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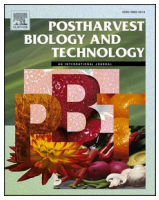

Chitosan inhibits the growth of *Penicillium digitatum* and *Penicillium italicum* and protects oranges from green and blue mold during postharvest

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ABSTRACT

Postharvest losses in oranges caused by *Penicillium* spp. infections are a major concern for the citrus industry worldwide. In this study, we examined the antimicrobial properties of chitosan against various *Penicillium* strains, including some resistant to fungicides. We also assessed the effectiveness of chitosan as a protective coating for navel oranges to prevent infections. Chitosan at 0.5 % concentration in media inhibited *Penicillium digitatum* growth, while *Penicillium italicum* required 1 % chitosan for complete inhibition. We then coated oranges by dipping them with chitosan, either alone or combined with commercial wax, to compare their effectiveness in controlling *Penicillium* infections against a conventional treatment with fungicide and wax. Additional controls included oranges treated with wax only or left untreated. Oranges were later contact-inoculated with *P. digitatum* and *P. italicum* strains under favorable conditions for disease development. Oranges coated with chitosan 1 %, 2 %, and 1 % + wax had a significantly lower cumulative incidence of infections than those treated with wax only or left untreated. Chitosan-based edible coatings also outperformed fungicide and wax treatments. Chitosan 2 % reduced the incidence of decay caused by fungicide-resistant *P. digitatum* and *P. italicum* strains by 68 % and 76 %, compared to conventionally treated oranges. Additionally, combining chitosan with wax extended the shelf life and enhanced the marketability of the oranges compared to all other treatments. These findings suggest chitosan as a viable complement or alternative to synthetic fungicides for managing green and blue mold on navel oranges in the postharvest supply chain.

1. Introduction

Fungal diseases are a major contributor to postharvest losses of fruit, presenting a global challenge from both economic and social perspectives (FAO: Food and Agriculture Organization,2014, FAO: Food and Agriculture Organization,2019, FAO: Food and Agriculture Organization,2022; Porat et al., 2018; Zhang et al., 2021). Fungal pathogens can remain quiescent in the fruit until conditions become suitable for their activation or until they spread through contact with other infected products during storage and transportation. Managing these pathogens is particularly difficult due to their wide host ranges, environmental resilience, capacity to infect after a latency period, and the potential to develop resistance to fungicides (Janisiewicz and Korsten, 2002; Petrasch et al., 2019a; Sánchez-Torres, 2021; Zhang et al., 2021). Furthermore, some pathogenic fungi can adapt their infection strategies based on the ripening stage of fruit (Petrasch et al., 2019b) or manipulate the ripening process to create a more favorable environment before colonization (Silva et al., 2023).

Oranges are highly susceptible to infections by *Penicillium digitatum* Sacc and *Penicillium italicum* Wehmer, the causal agents of green and blue mold, respectively (Bhatta, 2022; Li et al., 2022). These pathogens pose a significant global threat to citrus production worldwide, and despite the risks related to their use, chemical fungicides application remain the primary strategy for managing *Penicillium* spp. infections (Kanashiro et al., 2020). Moreover, resistance phenomena to key synthetic fungicidal active ingredients, such as imazalil, were observed more than 30 years ago (Eckert et al., 1994). Given these challenges, there is a pressing need for effective and sustainable alternatives.

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Regulatory restrictions on synthetic fungicides, along with growing consumer concerns about pesticide residues, have spurred the search for alternative disease management strategies (Romanazzi et al., 2022). Among these alternative solutions, chitosan, a biopolymer derived from chitin, has gained attention as a potential edible coating to enhance or even replace traditional disease control methods (Priya et al., 2023; Romanazzi and Moumni, 2022). Chitosan is classified as a Generally Recognized as Safe (GRAS) substance by the United States Food and Drug Administration (FDA) and was one of the first basic substances approved under European Regulation (EC) 1107/2009. Besides forming a protective film on fresh produce, chitosan activates the plant's natural defense mechanisms and exerts direct toxicity against pathogens (Prusky and Romanazzi, 2023; Romanazzi et al., 2009, 2018). Its antimicrobial action is mainly attributed to its positive charge, which can alter fungal cell membrane permeability, potentially leading to cell death (Goy et al., 2009; Kong et al., 2010; Rabea et al., 2003). While the antimicrobial properties of chitosan are influenced by various factors, the exact mechanism behind its effectiveness remains somewhat elusive (Rajestary et al., 2021; Riseh et al., 2022). Chitosan postharvest treatments are well-established in general fruit research and have shown promising results against *Botrytis cinerea* in table grapes (Romanazzi et al., 2002), *Monilinia fructicola* in peaches (Casals et al., 2012), and other fungal pathogens affecting sweet cherries and strawberries (Feliziani et al., 2013; Romanazzi, 2010; Romanazzi et al., 2013). However, its specific postharvest use for preserving the shelf life of citrus requires further investigation to optimize concentrations and application methods, as *Penicillium* spp. are less sensitive to this biopolymer (Rajestary et al., 2021; Romanazzi et al., 2018). Chitosan-based coatings, when enriched with other natural bioactive compounds, represent a promising strategy for the postharvest preservation of citrus. This approach combines antifungal effectiveness with the ability to deliver other functional ingredients (Li et al., 2024; Palou et al., 2011; Usman et al., 2025).

In this study, we assessed the antimicrobial activity of chitosan against various wild-type (WT) and fungicide-resistant (FR) strains of *Penicillium* spp. *in vitro*. Our primary focus was on its effectiveness in preventing green and blue mold on oranges when used as a coating. We then utilized a reliable inoculation method that simulated the contact inoculations typically occurring during the storage and distribution of citrus fruits. This approach demonstrated the potential of chitosan as a practical and low-risk solution for managing postharvest diseases in citrus, thereby reducing the reliance on synthetic fungicides.

2. Materials and methods

2.1. Fungal and plant material

The *in vitro* antimicrobial activity of chitosan was evaluated against several *Penicillium* spp. isolates from California, U.S. (Table 1). Two independent trials were conducted: one to assess chitosan effectiveness against ungerminated spores and another against actively growing mycelia. In the first trial, *P. italicum* (WT 3212 and FR 6125) and *P. digitatum* (WT 2388 and FR 3189) were evaluated. In the second trial, *P. italicum* (3212 WT and 6125 FR), *P. digitatum* (WT 2388 and FR strains 3189, 2850, 2851, 3455), and *P. expansum* (2239 WT and FR strains 3400, 2236, 2963) were included. The FR strains were confirmed to be imazalil-resistant using the spiral gradient dilution technique (Section 2.2.1).

Washington navel oranges, *Citrus sinensis* L., were collected from the Johnston Farms packing house in Bakersfield, California. Untreated fruit were collected immediately before entering the packing house processing line, with a maximum storage duration of one day at approximately 10 °C. Oranges showing signs of mechanical damage, incorrect maturity, physical damage, or diseases were discarded.

2.2. In vitro trials

2.2.1. Spiral gradient dilution (SGD) method to determine fungal resistance to imazalil

The effective concentrations of imazalil required to inhibit fungal growth by 50 % (EC_{50}) and 99 % (EC_{99}) were determined for all strains using the SGD method (Förster et al., 2004). Briefly, 150-mm PDA plates (3.3 mm thick) were treated with 50 µL of 400 ppm imazalil in a spiral pattern to create a radial concentration gradient; controls received sterile Milli-Q water. Plates were incubated at 25 °C for 4 h to allow absorption, and the central 28 mm was removed to prevent bridging growth. A 10 µL spore suspension (1000 spores μL^{-1}) was applied at the plate periphery and spread toward the center, with two replicates per strain. EC_{50} and EC_{99} values were calculated using the R package ECX (Torres-Londoño et al., 2016) with plate and solution parameters. Fungicide-resistant strains *P. digitatum* 3189 and 3455 and *P. italicum* 6125 showed no inhibition at the tested range (0.03–2.27 µg mL^{-1}) (Figure S1) and were selected for in vivo trials. The remaining strains displayed varying susceptibility, with *P. digitatum* and *P. italicum* showing approximately fourfold lower EC_{50} and EC_{99} values than *P. expansum* (Table S1).

2.2.2. Fungal inoculations

Chitosan antimicrobial activity was evaluated using two *in vitro* trials: one with direct spore inoculation and another targeting actively growing mycelia (i.e., fungi that have passed the germination stage). This distinction is important because fungi tend to be more aggressive and resistant to antimicrobial compounds once germination is complete.

For the spore inoculation trial, 10 µL of spore suspension (1000 spores μL^{-1}) was dispensed onto Petri dishes containing PDA amended with chitosan.

A novel approach, the Spore-plug transfer method (Figure S2), was developed to assess chitosan effectiveness against actively growing mycelia. A PDA plug (6.65 mm diameter and 1.2 mm high, excised from a plate with 10 mL of PDA) was placed in the center of the plate and a

Table 1
Characteristics of the *Penicillium* spp. isolates from California used in this study and their fungicide sensitivity.

Species	Isolate number	Host species	Isolation year	Symptoms	Information on fungicide sensitivity[a]
P. digitatum	2388 (WT)	*Citrus limon*	2001	Fruit rot	Sensitive to all fungicides
P.digitatum	3189	(lemon)	2003	Fruit rot	IMZ and TBZ-resistant
P. digitatum	2850		2003	Fruit rot	PYR-resistant
P. digitatum	2851		2003	Fruit rot	
P. digitatum	3455		2008	Fruit rot	IMZ-resistant
P. italicum	3212 (WT)		2003	Fruit rot	IMZ-sensitive
P. italicum	6125		2020	Fruit rot	PRO, CYP, IMZ, and AZO-resistant; FLU-sensitive
P. expansum	2239 (WT)		2001	Fruit rot	Sensitive to all fungicides
P. expansum	3400	n.a.	2008	n.a.	PYR-resistant
P. expansum	2236	*Pyrus communis* (pear)	2001	Fruit rot	TBZ-resistant
P. expansum	2963		2006	Fruit rot	PYR-resistant

[a] IMZ: imazalil, TBZ: thiabendazole, PYR: Pyrimethanil, PRO: propiconazole, CYP: cyprodinil, AZO: azoxystrobin, FLU: fludioxonil. **n.a.**: not available.

