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Mangifera indica postharvest: evaluation of the potential of alternative treatments to control anthracnose

Pós-colheita de *Mangifera indica*: avaliação do potencial dos tratamentos alternativos no controlar da antracnose

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ABSTRACT

The *Mangifera indica* L. is a fruit with a pleasant aroma, taste and color, with a high source of carotenoids, minerals, carbohydrates, fiber, antioxidants and vitamins A, B, C, E and K, being among the most economically expressive tropical fruits in the international market. However, several factors interfere with commercialization, mainly the damage caused by pathogens, such as *Colletotrichum gloeosporioides*, which is the pathogen responsible for losses in the postharvest phase. Among the alternatives to conventional control, resistance induction (biotic and abiotic elicitors) and fungistatic action have been promising for the production of high quality fruits, free from contamination by pesticides. The objective of this work was to evaluate the potential of *M. charantia* and Chitosan® extract in the natural incidence of *C. gloeosporioides*, severity and postharvest quality of *M. indica*. The fruits were obtained in CEASA-PB, Brazil, according to the mango maturity scale, in stage 3, in which the fruits were harvested for sale. *M. charantia* leaves were collected in the city of Areia-PB, Brazil, prepared at the Phytopathology Laboratory, on Campus II of UFPB, and sent to the Natural Products Chemistry Laboratory, Campus I, UFPB, for the production of ethanolic extract. The treatments were the extract of *M. charantia* in the concentrations: 1000, 1500 and 2000 µg.mL^{-1}, Chitosan®: 1.0%, 1.5% and 2.0%, acibenzolar-S-methyl (ASM) (0.1 gL^{-1}), fungicide (Tiabendazole) (4.0 mL.L^{-1}) and sterile distilled water (control), with four replicates and three fruits. The enzymatic analyzes (peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase) were carried out on the day of the fruit harvest and eight days after the application of the treatments. Physical and physical-chemical analyzes such as loss of mass, firmness, total soluble solids (SST) content, pH determination, total titratable acidity and SST/ATT ratio in all Chitosan® concentrations provided the lowest natural incidence of pathogens, reduced the severity of anthracnose and Influenced the high enzymatic activities of Peroxidase, Polyphenoloxidase and Phenylalanine ammonia-lyase, preserving the postharvest quality in *Mangifera indica* (Tommy Atkins) fruits during the entire storage period.

Keywords: Tommy Atkins, *Colletotrichum gloesporioides*, Fruit quality.

RESUMO

A *Mangifera indica* L. é uma fruta com aroma, sabor e cor agradáveis, com alta fonte de carotenóides, minerais, carboidratos, fibras, antioxidantes e vitaminas A, B, C, E e K, sendo uma das frutas tropicais mais expressivas economicamente no mercado internacional. Entretanto, vários fatores interferem na comercialização, principalmente os danos causados por patógenos, como o *Colletotrichum gloeosporioides*, que é o patógeno responsavel pelas perdas na fase pós-colheita. Entre as alternativas ao controle convencional, a indução de resistência (elicitores bióticos e abióticos) e a ação fungistática têm sido promissoras para a produção de frutos de alta qualidade, livres de contaminação por pesticidas. O objetivo deste trabalho foi avaliar o potencial de extrato de *M. charantia* e Chitosan® na incidência natural de *C. gloeosporioides*, severidade e qualidade pós-colheita de *M. indica*. Os frutos foram obtidos na CEASA- PB, Brasil, de acordo com a escala de maturidade das mangas, no estágio 3, no qual os frutos foram colhidos para comercialização. As folhas de *M. charantia* foram coletadas na cidade de Areia-PB, Brasil, preparadas no Laboratório de Fitopatologia, no Campus II da UFPB, e enviadas ao Laboratório de Química de Produtos Naturais, Campus I, UFPB, para produção do extrato etanólico. Os tratamentos foram o extrato de *M. charantia* nas concentrações: 1000, 1500 e 2000 µg.mL^{-1}, Chitosan®: 1,0%, 1,5% e 2,0%,

