



Plant Protection - Original Article - Edited by: Everaldo Antonio Lopes

Biological control of anthracnose in passion fruit

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Abstract: The biological products use as a disease control alternative has been studied to reduce the impacts to the environment, men and animals, showing satisfactory results in postharvest. This study aimed to evaluate the biological agents effect in the control of *Colletotrichum* spp. and on postharvest quality of yellow passion fruit. The treatments were *Trichoderma asperellum* and *Saccharomyces cerevisiae* species at concentrations of 0,5; 1,0; 1,5; 2,0 (g. L⁻¹); Mancozeb fungicide (Dithane® 2 g i.a. L⁻¹ water) and control (sterile distilled water). The fruits were immersed for 2 min in each treatment and then were drought. Five replications of three fruits were used to anthracnose severity analysis and yellow passion fruit physical-chemical quality in. The fruit inoculation was made with the deposition of *Colletotrichum* spp. on the surface of the fruit previously treated using holes which were made with the aid of a flamed perforator. In the research was evaluated: pH, total soluble solids and titratable acidity. Biological treatments reduced the anthracnose severity in yellow passion fruit. Fruit Post-harvest quality was not influenced by the biological control application. It is a viable alternative to postharvest management of anthracnose on yellow passion fruit under the studied conditions.

Index terms: *Colletotrichum* spp.; Postharvest Disease; Postharvest Quality; *Saccharomyces cerevisiae*; *Trichoderma asperellum*.

Controle biológico da antracnose em fruto de maracujá

Resumo: O uso de produtos biológicos como alternativa no controle de doenças tem sido estudado para diminuir os impactos ao meio ambiente, aos homens e animais, mostrando resultados satisfatórios na pós-colheita. O objetivo do trabalho foi avaliar o efeito de agentes biológicos no controle do *Colletotrichum* spp. e na qualidade pós-colheita em frutos de maracujazeiro-amarelo. Os tratamentos foram compostos por *Trichoderma asperellum* e *Saccharomyces cerevisiae* nas concentrações de 0,5; 1,0; 1,5; 2,0 (g. L⁻¹); fungicida Mancozebe (Dithane® 2 g i.a. L⁻¹ de água)

Rev. Bras. Frutic., v.45, e-997 DOI: <https://dx.doi.org/10.1590/0100-29452023997>

Received 29 Aug, 2021 • Accepted 05 May, 2023 • Published Sep/Oct, 2023. Jaboticabal - SP - Brazil.



e testemunha (água destilada esterilizada). Os frutos foram imersos durante 2 min em cada tratamento e, posteriormente, submetidos a secagem. Foram utilizadas cinco repetições de três frutos para as análises de severidade da doença e pós-colheita. As variáveis analisadas foram severidade da antracnose e qualidade química dos frutos. A inoculação dos frutos foi feita com a deposição de discos de micélio do *Colletotrichum* spp., e na superfície do fruto previamente tratado foram realizados orifícios com auxílio de um perfurador flambado. Foram avaliados os parâmetros químicos: pH, sólidos solúveis totais e acidez titulável. Os tratamentos biológicos reduziram a severidade da antracnose, e a qualidade pós-colheita dos frutos não foi influenciada pela aplicação do controle biológico. O controle biológico constitui alternativa viável para o manejo pós-colheita da antracnose em maracujazeiro-amarelo, nas condições estudadas.

Termos para indexação: *Colletotrichum* spp.; Doença pós-colheita; Qualidade pós-colheita; *Sacharomyces cerevisiae*; *Trichoderma asperellum*.

Introduction

Fruit crops represent more than 40% of agricultural production in Brazil (MACHADO, 2014). Of this total, the yellow passion fruit (*Passiflora edulis* Sims, f. *flavicarpa* Deg.) corresponds approximately 98% (AGRIANUAL, 2015). Occurrence is one of the main factors limiting the expansion of areas cultivated with passion fruit (FREITAS et al., 2016). However, there are several strategies used in the adoption of methods aimed at the diseases biological control, such as the substances use extracted from plants and the use of antagonistic microorganisms (RUFINO et al., 2018).

