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## **Antifungal Activity of Chitosan against Postharvest Fungi of Tropical and Subtropical Fruits**

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### **Abstract**

In the present chapter, results about the efficacy of chitosan (Chi) on sporulation, mycelial growth, germination, as well as quality parameters on fruits are shown. The results demonstrate that chitosan can control various phytopathogen isolates from diverse fruits. The pathogens in the genera *Colletotrichum*, *Fusarium*, and *Rhizopus* are involved in important postharvest disease losses throughout the world. In Nayarit, producers had reported high postharvest losses not only at field but also during the commercial chain with their products, besides the resistance of several pathogens to fungicides, which traditionally are applied for controlling diseases. In this sense, the aim of this research group is focused on the research of alternative and effective methods for controlling postharvest diseases. In vivo results are promising due to a good control in important tropical fruits like banana, avocado, mango, and jackfruit. An enhancement in the chitosan antimicrobial activity is reported with the combination with GRAS substances, as well as the use of nanotechnology. Chitosan can be an environment-friendly alternative to the use of chemical fungicides for controlling postharvest diseases in fruits.

**Keywords:** chitosan, fruits, fungal growth, vegetables

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## 1. Introduction

Mexico is an important exporter of fruits worldwide [1]. However, important postharvest losses have been reported. In this sense, postharvest diseases represent a major factor of losses during storage and shelf life of produce, due to the deterioration of quality and microbial contamination [2]. In many countries traditionally, postharvest decay control is obtained using chemical fungicides, but nowadays consumers are concerned about food safety and environmental issues [3]. The use of antimicrobial packaging can be effective during the storage period, handling, or transport of fruits [4]. In recent years, various investigations have reported the efficacy of the application of chitosan (Chi) in the control of postharvest pathogenic microorganisms, due to the diverse properties like the ability to form films, biodegradability, antimicrobial properties, and the elicitor function [5]. Chitosan 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 [6]. In a previous study, chitosan was applied successfully on strawberry (*Fragaria x ananassa*); the coating decreased the respiratory rate, reduced water losses, as well as preserved the firmness during storage time on treated fruit [7]. Besides, previous studies reported resistance-inducing properties of chitosan in the form of defense responses (enzymes POD, PPO, and PAL) in fruits [8]. The objective of this chapter article was to summarize information about the application of chitosan with other alternative methods, including GRAS substances and the use of nanotechnology, against important fungi that affect tropical and subtropical fruits.

### 1.1. Fruits and vegetables

#### 1.1.1. Health issues

Nowadays, an interest in the health benefits of fruit and vegetable consumption is increasing. The easy consumption, the good taste, and the nutritional value of fresh fruits and vegetables are important characteristics that have allowed consumers to be more aware about the benefits of a healthy diet. The consumption of fruits and vegetables contributes to the wellness and nutritional health of consumers, due to their high content of phytochemicals as well as other components that may act synergistically with phytochemicals (ascorbic acid, carotenoids, and phenolic compounds) [9].

#### 1.1.2. Production

Worldwide, the main tropical fruit producers and export countries are the Far East, Latin America, and the Caribbean, most of which are developing countries, while a high percentage of developed countries are importers of these fruits. The main tropical fruits for exportation are mango, pineapple, papaya, and avocado, which represent approximately 75% of the exportation of fresh tropical products [10]. The postharvest losses of fruits and vegetables caused by microorganisms worldwide are of the order of 5–25% in developed countries and 20–50% in developing countries; in developed countries they have technologies that allow to diminish or avoid the attack of microorganisms.

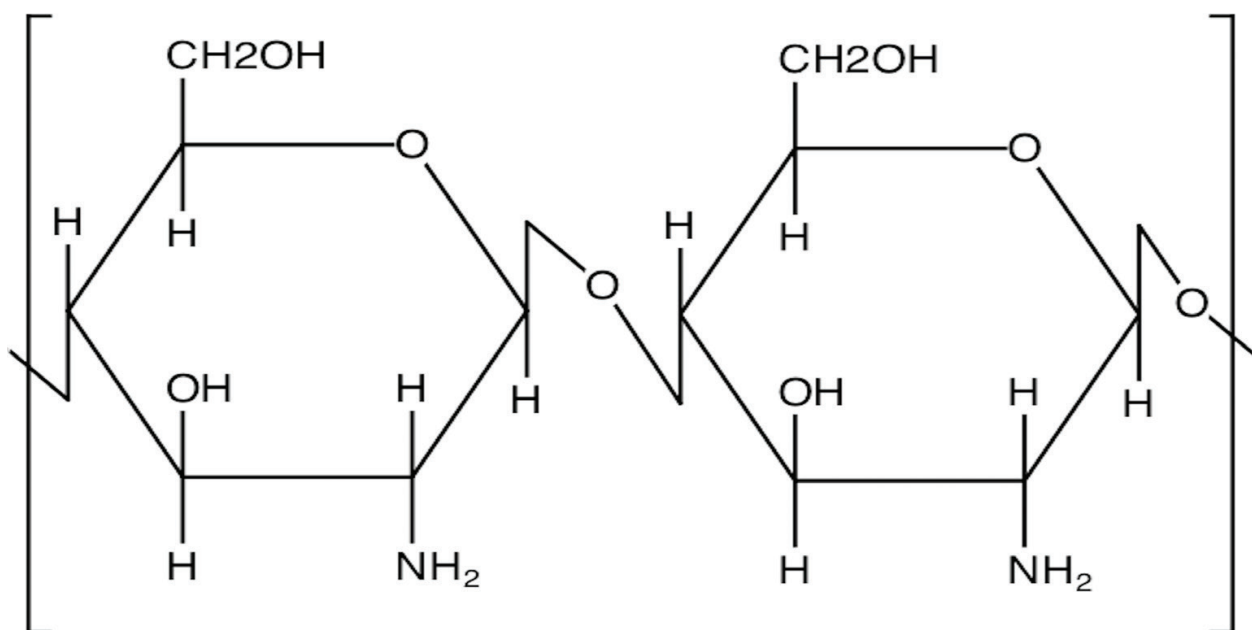
### 1.1.3. Postharvest losses

The main causes of quantitative postharvest fruit losses are classified as crop and harvest practices, availability and conditions of transport, pests and infections, climatic conditions, consumer preferences and attitudes, infrastructure, as well as financial availability of the markets [11]. Fruits can be infected at field or during the postharvest management [12]. Diseases are the principal cause of postharvest losses in tropical and subtropical fruits; anthracnose is the main postharvest disease in various tropical fruits caused by *Colletotrichum gloeosporioides* [13].

