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Article

Chitosan for controlling *Lasiodiplodia* and *Rhizopus* in *Carica papaya* L.

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Abstract

The study aimed to evaluate the effect of chitosan on controlling *L. theobromae* and *R. stolonifer*, fungi associated with postharvest diseases in papaya. *In vitro* and *in vivo* tests were conducted using different concentrations of chitosan and reference fungicide. The variables evaluated included the percentage of mycelial growth inhibition, the lesion area, and the disease severity. Chitosan concentrations between 0.75% and 1% showed high efficacy against both pathogens, with inhibition levels comparable to those of the synthetic fungicide, with no statistically significant differences in most treatments.

Keywords:

fungal pathogens, mycelial inhibition, postharvest.

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Introduction

Papaya (*Carica papaya* L.) represents a fruit of great value, both for local consumption and for international trade, in Costa Rica. Nevertheless, during the postharvest stage, it faces significant health challenges, mainly due to diseases caused by the fungi *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. and *Rhizopus stolonifer* (Ehrenb.) Vuill., which can cause losses of more than 50% of production.

Traditionally, the management of these pathogens has depended on the use of synthetic fungicides; however, in recent years, more sustainable alternatives, such as chitosan-based coatings, have gained relevance, capable of forming a semipermeable film that limits the action of pathogens on the surface of the fruit (Romanazzi *et al*., 2017; Ayón *et al*., 2022; Uclaray *et al*., 2022; Heng-Tan *et al*., 2023; Singh *et al*., 2024).

Chitosan is a biopolymer derived from chitin, exhibiting antifungal, antibacterial, film-forming, and biodegradable properties, the effects of which vary according to its molecular weight and degree of deacetylation (Islam *et al*., 2017; Kumar *et al*., 2020; Singh *et al*., 2024). Several studies have demonstrated its effectiveness in preserving fruit and vegetable products, including papaya, strawberries, and tomatoes, by reducing fungal load and improving shelf life (Badawy and Rabea, 2021; Hernández-Montiel *et al*., 2023).

The research aimed to evaluate the antifungal activity of chitosan against *L. theobromae* and *R. stolonifer*, through *in vitro* and *in vivo* assays, as an alternative strategy in the postharvest management of papaya fruits.

Materials and methods

Plant material, localization and isolation of pathogens

The study was conducted at the Phytopathology Laboratory of the School of Agrarian Sciences of the National University (UNA), by its Spanish acronym, in Heredia, Costa Rica, using papaya fruits of the 'Pococí' hybrid. The fruits were collected at the packing plant of the Association of Export Papaya Producers (ASOPROPA), by its Spanish acronym, located in Jiménez de Guácimo, Limón, Costa Rica (10° 24' 64.86" north latitude, 83° 73' 64.77" west longitude; altitude of 222 m) and were transferred under controlled conditions to the laboratory for processing.

To isolate the fungi *L. theobromae* and *R. stolonifer*, the potato dextrose agar (PDA) medium was used, following the protocol described by Samithri *et al*. (2020). Dishes were incubated at 26 ± 2 °C in darkness for seven days, and pathogenicity tests were performed to confirm the virulence of the isolates before starting experimental trials.

Evaluation of the antifungal effect at the *in vitro* level

Chitosan solutions and PDA medium were prepared according to Edirisinghe *et al*. (2014) (Table 1). The experimental unit was a Petri dish (90 × 20 mm), in which 15 ml of each treatment was deposited, evenly distributed on the surface of the still liquid medium.

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Table 1. Description of the treatments established at the *in vitro* level.

Treatments	Code	No. of replications
1. Chitosan (1%)	Tiv1	5
2. Chitosan (0.75%)	Tiv2	5
3. Chitosan (0.5%)	Tiv3	5
4. Chitosan (0.25%)	Tiv4	5
5. Prochloraz (550 µl L^{-1})	Tiv5	5
6. Prochloraz (1 000 µl L^{-1})	Tiv6	5
7. Acetic acid (0.5%)	Tiv7	5
8. PDA (39 g L^{-1})	Tiv8	5

Once the media were solidified, a 0.5 cm diameter disc with active mycelium from each fungus was placed in the center of the dish. The dishes were sealed with Parafilm and incubated at 26 ±2 °C until the negative control (PDA only) reached the edge of the dish. Mycelial growth measurements were performed every 24 h in two orthogonal directions, averaging the diameter of the colony (Hernández *et al*., 2007).

