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FREQUENCY OF QUIESCENT FUNGI AND POST-HARVEST ALTERNATIVE MANAGEMENT OF STEM END ROT IN PAPAYA[1]

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ABSTRACT - The aim of this study was to evaluate the frequency of quiescent fungi and the effect of phosphites under modified atmosphere on *Lasiodiplodia theobromae* in papaya. The fruits were treated with a range of doses of phosphites and their actions evaluated under conditions of ambient and modified atmosphere. Of the eight fungal genera found, *Lasiodiplodia* was the most common. No interaction was observed between the evaluated factors and only atmosphere and dose were independently significant. The usage of phosphites and modified atmosphere reduced the severity of the disease, and did not affect the chemical properties of the fruits.

Keywords: *Carica papaya*. Alternative control. Phosphites. Post-harvest quality.

FREQUÊNCIA DE FUNGOS QUIESCENTES E MANEJO ALTERNATIVO DA PODRIDÃO PEDUNCULAR EM MAMÃO NA PÓS-COLHEITA

RESUMO - O objetivo deste trabalho foi avaliar a frequência de fungos quiescentes e o efeito de fosfitos sob atmosfera modificada sobre *Lasiodiplodia theobromae* em mamões. Os frutos foram tratados com várias doses de fosfitos e suas ações foram avaliadas em condições de atmosfera ambiente e atmosfera modificada. Dos oito gêneros fúngicos encontrados, *Lasiodiplodia* sp. foi mais frequente. Não houve interação entre os fatores avaliados e apenas os fatores atmosfera e dose independentemente foram significativos. O uso de fosfitos e atmosfera modificada reduziram a severidade da doença e não alteraram os atributos químicos dos frutos.

Palavras-chave: *Carica papaya*. Controle alternativo. Fosfitos. Qualidade pós-colheita.

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INTRODUCTION

Papaya (*Carica papaya* L.) is widespread in tropical and subtropical regions, with Brazil being one of the largest producer, producing 1.58 million tons per year (REETZ et al., 2015). The fruit has good acceptance in the international market owing to its nutritional value and sensory qualities (SHINAGAWA et al., 2013). The papaya fruit losses that occur at the post-harvest stage can have various causes, notably diseases, especially those caused by fungi that are activated from the quiescent phase during the ripening of the fruit (PRUSKY et al., 2013).

In general, post-harvest decay causative agents exhibit common features, namely, the ability to establish in the immature fruit and to remain in a quiescent state until favorable conditions arise for the infection process to occur. Studies on quiescent infections are of key agricultural and economic importance for the development of new approaches related to the detection of quiescence and the subsequent control of post-harvest diseases (PRUSKY; KOBILER, 2012; PRUSKY et al., 2013).

Stem end rot caused by the fungus *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. (syn. *Botryodiplodia theobromae* Pat.) is one of the main diseases in the post-harvest of papaya. The pathogen can survive in the quiescent form in its host until, usually at the beginning of maturation, the first symptoms appear in the stem-cutting region, affecting the basal portion of the fruit (VENTURA; COSTA; TATAGIBA, 2004; LIMA et al., 2013; PRUSKY et al., 2013). In order to reduce the significant losses caused by post-harvest rots, fungicides such as imazalil, thiabendazole, and prochloraz, are still intensively used (BRASIL, 2016). Nevertheless, the use of phosphites in fruit and vegetables in the post-harvest stage has become a common alternative to the use of fungicides, because these salts exhibit antifungal activity and have no grace period, therefore representing a control tool in the disease management (MILLER et al., 2006; DELIOPOULUS; KETTLEWELL; HARE, 2010; CERIONI et al., 2013; ALEXANDRE et al., 2014; YOUSSEF; ROBERTO, 2014). According to Buffara et al. (2013), phosphites exhibit a systemic mode of action, apo-symplastic movement, low toxicity, low cost, and act directly on the fungi.