Colletotrichum spp. is the fungus responsible for the major passion fruit postharvest losses (FISCHER et al., 2005). The pathogen infects the fruit before harvest, remaining quiescent until maturation, when physical and physiological phenomena occur which favor the anthracnose development (BARKAI-GOLAN, 2001). In search for alternative disease control methods, biological control can contribute to sustainable production (GAUR; SHARMAM, 2010).

Saccharomyces cerevisiae fungus has been used to biocontrol plant diseases, due to ability to synthesize antibiotics and to compete for space and nutrients with pathogens (PICCININ et al., 2005). Moreover, *Trichoderma* spp. are the most used microorganisms as phytopathogenic fungi biocontrol agents (WIJESINGHE et

al., 2011; MARTÍNEZ-MEDINA et al., 2014). It suggests that these fungi can control the anthracnose in passion fruit.

Several studies, with biocontrol in passion fruit, are carried out and have promising results, studies carried out by Bulhões (2019) using bacterial isolates, *Bacillus alcalophilus*, *Phototrhobdus luminescens* and *Yersinia bercovieri* in the control of anthracnose in yellow passion fruit proved to be efficient in inhibiting the development of the pathogen, with control levels varying between 29,9% and 43,6% and with emphasis on *B. alcalophilus* and *P. luminescens*, with 43,6% relative control.

Therefore, this work aimed to evaluate the use of *S. cerevisiae* and *T. asperellum* in postharvest biological control of *Colletotrichum* spp. in yellow passion fruit.

Material and Method

The fungi *S. cerevisiae* (obtained from fresh yeast dough salt, Fleischmann®) and *T. asperellum* (as the commercial product Quality® WG, Farroupilha Group), both at different concentrations (0,0; 0,5; 1,0; 1,5; 2,0 g. L⁻¹), were evaluated as biological control of *Colletotrichum gloeosporioides* (Penz.) in yellow passion fruit (*P. edulis* f. *flavicarpa*) on the postharvest. To compare these products efficacy, Mancozeb fungicide (Dithane® 2 g i.a. L⁻¹ water) and distilled and sterilized water (DSW) were used.

Colletotrichum gloeosporioides (Penz) isolate was obtained from yellow passion fruit with anthracnose symptoms. Fragments (1 cm in diameter) were removed from the fruit peel at border region of the lesions. Disinfestation was carried out in a 70% alcohol solution for one minute, 5% sodium hypochlorite for three minutes, and finally, the fragments were washed with sterilized distilled water. After that, the disinfected fragments were incubated (8 days, 25 ± 2 °C and 65 ± 1% R.U.) in Petri dishes (containing BDA medium). Then, discs (7 mm in diameter) from the fungal colony were removed, and another incubation (under previously described conditions) was made to obtain the pathogen inoculum.

Yellow passion fruits at maturity stage 3 (SILVA et al., 2008) with no anthracnose symptoms, deformation and with same peel color, uniformity and maturation stage were used to test the biological control treatments. The fruits were obtained from a fruit distribution center located in Campina Grande, Paraíba State in Brazil. After sanitization by immersion in sodium hypochlorite solution (1%; 3 minutes), and then washed with distilled and sterilized water, the fruits were dried by environmental conditions (25 ± 2 °C; 10 min) in plastic trays with paper towel.

After that, the fruits were immersed (2 min) in the different treatment solutions (*S. cerevisiae*, *T. asperellum*, Mancozeb and DSW) using polyethylene containers (10 L capacity), and then conditioned on paper towels at environmental temperature (25 ± 2 °C; 10 min). Of the previously isolate pathogen were inoculated on the surface of the healthy passion fruits, in three equidistant wounds (3 mm deep; 1 mm in diameter) made by a flamed perforator. Then, the fruits were conditioned in humid chamber (25 ± 2 °C; 24 h), made of plastic trays covered by polystyrene bags previously sprayed with DSW. The fruits were maintained by environmental conditions (25 ± 2 °C) for disease and quality evaluations.

