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Bacillus subtilis and *Trichoderma harzianum* on the quality and occurrence of anthracnose in bananas

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Abstract

The objective of this study was to assess the efficiency of *Trichoderma harzianum* and *Bacillus subtilis* on postharvest control of anthracnose, the effects on postharvest quality in 'Nanicão' bananas, and the sensory acceptance of fruits. *In vitro* and *in vivo* tests were performed. The *in vitro tests* included the following treatments: control (distilled water), *T. harzianum, B. subtilis,* and thiabendazole. *In vitro* tests consisted of pairing *Colletotrichum musae* with *T. harzianum* and *B. subtilis* by daily measuring the mycelial growth of *C. musae* and by counting the spore germination of *C. musae*. For *in vivo* tests, the treatments were control (distilled water), *T. harzianum*, *B. subtilis*, *T. harzianum* + *B. subtilis*, and thiabendazole. *In vivo* tests consisted of a daily evaluation of the incidence and severity of anthracnose in 'Nanicão' bananas. Some physicochemical characteristics of fruits were evaluated (weight loss, color, pulp firmness, soluble solids (SS), titratable acidity (TA), pH, and SS/TA ratio), as well as the sensory acceptance of bananas. Based on the results, the application of *T. harzianum* in 'Nanicão' bananas was efficient in decreasing the severity of anthracnose caused by *C. musae*, but treatments with biological control agents were inefficient in this process. On postharvest quality, treatments with biological control agents seem to have accelerated the maturation process. However, results from the sensory analysis showed that fruit acceptance was not affected.

Keywords: biological control agents, *Colletotrichum musae,* disease, *Musa*.

Introduction

The banana is one of the main crops in the world and is first ranked among the most-produced fruit, with a world production of 116 million tons (Fao, 2018). Its production chain faces phytosanitary problems, responsible for postharvest losses. Among them, the pathogenic rot stands out, such as anthracnose, caused by the fungus *Colletotrichum musae* (Berk. & MA Curtis) Arx (1957), responsible for fruit rots (Agrofit, 2020).

The application of chemical fungicides is the most used method for the postharvest control of anthracnose in bananas. These products protect against the attack of the pathogen by systemic action. The fungicides registered for these controls are thiabendazole, imazalil, and azoxystrobin + fludioxonil (Agrofit, 2020). Although postharvest pathogen control still depends on the use of fungicides, there are strong restrictions on the use of these chemicals due to environmental and human health impacts (Zhou et al., 2020; Bhagat et al., 2021).

The use of alternative treatments instead of fungicides has shown promising results in the control of *C. musae*, among them the application of oils, extracts (Negreiros et al., 2013), and biological control agents (Oliveira et al., 2016; Bonett et al., 2020).

Biological control with antagonistic microorganisms can be applied both in the pre-harvest and in the postharvest periods; however, when applied in the postharvest, it has been more successful in the control of diseases. The *Trichoderma* and *Bacillus* genera are widely used; both can synthesize antibiotics and act through mechanisms of antibiosis, parasitism, and competition for nutrients and substrate (Dukare et al., 2018).

The effect of these biological agents on the control of postharvest phytopathogens has been studied in some crops, such as peaches (Casals et al., 2012),

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bananas (Oliveira et al., 2016) and pepper (Amaro et al., 2018). Some studies have verified the reduction of rot losses after the application of biocontrol agents (Alvindia, 2013; Vilaplana et al., 2020); however, much of the works do not evaluate the postharvest quality of fruits after this treatment (Dukare et al., 2018).

As banana is a fruit highly appreciated by consumers and widely marketed in its natural form, guaranteeing fruit quality in the postharvest period is important. There are few studies on using biological agents to control anthracnose in bananas, so this application becomes an interesting alternative to be tested. In addition, it is very important to evaluate postharvest quality after the application of antagonists and the sensory acceptance of fruits by consumers. The objective of this study was to assess the efficiency of *Trichoderma harzianum* and *Bacillus subtilis* on postharvest control of anthracnose (*Colletotrichum musae*), the effects on postharvest quality of 'Nanicão' bananas, and the sensory acceptance of fruits.

