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Evaluation of chitosan for *in vitro* control of *Colletotrichum tamarilloi* and anthracnose on scarlet eggplant fruit

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ABSTRACT

Scarlet eggplant (*Solanum aethiopicum* var. *gilo*) is a Solanaceae with an appreciated peculiar bitter taste, which plays a significant role in family farming in Brazil. Fruit anthracnose is the main pre- and postharvest disease that affects scarlet eggplant in Brazil. This study aimed to evaluate the effect of chitosan-based coating on *in vitro* inhibition of *Colletotrichum tamarilloi* and anthracnose control of scarlet eggplant fruits. Chitosan was dissolved in a 2% citric acid solution at 40°C and then homogenized with potato dextrose agar (PDA), and poured into Petri dishes, as follows: 1) PDA + 0.1% chitosan, 2) PDA + 0.2% chitosan, 3) PDA + 0.3% chitosan, 4) PDA + 0.4% chitosan, and 5) pure PDA as control. Discs of 5 mm diameter of pure fungus culture were placed on the center of the culture medium in the plates. The plates were then maintained in BOD at 25°C and 12-h photoperiod for 10 days. Colony characteristics, mycelial growth rate, and mycelial growth inhibition were evaluated. Afterward, the effect of chitosan coating was evaluated in fruit inoculated or not with *C. tamarilloi*. The treatments were: T1) uncoated and injured uninoculated fruits, T2) uncoated and inoculated fruit, T3) fruits coated with 0.1% chitosan and inoculated, T4) fruits coated with 0.2% chitosan and inoculated, and T5) fruits coated with chitosan at 0.3% and inoculated. For inoculation, 15 µL of a conidial suspension (2 x 10^5 conidia/mL) were deposited on an injury caused by a needle, and the fruits were coated by immersion into the different concentrations of chitosan gel. Fruits were placed on expanded polystyrene trays. Fresh weight loss, the mean incidence of disease, and lesion diameter were measured. All concentrations of chitosan reduced the *in vitro* growth of *C. tamarilloi*. The treatment T4 reduced the severity of anthracnose but did not prevent its incidence in scarlet eggplant fruits.

Keywords: *Solanum aethiopicum*, postharvest disease, vegetable, polysaccharide-based coating.

RESUMO

Avaliação da quitosana no controle *in vitro* de *Colletotrichum tamarilloi* e antracnose em frutos de jiló

O jiló (*Solanum aethiopicum* var. *gilo*) é uma Solanaceae apreciada por seu peculiar sabor amargo, e que desempenha um papel relevante para a agricultura familiar no Brasil. A antracnose dos frutos é a principal doença pré- e pós-colheita do jiló no Brasil. Este estudo teve como objetivo avaliar o efeito da quitosana na inibição *in vitro* de *Colletotrichum tamarilloi* e no controle da antracnose em frutos de jiló. A quitosana foi dissolvida em uma solução de ácido cítrico a 2% a 40°C e, em seguida, homogeneizada com o meio de cultura batata dextrose ágar (BDA) e colocado em placas de Petri, como segue: 1) BDA + quitosana 0,1%, 2) BDA + quitosana 0,2%, 3) BDA + quitosana 0,3%, 4) BDA + quitosana 0,4% e 5) BDA puro como controle. Discos de 5 mm de diâmetro de cultura de fungo puro foram colocados no centro do meio de cultura nas placas. As placas foram então mantidas em incubadora BOD a 25°C e fotoperíodo de 12 h por 10 dias. As características da colônia, a taxa de crescimento e a inibição do crescimento micelial foram avaliadas. Em seguida, foi avaliado o efeito do recobrimento de quitosana em frutos inoculados ou não com *C. tamarilloi*. Os tratamentos foram: T1) frutos não revestidos e feridos e não inoculados, T2) frutos não revestidos e inoculados, T3) frutos revestidos com quitosana 0,1% e inoculados, T4) frutos revestidos com quitosana 0,2% e inoculados e T5) frutos revestidos com quitosana 0,3% e inoculado. Para a inoculação, 15 µL de uma suspensão de conídios (2 x 10^5 conídios/mL) foram depositados sobre um ferimento causado por uma agulha, e os frutos foram revestidos por imersão nas diferentes concentrações de gel de quitosana. Os frutos foram colocados em bandejas de poliestireno expandido. Foram medidos a perda de massa fresca, a incidência média da doença e o diâmetro da lesão. Todas as concentrações de quitosana reduziram o crescimento *in vitro* de *C. tamarilloi*. O tratamento T4 reduziu a severidade da antracnose, mas não impediu sua incidência em frutos de jiló.