In vitro evaluated treatments

Based on the measurements of mycelial growth in each treatment described in Table 1, the percentage of mycelial growth inhibition (PGI) was calculated using the formula:

$$PGI = \frac{D1 - D2}{D1} x100$$

Where: D1= diameter of the control colony; D2= diameter of the colony in treatment.

Evaluation of the antifungal effect at the *in vivo* level

Four treatments, each with four replications, were evaluated (Table 2). Each fruit was wounded four times at four points using a sterile cork borer, immersed in the solutions for five minutes, and then left to stand at 26 °C for one hour to remove excess moisture (Hernández *et al*., 2020; Ayón *et al*., 2022).

Table 2. Description of the treatments established at the *in vivo* level.

Treatments	Code	No. of replications
1. Chitosan (1%)	Tev1	4
2. Chitosan (0.75%)	Tev2	4
3. Prochloraz (1 000 µl L^{-1})	Tev3	4
4. Sterile distilled water	Tev4	4

Immediately, each fruit was inoculated with four agar discs (5 mm in diameter) containing active mycelium of *L. theobromae* or *R. stolonifer*, with six days of development in PDA. To ensure inoculum homogeneity, an approximate concentration of 1 × 10# ml^{-1} conidia was estimated by Neubauer chamber counting in previous culture samples. The fruits were placed in sterile plastic containers with a capacity of 3 L and airtight lids and kept at 24 ±2 °C in dark conditions. The severity of the disease was assessed every 24 h post-inoculation by measuring the diameter of the lesions in two perpendicular directions, using a ruler (Karpova *et al*., 2021).

Statistical analysis

The statistical software InfoStat (Di Rienzo *et al*., 2020) was used for analyzing the data on incidence and percentage of mycelial growth inhibition (PGI) of pathogens. Measures of central tendency

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and dispersion (mean, deviation, and standard error of the mean) were calculated. Subsequently, a repeated measures analysis of variance (Anova) was performed, followed by a separation of means using the Di Rienzo, Guzmán, and Casanoves test (DGC) ($p \leq 0.05$), under a completely randomized design (CRD).

Results and discussion

In vitro evaluations, effect of chitosan on *R. stolonifer*, mycelial growth inhibition and percentage of growth inhibition

The chitosan concentrations evaluated (0.25%-1%) significantly inhibited the mycelial growth of *R. stolonifer*, with PGI values above 85%, comparable to those obtained with the synthetic fungicide prochloraz (Figures 1 and 2). In contrast, treatments with acetic acid and PDA medium allowed complete colony development in four days (Figure 3). The differences between treatments were statistically significant ($p \leq 0.05$), according to the Di Rienzo, Guzmán, and Casanoves (DGC) means comparison test.

Figure 1. *In vitro* mycelial growth (cm) of *R. Stolonifer* under the effect of the treatments.

