

Postharvest control of anthracnose in avocado with cassava starch and corn starch films

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Abstract

The objective of this study was to evaluate the effect of cassava starch and corn starch films on the control of anthracnose in avocado. The fruits were inoculated with conidia suspension and were then immersed in solutions with cassava starch and corn starch alone or in combination at concentrations of 1, 2, 3 and 4% under curative and preventive methods. The variables analysed included mean damaged area, area under the disease progress curve (AUDPC) and fruit fresh weight. In the preventive control method, cassava starch at concentrations of 2.18 and 2.24% provided lower AUDPC values with an average of 108.26 and average injured area of 4.23 cm², respectively. For corn starch, lower values of AUDPC and average injured area were observed at a concentration of 4% with averages of 1.84 and 0.47 cm², respectively. In the curative control method, the AUDPC and mean injured area were lower when the fruits were treated with cassava starch at concentrations of 1 and 4%. When corn starch was used, the smallest mean injured area was observed at a concentration of 1% with a value of 0.075 cm² and AUDPC of 1.68. In the curative method, in the combination starch + 1% starch, higher weight losses of 11.04 and 15.63% were observed, respectively. In the preventive form, there was no statistical difference between treatments. Cassava and corn starch films at the individual concentrations of 1 and 4% and the combinations of starch + corn starch at 2, 3 and 4% formed a protective layer deposited on the peel of the fruits.

Keywords: *Persea americana*, alternative control, postharvest diseases

Introduction

The avocado (*Persea americana* Mill.), which is native to Central America and Mexico, is cultivated in tropical and subtropical regions of the world. Avocado fruits have nutritional value because they are rich in proteins, fibre, minerals, vitamin A, vitamin B complex, vitamin C and vitamin E and have high concentrations of unsaturated fatty acids (Daiuto et al., 2013; Tremocoldi et al., 2018).

In 2019, Mexico was the world's largest avocado producer and exporter, with production of 3,300,889 tons, and Brazil was the seventh largest producer, with a production of 242,932 tons (Faostat, 2021). However, this production has been limited by phytosanitary problems, such as postharvest diseases. Anthracnose is considered the main disease among postharvest avocado diseases. This disease reduces fruit quality and affects consumer acceptance and market value (Oliveira et al., 2017b;

Sefu et al., 2015). This pathogen attacks leaves, flowers and fruits. Symptoms begin with small, dark brown, sunken, circular-shaped spots. With time, the spots progress to necrosis and may affect the entire fruit, making it unfit for consumption. At a more advanced stage of disease, the fruit pulp is affected, and in the presence of high moisture, a salmon-coloured mucilaginous mass with reproductive pathogen structures is formed (Kimaru et al., 2018). The causal agent of avocado anthracnose is the *Colletotrichum gloeosporioides* species complex (Soares et al., 2021; Tozze Júnior et al., 2015).

Control of the disease starts in the orchard through the application of fungicides between flowering and fruiting (Fischer et al., 2011). Although fungicide application is effective, this practice increases production costs and can cause environmental and human contamination. Alternative products such as biodegradable starch-based films stand out as a viable

form of postharvest control as a substitute for chemical products. In addition, edible coatings used in postharvest are inexpensive and are developed from renewable sources, which aim to maintain the quality of useful life of the fruits, avoid environmental pollution and create a resistant and transparent barrier, giving the fruits a bright and attractive appearance (Campos et al., 2011; Jiménez et al., 2012; Mardigan et al., 2014; Oliveira et al., 2017a).

Starch is a polysaccharide and is used in the formation of edible coatings because it is abundant, easy to use and cost effective (Vargas et al., 2008). Starch is composed of amylose and amylopectin chains with favourable properties due to its physiological safety and biodegradability (Weber et al., 2009). Starch serves as an energy reserve in many plants, including cereals, tubers, roots, fruits and seeds. In addition, starch is used in food product applications, such as adhesives, binders and film formers, and acts as a gelling agent, thickener and moisture retainer (Weber et al., 2009). The starch coatings most used in postharvest disease control are cassava starch and corn starch.

Several studies have demonstrated the efficacy of using starch in the control of postharvest diseases and the ability of starch to increase the shelf life in various fruits, such as mango, strawberry and papaya (Campos et al., 2015; Moreira et al., 2017; Oliveira et al., 2016; Santos et al., 2011). Therefore, this study aimed to evaluate the control of anthracnose in avocado fruits coated with cassava starch and corn starch films.

Materials and Methods

Experimental site

The experiment was conducted at the Laboratory of Electron Microscopy and Ultrastructural Analysis of the Federal University of Lavras (UFLA) - MG. Avocados of the Breda cultivar were obtained from the Bonela farm located on the banks of highway BR 381 (km 727) in the municipality of Carmo of Cachoeira - MG. Cassava starch and corn starch were purchased from the local market, and the Tecto® SC fungicide, which contains thiabendazole as the active ingredient, was provided by Syngenta.

Isolate and monosporic culture

The *Colletotrichum bredae* (CML 4027) isolate, which was previously deposited in the Lavras Mycological Collection (Department of Phytopathology, UFLA), was used to inoculate the avocado fruits. The monosporic isolate culture was prepared by adding 10 mL of sterile distilled water to dishes containing a colony. A 10- μ L

aliquot was extracted, deposited into Petri dishes (9-cm diameter) containing agar-water medium and spread with a Drigalski loop. The Petri dishes were placed in an incubator (BOD) at 25 °C for 12 h under fluorescent light. Subsequently, germinated conidia were transferred to new Petri dishes containing potato dextrose agar (PDA).

Pathogenicity

For pathogenicity tests, avocados of the Breda cultivar were previously washed in running water and disinfested in 2% sodium hypochlorite (NaOCl) for 3 min. After this step, five holes (depth of 2 mm) were prepared with sterilized multi-needles, and 20 μ L of suspension was applied at three different points at a concentration adjusted to 10⁶ conidia mL⁻¹ (Soares et al., 2017). The fruits were wrapped in plastic bags and incubated at 25 °C and 90% relative humidity. The isolate was pathogenic to the avocado fruits and caused dark sunken spots, delimiting all points inoculated on the external surface of the fruits compared to the control, which showed no symptoms. Eight days after inoculation at a more advanced stage of the disease, the spots showed masses of salmon-coloured conidia.

In vivo evaluation of starch coatings for preventive and curative control

The effects of cassava starch and corn starch alone and in combination at concentrations of 1, 2, 3 and 4% were evaluated in avocados for curative and preventive control.

