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Antifungal activity and sensory analyses of chitosan aromatized with essential oils for the protection of fresh oranges and apples

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ABSTRACT

Postharvest diseases caused by fungal and oomycete pathogens represent a major threat to fruit preservation, especially in the absence of effective and sustainable alternatives to synthetic fungicides. This study evaluated the antimicrobial potential of ten chitosan-based formulations, each containing a different essential oil (EO), for the control of a broad spectrum of fungal and oomycete plant pathogens. *In vitro* assays, including agar diffusion, MIC/MFC determination, and volatile organic compound (VOC) inhibition tests, identified the two formulations ID-F-03 (containing *Origanum vulgare* EO) and ID-F-06 (containing *Cinnamomum verum* EO) as the most effectives. These formulations were further characterized by GC–MS analysis and tested *in vivo* on artificially inoculated apples and oranges. The results showed that both formulations significantly reduced disease incidence in both fruit, with ID-F-03 being particularly effective in limiting bitter rot development in apples and ID-F-06 in reducing green mold symptoms in oranges. GC–MS analysis identified carvacrol (ID-F-03) and (*E*)-cinnamaldehyde/eugenol (ID-F-06) as the major volatiles. Sensory evaluation indicated a good overall acceptability of treated fruit, with formulation–fruit combinations showing specific sensory responses; specifically, ID-F-06 maintained higher hedonic scores in apples, whereas ID-F-03 was better accepted in oranges. The study confirms the potential of chitosan–EO combinations as promising tools for the management of postharvest diseases of apples and oranges, with their application as active components in packaging materials offering a viable strategy to balance antimicrobial efficacy and sensory quality.

1. Introduction

Pre- and postharvest diseases caused by plant pathogenic fungi and oomycetes are among the leading causes of agricultural product losses (Benigno et al., 2025; Cacciola et al., 2012; Erwin and Ribeiro, 1996; Fernández-Fernández et al., 2019; Parlascino et al., 2025; Rovetto et al., 2024b). In particular, losses occurring along the postharvest supply chain "of fruit crops", from harvesting to final distribution, have significant economic and social impacts, especially in countries with agriculture-based economies (Riolo et al., 2023b; Rovetto et al., 2024c;

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Santilli et al., 2020). Examples span multiple pathosystems. The Alternaria heart rot of pomegranate, mainly caused by *A. alternata* and *A. arborescens*, often remains latent until harvest and can taint juice quality (Aloi et al., 2021b; Luo et al., 2017; Riolo et al., 2025). *Colletotrichum* spp., among which *C. gloeosporioides* and *C. acutatum* are recognized as the predominating causative agents of the apple bitter rot (Khodadadi et al., 2020; Trkulja et al., 2024). *Botrytis cinerea*, which causes gray mold, a major postharvest disease of strawberries and stored apples (Konstantinou et al., 2011; Yousef et al., 2024). *Fusarium* spp., including *F. proliferatum* and *F. sacchari*, which are associated with banana fruit rot (Riolo et al., 2020; Xie et al., 2023). *Monilinia fructicola* which causes brown rot, a destructive fungal disease affecting stone fruits (Duchoslavová et al., 2007; Landi et al., 2025; Oliveira Lino et al., 2016). The brown rot of citrus fruit, a disease incited by several *Phytophthora* spp., notably *Ph. citrophthora*, *Ph. nicotianae*, *Ph. palmivora*, *Ph. hibernalis*, *Ph. mekongensis* and *Ph. prodigiosa* (Crous et al., 2017; Puglisi et al., 2017; Rovetto et al., 2024b). *Penicillium* spp. are likewise pivotal postharvest pathogens: *P. expansum* causes blue mold of apples, whereas *P. digitatum* (green mold) and *P. italicum* (blue mold) dominate on citrus fruit (La Spada et al., 2021; Luciano-Rosario et al., 2020; Rovetto et al., 2024b). Among these diseases, molds of citrus fruit caused by *Penicillium* spp. and the apple bitter rot caused by *Colletotrichum* spp. are recognized as the major causes of product losses in the postharvest supply chains of citrus and apple, respectively (Ismail and Zhang, 2004; La Spada et al., 2021; Trkulja et al., 2024). Traditionally, chemical active ingredients have been the most widely used means to prevent such losses (Boeckman et al., 2025; Ismail and Zhang, 2004; Migheli et al., 2009). However, due to their harmful effects on both human health and the environment, in recent years research has increasingly focused on identifying safer alternatives (du Jardin, 2015; Prusky and Romanazzi, 2023). In this context, natural solutions, such as biological control agents, plant extracts, processed by-products, and essential oils (EOs), are emerging as promising alternatives to achieve an effective and environmentally sustainable control of plant decays (El boumlasy et al., 2022, 2021; Hou et al., 2022; La Spada et al., 2024, 2020; Riolo et al., 2024, 2023a; Rovetto et al., 2024c). Among these, EOs are well known, and their efficacy for controlling pathogens and pests has been extensively documented in the literature (Álvarez-García et al., 2023, 2021; Amini et al., 2016; Ayllón-Gutiérrez et al., 2024; Bi et al., 2012; Hou et al., 2022; Lee et al., 2008; Tripathi et al., 2009). Despite their effectiveness, EOs present certain limitations that restrict their widespread adoption as reliable means of controlling postharvest issues (Farina and Conti, 2024). In particular, their high volatility reduces persistence and efficacy over time, and their strong aroma, especially when applied in pure form, may significantly affect the sensory qualities of treated food products (Adetunji and Sharifi-Rad, 2023; Bedini et al., 2024; Servili et al., 2017). To address these issues, recent studies have explored the use of chitosan as a carrier matrix capable of incorporating active substances, including EOs, modulating their release and reducing their volatility (Arslan et al., 2024; Djebbi et al., 2024; Farina et al., 2025). Chitosan, a biopolymer with intrinsic antimicrobial activity, finds wide applications in the agri-food sector (Abenaim and Conti, 2023; Bautista-Baños et al., 2016; Eddya et al., 2020; Romanazzi et al., 2018; Zargar et al., 2015). Given the established feasibility of chitosan–EO combinations in postharvest applications, combining chitosan with controlled amounts of EOs is a practical strategy to enhance disease control while tempering volatility and sensory impact. This feasibility is illustrated by practical applications: on citrus, postharvest application of chitosan coatings combined with clove EO significantly reduced green mold caused by *Penicillium digitatum in vivo*, supporting the feasibility of chitosan–EO systems for citrus preservation (Shao et al., 2015); on table grapes, chitosan–EO nanoemulsion coatings (*e.g.*, *Angelica archangelica* EO) reduced *B. cinerea* decay and helped maintain quality during storage (Das et al., 2023); for strawberries, chitosan carriers incorporating lavender or red thyme EOs suppressed *B. cinerea* and extended shelf life in packaging systems (Sangsuwan et al., 2016). In apples, chitosan–EO composite coatings (e.g., cinnamon EO in chitosan matrices) inhibited postharvest diseases *in vivo* (Zhang et al., 2023). Against this background, the present study evaluated chitosan–EO formulations as integrated systems, testing their ability to inhibit the *in vitro* growth of fungal and oomycete plant pathogens commonly associated with postharvest diseases of fruit crops. The two formulations that showed the most promising *in vitro* efficacy were furtherly characterized to assess the following aspects: *in vivo* effectiveness, verified as attitude of the formulations of reducing the incidence of two widespread postharvest diseases of fruit, the "apple bitter rot caused by *Colletotrichum gloeosporioides*" and "green mold of oranges caused by *Penicillium digitatum*"; the volatile composition of the corresponding EOs (gas chromatography–mass spectrometry – GC–MS – analysis was conducted); and the impact of postharvest application on the sensory profile of fresh fruit (a sensory panel test was conducted).

2. Materials and methods

2.1. Essential oils (EOs)

The tested EOs were chosen on the basis of their effectiveness (Bouqellah et al., 2025; Campos et al., 2025; Mahmutović-Dizdarević et al., 2023; Mohamed et al., 2025) availability, and affordability. All the EOs were 100 % pure and purchased from commercial suppliers: *Ocimum basilicum* L., *Cinnamomum verum* J.Presl, *Piper nigrum* L., and *Angelica archangelica* L. from Sigma-Aldrich (St. Louis, MO, USA); *Allium sativum* L. from Vis Medicatrix Naturae s.r.l. (Florence, Italy); *Origanum vulgare* from Herbal Products Italia S.r.l. (Sant'Elena, Italy); *Citrus aurantium* from Flora S.r.l. (Lorenzana, Italy); *Foeniculum vulgare* from KOS S.r.l. (Carmignano, Italy); *Laurus nobilis* L. from Giorgini Dr. Martino (Marradi, Italy); *Citrus limon* from Arkofarm S.r.l. (Ventimiglia, Italy).

2.2. Preparation of the chitosan-based formulations containing an essential oil (EO)

Each chitosan-based formulation containing an EO was prepared as follows. First, a 1 % (w/v) stock solution was prepared using highly viscous chitosan derived from crab shells (Sigma-Aldrich, CAS No: 9012–76–4) following the protocol outlined by Parichanon et al. (2024). Specifically, to prepare 100.0 mL of the stock solution, 1.0 g of chitosan was dispersed in an aqueous solution containing 1 % (v/v) lactic acid (Carlo Erba Reagents S.r.l., Cornaredo, Italy) until reaching 100 mL and stirred at 250 rpm and 25 °C for 2 h. Subsequently, each chitosan-based formulation containing an EO was prepared by adding to the chitosan stock solution the EO (2.0 % v/v), vegetal glycerol (0.5 % v/v; A.C.E.F. S.p.A., Fiorenzuola d'Arda, Italy), and Tween 80 (0.6 % v/v; Sigma-Aldrich). EO concentration was set at 2.0 % (v/v), in line with previous studies (Landi et al., 2021; Maswanganye et al., 2025; Ojagh et al., 2010; Silva et al., 2024; Zheng et al., 2023).

Each resulting formulation was stirred at 500 rpm and 18 °C for 10 min. All reagents employed were of food-grade quality. The obtained formulations were stored at 4 °C and heated to 18 °C before use. In total, the ten chitosan-based formulations obtained contained the EOs of: i. *Ocimum basilicum* (ID-Formulation – F – 01); ii. *Allium sativum*-EO (ID-F-02); iii. *Origanum vulgare* (ID-F-03); iv. *Citrus aurantium* (ID-F-04); v. *Foeniculum vulgare* (ID-F-05); vi. *Cinnamomum verum* (ID-F-06); vii. *Laurus nobilis* (ID-F-07); viii. *Piper nigrum* (ID-F-08); ix. *Angelica archangelica* (ID-F-09); x. *Citrus limon* (ID-F-10).