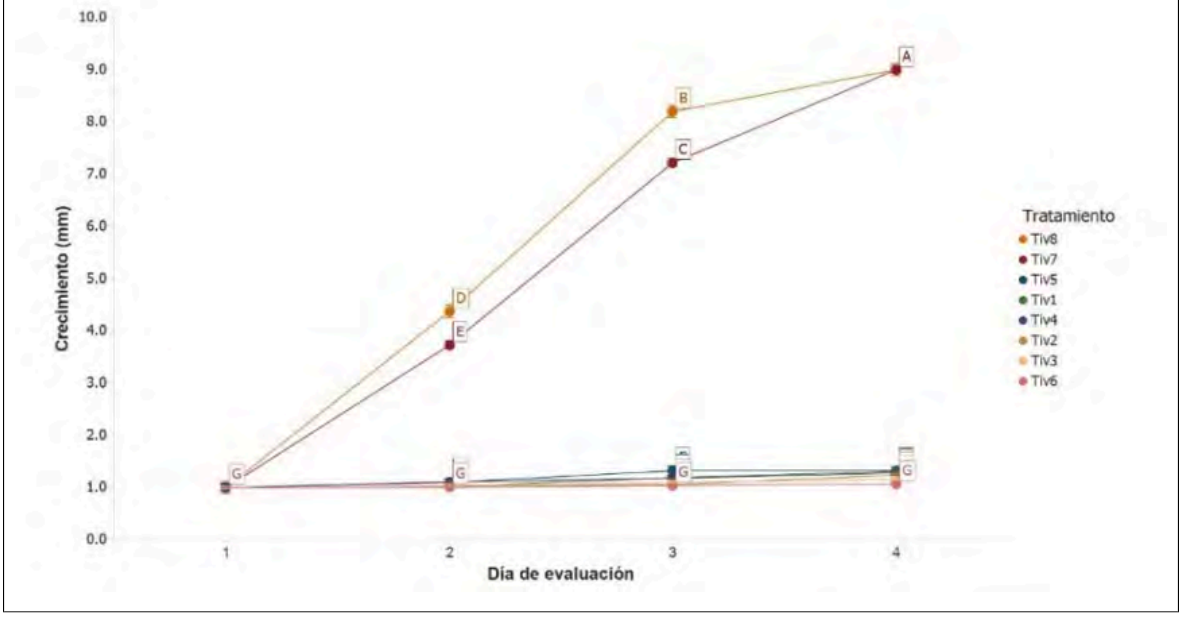

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Figure 2. Percentage of mycelial growth inhibition (PGI) of *R. stolonifer*.

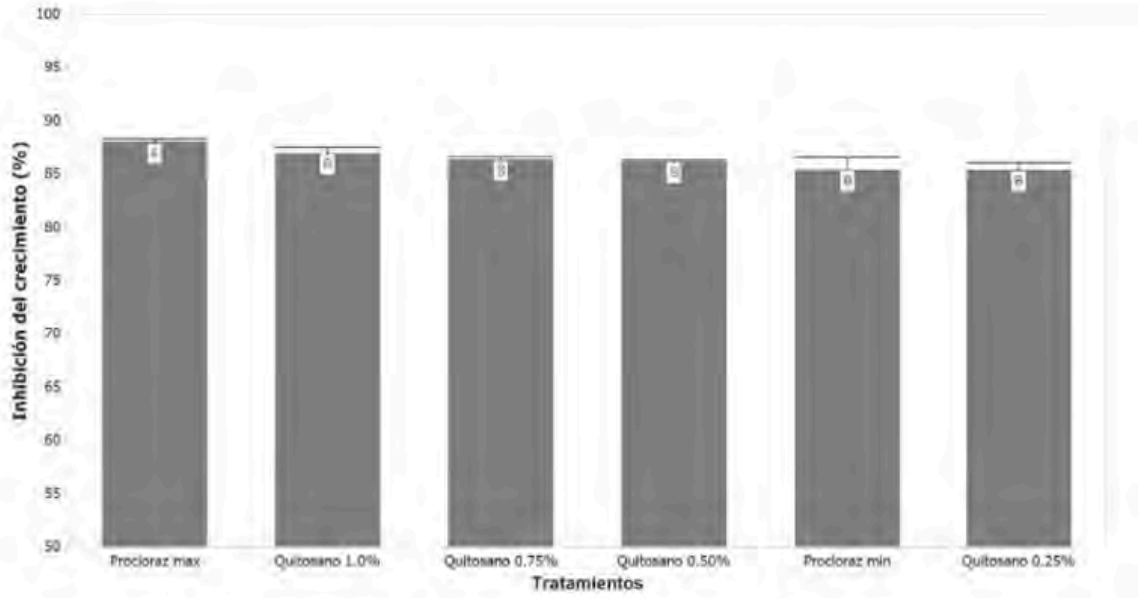
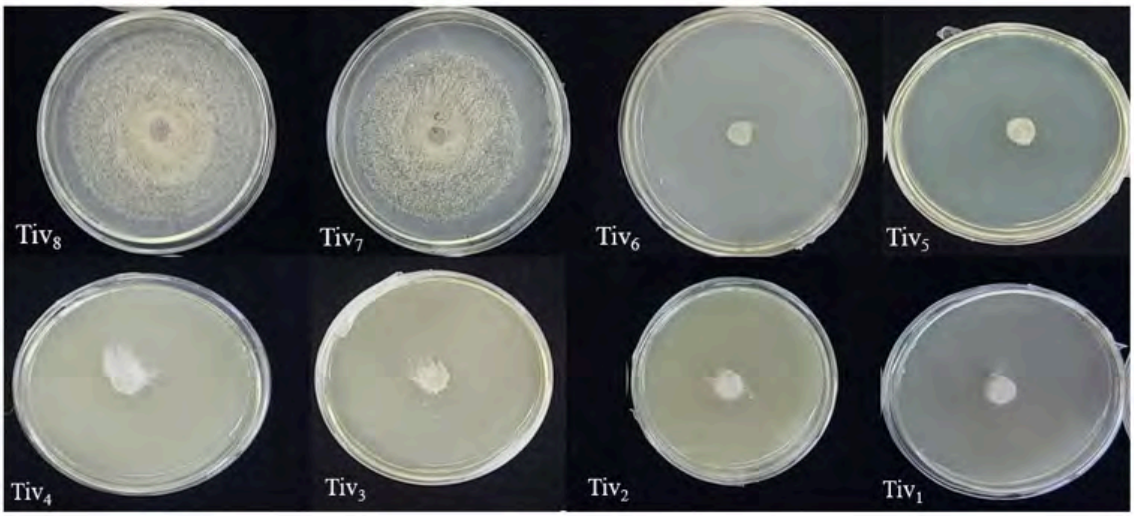