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acibenzolar-S-metil (ASM) (0,1 gL $^{-1}$), fungicida (Tiabendazol) (4,0 mL.L^{-1}) e água destilada estéril (controle), com quatro repetições e três frutos. As análises enzimáticas (peroxidase, polifenoloxidase e fenilalanina amônia-liase) foram realizadas no dia da colheita dos frutos e oito dias após a aplicação dos tratamentos. As análises físicas e físico-químicas, como perda de massa, firmeza, teor de sólidos solúveis totais (SST), determinação de pH, acidez total titulável e razão SST/ATT em todas as concentrações da Chitosan® forneceram a menor incidência natural de patógenos, reduziram a severidade da antracnose e influenciaram nas altas atividades enzimáticas da Peroxidase, Polifenoloxidase e Fenilalanina amônia-liase, preservando a qualidade pós-colheita em frutos de *Mangifera indica* (Tommy Atkins) durante todo o de armazenamento.

Palavras-chave: Tommy Atkins, *Colletotrichum gloesporioides*, Qualidade dos frutos.

1 INTRODUCTION

The *Mangifera indica* L. is a fruit that has a pleasant aroma, taste and color, exotic appearance, and with a rich source of carotenoids, minerals (calcium, zinc, potassium, iron and magnesium), carbohydrates, fiber, antioxidants and vitamins A, B, C, E and K. For this reason, it is part of the list of tropical fruits of great national and international economic importance (SANTOS *et al.*, 2018).

Brazil is the fourth largest exporter of fruit, behind India, Mexico and the Netherlands (FAO, 2018). In 2018, approximately 1.163,000 tons were produced. However, about 30 to 40% of this production has no commercial value, resulting in significant losses for both domestic and export (IBGE, 2013).

These losses are related to several factors, among them, the damage caused by pathogens, such as anthracnose, affected by *Colletotrichum gloeosporioides* Penz, which is one of the most prominent diseases in the culture of *M. indica*, infecting all organs of the plant, in fruits, typical symptoms such as large rounded necrotic patches with depression on the surface of the rind progressing to the pulp, playing a key role in postharvest, thus reducing the quality of the fruit and consequently making commercialization unfeasible (OLDONI *et al.*, 2018).

The excessive use of fungicide to combat the damage caused by pathogens, has caused the accumulation of chemicals in fruits and burdening production costs. Therefore, the use of pesticides by alternative products has been reduced and or replaced, for example the use of *Momordica charantia* L. plant extract are useful sources of fungitoxic (OLIVEIRA *et al.*, 2020).

And fungistatic substances such as momordicin, alkaloid, flavonoid, saponins, glycosides, phenolic constituents, phenylalanine, arginine, lignan-calceolarioside, triterpenes-momordicinase alkaloid zeatin, present in their chemical structure, widely used in disease control, due to the

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production of bioactive substances, and may have an inhibitory effect on the action of several pathogens (MARTINS-RAMOS *et al.,* 2010).

Chitosan® is another alternative method widely used in postharvest because it is a nontoxic and biodegradable polymer found naturally in the crustacean exoskeleton (DASH *et al*., 2011) and has been widely spread by colorless, edible and gelatinous biofilm, protects the outer surface of fruits, responsible for the thickening of the cell wall of host tissues, forming papillae that occupy intracellular space with amorphous substances that can induce defense mechanisms (COSTA *et al*., 2012).

Plant protection through resistance induction occurs through the activation of genes that encode several plant defense responses, activating the mechanisms through biotic or abiotic external agents (inducers) (SCHWAN-ESTRADA; STANGARLIN, 2005), which may increase the production of proteins related to pathogenesis or activity of enzymes such as peroxidase, β-1,3-glucanase, phenylalanine ammonia and polyphenoloxidase, capable of controlling or inhibiting the development of pathogens, as well as reducing environmental impacts and damage to humans (SILVA *et al*., 2012).

To complement the studies of phytopathology, postharvest fruits is also a research of great importance, because it aims to evaluate the balance of metabolic transformations (physical, physicochemical, chemical and biochemical) that occur in the life cycle, checking the integrity of the final product and preferably with high quality, providing healthy, colorful, aromatic, tasty fruits with high nutritional value and, consequently, increasing the storage time, without, however, changing their physical, organoleptic and nutritional characteristics, contributing to meet growing national and international demand with high quality fruits (SOUSA et al., 2015).

