



Article

Synergistic Postharvest Application of Low-Molecular-Weight Chitosan and Salicylic Acid Suppresses *Penicillium digitatum* Decay and Preserves the Quality of Egyptian Navel Orange (*Citrus sinensis*)

Hamam H. Youns¹, Marwa I. Mohamed¹, Wael M. Ibrahim² and Mohamed F.A. Ahmed^{2*}



¹ Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

² Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Center (ARC), Giza, Egypt.

<https://doi.org/10.37229/fsa.fja.2026.10.12>

*Corresponding author: mohamed_faah@yahoo.com

^{2*}<https://orcid.org/0000-0002-0118-3422>

Future Science Association

Available online free at
www.futurejournals.org

Print ISSN: 2687-8151

Online ISSN: 2687-8216

Received: 8 August 2026

Accepted: 27 September 2026

Published: 12 October 2026

Publisher's Note: FA stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Abstract: This study evaluated the postharvest application of salicylic acid (SA; 4, 8, and 16 mM) and chitosan (CS; 12.4, 31, and 62 mM) for controlling green mold (*Penicillium digitatum*) and maintaining the quality of Navel oranges during cold storage and subsequent shelf-life. *In vitro* assays demonstrated concentration-dependent inhibition of *P. digitatum* mycelial growth, with 16 mM SA and 62 mM CS reducing colony growth by 84.4% and 78.9%, respectively. Scanning electron microscopy confirmed structural damage to fungal hyphae, including deformation, shrinkage, and collapse, particularly following chitosan treatment. On fruit, postharvest dips at the highest concentrations significantly reduced disease incidence and lesion expansion under both natural infection and artificial inoculation conditions. Specifically, 16 mM SA maintained natural disease incidence at 1.67% after 30 days of cold storage plus 5 days of shelf-life, whereas control fruit experienced severe infection approaching 100% under artificial inoculation. Mechanistically, SA and CS treatments induced biochemical host defenses in the fruit flavedo by increasing total phenolic content and elevating the activity of defense-related enzymes, peroxidase (POD) and polyphenol oxidase (PPO). Furthermore, treatment with 16 mM SA and 62 mM CS effectively maintained fruit physical quality by reducing weight loss (3.44–4.23% vs. 9.82% in control after 30 days) and delaying loss of firmness (up to 5.49 kgf vs. 2.79 kgf in control). Physicochemical analysis after 30 days showed higher titratable acidity and improved retention of ascorbic acid (up to 53.90 mg/100 g FW with 16 mM SA), alongside lower TSS/TA ratios compared to untreated controls. Overall, high-concentration postharvest applications of SA (16 mM) and CS (62 mM) represent effective elicitor-based strategies for suppressing green mold,

enhancing biochemical defenses, and preserving the physical and nutritional quality of Navel oranges during prolonged storage.

Keywords: Salicylic acid, Chitosan, *Penicillium digitatum*, Navel orange, Green mold, Postharvest decay, Defense enzymes, Fruit quality.

1. Introduction

Egypt is one of the world's leading citrus-producing countries, with total citrus production reaching approximately 5.86 million tonnes in 2024 (El-Sayed, *et al.*, 2012). Moreover, Egypt has maintained its position as the world's leading exporter of fresh oranges, underscoring the strategic importance of citrus production and international trade to the Egyptian agricultural economy (FAO, 2026; USDA-FAS, 2025). Citrus fruits, particularly sweet orange (*Citrus sinensis*), are economically important but highly perishable, making postharvest quality preservation a major challenge. Mechanical injuries facilitate fungal infection, with *Penicillium* spp. being major causes of postharvest decay, quality deterioration, shortened shelf life, and economic losses (Droby *et al.*, 2009; Palou, 2014; Zhu, 2016; Liu *et al.*, 2019). However, post-harvest losses from fungal rots, particularly green mold caused by *Penicillium digitatum* (Pers.: Fr.) Sacc., can reach 20-80% under favorable storage conditions (high humidity, with range of 20-28°C), severely impacting economic viability in subtropical regions like Egypt's Nile Delta (Macarisin, *et al.*, 2020).

The high infection potential of *P. digitatum* and its ability to spread rapidly among stored fruits make effective postharvest management essential (Cheng *et al.*, 2020; Bhatta, 2022; Wang *et al.*, 2022).

Repeated fungicide applications have accelerated the emergence of resistant *Penicillium digitatum* populations, compromising conventional postharvest disease control and increasing concerns regarding pesticide residues, environmental impacts, and consumer safety (Chen *et al.*, 2023). These challenges underscore the need for effective, environmentally sustainable alternatives that can be integrated into modern integrated pest-management strategies for safer and more sustainable citrus production (Strano *et al.*, 2022).

Salicylic acid (SA) and chitosan (CS) are promising residue-minimizing elicitors for sustainable postharvest disease management in citrus. SA enhances systemic acquired resistance through NPR1-associated signaling and stimulates defense-related enzymes (Ding, *et al.*, 2022), whereas CS combines direct antifungal activity with induction of phenolic accumulation, oxidative enzymes, and cell-wall reinforcement (Romanazzi *et al.*, 2016, 2018; Saleem *et al.*, 2021). Importantly, combined SA-CS treatments can enhance suppression of *Penicillium digitatum* while increasing POD, PPO, PAL, chitinase, β -1,3-glucanase, and phenolic defenses, with concomitant preservation of fruit quality and storage life (Shi *et al.*, 2018; Ibrahim *et al.*, 2024; Piancatelli *et al.*, 2026; Wu *et al.*, 2026). Collectively, these findings support SA-CS integration as a promising sustainable strategy for reducing citrus postharvest decay and improving fruit storability.

In Egypt, the potential of biological and elicitor-based approaches for citrus postharvest disease management remains insufficiently explored, particularly for Egyptian Navel orange under commercial storage and export conditions (El-Garhy *et al.*, 2021). Accordingly, this study evaluated combined chitosan (CS) and salicylic acid (SA) treatments against *Penicillium digitatum*, assessing disease development, defense-related enzymes (POD, PPO, and CAT), total phenolics, and fruit quality during storage at 10 °C and 85% RH. The findings provide evidence for the potential of CS-SA integration as a sustainable strategy to suppress green mold, enhance host defense, preserve fruit quality, and extend the marketable storage life of Egyptian citrus (Moosa *et al.*, 2022).

2. Materials and Methods

2.1. Isolate, fruits, and chemical materials

2.1.1. Pathogen isolate and inoculum

A virulent isolate of *Penicillium digitatum* was obtained from naturally infected navel orange fruits (*Citrus sinensis* L. Osbeck cv. Navel) exhibiting typical green mold symptoms. Symptomatic fruits were collected from commercial packinghouses in Sadat City, Egypt. For pathogen isolation, approximately 5-mm sections were excised from the advancing margins of active lesions, surface-disinfested with 1% sodium hypochlorite (NaOCl) for 1 min, and rinsed three times with sterile distilled water. The disinfested tissues were subsequently blotted dry on sterile filter paper and aseptically transferred onto potato dextrose agar (PDA). The plates were incubated at 25 ± 1 °C in darkness for 7 days. Emerging fungal colonies were subcultured onto fresh PDA to obtain pure cultures. The isolate was provisionally identified as *P. digitatum* based on its characteristic green sporulation and microscopic morphological features, following the diagnostic criteria described by **Qian *et al.* (2019)**.

2.1.2 Used Chemical

Chitosan, purchased from Sigma-Aldrich (St. Louis, MO, USA), with a degree of deacetylation of $89.7 \pm 0.27\%$ and a low molecular weight of 45.87 kDa, was used in this study. The intrinsic viscosity of the chitosan was $264.6 \text{ g} \cdot \text{mol}^{-1}$. Chitosan was solubilized in water acidified with 10% vinegar under continuous magnetic stirring at 50–60 °C, following the procedure described by **Jabnoun-Khiareddine *et al.* (2015)**.

Salicylic acid was also obtained from Sigma-Aldrich (St. Louis, MO, USA). Glacial acetic acid (99–100% purity) was purchased from Al-Gomhouria Co., Cairo, Egypt.

Table (1). Chemical characteristics of salicylic acid, chitosan, and acetic acid used in the study

Compound	Chemical formula	Molecular weight
Salicylic acid	$\text{C}_7\text{H}_6\text{O}_3$	$138.12 \text{ g mol}^{-1}$
Chitosan	$(\text{C}_6\text{H}_{11}\text{NO}_4)_n$	45.87 kDa ($45,870 \text{ g mol}^{-1}$)
Acetic acid	CH_3COOH	60.05 g mol^{-1}

2.2. In vitro antifungal activity of chitosan and salicylic acid against *Penicillium digitatum*

The antifungal activities of chitosan (CS) and salicylic acid (SA) against *Penicillium digitatum* were evaluated using the poisoned food technique following **Moosa *et al.* (2022)**, with minor modifications. PDA was amended with CS at 0.5, 1.0, and 2.0 g L^{-1} (0.05, 0.10, and 0.20%, w/v), after dissolution in 1% acetic acid, and with SA at 0.138, 0.276, and 0.553 g L^{-1} (0.01, 0.03, and 0.06%, w/v). CS-containing media were autoclaved, whereas SA was filter-sterilized and incorporated into cooled molten PDA. Unamended PDA and the corresponding acetic acid treatment served as controls. Approximately 10 mL of each medium was dispensed into 90-mm Petri dishes (five replicates per treatment). A 5-mm mycelial plug from a 7-day-old *P. digitatum* culture was centrally placed on each plate, followed by incubation at 25 ± 1 °C in darkness for 7 days. Colony diameter was then measured along two perpendicular axes, and the percentage inhibition of mycelial growth was calculated according to Equation (1).

$$\text{Mycelial radial reduction (\%)} = \frac{D_c - D_t}{D_c} \times 100$$

where D_c = control diameter, D_t = treatment diameter (**Shi, *et al.*, 2018; Ibrahim, *et al.*, 2024**).