At 8 days after pathogen inoculation, the anthracnose incidence was calculated as per-

centage of fruits with symptoms, and the severity was daily evaluated by the mean diameter of lesions (mm), measured (by digital caliper) in two diametrically opposite sites, until the 8th day. The analysis of the anthracnose progression was carried out daily, until the 5th day after the symptoms appearance (third day after the pathogen inoculation). The Area Under the Disease Progress Curve (AUDPC) was calculated by trapezoidal payload method (SHANER; FINNEY, 1977):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_1)$$

Where: n = number of evaluations; y = disease intensity; t = disease intensity at the evaluated time; (y_i + y_{i+1}) = rectangle mean height between points y_i and y_{i+1}; and t_{i+1} = difference of the base of the rectangle between points t_{i+1} and t_i.

Each two days (0, 2, 4, 6, 8, 10 days) after the biological treatments application a fruit sample was taken to evaluate: soluble solids (SS; °Brix) by refractometer (PR-100, Pallete, Atago Co., LTD., Japan); titratable acidity (TA; %) according to AOAC (1994); and pH by digital pHmeter (Digimed DMPH-2).

A completed randomized design was used with five replications in a factorial scheme (2 x 4 + 2) (biological control x concentrations + DSW and fungicide). Data were submitted to regression analysis (p<0.05) and the difference between AUDPC means were compared by Mann-Whitney test (p<0.05) using SAS® software v 9.0.

Results and discussion

The biological products reduced the anthracnose severity-in passion fruits, *T. asperellum* and *S. cerevisiae* (2,0 g. L⁻¹) promoted a greater reduction of disease progression (110,45), in relation to control (270,38), with lower rates of disease severity progress (Figure 1).