Another alternative to fungicides is the application of modified atmosphere (MA) through the use of flexible films, such as polyvinyl chloride (PVC). The MA establishes a gaseous composition in the interior of the package, distinct from the ambient air, by reducing the concentration of oxygen and increasing the concentration of carbon dioxide. This reduces respiration and production rates of ethylene, delaying the senescence of these products (KADER, 2010). Thus, the MA delays the maturation of the product, avoiding the ideal conditions for reactivation of possible pathogens in quiescence (JAYATHUNGE et al., 2014; MARTINS et al., 2015; OZKAYA et al., 2016).

Thus, the aim of the present study was to evaluate the frequency of quiescent fungi and the effect of phosphites combined with MA on post-harvest stem end rot in papaya cv. Sunrise Solo.

MATERIAL AND METHODS

The experiments were conducted in the Postharvest Pathology Laboratory of the Universidade Federal Rural of Pernambuco, using healthy papayas cv. Sunrise Solo in stage two of commercial maturity (RITZINGER; SOUZA, 2000), obtained from the Supply Company and General Warehouses of the State of Pernambuco - CEAGEPE - Recife, Pernambuco, in 2013.

Quiescent fungi frequency

The fruits were washed in tap water, air dried, and placed in plastic trays covered with moistened paper towels inside plastic bags (constituting the wet chamber). After 48 h, the fruits were placed in ambient air (25±2°C), remaining there until the end of the evaluations. The fungi frequency analysis was calculated as the percentage of disease symptoms and/or signs of pathogens on each fruit. The assessments were performed every 48 hours by removing fragments of the damaged areas of the fruits with symptoms of rottenness. This procedure was repeated until all the fruits had rotted which occurred on the tenth day, so determining the final period of assessment. The fragments were disinfected with 70% ethanol for one minute, 2% sodium hypochlorite for two minutes, and then rinsed in distilled water for two minutes. After that, the etiologic agent from the lesions was isolated in petri dishes containing potato dextrose agar (PDA). The plates were incubated in a BOD incubator for seven days at 26 °C. In some cases, it was possible to remove structures of the pathogens directly from the lesions and identify the pathogen using direct microscopic preparations with a light microscope (×40), model N101-B (Coleman Equipamentos para Laboratório Com. e Imp. Ltda., Santo André, São Paulo), based on micro-morphological characteristics, using classification keys (ELLIS, 1971; BARNETT; HUNTER, 1999). The incidence and frequency of fungi was expressed as the percentage of each fungal genus found in the fruits.

Post-harvest treatment of stem end rot in papaya

The isolate *L. theobromae* from the collection of cultures of Postharvest Pathology Laboratory of the Universidade Federal Rural of Pernambuco with morphological and molecular identification, was used in this study. The fungus was grown in petri dishes containing potato dextrose agar (PDA) culture medium for 25 days. After this period, the suspension was prepared by adding 10 mL of sterile distilled water to the surface of the actively growing colonies and the suspension obtained was filtered with double sterile gauze. The spores were then counted in a Neubauer chamber adjusting the concentration to 10^6 conidia.mL^{-1}.

Surface disinfection of the fruits was carried out using the same methodology as mentioned above. The fruits were then immersed in solutions of calcium phosphite, potassium phosphite, ammonium phosphite, and calcium and boron phosphites, in the doses of 0.3, 0.6, 0.9, 1.2, and 1.5 g.L^{-1}, in containers with four liters of distilled water, for 10 minutes. The control consisted of fruit immersed in distilled water alone without phosphites. The fruits were left to dry at room temperature (25 ºC) and then perforated with a five-pointed puncher (5 mm in diameter and 2 mm deep). After that, the fruits were inoculated with 10 µL of the fungal spore suspension *L. theobromae* in the equatorial region. After 48 hours in a humid chamber, half of the trays containing the treated fruits were wrapped in 7-µm-thick PVC film. The control consisted of fruits immersed in distilled water for the same period of time under conditions of ambient atmosphere and MA. The trays were kept at a temperature of 28 ± 2 °C and relative humidity of 84%.