Material and Methods

Banana 'Nanicão' bunches were used to perform *in vivo* experiments, harvested from plants with a spacing of 2.5 m x 2.0 m, in an orchard of 17 years of age, located in Novo Itacolomi, PR, Brazil (23° 45' 41" S and 51° 30' 22" W and 620 meters of altitude). Fruits were harvested in the pre-climacteric stage, in the color stage 1 (totally green peel). Three commercial products were used in the *in vitro* and *in vivo* tests: *Trichoderma harzianum* (Trichodermil®, Koppert Biological Systems, Piracicaba, SP, Brazil) which shows a concentration of spores of 48 g L^{-1}; *Bacillus subtilis* (Serenade®, Bayer S/A, Socorro, SP, Brazil) which presents Colony Forming Units (CFU) concentration of 13.68 g L^{-1}, and Thiabendazole (Tecto®, Syngenta, Paulínia, SP, Brazil) with benzimidazole concentration of 485 g L^{-1}.

Isolates of *C. musae* were provided by the Phytopathology Laboratory of Universidade Estadual do Centro-Oeste, Guarapuava, PR, Brazil, for *in vitro* and *in vivo* tests. Mycelium fragments were inoculated on Petri dishes containing PDA (potato/dextrose/agar) culture medium to recover these isolates. These plates were then sealed with parafilm and incubated in a BOD-type growth chamber at 28 °C ± 2 °C, without photoperiod. After seven days, disks with the culture medium and pathogen structures were transferred to the center of new plates with PDA medium and again incubated under the same conditions described above for seven days. After confirmation of *C. musae*, based on colony morphology and spore characters according to Sutton and Waterson (1970), the fungus was multiplied in a PDA culture medium and stored at 28 °C ± 2 °C for testing.

The *in vitro* tests consisted of pairing tests and spore germination trials, both performed in duplicate. The design was completely randomized with 12 treatments and 10 replicates. The pairing tests were conducted using direct confrontation according to the methodology described by Campanile et al. (2007), calculating the antagonism index. The pairing tests used the agar dilution method, which consisted of adding the treatments to the culture medium. The following treatments were used: *T. harzianum* (0; 1; 1.75; 2.5; 3.25; 4 mL L^{-1}); *B. subtilis* (0; 0.5; 0.75; 1; 1.25; 1.5 mL L^{-1}) and thiabendazole (standard control) at the dose of 0.41 mL L^{-1}. The concentration of 0 mL L^{-1} refers to the control treatment.

All products were added to 200 mL of PDA medium, with the culture medium still flux, previously sterilized at 121 °C for 15 minutes, and poured into the Petri dishes. In the control treatment, no product was added to the culture medium. After preparing the Petri dishes, 8 mm diameter mycelial disks of *C. musae* were transferred to the center of the Petri dishes. These plates were sealed with parafilm and incubated in a BOD-type growth chamber at 28 °C ± 2 °C without photoperiod for five days. Petri dishes containing only PDA culture medium were used as an absolute control, and those added with the fungicide thiabendazole (0.41 mL L^{-1}) were used as the standard control.

The antagonistic activity of the treatments was determined by the evaluation of the mycelial growth, performed daily through measurements of the mycelial radius (mm) of the colony with a digital caliper. Plates were marked externally in a perpendicular direction to measure the radial growth of the colony in two orthogonal axes, calculating the average values per plate. The growth was measured until the mycelium of the control plate filled the edges of the plate. The antagonism index was evaluated according to equation 1, described by Campanile et al. (2007).

$$AI\ \% = \frac{(MR-mr)}{MR} \times 100 \qquad (1)$$

In which:

AI%: Antagonistic index percentage;

MR: Mycelial radius without the presence of the antagonist (control);

mr: Mycelial radius with the presence of the antagonist;

During the spore germination tests, suspensions of pathogen conidia obtained from pure colonies of the *C. musae* isolate grown in PDA medium were initially

prepared with the addition of distilled water + Tween 20, scraping of the Petri dish with the Drigalski's loop and gauze filtration. The suspension was calibrated to 1 x 10^4 mL^{-1} conidia with the Neubauer chamber under an optical microscope. Aliquots of 40 µL of the conidia suspension were added into individual cavities of Elisa test plates, where 40 µL of each treatment was also placed. Afterward, the plates were kept in a BOD-type growth chamber at 25 °C without photoperiod. After 24 hours of incubation, the conidia germination was stopped by adding 20 µL of the blue lactophenol cotton dye to each well. Spores were considered when they presented emission of the germ tube, regardless of its size. In an optical microscope, 50 spores per cavity were quantified randomly. Data were expressed as the percentage of spore germination on total spores.

