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Post-harvest treatment with Ca-phosphite reduces anthracnose without altering papaya fruit quality

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ABSTRACT
This study assessed the effect of phosphites (Mg, Zn, Ca, K) on papaya (‘Sunrise Solo’) anthracnose (Colletotrichum gloeosporioides). Surface-sterilised wounded (2mm) fruits were inoculated (50µL; 10⁵ con/ml) with C. gloeosporioides and then the products were applied. The lesion diameters (LD) and the physico-chemical properties were analysed. Assays in vitro and in vivo were carried out with phosphites. The results in vitro indicated that the phosphites were effective in reducing the mycelial growth and conidia production for all doses [Phosphite Mg - 0.75, 1.5, 3 ml/l (40% P₂O₅ + 6% Mg) Fitofos Mg; Phosphate Zn - 1.25, 2.5, 5 ml/l (40% P₂O₅ + 10% Zn); Phosphate Ca - 1.5, 3, 6 ml/l (30% P₂O₅ + 7% Ca); Phosphate K - 1.25, 2.5, 5 ml/l (40% P₂O₅ + 20% K₂O)]. Concentration experiments on disease control showed that Phosphate-Ca at 6 ml/l (30% P₂O₅ + 7% Ca) significantly reduced the LD. Fruit treated with phosphate-Ca maintained physico-chemical properties of papaya fruits.

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KEYWORDS
Disease control; fruit; lesion; losing fruit weight; ripeness; Colletotrichum gloeosporioides; fruit pathology; post-harvest diseases

Introduction
Papaya (Carica papaya L.) is a typical fruit tree in tropical areas, with the largest production reported in Brazil. One of the main problems affecting papaya orchards is inappropriate post-harvest management, which harms fruit quality and increases disease severity at this production stage (Jacomin, Bron, & Kluge, 2003). A factor that affects the post-harvest papaya quality is the fruit anthracnose, which is considered a severe disease in Brazil and other papaya-producing countries. The anthracnose is mainly caused by Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., but recently other Colletotrichum species have been reported to cause fruit rot, such as: C. acutatum, C. dematium, and C. circinans (Santos Filho, Barbosa, & Nickel, 2003). Colletotrichum can be pathogenic to other cultivated plants, such as mango (Mangifera indica), guava (Psidium guajava), and banana (Musa acuminata) (Singh, 2011; Zakaria, Sahak, Zakaria, & Salleh, 2009).

When young fruits are infected, their development ceases and they shrivel and fall. Higher precipitation and relative humidity induces the appearance of depressed black spots on fruit peels, which then increase up to 5 cm in diameter. Around these spots, a halo of aqueous tissue forms with a distinguished central colour (Santos Filho et al., 2003; Ventura, Costa, & Tatagiba, 2003). At the post-harvesting stage, physical control is an efficient prevention method; this includes cooling the storage temperature, and applying heated steam, heated air, or hot water (Benato, Cia, & Souza, 2001). Phosphites and their related products are derived from phosphorous acid (phosphonate) and function as an alternative to conventional fungicides for plant disease control (Blum et al., 2007). The use of phosphate-based products in Brazilian agriculture has grown considerably due to the increase in productivity and final quality of the products (Franzini & Gomes Neto, 2007). Numerous phosphate products are utilised in field crops, being registered as fungicides and/or fertilisers, where some of them are sold as a ‘nutritional compound with antifungal action’ (Lobato, Olivieri, Daleo, & Andreu, 2010). The use of phosphites could be included in an integrated disease management plan to reduce the intensive use of fungicides and the production costs. Some advantages of phosphite use in agriculture are the rapid phosphorus uptake by the plant as compared to other phosphate-based products, the relative low cost of the raw material, the extension of the storage shelf-life of fruit after harvesting, and, finally, the double action of phosphite, i.e. as a fertiliser and a fungicide (Brackmann et al., 2005; Franzini & Gomes Neto, 2007). Phosphites act by inhibiting pathogen mycelial growth and sporulation as well as inducing...
the production of enzymes, such as phenylalanine ammonia-lyase, and other compounds such as ethylene and lignin, which activate the host plant’s defence mechanism against pathogen (Dempsey, Wilson, & Spencer-Phillips, 2014; Lobato et al., 2010).