For the curative control method, avocado fruits were disinfested with 2% NaOCl, inoculated with spore suspension at a concentration of 10⁶ conidia mL⁻¹, dried for 12 h and then immersed in cassava starch and/or corn starch solutions (alone or combination) at concentrations of 1, 2, 3 and 4%. For the preventive method, the disinfested fruits were initially immersed in the solutions (Table 1), subsequently dried for 12 h and then subjected to inoculation. Avocados were inoculated with a suspension at three different points in the median region of the fruits using the five-hole method according to Soares et al. (2017). With the aid of an automatic pipette, 20 μ L of the suspension adjusted to a concentration of 10⁶ conidia mL⁻¹ was deposited at each point. Next, the fruits were wrapped in plastic bags, which formed a humidity chamber of 90% humidity and 25 °C, for 48 h of wetting. After removing the plastic bags, the avocados remained on a bench for 12 h for drying at 26 \pm 2 °C and a mean relative humidity of 60 \pm 5%. The controls included inoculated fruits immersed in distilled water and inoculated fruits treated with the Tecto® SC fungicide.

The experiments were conducted in a completely randomized design with 14 treatments, four replications and one fruit per plot (Table 1).

Table 1. Treatments used in the curative and preventive experiments for anthracnose control in the Breda avocado cultivar.

Curative and preventive treatments	
T1: Control (distilled water)	T8: Corn starch (2%)
T2: Fungicide (Tecto® SC)	T9: Corn starch (3%)
T3: Cassava starch (1%)	T10: Corn starch (4%)
T4: Cassava starch (2%)	T11: (Ca) Cassava (1%) + (Co) Corn (1%)
T5: Cassava starch (3%)	T12: (Ca) Cassava (2%) + (Co) Corn (2%)
T6: Cassava starch (4%)	T13: (Ca) Cassava (3%) + (Co) Corn (3%)
T7: Corn starch (1%)	T14: (Ca) Cassava (4%) + (Co) Corn (4%)

Preparation of coating solutions

The solutions containing cassava starch and corn starch alone at concentrations of 1, 2, 3 and 4% and solutions containing combinations of cassava starch and corn starch at concentrations of 1, 2, 3 and 4% were prepared in 1 L of water, heated in a microwave (90 °C for corn starch and 70 °C for cassava starch) and shaken every 10 seconds to obtain transparent, nongranular gels. After cooling the gels at room temperature, avocados were immersed in the solutions for 1 min, drained and dried for 3 min, and the process was repeated three times (Oliveira et al., 2016). Later, the fruits were placed on supports and placed on the laboratory bench at 25 ± 2 °C and a mean relative humidity of 60 ± 5% to reproduce commercialization conditions for 16 days (durability of control treatment).

Fresh weight loss

The loss of fresh matter was obtained from the difference between fruit weight on day 0 (initial weight) and that on the last day of evaluation (final weight) using the following equation by Coelho et al. (2017):

$$FWL = [(Wi - Wf) / Wi] * 100$$

where FWL is the fresh weight loss (%); Wi is the initial weight (g); and Wf is the final weight (g). The fruits were weighed on an analytical balance with 0.01 g precision, and the results are expressed as a percentage of the initial weight.

Anthracnose spots and statistical analyses

Evaluations were performed every two days by measuring the spots in two perpendicular directions with a digital calliper. The mean damaged area was then calculated in cm² at the inoculated points. The formula adapted by Soares et al. (2017) was used to calculate the circle area:

$$A = \pi r^2$$

where A is the mean damaged area; π is a constant (3.1416); and r is the mean radius of the spot.

The area under the disease progress curve (AUDPC) was also calculated using the formula proposed by Shaner & Finney (1977):

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

where yi is the value of the disease in the i-th observation; ti is the time in days in the i-th observation; and n is the total number of observations.

The Shapiro-Wilk test (Shapiro & Wilk, 1965) was applied to the data (variables mean damaged area and AUDPC) to evaluate the normal distribution using R software. In the absence of normality, the data were transformed using the log₁₀ function. Next, the data were analysed and subjected to analysis of variance using Sisvar software (Ferreira, 2011). The significant variables in the F test ($p \leq 0.05$) for the control with cassava starch or corn starch alone at concentrations 1, 2, 3 and 4% were compared by regression, and the combined treatments (cassava starch + corn starch) had the means grouped by the Scott-Knott test at 5% probability. The graphs were plotted using Sigma Plot® software.

Analysis of the film action using scanning electron microscopy (SEM)

At the end of the experiment, samples (diameter of 5 mm) of the fruits treated with the films were collected and immersed in Karnovsky's fixative solution (2.5% glutaraldehyde; 2.5% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2; and 1% 0.1 M CaCl₂) for a minimum period of 24 h. The samples were then washed three times for 10 min in 0.02 M sodium cacodylate buffer and subjected to dehydration in a series of acetone solutions at concentrations of 25, 50, 75, 90 and 100%. After dehydration, samples were taken to the critical

point using the BAL-TEC CPD 030 to replace acetone with CO₂. The obtained specimens were fixed in metal stubs with double-sided carbon tape, coated with aluminium foil, covered with gold in the BAL-TEC 050 evaporator and stored in a desiccator containing silica gel for observation using a Levo EVO 40 XVP SEM. Sample images were digitally generated and recorded under working conditions of 20 Kv and working distance of 10 mm, and images were edited using Corel Draw 12 software.

Results

Effect of cassava starch and corn starch alone on anthracnose control in avocado

There was a significant interaction between the variables (AUDPC and mean damaged area) and starch concentrations. All coatings inhibited the mean damaged area and the AUDPC under the curative and preventive methods (Figure 1).

In the curative control method, the AUDPC and mean damaged area were lower when the avocado fruits were treated with cassava starch at concentrations of 1 and 4% (Figure 1A). With the aid of the first-order derivative of the adjusted regression model, higher AUDPC and mean damaged area values were found at the 2.40 and 2.50% concentrations, with means of 190.71

and 14.46 cm², respectively (1A). When corn starch was used, the smallest mean damaged area was observed at a concentration of 1%, with a value of 0.075 cm² and an AUDPC of 1.68 (Figure 1B). The concentration of 2.94% provided a higher AUDPC value of 2.26 and a mean damaged area of 1.15 cm² (Figure 1B).