All formulations were tested both as such and at different concentrations diluted in sterile distilled water (sdw); the sole sdw served as the negative control.

2.3. Fungal and oomycete isolates

Fungal and oomycete isolates included in this study were specimens of ascomycetes and *Phytophthora* species belonging to the Molecular Plant Pathology laboratory of the Department of Agriculture, Food and

Environment (University of Catania, Italy). The complete list of isolates included: *A. alternata*, 646; *B. cinerea*, B05.10; *C. acutatum*, UWS149; *C. gloeosporioides*, C2; *F. proliferatum*, CBS 145950; *F. sacchari*, CBS 145949; *M. fructicola*, AN13; *P. expansum*, A8; *P. digitatum*, P1PP0; *P. italicum*, CECT 20909; *Ph. citrophthora*, Ax1Ar; *Ph. nicotianae*, T3-B-K1A; *Plenodomus tracheiphilus*, Pt2.

All isolates were characterized at species level previously (Aloi et al., 2021a; La Spada et al., 2021, 2020; Riolo et al., 2020; Riolo et al., 2021; Rovetto et al., 2024a). Fungal and oomycete isolates were maintained on Potato Dextrose Agar (PDA) at 25.0 ± 1.0 °C, in the dark, until use for tests.

2.4. Determining in vitro anti-fungal and anti-oomycete activity of chitosan-based formulation containing EO

The growth inhibitory activity of the liquid fraction of each chitosan-based formulation containing EO toward any fungal and oomycete pathogen was assessed qualitatively by using agar diffusion test assays performed as in El boumlasy et al. (2021). Each chitosan-based formulation containing EO was diluted in sdw and tested at the following concentrations: 25.0, 50.0, 75.0, and 100.0 % (sdw was used as negative control). For each ascomycete or *Phytophthora* sp., the agar diffusion test was carried out in triplicate. Results from this test, assessed on the bases of the diameter of the halo of inhibition of culture growth determined by the chitosan-based formulations containing EO or by sdw (controls), were scored according to the following empirical scale: (+), for a mean diameter of inhibition halo < 8 mm; (++), for a mean diameter of inhibition halo between 8 and 10 mm; (+++), for a mean diameter of inhibition halo between 10 and 89 mm; (++++), for a mean diameter of the inhibition halo exceeding 89 mm.

The biocidal activity of each chitosan-based formulation containing EO was also assessed quantitatively, evaluating the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Tests for the evaluation of the MIC and MFC were carried out following the methodology outlined by El boumlasy et al. (2021). For MIC determination, a pre-diluted stock of each chitosan-based formulation containing EO was prepared in sdw. For each pathogen, 400.0 µL of this stock were mixed with 400.0 µL sterile PDB and 200.0 µL of spore/zoospore suspension (1×10^4 mL^{-1}) in a 2.0 mL tube to a final volume of 1.0 mL. The stock concentration was set so that the top tube contained 15.0 % (v/v) of the complete formulation; serial dilutions in inoculated PDB were then prepared to yield a ten-point dilution series down to 1.5 % (v/v). sdw alone was used as the negative control. The tubes were incubated at 25.0 ± 1.0 °C for 3 days. After the incubation period, the MIC was assigned to the lowest concentration where no cloudiness was visible in the tubes, which means that no pathogen growth was observed. The MFC of each chitosan-based formulation containing EO toward each pathogen was evaluated by culturing, on drug-free PDA at 25.0 ± 1.0 °C for 3 days, 10.0 µL of liquid culture collected from the well corresponding to the concentration of the MIC, as well as from wells at higher concentrations, deposited as a spot and allowed to soak and dry completely. The inoculum was then streaked away from the deposition spot with a sterile loop to separate cells from any residual formulation (anti-carryover) (Cantón et al., 2003). Then, the MFC was determined as the lowest concentration of each chitosan-based formulation containing EO that completely inhibited any mycelial growth. For each test pathogen and chitosan-based formulation containing EO, experiments for evaluating MIC and MFC were repeated three times.

2.5. Determining the growth inhibitory activity of volatile organic compounds (VOCs) released by chitosan-based formulations containing EO

The growth inhibitory activity of VOCs released by each chitosan-based formulation containing EO was assessed through the following test on all pathogens reported at paragraph 2.3. For each chitosan-based formulation containing EO and pathogen, an agar plug (5.0 mm in size) excised from the growing edge of a 7-day-old culture grown on PDA was transferred to the center of a 9 cm diameter PDA Petri plate. Then, 500.0 µL of a chitosan-based formulation containing EO (concentration as such) or sdw (negative control) were suspended in 500.0 µL of 0.5 % water agar and placed on the top of a microscope slide. The microscope slide was transferred inside the lid of the Petri dish inoculated with the pathogen, and the plate was sealed with Parafilm (Amcor, Zürich, Switzerland). Then, plates were incubated at 25.0 ± 1.0 °C for 7 days. After incubation, the anti-fungal or anti-oomycete activity of the VOCs released by each chitosan-based formulation containing EO was evaluated as the percentage of inhibition of mycelial growth, calculated by using the following formula:

$$I(\%) = \frac{D1 - D2}{D1} x100 \quad (1)$$

where I (%) represents the percentage of growth inhibition, D1 is the mean diameter of a control colony of the pathogen, and D2 is the mean diameter of the colony of the pathogen grown in presence of VOCs released by the chitosan-based formulation containing EO. For each test pathogen and chitosan-based formulation containing EO, the experiment was repeated three times.

2.6. Gas Chromatography – Mass Spectrometry (GC-MS) Analysis of EOs

The chitosan-based formulations ID-F-03 and ID-F-06, containing *O. vulgare* and *C. verum* EOs, respectively, were selected for volatile compound characterization. The GC–MS analysis was performed exclusively on the EO component of these formulations, and not on the full chitosan-based mixture. This option was selected since chitosan was included at the same ratio across all formulations and does not contribute to the volatile profile. Therefore, the volatile compounds detectable are attributed solely to the EO fraction. For the analysis, the two EOs were individually diluted (5.0 %) in HPLC-grade *n*-hexane and then injected into a GC–MS apparatus. The analyses were performed with an Agilent 7890B gas chromatograph (Agilent Technologies Inc.) equipped with an Agilent HP-5 MS (Agilent Technologies Inc.) capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) and an Agilent 5977B single quadrupole mass detector (Agilent Technologies Inc., Santa Clara, CA, USA). Analytical conditions were as follows: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60.0 to 240.0 °C at 3 °C/min; carrier gas helium at 1.0 mL/min; injection of 1.0 µL; split ratio 1:25. The acquisition parameters were as follows: full scan; scan range: 30–300 *m/z*; scan time: 1.0 sec. The identification of the constituents was based on the comparison of their retention times with those of the authentic samples (when available), comparing their linear retention indices relative to the series of *n*-hydrocarbons (C6-C25). Computer matching was also used against a commercial (National Institute of Standards and Technology, 2014) and a laboratory-developed mass spectra library built up from pure substances and components of commercial EOs of known composition and MS literature data (Adams, 2007).

Quantitative comparisons of relative peaks areas (%) were performed between the same chemicals in the different samples.

2.7. Evaluating the in vivo effectiveness of chitosan-based formulations containing EO ID-F-03 and ID-F-06

To evaluate the *in vivo* antifungal effectiveness of chitosan-based formulations ID-F-03 (containing *O. vulgare* EO) and ID-F-06 (containing *C. verum* EO), selected for their superior antifungal activity in both liquid and volatile phases, experiments were conducted on two fruit pathosystems: apple bitter rot caused by *C. gloeosporioides* and green mold of oranges caused by *P. digitatum*. Mature apples (*Malus domestica* var. Morgenduft) and oranges (*Citrus* × *sinensis* var. Tarocco) were

surface-disinfected with 1 % NaClO, rinsed, and air-dried. Apples were inoculated with a 10.0 µL drop of a *C. gloeosporioides* isolate C2 conidial suspension (10^4 conidia/mL), incubated for 24 h at 21.0 ± 1.0 °C, and then treated by spraying 1.0 mL of either ID-F-03 or ID-F-06 at concentrations of 1 × or 2 × the MIC (3.5 %, 7.0 % for ID-F-03; 8.5 %, 17.0 % for ID-F-06). For the inoculation of oranges, the peel of each fruit was wounded at the center of the equatorial surface using a 2.0 mm sterile nail; subsequently, each wound was inoculated with a 10.0 µL drop of a *P. digitatum* isolate P1PP0 conidial suspension (10^4 conidia/mL) and treated by spraying the same formulations at respective MIC levels (5.0 %, 10.0 % for ID-F-03; 5.5 %, 11.0 % for ID-F-06). Each apple and orange control fruit were treated by spraying 1.0 mL of sdw. All fruit were air-dried post-treatment and incubated (apples for 20 days, oranges for 6 days) at 21.0 ± 1.0 °C. Treatments were applied to five batches of 10 fruit each, and each experiment was independently repeated three times. The effectiveness of the formulations was assessed at the end of the incubation period by measuring the reduction in disease incidence (%) in comparison to controls.

2.8. Evaluation of the impact of treatments with EOs on the sensory profile of fresh fruit

The chitosan-based formulations containing EO ID-F-03 and ID-F-06 were also evaluated for their sensory impact on fresh apples (*Malus domestica* var. Morgenduft) and oranges (*Citrus* × *sinensis* var. Tarocco) through a panel test.

For the treatments, raw fruit were first rinsed with sdw, air-dried at room temperature, and then homogeneously sprayed using the same procedure described for the *in vivo* assays with either formulation ID-F-03 (diluted in sdw at C = 10 %) or ID-F-06 (diluted in sdw at C = 19 %). These concentrations were selected since they represent the maximum effective doses required to achieve *in vitro* inhibition of all target pathogens. Specifically, they correspond to 2 × MIC observed for *P. digitatum* and *M. fructicola*, the two pathogens least sensitive to ID-F-03 and ID-F-06, respectively (see Table 2). Raw apples and oranges rinsed with sdw and air-dried at room temperature were used as negative controls. Following treatment, fruit were placed on a plastic mesh grill and let dry at room temperature for 12 h. Finally, they were stored in plastic lidded containers (150 cc, Cuki Cofresco Spa, Turin, Italy) with air as the storage atmosphere for 30 min before tasting. Right before the assessment, apples and oranges were peeled and sliced. Each treatment was replicated three times.