Considering the economic importance of papaya, the negative impact of anthracnose as a post-harvest disease, and the effects of some compounds on fruits during this stage, this study aimed to evaluate the effect of phosphites on disease and fruit quality.

Materials and methods

Inoculum preparation

Papaya fruits (‘Sunrise Solo’), obtained from CEASA-DF, Brazil, were placed in wet chambers (plastic trays with wet paper towels inside, closed with parafilm) and kept at room temperature (25 ± 3°C) for 24 h. Fungal structures, characterised by orange mass of spores, were aseptically transferred to petri dishes containing 50% Potato Dextrose Agar (PDA). These dishes were kept in the growth chamber at 25°C, with a photoperiod of 12 h/day, for 15 days. Ten milliliters of sterile water were added to each petri dish. C. gloeosporioides was isolated from the anthracnose infections from guava fruits, and morphologically identified (Weir, Johnston, & Damm, 2012). The conidia suspension was filtered through a double layer of gauze, and the conidia concentration was estimated using a Neubauer plate and adjusted to 10⁶ spores/mL (Bautista-Baños, Hernández-López, Bosquez-Molina, & Wilson, 2003).

Fruit inoculation

The papaya fruits were sourced from CEASA-DF, Brazil, and were selected on ripening stage 0–2, i.e. up to 25% yellowish (Bicalho, Chitarra, Chitarra, & Coelho 2000; Cia 2005). The fruits were sterilised by immersing in 10% alcohol (1 min), followed by 1% sodium hypochlorite (1 min), and then rinsed in autoclaved distilled water for 1 min. The fruits were wounded with a sterilised Philips screwdriver at a 2-mm deep, at 5 points on their surface. In the holes, 50 μL of the conidial suspension (10⁶/mL) was inoculated (Martins & Blum, 2014). In the control fruits, sterilised water was added to each hole. After inoculation, the fruits were incubated in a wet-chamber (25 ± 3°C) for 24 h (closed plastic boxes containing wet paper).

Effect of the phosphites on in vitro growth of C. gloeosporioides

The phosphites of Mg = 40% P₂O₅ + 6% Mg (Fitofós-Mg – 1,50 mL/L), Zn = 40% P₂O₅ + 10% Zn (Phytogard Zn – 2,50 mL/L), Ca = 30% P₂O₅ + 7% Ca (Phytogard Ca – 3 mL/L), and K = 30% P₂O₅ + 20% K₂O (Phytogard K – 2,50 mL/L), were applied in 3 concentrations (50%, 100%, and 200% of the recommended manufacturer’s concentration – CRM), and Carbendazim (Derosal – 1 mL/L). These products were added to the PDA media while still in the liquid phase and poured into dishes. The control treatment contained only PDA. After 24 h, a 3-mm fungi colony disk was inoculated onto the centre of the dish. Two days after the transfer, the colony diameter was measured to evaluate growth, and after 3 weeks, spore counting was performed using a Neubauer chamber. Four dishes per treatment were prepared and this experiment was performed twice.

Effect of phosphites on anthracnose in papaya fruits

Two assays with replicates were carried out with the Solo (cultivar Sunrise Solo) variety. First assay: Carbendazim (Derosal – 1.0 mL/L), and 9 concentrations of phosphites at the CRM for leaf disease in tropical fruits were used: Mg phosphite – 40% P₂O₅ + 6% Mg (Fitofós-Mg – 1.50 mL/L); 30% P₂O₅ + 4% Mg (Phytogard Mg – 3.00 mL/L); Phosphate K – 30% P₂O₅ + 20% K₂O (Fitofós-K Plus – 1.50 mL/L); 30% P₂O₅ + 20% K₂O (Nutrex Premium 0–30–20 – 1.75 mL/L); 20% P₂O₅ + 20% K₂O (Nutrex Premium 0-20-20 – 2.00 mL/L); 20% P₂O₅ + 20% K₂O (Hortifós PK – 3.00 mL/L). Phosphate Ca – 10% P₂O₅ + 6% Ca (Phitophos-Ca – 1.50 mL/L), 30% P₂O₅ + 7% Ca (Phytogard Calcium – 3.00 mL/L) and Phosphate-Zn – 40% P₂O₅ + 10% Zn (Phytogard Zn – 2.50 mL/L). Second assay: Phosphate-K – 30% P₂O₅ + 20% K₂O (Fitofós-K Plus) and Phosphate-Ca – 30% P₂O₅ + 7% Ca (Phytogard Ca), at 4 concentrations (25, 50, 100 and 200% of CRM).