A completely randomized design in a factorial arrangement (2 atmospheres x 5 treatments x 5 doses) was used. Each treatment consisted of five replicates with the experimental unit corresponding to a fruit. The evaluations were performed 10 days after inoculation by measuring the diameter of the lesions, expressed in millimeters (mm), with a digital caliper, in two directions.

At the end of the assessments, the chemical characteristics were determined after trituration of the pulp, performing three replicates for each fruit. In order to determine the total soluble solids (TSS) content, 10 µL of fresh sample was deposited on a refractometer Model EXACTA + OPTECH GmbH (0−32 °Brix) and the results expressed in °Brix. The total titratable acidity (TTA) was determined by weighing 5.0 g of fresh pul pand titrating with 0.1 M NaOH solution using phenolphthalein as an indicator, and expressed in g of citric acid $100g^{-1}$ of pulp. To determine the pH, 10g of fresh pulp was used, followed by direct reading in a potentiometer Quimis Model Q-400A. The analysis of these variables was performed according to A.O.A.C. (1990) methodology.

The data were subjected to analysis of variance (ANOVA) using the program Statistix 9 (Tallahassee, FL, USA). For the mean indices of severity, a regression analysis relating the severity as a function of the phosphites' concentration and atmosphere conditions was performed. The results of the chemical analysis were compared with the control by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

Quiescent fungi frequency

The genera of quiescent fungi identified in papaya fruits cv. Sunrise Solo after 10 days of incubation under ambient atmosphere conditions were *Aspergillus*, *Penicillium*, *Fusarium*, *Geotrichum*, *Corynespora*, *Cladosporium*, *Colletotrichum* and *Lasiodiplodia* (Figure 1).

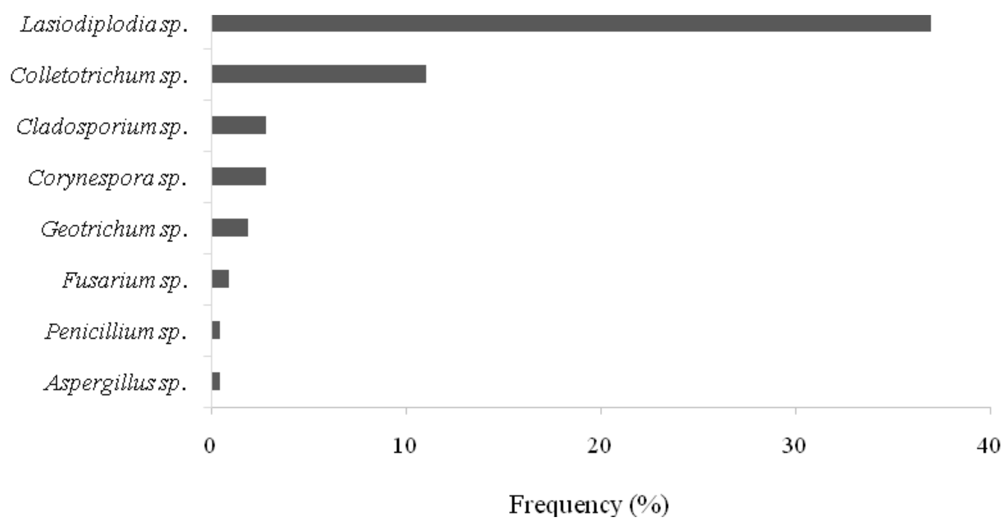

Figure 1. Frequency of quiescent fungi detected under ambient atmosphere in papayas cv. Sunrise Solo from the Supply Company and the General Warehouses of the State of Pernambuco - CEAGEPE - Recife.