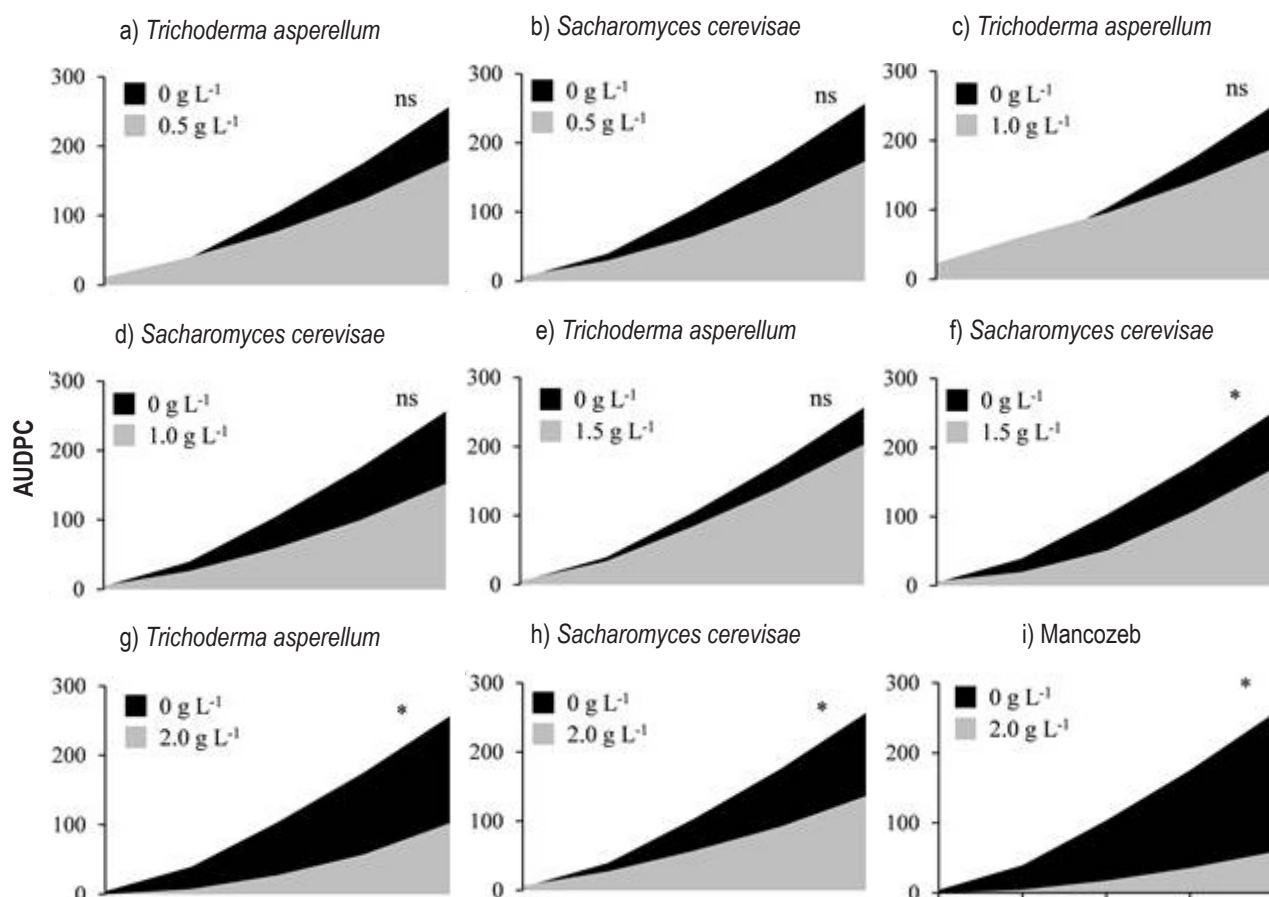


Figure 1. Area Under the Disease Progress Curve (AUDPC) of anthracnose (*Colletotrichum* spp.) in passion fruit (*Passiflora edulis* f. *flavicarpa*). AUDPC was calculated after immersion of the fruits in solutions (0,0; 0,5; 1,0; 1,5; 2,0 g L⁻¹) of *Sacharomyces cerevisiae*, *Trichoderma asperellum* and Mancozeb® fungicide, and then stored for 10 days at 25 ± 2 °C. *AUDPC for the treatment is significantly different from control (Mann-Whitney U test, p ≤ 0.05). ns: non-significant.

Both *T. asperellum* and *S. cerevisiae* showed to be beneficial for passion fruit, reducing the anthracnose symptoms. *Trichoderma* genus is known to promote plant growth, and to induce diseases resistance. This fungus acts as an elicitor, being able to trigger defense reactions in plants against the attack of pathogenic fungi (FRISCHMANN et al., 2012; GOMES et al., 2015; SALAS-MARINA et al., 2015). Furthermore, *S. cerevisiae* also shown to be another beneficial fungus species, which competes with pathogenic fungi for the same growth factors, as from external sources energy to conidia to germinate (PICCININ, 2005).

Mancozeb belongs to the group of ethylene-bisdithiocarbamates (EBDC) fungicides (CENGIZ; CERTEL, 2014). Its multisite nature in the mode of action shows activity against

a wide range of fungi, including ascomycetes, oomycetes, basidiomycetes and imperfect fungi, inhibiting spore germination (GULLINO et al., 2010).

Biological products can use different mechanisms of action, such as competition for nutrients, induction of resistance in hosts, secretion of hydrolytic enzymes that degrade the fungus cell wall, biofilm formation and production of volatile compounds, in the control of postharvest diseases (DROBY et al., 2002; BAR-SHIMON et al., 2004; GIOBBE et al., 2007; HUANG et al., 2011). Competition for nutrients (e.g. carbohydrates, nitrogen, oxygen) and space has been considered the main mode of action of antagonistic against postharvest fungal pathogens (ZHANG et al., 2011; SPADARO; DROBY, 2016).

