (Palou et al., 2001) and *Penicillium digitatum* (Cocco et al., 2008; Schirra et al., 2008) in citrus. In addition, heat treatments may induce defense responses in fruit tissues, contributing to reduced pathogen growth (De Costa & Gunawardhana, 2012).

Hot water is generally considered more effective as a curative treatment because it primarily reduces or eliminates fungal inoculum (Alvindia, 2012). It also exerts a direct effect by inhibiting germ tube elongation or inactivating fungal conidia (Di Francesco et al., 2018). Furthermore, hot water may indirectly suppress fungal development by triggering physiological responses in fruit tissues (Schirra et al., 2000) and by modifying the chemical environment of the fruit rind through the activation of antimicrobial compounds (De Costa & Erabadupitiya, 2005). However, although hot water acts as a fungistatic agent, it lacks residual activity to prevent fungal development after thermal treatment (Alvindia, 2012), which may explain the limited induction of defense mechanism observed in preventive treatments using hot water alone against *Colletotrichum musae*. In the present study, the combined application of hot water and SB significantly reduced both CR incidence and severity compared with individual treatments, consistent with the findings of Alvindia (2013), who demonstrated that thermal treatment enhances the efficacy of salts.

The direct or indirect effects of bicarbonate salts on microorganisms are likely associated with reduced turgor pressure within fungal cells, leading to hyphal and conidial collapse (Ilić et al., 2018). Additionally, bicarbonate ions may increase membrane permeability, promoting the leakage of ionic species and further reducing turgor pressure within fungal cells (Fallik, 2004).

When the effect of SB applied alone or in combination with a low dose of TBZ was evaluated against CR under curative conditions, the combined treatment of 500 mM SB and 225 μL L^{-1} TBZ (SB+TBZ) not only improved CR severity control (100 % reduction) but also completely suppressed CR incidence (100 % reduction). These results suggest that SB may have enhanced the effectiveness of TBZ, potentially by facilitating its diffusion or penetration through the fruit cuticular wax (Da Silva França et al., 2024; Schirra et al., 2008). Furthermore, the results suggest a synergistic interaction between SB and TBZ, since SB applied alone was less effective and TBZ applied alone at 225 μL L^{-1} did not significantly reduce CR incidence. The combined treatment (SB + TBZ) was more effective than the commercial TBZ dose (450 μL L^{-1}), and slightly more effective than the regional dose (600 μL L^{-1}).

Synergistic effects have previously reported for the combination of 400 mg L^{-1} of TBZ and 0.5 % SB at 40 °C to control green mold caused by *P. digitatum* in citrus, showing high efficacy in both TBZ-sensitive (85.8 %) and TBZ-resistant (83.3 %) strains (Schirra et al., 2008). Similarly, the combination of butylated hidroxyanisole with TBZ exhibited synergistic effects, inhibiting up to 96 % of mycelial growth and conidial germination of *Colletotrichum musae* (Khan et al., 2001). Moreover, the combination of gum Arabic and chitosan was reported to completely inhibit mycelial growth and conidial germination of *C. musae*, achieving a 92.5 % reduction (Maqbool et al., 2010). In contrast, the findings of the present study differ from those reported by Vidal-Vergara et al. (2022), who did not observe synergistic effects when combining potassium silicate with TBZ to control postharvest anthracnose in papaya caused by *Colletotrichum brevisporum*.

On the other hand, the results obtained in this study suggest reduced sensitivity of *Colletotrichum musae* to TBZ at the commercial dose (450 µL L^{-1}) under the evaluated conditions in Tabasco, Mexico. Consequently, banana producers in this region reportedly use higher TBZ doses (600 µL L^{-1}), which are considered effective under local conditions. However, confirmation of fungicide resistance would require dedicated sensitivity and molecular analysis. Previous studies have documented resistance to benzimidazole (the chemical group to which TBZ belongs) associated with mutations in the TUB-2 gene (Torres-Calzada et al., 2015) and other resistance-related mechanisms in fungal species (Downing, 2000).

Conclusion

SB demonstrated curative activity against both the incidence and severity of CR in primary *in vivo* experiments. Its effectiveness was further improved when applied as a dip treatment at 40 °C. Under curative conditions, the combined application of SB with low dose of TBZ provided highly effective control of CR. These findings suggest that integrating SB with reduced TBZ doses may represent a promising strategy for postharvest disease management, particularly in banana packinghouses operations.

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