Concentrations with the best results during the *in vitro* tests were used to evaluate their effect on anthracnose control and postharvest conservation of bananas during storage. The bananas were harvested in the orchard in the pre-climacteric stage and went through a ripening chamber with ethylene for three days, ensuring that all were in the same ripening stage. On the fourth day after harvesting, the banana bunches were separated into bouquets of four fruits. Bananas were washed in a tank with 0.2% neutral detergent (2 mL L^{-1}) for latex removal and surface cleaning, sanitized in 0.5% sodium hypochlorite solution (5 mL L^{-1}) for three minutes, and then washed with water.

After, bananas were immersed in the treatments for one minute, in constant movement. To evaluate the effect of treatments on the anthracnose control, the following treatments were applied: absolute control without inoculation (distilled water); Inoculated control (distilled water); *T. harzianum* at 1 mL L^{-1} (0.048 g a.i.); *B. subtilis* at 0.5 mL L^{-1} (0.00684 g a.i.); *T. harzianum* at 1 mL L^{-1} (0.048 g a.i) + *B. subtilis* at 0.5 mL L^{-1} (0.00684 g a.i); and Thiabendazole fungicide at 0.41 mL L^{-1}.

After 24 hours, the fruits were inoculated with *C. musae* suspension (1 x 10^4 mL^{-1} conidia) by spraying (hand sprayer), except for the treatment (absolute control), which were not inoculated with conidia fungi. Fruits treated with distilled water, not inoculated, were considered absolute control; and fruits treated with distilled water and inoculated with the conidia of *C. musae* were considered inoculated control. Fruits were placed on plastic trays and maintained at ambient conditions. After 48 hours of treatment application, the incidence of the disease was evaluated daily (four evaluations in total), observing the percentage of fruits with symptoms of anthracnose, based on equation 2.

$$I = \frac{(NFL)}{TNF} \times 100 \quad (2)$$

In which:

I: Incidence (%);

NFL: Number of fruits with lesions;

TNF: Total number of fruits.

Disease severity was evaluated daily, which refers to the percentage of the fruit peel area with anthracnose symptoms, determined from a specific diagrammatic scale for anthracnose in bananas, ranging from 0.5% to 64% of the fruit-injured area (Moraes et al., 2008). Subsequently, the area under the disease progression curve (AUDPC) was calculated according to the equation presented by Campbell and Madden (1990). The experimental design was completely randomized with six treatments and four replicates, composed of bouquets of four fruits.

To evaluate the postharvest quality of bananas during storage, the fruits were previously cleaned and the following treatments were applied: Control (distilled water); *T. harzianum* at 1 mL L^{-1} (0.048 g a.i.); *B. subtilis* at 0.5 mL L^{-1} (0.00684 g a.i.); *T. harzianum* at 1 mL L^{-1} (0.048 g a.i) + *B. subtilis* at 0.5 mL L^{-1} (0.00684 g a.i); and Thiabendazole fungicide at 0.41 mL L^{-1}.

Physicochemical characteristics evaluations were performed four and seven days after harvesting, during the storage period of the fruits under ambient conditions. Weight loss was determined using a semi-analytical balance, weighing the bouquets. Results were expressed as of initial weight of the bouquets. Analyses of color were performed on the peel of fruits by the CIEL *a *b system, using the Croma Meter CR-400/410 colorimeter (Konica Minolta, 1998).

Pulp firmness was measured at two points on opposite sides of the fruits without peel, using a digital penetrometer (FR-5120, Lutron Electronic Enterprise, Taipei, Taiwan) with an 8.0 mm diameter ferrule. Results were expressed in Newtons (N). Soluble solids (SS) content was determined by direct reading at room temperature in a digital refractometer (Pocket refractometer Pal1, Atago, Tokyo, China), and results expressed in ºBrix (Ial, 2008).

Titratable acidity (TA) was determined in an aqueous solution of 10 g of fruit pulp and 90 mL of distilled water with a standardized solution (0.1M NaOH) (Ial, 2008). Results were expressed as a percentage of the malic acid 100 g^{-1}. The pH was obtained by a digital pH

meter in the same aqueous extract (Ial, 2008). The ratio was determined by the relation between (SS) and (TA). The experimental design was completely randomized with five treatments and four replicates composed of a bouquet of four fruits.