The organoleptic profiles of the apples and oranges sprayed with the two chitosan-based formulations containing EO, including smell, taste, and touch (rheological properties during chewing), were assessed by a panel of 10 trained experts (6 females and 4 males) aged from 23 to 65 years. The assessors were selected based on their availability from a larger pool of judges who regularly collaborate with the Department of Agriculture, Food, and Environment (DAFE) of the University of Pisa. All judges underwent standardized training to enhance their ability to recognize, describe, and quantify tastes, odors, and texture properties in accordance with ISO 8586 standards. All participants were primarily experts in the evaluation of food and EOs, and before the start of the study, they had given their informed consent.

A final set of 20 descriptive parameters (Fig. 1), including both quantitative and hedonic attributes, was evaluated on a 0–9 scale.

The tasting sessions were conducted in the morning in a well-ventilated, quiet room (ISO-certified Sensory Laboratory at the Department of Agriculture, Food and Environment (DAFE), University of Pisa) with a relaxed atmosphere. To prevent cross-contamination, apples and oranges were evaluated at separate times during the same session by the same panel of assessors. Each panelist received 3–5 fruit slices per session, depending on the fruit size, with no information provided about the treatment provided. Samples with different treatments for each fruit

Fig. 1. Sensory wheel used to describe the smell, taste, and touch of apples and oranges treated with chitosan-based formulations containing EO.

type were assessed separately on the same morning, with a 5-minute interval between evaluations. Additionally, all treated fruit were removed from the room before the beginning of a new tasting session.

The overall organoleptic quality of treated fruit was quantified using the Overall Hedonic Index (OHI). This index was calculated as the average of the scores attributed to the hedonic parameters (fineness of smell, frankness of smell, and overall pleasantness, Fig. 1) during the panel tests, using the formula:

$$OHI = \text{Average [Hedonic indexes]} \times 1.11 \quad (2)$$

2.9. Statistical analyses

Data analysis was performed by using RStudio v.1.2.5 (R). The *in vivo* tests statistical comparisons among treatments were carried out using the one-way ANOVA followed by Tukey's HSD (Honestly Significant Difference) *post hoc* with $p \leq 0.05$.

The reliability of the panel test assessments was analyzed by two-way completely randomized Friedman's ANOVA with panelists and EO-containing treatments as fixed factors through the software Big Sensory Soft 2.0 (ver. 2018, Centro Studi Assaggiatori, Brescia (BS), Italy), which is specifically developed for sensory analysis.

3. Results

3.1. Qualitative biocidal activity of chitosan-based formulations containing EO

In the agar diffusion test, all tested chitosan-based formulations containing EO demonstrated inhibitory activity against all tested pathogens (Tables 1, 2, S1–S8). Formulations ID-F-02, ID-F03, and ID-F06 were effective at all tested concentrations (from 25 % to 100 %) against most of the test pathogens. Specifically, ID-F-02 at concentrations ranging from 50 % to 100 % determined growth inhibition halos with diameters ranging from 10 to 89 mm (+++) in all fungal pathogens, except for *M. fructicola* and *B. cinerea*, which were the least inhibited. At these concentrations, the oomycetes *Ph. citrophthora* and *Ph. nicotianae* were less susceptible than most of fungi, with maximum growth inhibition halos of 10 mm (++) (Table S2). When tested at a concentration of 25 %, ID-F-02 produced halos of growth inhibition against all tested pathogens except *B. cinerea*, *F. proliferatum*, and *P. italicum* (Table S2). ID-F-03 and ID-F-06 were the most effective formulations, determining a marked growth inhibition at all tested concentrations in most of the tested pathogens (Tables 1 and 2). In this respect, it is noteworthy that *B. cinerea*, *M. fructicola*, and *P. expansum* were the pathogens least inhibited by ID-F-03 and ID-F-06 (Tables 1 and 2). ID-F-04, ID-F-08, and ID-F-09 were the least effective formulations, determining halos of growth inhibition in only a limited number of the tested pathogens and only at concentrations above 75 % (Tables S3, S6 and S7). Similarly to the previous result, all the other tested formulations, *i.e.* ID-F-01, ID-F-05, ID-F-07, and ID-F-10, were effective against only some of the tested pathogens, although at a wider range of concentrations (*i.e.*, from 25 % to 100 %) (Tables S1, S4 S5 and S8). Overall, with the exception of ID-F-03 and ID-F-06, which inhibited all tested fungi and oomycetes, the remaining formulations inhibited the mycelial growth of only a subset of pathogens, including some fungi and consistently both oomycetes, *Ph. citrophthora* and *Ph. nicotianae*.

MIC and MFC values determined by each chitosan-based formulation containing EO toward any fungal and oomycete pathogens are reported at Tables 3 and S9. Noteworthy are values of MIC and MFC recorded for ID-F-03, which resulted in the most performing formulation (Table 3). Specifically, its MIC ranged from a minimum of 1.5 % (recorded for *C. acutatum* and *Pl. tracheiphilus*) to a maximum of 5.0 % (recorded for *P. digitatum*), while MFC values ranged from 1.5 % (recorded for *Pl. tracheiphilus*) to 5.5 % (recorded for *P. digitatum*) (Table 3). Although less effective than ID-F-03, formulation ID-F-06 also showed promising activity in inhibiting several plant pathogens (Table 3). Its MIC values ranged from 1.5 % (effective against *F. sacchari* and *Pl. tracheiphilus*) to 9.5 % (against *M. fructicola*), while MFC values ranged from 1.5 % (*Pl.*

Table 1
Halos of growth inhibition determined by the chitosan-based formulation containing EO ID-F-03 water diluted at different concentrations (or by sterile distilled water – control) toward fungal and oomycete test strains in the agar diffusion test.

Test pathogen (species, strain)	Control	ID-F-03 (C = 25 %)	ID-F-03 (C = 50 %)	ID-F-03 (C = 75 %)	ID-F-03 (C = 100 %)
A. alternata, 646	–	+ + + +	+ + + +	+ + + +	+ + + +
B. cinerea, B05.10	–	–	–	+	+
C. acutatum, UWS149	–	+ + +	+ + + +	+ + + +	+ + + +
C. gloeosporioides, C2	–	+ + +	+ + +	+ + + +	+ + + +
F. proliferatum, CBS 145950	–	+ + +	+ + +	+ + +	+ + +
F. sacchari, CBS 145949	–	+ + +	+ + +	+ + +	+ + +
M. fructicola, AN13	–	–	+	+ +	+ + +
P. expansum, A8	–	–	–	–	–
P. digitatum, P1PP0	–	+ + + +	+ + + +	+ + + +	+ + + +
P. italicum, CECT 20909	–	+ + + +	+ + + +	+ + + +	+ + + +
Ph. citrophthora, Ax1Ar	–	+ + + +	+ + + +	+ + + +	+ + + +
Ph. nicotianae, T3-B-K1A	–	+ + + +	+ + + +	+ + + +	+ + + +
Pl. tracheiphilus, Pt2	–	+ + +	+ + + +	+ + + +	+ + + +

Values were scored on the basis of the following empirical scale: (+), mean diameter of inhibition halo < 8 mm, (++), mean diameter of inhibition halo between 8 and 10 mm, (+++), mean diameter of inhibition halo between 10 and 89 mm, (++++), mean diameter of the inhibition halo exceeding 89 mm, no inhibition (–).

Table 2
Halos of growth inhibition determined by the chitosan-based formulation containing EO ID-F-06 water diluted at different concentrations (or by sterile distilled water – control) toward fungal and oomycete test strains in the agar diffusion test.

Test pathogen (species, strain)	Control	ID-F-06 (C = 25 %)	ID-F-06 (C = 50 %)	ID-F-06 (C = 75 %)	ID-F-06 (C = 100 %)
A. alternata, 646	–	+ + + +	+ + + +	+ + + +	+ + + +
B. cinerea, B05.10	–	+ +	+ +	+ +	+ + +
C. acutatum, UWS149	–	+ + + +	+ + + +	+ + + +	+ + + +
C. gloeosporioides, C2	–	+ + + +	+ + + +	+ + + +	+ + + +
F. proliferatum, CBS 145950	–	+ + + +	+ + + +	+ + + +	+ + + +
F. sacchari, CBS 145949	–	+ + + +	+ + + +	+ + + +	+ + + +
M. fructicola, AN13	–	+ +	+ +	+ +	+ +
P. expansum, A8	–	+ +	+ +	+ +	+ + +
P. digitatum, P1PP0	–	+ + + +	+ + + +	+ + + +	+ + + +
P. italicum, CECT 20909	–	+ + + +	+ + + +	+ + + +	+ + + +
Ph. citrophthora, Ax1Ar	–	+ + + +	+ + + +	+ + + +	+ + + +
Ph. nicotianae, T3-B-K1A	–	+ + + +	+ + + +	+ + + +	+ + + +
Pl. tracheiphilus, Pt2	–	+ + + +	+ + + +	+ + + +	+ + + +

Values were scored on the basis of the following empirical scale: (+), mean diameter of inhibition halo < 8 mm, (++), mean diameter of inhibition halo between 8 and 10 mm, (+++), mean diameter of inhibition halo between 10 and 89 mm, (++++), mean diameter of the inhibition halo exceeding 89 mm, no inhibition (–).

Table 3
Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the chitosan-based formulations containing EO ID-F-03 and ID-F-06 toward fungal and oomycete pathogens.

Test pathogen (species, strain)	ID-F-03 (C %)		ID-F-06 (C %)	
	MIC	MFC	MIC	MFC
A. alternata, 646	2.0	3.5	3.0	3.0
B. cinerea, B05.10	3.0	3.0	8.0	9.0
C. acutatum, UWS149	1.5	2.0	3.0	3.0
C. gloeosporioides, C2	3.5	3.5	8.5	10.0
F. proliferatum, CBS 145949	3.5	5.0	2.0	3.0
F. sacchari, CBS 145949	2.0	2.0	1.5	2.0
M. fructicola, AN13	3.0	3.5	9.5	10.0
P. expansum, A8	4.5	4.5	7.5	8.0
P. digitatum, P1PP0	5.0	5.5	5.5	5.5
P. italicum, CECT 20909	2.5	4.5	5.5	5.5
Ph. citrophthora, Ax1Ar	3.0	4.5	3.5	5.0
Ph. nicotianae, T3-B-K1A	3.0	5.0	2.5	5.0
Pl. tracheiphilus, Pt2	1.5	1.5	1.5	1.5

tracheiphilus) to 10 % (effective against *C. gloeosporioides* and *M. fructicola*) (Table 3). All other formulations were less effective, especially against *B. cinerea*, *C. acutatum*, *C. gloeosporioides*, *M. fructicola*, and *P. expansum*, whose MIC and MFC values were always above 7.5 % (Table S9).

