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EFFECTS OF CHITOSAN IN THE CONTROL OF POSTHARVEST ANTHRACNOSE OF SOURSOP (*Annona muricata*) FRUIT
EFECTOS DEL QUITOSANO EN EL CONTROL POSTCOSECHA DE LA ANTRACNOSIS EN FRUTOS DE GUANÁBANA (*Annona muricata*)

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Abstract
The disease reducing activity of eco-friendly chitosan (molecular weight: 250 kDa, % deacetylation: 75-85, Sigma-Aldrich, MO, USA) compound against postharvest anthracnose caused by *Colletotrichum* gloeosporioides was investigated in *in vitro* and *in vivo* conditions. In this study, *C. gloeosporioides* was isolated in pure culture from soursop (*Annona muricata*) tropical fruit, identified by morphological and molecular methods and then used in experimental tools. In the *in vitro* tests, the inhibition of *C. gloeosporioides* mycelial growth, sporulation and conidial germination by chitosan at different concentrations, 0, 0.1, 0.5, 1.0 and 1.5% was evaluated on potato dextrose agar. The application of 1.0 and 1.5% chitosan decreased mycelial growth and sporulation by 90%, and completely inhibited spore germination. The 1.0% of chitosan solution shown the best performance *in vitro*. In the *in vivo* tests, a coating of 1.0% chitosan was applied on artificially inoculated soursop fruits during evaluations carried out after 3, 6, and 9 days. Chitosan completely inhibited anthracnose and reduced weight loss of fruit. Quality parameters of fruit (pH, total soluble solids, firmness and titratable acidity) were not affected by chitosan application. Therefore, coatings with 1.0% chitosan can be recommended as on alternative treatment to the application of synthetic fungicides for keeping quality and controlling anthracnose of soursop during storage and shelf life.
Keywords: anthracnose, antimicrobial activity, *Colletotrichum* gloeosporioides, resistance inducers, tropical fruit.

Resumen
La reducción de enfermedad por actividad de quitosano (medio peso molecular: 250 kDa, % deacetilación: 75-85, Sigma-Aldrich, MO, USA) contra antracnosis en postcosecha causada por *Colletotrichum* gloeosporioides se investigó mediante pruebas realizadas *in vitro* e *in vivo*. En este estudio, se aisló *C. gloeosporioides* en cultivo puro de fruta tropical de guanábana (*Annona muricata*), se identificó por métodos morfológicos y moleculares. La inhibición del crecimiento micelial de *C. gloeosporioides*, esporulación y germinación por efecto del quitosano a diferentes concentraciones, 0, 0.1, 0.5, 1.0 y 1.5% se evaluó en agar papa dextrosa. La aplicación 1.0 y 1.5% quitosano disminuyó el crecimiento micelial y esporulación un 90%, e inhibió completamente la germinación de esporas. La solución 1.0% quitosano mostró el mejor rendimiento en pruebas *in vitro*. Para las pruebas *in vivo* se aplicó un recubrimiento de 1.0% quitosano en frutos de guanábana inoculados artificialmente con el patógeno, se realizaron evaluaciones los 0, 3, 6, y 9 días. El quitosano inhibió completamente la antracnosis y disminuyó la pérdida de peso de la fruta. Los parámetros de calidad en fruta (pH, sólidos solubles totales, firmeza y acidez titulable) no fueron afectados por aplicación de quitosano. Por lo tanto, recubrimientos con 1.0% quitosano podrían recomendarse como tratamiento alternativo a la aplicación de fungicidas sintéticos para mantener la calidad y controlar la antracnosis en guanábana durante el almacenamiento postcosecha.
Palabras clave: antracnosis, actividad antimicrobial, *Colletotrichum* gloeosporioides, inductor resistencia, fruta tropical.