In the preventive control method with increasing concentrations of 1 to 4% cassava starch, the AUDPC increased from 166.82 to 247.22, and the mean damaged area increased from 14.45 cm² to 24.63 cm² (Figure 1C). The first-order derivative of the regression model showed that cassava starch at concentrations of 2.18 and 2.24% provided lower AUDPC values, with a mean of 108.26 and a mean damaged area of 4.23 cm², respectively (Figure 1C). For corn starch, lower AUDPC values and mean damaged area were observed, with values of 1.84 and 0.47 cm² at 4% concentration, respectively (Figure 1D).

In addition to inhibiting anthracnose spots in avocados (Figures 2A and 2B), the coatings also kept the fruit pulp intact relative to the control. Figures 2C and 2D show healthy avocado pulp treated with 4% cassava starch film or 1% corn starch film under the curative and preventive methods, respectively, compared to the untreated control immersed only in distilled water.

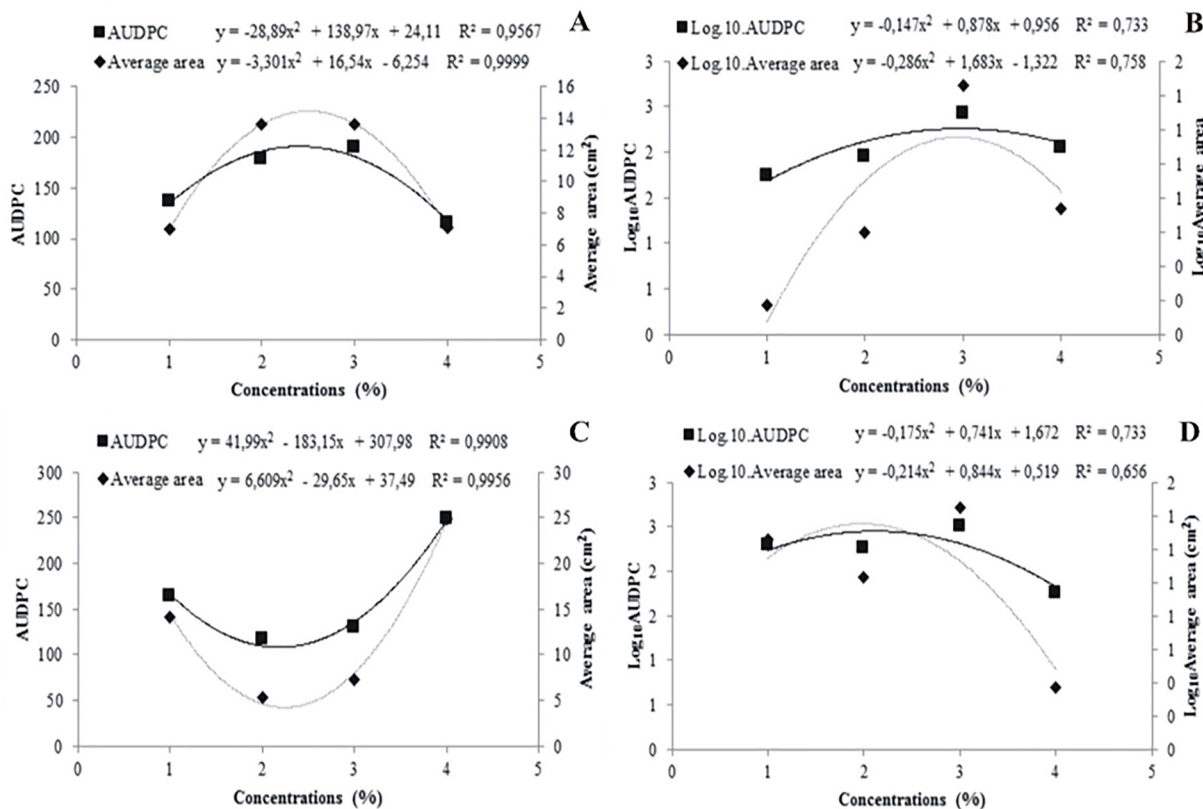


Figure 1. Mean damaged area by *Colletotrichum bredae* and area under the disease progress curve (AUDPC) in avocado fruits treated for anthracnose control. Curative form: cassava starch (A) and corn starch (B); Preventive form: cassava starch (C) and corn starch (D).