The sensory acceptance test was performed in individual booths, with 36 consumers of both genders, aged between 18 and 30 years. Bananas were sanitized and the following treatments were applied: Control (distilled water); *T. harzianum* at 1 mL L^{-1} (0.048 g a.i.); *B. subtilis* at 0.5 mL L^{-1} (0.00684 g a.i.); *T. harzianum* at 1 mL L^{-1} (0.048 g a.i) + *B. subtilis* at 0.5 mL L^{-1} (0.00684 g a.i); and Thiabendazole fungicide at 0.41 mL L^{-1}. When the control fruits were in the ideal stage for consumption (total yellow peel), they were evaluated by consumers. The sensory acceptance of bananas was performed using a nine-point hedonic scale (Ial, 2008). Samples were coded with three random digits, and the order of presentation was randomized among consumers. Consumers were instructed to clean their palate with water and cream cracker biscuits between samples. The project was approved by the Human Research Ethics Committee of Universidade Estadual do Centro-Oeste, Brazil (Certificate of Ethical Evaluation Presentation 3.185.727/2019).

All data were submitted to the Shapiro-Wilk normality tests, homogeneity of variance by Cochran C, and ANOVA. Germination data were analyzed by linear and polynomial regression and by the Tukey test ($p \leq 0.05$) using the Sisvar software (Ferreira, 2014). Severity data were compared by the Tukey test ($p \leq 0.10$) using the software R (R Core Team, 2013). Data on physicochemical characteristics did not show normality, so it used gamma probability distribution with log binding function and interchangeable matrix, and were analyzed by the Generalized Estimating Equations (GEE) model and by the Bonferroni test ($p \leq 0.05$) using the SPSS 18.0.0 software. Sensory acceptance data were compared by the Tukey test ($p \leq 0.05$) using the Sisvar software (Ferreira, 2014).

Results and Discussion

Results showed that, when *T. harzianum* or *B. subtilis* was added to the culture medium, all the concentrations tested inhibited the mycelial growth of *C. musae*, as well as the treatment with thiabendazole (**Figure 1**).

The species of the genus *Trichoderma* is known for its biocontrol activity on pathogenic fungi (Dukare et al., 2018). *C. musae* is one of the fungi antagonized by the genus *Trichoderma* in the cultivation of paired cultures, presenting rapid growth, a characteristic that gives it an advantage in the competition for nutrients and space (Bonett et al., 2013).

In this study, *T. harzianum* showed 100% inhibition of phytopathogen. Oliveira et al. (2016) also observed a high percentage of inhibition of mycelial growth of *C. musae* by *Trichoderma* spp. (84%), while in the Bonett et al. (2013) study, the *T. harzianum* species antagonized *C. musae* in 56.7% by the rapid growth, beating the pathogen by competition for space and nutrients.

The species of *Bacillus* is known for its antagonistic activity, with versatility to overcome phytopathogens. In general, antagonistic bacteria act by antibiosis and occasionally by parasitism and competition (Dukare et al., 2018). *C. musae* is one of the fungi antagonized by the genus *Bacillus* in the cultivation of paired cultures (Oliveira et al., 2016).

Based on the results, *B. subtilis* also presented 100% inhibition of the phytopathogen. Similarly, Oliveira et al. (2016) found a 74% inhibition index for *B. subtilis* on *C. musae,* and Amaro et al. (2018) reported that some isolates of the *B. subtilis* antagonized *C. gloeosporioides* in 100% by the mechanism of antibiosis on the phytopathogen.

Chemical treatment with thiabendazole also controlled the growth of *C. musae* efficiently, with 100% of phytopathogen inhibition. This action was already expected since this is a fungicide registered to control *C. musae* (Agrofit, 2020).

Regarding spore germination, this physiological process showed a quadratic effect as the concentrations of *T. harzianum* increased, and there was a statistical difference between these concentrations in the control and thiabendazole treatments (**Figure 2**).

For the treatment with *B. subtilis*, there was a linear increase in spore germination as a function of increasing concentrations of the biological product tested, and there was a statistical difference between these concentrations in the control and thiabendazole treatments (**Figure 3**).

The low germination of spores in water detected in this study (13.6%) was similar to that found by other authors, where the isolate of *C. musae* obtained 15.7% germinated conidia in distilled water when incubated at 27 °C (Couto & Menezes, 2004). The low germination of spores in the chemical treatment with thiabendazole was already expected. As mentioned previously, this is a systemic fungicide registered for the control of anthracnose during the banana postharvest period (Agrofit, 2020).