1 Introduction

Soursop (*Annona muricata* L.) is a tropical fruit with great economic potential. In addition to being an important source for the food industry, *A. muricata* is known as indigenous traditional medicinal plant, and provide a wide spectrum of phytochemical and biological activities (Moghadamtousi *et al*., 2015). Nayarit is the soursop largest production area in Mexico (SIAP/SAGARPA, 2016). However, there is a high rate of postharvest losses caused mainly by fungal pathogens (Andrades *et al*., 2009). *Colletotrichum* spp. causal agent of anthracnose is the most important postharvest pathogen of soursop. Due to the climatic

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conditions, the infection can take place in the orchards, affecting the postharvest fruit quality. The most widely method used for the control of postharvest diseases is the application of synthetic fungicides. However, awareness of the negative effects on human health and the environment, have been promoting the development of non-polluting strategies to control phytopathogens (Walters *et al*., 2013; Burketova *et al*., 2015). Actually, new strategies such as the use of antagonistic microorganisms, application of salt solutions as well as essential oils have been used for postharvest disease control (Youssef and Roberto, 2014; Sotelo-Boyás *et al*., 2015; González-Estrada *et al*., 2017a, b; Servili *et al*., 2017). The effectiveness of eco-friendly postharvest treatment to control anthracnose disease were shown in important tropical fruit plants including avocado (Sivakumar and Bautista-Baños, 2014) mango (Berumen-Varela *et al*., 2015), banana (Khleekorn *et al*., 2015), but little is known about the effectiveness of alternative fungicides in soursop postharvest disease control. Researchers have been explored the application of substances of biological origin, such as chitosan, a natural linear polysaccharide of glucosamine and N-acetylglucosamine units joined by β-1,4-glycosidic links derived from the deacetylation of chitin. Chitosan is a polysaccharide of high molecular weight, biodegradable and non-toxic. It is obtained mainly from the exoskeleton of crustaceans such as crabs and shrimp (El-Ghaouth *et al*., 2000). It has become a useful compound due to its fungicidal effect and its induction of plant defense mechanisms for controlling postharvest diseases of fruit and vegetables (Hernández-Ochoa *et al*., 2011; Salazar-Leyva *et al*., 2014; Landi *et al*., 2017). Several studies have evaluated the potential of chitosan at different concentration in controlling postharvest fungal pathogens (Berumen-Varela *et al*., 2015; Romanazzi *et al*., 2017). The objectives of this research were (i) to identify by molecular techniques causal agent of soursop anthracnose; (ii) to assess the inhibitory activity of chitosan on *C. gloeosporioides*; (iii) to evaluate the effectiveness of postharvest chitosan application to control anthracnose, and (iv) to evaluate the effects of chitosan on preserving postharvest fruit quality.

2 Materials and methods

2.1 Isolation, purification and identification of postharvest pathogens

Soursop fruits were harvested at physiological maturity from orchards in the municipality of Compostela, Nayarit, Mexico (21°04'20.9"N 105°13'20.1"O). Fruits were placed in chambers with high relative humidity (90- 95%) at 25 °C to stimulate disease development. After 8 days, cuts (1 × 1 cm) 50% affected surface and 50% healthy surface were made in the fruits with severe disease symptoms. Tissue sections were superficially disinfected for 1 min with 2% sodium hypochlorite rinsed with sterile distilled water and placed in the middle of PDA Petri dishes (85 mm Ø) then incubated at same temperature. The fungus isolated from damaged tissues was purified and monosporic isolation was performed. Then the Koch's postulates was evaluated in order to confirm the fungus pathogenicity. Briefly, healthy fruits were artificially infected with 40 μl of spore suspension (10^6 conidia ml^{-1}) using a BD ultra fine syringe (Becton, Dickinson and Company, Franklin, Lakes, NJ USA), the control fruits were inoculated with sterile distilled water. Subsequently, the fungal reisolation was performed with the same procedure used to isolate the pathogen.