The objective of this work was to evaluate the potential of *M. charantia* and Chitosan® extract on the natural incidence of *C. gloeosporioides*, anthracnose severity and postharvest quality in *M. indica* (Tommy Atkins) fruits.

2 MATERIALS AND METHODS

The fruits were obtained at the State Anonymous Supply Center (CEASA-PB) in Campina Grande-Paraíba, Brazil, Latitude: 07º 13 '50 "S and Longitude: 35º 52 '52 "W, with intermediate maturation stage, pulp scale scale 3 (ASSIS, 2010). Then, they were taken to the Laboratory of Phytopathology, Centro de Ciências Agrárias, Universidade federal da Paraíba, CCA/UFPB, Areia-Paraíba, Brazil, where they were washed with water, soap and 1.0% sodium hypochlorite and separated for the three experiments to be performed.

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The plant extract was obtained from leaves of *M. charantia*, collected from native plants of Areia-Paraíba, Brazil, Latitude: 06º 57 '48 "S and Longitude: 35º 41' 30" W. After collection, they were placed in Kraft paper bags and transported to the Phytopathology Laboratory, CCA/UFPB, Areia-Paraíba, Brazil, where they were placed for drying in an oven at a constant temperature of 40 ºC until continuous weight (≈72h) (CARRERA *et al*., 2014) and diluted in the following concentrations: 1000, 1500 and 2000 $\mu g.mL^{-1}$.

Subsequently, the leaves were ground in a knife mill and stored in polyethylene bags at room temperature (25 ± 5 ºC). Then, they were taken to the Laboratory of Natural Products Chemistry - LQPN, Universidade Federal da Paraíba, Campus I, João Pessoa, Paraíba, Brazil, where they were placed in 5 L containers and containing absolute ethanol, being stirred for 72 hours.

After extraction, it was transferred to the rotary evaporator for solvent removal at a temperature of 40 °C, and then the crude ethanol extract was transported to open glass containers for complete solvent removal.

The medium molecular weight Chitosan® solution (Qmpm; 190-310 KDa) purchased from Sigma-Aldrich Chemicals, São Paulo, Brazil was prepared according to the methodology of (CIA *et al*., 2010), solubilizing the polysaccharide in 1% acetic acid at room temperature (25 ± 5 °C), followed by constant stirring for a period of two hours to allow complete dissolution of the product. Then, dilutions were made at concentrations: 1.0%, 1.5% and 2.0% with 75-85% deacetylation.

For the naturally occurring experiment, the mango fruits, after washing and disinfestation, were treated with the respective treatments and incubated in a humid chamber made of polyethylene bags, containing moist hydrophilic cotton wads moistened with SDW (Sterile Distilled Water) for 24 hours at room temperature (25 ± 5 °C) for five days.

After this period, the natural incidence of the pathogen was performed in mango fruits with symptoms of the disease, where they were washed with soap and water, disinfected with 50% alcohol, 1.0% sodium hypochlorite for one minute each, and two washes in SDW.

Subsequently, they were placed in filter paper for drying at room temperature (25 ± 5 °C), and then the 5 mm diameter fragments were removed from the injured areas and incubated in Petri dishes containing PDA (200 g potato, 20 g dextrose, 20 g L^{-1} agar) in an equidistant position. After isolation the plates were incubated in B.O.D. (Biochemical Oxygen Demand) at (25 ± 2 °C), 12h photoperiod for seven days.

Next, we observed the pigmentation, texture, consistency and shape of the reverse and reverse colonies developed *in vitro*. For optical microscope analysis, smears were prepared on microscopy slides, which were stained with methylene blue. Identification was performed by

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observing structures such as mycelium and spores, and confirmed with the aid of optical microscopy and specialized literature (KIMATE *et al.*, 1997).

To evaluate the severity, the fruits were disinfected, emerged for five minutes in solutions containing the treatments, incubated in a humid chamber, as previously described. After 24 hours, the fruits were drilled with the aid of a flanged punch at one point (fruit stalk lesion) with a depth of 2 mm and inoculated with discs measuring 5 mm diameter of *C. gloesporioides* colony.

The fruits were kept at room temperature (25 ± 5 ºC) and the lesion sizes were measured daily with the aid of a digital caliper, in direction stalk, until most fruits were completely taken by the lesions approximately seven days of storage.