Figure 3. *In vitro* control effect of chitosan on *R. stolonifer*.



These data are consistent with reports by El-Araby *et al*. (2024), who documented that chitosan (molecular weight 150-190 kDa, 85% deacetylation) applied at concentrations between 2 and 3% managed to inhibit the growth of *R. stolonifer* in a range of 70-81.4%. Likewise, Coronado *et al*. (2023) found that concentrations of 1.5 and 2.5% chitosan reduced the mycelial growth of *R. stolonifer* by 44 to 48%.

The antifungal effect of chitosan can be attributed to its ability to alter plasma membrane permeability, cause cellular potassium loss, and decrease the activity of key enzymes in fungal metabolism, such as chitinase or β-glucanase (Kong *et al*., 2010; Rabea *et al*., 2020; Xing *et al*., 2021; Poznanski *et al*., 2023). This mode of action is intensified as the degree of deacetylation increases and the molecular weight decreases, which favors electrostatic interaction with the cell

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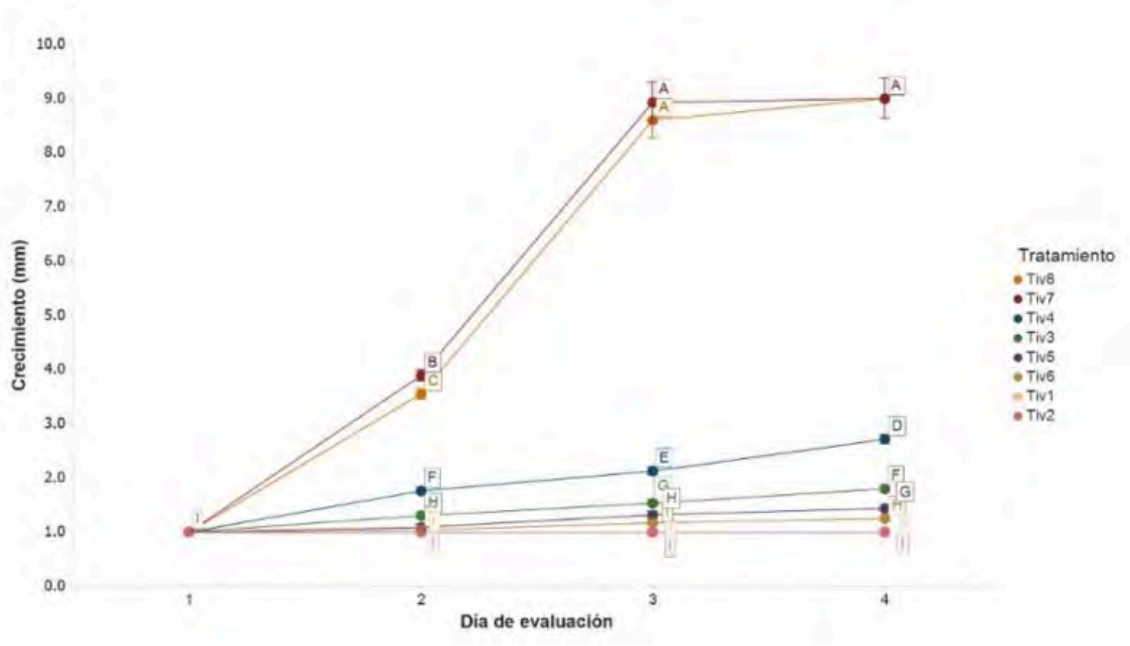
membrane of the pathogen. Additionally, the formation of a semipermeable barrier over the culture medium limits oxygen diffusion, which contributes to the inhibition of mycelial growth (Edirisinghe *et al*., 2014; Hernández-Montiel *et al*., 2023).