The identification at genus level of the pathogen was based on a microscopic analysis of conidia and mycelium, using a Motic BA300 optical microscope according to dichotomous keys (Suárez-Quiroz *et al*., 2013). The species identification was performed using PCR molecular tests according to amplification of Internal Transcribed Spacer (ITS) of the ribosomal DNA (rDNA) regions, widely used for systematic pathogen identification (Álvarez *et al*., 2001). DNA was extracted from the mycelium according to Álvarez *et al*., procedure (2011), with some modifications. Briefly, the universal primers ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' were used to amplify the region found between the genes coding for 18S and 28S rRNA (White *et al*.,1990). The PCR reaction, was carried out in a total volume of 25 μL contained Invitrogen® (Life Technologies, Carlsbad, USA) reagents. In detail: total DNA from the samples, 50 ng/ μL, 2 μL, 10× Buffer, 2.5 μL, dNTPs 10 mM, 0.5 μL, $MgCl_2$ 50 mM, 1.2 μL, primers ITS1 and ITS4, 10 μM, 1.5 μL respectively, Taq enzyme

polymerase, 0.2 U/μL, 0.2 μL, and H_2O 15.6 μL. The PCR was performed with the follows conditions: initial denaturation step for 5 min at 95 °C, followed by 30 cycles of 1 min denaturation at 95 °C, 30s annealing at 45°C and 1 min elongation at 72 °C. In addition, the final elongation at 72 °C for 10 min was performed. To detect the fungal specie, the PCR amplicons were sequenced by Macrogen Humanizing Genomics (Seoul, Korea) and the identification based on BLASTn was performed using NCBI (National Center for Biotechnology Information) database.

2.2 *Chitosan preparation*

A stock solution was performed with 1.5 g of chitosan (molecular weight: 250 kDa, % deacetylation: 75-85) (Sigma-Aldrich, MO, USA). From the stock solution, 0.1, 0.5, 1.0 and 1.5%, concentration were prepared and adjusted to pH 5.6 with 1N of NaOH (El Ghaouth *et al*., 1991). Chitosan solutions and PDA were sterilized separately, then mixed after PDA cooling at 45°C and following dispersed in Petri dishes (85 mm Ø) (El Ghaouth *et al*., 1991).

2.3 *In vitro tests: determination of mycelial growth, sporulation and conidial germination of C. gloeosporioides*

In order to evaluate the effects of chitosan on mycelial growth, plugs (5 mm) were cut from 8 days-old PDA cultures of *C. gloeosporioides* and then were replaced in Petri dishes with PDA medium containing different chitosan concentrations (0, 0.1, 0.5, 1.0 and 1.5%). Plates were incubated at 25±1 °C for 12 days, and colony diameter was daily detected. The results were reported as the percentage of mycelial growth inhibition as compared to the control.

To determine fungal sporulation, 10 ml of sterile distilled water were added to mycelial growth on Petri dishes. With a sterile glass rod fungal lawn was rubbed. Suspension was filtered through sterile cheesecloth to retain the mycelium. Finally, the spore concentration was determined by microscopic counting using hemocytometer. A total of 100 observations per treatment using a Motic BA300 optical microscope (Motic Instruments Inc., Canada) were performed. Values were expressed as number of spore's ml^{-1}.

The effectiveness of chitosan on conidial germination was evaluated as follows: 50 μL of the spore suspension were placed on PDA dishes (20 mm Ø) with the different chitosan concentrations (0, 0.1, 0.5, 1.0, and 1.5%). The samples were observed by microscope after 8 hours to quantify the germinated spores. Spores were considered germinated when the length of the germinative tube was at least twice the spore diameter. The germination process was stopped by adding one drop of lactophenol-safranin.