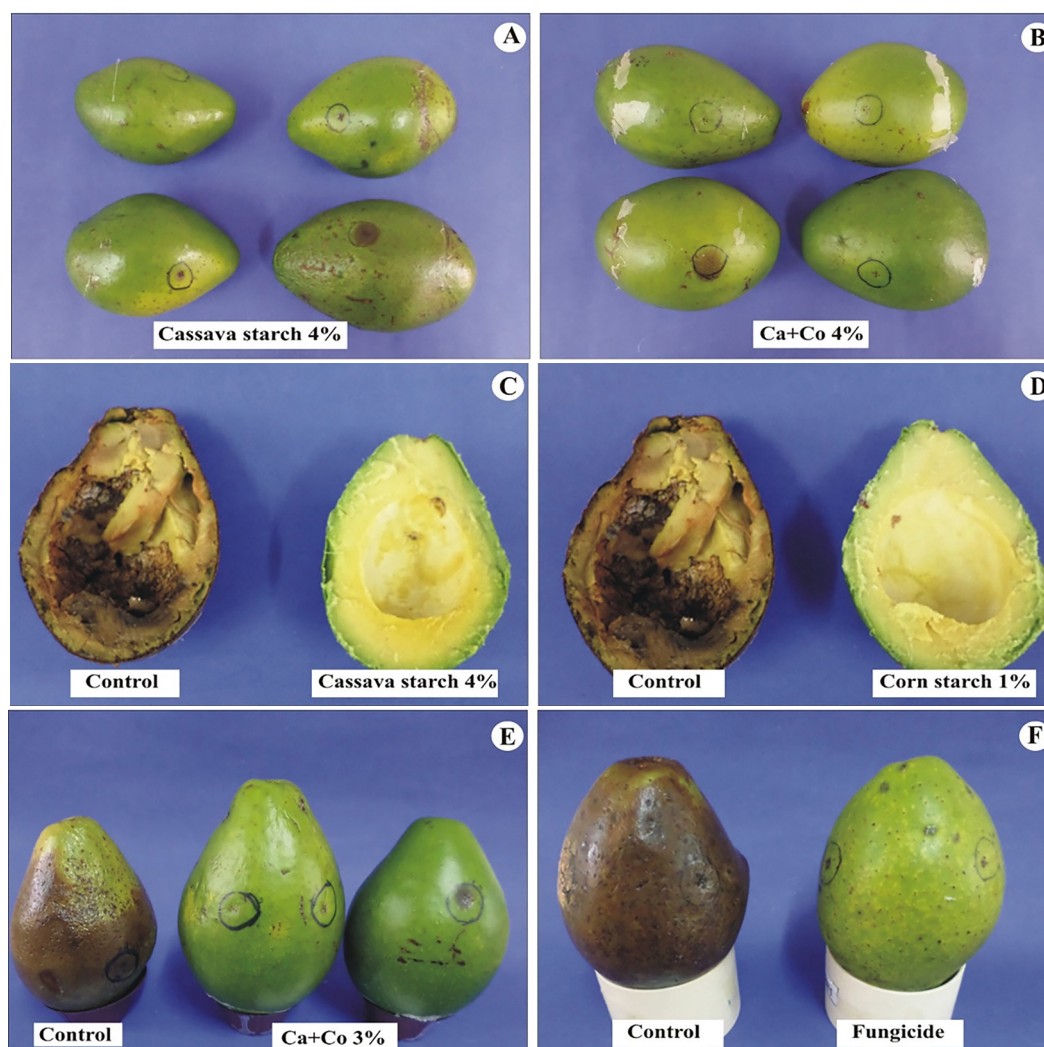


Figure 2. Photographs of Breda cultivar avocados treated curatively with 4% cassava starch to control Anthracnose 16 days after inoculation. (A and B) Fruits treated curatively with 4% cassava starch alone or 4% cassava starch + corn starch in combination, respectively. (C) Avocado pulp treated curatively with control, 4% cassava starch alone or 1% corn starch alone. (D) Preventive treatment combining 3% cassava starch + corn starch and the control (E). Symptoms of anthracnose in fruit inoculated and untreated and fruit inoculated and treated with fungicide Tecto® SC at 16 days after inoculation (F).

Combined effect of cassava starch + corn starch in anthracnose control in avocado

The combination of cassava starch and corn starch in the curative and preventive forms delayed the ripening of the fruits kept at room temperature and reduced the appearance of anthracnose spots at 16 days (Figure 2E). The AUDPC and the size of the spots showed the same behaviour for the fruits treated curatively with the cassava starch + corn starch combination at concentrations 2, 3 and 4%, and they did not differ statistically (Figure 3). The films combined at 2, 3 and 4% provided inhibition at the mean damaged area of 3.93, 1.79 and 2.34 cm² and AUDPC of 99.00, 73.61 and 67.93, respectively (Figures 3A and 3B). In addition, the results for the films did not differ from those of the Tecto® SC fungicide, which inhibited anthracnose spots with a mean damaged area of 5.31 cm² and AUDPC

of 101.11 (Figures 3A and 3B), resulting in improved fruit appearance compared to control fruits (Figure 2F).

In the preventive control method, the combinations of 2 and 3% cassava starch + corn starch were more efficient in anthracnose control and were significantly different from the Tecto® SC fungicide (400 mL for each 100 L of water), which was not as efficient and restricted the growth of anthracnose spots to a mean damaged area of 18.73 cm² and AUDPC of 206.08 (Figures 3C and 3D).

No inhibition of the mean damaged area and AUDPC was found for the curative method with the combination of 1% cassava starch + corn starch (Figures 3A and 3B). However, smaller spots of 9.70 cm² (Figure 3C) and a lower AUDPC of 133.43 were observed for the preventive form for this treatment compared to the control (Figure 3D).

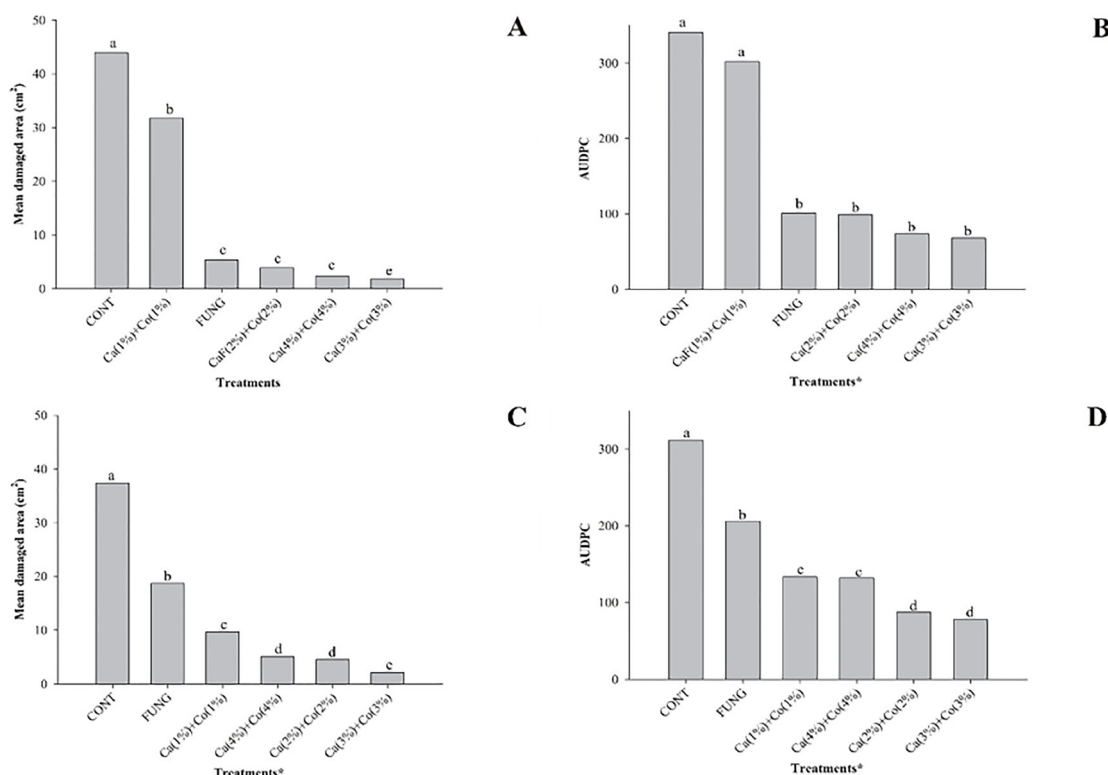


Figure 3. Average avocado damaged area under the curative method (A) and area under the disease progress curve (AUDPC) (B). Mean damaged area in avocado fruits under the preventive method (C) and AUDPC (D). TEST: control; FUNG: Tecto® SC fungicide; Ca (1%) + Co (1%): 1% cassava starch + corn starch; Ca (2%) + Co (2%): 2% cassava starch + corn starch; Ca (3%) + Co (3%): 3% cassava starch + corn starch; Ca (4%) + Co (4%): 4% cassava starch + corn starch. *Treatments followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability.