10 μL drop of spore suspension was applied directly on top of the plug. This allowed the fungus to germinate and establish active mycelial growth while minimizing the risk of spore spread during plug transfer, which could lead to inaccurate radial growth measurements. All inoculations were performed under a laminar flow hood.

2.2.3. Treatments

Substrates were prepared by amending PDA with chitosan (Chitosano denso: chitosan hydrochloride 50 % with molecular weight 47–65000 Da and degree of deacetylation ≈75 %, Agrilaete s.r.l., Italy) according to Moumni et al. (2021). Water and chitosan solutions were added to the cooled, double-concentrated PDA suspension before agar solidification. A 4 % (w v^{-1}) chitosan mother solution (see Section 2.3.2) was prepared and then diluted to obtain 30 mL of substrates per plate (Petri dishes, diameter 90 mm). Concentrations below and above 1 % were selected, considering 1 % as the reference value in the literature for postharvest pathogen control (Rajestary et al., 2021). In the first trial (spore inoculations), the following chitosan concentrations of 0.1 %, 0.25 %, 0.5 %, 0.75 %, 1 %, 1.5 %, and 2 % were tested, while in the second trial involving all strains, concentrations of 0.25 %, 0.5 %, 1 %, 1.5 %, and 2 % chitosan were evaluated. The final PDA concentration was maintained at 40 g L^{-1} in all treatments. Sterilized distilled water was used in all preparations, and the chitosan + PDA mixtures were homogenized using stir bars and magnetic stirrers. Control treatment consisted of 40 g of PDA dissolved in 1 L of sterilized distilled water. Three replicates per concentration and treatment were performed in each trial.

2.2.4. Evaluations and data analysis

The mean colony diameters were measured daily from 2 days post inoculation (dpi) using two orthogonal measures. Assessments continued until control plates were completely covered by mycelium. For plates inoculated with PDA plugs, the plug diameter was subtracted from the total colony diameter. All inoculated plates were sealed with micropore tape (3 M Company, St. Paul, MN, US) and stored at room temperature (21 ± 2 °C). The daily growth rates of the *Penicillium* spp. strains were calculated from the following formula:

$$\text{Growth rate}(mm\ day^{-1}) = \frac{d_t - d_{t-1}}{t - (t-1)}$$

where t represents the dpi and d_t represents the mycelial growth diameter at dpi t. Additionally, mycelial growth inhibition (%) was calculated after 10 dpi.

2.3. In vivo trials

2.3.1. Treatments

Three chitosan-based treatments were evaluated: chitosan 1 %, 2 %, and 1 % combined with wax. These treatments were tested for preventing infections caused by *P. digitatum* 2388 WT, *P. italicum* 3212 WT, and FR *P. digitatum* 3189 and *P. italicum* 6125 strains. Control treatments were imazalil (400 ppm) combined with wax, wax alone, and untreated oranges.

Before application, oranges were washed in a 10 % chlorine solution and then rinsed three times with water. Once treated, oranges were dried at room temperature before inoculation. The imazalil + wax coating was applied following a protocol that simulates commercial packing house conditions: oranges were first dipped in a 400 ppm imazalil solution (heated at 50 °C) for 20 s, then sprayed and massaged to evenly distribute the wax, leaving ≈ 0.9 mL per orange.

2.3.2. Chitosan coatings application

Chitosan solutions were prepared in a laminar flow hood using sterile distilled water and autoclaved tools. The same formulation described in Section 2.2.3 was applied to oranges. Since this formulation is separated into two phases, thorough homogenization is required prior to use. Once a homogeneous paste is obtained at a 50 % concentration, 80 g of the paste were slowly added in 1 L of sterilized distilled water under vigorous agitation to obtain a mother solution of 4 % (w v^{-1}) active ingredient. This 4 % solution was diluted to get the desired concentrations and volumes.

Stand-alone chitosan coatings were applied by dipping and rubbing the fruit in solutions for 30 s. Combining chitosan and carnauba wax was not feasible due to the sedimentation of the biopolymer once mixed with the liquid wax. To overcome this, a bilayer approach was adopted, inspired by Gutiérrez-Pacheco et al. (2020): chitosan-treated oranges were dried in a ventilated oven at 37 °C to set the film before being dipped in wax.

2.3.3. Inoculation and assessment of target fruit

Fruit inoculation was performed following the non-wounding contact inoculation method developed by Sbodio et al. (2024), with some modifications in the inoculation, contact, and storage times. In this study, source oranges were kept at room temperature overnight before being stored at 10 °C, resulting in a higher yield of successful infections. Also, source oranges were removed after 48 h of contact, and target fruit were stored at 12.5 °C under high (95 %) relative humidity (RH) for 12 days.

Each source orange was wounded and inoculated with 10 μL of spore suspension (1000 spores μL^{-1}) at three different points. Inoculated oranges were stored at 10 °C under high RH (95 %) until the lesion reached diameters of 30–40 mm. At that point, each source orange was placed atop three either untreated or treated, non-wounded target oranges for contact inoculation under greenhouse conditions (24 °C ± 1 and 95 % RH).

Target oranges were assessed for disease development every two days post contact-inoculation (dpci), starting at 2 dpci, when source oranges were removed. Four replicates of seven or eight fruits were evaluated, resulting in 30 oranges in total per treatment. The 30 contact-inoculated oranges were stored divided in four boxes at 12.5 °C with RH close to 100 %. At each assessment, disease incidence and severity were recorded, and fruit were photographed to monitor the disease development using a Nikon D5100 DSLR Camera fitted with an 18–55 mm f/3.5–5.6 lens. Disease incidence was calculated as the percentage of infected oranges per box. Disease severity was estimated using a caliper, measuring the mean diameters of the lesions with two orthogonal measures. The percentage reductions of cumulative disease incidence were also calculated compared to controls. To evaluate treatment effects on the infectious process, new symptom appearances on oranges, new external mycelial manifestation (NEM), and new sporulation (NS) were determined on diseased oranges for each time point. NEM consisted of the appearance of white hyphae growth, while NS was characterized by the formation of green (*P. digitatum*) or blue (*P. italicum*) mycelia coloration. Fruit exhibiting infections outside the contact area or caused by decay organisms other than the inoculated strains were excluded from the experiment.

Multispectral images were also taken of oranges treated with chitosan + wax and imazalil + wax and contact-inoculated with *P. italicum* FR strains. Pictures were compared with a negative control that was not contact-inoculated. A VideometerLab4 system (Videometer, Hørsholm, Denmark), equipped with a high-resolution monochromatic camera and LED-based multispectral capabilities, captured 19 monochrome images across wavelengths from 365 to 970 nm, including longpass filters, totaling 50 spectral bands. Images were segmented to isolate the oranges and remove the background. Pixels from both infected and healthy areas were collected, and their reflectance values were used to generate a normalized Canonical Discriminant Analysis (nCDA) transformation that accentuated class disparities while reducing within-class variations. This transformation was then applied to classify the remaining samples as healthy or infected. All steps were performed using VideometerLab software version 3.24.11.

2.3.4. Effects of the treatments on fruit quality and marketability

Oranges coated with chitosan 1 %, 2 %, and chitosan 1 % + wax were also evaluated for weight loss during shelf life storage at room temperatures (21 ± 2 °C). Fruit treated with imazalil + wax, wax alone, and untreated oranges were used as controls. For each treatment, 30 oranges were weighed daily, starting one day post-treatment (dpt). At 14 dpt, a visual quality perception test was conducted with 21 people using oranges from all treatments except chitosan 1 %, simulating the time of purchase. Each participant rated a tray containing ten oranges per treatment on a seven-point scale: 1 (dislike extremely), 2 (dislike very much), 3 (dislike slightly), 4 (neither like nor dislike), 5 (like slightly), 6 (like very much), 7 (like extremely).

2.4. Statistical analyses

Data were analyzed using ANOVA at a 95 % confidence level, with Tukey post hoc test ($p \leq 0.05$) used to determine significant differences among treatments. The assumptions of ANOVA, including residual normality and homogeneity, were assessed using the Shapiro-Wilk test ($p \leq 0.05$) and the Levene test ($p \leq 0.05$), respectively. For datasets that did not meet ANOVA assumptions, a Box-Cox transformation was applied. If normality was still not achieved, the Kruskal-Wallis test ($p \leq 0.05$) and the nonparametric multiple comparison LSD test were performed. Additionally, for the *in vitro* trials, hierarchical clustering analysis was conducted (Figure S3).

3. Results

3.1. Chitosan inhibits Penicillium spp.spore germination and mycelial growth in vitro

All chitosan concentrations significantly reduced growth rates compared to the PDA control plates following spore inoculation, demonstrating a strong antifungal activity (Figure S4). Even the lowest concentration tested (0.1 %), effectively limited fungal growth. No significant differences were found between chitosan concentrations of 0.5 % and higher, resulting in similar growth rate control (Figure S4). Complete fungal inhibition was observed at 0.75 % chitosan for most strains, except for *P. italicum* WT, which required a 1 % concentration for full inhibition (Table S2).