2.4 *In vivo test: severity infection and fruits quality parameters determination*

For postharvest treatment, the 1.0% chitosan solution, which showed the best performance in the *in vitro* assays, was selected. The soursop fruits were harvested at physiological maturity from orchards previous described. Before the treatments the fruits were selected on the basis of size uniformity, absence of defects and mechanical injuries. The fruits were washed with water, disinfected with a 2% of sodium hypochlorite for 1 min and then left to dry at room temperature in a biosafety hood. Three treatment were performed: *C. gloeosporioides* + chitosan (chi-patho), chitosan (chi) and *C. gloeosporioides* (pathogen). The soursop fruit involved in the chi-patho and pathogen treatments were before inoculated with 40 μL of *C. gloeosporioides* conidial suspension (10^6 spores/mL) at the depth of 3 mm and maintained for 30 min at room temperature in a biosafety hood. Following, the fruits involved in the chi-patho and chi treatments were immersed for 60 s in 1.0% chitosan solution. Distilled water was inoculated in the control fruits. After treatments the fruits were air dried for 4 h and stored at 25 °C, with high relative humidity (90-95%), for 10 days.

The severity of the infection was calculated using the equation proposed by Vero and Mondino (1999). Several quality parameters such as change of weight, firmness, Total Soluble Solids (TSS), and titratable acidity, have been evaluated in the treated fruits. The changes on weight loss, of each fruit were recorded initially and during storage using a digital balance (Sartorius BL 3100). Weight loss was expressed as percentage loss of the initial weight. Firmness was analyzed using a universal Digital Force Gauge (Shimpo FGE-50, Japan), employing the penetration test at three points along the fruit with the husk (ends and middle). The results were expressed in Newton (N). Total Soluble Solids (TSS) was expressed in °Brix and evaluated with an Abbé refractometer (Hanna instruments, Rhode Island, USA). The pH was evaluated with a pH 300 potentiometer (Hanna Instruments, Maharashtra, India).

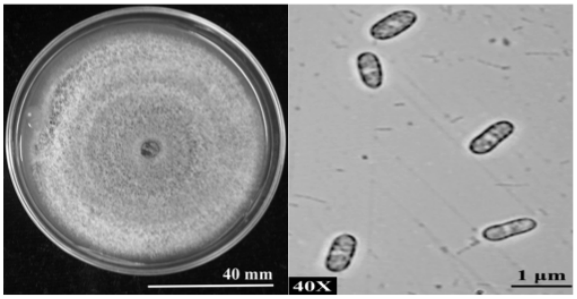

Fig. 1. Macroscopic and microscopic characteristics of *C. gloeosporioides*.

The titratable acidity was evaluated with the titration method that consist of homogenized 5 g sample and titrant with 0.1 N NaOH. The calculations were reported as percentage of malic acid on the AOAC, (1990). All *in vivo* tests were recorded at 0, 3, 6 and 9 days from treatments.

2.5 *Statistical analysis*

in vitro tests were achieved in four Petri dishes per treatment, with three replicates. *in vivo* tests consisted of 25 fruits per treatment with four replicates and the physicochemical evaluations were made on five fruits per treatment with four replicates. Each experiment was repeated at least twice, using a completely randomized block design. Data were subjected to analysis of variance (ANOVA) and a Tukey test ($P \leq 0.05$) was used for the comparison of means.

3 Results

3.1 *Isolation, purification and identification of postharvest pathogens*

The causal agent of anthracnose in soursop fruit was isolated and identified. The characteristics white mycelium and cylindrical conidia from 11 to 16 μm long from 2.7 to 5.4 μm wide, were recorded (Fig. 1). Based on the taxonomic keys used, it was possible to identify the pathogen as belonging to the genus *Colletotrichum* sp. (Barnet and Hunter, 1998). The molecular analysis of the intergenic (ITS) region of *Colletotrichum* DNA shown a PCR fragment of 563 bp. The conserved region of the *Colletotrichum* sequence was long 240 bp, showing that the comparative analysis of this region is 100% aligned with isolate *C. gloeosporioides* LSC-120, with Genbank accession number KU097233.1 (Kashyap *et al*., 2015).