Palavras-chave: *Solanum aethiopicum*, doença pós-colheita, hortaliça, revestimento à base de carboidrato.

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Scarlet eggplant (*Solanum aethiopicum* var. *gilo*) is a Solanaceae of significant economic impact on family farming in Brazil. Brazilian production was approximately 79.0 thousand tons (kt) in 2017, with the Southeast region accounting for 71.2% of the total national production. Rio de Janeiro state was the leading producer, contributing with 20.4 kt (26%), followed by Minas Gerais, 17.9 kt (23%), and São Paulo, 14.2 kt (18%)

(IBGE, 2023). In a global perspective, the largest cultivation of *S. aethiopicum* is concentrated in Africa and also in certain regions of the Caribbean and southern Italy, in addition to Brazil (Aguessy *et al*., 2021).

Several diseases have been reported in scarlet eggplant (Pavan *et al*., 2016; Mendes & Urben, 2023). Anthracnose, caused by fungal species of the genus *Colletotrichum*, causes the greatest economic losses in scarlet eggplant cultivation (Reis *et al*., 2009). To minimize these yield losses, farmers use fungicides as the only effective control practice (Pavan *et al*., 2016; Brasil, 2023). However, pesticides, such as fungicides, may be harmful to humans and the environment (Kim *et al*., 2016; Samsidar *et al*., 2018). Additionally, the frequent use of fungicides can lead to the selection of fungicide-resistant fungal populations, mainly for those with a single mode of action (Kuck *et al*., 2012).

Natural polymers can be an alternative to mitigate the use of pesticides for the control of plant pathogens in fruits (Gomes *et al*., 2020; Peralta-Ruiz *et al*., 2020; Avila *et al*., 2022). Polysaccharides are polymers widely used to develop edible films and coatings as they are abundant supply in nature and relatively low-cost biomaterials (Altaf *et al*., 2022). Moreover, polysaccharide-based coatings have been shown to be effective in preserving fresh produce (Wu *et al*., 2022).

Chitosan, a hydrophilic polysaccharide derived from chitin and soluble in most organic acids, is feasible to be incorporated into the edible coating formulation (Hirano, 1999; Pillai *et al*., 2009). Chitosan-based coating, alone or combined with other natural materials, has been widely used to delay fruit ripening during postharvest (Souza *et al*., 2011; Pilon *et al*., 2013; Suseno *et al*., 2014) and for having broad antimicrobial activity. It has been reported as a bactericidal or bacteriostatic, and an efficient inhibitor of spore germination, germ tube elongation, and mycelial growth of fungal phytopathogens (Liu *et al*., 2007; Goy *et al*., 2009; Meng *et al*., 2010).

The antimicrobial activity of chitosan has been efficient in controlling plant diseases in several pathosystems, such as gray mold (*Botrytis cinerea*) in grapes (Camili *et al*., 2007), anthracnose (*Colletotrichum lindemuthianum*) in common bean (Piero & Garda, 2008), anthracnose (*C. gloeosporioides*) in mango (Zhu *et al*., 2008), bitter rot (*C. acutatum*) in apple (Felipini & Piero, 2009), brown rot (*Monilinia fructicola*) in peach (Yang *et al*., 2012), blue mold (*Penicillium expansum*) in apple (Darolt *et al*., 2016), and late blight (*Phytophthora infestans*) in potato (Huang *et al*., 2021).

Therefore, since chemical fungicides are not registered in Brazil for this crop for postharvest treatment, this study aimed to assess the effectiveness of a chitosan-based coating as an alternative method on the control of *C. tamarilloi in vitro* and anthracnose in scarlet eggplant fruits.