Fresh weight loss

The fresh weight loss was influenced by the types of coatings (Figure 4). In the curative method, the fruits coated with cassava starch and corn starch films (both alone and in combination) showed reduced fresh weight loss at 16 days of storage. The exception was for treatments with 3% cassava starch alone and 1% cassava starch + corn starch in combination, which showed weight loss of 11.04 and 15.63%, respectively, which did

not differ from the control (12.91%) (Figure 4A).

Fruits treated preventively had a reduction in weight loss that did not differ from that of the control, which had a weight loss percentage of 9.74%. The most pronounced loss was observed in fruits treated with corn starch (3%), presenting a weight loss of 19.81%, which was significantly different from that of the other coatings (Figure 4B).

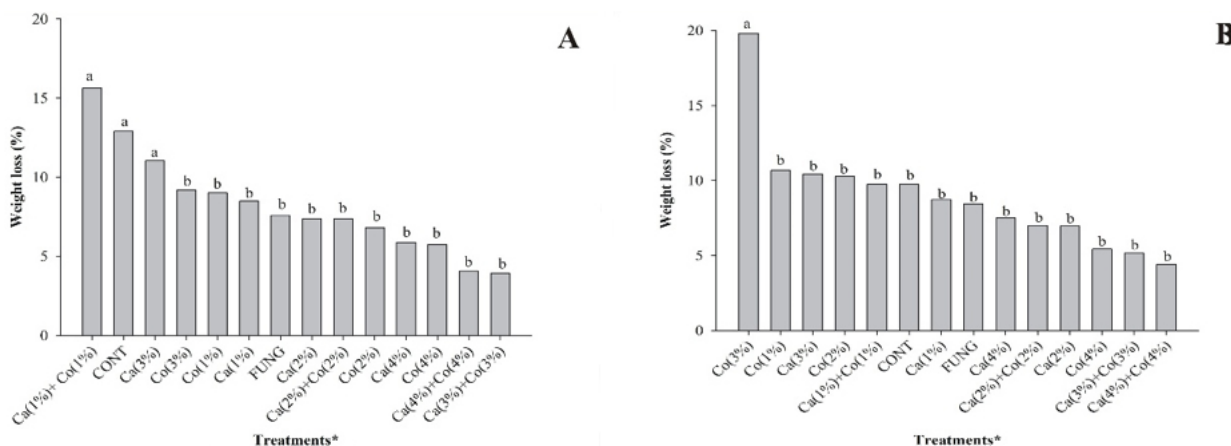


Figure 4. Fresh weight loss in avocados treated curatively (A) and preventively (B) at 16 days of storage. CONT: Control (water); FUNG: fungicide (Tecto® SC); Ca (1%): 1% cassava starch; Ca (2%): 2% cassava starch; Ca (3%): 3% cassava starch; Ca (4%): 4% cassava starch; Co (1%): 1% corn starch; Co (2%): 2% corn starch; Co (3%): 3% corn starch; Co (4%): 4% corn starch; Ca (1%) + Co (1%): 1% cassava starch + 1% corn starch; Ca (2%) + Co (2%): 2% cassava starch + 2% corn starch; Ca (3%) + Co (3%): 3% cassava starch + 3% corn starch; Ca (4%) + Co (4%): 4% cassava starch + 4% corn starch. * Treatments followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability.

Scanning electron microscopy (SEM) analysis of the action of films on fruits

The following films formed a protective layer deposited on the peel of the fruits: films composed of either cassava starch or corn starch alone at concentrations of 1% or 4% used in a curative manner; films composed of cassava starch + corn starch in combination at concentrations of 2%, 3% or 4% used in a curative manner; preventive application of 2% or 3%

cassava starch alone; preventive application of 2% or 4% corn starch alone; and preventive application of 2% or 3% cassava starch + corn starch in combination (Figures 5A and 5B). This protective layer covered the stomata and spots, reducing gas exchange and fruit ripening and preventing the penetration of the germ tube of the fungus. The films also inhibited conidial germination and caused hyphal deformation, leading to delayed tissue colonization (Figures 5C-F).