Physico-chemical analysis of fruits

Physico-chemical analysis of fruits was conducted at the end of each experiment with these parameters: Fresh mass loss (% FWL): Weight lost by fruits before the inoculation and at the end of the experiment was monitored using a precision semi-analytical balance (0.5 g precision – ‘Filizola’ model BP-15). The following formula was used to estimate the % FWL = [(initial weight – final weight)/initial weight] × 100. Ripening stage: The degree of ripening assessed with a scale ranging from 1 to 5 (Bicalho et al. 2000, Cia 2005). Firmness: This was determined using a hand penetrometer (Fruit Pressure Tester, FT 327, 8 mm tip) using the formula: P = F/A. Where P = Firmness (kg/cm²), F = penetration force (kg), and A = area of tip (cm²). pH: A centrifuged pulp sample (~ 50–100 mL) was used to determine the pH (digital
pH meter ‘Quimis’ template 400M1-Q/2). The temperature was recorded for later correction of total soluble solids (°Brix). **Total soluble solids** (TSS-°Brix): This was determined using a portable refractometer (Hand Held refractometer ‘Atago’, model N-1E), by placing a small amount of fresh pulp on the prism, which then marks the level of light refraction on a metric ruler that represents the TSS. These values were obtained after the adjustment of °Brix using a correction table (Instituto Adolfo Lutz, 2008).

**Titratable acidity** (TA): Representing the percentage of citric acid, was determined by diluting 20 g of a pulp sample in 100 mL of distilled water, then 3 drops of phenolphthalein (1%) was added to a 10-mL aliquot of the pulp mixture, which was then titrated with 0.01 N NaOH (standard solution) until the solution turned pink in colour. The TA, expressed as % of citric acid, was estimated using the following equation: TA = Vg × N × f × Eq Ac/10 × g, where: Vg = volume of NaOH used (mL); N = normality of NaOH (0.01N); f = correction factor obtained for NaOH standardisation; Eq. Ac. = Acid Equivalent (papaya = 64); g = sample weight.

**Experimental design**

All experiments were conducted twice with 5 fruits per treatment and a separated portion was also prepared for comparison in the physico-chemical analysis. The statistical design was a randomised block with 5 replications, where the average of the first and second experiments was considered 1 replicate. Data were subjected to analysis of variance, and the treatment means were compared using Fisher’s LSD test (P ≤ 0.01). Statistical analyses of the data were performed using SigmaStat 2.0 (Jandel Corporation 1992–1995) software.

**Results**

**Effect of phosphites on in vitro growth of C. gloeosporioides and on anthracnose severity**

The treatments had effect on mycelium diameter (Figure 1(a,c)) and number of conidia (Figure 1(b, d)). All phosphites effectively reduced mycelial growth and the production of conidia in vitro at all doses tested, indicating a direct action of phosphites on the pathogen. Phosphites were more efficient than the carbenzamid (‘Derosal’). The Phosphites Mg – 40% P₂O₅ + 6% Mg (‘Fitofós-Mg’) and K 30% + 20% P₂O₅ K₂O (‘Phytogard K’) at 50% of CRM (1.50 and 2.50 mL/L, respectively) were less effective at decreasing mycelial growth when compared to other phosphites.