An additional *in vitro* trial was conducted before selecting concentrations for *in vivo* testing on fruit to assess the effectiveness of chitosan against actively growing mycelia, adopting the "Spore-plug transfer" method (Section 2.2.2, Figure S2). We observed that all chitosan concentrations significantly reduced the growth rates of all *Penicillium* spp. strains compared to the PDA control (Fig. 1**A-B**), including the lowest tested concentration (0.25 %). Concentrations of 1 % or higher (1.5 % and 2 %) did not differ significantly in effectiveness, suggesting the growth rate plateaus at this threshold. PDA control, chitosan 0.25 %, and chitosan 0.5 % were significantly different from all other treatments (Fig. 1**B**). Inhibition trends further confirmed this concentration-dependent pattern: for all strains, chitosan concentrations of 0.5 % or higher exhibited significantly greater inhibition than 0.25 %, with a threshold of inhibition at 1.5 % concentration (Table 2). Complete inhibition of *P. digitatum* strains was achieved at 0.5 %, except for *P. digitatum* 3189, which required 1 % chitosan. Similarly, *P. italicum* strains were fully inhibited at concentrations of 1 % or higher. *P. expansum* strains exhibited greater resistance to chitosan, showing variable inhibition levels without ever achieving complete inhibition. The highest inhibition observed for *P. expansum* was nearly 65 % compared to the PDA control in strain 2236 when exposed to chitosan 2 %. Overall, *P. expansum* demonstrated significantly lower sensitivity to chitosan than *P. digitatum* and *P. italicum*, reinforcing the need for higher concentrations or alternative strategies for effective control (Fig. 1).

3.2. Chitosan-based edible coatings prevent fruit decay due to green and blue mold on oranges

The spiral gradient dilution method (Section 2.2.1) confirmed the strong resistance of *P. digitatum* 3189 and *P. italicum 6125* strains to imazalil (Table S1), supporting their selection for subsequent *in vivo* efficacy trials. Based on the *in vitro* results, chitosan 1 % and 2 % concentrations were chosen for testing, with 1 % evaluated as a stand-alone treatment and combined with commercial carnauba wax. Although chitosan 2 % exhibited the highest efficacy, it requires greater amounts of product (and thus a higher cost), making it necessary to assess if the 1 % concentration could provide comparable protection. In untreated fruit, the cumulative disease incidence was nearly 100 % for all strains. All chitosan-based treatments significantly reduced the cumulative disease incidence on oranges at 14 dpci compared to both untreated and wax-coated controls. Notably, when contact inoculations were performed with FR strains, chitosan-based coatings also outperformed imazalil-treated fruit (Fig. 2), always with significant differences. Chitosan 1 % reduced cumulative disease incidence compared to untreated fruit by 50 % on oranges contact-inoculated with *P. digitatum* WT, 73 % with *P. digitatum* FR, 50 % with *P. italicum* WT, and 52 % with *P. italicum* FR. Chitosan 2 % further reduced disease incidence by 54 %, 76 %, 71 %, and 80 % for *P. digitatum* WT, *P. digitatum* FR, *P. italicum* WT, and *P. italicum* FR, respectively. Combining chitosan 1 % with wax resulted in reductions of 54 % (*P. digitatum* WT), 64 % (*P. digitatum* FR), 60 % (*P. italicum* WT), and 66 % (*P. italicum* FR) (Fig. 2).

To evaluate whether chitosan prevents infections and to support visual assessments, multispectral imaging (MSI) was used on six oranges per treatment to detect early fungal development and observe disease progression. Multispectral images transformed by nCDA revealed clear distinctions in reflectance spectra between infected and healthy tissues. While the highest separation potential (eigenvalue of 18) was achieved using a combination of 17 wavelengths, we selected a subset of six wavelengths: 365, 490, 570, 645, 880, and 940 nm, spanning the UV, visible, and near-infrared regions, yielding an eigenvalue of 13.54. For example, Fig. 3 shows a comparison between oranges coated with chitosan 1 % + wax and imazalil + wax (positive control) after contact-inoculation with a *P. italicum* FR strain. The absence of red-scale pixels in both negative control and chitosan + wax transformed pictures confirmed that the visually healthy tissue was indeed healthy. The infection was halted in oranges treated with chitosan and wax after 48 h of contact inoculation. In contrast, the presence of dark red pixels in the imazalil-treated fruit indicated active fungal growth (*P. italicum* FR) despite fungicide application.

3.3. Chitosan-based edible coatings extend the shelf life of diseased fruit by delaying symptoms onset

Although chitosan treatments significantly reduced overall disease incidence, some oranges still developed symptoms. However, in all cases, symptom onset was delayed, especially compared to untreated fruit, which rapidly developed infections soon after removal of the source oranges used for contact inoculation. Considering all the strains together, 66 % of the untreated oranges considered infected at 14 dpci appeared symptomatic as early as 2 dpci, immediately after the source fruit were removed. In contrast, most infected oranges treated with chitosan 2 % remained asymptomatic until 6 dpci. Wax coatings also delayed symptom appearance, with most infections occurring by 4 dpci or, in some cases, extending to 6 dpci.

The delay in disease progression varied by pathogen and treatment type. In fruit contact-inoculated with *P. digitatum* WT, all chitosan-based treatments significantly delayed the appearance of first symptoms (Fig. 4A), the new external mycelial manifestation (NEM) (Fig. 4B), and the new sporulations (NS) (Fig. 4C) compared to the untreated control. A similar trend was observed on oranges infected with *P. italicum* WT, except that chitosan 1 % + wax treatment did not produce a significant

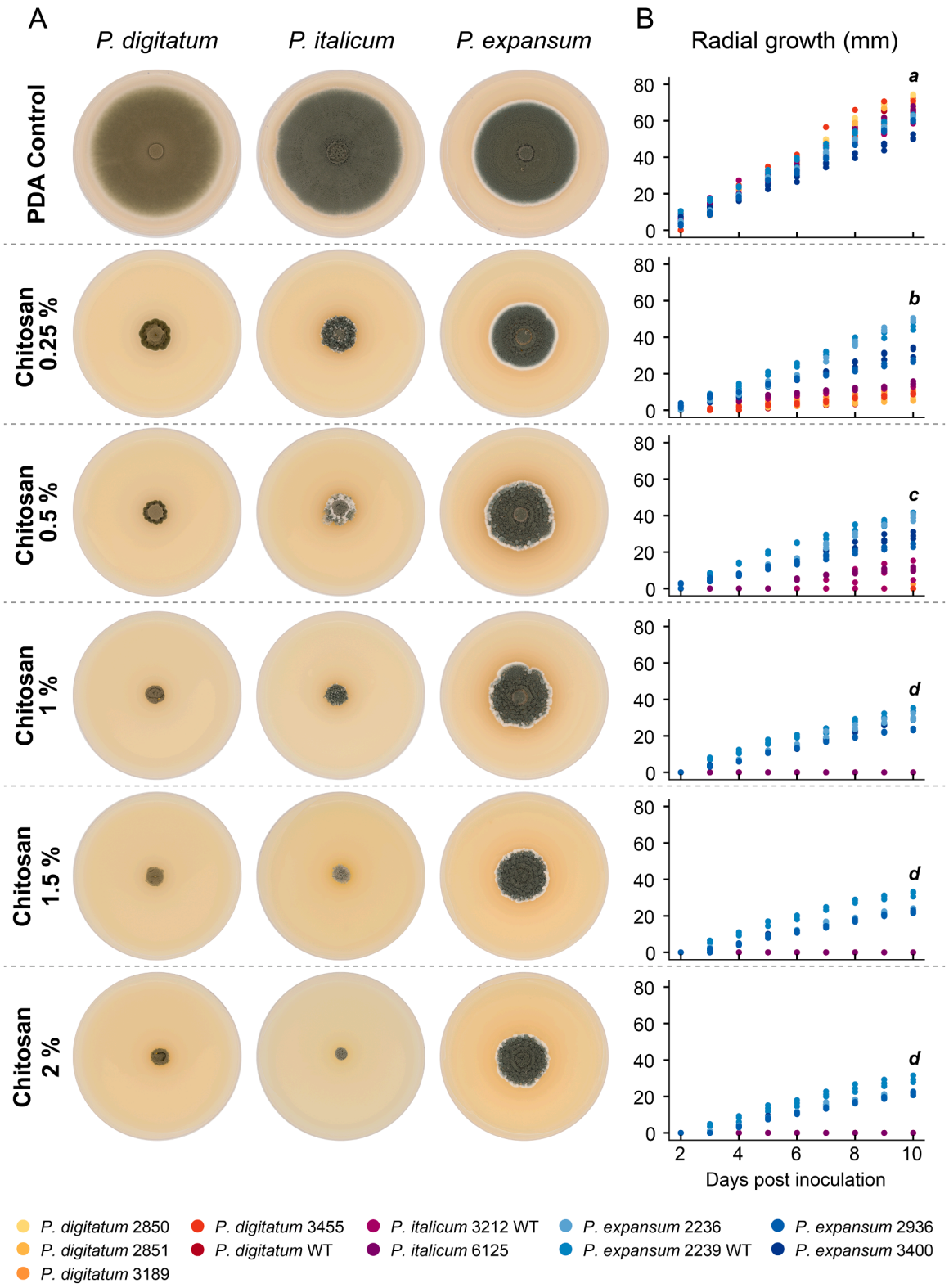

Fig. 1. Chitosan inhibited the growth of *Penicillium* spp. on PDA growth medium, both using fungicide-susceptible and fungicide-resistant strains. (A) Representative examples of *P. digitatum*, *P. italicum*, and *P. expansum* strains at 10 dpi (scale bar 20 mm). Plates were inoculated using the Spore-plug transfer method and stored at 21 ± 2 °C. The growth medium was amended with the following chitosan concentrations: 0.25 %, 0.5 %, 1 %, 1.5 %, and 2 %. (B) Radial growth (mm) over time of *P. digitatum*, *P. italicum*, and *P. expansum* strains on chitosan-amended PDA plates after inoculation with the Spore-plug transfer method (stored at 21 ± 2 °C). Data are reported as the mean of two orthogonal measurements per replicate (each colored point in the graph corresponds to one replicate). Different letters indicate significant differences in growth rates between treatments, according to ANOVA analysis and Tukey post hoc test ($p \leq 0.05$).