2.3. Scanning electron microscopy (SEM)

In order to perform scanning electron microscopy (SEM), 6-mm-diameter mycelial plugs were excised from 4-day-old *Penicillium digitatum* cultures grown on PDA supplemented with the respective treatments at $20 \mu\text{g mL}^{-1}$ or maintained without treatment as a control. The samples were fixed overnight

at 4 °C in a solution containing 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). The fixed specimens were washed three times with the same buffer for 10 min per wash and subsequently dehydrated through a graded ethanol series (30, 50, 70, 90, and 100%). Following dehydration, the samples were critical-point dried using CO₂, sputter-coated with gold, and examined using a Quanta FEG 250 scanning electron microscope (Czech Republic), according to **Simionato *et al.* (2017)**.

2.4. *in vivo* assays

2.4.1 Pathogen inoculum

Penicillium digitatum was isolated from naturally infected navel orange fruits and identified based on characteristic morphological features, including green conidia and monoverticillate conidiophores. The isolate was maintained on potato dextrose agar (PDA) and incubated at 25 ± 1 °C in darkness for 7 days. Conidia were harvested from actively sporulating cultures by gently rinsing the colony surface with sterile distilled water containing 0.01% Tween 80 and filtering the resulting suspension through sterile cheesecloth to remove mycelial fragments. The conidial concentration was determined using a hemocytometer and adjusted with sterile 0.01% Tween 80 solution to 1 × 10⁶ conidia mL⁻¹, following **Shi *et al.* (2018)**.

2.4.2 Fruit and treatment preparation

Navel oranges (*Citrus sinensis* L. Osbeck cv. Navel), uniform in size (200–250 g) and maturity and free from visible defects, were harvested from commercial orchards in Sadat City, Egypt, and transported to the laboratory within 24 h. Fruits were surface-disinfected by immersion in 1% sodium hypochlorite (NaOCl, v/v) for 2 min, rinsed twice with sterile distilled water, and air-dried at room temperature. For each treatment, concentration, and infection condition, four biological replicates were established, each comprising 30 fruits (120 fruits per treatment).

Two infection conditions were evaluated. For the natural-infection treatment, surface-disinfected fruits were placed in perforated plastic crates under high relative humidity (90–95%) without artificial wounding or pathogen inoculation, thereby providing conditions conducive to natural postharvest infection. For artificial inoculation, a single wound approximately 2 mm deep was made at the equatorial region of each fruit using a sterile scalpel, and 10 µL of a *Penicillium digitatum* conidial suspension (1 × 10⁶ conidia mL⁻¹) was applied directly to each wound. A conidial concentration of 1 × 10⁶ conidia mL⁻¹ is consistent with established experimental inoculation procedures for *P. digitatum* on citrus fruit (**Shi *et al.*, 2018; Qian *et al.*, 2019**).

Following inoculation, the fruits were maintained at 25 ± 1 °C for 4 h to allow wound curing and initial pathogen establishment. They were subsequently stored at 10 ± 1 °C and 85–90% relative humidity for 30 days. At the end of cold storage, the fruits were transferred to 25 ± 1 °C and 85–95% relative humidity for an additional 5 days (days 31–35) to assess post-storage disease development and shelf-life performance. Similar postharvest studies have used controlled temperature and humidity regimes to evaluate the development of *P. digitatum* and the efficacy of postharvest treatments on citrus fruit (**Shi *et al.*, 2018**).

2.4.3. Effects of chitosan (CS) and salicylic acid (SA) on disease incidence and lesion diameter of *P. digitatum* in navel orange fruit under natural infection and artificial inoculation

Chitosan (CS; Mw = 45.87 kDa; 85% degree of deacetylation) was prepared at 2.0, 5.0, and 10.0 g L⁻¹ (0.2, 0.5, and 1.0%, w/v; approximately 12.4, 31.0, and 62.0 mM monomer equivalents, respectively) in 1% (v/v) glacial acetic acid, with the pH adjusted to 5.6 using 1 M NaOH, followed by autoclaving at 121 °C for 15 min. Salicylic acid (SA) was prepared at 0.55, 1.10, and 2.21 g L⁻¹ (0.055, 0.11, and 0.221%, w/v; 4, 8, and 16 mM, respectively) in sterile distilled water and filter-sterilized through a 0.22-µm membrane. The preparation and postharvest use of CS and SA were based on previously reported antifungal applications, particularly against *Penicillium digitatum* on citrus fruit (**Jabnoun-Khiareddine *et al.*, 2015; Shi *et al.*, 2018**).

Disease incidence was assessed for each treatment at each sampling time during storage by recording the number of fruits exhibiting visible green or blue mold symptoms evaluated and calculated

according to the following equation, as described for postharvest citrus decay assessment by **Shi *et al.* (2018)**:

$$\text{Disease incidence (\%)} = \frac{\text{Number of rotten fruit}}{\text{Total Number of Fruit}} \times 100$$

Disease severity was assessed by measuring the diameter of the decay lesion on each fruit using a digital Vernier caliper. Disease severity was expressed according to the equation described by **Masood *et al.* (2010)**:

$$\text{Disease severity (\%)} = \frac{\text{Lesion diameter of fruit}}{\text{Total diameter of Fruit}} \times 100$$

2.4.4. Effects of Chitosan and Salicylic Acid on Total Phenolic Content and Defense-Related Enzyme Activities in Navel Orange Fruit

2.4.4.1. Samples preparation:

Flavedo samples (4 g) were collected from the equatorial region of the fruit peel at 15 and 30 days after treatment, immediately frozen, and stored at -20°C until analysis. Samples were homogenized in 0.2 M potassium phosphate buffer (pH 6.8), filtered through a sintered-glass funnel, and centrifuged at $12,298 \times g$ for 10 min at 4°C . The resulting supernatants were used to determine defense-related enzyme activities and total protein content following **Coseteng and Lee (1987)**.

2.4.4.2. Measurement of total phenolics compounds content

Total phenolic content was determined spectrophotometrically using the Folin–Ciocalteu reagent according to the method described by **Yang *et al.* (2011)**. Results were expressed as grams of gallic acid equivalents (GAE) per gram of fresh weight ($\text{g GAE g}^{-1}\text{FW}$).

2.4.4.3 Assay of Defense-related Enzymes Activities

Peroxidase (POD) activity was assayed by monitoring guaiacol oxidation at 470 nm. The reaction mixture contained 200 μL enzyme extract, 3.0 mL of 25 mM guaiacol in 50 mM sodium acetate buffer (pH 5.5), and 200 μL of 0.5 M H_2O_2 in the same buffer. One unit of POD activity was defined as a 0.01 increase in absorbance at 470 nm and expressed as $\text{U min}^{-1} \text{g}^{-1}\text{FW}$ (**Chen *et al.*, 2019**).

Polyphenol oxidase (PPO) activity was determined using catechol as the substrate by monitoring the increase in absorbance at 420 nm for 5 min. The reaction mixture comprised 0.5 mL enzyme extract, 1.0 mL of 50 mM catechol in 100 mM sodium acetate buffer (pH 5.5), and 4.0 mL of 50 mM sodium acetate buffer (pH 5.5). One unit of PPO activity was defined as a 0.01 increase in absorbance at 420 nm per hour and expressed as $\text{U h}^{-1} \text{g}^{-1}\text{FW}$ (**Chen *et al.*, 2019**).

2.4.5. Evaluate citrus fruit quality parameters during nature infection storage

2.4.5.1. Weight loss measurement

Five fruits from each replicate were individually weighed before treatment (initial weight) and again at the end of the storage period (final weight). Weight loss was calculated as the percentage reduction in fruit mass using the following equation according to **Wang *et al.* (2020)**.

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.4.5.2. Fruit firmness measurement

Firmness was measured at 0, 15, and 30 days of storage using a fruit penetrometer (Bishop, FT327, Italy) fitted with a 3-mm probe. Five fruits per replicate were measured at three equidistant points on the equatorial region after removing a small area of peel. The probe was inserted to a depth of 10 mm, and firmness was expressed as kgf. The mean of the three readings per fruit was calculated, and

the average of the five fruits per replicate was used for statistical analysis (Herrera-González *et al.*, 2024).