MATERIAL AND METHODS

Study sites, reagents and sample material

Experiments were carried out at Embrapa Hortaliças, in the Federal District, Brazil, in the Plant Pathology Laboratory (*in vitro* experiments) and Food Science and Technology Laboratory (*in vivo* experiments). Potato-dextrose-agar (PDA) used for *in vitro* studies was purchased from Merck (Darmstadt, Hessen, Germany), Medium molecular weight chitosan with 75-85% degree of deacetylation and viscosity of 200-800 cP was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and citric acid PA used to dissolve the chitosan and prepare chitosan gel was purchased from Vetec Química Ltda. (Rio de Janeiro, RJ, Brazil). Scarlet eggplant fruits long light green type cv. Tinguá, ecotype Gilo, produced in Valparaíso de Goiás-GO, were purchased from a farmers' market in Ceilandia-DF. Liquid alkaline soap (coconut babassu-based) donated by Veros Produtos Químicos Ltda. (São Paulo-SP, Brazil) and chlorinated sanitizing detergent purchased from Ecolab Química Ltda. (Barueri-SP, Brazil) were used to wash and sanitize the fruits respectively before dipping them in chitosan gels.

Preparation of chitosan coating for *in vitro* and *in vivo* experiments

Chitosan concentrations of 0.1%, 0.2%, 0.3% and 0.4% were prepared by dissolving chitosan in 2% citric acid solution under constant stirring for 12 h (Pilon *et al*., 2013). The 2% citric acid solution was prepared by dissolving citric acid (20 g/L) in distilled water. The mixtures were heated to 40°C to accelerate the homogenization process. For the respective concentrations, pHs were 3.4, 3.9, 4.2 and 4.6.

Mycelial growth of *Colletotrichum tamarilloi* in PDA-chitosan

Synthetic PDA medium (Merck) was prepared according to the manufacturer's instructions in distilled water. The medium was autoclaved at 121°C for 15 min and stored at room temperature for further use. The fusion of 500 mL of solidified PDA medium was performed by heating in a microwave oven at 80°C for 5 min and then kept cooling until it reached 40°C. An amount of 50 mL of the medium was transferred to a sterilized Becker, which contained 50 mL of chitosan at each of the following concentrations: 0.1, 0.2, 0.3 and 0.4%. The PDA-chitosan was homogenized with a sterile glass rod. Then, 20 mL of the PDA-chitosan was poured into 90 mm diameter plastic Petri dishes for each of the five treatments. Treatments were as follow: 1) PDA + 0.1% chitosan (PDAC1), 2) PDA + 0.2% chitosan (PDAC2), 3) PDA + 0.3% chitosan (PDAC3), 4) PDA + 0.4% chitosan (PDAC4) and 5) potato-dextrose-agar (PDA) as control.

The isolate of *C. tamarilloi* (Coll-265) was obtained from a diseased scarlet eggplant fruit in São Joaquim de Bicas-MG, and previously identified by morphological and molecular markers (Oliveira, 2017). A pure culture of the *C. tamarilloi* isolate was grown in PDA medium for six days at 25°C. Agar discs were made using a 5 mm-diameter cork punch from the margin of the pure culture. The discs were removed with

a histological needle and transferred to the center of 90 mm diameter plastic Petri dishes, which contained 20 mL of the respective PDA-chitosan media to be tested. Then, the Petri dishes were stored at 25°C and 12-h photoperiod for 10 days in a Bio-Oxygen Demand incubator (BOD).

The experiment was performed twice at two different times, under the same conditions. A completely randomized design with five treatments and five replicates was used in both trials. The experimental unit was a 90 mm diameter plastic Petri dish, with a total of 25 dishes per experiment.

Visual evaluations of the colony's cultural characters began after 48 h. Observations and measurements of the diameter were taken every 48 h for a total of 10 days of assessment. To analyze mycelial growth, two diametrically opposite measurements were taken using a digital caliper on the back of the Petri dish. The diameter of the PDA disc was subtracted from the measurement and the mean was calculated after each measurement was taken. The mycelial growth rate (MGR) and percent mycelial growth inhibition (MGI) were calculated using the formulas: MGR = [(Final colony diameter/incubation days) x 100] and MGI = [diameter of control (PDA) - diameter of treatment (PDACx) x 100].