Table 2
Inhibition (%) of mycelial growth (mm) at increasing chitosan concentrations for *P. italicum*, *P. digitatum, and P. expansum* strains on chitosan-amended PDA plates. The measurements were taken after 10 days post-inoculation following the Spore-plug transfer method. Plates were stored at 21 ± 2 °C. Data are reported as the mean percent inhibition ± standard error for each strain-concentration combination. Lowercase letters denote significant differences in percent inhibition between the treatments for each strain, while uppercase letters indicate the significant differences in treatments when considering all strains, based on two-way ANOVA analysis and Tukey post hoc test ($p \leq 0.05$).

Strains	Chitosan concentrations				
	0.25 % A	0.5 % B	1.00 % C	1.50 % D	2.00 % D
P. digitatum 2850	91.4 ± 0.3 a	100 b	100 b	100 b	100 b
P. digitatum 2851	92.8 ± 0.0 a	100 b	100 b	100 b	100 b
P. digitatum 3189	83.5 ± 0.8 a	98.7 ± 1.3 b	100 b	100 b	100 b
P. digitatum 3455	86.6 ± 0.3 a	100 b	100 b	100 b	100 b
P. digitatum WT	91.2 ± 0.2 a	100 b	100 b	100 b	100 b
P. italicum 6125	77.5 ± 0.7 a	83.8 ± 0.9 b	100c	100c	100c
P. italicum 3212 WT	78.6 ± 0.6 a	84.0 ± 5.0 b	100c	100c	100c
P. expansum 2236	20.3 ± 0.7 a	37.8 ± 1.7 b	51.2 ± 1.6c	61.8 ± 0.5 d	64.8 ± 0.8 d
P. expansum 2936	55.2 ± 0.6 a	60.1 ± 1.0 b	60.7 ± 0.5 b	63.0 ± 0.7 b	63.6 ± 1.0 b
P. expansum 3400	36.5 ± 3.2 a	42.8 ± 2.2 b	43.0 ± 0.5 b	55.5 ± 1.4c	58.0 ± 0.7c
P. expansum 2239 WT	25.9 ± 1.9 a	34.8 ± 1.4 b	45.0 ± 1.0c	48.3 ± 1.3 cd	52.5 ± 1.7 d

delay. For fruit contact-inoculated with *P. digitatum* FR, chitosan 2 % and chitosan 1 % + wax significantly delayed symptom onset and NEM (Fig. 4A-B). For fruit contact-inoculated with *P. italicum* FR, all chitosan treatments effectively delayed initial symptom expression (Fig. 4A), but no significant differences were observed for NEM and NS (Fig. 4B-4C). Oranges treated with imazalil + wax generally exhibited intermediate disease development, performing better than untreated controls but less effectively than chitosan treatments (Fig. 4). Once symptoms first appeared, disease severity (measured by lesion diameter and progression) was similar across all treatments, except for chitosan 2 % against *P. italicum* WT, which showed a significant reduction in lesions size (Figure S5).

3.4. Combined coating of chitosan and wax limited the cumulative weight loss and improved oranges' visual appeal

All the treatments significantly reduced the cumulative weight loss compared to untreated oranges (Fig. 5A). While stand-alone chitosan coatings showed significantly lower weight loss compared to untreated oranges, they still resulted in significantly greater weight loss compared to wax and standard imazalil + wax treatments, suggesting that chitosan alone may not provide sufficient moisture retention. No significant differences were observed between chitosan 1 % and 2 %, or between imazalil + wax and wax alone. However, the combination of chitosan 1 % + wax outperformed conventional treatments, providing the most effective weight loss limitation. Specifically, chitosan 1 % + wax reduced the cumulative weight loss by 50 %, 49 %, 10 %, 8 %, and 51 % compared to chitosan 1 %, chitosan 2 %, imazalil + wax, wax alone, and untreated fruit, respectively (Fig. 5A).

In addition to weight loss reduction, all treatments significantly improved the perceived quality of the oranges in the sensory evaluation compared to the untreated control, which was rated with the low-quality rate of 3 by 43 % of participants. Almost half (48 %) of participants rated chitosan 1 % + wax trays with a perceived quality score of 6, and, similarly, 43 % assigned the same value to the imazalil + wax trays, making these treatments comparable. For chitosan 2 % and wax-alone trays, the moderately high score of 5 received the highest response rate (38 % and 28 %, respectively) (Fig. 5**B**).

4. Discussion

Consumer awareness regarding pesticide residues in food has increased, driving farmers and research toward minimizing chemical inputs (Romanazzi and Moumni, 2022; Schäufele and Hamm, 2017). Previous authors studied chitosan for citrus postharvest disease management, both as a stand-alone coating and in combination with other antifungal ingredients, reporting promising results (Ipinza-Concha et al., 2024; Palou et al., 2015). However, its effectiveness was often obtained using wound-based inoculation methods that may not always accurately reflect infection processes in commercial settings.

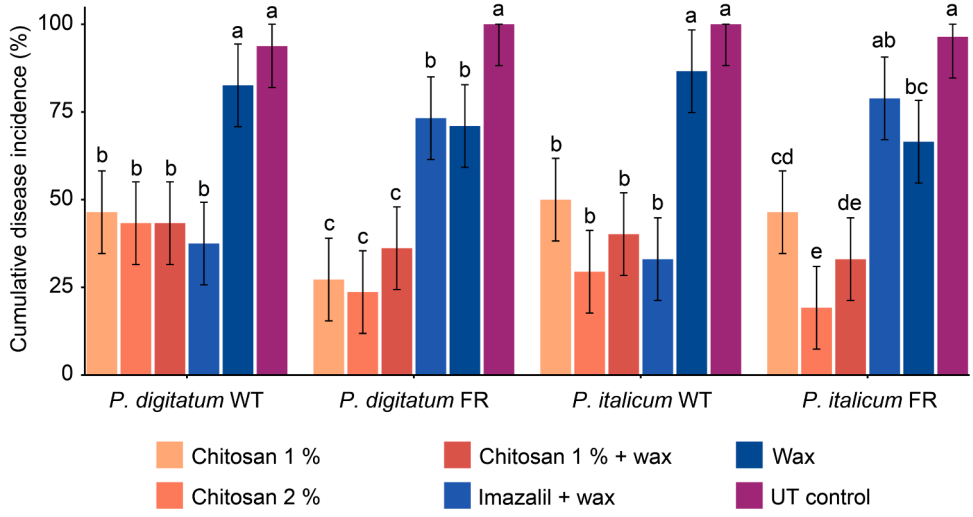

Fig. 2. Chitosan postharvest application reduced green and blue mold incidence regardless of fungicide resistance in *Penicillium* spp. strains. Cumulative disease incidence (%) after 14 dpci at 12.5 °C and under high (95 %) relative humidity was assessed in oranges treated with chitosan 1 %, 2 %, chitosan 1 % + wax, imazalil + wax, and wax alone following contact-inoculation with *P. digitatum* and *P. italicum* fungicide-susceptible (wild type: WT) and fungicide-resistant (FR) strains. Number of oranges per treatment: 30. Data are expressed as means ± standard error. Different letters indicate significant differences among treatments within strains as determined by two-way ANOVA followed by Tukey post hoc test ($p \leq 0.05$). **UT control:** untreated control.