Effect of chitosan on *L. theobromae*

The concentrations of chitosan at 0.75% and 1% showed the lowest values of mycelial growth of *L. theobromae*, with no statistically significant differences between them (Figure 4).

Figure 4. *In vitro* mycelial growth (cm) of *L. theobromae*, under the effect of treatments.



Chitosan concentrations at 0.75% and 1% also presented the highest average inhibition values (89%), which evidences its high antifungal efficacy under *in vitro* conditions (Figure 5). On the other hand, the doses of 0.5% and 0.25%, as well as the treatment with the minimum dose of prochloraz, partially inhibited mycelial growth, reaching PGI values of 80% and 70%, respectively. Prochloraz showed an inhibition ranging from 84% to 86%, without exceeding the effect of chitosan at high doses.

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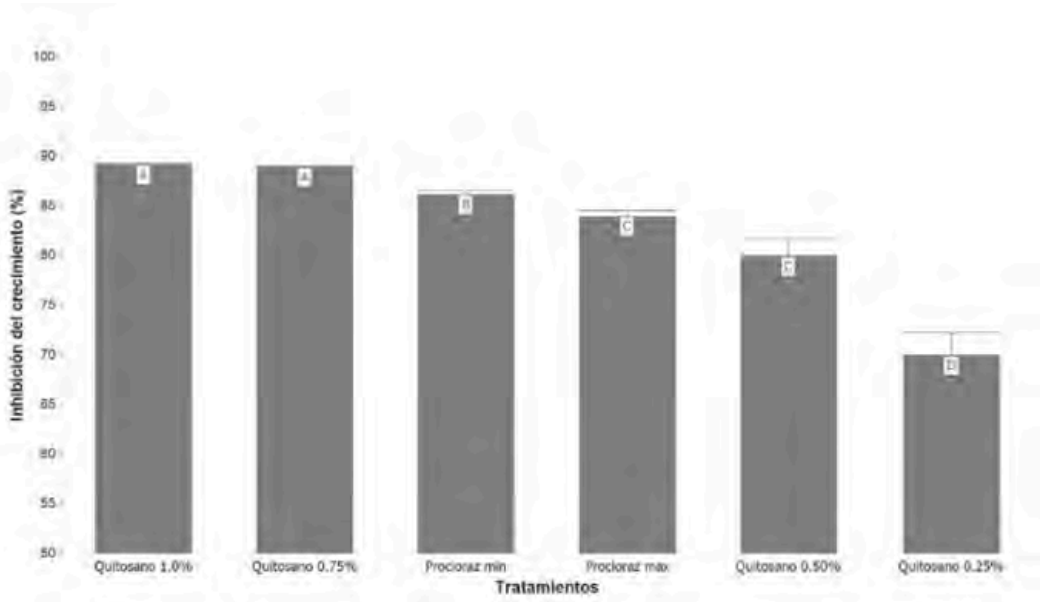
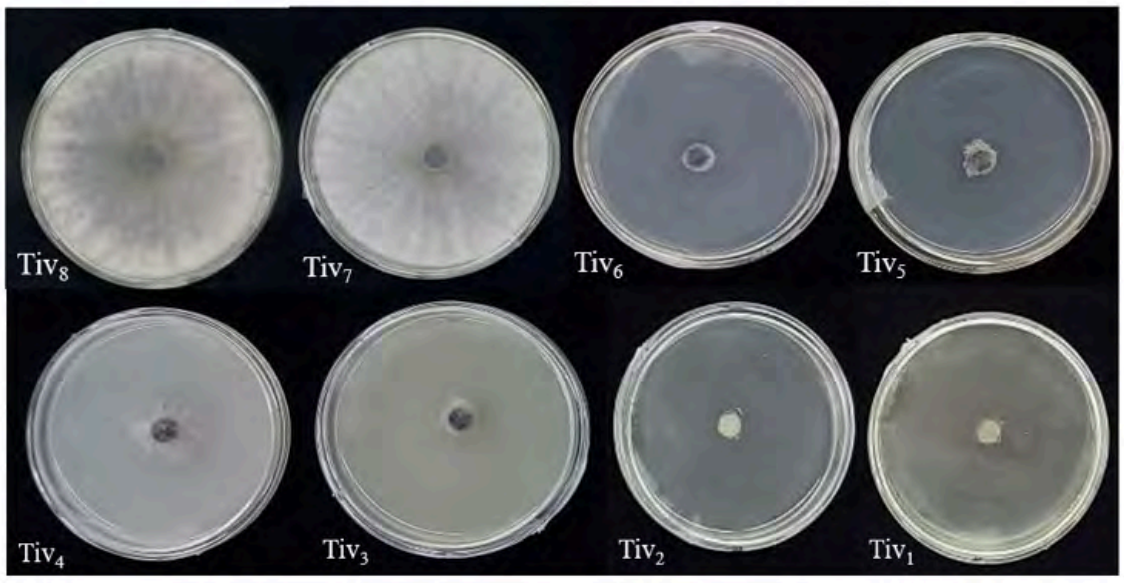
Figure 5. Percentage of mycelial growth inhibition (PGI) of *L. theobromae*.