## 1.2. Chitosan: origin, structure, and antimicrobial properties

Chitosan is a polysaccharide derived from chitin, which is the second most abundant polysaccharide in the world, after cellulose [14]. Chitin is a polysaccharide of animal origin and is the main constituent of the outer skeleton of insects and crustaceans like shrimp, crabs, and lobster [15]. Chitosan is the *N*-deacetylated derivative of chitin [16]. The molecular weight of chitosan ranged between 300 and 1000 kDa depending on the source of chitin. Chitosan is a copolymer of *N*-acetyl-*D*-glucose amine and *D*-glucose amine as shown in **Figure 1**.

Important chemical properties of chitosan are as follows: linear polyamine, reactive amino groups, reactive hydroxyl groups available, and chelates metal ions, specially transition metals. Between the biological properties of the chitosan, the most important one is the biocompatibility (natural, biodegradable, safe, and nontoxic) [17]. Various mechanisms of action have been proposed; however, this process is not fully understood. It is important to mention that the antimicrobial activity of the chitosan on pathogenic microorganisms depends on different factors like the strain, molecular weight, concentration, degree of deacetylation, and type of chitosan, among others [5]. The interaction of chitosan with the microorganism



**Figure 1.** Chemical structure of chitosan.

results in different changes: (a) changes on cell permeability, due to the polycationic nature of the chitosan amino group and the electronegative charges in the outer surface of the fungal or bacteria membrane [18]; (b) affectation on homeostasis ( $K^+$ ,  $Ca^{2+}$ ), leading to the efflux of small molecules affecting fungal respiration [19]; (c) microbial starvation, when chitosan acts as chelating agent of metals and essential nutrients affecting microbial development [20]; and (d) inhibition on synthesis of mRNA and proteins, related to their ability to pass through the cell membrane of a microorganism and subsequently bind to DNA [21].

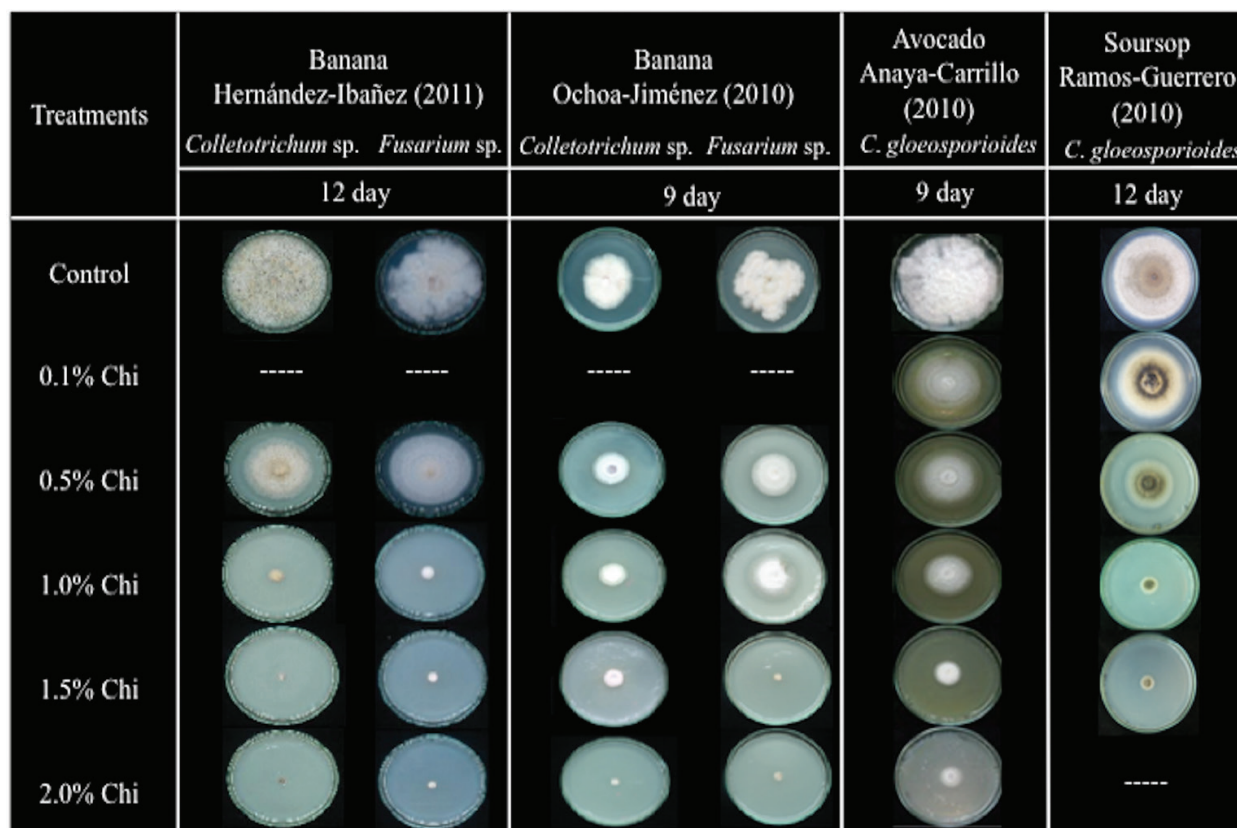
### 1.2.1. Effects of the antifungal activity of chitosan in the control of postharvest pathogens isolated from various fruits

Chitosan is considered one of the most promising products for the control of several important postharvest fungi in fruits and vegetables [22–25]. In vitro tests used 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. In our research, the molecular weight of chitosan as well as the concentration used plays an important role in antifungal efficacy against the pathogens tested. Several factors influence the antimicrobial activity of chitosan; among them are the type of chitosan and the concentration [21]. It is reported that the antimicrobial activity of chitosan also depends of the molecular weight, is better when chitosan of low molecular weight and oligochitosan instead of high molecular weight chitosan is applied. In this sense, high molecular weight chitosan cannot pass through the microbial membrane and acts against microbial development [21]. Concerning to the efficacy of chitosan at different concentrations is reported that at lower concentrations, chitosan binds cell surface of microorganisms (negatively charged), disturbing the cell membrane, and causes death of microbial cell by leakage of the intracellular components; however, at higher concentrations, chitosan may coat the microbial surface and prevent the leakage of intracellular components [21].