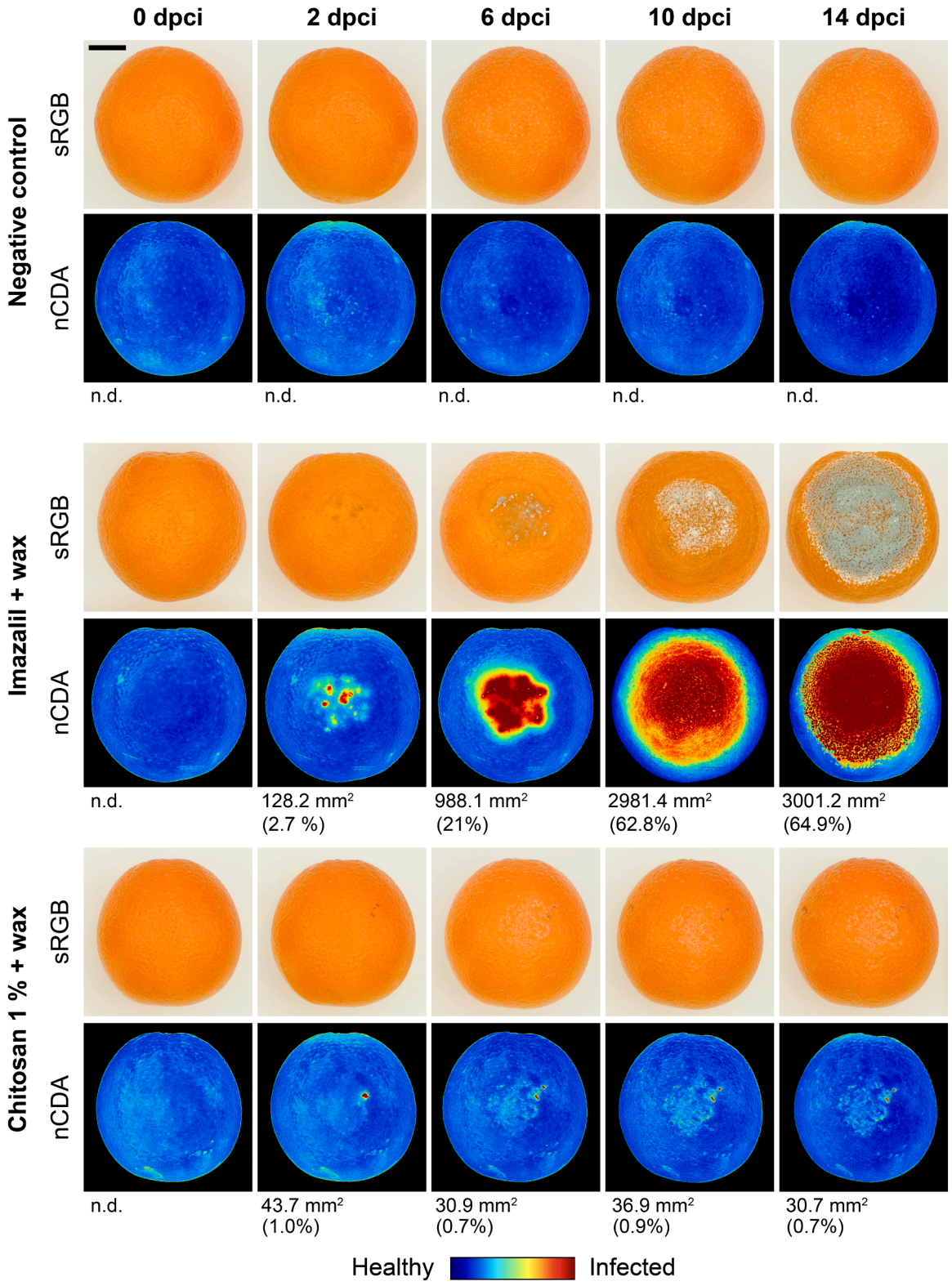

Fig. 3. Multispectral imaging of disease progression in oranges. The images compare a negative control (not inoculated) with fruits treated with chitosan 1 % + wax or imazalil + wax and contact-inoculated with *P. italicum* FR. The chitosan 1 % + wax coating protected the orange from the infection after contact-inoculation. Images of oranges at different time points are shown in raw (sRGB) and transformed (nCDA) formats. Fruit were stored at 12.5 °C and ≈ 95 % relative humidity. The color scale represents transformed pixel values, ranging from healthy (blue or –2) to infected (red or 2); scale bar = 20 mm. Below each orange, infected area is reported in mm^2 and as a percentage of infected tissue. **dpci:** days post contact inoculation. **2 dpci:** 48 h after the first contact with the source of inoculum. **nCDA:** non-canonical discriminant analysis. **sRGB:** standard red, green, blue. **Negative control:** fruit treated with imazalil + wax and mock-contact inoculated, with healthy fruit as "source".

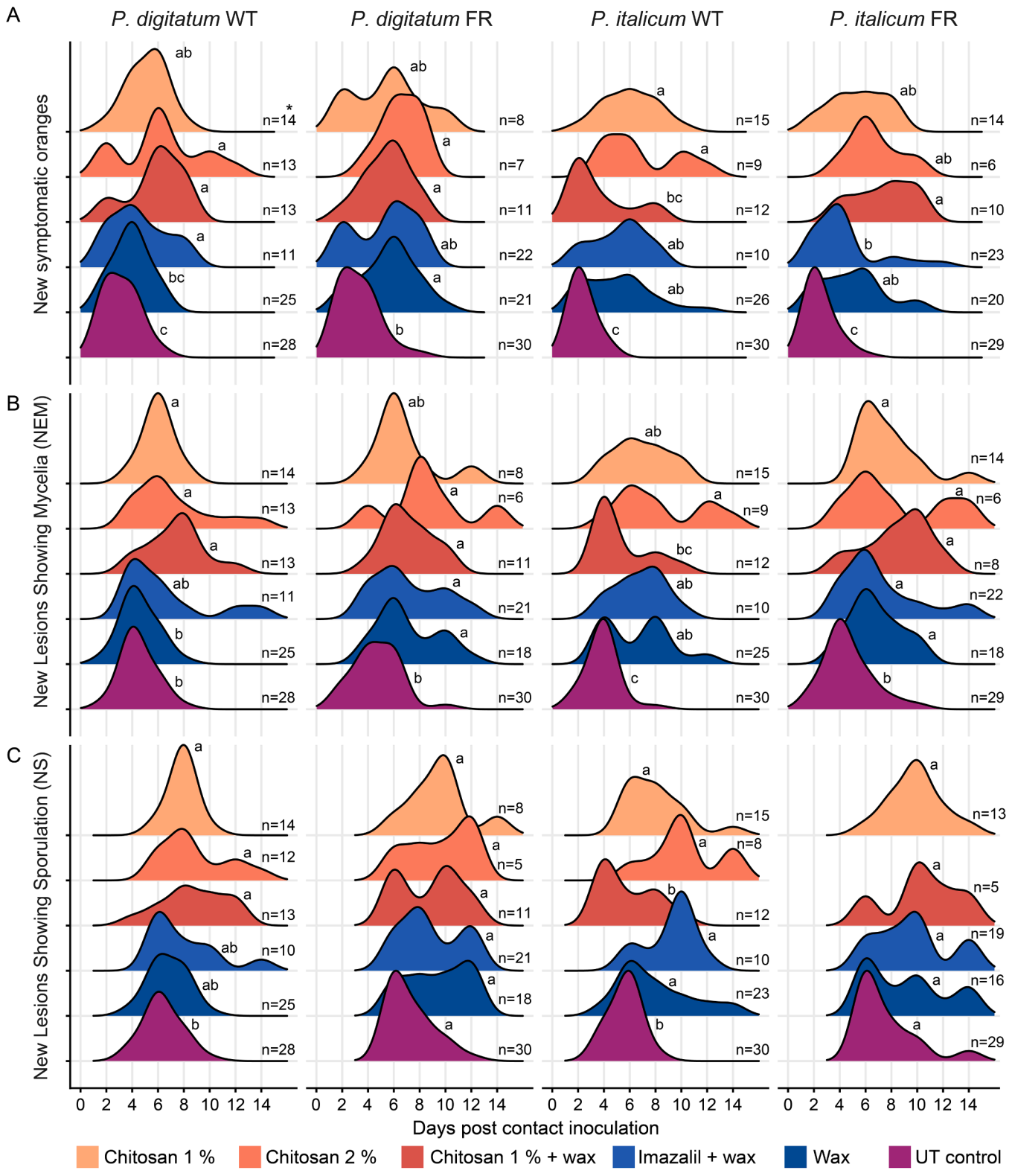

Fig. 4. Chitosan-based postharvest treatments delayed symptoms development in infected oranges, effectively extending their shelf life. (A) Distribution density plot showing the number of new symptomatic oranges at each days post-contact inoculation (dpci). (B) Distribution density plot of new lesions showing external mycelia (NEM), and (C) sporulation (NS). Oranges were stored and monitored for 14 dpci at 12.5 °C and ≈ 95 % relative humidity. Data are grouped by strains and subdivided by treatments within each strain. Different letters indicate significant differences among the treatments according to the Kruskal-Wallis test ($p \leq 0.05$) and the nonparametric multiple comparison nLSD test. ***n** = total **n**umber of infected oranges, out of 30, at the last time point (14 dpci). **UT control:** untreated control.

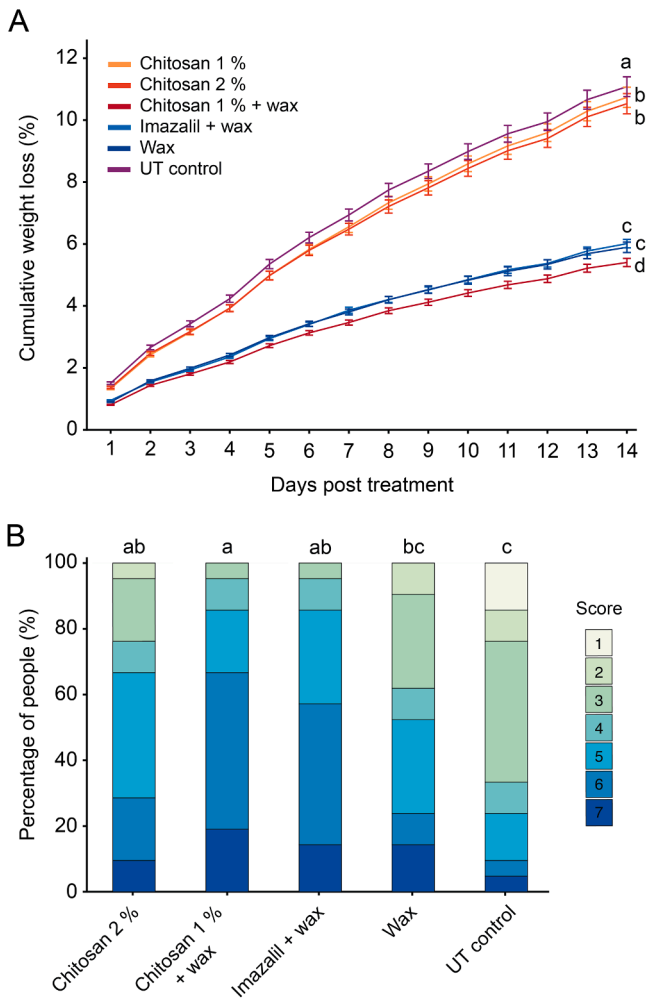

Fig. 5. Chitosan 1 % + carnauba wax improved fruit quality and marketability after 14 days at room temperature. This combined coating limited the weight loss, improving the shelf life of fruit compared to standard commercial oranges. (A) Cumulative weight loss (%) after 14 days post treatment (dpt) at 21 ± 2 °C for oranges treated with chitosan 1 %, 2 %, chitosan 1 % + wax, imazalil + wax, and wax alone. Data are expressed as means ± standard error of 30 oranges per treatment at each time point. Different letters indicate significant differences according to Tukey post hoc test ($p \leq 0.05$) after Box-Cox transformation (with $\lambda = -0.141$). (B) Perceived quality scores expressed as percentages (out of 21 responses per treatment) show that treatments did not affect fruit marketability after 14 dpt at 21 ± 2 °C. Different letters indicate significant differences according to one-way ANOVA and Tukey post hoc test ($p \leq 0.05$). **UT control:** untreated control. **Score:** 1 dislike extremely, 2 dislike very much, 3 dislike slightly, 4 neither like nor dislike, 5 like slightly, 6 like very much, 7 like extremely.