3.2 *In vitro antimicrobial activities of the chitosan versus C. gloeosporioides*

The reduction of colony growth diameter of *C. gloeosporioides* was relative to increases of chitosan concentration. The best results were recorded using the 1.0 and 1.5% chitosan concentrations, with significant difference respect to the control (Fig. 2). Using the 1.0 and 1.5% chitosan concentration, the *C. gloeosporioides* mycelial growth was inhibited up to 95%. In the sporulation assessment, with the application of chitosan at 1.0%, the spore concentration (1.38×10^6 conidia/mL) decreased significantly compared to the control (3.39×10^6 conidia/mL) (Table 1). However, no significant difference was detected between 1.0 and 1.5% chitosan concentrations. Using chitosan at 1.0 and 1.5%, a total inhibition of *C. gloeosporioides* germination was observed (Table 1). While, using chitosan at 0.1 and 0.5%, the reduction in conidial germination of 56% and 80% was observed respectively.

3.3 *In vivo test: severity infection and fruits quality parameters determination*

Fruits inoculated with *C. gloeosporioides* presented 100% disease incidence. The results showed that anthracnose was inhibited by 85%, compared to control fruit (Fig. 3). In control fruits, at 6 day from treatment the 13% of weight loss was observed with respect to 0 day, whereas fruit treated with 1.0% of chitosan showed only 12% weight loss until the 9 day from treatment. After 9 days of storage, fruits treated with chitosan were in a maximum degree of maturation, then they were not possible to manipulate. A significant difference on weight loss between fruit treated with chitosan and control was observed (Fig. 4). At day 0 the firmness data ranged from 120 to 150 N for all treatments (Table 2). From the third day, the behaviour of each of the treatments was similar since all tended to decrease gradually throughout the storage period (data not shown). However, at the end of the storage time, chitosan-treated fruits had significantly higher values (25.6 N) compared with control and soursop just challenged with *C. gloeosporioides*, that showed lower values (7 and 6.4 N, respectively).