The systemic fungicides are absorbed and can move in the plant through the conducting system,

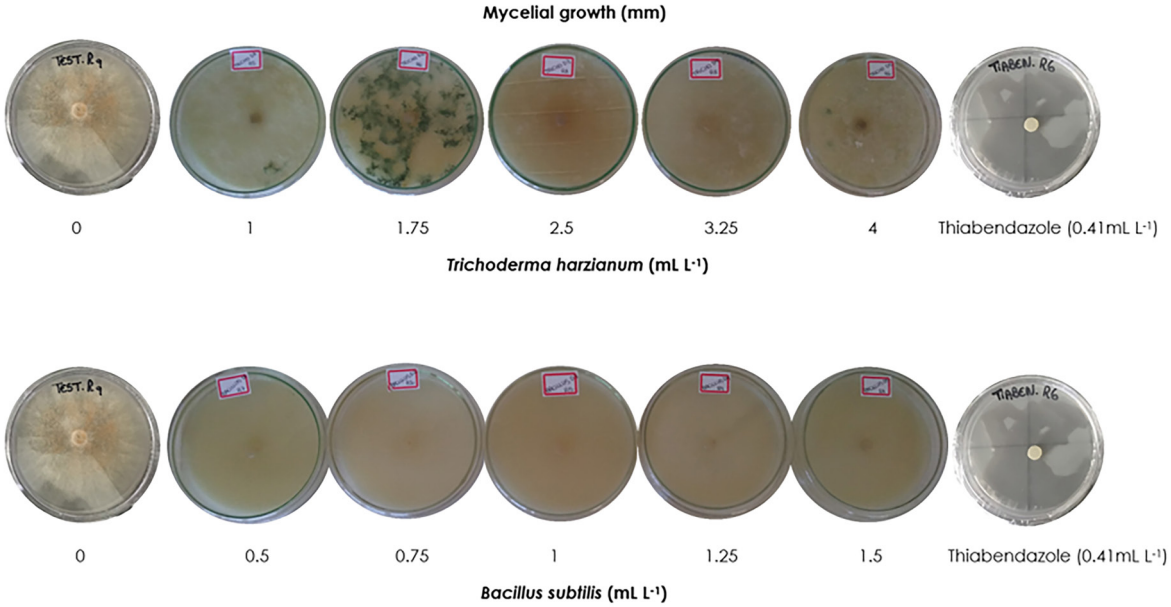

Figure 1. Mycelial growth (mm) of *T. harzianum* and *B. subtilis* on *C. musae*.

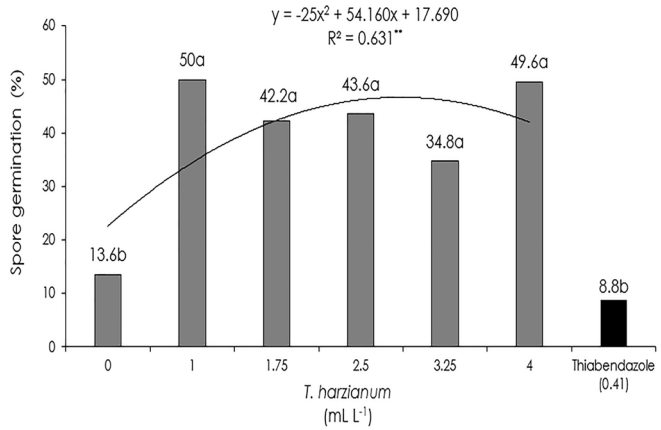

Figure 2. Percentage of germination of *C. musae* spores treated with *T. harzianum*. Assay performed in duplicate. Means (n = 10) followed by different letters, in the same evaluation, differ by the Tukey test at the 5% probability level ($p \leq 0.05$) with F value (0.0000).

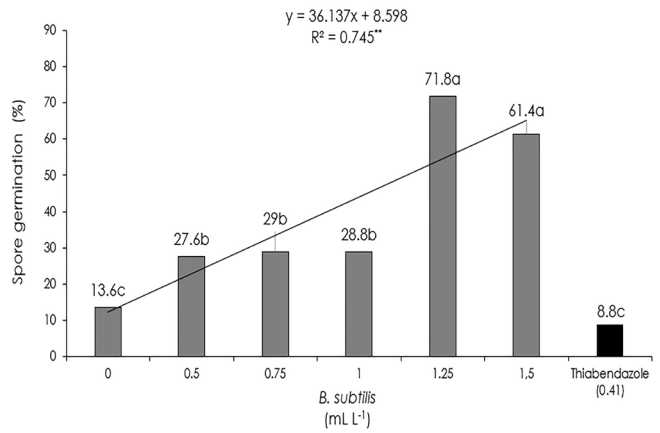

Figure 3. Percentage of germination of *C. musae* spores treated with *B. subtilis*. Assay performed in duplicate. Means (n = 10) followed by different letters, in the same evaluation, differ by the Tukey test at the 5% probability level ($p \leq 0.05$) with F value (0.0000).

providing a prolonged protective action (Reis et al., 2019). Thiabendazole is a systemic fungicide that is part of the group of benzimidazoles; these compounds act in the inhibition of mitosis during the cell division in the metaphase phase and tubulin biosynthesis (Agrofit, 2020; Frac, 2020), thus interfering in the development of the pathogen.