Treatments with 0.5% acetic acid and control with distilled water allowed a complete mycelial growth of *L. theobromae* in the Petri dish from the fourth day after inoculation, covering 100% of the available surface area (Figure 6). The statistical analysis includes error bars (standard deviation) and homogeneous groups by the DGC test ($p \leq 0.05$), which confirm significant differences between treatments.

Figure 6. *In vitro* control effect of chitosan on *L. theobromae*.



The antifungal effect of chitosan on *L. theobromae* can be attributed to mechanisms including cell membrane perturbation, inhibition of DNA synthesis, and the generation of oxidative stress, promoting programmed cell death in fungal structures (Xing *et al*., 2021). Studies such as those

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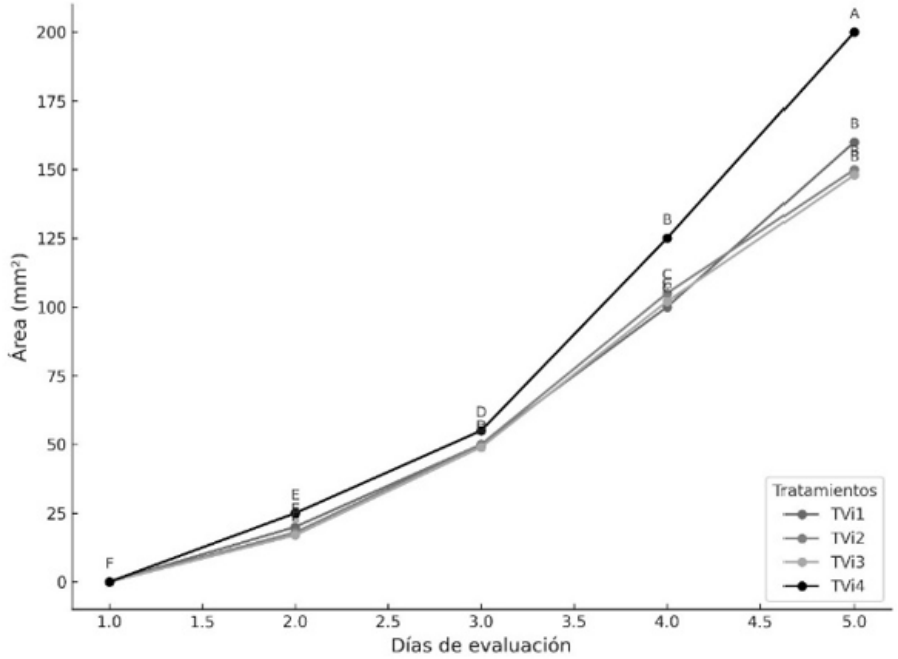
by El-Araby *et al*. (2024); Coronado *et al*. (2023) have documented similar results with other phytopathogenic fungi under *in vitro* conditions, highlighting that chitosan, at doses greater than 0.75%, can be as effective as conventional fungicides. In this study, the efficacy of chitosan was concentration-dependent, and the results support its potential as an eco-friendly alternative for postharvest management of tropical fruits.