#### 1.2.1.1. Mycelial growth

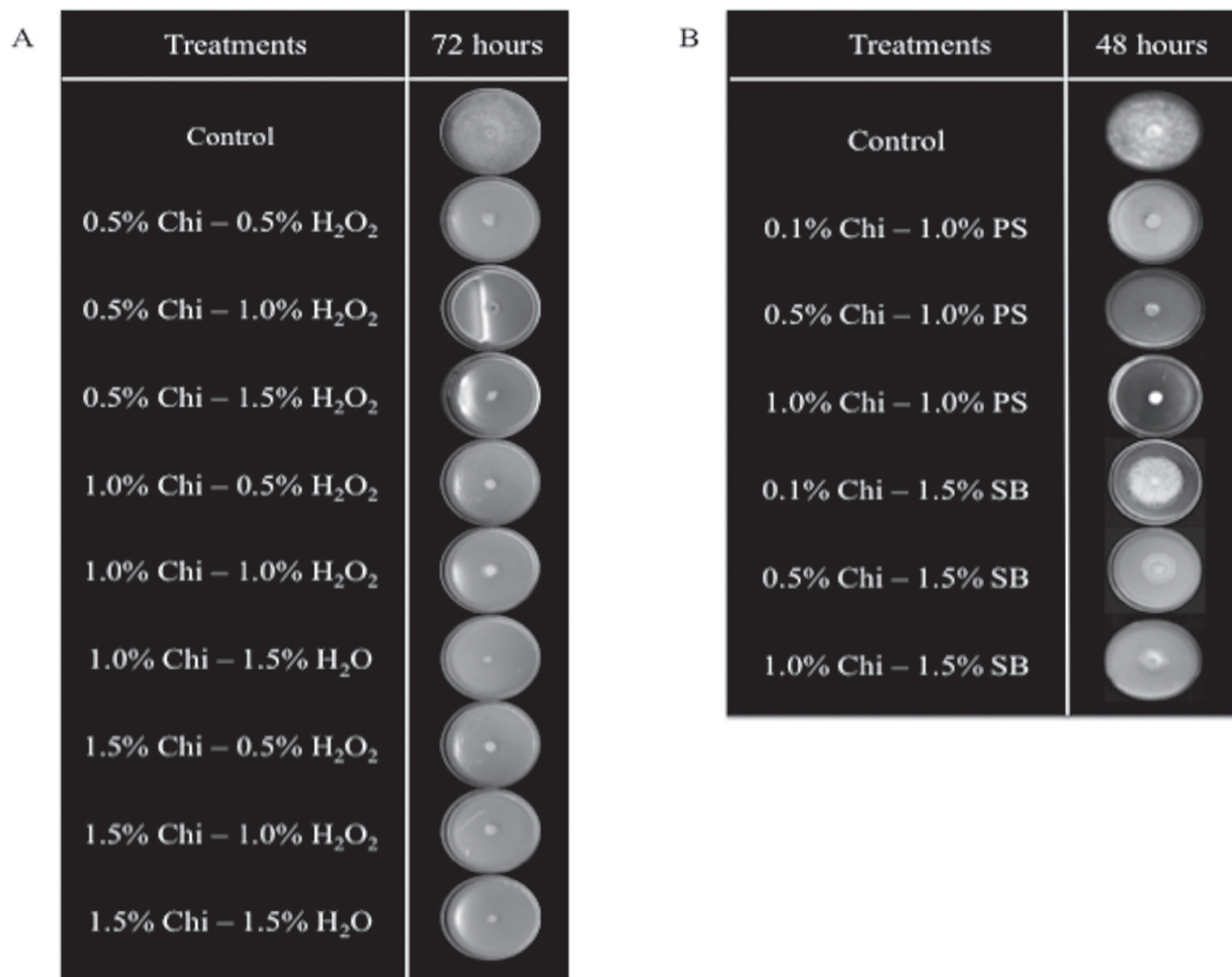
The application of chitosan (Chi) at 1.0, 1.5, and 2.0% was effective against the pathogen *Colletotrichum* sp. isolated from banana (*Musa paradisiaca*), 100% of mycelial growth inhibition was observed ( $p \leq 0.05$ ). Conversely, for the pathogen of *Fusarium* sp. isolated from banana, higher concentrations of the chitosan (1.5 and 2%) were applied to obtain a good inhibition (93.2 and 100%, respectively ( $p \leq 0.05$ )) (**Figure 2**) [26]. In a study, against *Colletotrichum* sp. isolated from banana (*Musa sapientum*), different concentrations where applied (1.0, 1.5 and 2.0%) obtaining a 78.94, 92.1, and 98.68%, respectively ( $p \leq 0.05$ ). Conversely, with the application of chitosan at 0.5%, only 49% of growth inhibition of *Colletotrichum* sp. was obtained ( $p \leq 0.05$ ) [27].