Enzyme evaluations were performed at the beginning and end of the experiment (seventh day), where three fruits were removed from the repetition of each treatment.

Peroxidase activity was determined by the reaction consisting of the addition of 750 µL of reaction buffer (sodium phosphate buffer (100 mM)) (pH 6.0), 250 µL of guaiacol (1.7%), 250 µL (H_2O_2) and 250 µL composed of: 150 µL of water + 100 µL of the extract, where guaiacol peroxidase converted guaiacol to tetraguaiacol. The reaction was stopped by the addition of 800 µL perchloric acid (2.0 M) and monitored for a period of two min and every 15 seconds readings were taken to verify the activity of the 470 nm wavelength peroxidase enzyme, and expressed in $UA.min^{-1}.mg^{-1}$ protein (RONCATO; PASCHOLATI, 1998).

Polyphenoloxidase was performed by converting catechol to quinone with addition of 250 µL catechol (60 mM), 750 µL 100 mM sodium phosphate buffer (pH 6.8) and 250 µL extract. The samples were heated in a water bath at 40 ºC for 15 min, followed by cooling. Soon after, the reaction was stopped by the addition of 800 µL perchloric acid (2.0 M), with the absorbance measured at 395 nm. Results were expressed in $UA.min^{-1}.mg^{-1}$ protein (DUANGMAL; APENTEN, 1999).

Phenylalanine Ammonia-lyase activity was determined by quantifying the trans-cinnamic acid released from phenylalanine (UMESHA, 2006). To this was added 250 µL extract, 1500 µL Tris-HCl buffer (100 mM) (pH 8.8), 500 µL phenylalanine (100 mM) and 750 µL SDW, incubated at 40 °C for 60 min. The reaction was stopped by the addition of 100 µL hydrochloric acid (5.0 M). The reading was performed in a quartz cuvette on the spectrophotometer (Lightwave II, WPA, Biochron) by means of a change in absorbance at wavelength 290 nm and expressed in $UA.min^{-1}.mg^{-1}$ protein.

Physical and physicochemical analyzes, such as weight loss, were made by weighing the fruits of *M. indica* (Tommy Atkins) in a semi-analytical balance, with capacity for 15 kg and sensitivity for 5 g, based on the initial weight, being performed daily until the last assessment day.

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Firmness was measured by penetration resistance using a penetrometer (Fruit Hardness Tester 5000 g model FR-5105) in equatorial region (three determinations per fruit) of the peeled fruit surface and the data transformed to Newtons (AOAC, 2005).

The hydrogenic potential (pH) was extracted from a 5 mL sample of *M. indica* (Tommy Atkins) juice, homogenized in 50 mL of SDW. Where the reading was made in a bench parameter with automatic correction of the values as a function of temperature (IAL, 2008). Total soluble solids (TSS) was performed with juice extracted from longitudinally cut *M. indica* (Tommy Atkins) fruit slices and determined using a digital refractometer with automatic temperature correction, the results being expressed in °Brix (IAL, 2008).

Titratable acidity was determined by titration with 25 mL of juice in 0.3125 N sodium hydroxide solution, using phenophthalein as an indicator and the result was expressed as a percentage of citric acid.100 g^{-1} of juice *M. indica* (Tommy Atkins) (IAL, 2008) and the SST/AT ratio was obtained by dividing the mean values of soluble solids content by the mean titratable acidity. The fruits were packed in polypropylene trays at room temperature (25 ± 2 ºC). And the analyzes were done at the beginning and end of the experiment.

The treatments used were: *M. charantia* extract at concentrations 1000, 1500 and 2000 $\mu g.mL^{-1}$, 1.0%, 1.5% and 2.0% Chitosan®, Acibenzolar-S-methyl (Bion®) (0.1 gL^{-1}), Thiabendazole fungicide (Tecto® SC) (4.0 mL. L^{-1}), SDW (control)

The design for all experiments was completely randomized with 9 treatments, 4 repetitions, 3 fruits per repetition, using the Scott-Knott Test ($p \leq 0,05$) and the data were analyzed using the R® Statistical Program (R DEVELOPMENT CORE TEAM, 2011).