2.4.5.3. Measurements of total soluble solids (TSS), titratable acidity (TA), and ascorbic acid / Efficacy of postharvest treatments on quality parameters of Navel Orange

Four fruits from each replicate were peeled on day 30 of storage, and the pulp was manually pressed through cheesecloth to obtain juice. The juice was analyzed for total soluble solids (TSS), titratable acidity (TA), and ascorbic acid content. TSS was determined at 20 °C using a digital refractometer (ATAGO PAL-3, Japan) and expressed as °Brix. Titratable acidity was determined by titrating 5 mL of juice with 0.1 M NaOH to an endpoint of pH 8.3 and expressed as citric acid percentage according to the equation described by Porat *et al.* (2000).

$$\text{Citric acid (\%)} = \frac{\text{Standard solution (N)} \times \text{Base solution (ml)} \times 0.06404}{\text{Total juice volume (ml)}} \times 100$$

Whereas: the equivalent weight of citric acid = 0.06404; Juice volume = 5 mL

TSS/TA ratio. The TSS/TA ratio was determined on day 30 of storage using juice obtained from four fruits per replicate for each treatment. TSS and titratable acidity (TA) were determined as described above, and the ratio was calculated as **TSS (°Brix)/TA (% citric acid)**, providing an indicator of the balance between sweetness and acidity and, consequently, fruit maturity and eating quality (Porat *et al.*, 2000).

$$\text{T.S.S./Acid (\%)} = \frac{\text{T.S.S}}{\text{Total acid content}} \times 100$$

Ascorbic acid content was determined by titration with 2,6-dichlorophenolindophenol (DCPIP), and the results were expressed as mg ascorbic acid per 100 g fresh weight (mg/100 g FW), following the method of Hughes (1983).

2.4.6. Statistical analysis

The collected data were subjected to analysis of variance (ANOVA). The treatment means were compared using the least significant difference (LSD) test at the 5% probability level ($p \leq 0.05$), according to Snedecor and Cochran (1989).

3. Results

In vitro mycelial growth inhibition:

The data presented in Table 2 demonstrate the effects of different concentrations of salicylic acid (SA; 4, 8, and 16 mM) and chitosan (12.4, 31, and 62 mM) on the mycelial growth of *Penicillium digitatum* on PDA medium, relative to the untreated control. Both compounds significantly inhibited fungal growth in a concentration-dependent manner. At the highest concentrations, SA and chitosan reduced colony diameter to 14 and 19 mm, corresponding to 84.4% and 78.9% inhibition of radial growth, respectively, compared with the control. These results demonstrate substantial concentration-dependent antifungal activity of both elicitors against *P. digitatum*, with maximum growth suppression observed at the highest concentrations tested.

Morphological effects of Salicylic acid (SA) and chitosan on *Penicillium digitatum* under scanning electron microscopy (SEM)

Scanning electron microscopy reveals that, distinct ultrastructural alterations in *Penicillium digitatum* following treatment with salicylic acid (SA) and chitosan compared with the untreated control (Fig. A–C). The untreated *P. digitatum* exhibited intact, smooth, and well-defined hyphae and conidiogenous structures, consistent with normal fungal morphology (Fig. 3A). In contrast, SA treatment induced evident surface irregularities, hyphal deformation, and structural collapse (Fig. B).

More pronounced abnormalities were observed following chitosan treatment, including marked hyphal deformation, shrinkage, surface collapse, and disruption of the normal fungal architecture (Fig. C). Overall, these SEM observations demonstrate substantial treatment-associated disruption of the morphological integrity of *P. digitatum*, particularly following chitosan exposure.

Chitosan caused more conspicuous morphological abnormalities, including hyphal shrinkage, collapse and disruption of the normal fungal architecture (Fig. C). These ultrastructural changes provide morphological evidence of the inhibitory effects of SA and chitosan on *P. digitatum*.

Table (2). *In vitro* mycelial growth inhibition of *Penicillium digitatum* on PDA medium amended with chitosan and salicylic acid

Treatment	Concentrations (mM)	<i>P. digitatum</i>	
		Colony Diameter (mm)	Mycelial Radial Reduction (%)
Salicylic acid	4	63.0	30.0
	8	36.0	60.0
	16	14.0	84.4
Chitosan	12.4	70.0	22.2
	31	44.0	51.1
	62	19.0	78.9
Control	Untreated	89.4	-
L.S.D at 1% for:	Pathogenic fungus (P)= 0.58	Treatment (T)= 0.77	T×P=1.32

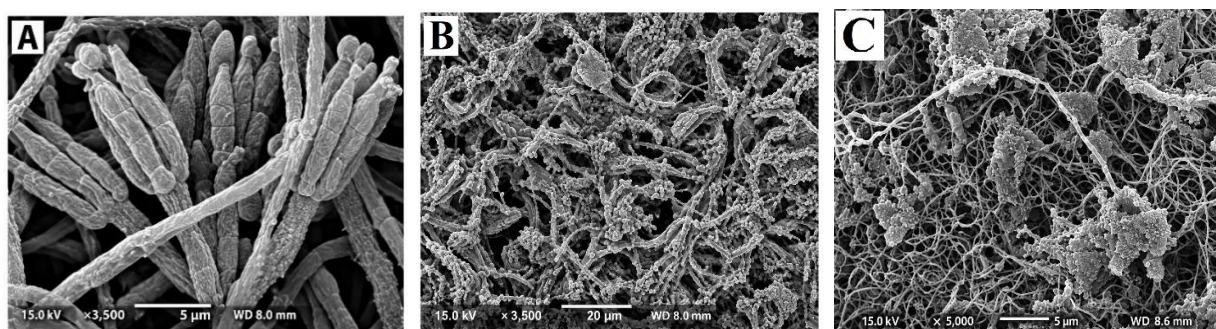


Fig. (A, B and C). Morphological effects of salicylic acid (SA) and chitosan on *Penicillium digitatum* as revealed by scanning electron microscopy (SEM)

Disease incidence under natural infection and artificial inoculation

Data presented in Table 3 illustrate, the efficacy of salicylic acid (SA) and chitosan (CS) in suppressing green mold incidence caused by *Penicillium digitatum* in Navel oranges under natural infection and artificial inoculation during cold storage and subsequent shelf-life. Under natural infection, SA at 16 mM consistently provided the greatest suppression of disease incidence of *P. digitatum*, with values of 0.00, 0.00, and 1.67% after 15 and 30 days of cold storage and 5 days of shelf-life, respectively. Similarly, under artificial inoculation, 16 mM SA resulted in disease incidences of 0.00, 14.17, and 28.33%, respectively, across the three assessment periods.

Chitosan also substantially reduced disease incidence, particularly at the highest concentration (62 mM). Under natural infection, 62 mM CS resulted in incidences of 0.00, 0.83, and 5.00%, whereas under artificial inoculation, the corresponding values were 4.17, 27.50, and 42.50%, respectively. Overall, both SA and CS treatments maintained markedly lower disease incidence than the untreated control throughout storage and shelf-life. Under artificial inoculation, disease incidence in control fruit

approached or reached 100% by the end of shelf-life, while treated fruit exhibited substantially lower incidence. These results demonstrate the concentration-dependent efficacy of SA and CS in suppressing *P. digitatum* development under both natural and artificial infection conditions.

Table (3). Disease incidence (%) of *Penicillium digitatum* in Navel oranges under natural infection and artificial inoculation following postharvest dipping treatments with chitosan and salicylic acid

Treatment	Concentration (mM)	(%) Disease incidence of <i>Penicillium digitatum</i>					
		Natural Infection			Artificial Infection		
		15 Days-Storage	30 Days-Storage	5 Days Shelf-life	15 Days-Storage	30 Days-Storage	5 Days Shelf-life
Salicylic acid	4	0.83	4.58	10.00	11.25	38.75	51.67
	8	0.00	2.50	7.50	5.00	27.50	50.83
	16	0.00	0.00	1.67	0.00	14.17	28.33
Chitosan	12.4	1.67	5.83	9.17	13.33	39.17	60.83
	31	0.83	3.33	6.67	8.33	30.83	50.00
	62	0.00	0.83	5.00	4.17	27.50	42.50
Control	Untreated	2.56	11.67	15.00	30.00	76.67	100.00
L.S.D. _{0.05}		0.11	0.44	0.86	0.88	2.26	2.71

Effect of chitosan and salicylic acid on *Penicillium digitatum* lesion diameter in Navel oranges

Data presented in Table 4 show that postharvest treatments with chitosan (CS) and salicylic acid (SA) markedly restricted the expansion of *Penicillium digitatum* lesions on Navel oranges under both natural infection and artificial inoculation. The inhibitory effect was particularly pronounced at the highest SA concentration (16 mM). Under natural infection, 16 mM SA maintained lesion diameters at 0.00, 0.00, and 2.88 mm after 15 and 30 days of cold storage and 5 days of shelf-life, respectively. Under artificial inoculation, the corresponding lesion diameters were 0.00, 0.00, and 25.32 mm.