Postharvest control of anthracnose in chitosan-coated fruit

In the laboratory, scarlet eggplant fruits were selected based on fruit quality, such as absence of detectable defects and disease symptoms, and uniformity in size and color. Using a digital caliper (MTX), the height of the fruits was measured from the base to the apex, and the diameter was measured at the equatorial zone of the fruit. Only fruits with similar characteristics were selected.

The fruits were washed with a 3% alkaline soap solution and then immersed in a 0.1% chlorinated sanitizer for 10 min. After sanitization, the fruits were rinsed with distilled water and left to dry on paper towels.

Chitosan based-coatings were prepared at concentrations of 0.1%, 0.2%, and 0.3% as described in the *in vitro* assay. The concentrations of chitosan used in this fruit coating experiment were selected based on the results of previous *in vitro* experiments.

The isolate Coll-265 of *C. tamarilloi*, was cultivated in 90 mm diameter Petri dishes containing PDA medium. After eight days of cultivation, 20 mL of sterile distilled water was placed in each plate and the water was spread throughout the colony with a sterile glass Drigalski loop to detach the fungal conidia. Subsequently, the suspension was filtered in sterile gauze folded four times to obtain only conidia. The spore concentration on the suspension was estimated in a hemacytometer and adjusted to 2.0 x 10^5 conidia/mL.

For fruit inoculation, 15 µL of inoculum was applied in a needle wound of 1.25-mm diameter on the fruit. The depth of the lesions was 4 mm reaching the top of the mesocarp and the inoculum was deposited with an automatic micropipette. All heat-resistant utensils were autoclaved at 121°C for 15 min and the non-resistant ones were disinfected with 70% ethanol.

Prior to coating the fruits, surfaces and materials that would come into contact with the fruits were previously washed and sanitized with 200 mg/L sodium hypochlorite solution. The temperature and relative humidity in the room were measured by TFA digital thermo-hygrometer.

The fruits were then subjected to the following treatments: T1) wounded and uncoated and uninoculated (as control 1), T2) uncoated and inoculated (as control 2), T3) inoculated and coated with 0.1% chitosan, T4) inoculated and coated with 0.2% chitosan, and T5) inoculated and coated with 0.3% chitosan.

Fruits of T3, T4 and T5 treatments were coated only 48 h after inoculation. The coatings were applied to the fruits after being cooled to room temperature. The fruits were immersed into the chitosan formulations for 1 min, followed by draining off the excess and the coatings were then formed by spontaneous evaporation at room temperature. After dried, the coated fruits were placed on expanded polystyrene trays, previously identified.

For the study on chitosan-coated fruit, the trials were conducted twice at separate intervals using the same conditions. A completely randomized design was implemented, which included five treatments and four replicates for each treatment. Each replicate consisted of two fruits, making the experimental unit.

Fresh weight loss, mean incidence of disease and severity of disease were analyzed. Fresh weight was assessed by weighing the fruit using a precision balance and the percentage loss per initial fruit weight for each sample was calculated as follow: [(Wi - Wf)/Wi x 100], where Wi is the initial fruit weight (g) at the beginning of the experiment and Wf is the fruit weight (g) at the end. The disease incidence and severity were assessed, every other day, after the appearance of the first symptoms in the fruits. The mean disease incidence was calculated as a percentage by the lesion count per treatment and, for the disease severity given by lesion diameter which was measured, obtaining the mean of two perpendicular measurements with a digital caliper. Disease severity values from each treatment were used to calculate the area under the disease progress curve (AUDPC) (Campbell & Madden, 1990).