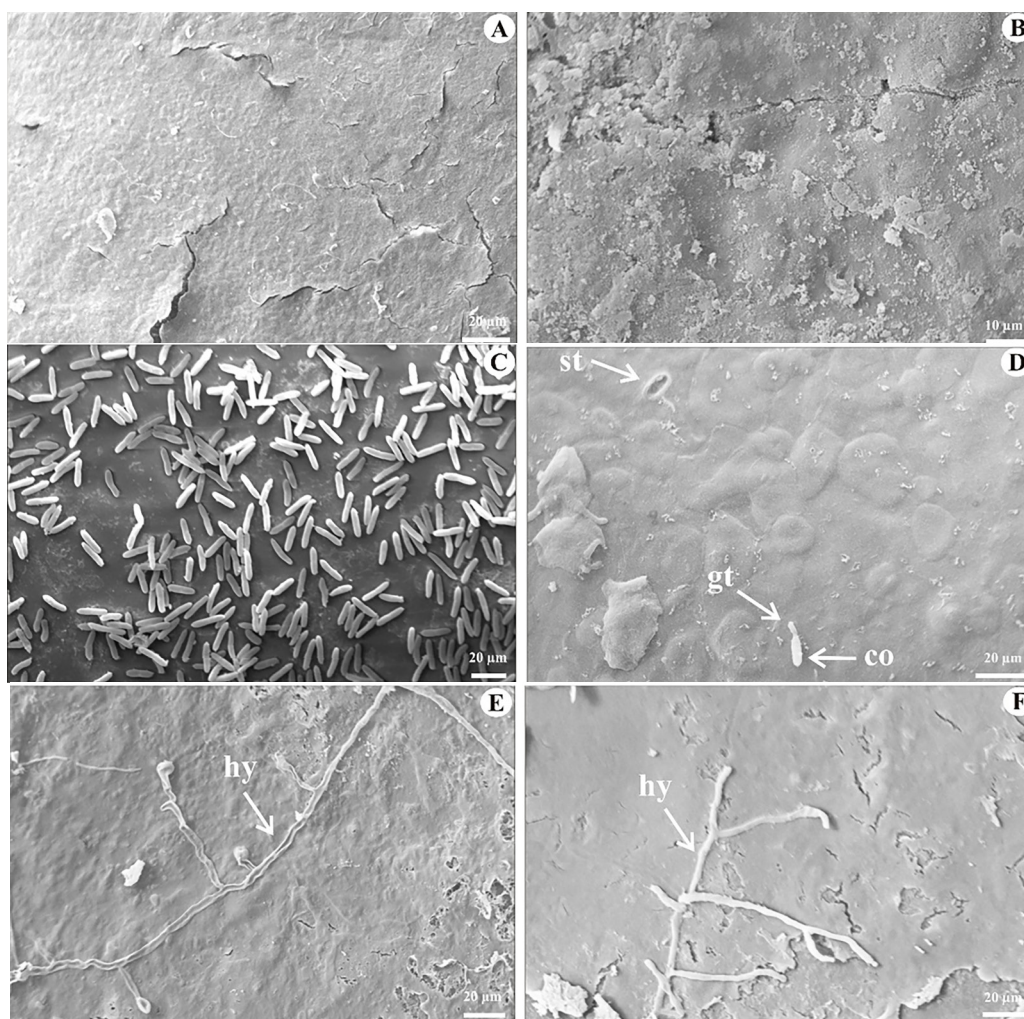


Figure 5. Scanning electron micrographs of avocado peel fragments infected with *Colletotrichum bredae*. (A and B) Protective layer on the surface of the fruits consisting of the films of curative 4% Ca alone and preventive 3% Ca alone; (C and D) Non-germinated conidia and slow germ tube growth on the surface of fruit treated with the combination of curative 4% Ca + 4% Co; (E and F) Deformed hyphae delaying colonization resulting from the action of films containing 2% Ca alone and 3% Ca alone. Ca: cassava starch; Co: corn starch; hy: hyphae; con: conidium; gt: germ tube; and st: stomata.

Discussion

Coating avocado fruits using films composed of 2, 3 and 4% cassava starch or corn starch alone and films composed of 2 and 3% cassava starch + corn starch in combination were effective against avocado anthracnose and maintained fruit quality for up to 16 days.

2, 3 and 4% cassava starch and corn starch films combined in the curative control, as well as the preventive combination of cassava starch + 2 and 3% corn starch were effective against anthracnose in avocado, in addition to maintaining the quality of the fruit for up to 16 days.

In addition to the combination coatings being

effective in controlling anthracnose, the use of 1 and 4% cassava starch alone and 1 and 2% corn starch alone also reduced the mean damaged area of the fruits when used curatively. Similar results have been observed for the preservation of postharvest fruits, such as bell pepper when treated with 4% cassava starch (Moreira et al., 2017) and mango fruits when treated with 2% cassava starch and 4% corn starch (Santos et al., 2011). Handayani et al. (2018) applied cassava starch coating at concentrations of 3, 4 and 5% on avocado fruits and observed good fruit appearance, firmness and pH and prolonged shelf life. In a preventive manner, the individual concentrations of 2% and 3% cassava starch and 2% and 4% corn starch promoted a smaller mean damaged area and AUDPC. Similarly, Oliveira et al. (2016) observed anthracnose control in papaya fruits treated with cassava starch at a 3% concentration.

When the Tecto® SC fungicide was applied in a curative manner, it was more efficient than the preventive method, and it minimized the spots in the avocado fruits compared to the control. However, the use of a combination of 2 and 3% of cassava starch + corn starch in a preventive manner was more efficient than the fungicide used to control the disease.

Surface coatings may decrease fruit peel permeability, modify the internal atmosphere, reduce water loss, lower the fruit respiration rate and delay senescence, which makes the fruit more vulnerable to pathogenic infection, resulting in loss of cell integrity or tissue (Tanada-Palmu & Grosso, 2005). Cassava starch forms a resistant, transparent and efficient film that reduces water loss, maintaining the quality of fruits and vegetables due to the preservation of its organoleptic characteristics and maintenance of a good visual presentation (Vicentino et al. 2011). Corn starch not only prolongs shelf life but also preserves quality during storage by providing selective barriers to moisture transfer, oxygen absorption, losses of volatile aromas and pleasant visual appearance (Ghosh et al., 2015).

The combination of more than one coating based on polysaccharides or based on polysaccharides and a lipid or protein has been the target of many studies. In the present study, combinations of 2% and 3% cassava starch + corn starch were efficient in restricting the increase in AUDPC and anthracnose spots in fruits treated with curative and preventive treatments. Several studies have also evaluated the effectiveness of alternative products in maintaining the postharvest quality of various fruits. Praseptiangga et al. (2017) observed the effectiveness of the edible coating of cassava starch with lemon

grass essential oil (1%) on papaya quality. Ambarsari et al. (2018) found that the incorporation of cassava starch at concentrations of 1, 2 and 3% with citric acid at concentrations of 0.5 and 1.0% prolongs the quality and shelf life of tomato fruits. A coating based on cassava starch and chitosan at 50% inhibits the mycelial growth of *Colletotrichum* isolates and maintains the quality of mango tree fruits (Oliveira et al., 2017b).

During the 16 days in which the fruits remained at room temperature, the treatments caused an increasing weight loss; and the lowest loss was 5%, which was observed in combinations of 3 and 4% of cassava starch + corn starch in the curative and preventive forms, respectively. Similar behaviours were observed by Coelho et al. (2017), who found a lower percentage of weight loss in treatments containing 3% cassava starch alone and cassava starch combined with antioxidants. Similarly, coatings with cassava starch at 4% resulted in lower weight loss in bell peppers, with maximum losses of 8.90% (Moreira et al., 2017). Lower mango weight loss was observed when mango fruits were treated with 2, 4 and 6% corn starch and 2, 4 and 6% cassava starch (Santos et al., 2011). Lower weight loss of 4.9% was observed in mango fruits coated with a combination of cassava starch and 5% chitosan (Camarati et al., 2017). According to Chitarra & Chitarra (2005), starch films increase the postharvest shelf life of fruits by minimizing the loss of water by transpiration, which may be due to the high hygroscopic nature of the starch. Moreover, fruit coated with polysaccharide films can delay the weight loss due to the gel applied, which allows the loss of moisture before the coated fruit is dried (Bourtoom, 2008). In addition, starch coatings have good mechanical properties and good linearity of amylose molecules in solution, which favours good parallel orientation for the formation of resistant and protective films (Mali et al., 2010).