As shown on **Figure 2**, *Colletotrichum gloeosporioides* T147 isolated from avocado (*Persea americana* mill.) c.v. Hass was successfully inhibited as the concentration of chitosan increased, and the percentage of inhibition ranged from 88.85 to 92.97% ( $p \leq 0.05$ ) [28]. In a recent study, the ability of chitosan-pepper tree (*Schinus molle*) essential oil biocomposites against *Colletotrichum gloeosporioides* was evaluated (data not shown) [29]. In this study, an important



**Figure 2.** Inhibition of the mycelial growth of pathogens isolated from various fruits by applying chitosan at different concentrations. Control, sterile distilled water; chi, chitosan; ----, concentration not analyzed.

inhibitory effect on the viability of *Colletotrichum gloeosporioides* increased proportionally to the used concentration; the authors concluded that the antifungal activity of the biocomposites against the pathogen can be associated to a synergic effect between the chitosan and pepper tree essential oil. The principal mechanism of pepper tree (*Schinus molle*) essential oil acts against the cytoplasmic membrane, causing it to lose its integrity by assisting the chitosan to enter the interior of the cell. This leads to dissipation of the proton-motive forces and the inhibition of the respiratory enzymes responsible for the cell wall synthesis; this in turn inhibits spore germination and germ tube elongation [30]. Good results were obtained with the application of chitosan at 1.0 and 1.5% against *Colletotrichum gloeosporioides* LSC-120 isolated from soursop (*Annona muricata* L.); up to 90% was achieved using 1.5% of the chitosan ( $p \leq 0.05$ ), whereas this strain was not totally controlled in vitro at low concentrations tested (0.1 or 0.5%) [31]. Similar results were obtained in a study with *Alternaria alternata* isolated from mango (*Mangifera indica* L.) c.v. Tommy Atkins; chitosan treatments at 0.05, 0.1, 0.5, and 1.0% inhibited the mycelial growth of the pathogen by 11.5, 23.1, 55.0, and 70.0%, respectively ( $p \leq 0.05$ ) [32]. *Rhizopus* sp. isolated from jackfruit (*Artocarpus heterophyllus* L.) was controlled (up to 98% ( $p \leq 0.05$ )) with the application of chitosan in combination with H<sub>2</sub>O<sub>2</sub> (peroxide hydrogen) in vitro tests (**Figure 3A**). Synergistic effects were reported with the combinations of chitosan with potassium sorbate or H<sub>2</sub>O<sub>2</sub>. The pathogen only was totally inhibited with the combinations of potassium sorbate and chitosan at high concentrations of the treatments (**Figure 3B**) [33].



**Figure 3.** Effect of the interactions of chitosan, hydrogen peroxide, potassium sorbate, and/or sodium bicarbonate at different concentrations in the inhibition of mycelial growth of *Rhizopus* sp. isolated from jackfruit (*Artocarpus heterophyllus* L.). (A) Arciniega-Castro (2014) and (B) Coronado-Partida (2015). Control, sterile distilled water; chi, chitosan; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; PS, potassium sorbate; SB, sodium bicarbonate.

#### 1.2.1.2. Sporulation

In a study with fungus isolated from banana (*Musa paradisiaca*), only the application of chitosan at concentrations of 1.0% the strains of *Colletotrichum* sp. and *Fusarium* sp. showed a decrease in the final concentration of spores [26]. For the fungus of *Fusarium* sp. isolated from banana (*Musa sapientum*), chitosan treatments of 0.5 and 1.0% showed a decrease on the final concentration of the spores, and this process was totally inhibited applying concentrations of 1.5 and 2.0% of chitosan (Table 1) [27]. In the same way, *Colletotrichum gloeosporioides* T147 isolated from avocado (*Persea americana* mill) c.v. Hass and *Annona muricata* L. was successfully inhibited using concentrations of chitosan at 1.5 and 2.0% and 1.0 and 1.5%, respectively [28, 31]. Chitosan at concentrations of 0.5, 1.0, and 1.5% in combination with H<sub>2</sub>O<sub>2</sub> at 0.5, 1.0, and 1.5% affected the development of the spores; this may be due to a synergistic effect between the mechanisms of actions of both compounds that affect the sporulation conditions of the pathogens. The application of combinations of chitosan and H<sub>2</sub>O<sub>2</sub> at concentrations greater than 0.5% decreased the final concentration of the spores of *Rhizopus* sp. isolated from

Treatments	Soursop Ramos-Guerrero (2012)	Avocado Anaya-Carrillo (2013)	Banana Hernández-Ibañez (2011)	Banana Ochoa-Jiménez (2010)		
	<i>C. gloeosporioides</i>	<i>C. gloeosporioides</i>	<i>Colletotrichum sp.</i>	<i>Fusarium sp.</i>	<i>Colletotrichum sp.</i>	<i>Fusarium sp.</i>
Control	3.4 × 10 <sup>6</sup> a	4.0 × 10 <sup>7</sup> a	2.5 × 10 <sup>7</sup> a	9.9 × 10 <sup>7</sup> a	3.8 × 10 <sup>7</sup> a	2.8 × 10 <sup>7</sup> a
0.5% Chi	2.2 × 10 <sup>6</sup> b	3.6 × 10 <sup>7</sup> b	1.0 × 10 <sup>7</sup> b	7.7 × 10 <sup>7</sup> b	1.5 × 10 <sup>7</sup> b	8.7 × 10 <sup>6</sup> b
1.0% Chi	1.4 × 10 <sup>6</sup> c	2.0 × 10 <sup>7</sup> c	6.4 × 10 <sup>6</sup> c	5.0 × 10 <sup>6</sup> c	1.1 × 10 <sup>7</sup> b	2.7 × 10 <sup>6</sup> c
1.5% Chi	1.2 × 10 <sup>6</sup> c	2.2 × 10 <sup>7</sup> c	3.8 × 10 <sup>6</sup> d	2.9 × 10 <sup>6</sup> d	6.0 × 10 <sup>6</sup> d	0 d
2.0% Chi	---	2.2 × 10 <sup>7</sup> c	1.8 × 10 <sup>5</sup> e	3.3 × 10 <sup>5</sup> e	3.7 × 10 <sup>6</sup> c	0 d

The values with different letters in columns are significantly different (Tukey's honestly significant difference;  $p \leq 0.05$ ). Control = Sterile distilled water; Chi = Chitosan; --- = not determined.

**Table 1.** Effect of chitosan at different concentrations on the sporulation of different pathogens isolated from different fruits.