Statistical analysis

For *in vitro* experiments, MGI results were analyzed using analysis of variance. For *in vivo* experiments, AUDPC data were previously transformed to $\sqrt{x+0.5}$ and then submitted to analysis of variance. Maxima F test showed that both *in vitro* and both *in vivo* experiments had the Mean Squared Error homogeneous and were, therefore, comparable. Therefore, both *in vitro* assay and also the *in vivo* assays were analyzed as one assay each. The interaction experiment*treatment was sliced by treatment to evaluate (by using the F test) if the treatment had the same behavior within each experiment. Regression analysis for MGI and AUDPC data were also performed. Statistical analyses were performed

by using the SAS Statistical Analysis System v 9.4 (SAS Institute Inc., Cary, NC, USA, 2013).

RESULTS AND DISCUSSION

Mycelial growth of *Colletotrichum tamarilloi* in PDA-chitosan

MGR of the *C. tamarilloi* isolate (Coll-265) on PDA medium, used as a control, was found to be 67.5% after 10 days on average, based on two experiments. Coll-265 showed circular, aerial and cottony mycelial growth. The colonies started out as white but gradually turned gray, with the appearance of gray-colored concentric rings. The conidial masses were concentrated at the center of the plate, where the disc was placed, and displayed an orange color when abundant. The colony coloring on the back side of the Petri dish was similar but more intense, ranging from white to dark gray. Over time, the colonies darkened to black.

Each of the tested chitosan concentrations showed positive effects on reducing fungal growth. However, there was not a proportional decrease in mycelia growth with increasing chitosan concentration. Cultural characteristics of the colonies could not be observed in the four treatments due to their small development or non-growth (Figure 1).

The highest percentage of mycelial growth inhibition of the *C. tamarilloi* isolate was observed at chitosan concentrations of 0.1%, 0.2% and 0.3%, with over 90% inhibition in both experiments (Table 1). Colony diameter of the fungus also displayed slow growth at a 0.4% chitosan concentration after 10 days of incubation at 25°C and 12-h photoperiod (Figure 1).

In assay #1 (E1), neither the linear nor the quadratic models fit the data, as evidenced by the low R^2 values of 0.13 and 0.35, respectively. In contrast, the quadratic model in assay #2 (E2) showed partial adjustment and was represented by the equation $y = 90.27 + 35.34 \text{ dose} - 94.16 \text{ dose}^2$ ($R^2 = 0.63$). Based on the quadratic regression of E2, the maximum (MGI) was estimated to be 93.59% at a chitosan concentration of 0.19% (Table 1).

After reaching a peak of maximal mycelial growth inhibition of *C. tamarilloi*, the subsequent decrease suggests that increasing chitosan concentration may not necessarily enhance its fungistatic effect (Table 1). Mendes *et al*. (2016) showed that lower concentrations of chitosan effectively inhibited the growth of *Penicillium expansum* in a liquid medium under *in vitro* conditions. However, under solid medium conditions, there was no inhibition efficacy of this fungus. In contrast, Botelho *et al*. (2010) and Freddo *et al*. (2014) observed a linear reduction in mycelial growth in solid medium with increasing chitosan concentrations for *Penicillium* sp. and *Rhizoctonia solani*, respectively. Thus, the effect of chitosan on pathogen mycelial growth may vary depending on the fungal species and the composition of the culture medium.

Citric acid used for dissolving chitosan and its concentration may affect fungal growth. Cia *et al*. (2010) found that *Rhizopus stolonifer* was able to develop, although slowly, in PDA medium when 0.5% and 1% citric acid were added. In contrast, 0.5% and 1% chitosan dissolved in citric acid completely inhibited the development of *R. stolonifer*. The inhibitory effect of chitosan on the *in vitro* mycelial growth seems to be a synergistic action of chitosan and organic acid, possibly in combination with the low pH (approximately 2.2) of the solution (Pilon *et al*., 2013; Spricigo *et al*., 2021).

Chitosan's antimicrobial activity is affected by various factors, such as its molecular weight (Jing *et al*., 2007; Hernández-Lauzardo *et al*., 2008), degree of acetylation (Andres *et al*., 2007), temperature during interaction (Hefian *et al*. 2010; Szymańska & Winnicka, 2015), and the pH of the medium (Felipini & Piero, 2009; Alburquenque *et al*., 2010). The local pH, specifically the pH of the coated produce, is also known to have an impact on chitosan's antifungal activity (Assis & Leoni, 2003).