The following treatments were not efficient in minimizing spots in avocados and showed greater weight loss: curative application of 3% of cassava starch alone; curative application of the combination of 1% cassava starch + corn starch; and preventive application of 3% corn starch alone. Similarly, guava fruits coated with 2% and 6% cassava starch film and stored for 12 days showed no reduction in weight loss, and a smaller reduction was observed with the application of 2 and 4% pectin coatings (Quirino et al., 2018). Souza et al. (2009) found a greater weight loss of 14.7% in fruits treated with 3% cassava starch film. Hog plums treated with 3% cassava starch film presented a greater weight loss of 10.76% compared with the control (Freitas et al., 2017).

Microscopy is an important tool to understand processes related to disease development in the host and to record the mode of action of alternative products on the structures of phytopathogens (Pereira & Pereira, 2007). Studies have reported barriers formed by starch coatings that cover the stomata, restricting CO₂ and O₂ gas exchange, which may delay fruit ripening and prevent the development of *C. breddae* (Assis, 2009). In the present study, efficient treatments to control anthracnose spots created a protective layer on avocado fruits, which developed some striae and cracking due to moisture loss. Oliveira et al. (2017a) observed barriers in cassava starch-coated papaya fruit that prevent fruit ripening and the development of *C. gloeosporioides*, and they observed a more complete protective layer without breaking the starch gel coating in a 3% film. Basiak et al. (2017) studied the properties of starch films and found greater thickness and stiffness in films with high amylose content in addition to forming a greater barrier to water content and gas exchange.

Starch coatings influence the germination and colonization stages of the pathogen, which may explain the inhibition of anthracnose spots in avocado. Similar results have been found in other studies. Pereira et al. (2011) demonstrated a direct activity in reducing *Cercospora coffeicola* mycelial germination and development due to the fungitoxic effect of citronella and cinnamon essential oils. Wall thinning, reduced hyphal diameter in *Aspergillus niger* and the absence of conidiophores were promoted by the action of orange essential oil (Sharma & Tripathi, 2006).

Conclusions

The curative or preventive coating of avocado pods with cassava starch and corn starch film provides a smaller average lesioned area of anthracnose and a reduction in the area under the disease progress curve (AUDPC). Furthermore, the coatings keep the fruit pulps intact, delay fruit ripening, reduce the loss of fresh weight, form a protective layer deposited on the fruit skin and influence the inhibition of conidia germination and hyphae deformation.

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References

- Ambarsari, I., Oktaningrum, G.N., Endrasari, R. 2018. Effectiveness of incorporating citric acid in cassava starch edible coatings to preserve quality of Martha tomatoes. *IOP Conf. Series: Earth and Environmental Science* 102: 1-8.
- Assis, O.B.G., Britto, D., Forato, L.A. 2009. O uso de biopolímeros como revestimentos comestíveis protetores para conservação de frutas in natura e minimamente processadas. EMBRAPA, São Carlos, Brasil. 23p.
- Basiak, E., Lenart, A., Debeaufort, F. 2017. Effect of starch type on the physico-chemical properties of edible films. *International Journal of Biological Macromolecules* 98: 348-356.
- Bourtoom, T. 2008. Edible films and coatings: characteristics and properties. *International Food Research Journal* 15: 237-248.
- Camatari, F.O.D.S., Santana, L.C.L.A., Carnelossi, M.A.G., Alexandre, A.P.S., Nunes, M. L., Goulart, M.O.F., Narain, N., Silva, M.A.A.P. 2017. Impact of edible coatings based on cassava starch and chitosan on the post-harvest shelf life of mango (*Mangifera indica* 'Tommy Atkins') fruits. *Food Science and Technology* 38: 86-95.
- Campos, R.P., Kwiatkowsk, A., Clemente, E. 2015. Conservação pós-colheita de morangos recobertos com fécula de mandioca e quitosana. *Ceres* 58: 554-560.
- Campos C.A., Gerschenson, L.N., Flores, S.K. 2011. Development of edible films and coatings with antimicrobial activity. *Food and Bioprocess Technology* 4: 849-875.
- Chitarra, M.L.F., Chitarra, A.B. 2005. Pós-colheita de frutos e hortaliças - Fisiologia e Manuseio. UFLA, Lavras, 785p.
- Coelho, D.G., Andrade, M.T.D., Neto, M., Ferreira-Silva, S.L., Simões, A., Nascimento, D. 2017. Application of antioxidants and edible starch coating to reduce browning of minimally-processed cassava. *Revista Caatinga*, 30: 503-512.
- Daiuto É.R., Vieites R.L., Tremocoldi, M.A., Carvalho, L.R.D., Fumes, J.G.F. 2013. Postharvest of 'Hass' avocados submitted to UV-C radiation. *Revista Colombiana de Ciências Hortícolas* 7: 149-160.
- Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia* 35: 1039-1042.
- Fischer, H.I., Tozze Júnior, H.J., Arruda, M.C., Massola Júnior, N.S. 2011. Pós-colheita de abacates 'Fuerte' e 'Hass': características físicas e químicas, danos e controle de doenças. *Semina: Ciências Agrárias* 32: 209-220.
- FAOSTAT. Food and Agriculture Organization of the United Nations. 2020. <http://faostat3.fao.org/home/E> <Accessed in 26 April 2021>
- Freitas, R.V.S., Souza, P.A., Coelho, E.L., Souza, F.X., Beserra, H.N.B.R. 2017. Storage of mombin fruits coated