Treatments	Jack fruit Arciniega-Castro (2014)	Treatments	Jack fruit Coronado-Partida (2015)
	<i>Rhizopus sp.</i>		<i>Rhizopus sp.</i>
Control	4.75 × 10 <sup>6</sup> a	Control	1.4 × 10 <sup>8</sup> a
0.5% Chi—0.5% H <sub>2</sub> O <sub>2</sub>	4.3 × 10 <sup>4</sup> f	0.1% Chi—1.0% PS	0 e
0.5% Chi—1.0% H <sub>2</sub> O <sub>2</sub>	2.83 × 10 <sup>5</sup> b	0.5% Chi—1.0% PS	0 e
0.5% Chi—1.5% H <sub>2</sub> O <sub>2</sub>	0 g	1.0% Chi—1.0% PS	0 e
1.0% Chi—0.5% H <sub>2</sub> O <sub>2</sub>	8.5 × 10 <sup>4</sup> d	0.1% Chi—1.5% SB	3.6 × 10 <sup>7</sup> b
1.0% Chi—1.0% H <sub>2</sub> O <sub>2</sub>	1.0 × 10 <sup>5</sup> c	0.5% Chi—1.5% SB	2.0 × 10 <sup>7</sup> c
1.0% Chi—1.5% H <sub>2</sub> O <sub>2</sub>	0 g	1.0% Chi—1.5% SB	1.2 × 10 <sup>7</sup> d
1.5% Chi—0.5% H <sub>2</sub> O <sub>2</sub>	6.6 × 10 <sup>4</sup> e	---	---
1.5% Chi—1.0% H <sub>2</sub> O <sub>2</sub>	1.0 × 10 <sup>5</sup> c	---	---
1.5% Chi—1.5% H <sub>2</sub> O <sub>2</sub>	0 g	---	---

The values with different letters in columns are significantly different (Tukey's honestly significant difference;  $p \leq 0.05$ ). Control = Sterile distilled water; Chi = Chitosan; H<sub>2</sub>O<sub>2</sub> = Hydrogen peroxide; PS = Potassium sorbate; SB = Sodium bicarbonate.

**Table 2.** Synergistic effect between chitosan, hydrogen peroxide, potassium sorbate and/or sodium bicarbonate at different concentrations on the sporulation of *Rhizopus sp* isolated from jackfruit (*Artocarpus heterophyllus L.*).

jackfruit (*Artocarpus heterophyllus L.*) [33]. The use of chitosan in combination with H<sub>2</sub>O<sub>2</sub> was effective to reduce the spore's production of *Rhizopus sp.* isolated from jackfruit (*Artocarpus heterophyllus L.*) (Table 2) [34]. In the same sense, the synergistic effect was also evidenced with the use of organic salts (potassium sorbate and sodium bicarbonate) applied at different concentrations with chitosan, obtaining good results on sporulation as shown in Table 2 [33].



### 1.2.1.3. Spore germination

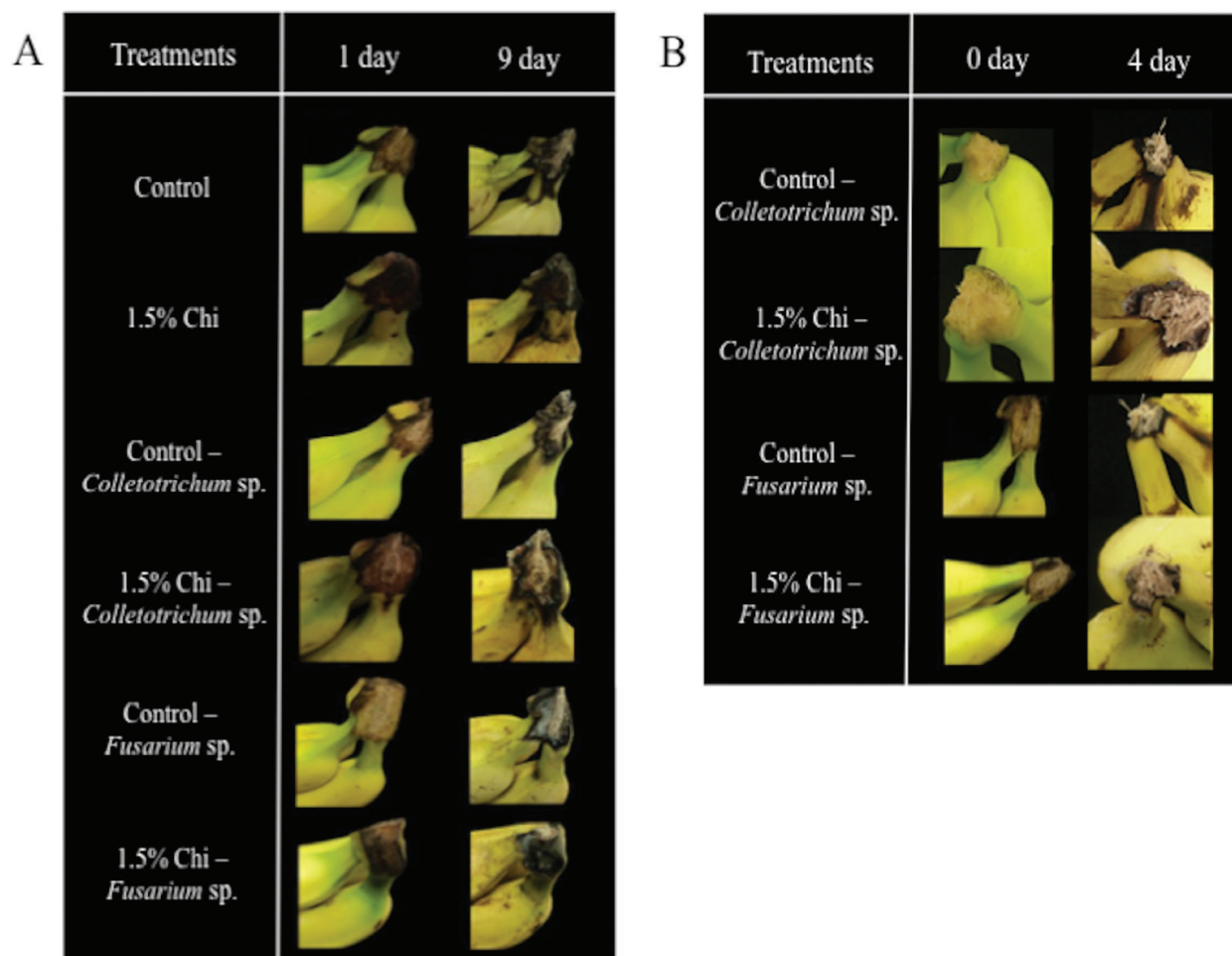
Promising results have been obtained in different investigations on *Colletotrichum* sp. (banana) and *Fusarium* sp. (banana), with the total inhibition on germination applying chitosan at different concentrations (0.5, 1.0, 1.5, and 2.0%) [26, 27]. Conversely, at concentrations of 0.5 and 1.0%, only 50% of the inhibition was obtained ( $p \leq 0.05$ ) against *Colletotrichum gloeosporioides* T147 isolated from avocado (*Persea americana* mill.) c.v. Hass [28]. Good results were reported by Chávez-Magdaleno and Luque-Alcaraz [29] with the application of biocomposites of chitosan loaded with essential oils against *Colletotrichum gloeosporioides* T147, reporting up to 95% of inhibition on spore development ( $p \leq 0.05$ ). Phytopathogens isolated from soursop (*Colletotrichum gloeosporioides* LSC-120), mango (*Alternaria alternata*), and jackfruit (*Rhizopus* sp.) were successfully controlled by the application of chitosan alone or in combination with organic salts [31–34].