Chitosan at 62 mM also substantially restricted lesion development. Under natural infection, lesion diameters were 0.00, 2.24, and 12.18 mm after 15 and 30 days of cold storage and 5 days of shelf-life, respectively, whereas artificial inoculation resulted in lesion diameters of 3.21, 5.77, and 37.82 mm at the corresponding assessment periods. In contrast, untreated control fruit developed substantially larger lesions, particularly following prolonged cold storage and subsequent shelf-life. Collectively, these findings indicate that the highest concentrations of SA and CS effectively suppressed lesion expansion and, consequently, reduced disease severity in Navel oranges under both natural and artificial infection conditions.

Total phenolics

Data presented in Table 5 show that postharvest treatments with salicylic acid (SA) and chitosan (CS) significantly increased the total phenolic content of Navel orange flavedo compared with the untreated control at both 15 and 30 days of cold storage. The magnitude of the response was concentration-dependent, with the highest SA concentration (16 mM) producing the greatest phenolic accumulation, reaching 2.200 ± 0.015 and 2.050 ± 0.012 mg GAE/g FW after 15 and 30 days, respectively. Chitosan at 62 mM also markedly enhanced phenolic content, with values of 1.800 ± 0.012 and 1.700 ± 0.012 mg GAE/g FW at the corresponding storage periods.

At 15 days, the untreated control exhibited the lowest phenolic content (0.845 ± 0.010 mg GAE/g FW), which further declined to 0.495 ± 0.010 mg GAE/g FW after 30 days. In contrast, all SA- and CS-treated fruit maintained substantially higher phenolic levels throughout storage. The observed treatment- and concentration-dependent enhancement of phenolic accumulation indicates that SA and CS promoted

the maintenance of phenolic compounds in the flavedo during cold storage, potentially contributing to the enhanced resistance of treated fruit to *Penicillium digitatum* infection.

Table (4). Lesion diameter (mm) caused by *Penicillium digitatum* on Navel oranges under natural infection and artificial inoculation following postharvest dipping treatments with chitosan and salicylic acid

Treatment	Concentration (mM)	Lesion diameter (mm)					
		Natural Infection			Artificial Infection		
		15 Days-Storage	30 Days-Storage	5 Days Shelf-life	15 Days-Storage	30 Days-Storage	5 Days Shelf-life
Salicylic acid	4	1.60	8.33	10.90	7.37	16.03	45.83
	8	0.00	5.45	9.29	3.85	6.73	44.87
	16	0.00	0.00	2.88	0.00	0.00	25.32
Chitosan	12.4	3.53	14.10	25.64	8.97	16.03	53.85
	31	1.28	7.69	21.47	6.09	10.90	43.91
	62	0.00	2.24	12.18	3.21	5.77	37.82
Control	Untreated	5.24	18.16	34.72	21.79	39.42	88.46
L.S.D. _{0.05}		1.12	0.62	1.22	1.70	1.20	2.13

Table (5). Effect of postharvest chitosan and salicylic acid treatments on total phenolic content (mg GAE/g FW) in Navel orange flavedo under natural infection

Treatment	Concentration (mM)	Total phenolic content (mg GAE/g FW)	
		15 Days-Storage	30 Days-Storage
Salicylic acid	4	1.400±0.012	1.250±0.012
	8	1.750±0.012	1.600±0.012
	16	2.200±0.015	2.050±0.012
Chitosan	12.4	1.200±0.011	1.050±0.012
	31	1.450±0.012	1.350±0.012
	62	1.800±0.012	1.700±0.012
Control	Untreated	0.845±0.010	0.495±0.010
L.S.D. _{0.05}		0.33	0.44

Peroxidase and polyphenol oxidase activities

Data presented in Table 6 demonstrate that postharvest application of salicylic acid (SA) and chitosan (CS) markedly enhanced peroxidase (POD) and polyphenol oxidase (PPO) activities in the flavedo of Navel oranges under natural infection compared with the untreated control. For both enzymes, activity increased progressively with increasing treatment concentration, while values were consistently higher after 15 days than after 30 days of cold storage.

Among the SA treatments, 16 mM produced the highest enzymatic activities, with POD reaching 42.00 ± 0.24 and 38.00 ± 0.24 U h⁻¹ g⁻¹ FW after 15 and 30 days, respectively. The corresponding PPO activities were 132.00 ± 0.98 and 120.00 ± 0.98 U h⁻¹ g⁻¹ FW. Chitosan at 62 mM also substantially enhanced both enzymes, resulting in POD activities of 35.00 ± 0.24 and 31.00 ± 0.24 U h⁻¹ g⁻¹ FW and PPO activities of 114.00 ± 0.98 and 102.00 ± 0.98 U h⁻¹ g⁻¹ FW at the respective storage periods.

In contrast, untreated fruit exhibited the lowest POD and PPO activities, declining from 12.00 ± 0.20 and 30.00 ± 0.49 U h⁻¹ g⁻¹ FW at 15 days to 8.00 ± 0.20 and 18.00 ± 0.49 U h⁻¹ g⁻¹ FW at 30 days, respectively. The treatment differences exceeded the corresponding LSD_{0.05} values for both enzymes, indicating significant treatment effects. Overall, SA and CS treatments, particularly at their highest concentrations, enhanced POD and PPO activities and maintained higher levels of these defense-associated enzymes during cold storage, consistent with an induced biochemical defense response in Navel orange flavedo.

Table (6). Effect of postharvest chitosan and salicylic acid treatments on peroxidase (POD) and polyphenol oxidase (PPO) activities (U h⁻¹ g⁻¹ FW) in Navel orange flavedo under natural infection

Treatment	Concentration (mM)	Peroxidase (POD) activity (U·h ⁻¹ ·g ⁻¹ FW)		Polyphenol oxidase (PPO) activity (U·h ⁻¹ ·g ⁻¹ FW)	
		15 Days-Storage	30 Days-Storage	15 Days-Storage	30 Days-Storage
Salicylic acid	4	26.00±0.20	22.00±0.20	78.00±0.49	66.00±0.49
	8	33.00±0.24	29.00±0.24	102.00±0.98	90.00±0.98
	16	42.00±0.24	38.00±0.24	132.00±0.98	120.00±0.98
Chitosan	12.4	22.00±0.20	18.00±0.20	60.00±0.49	48.00±0.49
	31	28.00±0.20	24.00±0.20	84.00±0.98	72.00±0.98
	62	35.00±0.24	31.00±0.24	114.00±0.98	102.00±0.98
Control	Untreated	12.00±0.20	8.00±0.20	30.00±0.49	18.00±0.49
L.S.D. _{0.05}		1.83	1.63	2.64	2.62

Effect of different treatments on post-harvest fruit quality parameters

1. Weight loss

Data presented in Table 7 show that postharvest treatments with salicylic acid (SA) and chitosan (CS) significantly reduced weight loss in Navel oranges during cold storage compared with the untreated control. Weight loss increased with prolonged storage in all treatments; however, treated fruit consistently exhibited lower weight loss than the control at both assessment periods.

Among the SA treatments, 16 mM resulted in the lowest weight loss, with values of $1.90 \pm 0.03\%$ and $4.23 \pm 0.05\%$ after 15 and 30 days of storage, respectively. Chitosan exhibited a similar concentration-dependent effect, with 62 mM producing the lowest weight loss among the CS treatments, at $1.53 \pm 0.04\%$ after 15 days and $3.44 \pm 0.04\%$ after 30 days. In contrast, untreated fruit showed substantially greater weight loss, reaching $4.30 \pm 0.06\%$ and $9.82 \pm 0.12\%$ at the corresponding storage periods.

The observed differences exceeded the respective LSD_{0.05} values (0.66 and 0.88% at 15 and 30 days, respectively), indicating significant treatment effects. Overall, the results demonstrate that both SA and CS, particularly at their highest concentrations, effectively limited weight loss during cold storage, with the effect becoming more pronounced as treatment concentration increased.

2. Effect of postharvest chitosan and salicylic acid dips on firmness retention of Navel oranges during storage

Fruit firmness declined progressively during storage in all treatments; however, the magnitude of softening differed markedly among treatments. At 0 days, firmness values were very similar among all treatments (7.08–7.13 kgf), indicating comparable initial fruit firmness. After 15 days of storage, both salicylic acid (SA) and chitosan treatments substantially reduced firmness loss compared with the untreated control, which declined to 4.49 kgf. The highest firmness at this stage was recorded for SA at 16 mM (6.29 kgf), followed by chitosan at 62 mM (6.09 kgf).

After 30 days of storage, treatment effects became more pronounced. SA at 16 mM maintained the highest fruit firmness (5.49 kgf), followed by chitosan at 62 mM (5.19 kgf) and SA at 8 mM (4.79 kgf), whereas the untreated control exhibited the greatest softening, reaching only 2.79 kgf. Thus, relative to the untreated control, SA at 16 mM preserved approximately 2.70 kgf more firmness after 30 days.

Overall, the results demonstrate a concentration-dependent protective effect, particularly at the highest concentrations tested. SA was generally more effective than chitosan in maintaining Navel orange firmness throughout storage. These findings suggest that postharvest SA and chitosan dips can delay firmness loss and thereby contribute to maintaining the textural quality of Navel oranges during storage.