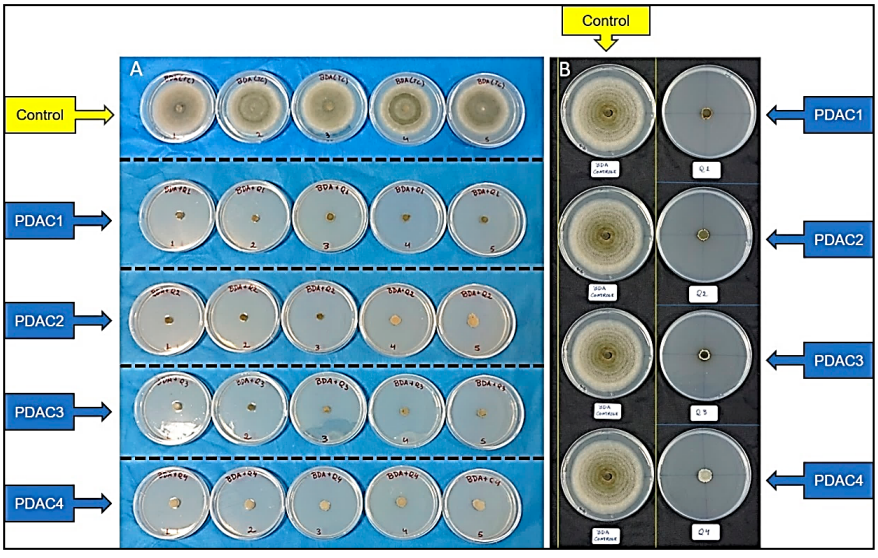

Figure 1. *Colletotrichum tamarilloi* (Coll-265) grown on potato-dextrose-agar (PDA) for 10 days. Control: PDA; PDAC1: 0.1% chitosan-PDA; PDAC2: 0.2% chitosan-PDA; PDAC3: 0.3% chitosan-PDA and PDAC4: 0.4% chitosan-PDA for control test. A) Assay 1 and B) Assay 2 (one Petri dish representing each treatment). Brasília, Embrapa Hortaliças, 2022.

Postharvest control of anthracnose in chitosan-coated fruit

During the 12-day *in vivo* assay 1, the temperature ranged from 23.5°C to 24.9°C, and relative humidity (RH) ranged from 56.4% to 69.3%. In the second assay, the temperature ranged from 23.1°C to 24.5°C, and RH ranged from 53.8% to 70%.

In both assays, all treatments showed a similar fresh weight loss of approximately 30% for fruits, regardless of whether they were coated or uncoated

with chitosan (data not shown).

Although hydrophilic formulations like chitosan are also recommended for preventing the loss of moisture in fruits and maintaining their shine (Assis & Brito, 2014), the coating formed by 0.3% chitosan on scarlet eggplant fruits did not reduce weight loss (Table 2). Both coated and uncoated fruits showed similar weight loss and changes in appearance.

All fruits from the treatments with pathogen inoculation in both assays exhibited symptoms of anthracnose on the final evaluation day (Figure 2). The T1 treatment, which was not inoculated or coated, showed no lesions (Figure 2 and Table 2).

The results of two experiments on scarlet eggplant fruit anthracnose control were jointly analyzed using an F-test, and all treatments showed similar results (Table 2). Upon analyzing the data from the first experiment, a significant regression result was obtained with an R^2 value of 0.66 and a quadratic model was fit to the data. The equation y = 234.97 - 2099.62 dose + 5050.94 dose² was adjusted to the data, yielding an estimated lowest severity of anthracnose (measured by AUDPC) of 16.77% at a concentration of 0.21% chitosan (Table 2). In contrast, the results of the second experiment did not fit the data for both linear and quadratic models, with low R^2 values of 0.03 and 0.17, respectively.

The chitosan-coated fruits at the concentration of 0.2% (T4) showed the lowest AUDPC values in both experiments (Table 2), indicating it is the most effective in reducing disease progression. However, it is worth noting that there was no linear reduction in disease severity, as represented by the AUDPC, with the increase in chitosan concentration, suggesting that factors other than chitosan concentration may be affecting its efficacy as a treatment.