### 1.2.2. Effects of the application of chitosan on postharvest disease control in fruits

The use of chitosan as a coating on fresh fruits is a real alternative on the control of postharvest diseases. An important biological function of the chitosan is like an inducer of defense mechanisms in fruit and vegetable products, causing a reduction and/or inhibition of the development of diseases [35–37]. Besides, the application of chitosan with other natural methods of biological control and nanoparticles is another promising alternative for controlling postharvest diseases [38]. Chitosan nanoparticles can improve the antimicrobial activity, which is associated with the position of the amino groups favoring the binding to the cell surface and an alteration with the normal functions of the membrane, thus inhibiting the growth of the pathogen [16]. There are several studies on the application of chitosan alone or in combination with natural methods such as resistance inducer or as a disease control agent [33, 34, 39, 40]. The analysis of disease incidence, severity, and quality parameters used a completely randomized block design. Data were subjected to analysis of variance (ANOVA), and a Tukey test ( $p \leq 0.05$ ) was used for a means of comparison.

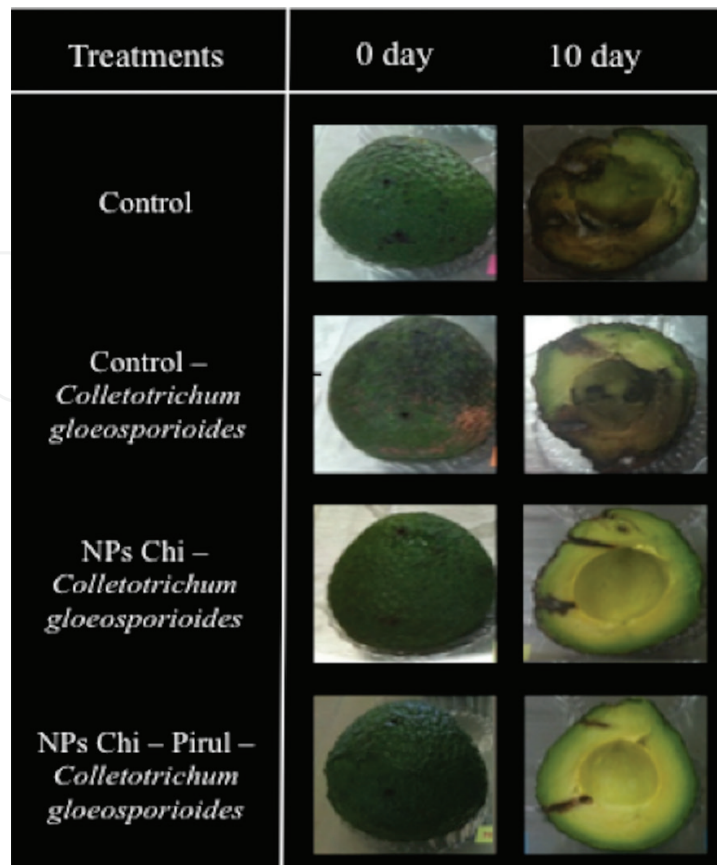
#### 1.2.2.1. Disease incidence and severity

In a study on banana fruits *Musa paradisiaca* and *Musa sapientum*, a total inhibition on infected fruits with *Colletotrichum* sp. and *Fusarium* sp. were reported with the application of chitosan at 1.5% compared to control (80% of incidence) [26, 27]. Related to severity, control fruits presented a damage around the crown on bananas; conversely, fruits treated do not present visible damage (**Figure 4A, B**). As shown in **Figure 4**, the absence was reported on avocado fruits c.v. Hass treated with chitosan (1.5%) and inoculated with *Colletotrichum gloeosporioides* T147. The application of biocomposites of chitosan-pepper tree (*Schinus molle*) essential oil in avocado fruits (*Persea americana* mill.) c.v. Hass infected with *Colletotrichum gloeosporioides* successfully controlled anthracnose disease in a preventive and curative way (**Figure 5**) [39]. In a study on soursop fruits (*Annona muricata* L.) artificially and naturally infected with *Colletotrichum gloeosporioides* LSC-120, a total control was obtained by the application of chitosan at 1.0% (**Figure 6**) [31]. The combination of chitosan with peroxide and GRAS substances in jackfruit (*Artocarpus heterophyllus* L.) was effective in inhibiting the



**Figure 4.** Crowns of banana fruits with 1.5% chitosan with and without inoculation of *Colletotrichum* sp. or *Fusarium* sp., on days 9 and 4 of storage. (A) Banana (*Musa paradisiaca*) stored at 15°C and (B) banana (*Musa sapientum*) stored at 25°C. Control, sterile distilled water; chi, chitosan.