The results showed the efficacy of *T. asperellum* and *S. cerevisiae* as biological control of passion fruit anthracnose after harvest. In addition to potential of reducing the environmental impact, these alternatives to chemical control did not alter fruit quality.

Soluble solids

The soluble solids (SS) content was higher in fruits treated with biological products and

fungicide, compared to fruits non-treated (DSW 0,0 g L⁻¹). The maximum SS content was found at fourth day of storage, coincident with the appearance of anthracnose. After that, the SS content reduced until 10th day. The maximum SS content of 13.4, 15.0 and 14.4% were found in fruits treated with *S. cerevisiae* (0,5-1,5 g L⁻¹) and *T. asperellum* (>0,5 g L⁻¹) and Mancozeb®, respectively (Figure 2).

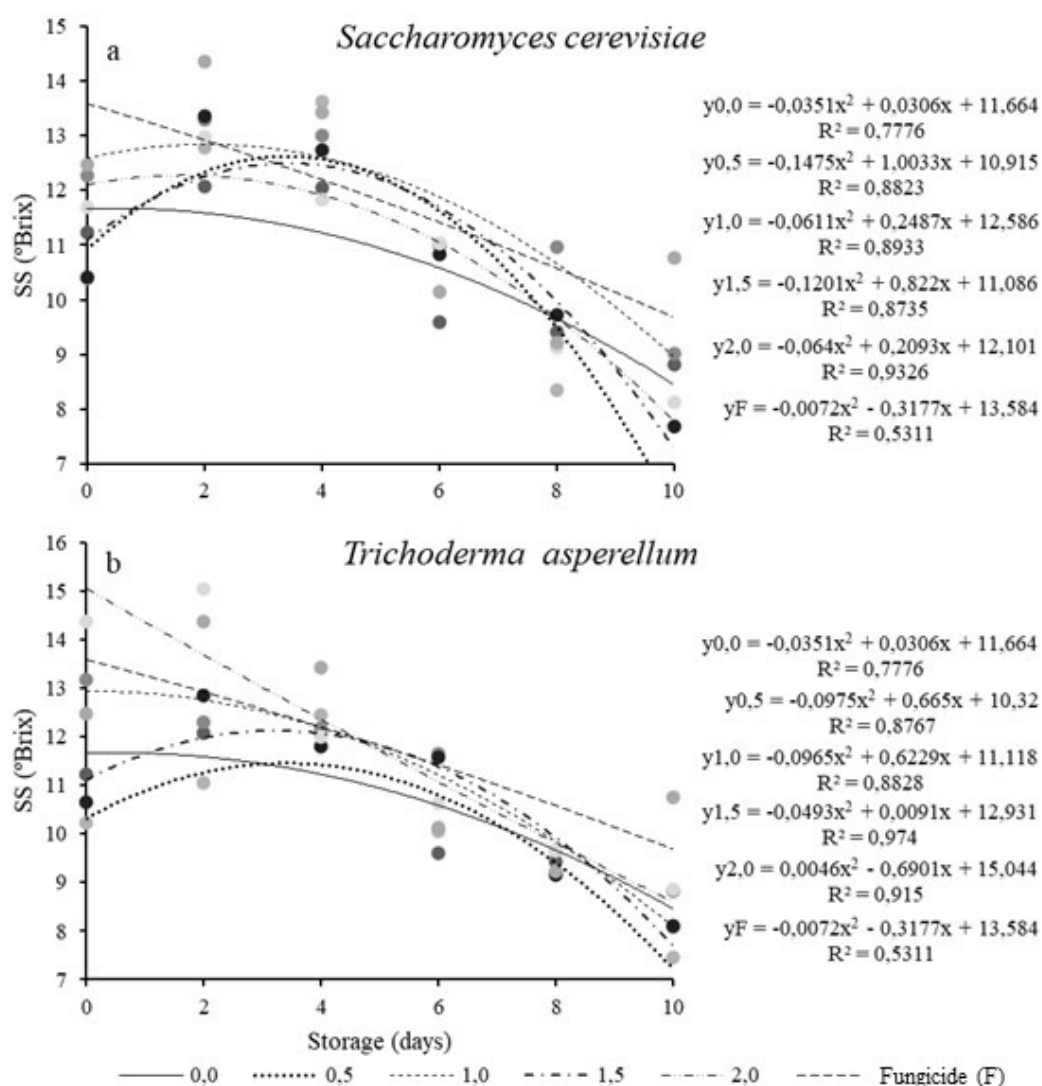


Figure 2. Soluble solids content (SS) of passion fruit (*Passiflora edulis* f. *flavicarpa*) during 10 days after fruit dipping in *Saccharomyces cerevisiae* or *Trichoderma asperellum* solutions (0,0; 0,5; 1,0; 1,5; 2,0 g L⁻¹), or Mancozeb fungicide (2,0 g L⁻¹).

Several factors interfere in the soluble solids content, such as: light intensity, temperature, rainfall, edaphoclimatic interactions, the har-

vest point, the harvest time (NASCIMENTO et al., 1998) and the storage time of the passion fruit. In the content of total soluble solids,

mainly under different environmental conditions (ARJONA et al., 1992).

The SS contents agree with those observed by other authors for passion fruit (PINHEIRO et al., 2006; MEDEIROS et al., 2009). Juice from yellow passion fruit in different degrees of maturation presented an increase in the value of SS, according to the increase in maturation degree (VIANNA-SILVA et al., 2008; JIMÉNEZ et al., 2011). The increase in SS content is dependent on the stage of maturity in which the fruit is harvested and generally increases during maturation by biosynthesis or degradation of polysaccharides (CHITARRA; CHITARRA, 2005).

Titrateable acidity

Titrateable acidity (TA) increased until the fourth day of storage. Then the TA decreased until the 10th day, similar to observed for SS content. The maximum 5.0% TA value was found in fruits treated with *S. cerevisiae* (0,5 g. L⁻¹) and *T. asperellum* (1,0 g. L⁻¹), respectively, similar to fruits treated with fungicide (Figure 3). The TA value was higher than the minimum (0.27%) recommended for passion fruit juice by MAPA (BRASIL, 2003). The results showed that the biological control maintained the passion fruit quality and controlled the anthracnose.