Lasiodiplodia sp. stood out among the other fungi, showing a frequency of 37%. According to Gomes et al. (2010) and Lins et al. (2011), the damage caused by *Lasiodiplodia* sp. compromises the quality of the product at retail and is one of the major causes of post-harvest loss. These authors found a high frequency with an incidence of 88% of stem end rot in mango.

The second most frequently detected genus was *Colletotrichum* with a frequency of 11%. It is one of the main post-harvest pathogens, and can remain quiescent for long periods of time in the fruit tissues, although immediately initiating necrotrophic development in ripe and senescent fruit (PRUSKY; KOBILER, 2012).

The genera *Lasiodiplodia* and *Colletotrichum*, which showed the highest frequencies, are the primary causative agents of fruit damage during transportation and storage (NETTO et al., 2014; ZAHID et al., 2012). The infection begins near the stem end, later moving through the fruit by direct penetration. The other pathogens were observed in places of injury, which is another form of penetration. It should be stressed that there are reports of several species of *Lasiodiplodia* and *Colletotrichum* surviving in a quiescent form in their hosts (YUJU et al., 2009; MYARA et al., 2010).

A frequency of 2.8% was observed for the genera *Corynespora* and *Cladosporium*. Fungi of the genus *Cladosporium* cause greenish-black spots on papayas. This pathogen is confined to the surface of the skin, only compromising the external appearance of the fruit, thus reducing its commercial value (SILVEIRA et al., 2001). The disease caused by *Corynespora* sp. may occur in the stem, petioles, leaves, and in the papaya fruit. Even in green fruits, several small circular spots appear which can evolve quickly. The damages are depressed and with a dark center where the fungal structures are observed. Lesions may coalesce to form a large area of irregular shape in the fruit (VENTURA; COSTA; TATAGIBA, 2004). The genera *Fusarium* sp. and *Geotrichum* sp. exhibited frequencies of 0.9% and 1.9%, respectively. Several species of *Fusarium* can cause rot in papayas, such as *F. verticillioides* (Sacc.) Niremberg (=*F. moniliforme* Sheldon), *F. solani* (Mart.) Appel and Wollenw., *F. oxysporum* Schlecht., *F. anthophilum* (A. Braun) Wollenw., *F. equiseti* (Corda) Sacc. and *F. semitectum* Berk. and Rav. (DANTAS et al., 2003). The rot associated with *Geotrichum* develops on the fruits creating soaked and softened areas that are easily perforated, facilitating the entry of saprophytic fungi. The rot spreads quickly and the skin of the fruit usually cracks in the affected area, which may become covered by a white mycelial growth (DANTAS; OLIVEIRA, 2006). Fatima et al. (2009) also observed the presence of these genera of fungi in pre-refrigerated papaya fruits.

The genera *Aspergillus* and *Penicillium* appeared at the end of the incubation period of the fruits in an advanced stage of decay with frequency of 0.4%. These fungi are opportunistic, and they commonly cause rot in several vegetable products (FATIMA et al., 2009). Lima et al. (2009) studied the frequency of fungi isolated from post-harvest Formosa papaya fruits. The authors observed that *Aspergillus* spp. appeared with the highest frequently, followed by C. *gloeosporioides*. The storage temperature (25°C ± 2 °C) was favorable to the development of these fungi.

Post-harvest treatment of stem end rot in papaya

The treatments used were effective in reducing the disease, showing less severe symptoms compared to the control group (Figure 2).