3.2. *In vitro inhibitory activity of VOCs released by the chitosan-based formulations containing EO*

The *in vitro* tests for evaluating the activity of VOCs released by the chitosan-based formulations containing EO showed a generalized inhibition of growth compared to control cultures (Figures 2, 3, S1, and S2). In detail, the formulations ID-F-03 and ID-F-06 were those that, among all formulations, determined the strongest growth inhibition in all tested pathogens (Figs. 2 and 3). Species such as *A. alternata*, *C. gloeosporioides*, *F. sacchari*, *P. nicotianae*, and *P. expansum* were non-sensitive to the inhibitory activity of VOCs of some formulations; these included ID-F-01 and ID-F-07 for *A. alternata* (Figure S1a), ID-F-05, ID-F-07, ID-F-08, ID-F-09, and ID-F-10 for *C. gloeosporioides* (Figure S1d), ID-F-01, ID-F-04, ID-F-07, ID-F-08, ID-F-09, and ID-F-10 for *F. sacchari* (Figure S1f), ID-F-01 for *P. nicotianae* (Figure S2c), and ID-F-09 and ID-F-10 for *P. expansum* (Figure S1h). Finally, it is noteworthy that some pathogens were stimulated in their growth by VOCs released by some formulations; these latter were ID-F-01, ID-F-04, ID-F-07, ID-F-08, ID-F-09, and ID-F-10 for *P. digitatum* (Figure S1i), ID-F-05 for *P. italicum* (Figure S2a), and ID-F-04, ID-F-05, ID-F-09, and ID-F-10 for *Ph. citrophthora* (Figure S2b).

3.3. *Gas chromatography – Mass Spectrometry Analysis of EOs*

The complete composition of the two EOs, *C. verum* and *O. vulgare*, included in the chitosan-based formulations containing EO ID-F-06 and ID-F-03, respectively, is reported in Table 4. Phenylpropanoids were detected as the most abundant chemical class in the *C. verum* EO. Among these, (*E*)-cinnamaldehyde and eugenol were the most represented compounds, accounting for 40.0 % and 36.0 %, respectively. The *O. vulgare* EO used in this study exhibited a carvacrol chemotype, with carvacrol as the predominant compound (71.3 %), followed by thymol at 4.7 %. Both compounds belong to the class of oxygenated monoterpenes.

3.4. *In vivo effectiveness of chitosan-based formulations containing EO ID-F-03 and ID-F-06*

The *in vivo* test for evaluating the efficacy of ID-F-03 and ID-F-06 in controlling the apple bitter rot by *C. gloeosporioides* highlighted a significant effectiveness of the two formulations in reducing the disease incidence compared to the control treatment (Fig. 4). Concerning ID-F-03, apples treated with this formulation at conc. 1 × *C. gloeosporioides*–MIC had a disease incidence of approximately 33 %, while fruit treated with 2 × *C. gloeosporioides*–MIC had a disease incidence of 13 % (Fig. 4). Fruit that were treated with ID-F-06 at conc. 1 × *C. gloeosporioides*–MIC had a disease incidence of 50 %, while it was about 30 % fruit treated with 2 × *C. gloeosporioides*–MIC (Fig. 4)

On oranges inoculated with *P. digitatum*, ID-F-03 significantly

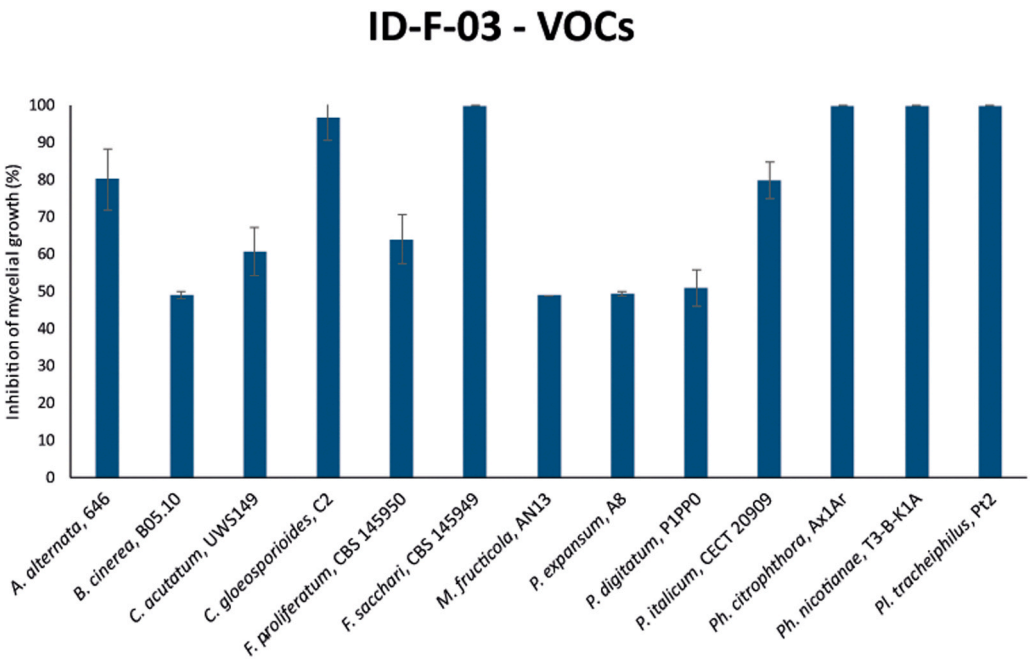

Fig. 2. *In vitro* inhibitory activity (evaluated as inhibition of mycelial growth - %) of volatile organic compounds (VOCs) released by the chitosan-based formulation containing EO ID-F-03 on the test strains *Alternaria alternata* 646, *Botrytis cinerea* B05.10, *Colletotrichum acutatum* UWS149, *Colletotrichum gloeosporioides* C2, *Fusarium proliferatum* CBS 145950, *Fusarium sacchari* CBS 145949, *Monilinia fructicola* AN13, *Penicillium expansum* A8, *Penicillium digitatum* P1PP0, *Penicillium italicum* CECT 20909, *Phytophthora citrophthora* Ax1Ar, *Phytophthora nicotianae* T3-B-K1A, and *Plenodomus tracheiphilus* Pt2. Bars represent standard deviation.

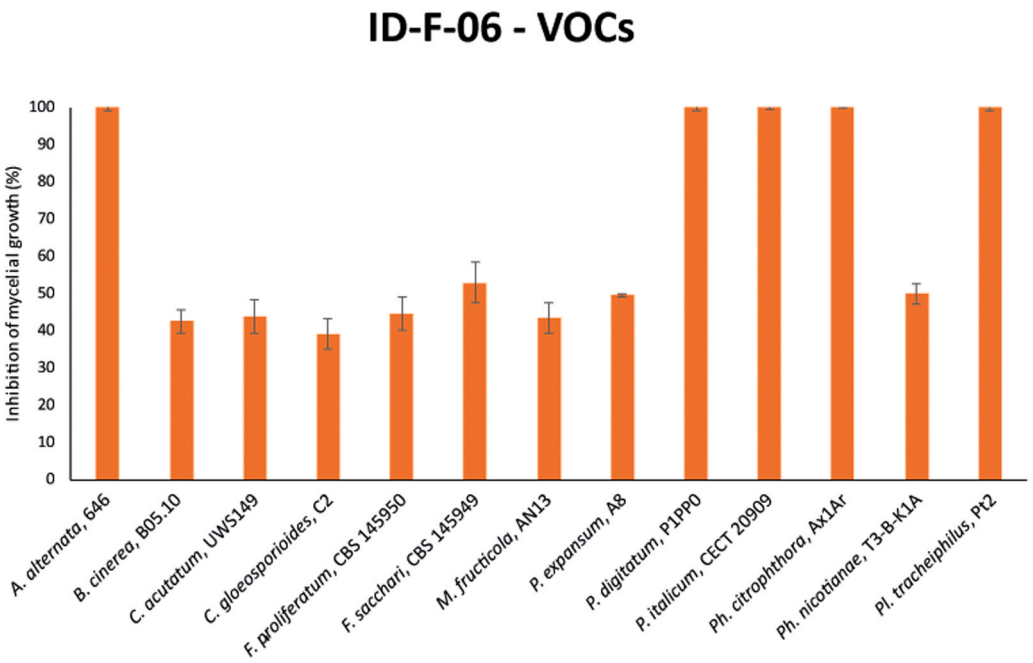

Fig. 3. *In vitro* inhibitory activity (evaluated as inhibition of mycelial growth - %) of volatile organic compounds (VOCs) released by the chitosan-based formulation containing EO ID-F-06 on the test strains *Alternaria alternata* 646, *Botrytis cinerea* B05.10, *Colletotrichum acutatum* UWS149, *Colletotrichum gloeosporioides* C2, *Fusarium proliferatum* CBS 145950, *Fusarium sacchari* CBS 145949, *Monilinia fructicola* AN13, *Penicillium expansum* A8, *Penicillium digitatum* P1PP0, *Penicillium italicum* CECT 20909, *Phytophthora citrophthora* Ax1Ar, *Phytophthora nicotianae* T3-B-K1A, and *Plenodomus tracheiphilus* Pt2. Bars represent standard deviation.

reduced disease incidence compared to the control, whereas ID-F-06 yielded intermediate values that did not differ significantly from either the control or ID-F-03 at the tested concentrations (Fig. 5). In detail, oranges treated with ID-F-03 at conc. 1 × *P. digitatum*–MIC had a disease incidence of approximately 60 %, while fruit treated with this formulation at conc. 2 × *P. digitatum*–MIC had a disease incidence of 50 % (Fig. 5). The treatment of orange fruit with ID-F-06 at conc. 1 × *P. digitatum*–MIC determined an incidence of green mold of about 70 %, while fruit that received the formulation at conc. 2 × *P. digitatum*–MIC showed symptoms of the disease with an incidence of approximately 63 % (Fig. 5).

3.5. Impact of treatments with EOs on the sensory profile of apples and oranges

A sensory evaluation was carried out to assess the impact of two chitosan-based formulations containing EO, ID-F-03 and ID-F-06, on the organoleptic characteristics of fresh apple and orange fruit. Both quantitative sensory descriptors (such as aroma intensity, bitterness, and sweetness) and hedonic parameters (such as harmony, retro-olfactory clarity, and overall acceptability) were considered. All fruit were tasted after peeling, in order to eliminate the influence of the outer surface on perception.