Table (7). Effect of postharvest chitosan and salicylic acid treatments on weight loss (%) of Navel oranges under natural infection during cold storage.

Treatment	Concentration (mM)	Weight loss (%) of Navel oranges	
		15 Days-Storage	30 Days-Storage
Salicylic acid	4	3.03±0.05	6.63±0.07
	8	2.50±0.04	5.49±0.06
	16	1.90±0.03	4.23±0.05
Chitosan	12.4	2.60±0.05	5.67±0.07
	31	2.05±0.04	4.54±0.06
	62	1.53±0.04	3.44±0.04
Control	Untreated	4.30±0.06	9.82±0.12
L.S.D. _{0.05}		0.66	0.88

Table (8). Effect of Chitosan and Salicylic Acid Dips on Fruit Firmness of Navel Oranges under Natural Infection during Storage

Treatment	Concentration (mM)	Fruit Firmness (kgf) of navel oranges		
		0 Days-Storage	15 Days-Storage	30 Days-Storage
Salicylic acid	4	7.09±0.04	5.39±0.04	4.09±0.04
	8	7.13±0.03	5.84±0.04	4.79±0.04
	16	7.09±0.04	6.29±0.04	5.49±0.04
Chitosan	12.4	7.08±0.03	5.29±0.04	3.89±0.04
	31	7.13±0.03	5.69±0.04	4.59±0.04
	62	7.08±0.03	6.09±0.04	5.19±0.04
Control	Untreated	7.09±0.04	4.49±0.04	2.79±0.04
L.S.D. _{0.05}		0.52	0.40	0.32

Effect of postharvest chitosan and salicylic acid treatments on quality attributes of Navel orange flavedo under natural infection after 30 days of storage.

Table 9 presents the effects of postharvest salicylic acid (SA) and chitosan treatments on TSS, titratable acidity (TA), the TSS/TA ratio, and ascorbic acid content in the flavedo of naturally infected Navel oranges after 30 days of cold storage. Overall, the treatments produced clear concentration-dependent changes in the measured physicochemical and nutritional attributes.

Total soluble solids (TSS): The untreated control recorded the highest TSS value (12.81 ± 0.04 °Brix). Both SA and chitosan treatments slightly reduced TSS, with the lowest value recorded at 16 mM SA (12.06 ± 0.04 °Brix), followed by 62 mM chitosan (12.18 ± 0.03 °Brix). The relatively small variation in TSS among treatments suggests that both treatments substantially maintained soluble-solids levels during storage.

Titrateable acidity (TA): In contrast to TSS, both SA and chitosan progressively increased TA with increasing concentration. The control exhibited the lowest TA ($0.588 \pm 0.005\%$), whereas 16 mM SA and 62 mM chitosan increased TA to $0.998 \pm 0.005\%$ and $0.934 \pm 0.005\%$, respectively. This indicates greater preservation of organic acids in treated fruits during storage.

TSS/TA ratio: The control had the highest TSS/TA ratio (2181.63 ± 27.57), whereas increasing concentrations of both treatments progressively decreased this parameter. The lowest ratios were recorded with 16 mM SA (1209.46 ± 11.02) and 62 mM chitosan (1304.42 ± 10.79). This reduction is consistent with the higher TA values observed in treated fruits and indicates altered sugar–acid balance during storage.

Ascorbic acid: A concentration-dependent increase in ascorbic acid content was observed following both SA and chitosan treatments. The untreated control contained 32.40 ± 0.30 mg/100 g FW, while the highest concentrations of SA (16 mM) and chitosan (62 mM) increased ascorbic acid to 53.90 ± 0.34 and 50.95 ± 0.25 mg/100 g FW, respectively. Thus, the treatments, particularly higher SA concentrations, were associated with enhanced retention of ascorbic acid in the flavedo.

Table (9). Effect of postharvest chitosan and salicylic acid treatments on total soluble solids (TSS), titrateable acidity (TA, % citric acid), TSS/TA ratio, and ascorbic acid content of Navel orange flavedo under natural infection after 30 days of storage

Treatment	Concentration (mM)	TSS	Titrateable acidity (%)	TSS/TA (%)	Ascorbic acid content (mg/100 g FW)
Salicylic acid	4	12.36 ± 0.04	0.831 ± 0.005	1487.95 ± 14.73	43.98 ± 0.23
	8	12.21 ± 0.04	0.908 ± 0.005	1345.58 ± 12.59	48.43 ± 0.26
	16	12.06 ± 0.04	0.998 ± 0.005	1209.46 ± 11.02	53.90 ± 0.34
Chitosan	12.4	12.41 ± 0.04	0.793 ± 0.005	1566.58 ± 16.20	42.00 ± 0.29
	31	12.31 ± 0.04	0.870 ± 0.005	1416.31 ± 13.88	46.40 ± 0.31
	62	12.18 ± 0.03	0.934 ± 0.005	1304.42 ± 10.79	50.95 ± 0.25
Control	Untreated	12.81 ± 0.04	0.588 ± 0.005	2181.63 ± 27.57	32.40 ± 0.30
L.S.D. _{0.05}		0.22	0.10	12.53	1.42

Overall Q1-style interpretation, the data in Table 9 indicate that postharvest SA and chitosan treatments modified the maturity-related and nutritional attributes of naturally infected Navel oranges after 30 days of storage. The progressive increase in TA and ascorbic acid, together with the reduction in the TSS/TA ratio, suggests that these treatments may contribute to the preservation of fruit quality during prolonged storage.

Among the tested concentrations, 16 mM SA produced the highest TA and ascorbic acid content and the lowest TSS/TA ratio, whereas 62 mM chitosan showed a similar but slightly less pronounced response. Importantly, interpretation of treatment differences should be supported by the corresponding statistical significance indicators (e.g., LSD/SE and letter groupings) before assigning significance to individual treatment comparisons. Important table-format point: the column currently labeled “TSS/TA (%)” should be corrected to “TSS/TA ratio” because the ratio is dimensionless and should not be expressed as a percentage. Also, if TSS is measured in °Brix, the column heading should preferably read “TSS (°Brix)”.

4. Discussion

The present study demonstrated that salicylic acid (SA) and chitosan significantly inhibited *Penicillium digitatum* growth in a concentration-dependent manner, with the highest concentrations of SA (16 mM) and chitosan (62 mM) achieving 84.4% and 78.9% radial growth inhibition, respectively. The stronger inhibition by SA under the tested conditions suggests differences in their antifungal effectiveness, whereas chitosan may suppress fungal growth by disrupting cell-wall and membrane integrity. These findings agree with previous reports highlighting the antifungal potential of chitosan and the role of SA in postharvest disease management (**Bautista-Baños *et al.*, 2006**; **Romanazzi *et al.*, 2016**). Moreover, **Piancatelli *et al.* (2026)** confirmed the inhibitory activity of chitosan against *P. digitatum* and *P. italicum* and its potential to reduce citrus postharvest decay. Overall, both compounds represent promising alternatives for sustainable green mold management; however, further fruit-based experiments are required to evaluate their effects on disease development, fruit quality, and storage life and to determine whether their combined application produces additive or synergistic effects (**Palou *et al.*, 2016**; **Piancatelli *et al.*, 2026**).

Scanning electron microscopy (SEM) revealed distinct ultrastructural alterations in *Penicillium digitatum* following treatment with salicylic acid (SA) and chitosan compared with the untreated control. Untreated fungal hyphae exhibited smooth, intact surfaces and well-preserved conidiogenous structures, whereas SA treatment induced surface irregularities, hyphal deformation, and structural collapse (**Shao *et al.*, 2015**). Chitosan caused more pronounced abnormalities, including marked hyphal shrinkage, collapse, and disruption of normal fungal architecture, suggesting greater morphological damage under the examined conditions (**Saeed and Ibrahim, 2025**). These observations support the antifungal effects of both compounds and are consistent with the reported ability of chitosan to interact with fungal cell walls and membranes, impair cellular integrity, and inhibit pathogen growth (**Bautista-Baños *et al.*, 2006**). Similar antifungal activity against *P. digitatum* has been reported by **Piancatelli *et al.* (2026)**, who demonstrated the potential of chitosan to suppress fungal growth and reduce citrus green mold. Nevertheless, SEM provides morphological rather than direct biochemical evidence of the underlying mechanisms; therefore, the observed structural damage should be interpreted as evidence of treatment-associated fungal disruption rather than definitive proof of a specific cellular mechanism (**De Azevedo *et al.*, 2024**).