This research was performed adopting the non-wounding contact inoculation method developed by Sbodio et al. (2024), which exposes chitosan-treated fruit to direct contact with infected fruit under conditions highly conducive to disease development. Indeed, when oranges are stored piled in large containers, significant losses can occur as fungal pathogens spread from infected fruit to adjacent healthy tissues or by soiling (Kader, 2002). This method enables a comprehensive assessment of chitosan as a postharvest treatment on orange under favorable conditions for disease development.

Postharvest fungal infections caused by *P. digitatum* and *P. italicum* pose a significant challenge in citrus storage, particularly when FR strains are involved. Chitosan reduced the growth rates of *Penicillium* species, regardless of fungicide resistance, notably inhibiting *P. digitatum* and *P. italicum* on media following spore and mycelia inoculations. The inhibitory effect of chitosan was found to be concentration-dependent, showing a positive correlation with increased concentration. The minimum concentration of chitosan tested in the spore inoculation trial was 0.1 %, while the concentration against actively growing mycelia was 0.25 %. Both concentrations significantly reduced the growth rate of all strains. Concentrations of chitosan at 0.5 % or higher for the spore trial, and 1 % or higher on the actively growing mycelia, did not produce additional reductions in growth rates. This indicates that higher concentrations of chitosan can reach a plateau of effectiveness. Complete inhibition of *P. digitatum* was observed at chitosan concentrations between 0.5 % and 0.75 %, while *P. italicum* required concentrations between 0.75 % and 1 % for full inhibition. These findings align with previous research; for instance, Waewthongrak et al. (2015) reported that a chitosan concentration of 1 and 5 mg mL^{-1} achieved 100 % inhibition of *P. digitatum*. In another study, chitosan-supplemented PDA plates (maximum 3.5 g L^{-1}) reduced *P. digitatum* growth by up to 69 %, although complete inhibition was not achieved (El Guilli et al., 2016). Additionally, chitosan at 0.8 % completely inhibited the spore germination of *P. italicum* and demonstrated enhanced efficacy against mycelial growth when combined with other compounds (Wardana et al., 2022). These results provide solid evidence supporting the use of chitosan in controlling *Penicillium* spp. Its antimicrobial activity increases with concentration until a plateau. Identifying the lowest effective concentration is therefore crucial to ensure both biological efficacy and economic sustainability for commercial application. Strategies such as nanoformulations or combination with other active ingredients may enhance its effectiveness at lower concentrations (Landi et al., 2021; Wang et al., 2023).

P. expansum exhibited greater resistance to chitosan compared to other *Penicillium* species, never reaching full inhibition. Beyond a certain concentration, increasing chitosan levels did not further enhance its inhibitory effects. This resistance has been noted in prior studies using lower chitosan concentrations or chitosan in nanoform (Abdel-Rahman et al., 2021). Moreover, *P. expansum* strains isolated from various crops were never fully inhibited with chitosan concentrations between 0.1 % and 1 % (Liu et al., 2007; Wang et al., 2014). The mechanisms underlying this resistance to chitosan warrant further investigation to develop more effective *in vivo* control strategies for *P. expansum*. While chitosan concentration is a key determinant of its effectiveness, additional and intrinsic factors, including molecular weight, degree of deacetylation, number of positively charged groups, pH, and the acid in which it is dissolved, as well as the organisms involved, can also significantly influence chitosan antimicrobial activity (Badawy and Rabea, 2011; Chien et al., 2007; Rabea et al., 2003; Romanazzi et al., 2009, 2018).

Higher chitosan concentrations were required to achieve high levels of efficacy *in planta*. Although chitosan 1 % has been effective in several pathosystems (Casals et al., 2012; Prusky and Romanazzi, 2023; Rajestary et al., 2021; Romanazzi, 2010; Romanazzi et al., 2002), Romanazzi et al. (2018) recommended utilizing concentrations above 1 % for managing *Penicillium* species. Consistently, in this study, a 2 % chitosan concentration was more effective than 1 % in reducing disease incidence, with significant differences noted for *P. italicum* FR. Overall, both chitosan concentrations delayed the onset of symptoms in infected fruit compared to the controls, thereby extending the shelf life of diseased fruit. A 2 % chitosan solution generally postponed the onset of symptoms in infected oranges more effectively than chitosan 1 %. These findings indicate that when chitosan was unable to prevent infection, it still played a crucial role in delaying disease onset, thereby potentially extending the marketability window of infected fruit before visible decay appears and minimizing the risk of cross-contamination (Kader, 2002). Our study supports the hypothesis that chitosan acts primarily as a physicochemical barrier. Once a pathogen breaches the chitosan barrier and infects the orange tissues, lesion development followed a similar progression across treatments. A comparable barrier effect was observed in wax-coated fruit, where the coating mechanically hindered fungal infection. While wax alone delayed symptom onset compared to

untreated oranges, it did not reduce the overall cumulative disease incidence.

Our findings corroborate and are consistent with other research, although many prior studies employed conventional wound inoculation methods, various chitosan formulations, non-fungicide-resistant strains, and did not always operate under high humidity conditions during the infection process. Chitosan concentrations of 0.5 % or greater have been reported as more effective than lower concentrations against green mold on citrus and were associated with enhanced biochemical defense responses in oranges (El Guilli et al., 2016; Panebianco et al., 2014). Our study did not reveal species-dependent differences in chitosan efficacy on fruit, whereas Zeng et al. (2010) reported higher effectiveness against *P. italicum* than *P. digitatum* in inoculated fruit. Chitosan at 1 % has also demonstrated efficacy against emerging orange pathogens, including *P. citrinum* and *P. mallochii* (Coutinho et al., 2020). Chitosan is recognized to possess three key activities on fruit: antimicrobial properties, elicitation of host defense mechanisms, and creation of a protective film. Based on literature, it has been reported that its effectiveness against postharvest fruit decay is attributed to approximately 30–40 % induction of host resistance, 35–45 % fungicidal activity, and 20–30 % film-forming ability (Romanazzi et al., 2018). Chitosan enhances fruit resistance by triggering plant defense mechanisms similarly to pathogen attacks, leading to the activation of defense-related enzymes, accumulation of reactive oxygen species, and synthesis of antifungal compounds like polyphenols, lignin, flavonoids, and phytoalexins. It can also slow ripening, priming the fruit for faster responses (Landi et al., 2014; Prusky et al., 2025). Chitosan's direct antifungal activity can be associated with multiple mechanisms, including disruption of cell membrane integrity, interference with nutrient uptake, chelation of essential metal ions, and inhibition of spore germination. Additionally, it can also interfere with gene expression and trigger oxidative stress in fungal cells (Badawy and Rabea, 2011; Rabea et al., 2003).

Overall, this research demonstrates that chitosan-based treatments have the potential to be applied in the orange industry as a support or alternative to synthetic fungicides. The observed reduction in disease incidence and the delayed symptom appearance on infected fruit under favorable conditions for the disease indicate that chitosan can contribute to reducing citrus losses and postharvest waste. Furthermore, this formulation's industrial production from crustacean carapaces, an abundant by-product of the seafood industry, yields a model example of a circular economy approach. Although both chitosan 1 % and 2 % concentrations significantly reduced the incidence of green and blue mold, standalone coatings were insufficient in mitigating weight loss compared to conventional treatments. The combination of chitosan with carnauba wax further enhanced moisture retention, outperforming standard fungicide-wax treatments. Additionally, sensory evaluations confirmed that the chitosan-wax coating preserved fruit quality without negatively impacting consumer perception. These findings reinforce previous results reported for other chitosan composite coatings on orange quality (Gao et al., 2018).

Additional research is necessary to validate these findings across various orange varieties and fungal strains. Large-scale experiments will be essential to devise effective strategies for the widespread application of this natural compound on oranges.

5. Conclusion

Chitosan is a promising tool for managing green and blue mold on oranges, particularly given its acceptance in organic farming and its proven effectiveness against fungicide-resistant strains, where conventional fungicides are ineffective. Our results show that chitosan at 0.25–1.5 % inhibits the growth of both fungicide-susceptible and fungicide-resistant *Penicillium* spp. strains, with strong effects starting at 1 %. Chitosan reduced disease incidence on oranges and helps extend the shelf life of infected fruit by delaying the onset of symptoms. However, when applied in stand-alone chitosan coatings, it could not limit fruit weight loss to the same level as oranges treated with commercial wax. Considering this, the best solution for treating oranges in our study was the combination of chitosan 1 % and wax, because standalone wax applications did not control fungal infections. The combination of chitosan and wax effectively controlled disease, improved moisture retention compared to standard treatments, and maintained marketability while being cost-effective compared to the 2 % concentration. These findings support chitosan's potential as a sustainable alternative to synthetic fungicides for oranges, while underscoring the need for further research to optimize concentration, formulation and application protocols for successful commercial-scale adoption.