The application of phosphites was ineffective in reducing diameter of inoculated fruit (Figure 2(a,b)). However, the Phosphite-Ca – 30% P₂O₅ + 7% Ca (‘Phytogard Calcium’) at 6 mL/L considerably reduced the diameter of anthracnose lesion when compared to the control (Figure 3(a,b)). None of the applied doses of Phosphite K-1 (K1) [0.375, 0.75, 1.5, 3 ml/l (30% P₂O₅ + 20% K₂O) Fitofós K Plus] reduced the diameter of the lesion (Figure 3(a,b)).

![Figure 1.](image1.png)  
Figure 1. Effects of phosphites (Mg, Zn, Ca, K) on the diameter of the mycelial growth (cm) (a and c) and on the number of conidia (10⁶ con/mL) (b and d) of Colletotrichum gloeosporioides in vitro (PDA). (a) and (b) Experiment 1. (c) and (d) Experiment 2. Con = control without products. Phosphite Mg [0.5, 1, 2 = 0.75, 1.5, 3 mL/L (40% P₂O₅ + 6% Mg) Fitofós Mg]. Phosphite Zn [0.5, 1, 2 = 1.25, 2.5, 5 mL/L (40% P₂O₅ + 10% Zn) Phytogard Zn]. Phosphite Ca [0.5, 1, 2 = 1.5, 3, 6 mL/L (30% P₂O₅ + 7% Ca) Phytogard Ca]. Phosphite K [0.5, 1, 2 = 1.25, 2.5, 5 mL/L (40% P₂O₅ + 20% K₂O) Phytogard K]. Car = Carbenzamid (1 mL/l of Derosal). * No mycelial growth or sporulation. Bars with the same letter did not differ by LSD Fisher’s Test (P ≤ 0.01).
Physico-chemical analysis of fruits

Compared to the control, Phosphite Ca-1, Phosphite Ca-2, Phosphite K-1, Phosphite K-2, Phosphite K-3, Phosphite K-4, Phosphite Mg-1, Phosphite Mg-2, and Phosphite Zn did not affect fruit pH (Table 1). However, fruit firmness was significantly reduced by Phosphite Ca-1 (10% P₂O₅ + 6% Ca, 1.5 ml/l), Phosphite K-2 (30% P₂O₅ + 20% K₂O, 1.5 ml/l), Phosphite K-3 (20% P₂O₅ + 20% K₂O, 2 ml/l), Phosphite K-4 (20% P₂O₅ + 20% K₂O, 3 ml/l), Phosphite Mg-1 (40% P₂O₅ + 6% Mg, 1.5 ml/l), Phosphite Mg-2 (30% P₂O₅ + 4% Mg, 3 ml/l), and Phosphite Zn (40% P₂O₅ + 10% Zn, 2.5 ml/l). Total Soluble Solids (TSS = °Brix) and Fruit Fresh Mass Loss (% – FML) were unaffected or barely affected by phosphite applications (Table 1).

Discussion

Most of the phosphite treatments had an effect on C. gloeosporioides growth in vitro, especially on reducing mycelial growth and the production of conidia; these facts could indicate a direct action of phosphites on the pathogen (Fenn & Coffey, 1989). Araújo, Valdebenito-Sanhueza, and Stadnik (2010) reported that phosphites in the formulation 0-40-20 (pH 3) interfered with mycelial development of C. gloeosporioides and reduced colony diameter by 94%. Ogoshi et al. (2013) reported that K-phosphite (5.0 and 10.0 mL.L⁻¹) was most efficient in the in vitro control of C. gloeosporioides. Silva et al. (2015) showed that potassium phosphite reduced mycelial growth and emission of germ tubes of C. lindemuthianum. These authors commented that potassium phosphite acted directly on the fungus by a fungistatic activity, probably related to the phosphate ion. Lobato et al. (2010) worked with K-phosphite and demonstrated that the phosphate anion has antimicrobial activity itself. These authors also reported that the increase in ionic strength after phosphite addition was not important in the antimicrobial activity of K-phosphite, and the activity of phosphites on
The germination of *Fusarium solani* conidia showed to be fungistatic rather than fungicidal. In general, post-harvest diseases begin in the field, thus all control methods should also start in the field, such as the application of protective fungicides to reduce the inoculum (Araújo et al., 2010). Adequate control might be achieved when field sprays are combined with post-harvest treatments, such as in the cases mentioned in this research (Singh, 2011).