3 RESULTS AND DISCUSSION

The natural incidence of pathogens in fruits of *M. indica* (Tommy Atkins) was evaluated, a statistical difference was observed, as the treatments: 1500 $\mu g.mL^{-1}$, 2000 $\mu g.mL^{-1}$ *M. charantia*, 1.0%, 1.5%, 2.0% Chitosan® and Acibenzolar-S-methyl presented the lowest incidence of all evaluated pathogens: *Aspergillus* spp., *Colletotrichum gloesporioides*, *Pestalotia* sp. and *Penicillium* sp., when compared with the treatments: 1000 $\mu g.mL^{-1}$ *M. charantia* and control during seven days storage the period.

Results similar to the present study were verified by Oliveira *et al.* (2017), when using Chitosan® at concentrations of 5.0 to 7.5 gL^{-1}, observed effective inhibition of mycelial growth of *Colletotrichum fructicola* (Prihastuti, L. Cai & KD Hyde) in fruits of *M. indica* (Tommy Atkins) during fifteen days storage the period.

Demartelaere *et al*. (2015), evaluating plant extracts in the control of anthracnose and quality conservation in papaya (*Carica papaya* L.) fruits, found that *M. charantia* 10, 100, 500 and 1000 µg.mL^{-1} extract influenced lower incidence of *C. gloesporiodes* in fruits.

Lemos *et al.* (2013), testing alternative products for postharvest anthracnose control in *M. indica* (Ubá) found that fruits treated with Chitosan® had the lowest percentage of *C. gloesporioides* incidence.

Celoto *et al*. (2011), studying the antifungal activity of *M. charantia* extract of *Colletotrichum musae* (Berk. & Curtis) Arx. In of in *Musa* spp., observed linear effect of the concentrations on the inhibition of mycelial growth of *C. musae*, that is, with the increased concentration of the extracts, greater inhibition of mycelial growth was observed.

The severity of anthracnose was evaluated in the region near the fruit stalk of *M. indica* (Tommy Atkins), and it was possible to verify statistical difference, since only Chitosan® in the three concentrations and and Acibenzolar-S-methyl presented the smallest development in lesion sizes in other treatments evaluated during the period seven days storage (Table 1).

Table 1. Evaluation of the severity of anthracnose near the stalk in fruits of *M. indica* (Tommy Atkins) treated with *M. charantia* extract, Chitosan®, Acibenzolar-S-methyl (ASM), Fungicide and Control, during seven days of storage.

Treatments	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
M. charantia 1000 µg.mL^{-1}	6.00	d	7.29	d	9.83	C	21.64	b	38.22	b	54.54	a	81.60	a
M. charantia 1500 µg.mL^{-1}	6.00	d	9.33	d	13.40	C	37.89	b	66.08	a	91.88	a	109.21	a
M. charantia 2000 µg.mL^{-1}	6.20	d	8.55	d	13.03	C	32.06	b	56.20	a	78.06	a	113.71	a
Chitosan® 1.0%	6.00	d	6.00	d	6.28	d	6.82	d	8.88	d	11.89	c	16.19	c
Chitosan® 1.5%	6.00	d	6.00	d	6.12	d	6.72	d	9.44	d	14.05	c	14.95	c
Chitosan® 2.0%	6.00	d	6.00	d	6.00	d	6.00	d	6.47	d	11.31	c	16.03	c
ASM	6.00	d	10.69	c	17.64	c	37.77	b	53.86	a	17.38	c	14.12	c
Fungicide	6.00	d	6.58	d	8.59	d	17.65	c	29.95	b	43.35	a	56.12	a
Control	7.34	d	10.45	c	15.87	c	31.23	b	48.02	a	55.39	a	77.86	a
CV (%)	16.00		39.68		61.01		59.98		55.37		46.95		48.40	

Averages followed by the same letter in the column do not differ by the Scott-Knott Test (p≤0,05).

Oliveira *et al.* (2017), using Chitosan® at concentrations of 5.0 to 7.5 g.L^{-1}, found a reduction in the severity of *C. fructicola* anthracnose in *M. indica* (Tommy Atkins) fruits during the fifteen days storage period.

Lima (2013), evaluating the effect of Chitosan®, observed that the higher the concentration, the lower the development of anthracnose lesions caused by *C. gloeosporioides* in fruits of *M. indica* (Tommy Atkins). Negreiros *et al.* (2013), evaluating alternative products to conventional pesticides in postharvest anthracnose control of *Musa* spp. (Silver), observed reduction in lesion sizes and maintained fruit quality.