Author statement

BB-U, and GR conceptualized the research. SP and AOS designed the experiments and developed the methodologies. SP, AOS, TBM, MT, MCC-R, EG-B performed the experiments and participated in data collection. SP, AOS, TBM and MT analyzed the data and produced the figures. SP, AOS, TBM, MT, MCC-R, and EG-B, wrote the manuscript. GR and BB-U reviewed the manuscript. SP, AOS, TBM, MT, MCC-R, EG-B, GR and BB-U participated in the discussion of the results. BB-U and GR provided resources. All authors have provided editorial support to the manuscript.

Author contributions

Barbara Blanco-Ulate and Gianfranco Romanazzi conceptualized the research. Simone Piancatelli and Adrian O. Sbodio designed the experiments and developed the methodologies. Simone Piancatelli, Adrian O. Sbodio, Thomas Bruno Michelon, Muyun Tsen, Mary Carmen Carranza-Rodriguez, and Elia Gutierrez-Baeza performed the experiments and participated in data collection. Simone Piancatelli, Adrian O. Sbodio, Thomas Bruno Michelon, and Muyun Tsen, analyzed the data and produced the figures. Simone Piancatelli, Adrian O. Sbodio, Thomas Bruno Michelon, Muyun Tsen, Mary Carmen Carranza-Rodriguez, and Elia Gutierrez-Baeza wrote the manuscript. Barbara Blanco-Ulate and Gianfranco Romanazzi reviewed the manuscript. All authors participated in the discussion of the results and provided editorial support to the manuscript. Barbara Blanco-Ulate and Gianfranco Romanazzi provided resources.

CRediT authorship contribution statement

Simone Piancatelli: Writing – original draft, Methodology, Investigation, Data curation. **Mary Carmen Carranza-Rodriguez:** Writing – original draft, Investigation. **Muyun Tsen:** Writing – original draft, Methodology, Investigation, Data curation. **Thomas Bruno Michelon:** Software, Formal analysis, Data curation. **Sbodio Adrian Oscar:** Writing – original draft, Software, Methodology, Investigation, Data curation. **Barbara Blanco-Ulate:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Gianfranco Romanazzi:** Writing – review & editing, Project administration, Conceptualization. **Elia Gutierrez-Baeza:** Writing – original draft, Investigation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2025.113955.

Data availability

Data will be made available on request.

References

Abdel-Rahman, F.A., Monir, G.A., Hassan, M.S.S., Ahmed, Y., Refaat, M.H., Ismail, I.A., El-Garhy, H.A.S., 2021. Exogenously applied chitosan and chitosan nanoparticles improved apple fruit resistance to blue mold, upregulated defense-related genes expression, and maintained fruit quality. Horticulturae 7, 224. https://doi.org/10.3390/horticulturae7080224.

Badawy, M.E., Rabea, E.I., 2011. A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. Int. J. Carbohydr. Chem. 2011, 460381. https://doi.org/10.1155/2011/460381.

Bhatta, U.K., 2022. Alternative management approaches of citrus diseases caused by *penicillium digitatum* (Green mold) and *penicillium italicum* (blue mold). Front. Plant Sci. 12, 833328. https://doi.org/10.3389/fpls.2021.833328.

Casals, C., Elmer, P.A.G., Viñas, I., Teixidó, N., Sisquella, M., Usall, J., 2012. The combination of curing with either chitosan or *bacillus subtilis* CPA-8 to control brown rot infections caused by *monilinia fructicola*. Postharvest Biol. Technol. 64, 126–132. https://doi.org/10.1016/j.postharvbio.2011.06.004.

Chien, P.-J., Sheu, F., Lin, H.-R., 2007. Coating citrus (*murcott tangor*) fruit with low molecular weight chitosan increases postharvest quality and shelf-life. Food Chem. 100, 1160–1164. https://doi.org/10.1016/j.foodchem.2005.10.068.

Coutinho, T.C., Ferreira, M.C., Rosa, L.H., De Oliveira, A.M., Oliveira Júnior, E.N.D., 2020. *penicillium citrinum* and *penicillium mallochii*: new phytopathogens of Orange fruit and their control using chitosan. Carbohydr. Polym. 234, 115918. https://doi.org/10.1016/j.carbpol.2020.115918.

Eckert, J.W., Sievert, J.R., Ratnayake, M., 1994. Reduction of imazalil effectiveness against citrus Green mold in california packinghouses by resistant biotypes of *penicillium digitatum*. Plant Dis. 78, 971–974.

El Guilli, M., Hamza, A., Clément, C., Ibriz, M., Ait Barka, E., 2016. Effectiveness of postharvest treatment with chitosan to control citrus Green mold. Agriculture 6, 12. https://doi.org/10.3390/agriculture6020012.

FAO: Food and Agriculture Organization, 2014. Food wastage footprint: full-cost accounting https://openknowledge.fao.org/server/api/core/bitstreams/6a266c4f-8493-471c-ab49-30f2e51eec8c/content (Accessed January 2024).

FAO: Food and Agriculture Organization, 2019. The state of food and agriculture 2019. Moving forward on food loss and waste reduction. (Accessed August 2024). https://openknowledge.fao.org/server/api/core/bitstreams/11f9288f-dc78-4171-8d02-92235b8d7dc7/content.

FAO: Food and Agriculture Organization, 2022. Tackling food loss and waste: a triple win opportunity. https://www.fao.org/newsroom/detail/FAO-UNEP-agriculture-environment-food-loss-waste-day-2022/en (Accessed August 2024).

Feliziani, E., Santini, M., Landi, L., Romanazzi, G., 2013. Pre-and postharvest treatment with alternatives to synthetic fungicides to control postharvest decay of sweet cherry. Postharvest Biol. Technol. 78, 133–138. https://doi.org/10.1016/j.postharvbio.2012.12.004.

Förster, H., Kanetis, L., Adaskaveg, J.E., 2004. Spiral gradient dilution, a rapid method for determining growth responses and 50% effective concentration values in fungus-fungicide interactions. Phytopathology 94, 163–170. https://doi.org/10.1094/PHYTO.2004.94.2.163.

Gao, Y., Kan, C., Wan, C., Chen, C., Chen, M., Chen, J., 2018. Quality and biochemical changes of navel Orange fruits during storage as affected by cinnamaldehyde-chitosan coating. Sci. Hortic. 239, 80–86. https://doi.org/10.1016/j.scienta.2018.05.012.

Goy, R.C., Britto, D.D., Assis, O.B.G., 2009. A review of the antimicrobial activity of chitosan. Polímeros 19, 241–247. https://doi.org/10.1590/S0104-14282009000300013.

Gutiérrez-Pacheco, M.M., Ortega-Ramírez, L.A., Silva-Espinoza, B.A., Cruz-Valenzuela, M.R., González-Aguilar, G.A., Lizardi-Mendoza, J., Miranda, R., Ayala-Zavala, J.F., 2020. Individual and combined coatings of chitosan and carnauba wax with oregano essential oil to avoid water loss and microbial decay of fresh cucumber. Coatings 10, 614. https://doi.org/10.3390/coatings10070614.

Ipinza-Concha, B.M., Dibona-Villanueva, L., Fuentealba, D., Pinilla-Quispe, A., Schwantes, D., Garzón-Nivia, M.A., herrera Défaz, M.A., Valdés-Gómez, H.A., 2024. Effect of chitosan–riboflavin bioconjugate on Green mold caused by *penicillium digitatum* in lemon fruit. Polymers 16, 884. https://doi.org/10.3390/polym16070884.

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40, 411–441.

Kader, A., 2002. Postharvest technology of horticultural crops, 3rd edition. University of California Agricultural and Natural Resources Publication, Richmond, California. 3311.

Kanashiro, A.M., Akiyama, D.Y., Kupper, K.C., Fill, T.P., 2020. *penicillium italicum*: an underexplored postharvest pathogen. Front. Microbiol 11. https://doi.org/10.3389/fmicb.2020.606852.

Kong, M., Chen, X.G., Xing, K., Park, H.J., 2010. Antimicrobial properties of chitosan and mode of action: a state of the art review. Int. J. Food Microbiol. 144, 51–63. https://doi.org/10.1016/j.ijfoodmicro.2010.09.012.

Landi, L., Feliziani, E., Romanazzi, G., 2014. Expression of defense genes in strawberry fruits treated with different resistance inducers. J. Agric. Food Chem. 62, 3047–3056. https://doi.org/10.1021/jf500292q.

Landi, L., Peralta-Ruiz, Y., Chaves-López, C., Romanazzi, G., 2021. Chitosan coating enriched with ruta graveolens L. Essential oil reduces postharvest anthracnose of papaya (*carica papaya* L.) and modulates defense-related gene expression. Front. Plant Sci. 12, 765806. https://doi.org/10.3389/fpls.2021.765806.

Li, X., Sheng, L., Sbodio, A.O., Zhang, Z., Sun, G., Blanco-Ulate, B., Wang, L., 2022. Photodynamic control of fungicide-resistant *penicillium digitatum* by vitamin K3 water-soluble analogue. Food Control 135. https://doi.org/10.1016/j.foodcont.2021.108807.

Li, Y., Guo, L., Wei, J., Yao, Y., Xu, L., Zhou, Z., 2024. Effect of polyethoxylated flavonoids (PMFs)-loaded citral and chitosan composite coatings on citrus preservation: from the perspective of fruit resistance. Food Chem. 22, 101417. https://doi.org/10.1016/j.fochx.2024.101417.

Liu, J., Tian, S., Meng, X., Xu, Y., 2007. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. Postharvest Biol. Technol. 44, 300–306. https://doi.org/10.1016/j.postharvbio.2006.12.019.