Phosphites of Ca and K applied post-harvest showed promising results in reducing severity of disease in other fruit species before and after harvesting (Blum et al., 2007; Cruz et al., 2015; Dianese & Blum, 2010; Moreira & May-de Mio, 2009). Madani, Mirshekari, Sofo, and Mohamed (2016) working with other Ca sources reported that there was a reduction in anthracnose lesion diameter in the infected fruit with Ca from CaCl₂ and Ca(NO₃)₂ applications. CaCl₂ at higher concentrations is effective in maintaining papaya fruit quality post-harvest (Madani, Mirshekari, & Yahia, 2015). These authors stated that the Ca content in papaya fruit peel and pulp increased at higher CaCl₂ levels, whereas ethylene production, anthracnose lesion diameter, and magnesium content decreased compared to control. However, in this study only phosphite of Ca significantly reduced anthracnose severity post-harvest.

Phosphite of K did not reduce the diameter of papaya anthracnose lesion as it did in other fruits post-harvest (Blum et al., 2007; Cruz et al., 2015; Dianese & Blum, 2010; Ferraz et al., 2016). This discrepancy in results might be associated with the aggressiveness of the pathogen, species of fruit, fruit maturation age, and pathogen inoculation method (Oliveira, Blum, Duarte, Carvalho, & Luz, 2016). Also, the characteristics of the papaya peels might favour more mechanisms of other phosphites than the phosphite of K. Sometimes, this phosphite itself is not enough to control the post-harvest disease in order to make the fruit acceptable in the market, so it needs to be associated with other fungicides (Dianese et al., 2008) and/or methods such as hot water (Cruz et al., 2015). Another phenomena that might occur is that the anthracnose tends to develop fungicide resistance, thus the rotation of applications with other classes could minimise the resistance risk (Tredway & Wong, 2012).

**Figure 3.** Effects of phosphites (K, Ca) on the diameter of the anthracnose lesion (mm) caused by *Colletotrichum gloeosporioides* in papaya fruits ('Sunrise Solo'). (a) and (b) Experiment 1 and, respectively. Phosphite K-1 (K1) [0.25, 0.5, 1, 2 = 0.375, 0.75, 1.5, 3 ml/l (30% P₂O₅ + 20% K₂O) Fitofós K Plus]. Phosphite Ca-2 (Ca2) [0.25, 0.5, 1, 2 = 0.75, 1.5, 3, 6 ml/l (30% P₂O₅ + 7% Ca) Phytogard Ca]. Car = Carbendazim (1 ml/l of Derosal). Con = control without products. Bars with the same letter did not differ by LSD Fisher’s Test (P ≤ 0.01).
Table 1. Effects of phosphites (Ca, K, Mg, Zn) on the physico-chemical properties [pH, firmness (Firm – kg/cm²), Total Soluble Solids (TSS = °Brix), Fruit Fresh Mass Loss (% – FML)] of papaya fruits (‘Sunrise Solo’) inoculated with Colletotrichum gloeosporioides.

<table>
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<th>Treatment</th>
<th>Active ingredient</th>
<th>Dose ml/l</th>
<th>pH</th>
<th>Firm</th>
<th>SST</th>
<th>%FML</th>
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<tbody>
<tr>
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<td>1.3</td>
<td>9.5</td>
<td>9.9</td>
</tr>
<tr>
<td>Fungicide</td>
<td>Carbendazim</td>
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<tr>
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<tr>
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<td>30% P₂O₅ + 20% K₂O</td>
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</table>

Notes: Comparison made with the untreated control [LSD Fisher’s Test (P ≤ 0.01)]. (*) Did not alter. (–) Altered negatively. (+) Altered positively.

Disclosure statement
No potential conflict of interest was reported by the authors.

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