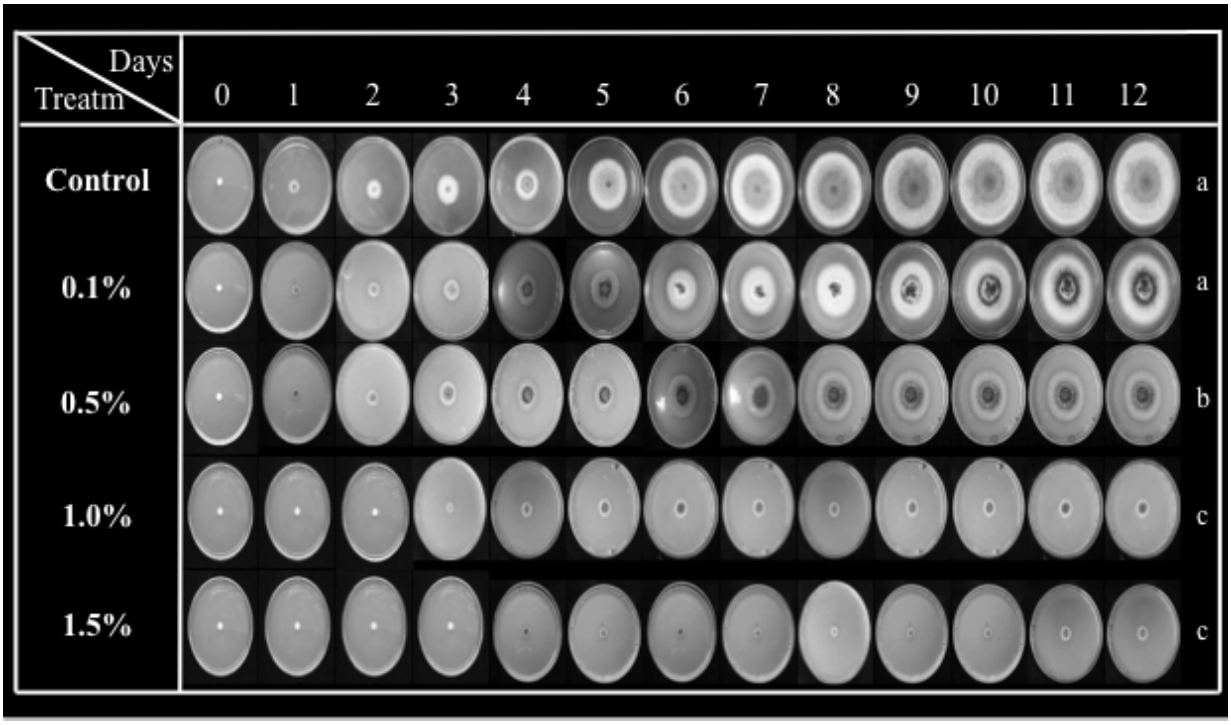

Fig. 2. Mycelial growth kinetics of *C. gloeosporioides* at different concentrations of chitosan for 12 days.

Table 1. Effect of chitosan at different concentrations on germination and sporulation of *C. gloeosporioides*.

Chitosan	Inhibition Germination (%)z Day 12	Sporulation (spores/mL)z At 8 hours
0.10%	56 ± 0.57 b	$3.02 \times 10^6 \pm 0.13$ a
0.50%	80 ± 0.57 c	$2.23 \times 10^6 \pm 0.12$ b
1.00%	100 d	$1.38 \times 10^6 \pm 0.02$ c
1.50%	100 d	$1.22 \times 10^6 \pm 0.09$ c
Control	0 a	$3.39 \times 10^6 \pm 0.50$ a

[z]Data are means ± standard deviation. Values followed by different letters in the same column are significantly different (Tukey's honestly significant difference; $P \leq 0.05$).

TSS, pH and titratable acidity in soursop fruits treated with chitosan showed no significant differences as compared to the control (data not shown). In both, control and fruits treated with chitosan, a gradual increase of TSS was observed with advance of ripening in soursop fruits. Moreover, titratable acidity decreased and pH increased as the storage progressed (data not shown).

4 Discussion

Chitosan is a biopolymer known to be effective in the control of postharvest decay of fruit, with multiple mechanisms of action including antimicrobial activity, film-forming properties and induction of host defences (Bautista-Baños *et al*., 2006; Romanazzi *et al*., 2017). The present study showed the potential use of chitosan for controlling both fungal growth and spore germination of *C. gloeosporioides*.

Table 2. Evaluation of firmness (N) during storage of soursop fruits with different treatments.

Treatments	Days[z]			
	0	3	6	9
Control	124.30 ± 4.41 bc	45.30 ± 2.57 bc	25.30 ± 2.36 b	7.02 ± 1.45 bc
Pathogen	130.40 ± 3.94 b	64.60 ± 2.63 b	24.00 ± 2.25 b	6.40 ± 1.26 c
Chi	149.50 ± 2.89 a	105.50 ± 1.93 a	75.77 ± 1.35 a	25.40 ± 0.89 a
Chi-Patho	124.00 ± 3.00 bc	114.50 ± 2.73 a	64.90 ± 1.65 a	25.60 ± 1.18 a

[z]Data are means ± standard deviation. Values followed by different letters in the same column are significantly different (Tukey's honestly significant difference; $P \leq 0.05$).