On the other hand, the higher germination of spores presented by treatments with *T. harzianum* and *B. subtilis* can be related to stress and competition situations, as pathogenic fungi are stimulated for their germination to perpetuate the species (Agrios, 2005).

There were no significant differences between treatments for disease incidence. For disease severity (AUDPC), fruits treated with *T. harzianum* showed a 48% reduction in the inoculated control (**Table 1**). Similarly, Oliveira et al. (2016) verified an antagonistic activity of *Trichoderma* spp. in the causal agent of anthracnose *in vivo* tests with bananas, with a 56% disease inhibition rate, differing from other microorganisms tested and from the control.

Firmness, SS, TA, pH, and SS/TA ratio showed a significant response between treatment and time by the Bonferroni test (≤ 0.05) (**Table 2**).

There was no difference between treatments for weight loss and fruit color. For pulp firmness, a difference was observed in the 2nd evaluation (seven days after harvest), when the treatment with *B. subtilis* showed the lowest pulp firmness but did not differ from the treatment with *T. harzianum* + *B. subtilis* (**Figure 4**).

The presence of microorganisms in the tissues

Table 1. Comparison of severity (AUDPC) of anthracnose in 'Nanicão' bananas treated postharvest with different products

	Absolute control	Inoculated control	*Trichoderma harzianum*	*Bacillus subtilis*	*Trichoderma harzianum* + *Bacillus subtilis*	Thiabendazole
Severity	45.12 ab	53.00 a	27.56 b	47.12 ab	41.56 ab	45.12 ab
Standard error	6.55	5.55	5.00	7.08	4.28	2.53
CV (%)	29.04	20.95	36.29	30.06	20.64	11.24

Means (n = 16) followed by different letters in the same line differ by the Tukey test ($p \leq 0.10$).

Table 2. Summary of Generalized Estimation Equations (GEE) for the variation sources in treatment and time

VARIABLES	Variation sources (F values)		
	Treatment	Time	Treatment * time
Firmness (N)	0.000*	0.000*	0.000*
Soluble solids (°Brix)	0.784	0.000*	0.000*
Titratable acidity (%)	0.000*	0.000*	0.000*
pH	0.025*	0.000*	0.000*
Ratio SS/TA	0.737	0.000*	0.000*

***** Significant by the Bonferroni test ($p \leq 0.05$).

Table 3. Multiple comparisons of the soluble solids in 'Nanicão' bananas treated postharvest with different products

TIMES (Days)	Control	Trichoderma harzianum	Bacillus subtilis	Trichoderma harzianum + Bacillus subtilis	Thiabendazole
1° (4 DAH)	18.25 ± 0.97 aB	18.30 ± 0.50 aB	17.97 ± 0.23 aB	19.32 ± 0.63 aB	19.27 ± 0.61 aB
2° (7 DAH)	23.60 ± 0.42 abA	23.55 ± 0.40 abA	24.05 ± 0.20 aA	23.55 ± 0.25 abA	23.12 ± 0.07 bA

DAH (days after harvest). Mean (n = 16) ± standard error. Different lowercase letters in the lines and different uppercase in the columns indicate differences by the Bonferroni test ($p \leq 0.05$).

can stimulate fruit senescence, increasing respiratory activity (Negreiros et al., 2013). This may be related to the lower pulp firmness in bananas treated with *B. subtilis* and *T. harzianum + B. subtilis*.

Analyzing pulp firmness under the same treatment at different times, there were significant differences since bananas lost firmness throughout the days of storage (Figure 4).

The decrease in firmness in bananas during ripening was also observed in the study by Aquino et al. (2017). After the harvest, the fruits presented higher values of firmness, but during ripening, changes in the texture occurred. Such changes can be explained by the pulp softening caused by the production of pectinolytic enzymes such as pectin methylesterase and polygalacturonase, which are responsible for the degradation of the cell wall and the solubilization of pectins (Barros et al., 2020).