Moumni, M., Romanazzi, G., Najar, B., Pistelli, L., Amara, H.B., Mezrioui, K., Karous, O., Chaieb, I., Allagui, M.B., 2021. Antifungal activity and chemical composition of seven essential oils to control the main seedborne fungi of cucurbits. Antibiotics 10, 1–16. https://doi.org/10.3390/antibiotics10020104.

Palou, L., Smilanick, J.L., Montesinos-Herrero, C., Valencia-Chamorro, S., Pérez-Gago, M. B., 2011. Novel approaches for postharvest preservation of fresh citrus fruits. In: Duane, A., Slaker, D.A. (Eds.), Citrus Fruits: Properties, Consumption and Nutrition. Nova Science Publishers Inc, New York, pp. 1–45.

Palou, L., Valencia-Chamorro, S., Pérez-Gago, M., 2015. Antifungal edible coatings for fresh citrus fruit: a review. Coatings 5, 962–986. https://doi.org/10.3390/coatings5040962.

Panebianco, S., Vitale, A., Platania, C., Restuccia, C., Polizzi, G., Cirvilleri, G., 2014. Postharvest efficacy of resistance inducers for the control of Green mold on important sicilian citrus varieties. J. Plant Dis. Prot. 121, 177–183. https://doi.org/10.1007/BF03356507.

Petrasch, S., Knapp, S.J., Van Kan, J.A.L., Blanco-Ulate, B., 2019a. Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *botrytis cinerea*. Mol. Plant Pathol. 20, 877–892. https://doi.org/10.1111/mpp.12794.

Petrasch, S., Silva, C.J., Mesquida-Pesci, S.D., Gallegos, K., Abeele, C. van den, Papin, V., Fernandez-Acero, F.J., Knapp, S.J., Blanco-Ulate, B., 2019b. Infection strategies deployed by *botrytis cinerea*, *fusarium acuminatum*, and *rhizopus stolonifer* as a function of tomato fruit ripening stage. Front. Plant Sci. 10, 223. https://doi.org/10.3389/fpls.2019.00223.

Porat, R., Lichter, A., Terry, L.A., Harker, R., Buzby, J., 2018. Postharvest losses of fruit and vegetables during retail and in consumers' homes: quantifications, causes, and means of prevention. Postharvest Biol. Technol. 139, 135–149. https://doi.org/10.1016/j.postharvbio.2017.11.019.

Priya, K., Thirunavookarasu, N., Chidanand, D.V., 2023. Recent advances in edible coating of food products and its legislations: a review. J. Agric. Food Res. 12, 100623. https://doi.org/10.1016/j.jafr.2023.100623.

Prusky, D.B., Bi, Y., Li, Y., Guillén, F., Romanazzi, G., 2025. Inducing resistance in fruit delays ripening and reduces postharvest decay. Postharvest Biol. Technol. 230, 113817. https://doi.org/10.1016/j.postharvbio.2025.113817.

Prusky, D., Romanazzi, G., 2023. Induced resistance in fruit and vegetables: a host physiological response limiting postharvest disease development. Annu. Rev. Phytopathol. 61, 279–300. https://doi.org/10.1146/annurev-phyto-021722-035135.

Rabea, E.I., Badawy, M.E.-T., Stevens, C.V., Smagghe, G., Steurbaut, W., 2003. Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4, 1457–1465. https://doi.org/10.1021/bm034130m.

Rajestary, R., Landi, L., Romanazzi, G., 2021. Chitosan and postharvest decay of fresh fruit: meta-analysis of disease control and antimicrobial and eliciting activities. Compr. Rev. Food Sci. Food Saf. 20, 563–582. https://doi.org/10.1111/1541-4337.12672.

Riseh, R.S., Hassanisaadi, M., Vatankhah, M., Babaki, S.A., Barka, E.A., 2022. Chitosan as a potential natural compound to manage plant diseases. Int. J. Biol. Macromol. 220, 998–1009. https://doi.org/10.1016/j.ijbiomac.2022.08.109.

Romanazzi, G., 2010. Chitosan treatment for the control of postharvest decay of table grapes, strawberries and sweet cherries. Fresh Prod. 4, 111–115.

Romanazzi, G., Feliziani, E., Santini, M., Landi, L., 2013. Effectiveness of postharvest treatment with chitosan and other resistance inducers in the control of storage decay

of strawberry, 75, 24-27 Postharvest Biol. Technol. 99, 44–49. https://doi.org/10.1016/j.postharvbio.2012.07.007.
Romanazzi, G., Feliziani, E., Sivakumar, D., 2018. Chitosan, a biopolymer with triple action on postharvest decay of fruit and vegetables: eliciting, antimicrobial and film-forming properties. Front. Microbiol. 9, 2745. https://doi.org/10.3389/fmicb.2018.02745.
Romanazzi, G., Gabler, F.M., Margosan, D., MacKey, B.E., Smilanick, J.L., 2009. Effect of chitosan dissolved in different acids on its ability to control postharvest gray mold of table grape. Phytopathology 99, 1028–1036. https://doi.org/10.1094/PHYTO-99-9-1028.
Romanazzi, G., Moumni, M., 2022. Chitosan and other edible coatings to extend shelf-life, manage postharvest decay, and reduce loss and waste of fresh fruits and vegetables. Curr. Opin. Biotechnol. 78. https://doi.org/10.1016/j.copbio.2022.102834.
Romanazzi, G., Nigro, F., Ippolito, A., Di Venere, D., Salerno, M., 2002. Effects of pre- and postharvest chitosan treatments to control storage grey mold of table grapes. J. Food Sci. 67, 1862–1867. https://doi.org/10.1111/j.1365-2621.2002.tb08737.x.
Romanazzi, G., Orçonneau, Y., Moumni, M., Davillerd, Y., Marchand, P.A., 2022. Basic substances, a sustainable tool to complement and eventually replace synthetic pesticides in the management of pre and postharvest diseases: reviewed instructions for users. Molecules 27, 3484. https://doi.org/10.3390/molecules27113484.
Sánchez-Torres, P., 2021. Molecular mechanisms underlying fungicide resistance in citrus postharvest Green mold. J. Fungi 7, 783. https://doi.org/10.3390/jof7090783.
Sbodio, A.O., Mesquida-Pesci, S.D., Yip, N., Alvarez-Rojo, I., Gutierrez-Baeza, E., Tay, S., Bello, P., Wang, L., Blanco-Ulate, B., 2024. Non-wounding contact-based inoculation of fruits with fungal pathogens in postharvest. Plant Methods 20, 83. https://doi.org/10.1186/s13007-024-01214-2.
Schäufele, I., Hamm, U., 2017. Consumers' perceptions, preferences and willingness-to-pay for wine with sustainability characteristics: a review. J. Clean. Prod. 147, 379–394. https://doi.org/10.1016/j.jclepro.2017.01.118.
Silva, C.J., Adaskaveg, J.A., Mesquida-Pesci, S.D., Ortega-Salazar, I.B., Pattathil, S., Zhang, L., Hahn, M.G., van Kan, J.A.L., Cantu, D., Powell, A.L.T., Blanco-Ulate, B., 2023. *botrytis cinerea* infection accelerates ripening and cell wall disassembly to promote disease in tomato fruit. Plant Physiol. 191, 575–590. https://doi.org/10.1093/plphys/kiac408.
Torres-Londoño, G.A., Hausbeck, M., Hao, J., 2016. ECX: an r package for studying sensitivity of antimicrobial substances using spiral plating technology. Plant Health Progr 17, 188–194. https://doi.org/10.1094/PHP-RS-16-0020.
Usman, I., Sana, S., Jaffar, H.M., Munir, M., Afzal, A., Sukhera, S., Boateng, D.I., Afzaal, M., Urugo, M.M., 2025. Recent progress in edible films and coatings: toward Green and sustainable food packaging technologies. Appl. Food Res., 101070 https://doi.org/10.1016/j.afres.2025.101070.
Waewthongrak, W., Pisuchpen, S., Leelasuphakul, W., 2015. Effect of *bacillus subtilis* and chitosan applications on Green mold (*penicillium digitatum* Sacc.) decay in citrus fruit. Postharvest Biol. Technol. 99, 44–49. https://doi.org/10.1016/j.postharvbio.2014.07.016.
Wang, L., Wu, H., Qin, G., Meng, X., 2014. Chitosan disrupts *penicillium expansum* and controls postharvest blue mold of jujube fruit. Food Control 41, 56–62. https://doi.org/10.1016/j.foodcont.2013.12.028.
Wang, S.Y., Herrera-Balandrano, D.D., Jiang, Y.H., Shi, X.C., Chen, X., Liu, F.Q., Laborda, P., 2023. Application of chitosan nanoparticles in quality and preservation of postharvest fruits and vegetables: a review. Compr. Rev. Food Sci. Food Saf. 22, 1722–1762. https://doi.org/10.1111/1541-4337.13128.
Wardana, A.A., Kingwascharapong, P., Wigati, L.P., Tanaka, F., Tanaka, F., 2022. The antifungal effect against *penicillium italicum* and characterization of fruit coating from chitosan/ZnO nanoparticle/Indonesian sandalwood essential oil composites. Food Packag. Shelf Life 32, 100849. https://doi.org/10.1016/j.fpsl.2022.100849.
Zeng, K., Deng, Y., Ming, J., Deng, L., 2010. Induction of disease resistance and ROS metabolism in navel oranges by chitosan. Sci. Hortic. 126, 223–228. https://doi.org/10.1016/j.scienta.2010.07.017.
Zhang, Z.-Q., Chen, T., Li, B.-Q., Qin, G.-Z., Tian, S.-P., 2021. Molecular basis of pathogenesis of postharvest pathogenic fungi and control strategy in fruits: progress and prospect. Mol. Hortic. 1, 1–10. https://doi.org/10.1186/s43897-021-00004-x.