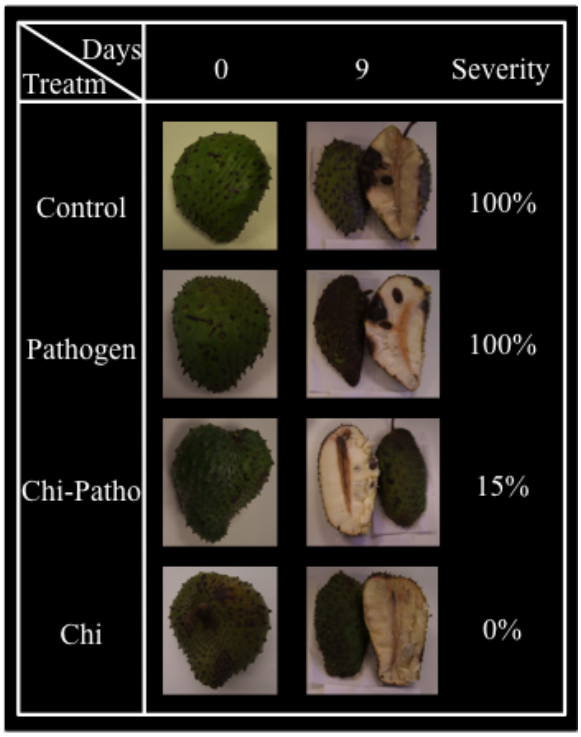

Fig. 3. Antrachnose severity in soursop fruits, treated or not with chitosan, and inoculated or not with *C. gloeosporioides*, stored 20 ºC at 9 day. Control = Water; Pathogen = *C. gloeosporioides*; Chi-Patho = Chitosan plus *C. gloeosporioides*; Chi = Chitosan 1.0%.

Our *in vitro* results confirm that the chitosan concentration is a key factor influencing the different development stages of *C. gloeosporioides*. At 1.0% concentration, total inhibition of spore germination was observed and 1.5% chitosan concentration has no further significant effect on fungal sporulation or mycelium growth. Then the 1.0% was the minimal inhibitory concentrations of chitosan compound against *in vitro C. gloeosporioides* germination and sporulation.

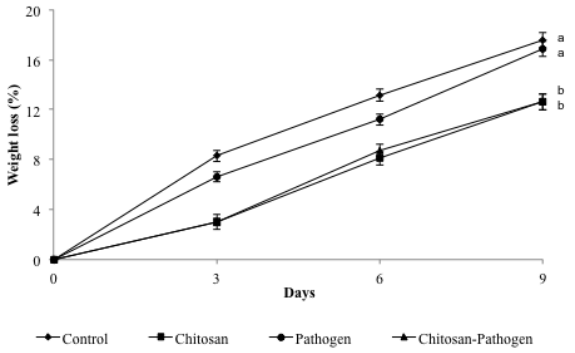

Fig. 4. Weight loss of soursop fruits with different treatments stored at 20°C.

Germination is a natural development process of the pathogen that is significantly affected by chitosan (Zakrzewska *et al*., 2005). The chitosan effects on fungal growth can be ascribed to its cationic character, where the free amino groups, positively charged in acid medium, interact with the negative residues of the macromolecules exposed on fungal cell wall, changing the permeability of the plasma membrane, leading to an alteration of its main functions, such as waste output and nutrient input (Bautista-Baños *et al*., 2006; Chávez-Magdaleno *et al*., 2018). Previous works related to plant pathogen sensitivity to chitosan treatments shown that the effectiveness depends by both, the chitosan concentration and the pathogen sensitivity. At this regard the inhibitory effect of chitosan on mycelial growth, sporulation and germination were detected related to postharvest pathogens affecting papaya, like *C. gloeosporioides*, *Fusarium* spp. and *Penicillium digitatum*, using chitosan at 1.5% (Bautista-Baños *et al*., 2004). Another work reported *Alternaria alternate* mycelial growth inhibition applying chitosan at 1.0% (López-Mora *et al*., 2013).