In vivo evaluations

Antifungal activity of chitosan on *R. stolonifer* in papaya fruits

At concentrations of 1% and 0.75%, chitosan showed an effectiveness comparable to that of the maximum dose of prochloraz in suppressing the mycelial growth of *R. stolonifer* under *in vivo* conditions. These concentrations significantly inhibited the progression of fungal colonization from the second day post-inoculation, remaining constant until the fifth day (Figure 7) (Pervin *et al*., 2020). Comparisons were made between treatments at each daily assessment point using a repeated-measures analysis of variance, followed by the DGC test ($p < 0.05$).

Figure 7. Effect of the treatments on the severity of mycelial growth (mm) of *R. stolonifer* in papaya for five days aft er applying the treatments. *In vivo* evaluation of: A) chitosan at 1%; B) chitosan at 0.75%; C) maximum dose of prochloraz; D) distilled water. Different letters indicate significant differences between treatments ($p < 0.05$).



Cortés-Rivera *et al*. (2021) also evaluated the antifungal activity of chitosan (1 and 1.5%) against *R. stolonifer*, obtaining PGI results of 83 and 87%, respectively. Figure 8 shows the appearance of papaya fruits five days after applying the treatments. Visually, fruits treated with 1% and 0.75% chitosan (panels B and D) have a lower number and size of necrotic lesions compared to fruits treated with distilled water (control, panel A) (Torres-Rodríguez *et al*., 2025).

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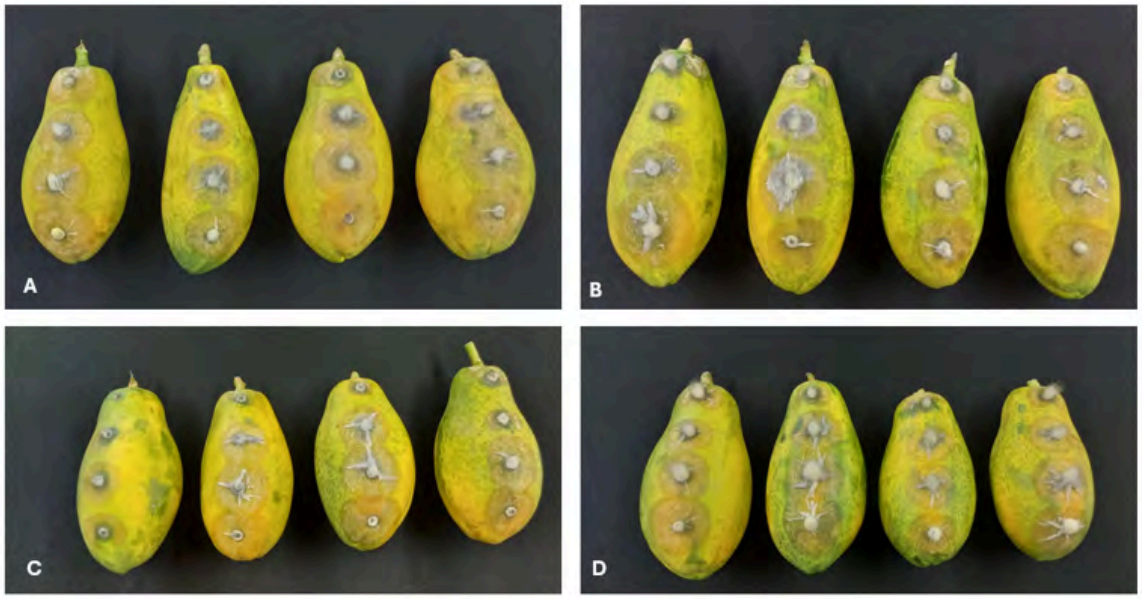
Figure 8. Appearance of the effect of chitosan on the control of *R. stolonifer* in papaya, five days after applying the treatments. A) distilled water (control); B) chitosan at 1%; C) maximum dose of prochloraz; D) chitosan at 0.75%.



This observation is consistent with quantitative values of the percentage of mycelial growth inhibition (PGI), where inhibitions of up to 87% were recorded for chitosan at 1%, compared to 85-88% for prochloraz (Figure 8C) (Silva *et al*., 2023). While the visual difference between chitosan and prochloraz is minimal, experimental data indicate that both alternatives offer similar control over *R. stolonifer* under *in vivo* conditions.