Friedman's ANOVA revealed statistically significant differences across the tested conditions, including both treatments and the untreated control, for most of the evaluated parameters. The effects of the treatments on quantitative sensory descriptors are presented in Fig. 6a for apples and in Fig. 6b for oranges.

In general, apples exhibited a more pronounced sensory alteration following the treatments compared to oranges. In both fruit, a significant reduction was observed in the perception of fruity and typical fruit aroma, accompanied by a marked increase in bitterness. A significant decrease in sweetness was recorded only in oranges, suggesting a higher susceptibility of citrus to changes in this attribute.

The analysis of hedonic parameters revealed a general reduction in panel appreciation following the treatments, affecting both fruit types. As shown in Fig. 7a for apples and Fig. 7b for oranges, this decline was primarily associated with reduced perceived harmony and a loss of retro-olfactory clarity. These effects were observed regardless of the formulation used, although the extent varied depending on the fruit.

The overall outcome of the hedonic evaluation is summarized in Figs. 8a and 8b, which report the Overall Hedonic Index for each treatment and fruit.

Interestingly, the impact of the formulations appeared fruit-specific. In apples, formulation ID-F-06 (containing *C. verum* EO) was associated with a more favorable hedonic response, suggesting better sensory compatibility compared to ID-F-03 (containing *O. vulgare* EO). In contrast, in oranges, formulation ID-F-03 preserved a higher level of acceptability than ID-F-06. This differential response highlights the importance of fruit type in determining the sensory tolerance to postharvest treatments based on EOs.

Overall, although both treatments altered the sensory perception of the fruit, their impact varied depending on the specific formulation–fruit combination. ID-F-06 was better tolerated in apples, whereas ID-F-03 showed a more favorable profile in oranges.

4. Discussion

Effective management of fruit diseases in the postharvest phase, where decay during storage and distribution leads to major economic losses, remains a central challenge for sustainable agriculture (Bhatta, 2022; Lahlali et al., 2022). Compared with synthetic fungicides, chitosan–EO formulations reduce residue and single-site resistance concerns (Ismail and Zhang, 2004; Migheli et al., 2009; Prusky and Romanazzi, 2023). Versus physical treatments (*e.g.*, heat or UV), they offer sustained headspace activity without thermal stress, although they do not provide the instantaneous sanitation of short, high-intensity interventions. Relative to biocontrol agents, they deliver immediate inhibition that is less dependent on establishment conditions, whereas biocontrols may achieve longer persistence but with higher variability (Romanazzi et al., 2018). Finally, compared with EO-only or chitosan-only coatings, the composite couples EO potency with the filmability and

Table 4
Complete composition (detection threshold ≥ 0.1 %) of the two essential oils included in the chitosan-based formulations containing EO ID-F-03 (*i.e. Origanum vulgare*) and ID-F-06 (*i.e. Cinnamomum verum*).

Compounds	l.r.i.[a]	Relative abundance (%)	
		Cinnamomum verum[2]	*Origanum vulgare*
α-thujene	931	-[3]	0.1
α-pinene	941	0.4	0.4
camphene	955	0.2	0.1
benzaldehyde	959	0.1	-
β-pinene	982	0.2	0.6
3-octanone	987	-	0.1
myrcene	993	-	0.3
α-phellandrene	1005	0.4	-
α-terpinene	1018	-	0.5
p-cymene	1027	0.7	5.8
limonene	1032	0.3	0.5
1,8-cineole	1034	-	0.8
γ-terpinene	1062	-	3.4
linalool	1101	1.4	2.8
camphor	1143	-	0.8
borneol	1165	-	1.1
4-terpineol	1178	-	0.6
α-terpineol	1189	0.1	0.7
carvacrol methyl ether	1244	-	0.3
(*E*)-cinnamaldehyde	1268	40.0	-
safrole	1288	0.9	-
thymol	1292	-	4.7
carvacrol	1298	-	71.3
eugenol	1358	36.0	0.2
hydrocinnamyl acetate	1370	0.1	-
α-copaene	1376	0.4	-
vanillin	1401	0.5	-
β-caryophyllene	1420	2.7	5.5
cinnamic acid	1434	0.7	-
(*E*)-cinnamyl acetate	1444	0.8	-
α-humulene	1456	0.4	0.6
β-bisabolene	1509	-	0.3
δ-cadinene	1525	0.1	-
eugenol acetate	1528	2.5	-
caryophyllene oxide	1581	0.6	0.8
4-hydroxy−3-methoxycinnamaldehyde	1741	1.6	-
(*E*)-coniferyl alcohol	1743	0.6	-
benzyl benzoate	1764	7.9	-
Monoterpene hydrocarbons		2.2	11.5
Oxygenated monoterpenes		1.5	83.1
Sesquiterpene hydrocarbons		3.7	4.3
Oxygenated sesquiterpenes		0.6	0.8
Phenylpropanoids		83.2	0.2
Non-terpene derivatives		8.6	0.1
Total identified (%):		99.7	100.0

[a] Linear retention index on a HP-5 MS capillary column; [2]Composition already published in Bedini et al. (2024); [3]Not detected.

release-modulating capacity of chitosan (Bautista-Baños et al., 2016; Quesada et al., 2016). Within this landscape, chitosan–EO systems are most suitable where residue constraints are stringent, or when integrated as active packaging to minimize direct contact while retaining vapor-phase control.

Although chitosan–EO systems have been widely investigated, integrated evidence that relates the volatile composition of EO–chitosan formulations to both contact- and vapor-phase inhibition across fungi and oomycetes, and verifies the translation to *in vivo* control together with sensory effects on fresh fruit, remains limited. Previous systems, such as chitosan–cinnamon EO coatings on postharvest decay on apples (Zhang et al., 2023) chitosan–spearmint EO nanoemulsions against green and blue molds on citrus, and chitosan/limonene active pads for controlling *B. cinerea* on strawberries (REF), have shown the efficacy of EO–chitosan formulations in specific pathosystems (Cefola et al., 2023; Maswanganye et al., 2025; Zhang et al., 2023). However, such studies typically address a single host and do not simultaneously relate volatile composition, contact- and vapor-phase inhibition, *in vivo* performance, and sensory effects. The present study addressed this gap by screening ten chitosan-based formulations (each containing a single EO) against representative fungal and oomycete plant pathogens with agar diffusion and MIC/MFC assays (contact) and VOC exposure tests (vapor), profiling EO volatiles by GC–MS to contextualize activity, and subsequently validating the two top performers (ID-F-03 and ID-F-06) *in vivo* on apples and oranges artificially inoculated; the sensory impact on fresh fruit was then assessed by a trained panel.

The *in vitro* evaluation of the ten chitosan-based formulations containing EOs through agar diffusion and MIC/MFC assays revealed a variable but generally strong inhibitory activity against the tested phytopathogens. In particular, several formulations exhibited pronounced inhibition zones and MIC/MFC values ranging from 1.5 % to 9.5 % (v/v), thus confirming the broad-spectrum potential of the EOs–chitosan combinations. Comparable antimicrobial performance has been documented in previous studies evaluating similar composite matrices. Chein et al. (2019) reported that chitosan films enriched with cinnamon EO achieved complete inhibition of *Aspergillus flavus* and *Penicillium citrinum*, with MIC values close to 40 μL/mL. A further study by Maswanganye et al. (2025) demonstrated that a chitosan coating incorporating 2 % spearmint EO completely inhibited *P. digitatum* and *P. italicum in vitro* using an agar diffusion assay. The observed antifungal activity is likely due to a synergistic effect between the EO components and the chitosan matrix, which is known to increase the solubility and bioavailability of hydrophobic volatile molecules, while contributing its own intrinsic antifungal properties (Ashraf et al., 2022).

The VOCs released by the chitosan-based formulations containing EO exhibited variable antifungal and anti-oomycete activity across the tested pathogens. Among all tested formulations, ID-F-03 and ID-F-06 induced the highest overall growth inhibition, although the magnitude of this effect varied depending on the pathogen. In contrast, several other formulations displayed poor or no inhibitory activity against specific species, and even stimulation of mycelial growth was observed in the case of *P. digitatum*, *P. italicum*, and *Ph. citrophthora*. The superior performance of ID-F-03 and ID-F-06 is likely related to their specific volatile profiles, as confirmed by the GC–MS analysis. The *C. verum* EO used in ID-F-06 was characterized by a high content of phenylpropanoids, particularly (*E*)-cinnamaldehyde (40.0 %) and eugenol (36.0 %), while the *O. vulgare* EO in ID-F-03 exhibited a carvacrol chemotype, with carvacrol accounting for 71.3 % of the total composition. These compounds are widely recognized for their antifungal activity in the vapor phase. Carvacrol, for instance, completely inhibited postharvest gray mold in strawberries in (Tančinová et al., 2022). Similarly, fumigation trials with eugenol inhibited *Aspergillus niger* and *Aspergillus ochraceus* in stored cereals (Ben Miri et al., 2023). Cinnamaldehyde has demonstrated strong vapor-phase activity against *A. niger* (Niu et al., 2022). These results support the potential of selected chitosan–EO combinations as effective systems for volatile-phase control of postharvest pathogens, with the chitosan matrix likely contributing to the modulation of volatility and sustained release of active compounds, as also supported by results from previous studies (Djebbi et al., 2024; Farina et al., 2025; González-Reza et al., 2021).