In this investigation, the *in vivo*-effectiveness of chitosan was confirmed in the postharvest treatments

of soursop fruits. Our results show a decrease of anthracnose disease, until 85%, in fruits treated with chitosan (chi) and *C. gloeosporioides* + chitosan (chi-patho) compared to control. Previous works reported a total postharvest protection by anthracnose in mango fruits treated with chitosan at 1.0 and 1.5% (Berumen-Varela *et al*., 2015) in agreement with the results of this research. However, in addition to antifungal activity, the reduction of disease can be due to the ability of the chitosan to induce resistance in the soursop fruits. It is now acknowledged the ability of chitosan to triggering plant defenses, in pre and postharvest production of specific crops (Zhang *et al*., 2015; Romanazzi *et al*., 2016; Landi *et al*., 2017). The defense response induced in the soursop fruits by chitosan and *C. gloeosporioides* need to be investigated. When chitosan was applied to soursop fruits, the physicochemical parameters (TSS, pH, titratable acidity) were not altered. The maintenance of qualitative of fruits is a key aspect for acceptability by consumers. Previous works show that the effect of chitosan treatment on the storage duration is greater without altering the quality (Feliziani *et al*., 2015). In soursop fruits treated with chitosan, a lower weight loss was observed. This result was in agreement with previous studies on bananas (Hernández-Ibáñez *et al*., 2013). The decreased weight loss in fruits after chitosan treatments is due to the fact that this compound forms a film on the surface fruit, acting as a physical barrier, that avoid the moisture losses (Bautista-Baños *et al*., 2018; Jacobi *et al*., 2000; Zhu *et al*., 2008; Romanazzi *et al*., 2018). Regarding to firmness, our data shown the ability to chitosan to maintain the highest value after 9 days from treatments in both, chi and chi + patho treatments. At this regard, according to chitosan treatment, the natural metabolic processes occurring at the level of the cell wall in maturation progress develop slowly due to the presence of the chitosan film on the surface of the fruit, modifying the atmosphere, altering the ethylene production and maturation process, resulting in an increase in the shelf life of the fruit.

In our study the TSS content on the soursop fruits not was affected by chitosan. These results are in agreement with the data reported on mango fruits treated with 1.0% chitosan (López-Mora *et al*. 2013), banana fruits inoculated with *Colletotrichum* sp and treated with 1.5% of chitosan (Ochoa *et al*., 2015). The °Brix performance is associated with fruit maturity. This is due to the reaction that occurs during maturation for the synthesis of sugars, which is the hydrolysis of starch to simpler carbohydrates of the disaccharide and monosaccharide types (glucose, sucrose and fructose), mediated by the action of enzymes such as α and β amylases (Romanazzi *et al*., 2016). Ojeda *et al*., (2007) report pH levels ranging from 3.9 to 4.3 at 22 °C in soursop fruit. Hernández-Ibáñez *et al*., (2013) obtained a decrease in total acidity from 0.75 to 0.23% in chitosan-treated banana fruits as storage days progressed. The decrease in acidity is probably due to the consumption of these organic molecules, in the different metabolic cycles (e.g., respiration) to provide the energy required by the fruit. In addition, many of the organic acids participate as precursors of volatile substances, which intensify their presence during this period (Feliziani *et al*., 2013).

Conclusions

The results of our investigations showed the effectiveness of chitosan as antifungal agent in controlling soursop anthracnose by *C. gloeosporioides*. The application of chitosan as coating can be a smart choice and a suitable alternative for control of postharvest *C. gloeosporioides* infections in soursop without affecting quality parameters. Increased knowledge on the mechanisms of action of chitosan in soursop can be useful to optimize application timing, and additional benefits can be obtained exploring the chance of combined application with other alternatives to synthetic fungicides.

Acknowledgements

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Nomenclature

NaClO	sodium hypochlorite
PDA	Potato dextrose Agar
ITS	Internal Transcribed Spacer
dNTP's	Deoxyribonucleotide triphosphate
$MgCl_2$	Magnesium chloride
rDNA	Ribosomal DNA
rRNA	Ribosomal RNA
cDNA	Complementary DNA

PCR	Polymerase chain reaction
NCBI	National Center for Biotechnology Information
Chi-Patho	*C. gloeosporioides* + chitosan
Chi	Chitosan
TSS	Total Soluble Solids
Ø	Diameter

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