Determination of the antifungal activity of chitosan on *L. theobromae* in papaya fruits

Under the *in vivo* conditions evaluated, prochloraz treatment showed the highest values of inhibition of mycelial growth of *L. theobromae*, followed by chitosan concentrations of 1% and 0.75%, which also exerted a significantly higher effect than that of the negative control (distilled water) (Figure 9 A-D).

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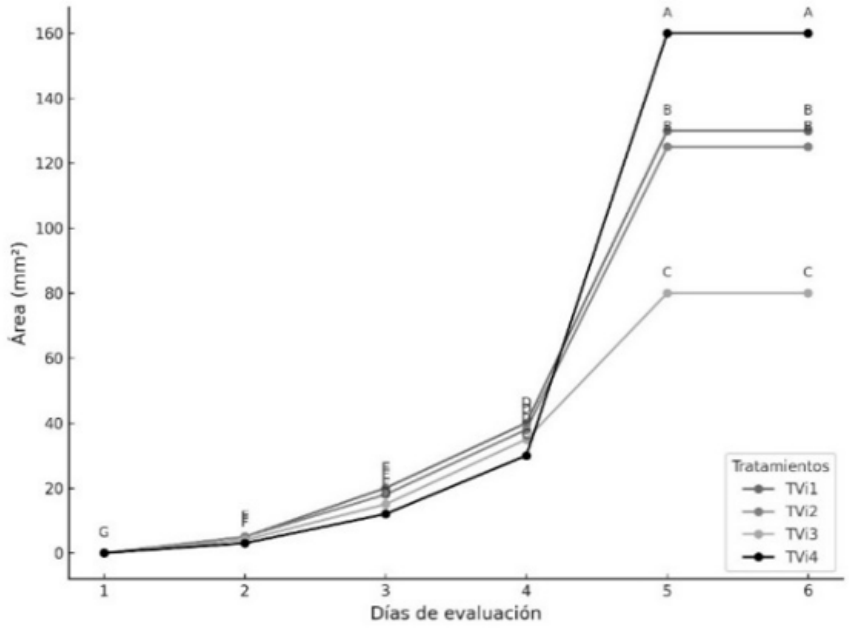
Figure 9. Effect of treatments on the severity of mycelial growth (mm) of *L. theobromae* in papaya for five days after applying the treatments. *In vivo* evaluation of: A) distilled water (control); B) chitosan at 0.75%; C) chitosan at 1%; D) maximum dose of prochloraz. Different letters indicate significant differences ($p < 0.05$).



At the visual level, a lower area and number of necrotic lesions were observed in the fruits treated with chitosan (Figure 10 A-D), which coincides with the quantitative measurements and statistical analysis (Figure 9 A-D). Differences between treatments were significant ($p < 0.05$) and were indicated by different letters on the growth charts (Figure 9 E).

Previous studies support these findings. Gomes *et al*. (2020) demonstrated that coatings with chitosan at concentrations of 1-5% inhibited the growth of several species of the Lasiodiplodia complex, including *L. theobromae*, in papaya, especially at concentrations of 4%. Cuong *et al*. (2022) reported that chitosan nanoparticles at 250 ppm completely suppressed *L. pseudotheobromae* symptoms in citrus under high moisture and 30 ±0.2 °C. These results confirm that chitosan can act effectively as an alternative control agent against *L. theobromae*, with results comparable to those of conventional fungicides, especially when applied in doses greater than 0.75% (Figure 10).

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Figure 10. Effect of chitosan on the control of *L. theobromae* in papaya five days after applying the treatments. A) Distilled water (control); B) chitosan at 0.75%; C) chitosan at 1%; D) maximum dose of prochloraz.

The study by Cuong *et al*. (2022) demonstrated *in vivo* that treatment with 250 ppm of chitosan nanoparticles completely suppressed the symptoms of the disease caused by *L. pseudotheobromae* in citrus fruits. This effect was observed after 12 days of incubation at 30 ±0.2 °C and under conditions of high relative humidity, confirming Chitosan's potential as an effective antifungal agent in postharvest environments (Chowdappa *et al*., 2020).

Conclusions

Chitosan demonstrated high antifungal efficacy against *L. theobromae* and *R. stolonifer* in papaya fruits, both *in vitro* and *in vivo*, especially at concentrations of 0.75% and 1%. Its effectiveness was comparable to that of the synthetic fungicide prochloraz, reinforcing its potential as an ecological and sustainable alternative in the postharvest management of fungal diseases.

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