The *in vivo* assays conducted on artificially inoculated fruit confirmed the antifungal potential of selected chitosan-based formulations containing EOs under real-pathosystem conditions. The application of ID-F-03 and ID-F-06 significantly reduced disease incidence in both apple and orange fruit, although with different degrees of effectiveness depending on the formulation, target pathogen, and dosage applied. In the apple bitter rot by *C. gloeosporioides* model, ID-F-03 was the most effective, reducing bitter rot incidence to 33.0 % and 13.0 % at 1 × and 2 × MIC, respectively, while ID-F-06 achieved reductions to 50.0 % and 30.0 % at corresponding concentrations. These values confirm the high efficacy of these chitosan–EO coatings against *Colletotrichum* spp., in agreement with previous studies. In this respect, Zhang et al. (2023) demonstrated that chitosan–cinnamon EO coatings

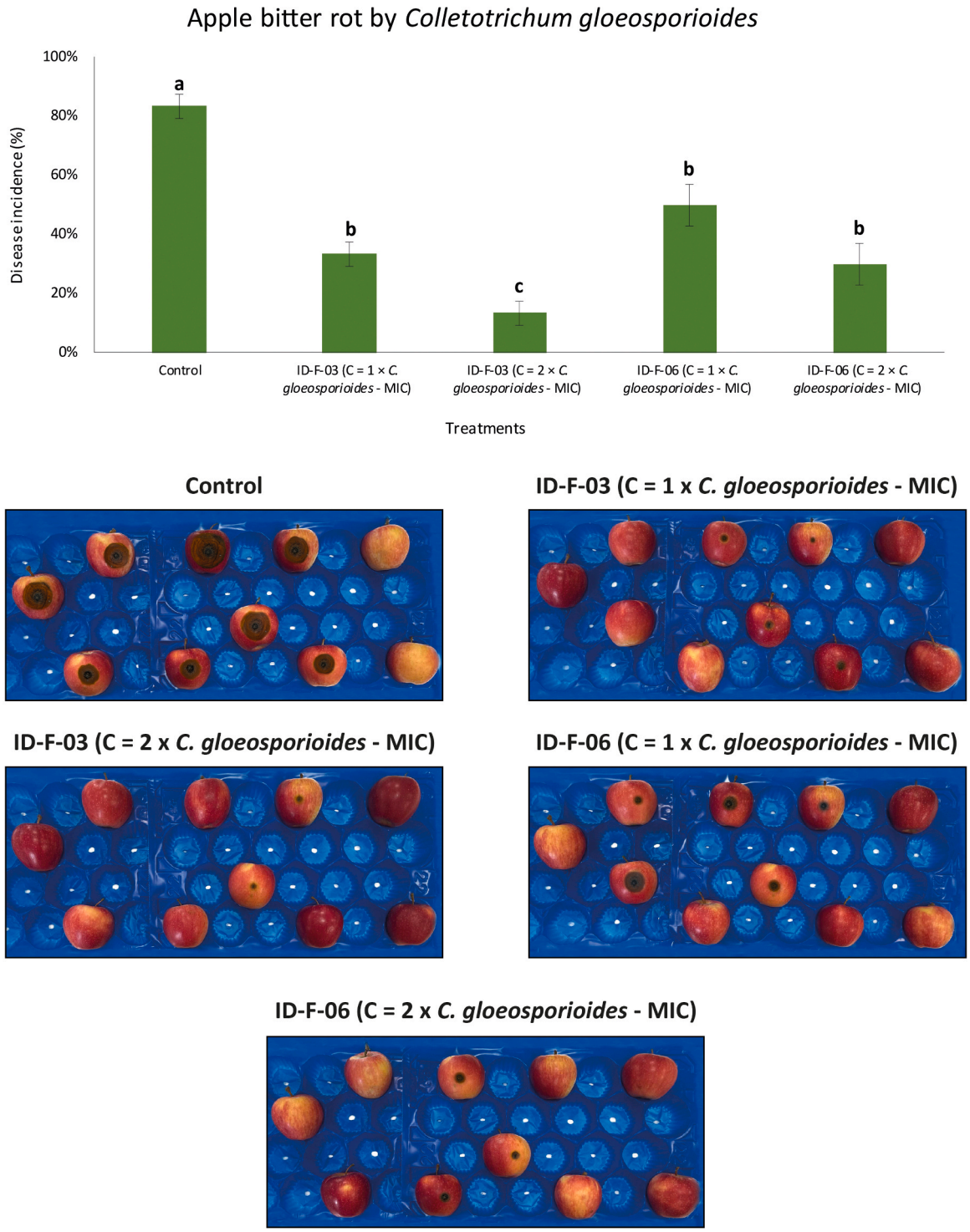

Fig. 4. Incidence of apple bitter rot by *Colletotrichum gloeosporioides* in apple fruit 21-days-post inoculation with *C. gloeosporioides* – UWS149 and treatment with sterile distilled water (sdw) (control), ID-F-03 at conc. 1 × *C. gloeosporioides*–MIC (*i.e.*, 3.5 %) or 2 × *C. gloeosporioides*–MIC (*i.e.*, 7.0 %), and ID-F-06 at conc. 1 × *C. gloeosporioides*–MIC (*i.e.*, 8.5 %) or 2 × *C. gloeosporioides*–MIC (*i.e.*, 17.0 %). Values sharing the same letters are not statistically different according to the Tukey's HSD (Honestly Significant Difference) test ($p \leq 0.05$). Bars represent standard deviation.

significantly controlled *P. expansum* in postharvest on apples, highlighting the suitability of *C. verum* EO in such systems. Similarly, Soppelsa et al. (2023) reported that chitosan–EO treatments applied on apples reduced *B. cinerea* incidence and delayed spoilage without compromising fruit quality. On oranges inoculated with *P. digitatum*, both formulations showed more modest, yet consistent, reductions in green mold incidence. ID-F-03 limited disease to 60.0 % and 50.0 % at 1 × and 2 × MIC, respectively, while ID-F-06 achieved corresponding values of 70.0 % and 63.0 %. Comparable results were reported by Maswanganye et al. (2025), where a chitosan coating loaded with spearmint EO nanoemulsion effectively reduced the incidence of *P. digitatum* and *P. italicum* in soft citrus fruit. Additionally, Ipinza-Concha et al. (2024) demonstrated the efficacy of advanced chitosan coatings against *P. digitatum* on lemon, even in the absence of EO

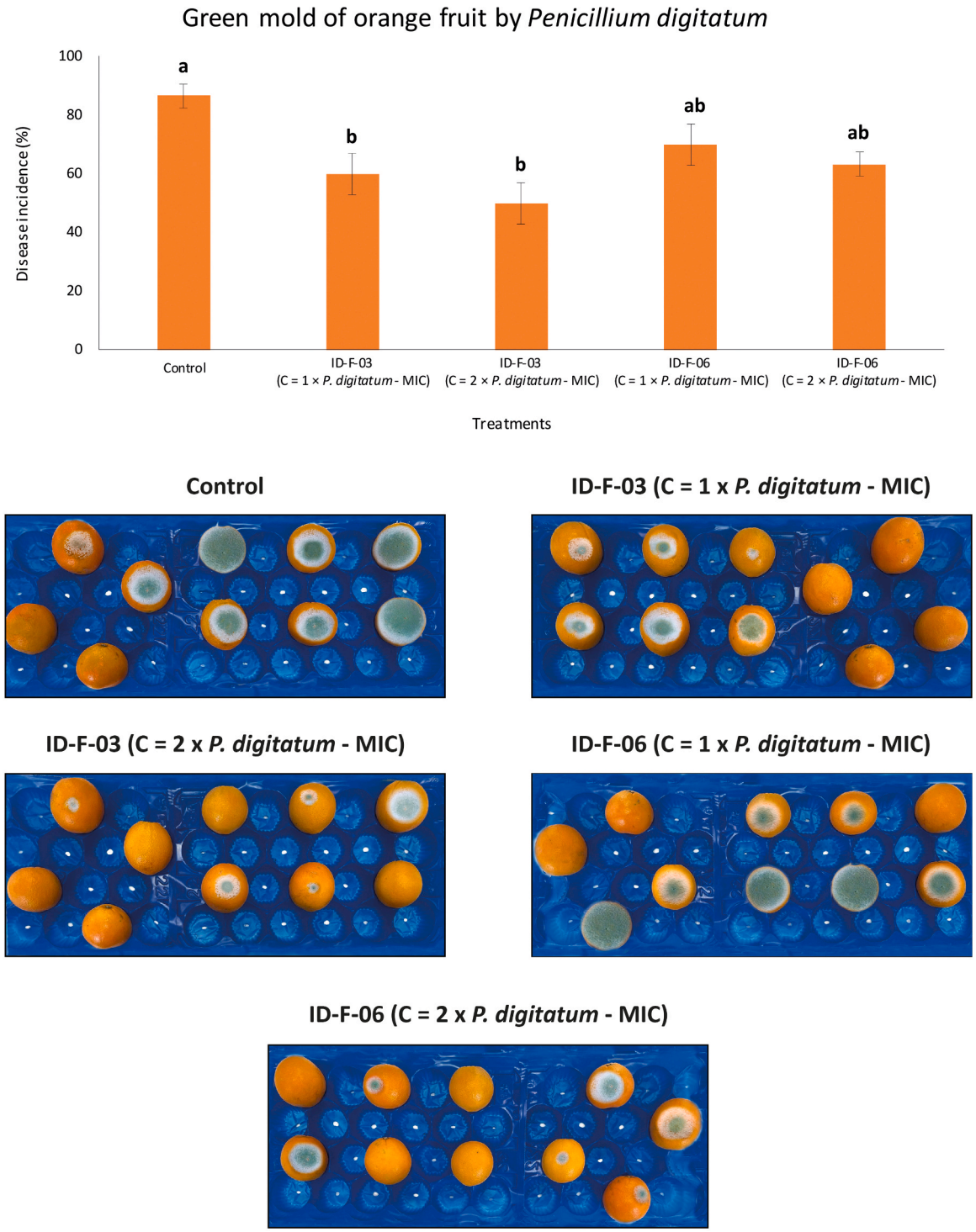

Fig. 5. Incidence of green mold of orange fruit by *Penicillium digitatum* in orange fruit 7-days-post inoculation with *P. digitatum* – P1PP0 and treatment with sterile distilled water (sdw) (control), ID-F-03 at conc. 1 × *P. digitatum*–MIC (*i.e.*, 5.0 %) or 2 × *P. digitatum*–MIC (*i.e.*, 10.0 %), and ID-F-06 at conc. 1 × *P. digitatum*–MIC (*i.e.*, 5.5 %) or 2 × *P. digitatum*–MIC (*i.e.*, 11.0 %). Values sharing the same letters are not statistically different according to the Tukey's HSD (Honestly Significant Difference) test ($p \leq 0.05$). Bars represent standard deviation.

enrichment, thus corroborating the functional role of the chitosan matrix in postharvest biocontrol strategies. The efficacy trends observed across both fruit models are also consistent with those previously described for other fruit–pathogen combinations. Munhuweyi et al. (2017) reported that chitosan–EO treatments on pomegranates resulted in substantial reductions in postharvest disease incidence, particularly when applied at appropriate concentrations.