- with cassava starch and PVC film. *Revista Caatinga* 30: 244-249.
- Ghosh, A., Dey, K., Bhowmick, N. 2015. Effect of corn starch coating on storage life and quality of Assam lemon (*Citrus limon* Burn.). *Journal Crop and Weed* 11: 101-107.
- Handayani, M.N., Karlina, S., Sugiarti, Y., Cakrawati, D. 2018. Application of edible coating from cassava peel-bay leaf on avocado. *Journal of Physics: Conference Series* 1013: 1-8.
- Jiménez, A., Fabra, M.J., Talens, P., Chiralt, A. 2012. Edible and biodegradable starch films: a review. *Food and Bioprocess Technology* 5: 2058-2076.
- Kimaru, S.K., Monda, E., Cheruiyot, R.C., Mbaka, J., Alakonya, A. 2018. Morphological and Molecular Identification of the Causal Agent of Anthracnose Disease of Avocado in Kenya. *International Journal of Microbiology* 1-10.
- Mali, S., Grossmann, M.V.E., Yamashita, F. 2010. Starch films: production, properties and potential of utilization. *Semina: Ciências Agrárias* 31: 137-155.
- Mardigan, L., Kwiatkowski, A., Castro, J., Clemente, E. 2014. Application of Biofilms on Fruits of Avocado (*Persea Americana* Miller) in Postharvest. *International Journal of Sciences* 3: 35-45.
- Moreira, E.G.S., Sanches, A.G., Silva, M.B., Macedo, J., Cordeiro, C.A.M., Costa, S.S.C. 2017. Use of edible film in the postharvest conservation of 'Magali' sweet pepper. *Scientia Agraria Paranaensis* 16: 120-126.
- Oliveira, B.F., Cruz, A.F., Alves, E. 2016. Cassava starch coatings for postharvest control of papaya anthracnose. *Phytopathologia Mediterranea* 55: 276-284.
- Oliveira, K.Á.R., Berger, L.R.R., Araújo, S.A., Câmara, M.P.S., Souza, E.L. 2017a. Synergistic mixtures of chitosan and *Mentha piperita* L. essential oil to inhibit *Colletotrichum* species and anthracnose development in mango cultivar Tommy Atkins. *Food Microbiology* 66: 96-103.
- Oliveira, T.A., Paiva, C.A., Silva, A.C., Costa, T.L.E., Nascimento, L.V., Leite, R.H.L., Aroucha, E.M.M. 2017b. Tommy Atkins mango (*Mangifera indica* L.) postharvest quality with cassava starch, chitosan and pectin based coatings. *African Journal of Biotechnology* 16: 1596-1610.
- Pereira, O.L., Pereira, J.F. 2007. Microscopia e suas aplicações no estudo das interações fungo-planta. In: Alfenas, A. C., Mafia, R. G. (ed.) *Métodos em Fitopatologia*. UFV, Viçosa, Brasil. p. 221-252.
- Pereira, R.B., Lucas, G.C., Perina, F.J., Resende, M.L.V.D., Alves, E. 2011. Potential of essential oils for the control of brown eye spot in coffee plants. *Ciência e Agrotecnologia* 35: 115-123.
- Praseptiangga, D., Utami, R., Khasanah, L.U., Evirananda, I.P. 2017. Effect of cassava starch-based edible coating incorporated with lemongrass essential oil on the quality of papaya MJ9. *IOP Conference Series: Materials Science and Engineering* 176: 1-6.
- Quirino, A.K.R., Costa, J.D.S., Neto, A.F., Sousa, C.M., Sánchez-Sáenz, C.M. 2018. Conservation of 'Paluma' guavas coated with cassava starch and pectin. *Dyna* 85: 344-351.
- Santos, A.E., Assis, J.A., Berbert, P.A., Santos, O.O., Batista, P.F., Gravina, G.A. 2011. Influência de biofilmes de fécula de mandioca e amido de milho na qualidade pós-colheita de mangas' Tommy Atkins'. *Revista Brasileira de Ciências Agrárias* 6: 508-513.
- Sefu, G., Satheesh, N., Berecha, G. 2015. Effect of essential oils treatment on anthracnose (*Colletotrichum gloeosporioides*) disease development, quality and shelf life of mango fruits (*Mangifera indica* L). *American-Eurasian Journal of Agricultural and Environmental Sciences* 15: 2160-2169.
- Shaner, G., Finney, R.E. 1977. The effect of nitrogen fertilization on the expression of slow-mildew resistance in Knox wheat. *Phytopathology* 67: 1051-1056.
- Shapiro, S.S., Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52: 591-611.
- Sharma, N., Tripathi, A. 2006. Fungitoxicity of the essential oil of *Citrus sinensis* on post-harvest pathogens. *World Journal of Microbiology and Biotechnology* 22: 587-593.
- Soares, M.G.O., Alves, E., Freitas, A.S. 2017. Influência da inoculação e do molhamento no desenvolvimento de lesões de *Colletotrichum musae* em banana. *Agrotrópica* 29: 213-218.
- Soares, M.G., Alves, E., Silveira, A.L., Pereira, F.D., Guimarães, S.S. 2021. *Colletotrichum siamense* is the main aetiological agent of anthracnose of avocado in south-eastern Brazil. *Plant Pathology* 70: 154-166.
- Souza, P.A., Aroucha, E.M.M., Souza, A.E., Costa, A.R., Ferreira, G.S., Bezerra Neto, F. 2009. Conservação pós-colheita de berinjela com revestimentos de fécula de mandioca ou filme de PVC. *Horticultura brasileira* 27: 235-239.
- Tanada-Palmu, P.S., Grosso, C.R.F. 2005. Effect of edible wheat gluten-based films and coatings on refrigerated strawberry (*Fragaria ananassa*) quality. *Postharvest Biology and Technology* 36: 199-208.
- Tozze Júnior, H.J., Firmino, A.C., Fischer I.H., Furtado, E.L., Massola Júnior, N.S. 2015. Characterization of *Colletotrichum* spp. isolates associated with fruit trees in the state of São Paulo. *Summa Phytopathologica* 41: 270-280.
- Tremocoldi, M.A., Rosalen, P.L., Franchin, M., Massarioli, A.P., Denny, C., Daiuto, É.R., Paschoal, J.A.R., Melo, P.S., Alencar, S.M. 2018. Exploration of avocado by-products as natural sources of bioactive compounds. *PloS one* 13: 209-220.
- Vargas, M., Pastor, C., Chiralt, A., McClements, D.J., Gonzalez-Martinez, C. 2008. Recent advances in edible coatings for fresh and minimally processed fruits. *Critical Reviews in Food Science and Nutrition* 48: 496-511.

Vicentino, S.L., Floriano, P.A., Dragunski, D.C., Caetano, J. 2011. Filmes de amidos de mandioca modificados para recobrimento e conservação de uvas. *Química Nova*, 34: 1309-1314.

Weber, F.H., Collares-Queiroz, F.P., Chang, K.Y. 2009. Caracterização físico-química, reológica, morfológica e térmica dos amidos de milho normal, ceroso e com alto teor de amilose. *Ciência e Tecnologia de Alimentos* 29: 748-753.

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