Loss of firmness can also occur due to the increase in pulp moisture caused by osmotic changes with the peel. The pulp sugars increase more rapidly during the ripening than the peel due to the consumption of carbohydrates in respiration, contributing to a differential change in osmotic pressure (Aquino et al., 2017).

As for soluble solids, there was a significant difference in the 2nd evaluation between the *B. subtilis* treatment, which presented the highest soluble solids value, and the thiabendazole treatment, which presented the lowest value (**Table 3**). Analyzing the soluble solids under the same treatment at different times, there were significant differences for all the treatments. Fruits increased the soluble solids content throughout the days of storage (Table 3).

Unripen bananas are characterized by presenting starch in their composition; during the ripening process, starch hydrolysis occurs and the soluble sugars increase to guarantee a pleasant flavor (Rocha & Uribe, 2018). In the present study, the increase in soluble solids content was observed, and bananas reached up to 24.05 °Brix for

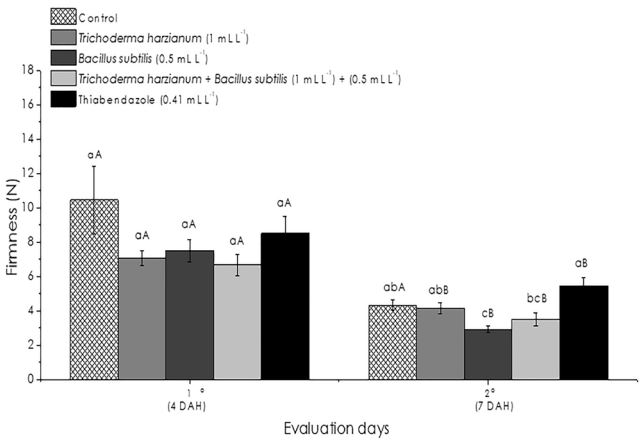

Figure 4. Firmness of pulp at 4 and 7 days after harvest (DAH) in 'Nanicão' bananas treated postharvest with different products. Bars indicate the standard error of the means (n = 16). Lowercase letters compare treatments at the same time. Uppercase letters compare the same treatment at different times. Different letters indicate differences by the Bonferroni test ($p \leq 0.05$)

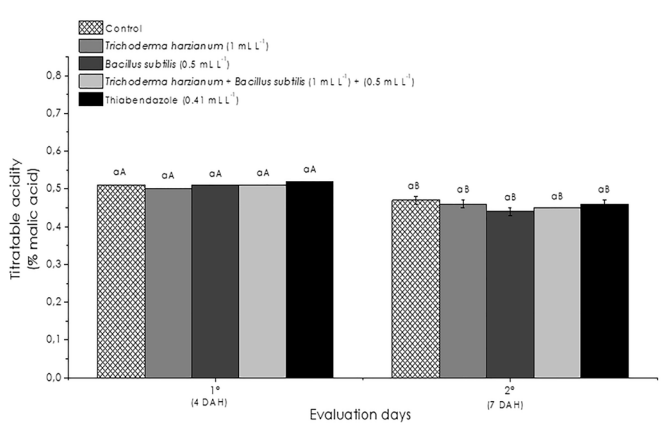

Figure 5. Titratable acidity at 4 and 7 days after harvest (DAH) in 'Nanicão' bananas treated postharvest with different products. Bars indicate the standard error of the means (n = 16). Lowercase letters compare treatments at the same time. Uppercase letters compare the same treatment at different times. Different letters indicate differences by the Bonferroni test ($p<0.05$).

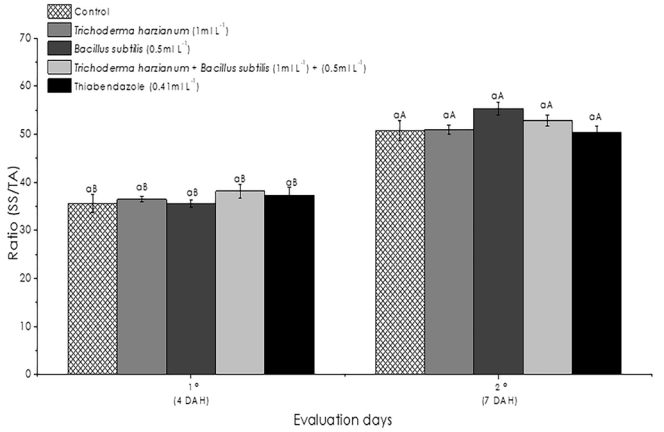

Figure 6. The SS/TA ratio at 4 and 7 days after harvest (DAH) in 'Nanicão' bananas treated postharvest with different products. Bars indicate the standard error of the means (n = 16). Lowercase letters compare treatments at the same time. Uppercase letters compare the same treatment at different times. Different letters indicate differences by the Bonferroni test ($p \leq 0.05$).