The sensory analysis revealed that both ID-F-03 and ID-F-06 induced moderate but perceptible alterations in the organoleptic profile of apples and oranges. Notably, panelists reported a decrease in fruity aroma and sweetness, particularly in oranges, as well as increased bitterness in both fruit. Hedonic parameters such as retro-olfactory frankness and overall harmony were also negatively affected, suggesting that the formulations, while microbiologically effective, may influence the sensory appeal of fresh produce. However, the degree of impact varied depending on the fruit–formulation combination: ID-F-06 was better tolerated in

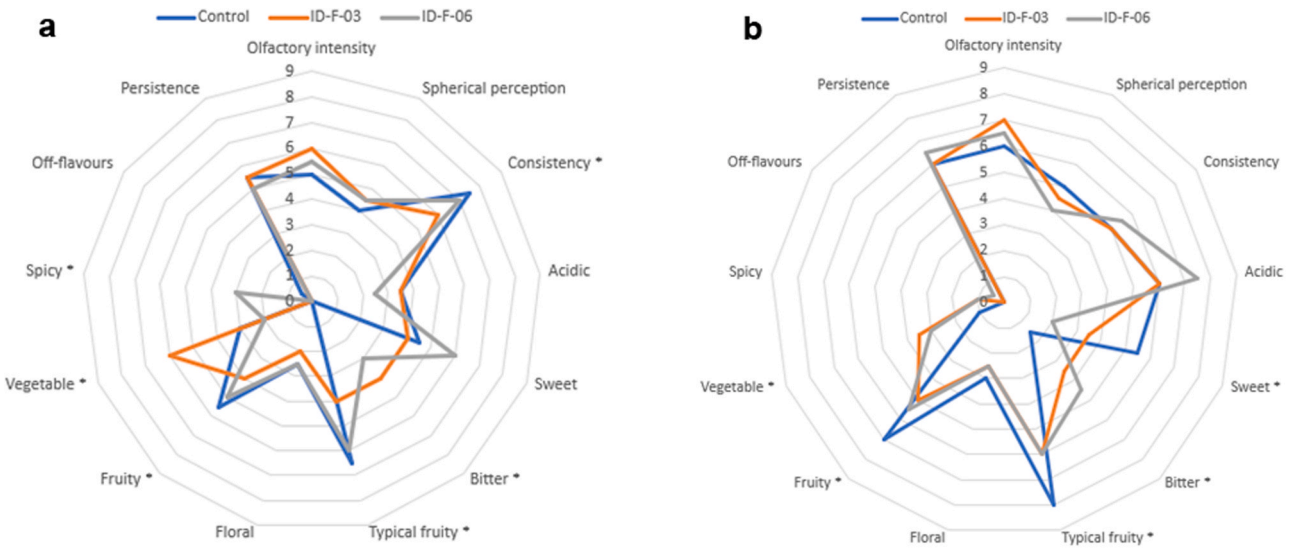

Fig. 6. Median values for quantitative parameters for treated apples (a) and oranges (b). *Statistically significant differences based on Friedman's ANOVA.

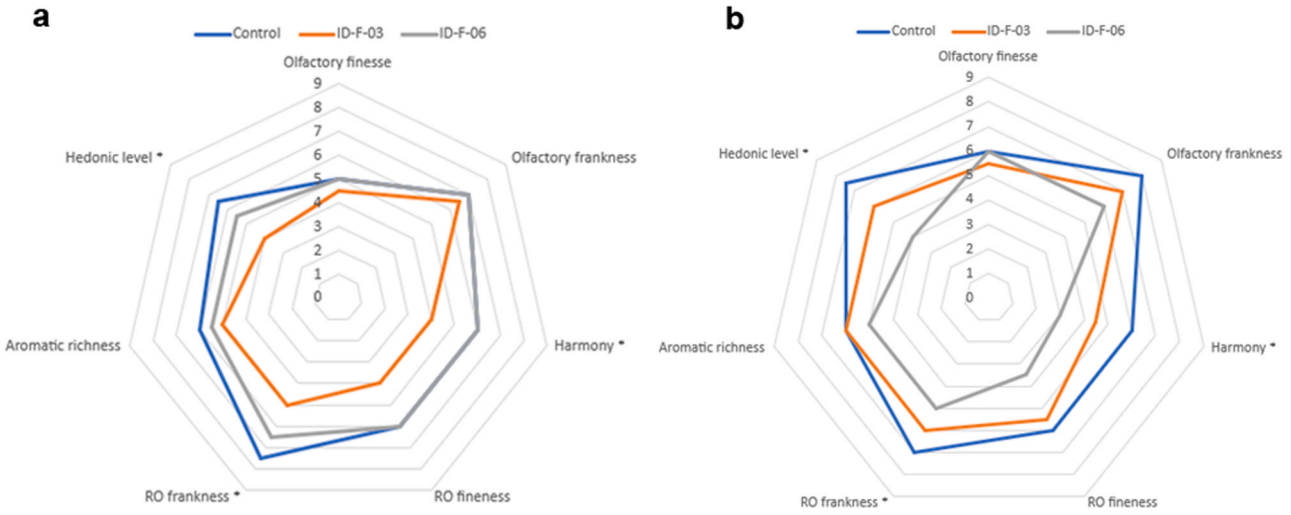

Fig. 7. Median values for hedonic parameters for treated apples (a) and oranges (b). *Statistically significant differences based on Friedman's ANOVA.

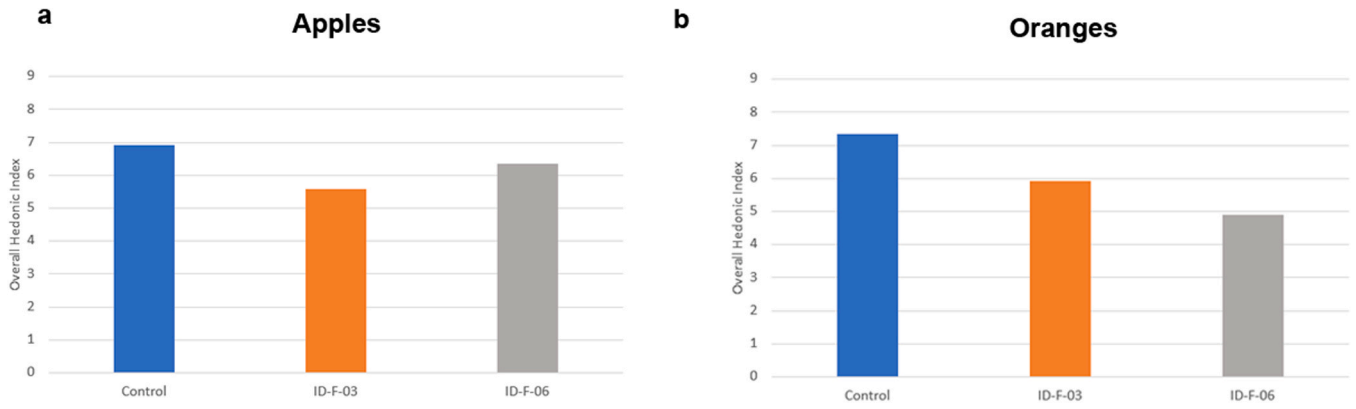

Fig. 8. Overall Hedonic Index for treated apples (a) and oranges (b).

apples, whereas ID-F-03 showed greater acceptability in oranges. Although these results highlight certain limitations, they do not contradict the broader potential of chitosan–EO coatings. Shah et al. (2025) demonstrated that sensory acceptability can be preserved or even improved in pears when optimized chitosan–EO formulations are employed, emphasizing the importance of formulation tuning. Likewise, Ma et al. (2024) reviewed multiple studies showing that the sensory outcomes of edible coatings are strongly dependent on the interaction

between coating composition, application strategy, and fruit matrix. In this context, the sensory alterations observed here may reflect formulation-specific imbalances or suboptimal dosage rather than intrinsic limitations of the chitosan–EO systems. In light of these findings, future applications may benefit from employing these chitosan–EO formulations as active components of postharvest packaging materials, rather than as direct coatings in fruit. Such an approach, already successfully tested in other chitosan-EO systems (Quesada et al., 2016), could enable the controlled release of antimicrobial volatiles into the storage atmosphere, limiting microbial growth while minimizing the absorption of EO compounds into fruit tissues.

Overall, ID-F-03 showed the most consistent performance across the two pathosystems evaluated, achieving the largest reductions in disease incidence relative to the control and outperforming ID-F-06 under the present experimental conditions, while inducing only moderate and acceptable sensory changes.

In conclusion, the findings of this study confirm the potential of chitosan-based formulations containing EOs as effective tools for postharvest control of fungal and oomycete pathogens. The selected formulations ID-F-03 and ID-F-06 demonstrated strong antimicrobial performance *in vitro* and *in vivo*, supported by their distinct volatile profiles, enriched in carvacrol (ID-F-03) and (*E*)-cinnamaldehyde/eugenol (ID-F-06). Across the two pathosystems evaluated, ID-F-03 provided the most consistent reductions in disease incidence relative to the control and the best overall balance between antimicrobial performance and sensory acceptability under the present experimental conditions. Although the sensory analysis highlighted some modifications in the organoleptic attributes of treated fruit, these changes were moderate and formulation–fruit dependent. However, because the panel evaluated peeled, sliced fruit rather than whole fruit, acceptance at the point of purchase, where appearance and surface aroma are critical, cannot be inferred from the present data and should be addressed in future consumer tests presenting whole fruit. Such outcomes underline the need for strategic application approaches that preserve the integrity of the fruit while maintaining antimicrobial efficacy. Because the real-world performance of EO–chitosan systems during storage hinges on the controlled emission and persistence of key volatiles rather than on nominal loading alone, a quantitative understanding of release–efficacy relationships is needed to optimize use conditions and packaging design. Accordingly, future studies should quantitatively characterize the release kinetics and persistence of active volatiles from chitosan–EO matrices under storage conditions (*e.g.*, time-resolved HS-SPME–GC–MS with kinetic modeling) and relate these profiles to the duration of antifungal and anti-oomycete efficacy. In this perspective, the use of chitosan–EO formulations as active components in postharvest packaging materials could offer an effective means of extending shelf life and controlling microbial decay, while minimizing direct contact with the fruit surface and thus preserving its sensory quality.

CRediT authorship contribution statement

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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.2025.114068.

Data availability

All data generated during this study are available within the article

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References

Abenaim, L., Conti, B., 2023. Chitosan as a control tool for insect pest management: a review. Insects 14, 949. https://doi.org/10.3390/INSECTS14120949.

Adams, R., 2007. Identification of essential oil components by Gas Chromatography/ Mass Spectrometry. Allured Pub. Corp, Carol Stream, Illinois, USA.

Adetunji, C.O., Sharifi-Rad, J., 2023. Applications of essential oils in the food industry. In: Applications of Essential Oils in the Food Industry. Elsevier, pp. 1–359. https://doi.org/10.1016/C2021-0-00817-8.

Aloi, F., Riolo, M., Parlascino, R., Pane, A., Cacciola, S.O., 2021a. Bot gummosis of lemon (*Citrus* × *limon*) caused by *Neofusicoccum parvum*. J. Fungi 7, 294. https://doi.org/10.3390/JOF7040294.