development of soft rot disease by *Rhizopus* sp. The application of 1.0% Chi–1.0% H<sub>2</sub>O<sub>2</sub> in jackfruit inoculated with the pathogen was able to totally inhibit the development of soft rot (**Figure 7A**) [34]. In other studies, the application of chitosan with potassium sorbate (SP) or sodium bicarbonate (BS) in jackfruit (*Artocarpus heterophyllus* L.) was effective for controlling the development of *Rhizopus* sp. The combination of 1.0% Chi–1.0% SP with and without inoculation of *Rhizopus* sp. fruits does not present the presence of infection (**Figure 7B**) [33]. Conversely, with the use of BS with chitosan, only 10% of severity reduction was obtained ( $p \leq 0.05$ ). It is concluded that the combination of chitosan with SP can be an alternative to control *Rhizopus* sp. infection in jackfruit. The principal mode of action of the bicarbonate ion is through its buffering capacity, whereby an alkaline environment is sustained, and inhibition of microorganisms occurs due to the use of energy from microbial cells to produce an acidic environment [41]. The antimicrobial activity of potassium sorbate is associated to an alteration of the activity of Krebs cycle enzymes as well as the integrity of cell membranes [42]. The effective conditions for the control of diseases were for bananas *Musa paradisiaca* (15°C) and *Musa sapientum* (25°C), and avocado (*Persea americana* mill.) is 1.5% chitosan with 90–95% of relative humidity. For fruits of soursop (*Annona muricata* L.)



**Figure 5.** Avocado fruits (*Persea americana* mill.) c.V. Hass coated with chitosan nanoparticles and chitosan-pepper tree essential oil with and without inoculation of *Colletotrichum gloeosporioides* T147 for 10 days at 25°C. Control, sterile distilled water; chi, chitosan, NPs, nanoparticles.

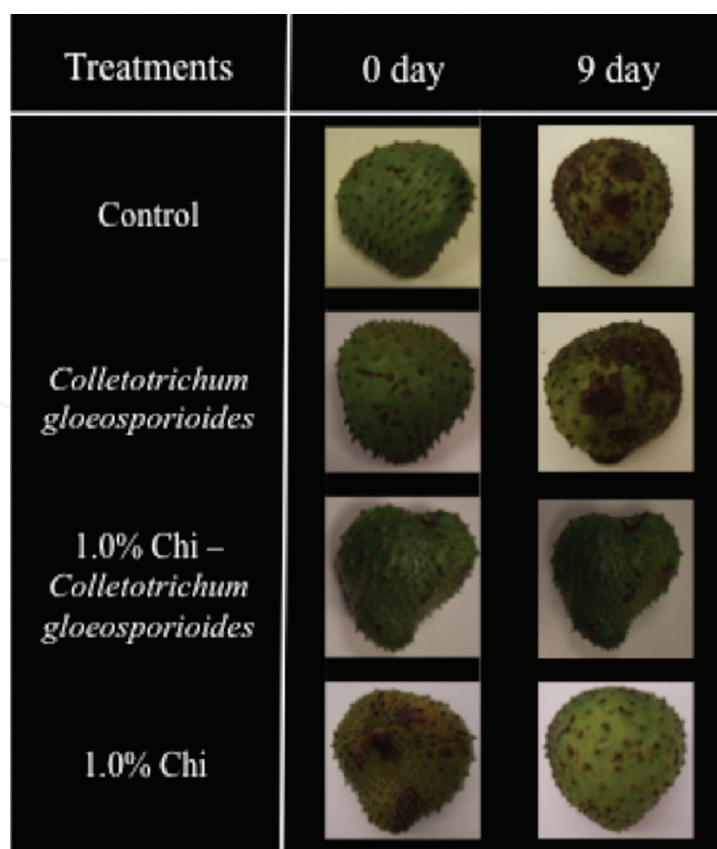
and jackfruit (*Artocarpus heterophyllus* L.), the effective concentration was 1.0% chitosan at 20°C with 90–95% of relative humidity.

The efficacy of chitosan for controlling postharvest diseases depends not only on its antimicrobial properties. Romanazzi and Sanzani [43] reported that the arrays of defense mechanisms are activated in fruits exposed to biotic or abiotic stress, including chitosan application with or without inoculation of the pathogen. Chitosan induces the synthesis of phenolic compounds (chlorogenic acid, caffeic acid) and hydrolase antifungal enzymes (chitinases and  $\beta$ -(1,3)-glucanases) that hydrolyze the main components of the cell wall of fungi causing inhibition of their growth [44]. On the other hand, chitosan coating can serve not only as a protective barrier to fungal infection but also as a barrier to gaseous exchange affecting the fungal development on fruits.

#### 1.2.2.2. Quality parameters

##### 1.2.2.2.1. Weight loss

The ability of chitosan to form coatings is well documented; this property is useful to preserve the quality of fruits and vegetables. Chitosan applied at 1.5% on banana fruits was useful to avoid water losses compared to control [26]. The same concentration of chitosan was effective on bananas (*Musa sapientum*) with lower water losses on fruits compared to control (Table 3) [27].