In fact, the effectiveness of different chitosan concentrations can vary depending on the specific conditions and pathogen being targeted. For example, in a study by Oliveira *et al*. (2016) on the use of cassava starch coating to control anthracnose in papaya, higher concentrations of the coating became rigid and cracked upon contact with the fruit, as revealed by scanning electron microscopy. This may explain why the 0.3% chitosan was found to be less effective than the 0.2% chitosan in reducing the severity of the scarlet eggplant anthracnose.

When scarlet eggplant fruits were coated with 0.1% chitosan, the resulting coating was less consistent and tended to drain better, which may have allowed the disease to develop and contributed to higher disease severity, as indicated by the higher AUDPC values. Thus, the concentration and composition of the coating material can significantly affect the coating performance and ultimately the preservation of fruits.

The effectiveness of chitosan as a fungicidal or fungistatic agent is highly dependent on the specific species of fungus being controlled, as well as the timing of its application (Camili *et al*., 2007; Maia *et al*., 2010; Qiu *et al*., 2014). Therefore, when considering the use of chitosan as a disease control agent, it is important to account for these

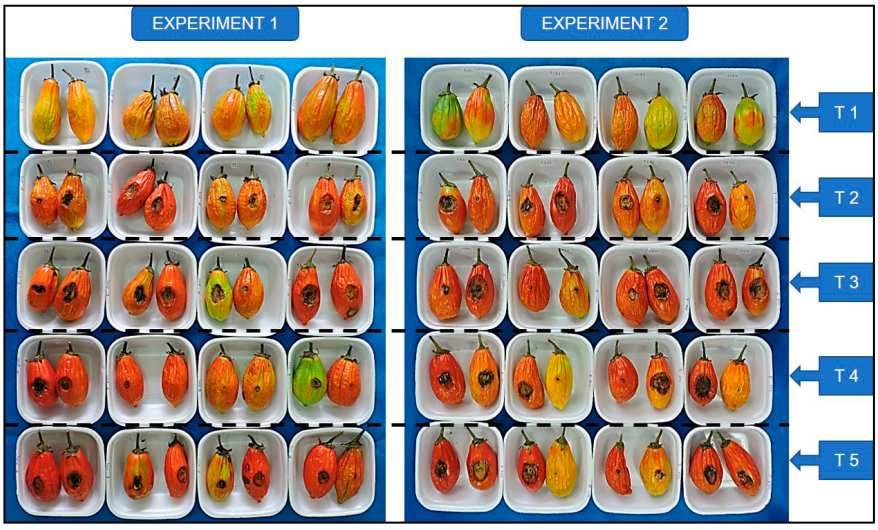

Figure 2. Scarlet eggplant fruits inoculated with *Colletotrichum tamarilloi* (Coll-265) and coated with chitosan at different concentrations at 12 days. T1: uncoated and uninoculated fruits; T2: uncoated and inoculated fruits; T3: fruits coated with 0.1% chitosan and inoculated; T4: fruits coated with 0.2% chitosan and inoculated and T5: fruits coated with 0.3% chitosan and inoculated. Brasília, Embrapa Hortaliças, 2022.

Table 1. Percentage of mycelial growth inhibition (MGI) of *Colletotrichum tamarilloi* (Coll-265) *in vitro* in PDA with chitosan at concentrations of 0.1%, 0.2%, 0.3% and 0.4%: separate (regression) analysis of experiment 2 and joint (F-test) of the two experiments performed with the same conditions. Brasília, Embrapa Hortaliças, 2022.