From a phytosanitary point of view, alternative products may have three main activities: antimicrobials (direct activity against phytopathogens, inhibiting mycelial growth, spore germination or multiplication of phytopathogens), resistance inducers (contains bioactive molecules capable of inducing or activating plant defense mechanisms) and biostimulants (STANGARLIN *et al*., 2011).

This fact can be explained because, as an example, *M. charantia* plants present bioactive substances such as alkaloids, flavonoids, saponins, glycosides, phenolic constituents and free acids that have activity on the metabolism of a living organism (pathogens), causing disorganization in cell membrane structures, inducing depolarization, physical and chemical changes and compromising fungal metabolic activities (ABDENOUR *et al*., 2012).

According to Stangarlin *et al*. (2011), the large accumulation of toxic substances (fungitoxic effect) may have occurred after infection, promoting morphological changes, structural changes, fungal molecular disorganization and interfering in growth, development and reproduction through the interaction between polysaccharide positive charges with negatively charged residues of macromolecules exposed on the cell surface of the pathogen, which may result in alteration of membrane permeability, with release of cell contents into the medium.

As for the indirect mechanism, according to El Hadrami *et al.* (2010), when using Chitosan® in fruits, observed the activation of defense responses, such as tissue lignification, inducing callose synthesis, phytoalexins production, increase in hydrogen peroxide (H_2O_2) production, chitinase and the synthesis of proteinase inhibitors. Functioning as a barrier to fungus penetration, increasing the resistance of the cell wall and consequently reducing the inoculum potential (GALO *et al.,* 2014).

Analyzing the enzymatic activity of peroxidase in fruits of *M. indica* (Tommy Atkins) no statistical difference was observed in all treatments, finding averages ranging from 1.8845 to 6.0485 UA.min^{-1}.mg^{-1} protein. For the activities of Polyphenoloxidase and Phenylalanine Ammonia-lyase, statistical differences were observed, as the treatment using Chitosan® at concentrations: 1.0, 1.5 and 2.0% had the highest averages: 1.3858, 1.5248, 1.1576 and 0.1846, 0.2344, 0.4397. UA.min^{-1}.mg^{-1}, respectively, when compared to the other treatments (Table 2).

Table 2. Enzyme activity of Peroxidase, Polyphenoloxidase and Phenylalanine ammonia-lyase (UA.min^{-1}.mg^{-1} protein) in *M. indica* (Tommy Atkins) fruits on collection day (day 0) and fruits treated with *M. charantia*, Chitosan®, Acibenzolar-S-methyl (ASM), Fungicide and Control, during seven days of storage.

Treatments	Peroxidase	Polyphenoloxidase	Phenylalanine Ammonia-lyase
Collect (0 Day)	0.8759	0.5964	0.1674
M. charantia 1000 µg.mL^{-1}	6.0485 a	0.8502 b	0.2297 b
M. charantia 1500 µg.mL^{-1}	4.7981 a	0.3336 b	0.1545 b
M. charantia 2000 µg.mL^{-1}	4.6230 a	1.3858 b	0.1057 b
Chitosan® 1.0%	4.2703 a	1.5248 a	0.1846 a
Chitosan® 1.5%	4.5001 a	1.1576 a	0.2344 a
Chitosan® 2.0%	5.9976 a	1.4734 a	0.4397 a
ASM	5.2335 a	0.6958 b	0.1341 b
Fungicide	4.3575 a	0.2728 b	0.1201 b
Control	1.8845 a	0.4756 b	0.1520 b
CV (%)	49.66	102.12	60.33

Averages followed by the same letter in the column do not differ by the Scott-Knott Test ($p \leq 0,05$).

Demartelaere *et al.* (2018), using alternative methods to control the brown spot of alternaria, affected by *Alternaria alternata* f. sp. citri in tangerine 'Dancy (*Citrus tangerina* hort. ex Tanaka) found that Chitosan® at all concentrations did not show statistical differences for enzymatic activity of peroxidase.

Felipini; Di Piero (2009), studying the application of Chitosan® in the postharvest control of *Malus domestica* L. against *Colletotrichum acutatum* (Simmonds) and the peroxidase activity, observed that the application of Chitosan® independent of pathogen inoculation did not change the activity of this enzyme.