Salicylic acid (SA) and chitosan (CS) effectively suppressed green mold caused by *Penicillium digitatum* in Egyptian navel oranges under both natural and artificial infection conditions. SA at 16 mM provided the strongest protection, maintaining disease incidence at 0.00% after 15 and 30 days of cold storage and 1.67% after an additional 5 days of shelf-life under natural infection. Under artificial inoculation, incidence increased to 28.33% by the end of the assessment period. Similarly, CS at 62 mM limited disease incidence to 0.00–5.00% under natural infection and 4.17–42.50% under artificial inoculation. The higher disease incidence following artificial inoculation and prolonged storage suggests that treatment efficacy depends on pathogen pressure and storage conditions. These findings agree with **Ding *et al.* (2022)** and **Ezzouggari *et al.* (2025)**, who reported that SA inhibited *P. digitatum* spore germination and mycelial growth while helping preserve citrus fruit quality, and with **Piancatelli *et al.* (2026)**, who demonstrated the effectiveness of chitosan coatings in reducing green and blue mold on oranges. Collectively, these results support the potential of SA and CS as alternatives or complements to conventional fungicides for postharvest citrus disease management. However, their comparative performance and any additional benefit from combined application should be evaluated under the specific treatment conditions used in this study (**Bhatta, 2022**; **Talibi *et al.*, 2014**).

Postharvest application of salicylic acid (SA) and chitosan (CS) significantly enhanced total phenolic content in navel orange flavedo during cold storage, with concentration-dependent effects. SA at 16 mM produced the highest values (2.200 and 2.050 mg GAE g⁻¹ FW after 15 and 30 days, respectively), followed by CS at 62 mM (1.800 and 1.700 mg GAE g⁻¹ FW), whereas the untreated control declined from 0.845 to 0.495 mg GAE g⁻¹ FW. These findings indicate that both treatments helped maintain phenolic compounds during storage, potentially supporting antioxidant activity and host defense against *Penicillium digitatum*. Similar effects have been reported for SA-induced accumulation of defense-associated metabolites in citrus and chitosan-mediated enhancement of postharvest disease resistance (**Zhu *et al.*, 2016**; **Shi *et al.*, 2018**; **Rajestary *et al.*, 2021**). However, because individual

phenolic compounds and their biosynthetic pathways were not assessed, the contribution of phenolic accumulation to disease suppression remains a plausible explanation rather than a confirmed causal mechanism.

Postharvest application of salicylic acid (SA) and chitosan (CS) significantly enhanced peroxidase (POD) and polyphenol oxidase (PPO) activities in navel orange flavedo under natural infection, with concentration-dependent effects and higher activities after 15 than 30 days of cold storage. SA at 16 mM produced the highest POD activities (42.00 and 38.00 U h⁻¹ g⁻¹ FW) and PPO activities (132.00 and 120.00 U h⁻¹ g⁻¹ FW) at 15 and 30 days, respectively, followed by CS at 62 mM. In contrast, enzyme activities declined markedly in untreated fruit during storage. These findings suggest that SA and CS may stimulate defense-related biochemical responses, supporting pathogen resistance through oxidative metabolism, phenolic oxidation, and potentially cell-wall reinforcement. Similar effects have been reported in citrus treated with SA and chitosan against *Penicillium digitatum* (Shi *et al.*, 2018; Moosa *et al.*, 2019, 2022). Overall, SA at 16 mM elicited the strongest response under the tested conditions, although further molecular analyses are needed to confirm the mechanisms linking enzyme activation to disease suppression.

Postharvest application of salicylic acid (SA) and chitosan (CS) significantly reduced weight loss in navel oranges during cold storage, with the greatest reductions observed at the highest treatment concentrations. CS at 62 mM was particularly effective, limiting weight loss to 1.53% and 3.44% after 15 and 30 days, respectively, compared with 4.30% and 9.82% in untreated fruit. SA at 16 mM also reduced weight loss to 1.90% and 4.23% at the corresponding assessment periods. The lower weight loss in treated fruit may reflect reduced transpiration and moisture loss, potentially through the formation of a semipermeable coating by chitosan that limits water-vapor transfer across the fruit surface. Chitosan-based coatings have been widely investigated for maintaining postharvest quality and reducing water loss in horticultural commodities (Bautista-Baños *et al.*, 2006; Romanazzi *et al.*, 2016). The increase in weight loss with storage duration is consistent with progressive water loss through transpiration and respiratory metabolism. Chitosan-based coatings can act as semipermeable barriers that reduce moisture transfer and modify gas exchange, thereby helping preserve fruit freshness and postharvest quality (Xing *et al.*, 2016; Adiletta *et al.*, 2021). Although SA also reduced weight loss, CS at 62 mM performed better numerically at both assessment periods in the present study. This difference suggests that the coating-related effects of CS may have contributed to moisture retention, whereas the precise mechanisms underlying the SA response require further investigation. Overall, the results identify high-concentration CS, followed by SA, as promising treatments for limiting postharvest weight loss in navel oranges during cold storage (Nikhil *et al.*, 2025).

Fruit firmness declined progressively during cold storage in all treatments, reflecting the gradual softening associated with postharvest ripening and senescence. However, salicylic acid (SA) and chitosan (CS) significantly mitigated this decline, particularly at the highest concentrations tested. SA at 16 mM maintained the greatest firmness after 15 and 30 days (6.29 and 5.49 kgf, respectively), followed by CS at 62 mM (6.09 and 5.19 kgf), whereas untreated fruit softened markedly to 2.79 kgf after 30 days. The superior performance of SA suggests that it may help preserve fruit texture by delaying senescence and influencing processes associated with cell-wall degradation (Khademi and Ershadi, 2013). Previous studies have shown that SA treatments can maintain firmness and delay quality deterioration in postharvest fruit, while chitosan coatings can reduce moisture loss and help preserve structural integrity during storage (Asghari and Aghdam, 2010; Xing *et al.*, 2016). These findings are consistent with Alshallash *et al.* (2022), who reported that nano-chitosan coatings maintained greater hardness and pulp firmness in Valencia oranges during storage. SA has also been reported to delay fruit softening by influencing ethylene production and the activity of cell-wall-degrading enzymes, including polygalacturonase and pectin methylesterase (Asghari and Aghdam, 2010). The maintenance of firmness in the present study may therefore reflect a combination of delayed physiological deterioration and reduced moisture loss. Because cell-wall enzyme activities and ethylene production were not measured here, these mechanisms should be considered plausible explanations rather than confirmed effects. Overall, SA at 16 mM was the most effective treatment for preserving navel orange firmness, followed by CS at 62 mM, supporting their potential for maintaining fruit texture during cold storage (Xing *et al.*, 2016; Gao *et al.*, 2018).

The results presented in Table 9 demonstrate that postharvest salicylic acid (SA) and chitosan (CS) treatments influenced the physicochemical and nutritional characteristics of naturally infected navel oranges after 30 days of cold storage. Both treatments reduced total soluble solids (TSS) relative to the untreated control, with the lowest values recorded at 16 mM SA (12.06 °Brix) and 62 mM CS (12.18 °Brix), compared with 12.81 °Brix in the control. These relatively modest differences suggest that the treatments helped maintain soluble-solids levels during storage, although TSS alone cannot establish whether fruit ripening was delayed. In contrast, titratable acidity (TA) increased with treatment concentration, reaching 0.998% with 16 mM SA and 0.934% with 62 mM CS, compared with 0.588% in the control. The corresponding reduction in the TSS/TA ratio indicates that treated fruit retained a higher acid content relative to soluble solids. These responses are consistent with the potential of postharvest treatments to modify fruit metabolism and preserve quality during storage (Asghari and Aghdam, 2010; Xing *et al.*, 2016).

Ascorbic acid content also increased markedly following both treatments. The highest concentration was recorded in fruit treated with 16 mM SA (53.90 mg/100 g FW), followed by 62 mM CS (50.95 mg/100 g FW), compared with 32.40 mg/100 g FW in the untreated control. This improvement may reflect better retention of vitamin C during storage and reduced deterioration of the fruit's antioxidant system. Salicylic acid has been reported to influence postharvest physiological processes and antioxidant responses, while chitosan-based coatings can help maintain fruit quality by modifying the fruit–environment interface and reducing physiological deterioration (Asghari and Aghdam, 2010; Bautista-Baños *et al.*, 2006; Xing *et al.*, 2016). Nevertheless, the present measurements do not establish the precise biochemical mechanisms responsible for the observed changes (Chen *et al.*, 2023).

Overall, 16 mM SA was the most effective treatment for maintaining TA and ascorbic acid content and producing the lowest TSS/TA ratio, while 62 mM CS generally produced the second-best response. These findings support the potential use of SA and CS to preserve selected quality attributes of navel oranges during cold storage. However, treatment differences should be described as statistically significant only where supported by the table's statistical tests and mean-separation results (Zhang *et al.*, 2024).

5. Conclusion

This study demonstrated the effectiveness of salicylic acid (SA) and chitosan (CS) in suppressing *Penicillium digitatum* and preserving Egyptian navel orange quality during storage. *In vitro*, 16 mM SA and 62 mM CS inhibited fungal growth by 84.4% and 78.9%, respectively, with scanning electron microscopy revealing marked hyphal damage. Both treatments reduced disease incidence and lesion development, while enhancing phenolic content and peroxidase and polyphenol oxidase activities. SA at 16 mM maintained the lowest natural disease incidence (1.67%) after cold storage and shelf-life and the highest ascorbic acid content (53.90 mg/100 g fresh weight). Chitosan at 62 mM was particularly effective in reducing weight loss (3.44% versus 9.82% in untreated fruit). Overall, these treatments offer promising alternatives for postharvest green mold management and quality preservation, although further validation under commercial conditions is required.