Treatments	E1	E2			F-test (Pr > f)
		VER[a]	AP[b]	D[c]	
PDAC1**	90.65	92.86	92.98	- 0.12	0.02 s*
0.19 % ***	-	93.59	-	-	-
PDAC2	91.10	93.57	93.21	0.36	0.04 s
PDAC3	91.69	91.40	92.76	- 1.36	0.27 ns
PDAC4	87.24	89.34	89.22	0.12	0.05 s
			R^2 = 0,63		CV (%) = 1.63

*Level of significance of the *in vitro* assays (E1 and E2) for each concentration (joint analysis) by the F-test, where s: significant and ns: not significant. **PDAC1: potato dextrose agar (PDA), 0.1% chitosan; PDAC2: PDA, 0.2% chitosan; PDAC3: PDA, 0.3% chitosan and PDAC4: PDA, 0.4% chitosan. ***Chitosan concentration estimated by the quadratic regression analysis of *in vitro* assay 2 (E2). Regression analysis: (a) VER: Value Estimated by Regression; (b) AP: Average of Points (real data) and (c) D: difference between VER and AP.

Table 2. Area under the disease progress curve (AUDPC, %) of anthracnose caused by *Colletotrichum tamarilloi* (Coll-265) in scarlet eggplant fruits coated or not with chitosan in different concentrations, with separate analysis (regression) of experiment 1 and joint (F-test) of the two experiments carried out in the same condition. Brasília, Embrapa Hortaliças, 2022.

Treatments	Exp. 1			Exp. 2	F-test (Pr > f)
	VER[a]	AP[b]	D[c]		
T1**	-	0.00	-	0.00	1.0000 ns*
T2	-	57.48	-	69.56	0.4434 ns
T3 (0.1)	75.52	74.90	0.62	73.46	0.9352 ns
T4 (0.2)	17.8	17.08	0.72	50.62	0.0618 ns
0.21 %***	-	16.77	-	-	-
T5 (0.3)	59.67	58.26	1.41	63.34	0.7331 ns
		$R^2 = 0.66$			CV (%) = 21.83

*Level of significance of the experiments for each concentration (joint analysis) by the F-test, where s: significant and ns: not significant. **T1: uncoated and uninoculated fruits; T2: uncoated and inoculated fruits; T3: fruits coated with 0.1% chitosan and inoculated; T4: fruits coated with 0.2% chitosan and inoculated and T5: fruits coated with 0.3% chitosan and inoculated. ***Chitosan concentration estimated by the quadratic regression analysis of experiment 1. Regression analysis: (a) VER: Value Estimated by Regression; (b) AP: Average of Points (real data) and (c) D: difference between VER and AP.

factors to ensure optimal performance.

The low effectiveness of chitosan coating in controlling anthracnose in fruits may be attributed to method of inoculation, in this case the pathogen being introduced into the fruit through a wound. Additionally, the inoculation preceded the chitosan coating and, in the interval between the inoculation and chitosan-based treatment, the pathogen initiated the colonization of fruit tissues.

It is known that chitosan has two disease control mechanisms. The first is the direct action of chitosan inhibiting the pathogen growth or spore germination (Goy *et al*., 2009; Qiu *et al*., 2014; Betchem *et al*., 2019). Another effect of chitosan is the induction of resistance that exerts on the plant (or fruit), making it resistant or partially resistant to the pathogen (Bautista-Baños *et al*., 2006; Zeng *et al*., 2010; Betchem *et al*., 2019). In the present research, these effects were not observed or only partially observed, as there was no pathogen-chitosan interaction or because the pathogen had already started to colonize fruit tissues when the chitosan coat was applied, thus suggesting it is not a curative treatment, but eventually a preventive one.

Several studies have shown an additive or synergistic effect of chitosan in controlling postharvest diseases in fruits when used in combination with other products. These studies have found that the antimicrobial activity and protective effect of chitosan can be significantly enhanced by combining it with other natural products. A mixture of chitosan and glycerol (Malmiri *et al*., 2011), cassava starch (Campos *et al*., 2011), propolis (Barrera *et al*., 2015), gelatin and thyme essential oil (Jovanovic *et al*., 2016), olive leaf extract (Khalifa *et al*., 2017), and *Ruta graveolens* essential oil (Peralta-Ruiz *et al*., 2020) have been found to be particularly effective, demonstrating the potential of chitosan as a fungicide or protective barrier in sustainable agriculture. Future research should explore the effectiveness of chitosan in combination with plant extracts or other fungicidal products for controlling scarlet eggplant anthracnose, taking into consideration the inoculation method.

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