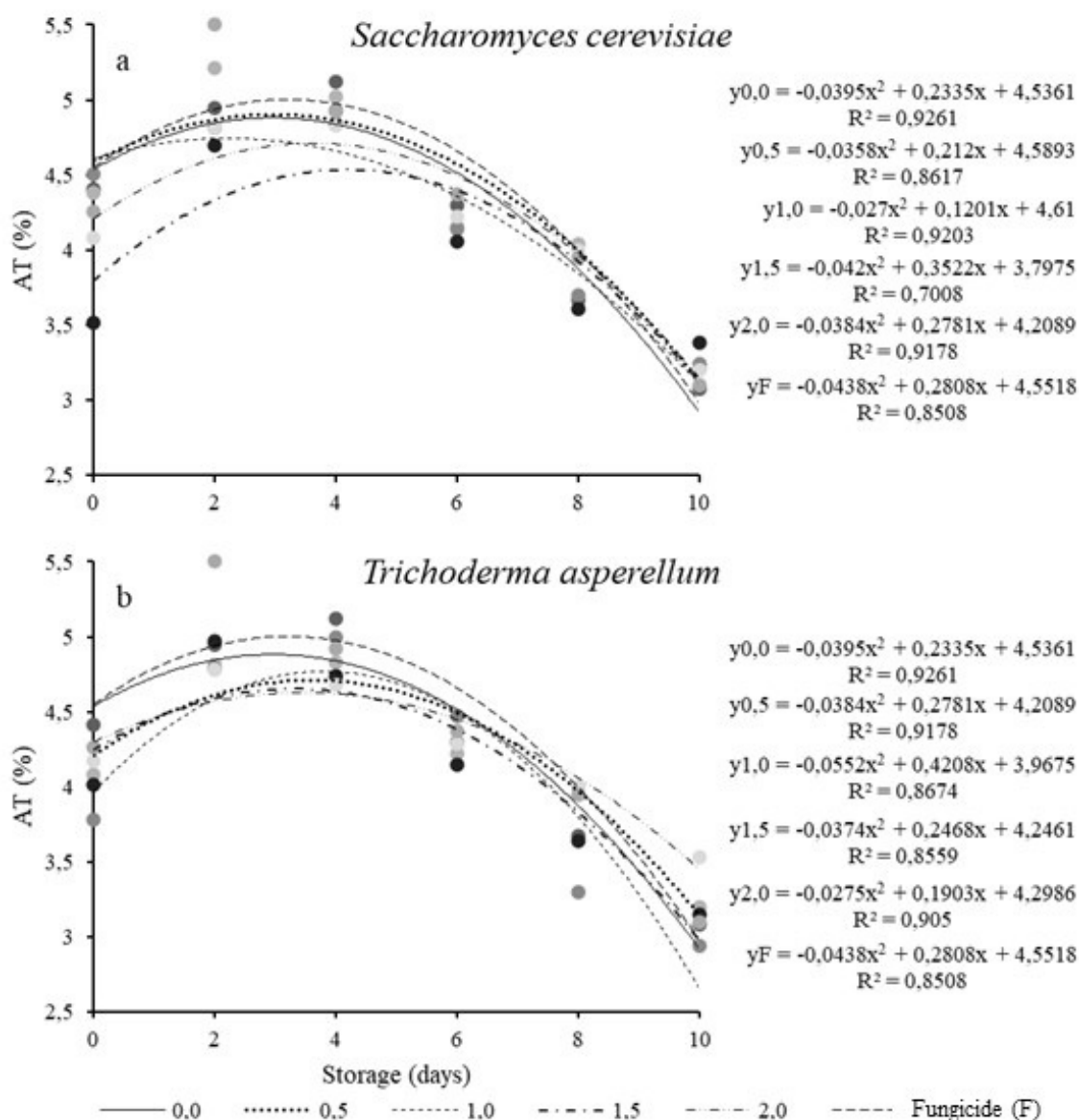


Figure 3. Titrateable acidity (TA) of passion fruit (*Passiflora edulis* f. *flavicarpa*) during 10 days after fruit dipping in *Saccharomyces cerevisiae* or *Trichoderma asperellum* solutions (0,0; 0,5; 1,0; 1,5, 2,0 g. L⁻¹), or Mancozeb fungicide (2,0 g. L⁻¹).

pH

The pH values increased until the 10 days of storage. An increase from 2.3 to 3.5 occurred in all fruits evaluated (Figure 4). These chang-

es can be attributed to the initial and subsequent degradation of organic acid synthesis with different potentials of ionic dissociation (ALMEIDA et al., 2006).