References

- Adiletta, G.; Di Matteo, M. and Petriccione, M. (2021). Multifunctional role of chitosan edible coatings on antioxidant systems in fruit crops: A review. *International Journal of Molecular Sciences*, 22(5), 2633. <https://doi.org/10.3390/ijms22052633>
- Alshallash, K.S.; Sharaf, M.; Abdel-Aziz, H.F.; Arif, M.; Hamdy, A.E.; Khalifa, S.M.; Hassan, M.F.; Abou ghazala, M.M.; Bondok, A.; Ibrahim, M.T.S.; Alharbi, K. and Elkelish, A. (2022). Postharvest physiology and biochemistry of Valencia orange after coatings with chitosan nanoparticles as edible for green mold protection under room storage conditions. *Frontiers in Plant Science*, 13, Article 1034535. <https://doi.org/10.3389/fpls.2022.1034535>

- Asghari, M. and Aghdam, M.S. (2010).** Impact of salicylic acid on post-harvest physiology of horticultural crops. *Trends in Food Science and Technology*, 21(10), 502–509. <https://doi.org/10.1016/j.tifs.2010.07.009>
- Bhatta, U.K. (2022).** Alternative management approaches of citrus diseases caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). *Frontiers in Plant Science*, 12: 833328. <https://doi.org/10.3389/fpls.2021.833328>
- Bautista-Baños, S.; Hernández-Lauzardo, A.N.; Velázquez-del Valle, M.G.; Hernández-López, M.; Ait Barka, E.; Bosquez-Molina, E. and Wilson, C.L. (2006).** Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection*, 25(2): 108–118. <https://doi.org/10.1016/j.cropro.2005.03.010>
- Chen, Y., Li, X., and Wang, Y. (2023).** Chlorine dioxide controls green mold caused by *Penicillium digitatum* in citrus fruits and the mechanism involved. *Journal of Agricultural and Food Chemistry*, 68(47): 13897–13905. <https://doi.org/10.1021/acs.jafc.0c05288>
- Chen, C.; Cai, N.; Chen, J. and Wan, C. (2019).** Clove essential oil as an alternative approach to control postharvest blue mold caused by *Penicillium italicum* in citrus fruit. *Biomolecules*, 9(5): 197. <https://doi.org/10.3390/biom9050197>
- Cheng, Y.; Lin, Y.; Cao, H. and Li, Z. (2020).** Citrus postharvest green mold: Recent advances in fungal pathogenicity and fruit resistance. *Microorganisms*, 8(3): 449. <https://doi.org/10.3390/microorganisms8030449>
- Chen, C.; Sun, C.; Wang, Y.; Gong, H.; Zhang, A.; Yang, Y.; Guo, F.; Cui, K.; Fan, X. and Li, X. (2023).** The preharvest and postharvest application of salicylic acid and its derivatives on storage of fruit and vegetables: A review. *Scientia Horticulturae*, 312, 111858. <https://doi.org/10.1016/j.scienta.2023.111858>
- Coseteng, M.Y. and Lee, C.Y. (1987).** Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *Journal of Food Science*, 52(4): 985–989. <https://doi.org/10.1111/j.1365-2621.1987.tb14257.x>
- De Azevedo, M.I.G.; Souza, P.F.N.; Monteiro Júnior, J.E. and Grangeiro, T.B. (2024).** Chitosan and chitoooligosaccharides: Antifungal potential and structural insights. *Chemistry and Biodiversity*, 21(6), e202400044. <https://doi.org/10.1002/cbdv.202400044>
- Deng, B.; Wang, W.; Ruan, C.; Deng, L.; Yao, S. and Zeng, K. (2020).** Involvement of CsWRKY70 in salicylic acid-induced citrus fruit resistance against *Penicillium digitatum*. *Horticulture Research*, 7, 157. <https://doi.org/10.1038/s41438-020-00377-y>
- Ding, P.; Wang, Z., and Huang, J. (2022).** Citrus postharvest green mold: Recent advances in fungal pathogenicity and fruit resistance. *Journal of Fungi*, 8(4), 367. <https://doi.org/10.3390/jof8040367>
- Droby, S.; Wisniewski, M.; Macarisin, D. and Wilson, C. (2009).** Twenty years of postharvest biocontrol research: Is it time for a new paradigm? *Postharvest Biology and Technology*, 52(2): 137–145. <https://doi.org/10.1016/j.postharvbio.2008.11.009>
- El-Garhy, M.A.; El-Sayed, A.M. and Abdel-Hafez, S.I. (2021).** Isolation, screening and identification of promising antagonistic isolates against *Penicillium digitatum* causing green mold of navel orange. *Egyptian Journal of Biological Pest Control*, 31: 45. https://journals.ekb.eg/article_14566.html
- El-Sayed, A.M.; Ibrahim, B.Y. and Mustafa, Y.F. (2012).** First report of *Penicillium ulaiense* as a postharvest pathogen of orange fruit in Egypt. *Australasian Plant Disease Notes*, 7: 237–239. <https://doi.org/10.1007/s13313-012-0165-4>
- Ezzouggari, R.; Bahhou, J.; Taoussi, M.; Boutagayout, A.; Goura, K.; Laasli, S.E. and Lahlali, R. (2025).** Salicylic acid treatment effectively controls green mold and preserves the postharvest quality of citrus fruit. *Food and Humanity*, 5: 100710. <https://doi.org/10.1016/j.foohum.2025.100710>
- Food and Agriculture Organization of the United Nations (FAO). (2026).** FAOSTAT: Crops and livestock products—Citrus fruit, total: Production quantity. Rome, Italy: FAO. [FAOSTAT database](https://www.fao.org/faostat)

- Gao, Y.; Kan, C.; Wan, C.; Chen, C.; Chen, M. and Chen, J. (2018).** Quality and biochemical changes of navel orange fruits during storage as affected by cinnamaldehyde–chitosan coating. *Scientia Horticulturae*, 239: 80–86. <https://doi.org/10.1016/j.scienta.2018.05.012>
- Herrera-González, J.A.; Ramos-Bell, S.; Bautista-Baños, S.; Velázquez-Estrada, R.M.; Rayón-Díaz, E.; Martínez-Batista, E. and Gutiérrez-Martínez, P. (2024).** Chitosan and GRAS substances: An alternative for the control of *Neofusicoccum parvum* in vitro, elicitor and maintenance of the postharvest quality of avocado fruits. *Horticulturae*, 10(7): 687. <https://doi.org/10.3390/horticulturae10070687>.
- Hughes, D.E. (1983).** Titrimetric determination of ascorbic acid with 2,6-dichlorophenol indophenol in commercial liquid diets. *Journal of Pharmaceutical Sciences*, 72(2): 126–129. <https://doi.org/10.1002/jps.2600720208>
- Ibrahim, B.Y.; Aljuboori, F.K.; Mheedi, R.N. and Baker, S.M. (2024).** Control of green mold disease in oranges caused by *Penicillium digitatum* by using chitosan and salicylic acid. *Mesopotamia Journal of Agriculture*, 52(1): 48–59. <https://doi.org/10.33899/mja.2024.144845.1308>.
- Jabnoun-Khiareddine, H.; El-Mohamedy, R.S.R.; Abdel-Kareem, F.; Aydi Ben Abdallah, R.; Gueddes-Chahed, M. and Daami-Remadi, M. (2015).** Variation in chitosan and salicylic acid efficacy towards soil-borne and air-borne fungi and their suppressive effect of tomato wilt severity. *Journal of Plant Pathology and Microbiology*, 6(11): 325–335. <https://doi.org/10.4172/2157-7471.1000325>
- Khademi, O. and Ershadi, A. (2013).** Postharvest application of salicylic acid improves storability of peach (*Prunus persica* L.) fruit. *International Journal of Postharvest Technology and Innovation*, 3(2), 151–160.
- Liu, Y.; Heying, E. and Tanumihardjo, S.A. (2012).** History, global distribution, and nutritional importance of citrus fruits. *Comprehensive Reviews in Food Science and Food Safety*, 11(6): 530–545. <https://doi.org/10.1111/j.1541-4337.2012.00201.x>
- Macarasin, D.; Cohen, E.; Alkan, N.; Prusky, D., and Sionov, E. (2020).** *Penicillium digitatum* suppressors of production of the antifungal metabolite pyruvic acid. *Applied and Environmental Microbiology*, 86(10): e00264–20. <https://doi.org/10.1128/AEM.00264-20>
- Masood, A.; Saeed, S.; Iqbal, N.; Malik, M.T. and Kazmi, M.R. (2010).** Methodology for the evaluation of symptoms severity of mango sudden death syndrome in Pakistan. *Pak. J. Bot.*, 42(2): 1289–1299.
- Moosa, A.; Sahi, S.T.; Khan, S.A. and Malik, A.U. (2019).** Salicylic acid and jasmonic acid can suppress green and blue moulds of citrus fruit and induce the activity of polyphenol oxidase and peroxidase. *Folia Horticulturae*, 31(1): 195–204. <https://doi.org/10.2478/fhort-2019-0014>
- Moosa, A.; Zulfiqar, F. and Siddique, K.H.M. (2022).** Transcriptional and biochemical profiling of defense enzymes in *Citrus sinensis* during salicylic acid and cinnamon mediated suppression of green and blue mold. *Frontiers in Plant Science*, 13: 1048433. <https://doi.org/10.3389/fpls.2022.1048433>
- Nikhil, Kaur Jawandha, S.; Gill, P.S.; Bajaj, K.; Dhiman, D.; Singh, H. and Arashdeep Singh. (2025).** Potassium sorbate-enriched chitosan coatings enhance antioxidant stability and preserve postharvest quality of lemons (*Citrus limon*). *Journal of the Science of Food and Agriculture*, 105(13), 7350–7361. <https://doi.org/10.1002/jsfa.14439>.
- Palou, L. (2014).** *Penicillium digitatum*, *Penicillium italicum* (green mold, blue mold). In: Bautista-Baños, S. (Ed.), *Postharvest Decay: Control Strategies*. Academic Press, pp. 45–102.
- Palou, L.; Ali, A.; Fallik, E. and Romanazzi, G. (2016).** GRAS, plant- and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. *Postharvest Biology and Technology*, 122, 41–52. <https://doi.org/10.1016/j.postharvbio.2016.04.017>
- Piancatelli, S.; Sbodio, A.O.; Michelon, T.B.; Tsen, M.; Carranza-Rodriguez, M.C.; Gutierrez-Baeza, E.; Romanazzi, G. and Blanco-Ulate, B. (2026).** Chitosan inhibits the growth of *Penicillium*