It is noteworthy that there were no changes in enzyme activity in relation to the concentrations studied, however, there was high activity of peroxidase in the evaluations performed from the beginning to the end of the experiment. This fact can be explained by Pedroza (2013), when they stated that the activity of peroxidase may be associated with lignin biosynthesis, which act on neighboring infected cells and wounds which direct the defense system in the wound area.

Buso *et al.* (2014), evaluating the effectiveness of Chitosan® on the quality of *Chaerophyllum bulbosum* L., verified that the application of the product promoted elicitor effect of biochemical defense responses, promoting a significant increase in the activity of polyphenoloxidase.

The treatments using Chitosan® in this research, obtained high activity of Polyphenoloxidase, evidencing the induction of resistance, because this enzyme, participates in the lignification process during invasion by pathogens, besides producing toxic compounds and

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phenols, which are responsible for the increase on plant resistance against pathogens. In these same treatments, it was also observed the high production of phenylalanine ammonia-lyase activity, which is a key enzyme, considered one of the main in the defense mechanism, making it a good indicator of the activation of fruit resistance (STANGARLIN *et al.,* 2011).

It is a precursor enzyme of pigments such as anthocyanins that help protect against abiotic and biotic stresses by the action of phytopathogens, promoting increased activity and consequently increasing the synthesis of substances such as phenolic compounds and signaling molecules including phytoalexins and lignins (4-coumaric acid, caffeic acid, ferulic acid, gallic acid and synapic acid) that may be toxic to pathogens (MARIANGEL *et al.,* 2013).

For the assessment of mass loss, a statistical difference was found, showing that the treatment of Chitosan® at different concentrations obtained the lowest averages compared to other treatments (Table 3).

Table 3. Percentage of accumulated mass loss of *M. indica* (Tommy Atkins) fruits treated with *M. charantia* extract, Chitosan®, Acibenzolar-S-methyl (ASM), Fungicide and Control, for five days of storage.

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5
M. charantia 1000 µg.mL⁻¹	0.46	1.45	2.32	3.05	3.73 b
M. charantia 1500 µg.mL⁻¹	0.51	1.64	2.35	3.80	4.50 b
M. charantia 2000 µg.mL⁻¹	0.68	1.45	2.16	2.90	3.64 b
Chitosan® 1.0%	0.63	1.25	1.89	2.59	3.13 a
Chitosan® 1.5%	0.53	1.29	2.03	2.73	3.25 a
Chitosan® 2.0%	0.55	1.36	2.00	2.61	3.09 a
ASM	0.86	1.61	2.39	3.25	3.86 b
Fungicide	0.45	1.52	2.31	2.92	3.50 b
Control	0.66	1.36	2.12	2.96	3.62 b
CV (%)					28.98

Averages followed by the same letter in the column do not differ by the Scott-Knott Test ($p \leq 0,05$).

Similar results were obtained by Souza *et al.* (2011), when evaluating Chitosan® on the influence of postharvest on *M. indica*, observed that fruits treated with accumulated mass loss of 3.28%. Untreated fruits lost 3.8% of their total mass over nine days.

Postharvest fruit loss is mainly due to water loss, so fruits with low weight loss tend to maintain visual quality, texture and nutritional value. According to Chitarra; Chitarra (2005), some products can be traded with up to 10% losses, but the level where there is already a decrease in product quality can be verified in the order of 3 to 6% of mass loss.

As with mass loss, all Chitosan® concentrations maintained firmness levels in the same proportion as the fruits evaluated at the time of collection, still green (Table 4), differing from other treatments. Through firmness it is possible to evaluate the stage of fruit maturation, the higher the firmness level, the greener the fruit will be, and the lower the firmness levels, the greater the loss of cell wall integrity, thus making it difficult to handle the fruit if it has reduced values (PONZO, 2014).

Table 4. Evaluation of firmness (N), pH, total soluble solids (°Brix), total titratable acidity (% citric acid), TSS/TTA of *M. indica* (Tommy Atkins) fruits on the day of collection (day 0) and fruits treated with *M. charantia*, Chitosan®, Acibenzolar-S-methyl (ASM), Fungicide and Control.