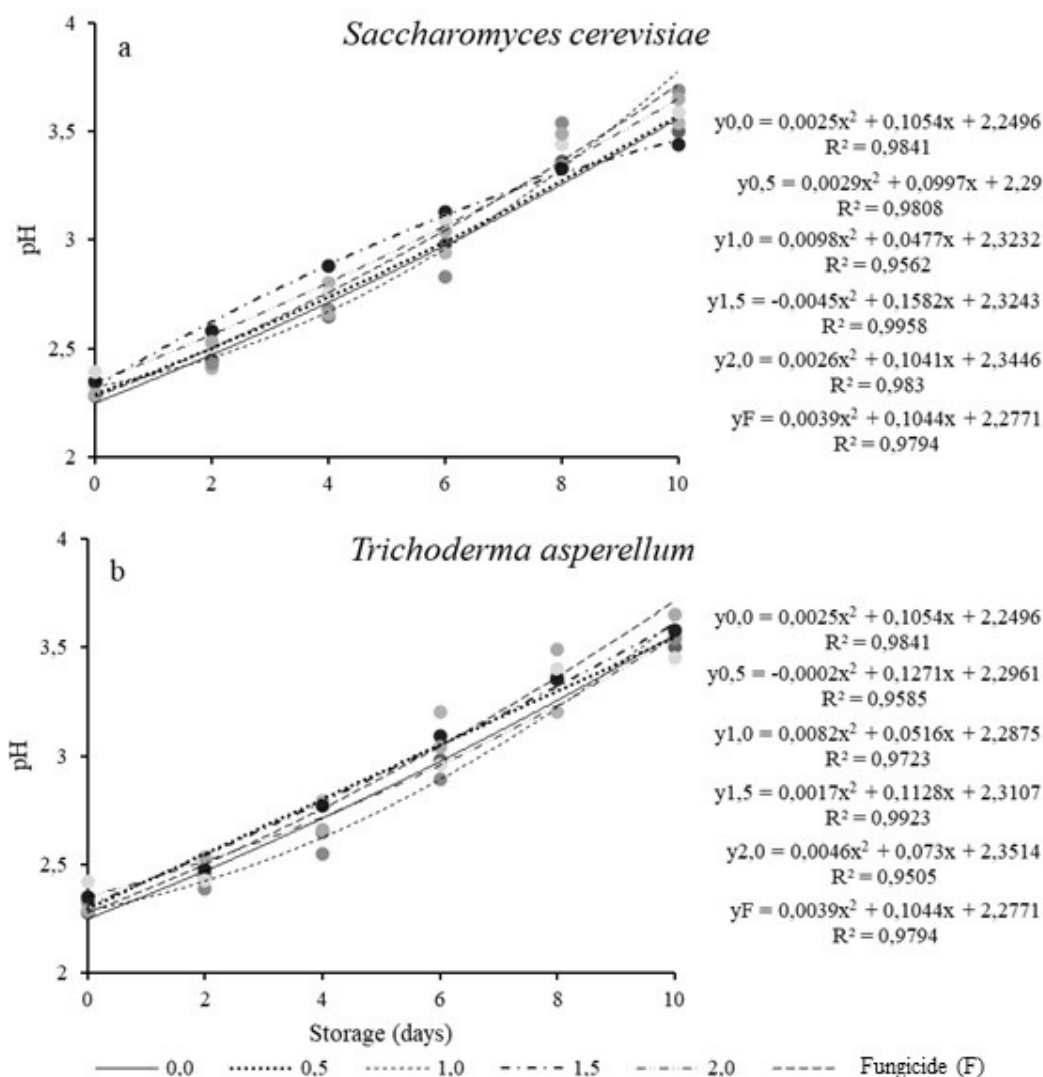


Figure 4. pH values from passion fruit juice (*Passiflora edulis* f. *flavicarpa*) during 10 days after fruit dipping in *Saccharomyces cerevisiae* or *Trichoderma asperellum* solutions (0,0; 0,5; 1,0; 1,5; 2,0 g. L⁻¹), or Mancozeb fungicide (2,0 g. L⁻¹).

The pH data were higher than the average values found by Coelho et al. (2010), in passion fruits, which obtained average values of 2.92. According to Campos et al. (2013) passion fruit with pH of the pulp between 2.5 and 3.5 are more suitable to the processing for production of concentrated juice.

Conclusion

The fungi *Saccharomyces cerevisiae* and *Trichoderma asperellum* reduce anthracnose severity in passion fruit (*Passiflora*

edulis f. *flavicarpa*), and do not alter the fruit quality. *Trichoderma asperellum* and *Saccharomyces cerevisiae* at 2,0 g. L⁻¹ promote anthracnose control similar to 2,0 g. L⁻¹ Mancozeb fungicide.

Acknowledgements

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES Brazil), for granting grants from the first author.

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