digitatum and *P. italicum* and protects oranges from green and blue mold during postharvest. Postharvest Biology and Technology, 232: 113955. <https://doi.org/10.1016/j.postharvbio.2025.113955>.

Porat, R.; Pavoncello, D.; Peretz, J.; Ben-Yehoshua, S. and Lurie, S. (2000). Effects of various heat treatments on the induction of cold tolerance and on the postharvest qualities of ‘Star Ruby’ grapefruit. Postharvest Biology and Technology, 18(2): 159–165. [https://doi.org/10.1016/S0925-5214\(99\)00075-7](https://doi.org/10.1016/S0925-5214(99)00075-7).

Rajestary, R.; Landi, L. and Romanazzi, G. (2021). Chitosan and postharvest decay of fresh fruit: Meta-analysis of disease control and antimicrobial and eliciting activities. Comprehensive Reviews in Food Science and Food Safety, 20(1), 563–582. <https://doi.org/10.1111/1541-4337.12672>

Romanazzi, G.; Sanzani, S.M.; Bi, Y.; Tian, S.; Gutiérrez Martínez, P. and Alkan, N. (2016). Induced resistance to control postharvest decay of fruit and vegetables. Postharvest Biology and Technology, 122: 82–94. <https://doi.org/10.1016/j.postharvbio.2016.08.003>.

Romanazzi, G.; Feliziani, E. and Sivakumar, D. (2018). Chitosan, a biopolymer with triple action on postharvest decay of fruit and vegetables: Eliciting, antimicrobial and film-forming properties. Frontiers in Microbiology, 9: 2745. <https://doi.org/10.3389/fmicb.2018.02745>.

Qian, X.; Yang, Q.; Zhang, Q.; Abdelhai, M.H.; Dhanasekaran, S.; Serwah, B.N.A.; Gu, N. and Zhang, H. (2019). Elucidation of the Initial Growth Process and the Infection Mechanism of *Penicillium digitatum* on Postharvest Citrus (*Citrus reticulata* Blanco). Microorganisms, 7(11): 485. <https://doi.org/10.3390/microorganisms7110485>

Saeed, H.M. and Ibrahim, B.Y. (2025). Postharvest control of orange green mold caused by *Penicillium digitatum* using chitosan and salicylic acid. NTU Journal of Agricultural and Veterinary Sciences, 5(4), 404–411. <https://doi.org/10.56286/gnfb5v29>.

Saleem, M.; Fariduddin, Q. and Castroverde, C.D.M. (2021). Salicylic acid: A key regulator of redox signalling and plant immunity. Plant Physiology and Biochemistry, 168: 381–397. <https://doi.org/10.1016/j.plaphy.2021.10.011>.

Shao, X.; Cao, B.; Xu, F.; Xie, S.; Yu, D. and Wang, H. (2015). Effect of postharvest application of chitosan combined with clove oil against citrus green mold. Postharvest Biology and Technology, 99, 37–43. <https://doi.org/10.1016/j.postharvbio.2014.07.014>.

Shi, Z.; Wang, F.; Lu, Y. and Deng, J. (2018). Combination of chitosan and salicylic acid to control postharvest green mold caused by *Penicillium digitatum* in grapefruit fruit. Scientia Horticulturae, 233: 54–60. <https://doi.org/10.1016/j.scienta.2018.01.039>.

Simionato, A.S.; Navarro, M.O.P.; de Jesus, M.L.A.; Barazetti, A.R.; da Silva, C.S.; Simões, G.C.; Balbi-Peña, M.I.; de Mello, J.C.P.; Panagio, L.A.; de Almeida, R.S.C.; Andrade, G. and de Oliveira, A.G. (2017). The effect of phenazine-1-carboxylic acid on mycelial growth of *Botrytis cinerea* produced by *Pseudomonas aeruginosa* LV strain. Frontiers in Microbiology, 8: 1102. <https://doi.org/10.3389/fmicb.2017.01102>

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State Univ. Press, Ames, Iowa USA, pp. 503.

Strano, M.C.; Altieri, G.; Allegra, M.; Di Renzo, G.C.; Paterna, G.; Matera, A. and Genovese, F. (2022). Post-harvest technologies of fresh citrus fruit: Advances and recent developments for the loss reduction during handling and storage. Horticulturae, 8(7), 612. <https://doi.org/10.3390/horticulturae8070612>

Talibi, I.; Boubaker, H.; Boudyach, E.H. and Ait Ben Aoumar, A. (2014). Alternative methods for the control of postharvest citrus diseases. Journal of Applied Microbiology, 117(1): 1–17. <https://doi.org/10.1111/jam.12495>

United States Department of Agriculture, Foreign Agricultural Service (USDA–FAS). (2025). Egypt: Citrus Annual. Global Agricultural Information Network (GAIN), Report No. EG2025-0031, Cairo, Egypt, 17 December 2025, 8 pp.

- Wang, Y.; Li, B.; Li, D. and Liu, C. (2020).** Effects of hot air treatments on postharvest storage of Newhall navel orange. *Plants*, 9(2), Article 170. <https://doi.org/10.3390/plants9020170>
- Wang, Z.; Sui, Y.; Li, J.; Tian, X. and Wang, Q. (2022).** Biological control of postharvest fungal decays in citrus: A review. *Critical Reviews in Food Science and Nutrition*, 62(4): 861–870. <https://doi.org/10.1080/10408398.2020.1829542>
- Wu, H.; Wang, X.; Gao, P.; Wang, Y.; Wang, T.; Chun, C.; He, Y. and Zhang, M. (2026).** Chitosan oligosaccharides reshape *Penicillium digitatum* infection responses in ‘Harumi’ fruit by reinforcing defense signaling and phenolic metabolism. *Postharvest Biology and Technology*, 239: 114410. <https://doi.org/10.1016/j.postharvbio.2026.114410>.
- Xing, Y.; Li, X.; Xu, Q.; Yun, J. and Lu, Y. (2016).** Chitosan-based coating with antimicrobial agents: Preparation, property, mechanism, and application effectiveness on fruits and vegetables. *International Journal of Polymer Science*, 2016, Article 4851730. <https://doi.org/10.1155/2016/4851730>
- Yang, Z.; Cao, S.; Cai, Y. and Zheng, Y. (2011).** Combination of salicylic acid and ultrasound to control postharvest blue mold caused by *Penicillium expansum* in peach fruit. *Innov. Food Sci. Emerg. Technol.* 12: 310–314.
- Zhang, X.; Liu, Y.; Zhang, W.; Yang, W.; An, S.; Guo, M. and Chen, G. (2024).** Salicylic Acid Treatment Ameliorates Postharvest Quality Deterioration in ‘France’ Prune (*Prunus domestica* L. ‘Ximei’) Fruit by Modulating the Antioxidant System. *Foods*, 13(18), 2871. <https://doi.org/10.3390/foods13182871>
- Zhu, H. (2016).** Postharvest biocontrol of green mold (*Penicillium digitatum*) in citrus. *Postharvest Biology and Technology*, 111, 1-10. <https://doi.org/10.1016/j.postharvbio.2015.09.005>
- Zhu, F.; Chen, J.; Xiao, X.; Zhang, M.; Yun, Z.; Zeng, Y.; Xu, J.; Cheng, Y. and Deng, X. (2016).** Salicylic acid treatment reduces the rot of postharvest citrus fruit by inducing the accumulation of H₂O₂, primary metabolites and lipophilic polymethoxylated flavones. *Food Chemistry*, 207, 68–74. <https://doi.org/10.1016/j.foodchem.2016.03.077>