The 1.2 and 1.5 $g.L^{-1}$ doses showed significant differences as compared to the control and the other doses (0.3, 0.6, and 0.9 $g.L^{-1}$), regardless of the phosphite. The effect of the phosphites did not differ significantly between them, all causing a reduction in the diameter of the lesion for increasing doses, under ambient atmosphere and MA (Figure 2). With respect to calcium phosphite, Cerioni et al. (2013) found that the treatment of citrus with calcium phosphite followed by inoculation with *P. digitatum* was highly efficient, although it left a slight residue on the fruit. Naradisorn et al. (2006) showed that treatment of strawberry plants with calcium chloride resulted in firmer fruits than those untreated. Youssef and Roberto (2014) tested different types of calcium salts on pre-harvest, post-harvest, and a combination of pre and post-harvest 'Italia' grape, and found that the salts significantly reduced the incidence of gray mold caused by *Botrytis cinerea*. This could possibly be explained by the different roles played by calcium in plant tissues, especially on the protection of membranes and cell walls as well as on the elicitation of responses to biotic or abiotic stresses (CHITARRA, 2006).

Similarly, potassium phosphate caused a reduction of the severity of the disease with increasing dose in both ambient atmosphere and MA. However, under MA, the values of the lesions' diameters were lower, showing the greater efficiency of the PVC film wrapping in maintaining the fruit for a longer period of time (Figure 2B). Alexandre et al. (2014) observed the protective effect of potassium phosphite in the control of anthracnose on jilo caused by *C. tamarilloi*. Cerioni et al. (2013) also reported the effect of potassium phosphite in controlling post-harvest rot on citrus caused by *P. digitatum*.