Treatments	Firmness	pH	TSS	TTA	TSS/TTA
Collect (day 0)	139.17	3.90	8.96	0.75	12.59
M. charantia 1000 µg.mL^{-1}	58.27 b	4.88 b	15.40 b	0.23 b	86.99 c
M. charantia 1500 µg.mL^{-1}	59.57 b	4.91 b	14.54 b	0.29 b	70.51 b
M. charantia 2000 µg.mL^{-1}	69.50 b	4.56 b	13.68 b	0.41 b	50.24 b
Chitosan® 1.0%	83.16 a	4.08 a	11.74 a	0.83 a	16.47 a
Chitosan® 1.5%	106.22 a	4.06 a	10.40 a	0.67 a	16.68 a
Chitosan® 2.0%	107.66 a	4.16 a	10.06 a	0.60 a	17.15 a
ASM	60.53 b	4.78 b	14.65 b	0.36 b	56.19 b
Fungicide	49.01 b	5.08 b	14.39 b	0.20 b	94.19 c
Control	65.50 b	5.01 b	14.15 b	0.32 b	86.10 c
CV (%)	36.86	9.38	12.29	39.57	61.24

Averages followed by the same letter in the column do not differ by the Scott-Knott Test (p≤0,05).

The pH of Chitosan® treated fruits in all concentrations presented statistical differences in relation to the other treatments (Table 4). Since they presented the lowest pH levels, showing one more satisfactory characteristic in postharvest conservation, because, the lower the pH, the lower will be the advance in fruit maturation, since it is directly related to the increase of acidity in the fruit, influencing the delay of fruit maturation, thus, pH is a necessary parameter for conservation (VENCESLAU, 2013).

The highest results of total soluble solids (TSS) content (°Brix) were observed only at Chitosan® concentrations. However, it was found that polysaccharide interfered with fruit metabolism, keeping sugar levels low (Table 4). Silva *et al*. (2009), evaluating the physical and chemical characteristics of fifteen varieties of *M. indica* in Minas Gerais-Brazil, observed that only the Tommy Atkins variety without the use of resistance inducers presented 14.7 °Brix.

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It can be stated that when fruits maintain low soluble solids content in postharvest storage, they tend to reduce the advance of ripening due to the delay in polysaccharide degradation or biosynthesis (BARANKEVICZ *et al.*, 2015).

The titratable acidity in fruits treated with Chitosan® presented the highest results in all concentrations when compared to the other treatments (Table 4). Souza *et al.* (2011), evaluating the postharvest of *M. indica* treated with Chitosan®, observed that the coated fruits had a small variation in the acidity levels. Evidencing that, during the storage period, the fruits ripened gradually, due to the reduction of acids, which were decelerated.

In relation to SST/ATT the lowest values were obtained when Chitosan® was used in relation to the other treatments. Since, the metabolism deceleration has been proven, which may have influenced the delay of fruit ripening, presenting high quality and maintaining the postharvest characteristics throughout the storage (CHITARRA; CHITARRA, 2005).

Treatments using Chitosan® in fruits of *M. indica* favored the best results, both for evaluations of anthracnose severity affected by *C. gloesporioides* and postharvest, proving that polysaccharide is a colorless, edible biofilm of gelatinous consistency that protects the outer surface of fruits by reducing the incidence of pathogens through resistance induction stimulated by the production of chitinase, β-1,3-glucanases, phenylalanine ammonia lyase and peroxidase (STANGARLIN *et al.,* 2011).

This fact justifies its potential, as it is a natural product, renewable and does not present any toxicity to man, besides replacing the pesticides used in the postharvest, preserves the physicochemical characteristics and prolongs the fruit preservation period. This polysaccharide stands out as an alternative to the management of postharvest diseases. And also for its potential in various uses, such as food, biotechnology, pharmaceuticals and agriculture (FREDDO *et al.*, 2014).

4 CONCLUSIONS

Chitosan® at all concentrations provided the lowest natural incidence of pathogens, reduced the severity of anthracnose caused by the pathogen *Colletotrichum gloeosporioides*. It influenced the highest enzymatic activities of Peroxidase, Polyphenoloxidase and Phenylalanine ammonia-lyase and preserved postharvest quality in *Mangifera indica* (Tommy Atkins) fruits throughout the storage period.

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