ripe fruit. According to Aquino et al. (2017), the soluble solids content for bananas, regardless of the cultivar, can vary from 22.01 to 29.53 °Brix for ripe fruit, and the ideal stage for consumption of 'Nanicão' bananas present soluble solids of 25.34 °Brix.

For titratable acidity, analyzing the same treatment at different times, there were significant differences for all the treatments because the fruits reduced the acidity throughout the days of storage (**Figure 5**).

During ripening, bananas have an increase in organic acids content, predominantly malic acid (Maduwanthi & Marapana, 2019). In this study, the reduction in banana acidity can be due to the use of organic acids in reactions during the respiratory process to produce ATP, which is necessary for the metabolism of the fruits during storage (Hazrati et al., 2017).

Titratable acidity varied from 0.44 to 0.47% in the 2nd evaluation when the fruits were with the peel all yellow. Other authors found a similar value of titratable acidity (0.37%) for 'Nanicão' bananas at the same ripening stage (Aquino et al., 2017).

For the pH, there was a significant difference in the 2nd evaluation between the control treatment, which presented the lowest pH value, and the *B. subtilis* treatment, which presented the highest value of the analyzed variable (**Table 4**). Analyzing the pH value of the same treatment at different times, there were significant differences for all treatments because the fruits increased the pH over the days of storage (Table 4).

Bananas show a decrease in pulp pH with ripening (Carvalho et al., 2011). This is due to the increase in the levels of organic acids, with the predominance of the malic acid (Maduwanthi & Marapana, 2019). However, the pH can increase in the final ripening stage, as verified in the present study, and also observed in the study by Carvalho et al. (2011). It may be associated with a reduction in titratable acidity.

For the SS/TA ratio of the same treatment at different times, there were significant differences for all treatments since the fruits gradually increased the SS/TA ratio over the days (**Figure 6**). The pleasant flavor of the fruits is guaranteed by the balance between sugar proportion and the number of acids. The higher the SS/TA ratio, the greater the balance between sweet and sour tastes, which makes the fruit more attractive for consumption (Bezerra & Dias, 2009).

In the present study, seven days after harvest at room temperature, when the fruits were ripe, the SS/TA ratio varied from 50.27 to 55.32. Vilaplana et al. (2020), using biological control agents in bananas, reported that the SS/TA ratio varied in the same range, from 50.2 to 59.5 after 21 days of cold storage, and that the use of biological control agents did not negatively affect

Table 4. Multiple comparisons of the pH in 'Nanicão' bananas treated postharvest with different products

TIMES (Days)	Control	*Trichoderma harzianum*	*Bacillus subtilis*	*Trichoderma harzianum* + *Bacillus subtilis*	Thiabendazole
1° (4 DAH)	4.68 ± 0.01 aB	4.67 ± 0.02 aB	4.67 ± 0.01 aB	4.70 ± 0.03 aB	4.74 ± 0.02 aB
2° (7 DAH)	4.89 ± 0.03 bA	4.95 ± 0.03 abA	5.04 ± 0.01 aA	4.96 ± 0.05 abA	4.99 ± 0.03 abA

DAH (days after harvest). Mean (n = 16) ± standard error. Different lowercase letters in the lines and different uppercase in the columns indicate differences by the Bonferroni test ($p \leq 0.05$).

fruity quality. Alvindia (2013) also showed that the use of *Trichoderma* spp. combined with sodium bicarbonate did not affect the ripening process of bananas, maintaining fruity quality.

In the acceptance test, all fruits were accepted (6.88 to 7.33), and there was no significant difference between the control and other treatments. Díaz et al. (2020) also reported no difference between the control and the antagonist treatments used during the lemon fruits postharvest.

Conclusions

Based on the results found under the conditions of this experiment, the application of *T. harzianum* in 'Nanicão' bananas was efficient in decreasing the severity of anthracnose (*C. musae*), while other treatments with biological control agents were inefficient in this process. On postharvest quality, treatments with biological control agents seem to have accelerated the maturation process. However, they did not affect the sensorial acceptance of the fruits.

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