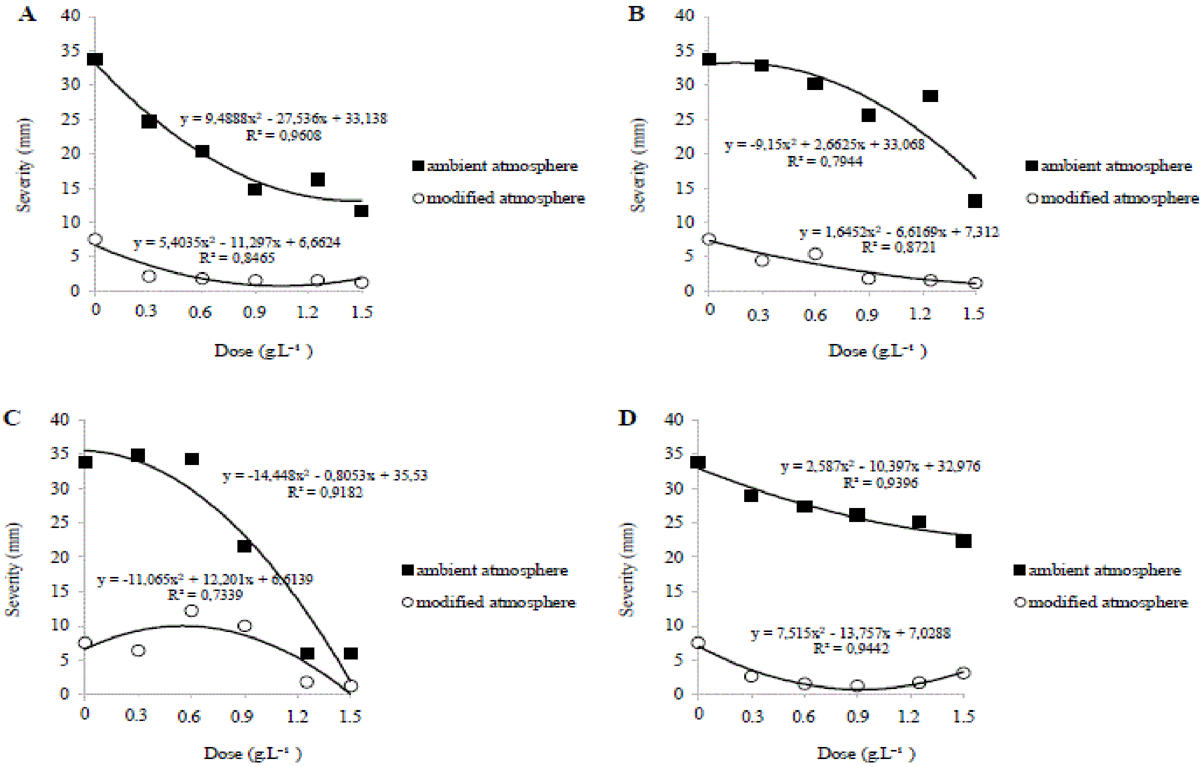

Figure 2. Effect of different phosphites at doses of 0.3, 0.6, 0.9, 1.2, and 1.5 g.L^{-1} and of modified atmosphere on the severity of stem end rot caused by *Lasiodiplodia theobromae* on papaya cv. Sunrise Solo. A: calcium phosphite; B: potassium phosphite; C: ammonium phosphite; D: calcium and boron phosphites.

The treatment with ammonium phosphite caused a significant reduction in the value of the diameter of the lesion under ambient atmosphere. The highest dose (1.5 g.L^{-1}) showed a 5.85 mm lesion size, significantly different from the control with 33.77 mm (Figure 2C). A more efficient reduction was observed under MA. Nunes et al. (2001) tested ammonium molybdate as a potential fungicide against blue and gray mold on apples cv. Golden Delicious observing a reduction of the diameters of the lesions caused by *P. expansum*, *B. cinerea*, and *R. stolonifer* by 84.88% and 100%, respectively. In addition, Miller et al. (2006) found a lower incidence and severity of late blight of potato, relative to the control group, when using ammonium phosphate against *Phytophthora infestans* and *P. erythrosepctica* in potato tubers.

Treatment with calcium and boron phosphites was also effective in reducing the severity of the disease under ambient and MA (Figure 2D). For the highest dose, a 33.8% reduction in lesion size under ambient atmosphere was observed. An increased content of these nutrients in leaves and fruits was reported in ‘Chandler’ strawberries, with increased fruit firmness and reduction of the incidence of gray mold, following application of calcium and boron phosphites (SINGH; SHARMA; TYAGI, 2007).

The use of MA caused a reduction in the aggressiveness of the fungus, showing a significant difference relative to the ambient atmosphere, with values close to zero for every phosphite and dose. Moreover, Moura et al. (2013) found that the combined use of MA and refrigeration increased the post-harvest shelf life of umbu fruit. Increasing the shelf life is important, since during post-harvest ripening and storage, the mechanism that protects the fruit from fungal infections is no longer effective enough. Jayathunge et al. (2014) verified the effectiveness of plastic film in maintaining the papaya external quality and the increase of the shelf life up to 19 days at 4 °C. According to Santos, Lins and Oliveira (2012), that may be linked to the action on the hosts cell walls. As the fruits ripen, the pectic substances of the middle lamella, which are in an insoluble form, protopectin, become more soluble and consequently, the tissues become softer. This softening is promoted by pectinolytic enzymes released by the pathogen. In addition to reducing the severity of the disease, it can be observed that the MA influences the quality of the fruit. González-Aguiar, Buta and Wang (2003) observed a reduction in water loss and the preservation of firmness in papayas subjected to MA.

Although the TSS and the pH of the papaya fruits treated with different phosphites and doses showed significant difference from those of the control (Table 1), these are in accordance with the standards required by the Ministry of Agriculture, without compromising the quality of the fruit. The TTA did not change, which is essential for maintaining the properties of the fruit for their commercialization.

Table 1. Total soluble solids (TSS) content expressed in °Brix; total titratable acidity (TTA) expressed in g of citric acid 100 g^{-1} pulp, and pH of papaya after treatments with phosphites.