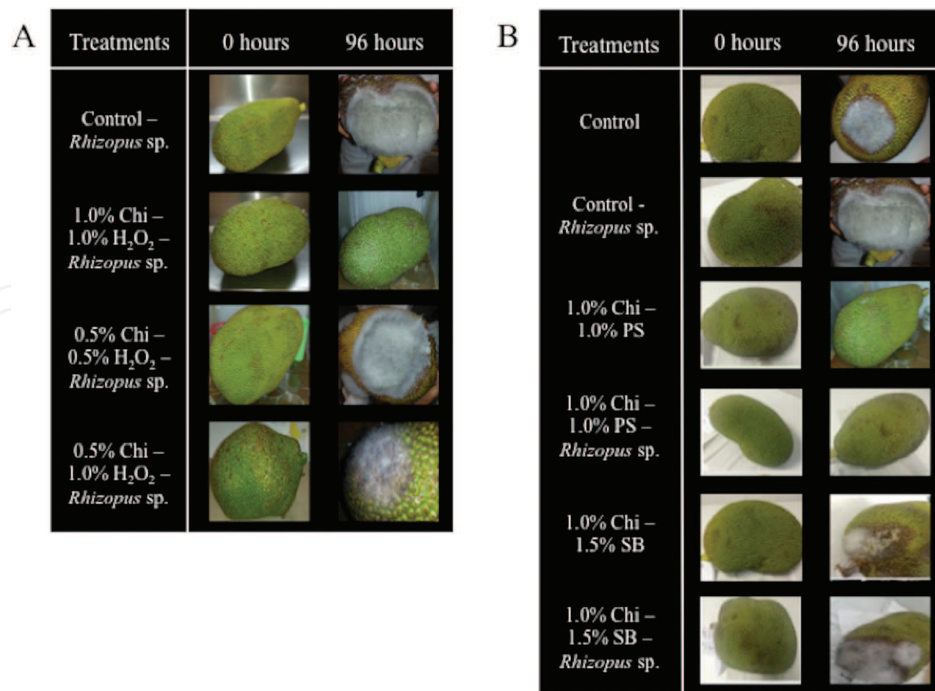


**Figure 6.** Anthracnose severity in soursop fruits treated with chitosan, inoculated or not with *C. gloeosporioides* LSC-120, and stored 20°C for 9 days. Control, sterile distilled water; chi, chitosan.

The ability to maintain the quality of fruits at different temperatures has been reported also for mango fruits (*Mangifera indica* L.) c.v. Tommy Atkins treated with chitosan at 1.0% and stored at 12°C. In the same sense, fruits treated with 1.0% chitosan and stored at 25°C showed the highest weight loss (10.5%) [32]. Novel formulations of chitosan like the use of nanoparticles have been applied with good results with the application of treatments (2.4%) compared to control (12.3%) [39]. The decreased weight loss in fruits is due to the presence of chitosan coating on the surface of the fruit, acting as a physical barrier to moisture loss and therefore delaying dehydration [45].

#### 1.2.2.2.2. Firmness, pH, and total soluble solids

The loss of firmness is a factor that affects the quality of the fruits. In fruits of soursop (*Annona muricata* L.) treated with chitosan, at the end of the evaluation, fruits had a greater firmness (25 N) compared to control fruits (6.4 N) ( $p \leq 0.05$ ). The total soluble solids (TSS) of the soursop fruits treated with 1.0% of chitosan and control showed a continuous increase (10–18% ( $p \leq 0.05$ )), and in the case of pH, in fruits treated with 1.0% of chitosan, an increase during storage was obtained, with a pH ranging from 3.49 to 4.10 ( $p \leq 0.05$ ) [31]. The coating formed in banana fruits (*Musa paradisiaca*) by chitosan (1.5%) maintains the losses of firmness compared to control (data not shown). The concentration used on fruits does not affect the TSS, pH, and titratable acidity [26]. Mango fruits treated with 1.0% of chitosan no significant changes on TSS, pH, and titratable acidity were reported. The use of chitosan nanoparticles resulted effectively to



**Figure 7.** Severity of soft rot infection in jackfruit (*Artocarpus heterophyllus* L.) treated with the combination of chitosan, potassium sorbate, and sodium bicarbonate inoculated with *Rhizopus* sp. at 25°C. (A) Arciniega-Castro (2014) and (B) Coronado-Partida (2015). Control, sterile distilled water; chi, chitosan; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; PS, potassium sorbate; SB, sodium bicarbonate.

Fruits	Temperature	Treatments	% Weight loss
Banana ( <i>Musa paradisiaca</i> )	15°C	Control	8 ± 0.23 <sup>a</sup>
		1.5% Chi	2 ± 0.12 <sup>b</sup>
Banana ( <i>Musa sapientum</i> )	25°C	Control	9.8 ± 0.34 <sup>a</sup>
		1.5% Chi	8 ± 0.47 <sup>a</sup>
Mango ( <i>Mangifera indica</i> L.) c.v. Tommy Atkins	12°C	Control	5 ± 0.25 <sup>a</sup>
		1.0% Chi	3 ± 0.39 <sup>b</sup>
	25°C	Control	15 ± 0.5 <sup>a</sup>
		1.0% Chi	10.5 ± 0.46 <sup>b</sup>
Soursop ( <i>Annona muricata</i> L.)	20°C	Control	18 ± 0.7 <sup>a</sup>
		1.0% Chi	13.3 ± 0.55 <sup>b</sup>
Avocado ( <i>Persea americana</i> mill.) c.v. Hass	25°C	Control	12.3 ± 0.5 <sup>a</sup>
		NPs Chi	2.4 ± 0.43 <sup>b</sup>
		NPs - Chi - P	1.5 ± 0.51 <sup>b</sup>

Data are means ± standard deviation. The values with different superscripts are significantly different (Tukey's honestly significant difference;  $p \leq 0.05$ ). Control = Sterile distilled water; NPs = Nanoparticles; Chi = Chitosan, P: Pepper tree essential oil.

**Table 3.** Weight loss of fruits at different temperatures treated with chitosan.

maintain firmness (29 N) on avocado fruits (*Persea americana* mill.) c.v. Hass compared to control (15 N) ( $p \leq 0.05$ ) [39]. In terms of firmness, it decreases as maturation progresses due to changes occurring at the level of the cell wall, where there is hydrolysis of the pectic compounds due to the action of the enzymes cellulase, pectin methylesterase, and polygalacturonase, which in turn degrade high molecular weight polymers such as cellulose and hemicellulose [46]. The coating of chitosan at different concentrations on fruits does not change the quality parameters (TSS, pH, and titratable acidity). This may be due to the fact that chitosan does not interfere in the metabolism cycles (synthesis of sugars, synthesis of organic molecules) [47].

## 2. Conclusions

The use of chitosan in agriculture commodities can be a suitable alternative to the use of fungicides for controlling postharvest diseases, as well as to preserve the quality of fruits.

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## Conflict of interest

Replace the entirety of this text with the “conflict of interest” declaration.

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