Treatments	Doses (g.L⁻¹)	Ambient atmosphere			Modified atmosphere		
		TSS	TTA	pH	TSS	TTA	pH
Control	0.00	12.0	0.1	5.3	11.0	0.1	5.0
Calcium phosphite	0.30	11.5	0.1	5.0*	11.0	0.1	5.3*
	0.60	12.0	0.1	5.0*	12.0*	0.1	5.0
	0.90	11.0*	0.1	5.3	12.0*	0.1	5.0
	1.25	11.5	0.1	5.1*	11.0	0.1	5.3*
	1.50	12.0	0.1	5.1*	11.0	0.1	5.4*
Potassium phosphite	0.30	11.5	0.1	5.1*	11.0	0.1	5.1*
	0.60	11.0*	0.1	5.1*	11.0	0.1	5.1*
	0.90	10.5*	0.1	5.1*	11.0	0.1	5.3*
	1.25	11.0*	0.1	5.2	12.0*	0.1	5.3*
	1.50	11.0*	0.1	5.4*	12.0*	0.1	5.4*
Ammonium phosphite	0.30	11.0*	0.1	4.9*	11.5	0.1	5.1
	0.60	12.0	0.1	5.2	11.0	0.1	5.0*
	0.90	10.0*	0.1	5.1*	13.0*	0.1	5.2*
	1.25	11.0*	0.1	5.6*	12.0*	0.1	5.1
	1.50	11.0*	0.1	5.1*	12.5*	0.1	5.1*
Calcium and boron phosphites	0.30	11.0*	0.1	4.9*	11.0	0.1	5.0
	0.60	12.0	0.1	5.0*	11.0	0.1	4.9
	0.90	11.0*	0.1	5.1*	12.0*	0.1	5.1
	1.25	11.0*	0.1	5.1*	11.5	0.1	5.3*
	1.50	11.5*	0.1	5.7*	11.0	0.1	5.3*

*=Mean values in the same column marked with an asterisk are significantly different from the control by Tukey test (p<0.05).

As can be observed from Table 1, fruits submitted to MA had higher TSS contents (11−13 °Brix) than those under ambient atmosphere, which ranged from 10−12 °Brix. This indicates that in addition to reducing the severity of stem end rot, the MA preserves the fruit quality. This result is also consistent with that found by Jayathunge et al. (2014) where papayas showed TSS contents around 11 and 12 ºBrix. The small fluctuation of soluble solid content during papaya ripening was attributable to the fact that, according to reports from Jacomino et al. (2007), the fruit does not have significant amounts of starch to be hydrolyzed during the maturation process. The TTA contents, on the other hand, remained constant, with an average value of 0.1 g of citric acid 100g^{-1} pulp. Commonly, fruits show a reduction in acidity during ripening, although in some cases, there may be an enhancement with increasing maturity (CHITARRA; CHITARRA, 2005).

The pH showed small variations, ranging from 4.9 to 5.7 under ambient atmosphere conditions and from 4.9 to 5.4 under MA. These values are within the range of pH considered optimal for the consumption of papayas ‘Solo’ in their natural state, according to Chan Junior et al. (1971). Further, these values are close to those found by Fagundes and Yamanishi (2001), which varied between 5.20 and 5.71.

With respect to the ambient atmosphere and MA, similar results were shown by Oliveira Junior, Coelho and Coelho (2006) with respect to the post-harvest life of papayas under ambient atmosphere and MA. The author found that the MA preserved the physical, chemical, and sensory characteristics, favoring the fruit quality.

Thus, the post-harvest diseases originating from quiescent or active infections observed in the present study demonstrate the need for the use of effective control measures at the production stage and in the subsequent post-harvest operations. The results obtained indicate the potential of phosphites in combination with MA in the management of stem end rot caused by *L. theobromae* in papayas.

CONCLUSION

Lasiodiplodia sp., *Colletotrichum* sp., *Cladosporium* sp., *Corynespora* sp., *Fusarium* sp., *Geotrichum* sp., *Aspergillus* sp., and *Penicillium* sp. are in a state of quiescence in papaya cv. Sunrise Solo, which may cause rot during fruit ripening.

Phosphite salts combined with MA are effective in the management of the disease caused by *L. theobromae* in post-harvest papayas, without compromising the quality of the fruits.

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