Aloi, F., Riolo, M., Sanzani, S.M., Mincuzzi, A., Ippolito, A., Siciliano, I., Pane, A., Gullino, M.L., Cacciola, S.O., 2021b. Characterization of *Alternaria* species associated with heart rot of pomegranate fruit. J. Fungi 7, 172.

Álvarez-García, S., Mayo-Prieto, S., Carro-Huerga, G., Rodríguez-González, Á., González-López, Ó., Gutiérrez, S., Casquero, P.A., 2021. Volatile organic compound chamber: a novel technology for microbiological volatile interaction assays. J. Fungi 7, 248. https://doi.org/10.3390/JOF7040248/S1.

Álvarez-García, S., Moumni, M., Romanazzi, G., 2023. Antifungal activity of volatile organic compounds from essential oils against the postharvest pathogens *Botrytis cinerea*, *Monilinia fructicola*, *Monilinia fructigena*, and *Monilinia laxa*. Front. Plant Sci. 14. https://doi.org/10.3389/FPLS.2023.1274770.

Amini, J., Farhang, V., Javadi, T., Nazemi, J., 2016. Antifungal effect of plant essential oils on controlling *Phytophthora* species. Plant Pathol. J. 32, 16–24. https://doi.org/10.5423/PPJ.OA.05.2015.0091.

Arslan, D., Tuccitto, N., Auditore, A., Licciardello, A., Marletta, G., Riolo, M., La Spada, F., Conti Taguali, S., Calpe, J., Meca, G., Pane, A., Cacciola, S.O., Karakeçili, A., 2024. Chitosan-based films grafted with citrus waste-derived antifungal agents: an innovative and sustainable approach to enhance post-harvest preservation of citrus fruit. Int. J. Biol. Macromol. 264, 130514. https://doi.org/10.1016/J.IJBIOMAC.2024.130514.

Ashraf, H., Gul, H., Jamil, B., Saeed, A., Pasha, M., Kaleem, M., Khan, A.S., 2022. Synthesis, characterization, and evaluation of the antifungal properties of tissue conditioner incorporated with essential oils-loaded chitosan nanoparticles. PLoS One 17, e0273079. https://doi.org/10.1371/JOURNAL.PONE.0273079.

Ayllón-Gutiérrez, R., Díaz-Rubio, L., Montaño-Soto, M., Haro-Vázquez, M. del P., Córdova-Guerrero, I., 2024. Applications of plant essential oils in pest control and their encapsulation for controlled release: a review. Page 1766 14 Agric 2024 14, 1766. https://doi.org/10.3390/AGRICULTURE14101766.

Bautista-Baños, S., Romanazzi, G., Jiménez-Aparicio, A., 2016. Chitosan in the preservation of agricultural commodities. Chitosan Preserv. Agric. Commod. https://doi.org/10.1016/C2014-0-03033-X.

Bedini, S., Djebbi, T., Ascrizzi, R., Farina, P., Pieracci, Y., Echeverría, M.C., Flamini, G., Trusendi, F., Ortega, S., Chiliquinga, A., Conti, B., 2024. Repellence and attractiveness: the hormetic effect of aromatic plant essential oils on insect behavior. Ind. Crops Prod. 210, 118122. https://doi.org/10.1016/J.INDCROP.2024.118122.

Ben Miri, Y., Nouasri, A., Herrera, M., Djenane, D., Ariño, A., 2023. Antifungal activity of menthol, eugenol and their combination against *Aspergillus ochraceus* and *Aspergillus niger in vitro* and in stored cereals. Foods 12, 2108. https://doi.org/10.3390/FOODS12112108/S1.

Benigno, A., Papini, V., La Spada, F.La, Rizzo, D., Cacciola, S.O., Moricca, S., 2025. *Phytophthora plurivora*: a serious challenge for english walnut (*Juglans regia*) cultivation in Europe. Microorganisms 13, 2094. https://doi.org/10.3390/MICROORGANISMS13092094.

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/XML.

Bi, Y., Jiang, H., Hausbeck, M.K., Hao, J.J., 2012. Inhibitory effects of essential oils for controlling *Phytophthora capsici*. Plant Dis. 96, 797–803. https://doi.org/10.1094/PDIS-11-11-0933.

Boeckman, N.J., Borba, M.C., Bernal, V.V., Khodadadi, F., Jurick, W.M., Aćimović, S.G., 2025. Apple bitter rot: biology, ecology, omics, virulence factors, and management of causal *Colletotrichum* species. Mol. Plant Pathol. 26. https://doi.org/10.1111/MPP.70050.

Bouqellah, N.A., Abdulmajeed, A.M., Rashed Alharbi, F.K., Mattar, E., Al-Sarraj, F., Abdulfattah, A.M., Hassan, M.M., Baazeem, A., Al-Harthi, H.F., Musa, A., Asseri, T.A. Y., Soliman, M.H., 2025. Optimizing encapsulation of garlic and cinnamon essential oils in silver nanoparticles for enhanced antifungal activity against *Botrytis cinerea* pathogenic disease. Physiol. Mol. Plant Pathol. 136, 102522. https://doi.org/10.1016/J.PMPP.2024.102522.

Cacciola, S.O., Faedda, R., Sinatra, F., Agosteo, G.E., Schena, L., Frisullo, S., Magnano di San Lio, G., 2012. Olive anthracnose. J. Plant Pathol. 94, 29–44.

Campos, S., Espinoza, J., Fuentes, J.M., Jofré-Fernández, I., Tortella, G., Navarro, D., Quiroz, A., Diez, M.C., Rubilar, O., Fincheira, P., 2025. The impact of essential oils derived from *Citrus* species to control *Botrytis cinerea* and their potential physiological actions. Plants 14, 1859. https://doi.org/10.3390/PLANTS14121859.

Cantón, E., Pemán, J., Viudes, A., Quindós, G., Gobernado, M., Espinel-Ingroff, A., 2003. Minimum fungicidal concentrations of amphotericin B for bloodstream *Candida* species. Diagn. Microbiol. Infect. Dis. 45, 203–206. https://doi.org/10.1016/S0732-8893(02)00525-4.

Cefola, M., Caputo, L., Quintieri, L., Cervellieri, S., Fancello, F., Netti, T., Lippolis, V., Palumbo, M., Ricci, I., Sorrentino, A., Pace, B., Zara, S., 2023. Innovative limonene and chitosan-based pads in improving shelf-life and preserving the volatile profile of fresh strawberries. Postharvest Biol. Technol. 204, 112430. https://doi.org/10.1016/J.POSTHARVBIO.2023.112430.

Chein, S.H., Sadiq, M.B., Anal, A.K., 2019. Antifungal effects of chitosan films incorporated with essential oils and control of fungal contamination in peanut kernels. J. Food Process. Preserv 43, e14235. https://doi.org/10.1111/JFPP.14235.

Crous, P.W., Wingfield, M.J., Burgess, T.I., Hardy, G.E.S.J., Barber, P.A., Alvarado, P., Barnes, C.W., Buchanan, P.K., Heykoop, M., Moreno, G., Thangavel, R., van der Spuy, S., Barili, A., Barrett, S., Cacciola, S.O., Cano-Lira, J.F., Crane, C., Decock, C., Gibertoni, T.B., Guarro, J., Guevara-Suarez, M., Hubka, V., Kolařík, M., Lira, C.R.S., Ordoñez, M.E., Padamsee, M., Ryvarden, L., Soares, A.M., Stchigel, A.M., Sutton, D. A., Vizzini, A., Weir, B.S., Acharya, K., Aloi, F., Baseia, I.G., Blanchette, R.A., Bordallo, J.J., Bratek, Z., Butler, T., Cano-Canals, J., Carlavilla, J.R., Chander, J., Cheewangkoon, R., Cruz, R.H.S.F., da Silva, M., Dutta, A.K., Ercole, E., Escobio, V., Esteve-Raventós, F., Flores, J.A., Gené, J., Góis, J.S., Haines, L., Held, B.W., Horta Jung, M., Hosaka, K., Jung, T., Jurjević, Ž., Kautman, V., Kautmanova, I., Kiyashko, A.A., Kozanek, M., Kubátová, A., Lafourcade, M., La Spada, F., Latha, K.P. D., Madrid, H., Malysheva, E.F., Manimohan, P., Manjón, J.L., Martín, M.P., Mata, M., Merényi, Z., Morte, A., Nagy, I., Normand, A.-C., Paloi, S., Pattison, N., Paw?owska, J., Pereira, O.L., Petterson, M.E., Picillo, B., Raj, K.N.A., Roberts, A., Rodríguez, A., Rodríguez-Campo, F.J., Romański, M., Ruszkiewicz-Michalska, M., Scanu, B., Schena, L., Semelbauer, M., Sharma, R., Shouche, Y.S., Silva, V., Staniaszek-Kik, M., Stielow, J.B., Tapia, C., Taylor, P.W.J., Toome-Heller, M., Vabeikhokhei, J.M.C., van Diepeningen, A.D., Van Hoa, N., Van Tri, M., Wiederhold, N.P., Wrzosek, M., Zothanzama, J., Groenewald, J.Z., 2017. Fungal Planet description sheets. Pers. Mol. Phylogeny Evol. Fungi 38, 558–624. https://doi.org/10.3767/003158517X698941.

Das, S., Chaudhari, A.K., Singh, V.K., Dwivedy, A.K., Dubey, N.K., 2023. *Angelica archangelica* essential oil loaded chitosan nanoemulsion as edible coating for preservation of table grape fruit against *Botrytis cinerea* contamination and storage quality deterioration. Postharvest Biol. Technol. 205, 112482. https://doi.org/10.1016/J.POSTHARVBIO.2023.112482.

Djebbi, T., Ascrizzi, R., Bedini, S., Farina, P., Sanmartin, C., Mediouni Ben Jemâa, J., Bozzini, M.F., Flamini, G., Conti, B., 2024. Physicochemical and repellent properties of chitosan films loaded with essential oils for producing an active packaging effective against the food pest *Sitophilus oryzae*. J. Stored Prod. Res. 106, 102297. https://doi.org/10.1016/J.JSPR.2024.102297.

du Jardin, P., 2015. Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hortic. (Amst.). 196, 3–14. https://doi.org/10.1016/J.SCIENTA.2015.09.021.

Duchoslavová, J., Širučková, I., Zapletalová, E., Navrátil, M., Šafářová, D., 2007. First report of brown rot caused by *Monilinia fructicola* on various stone and pome fruits in the Czech Republic. –907 Plant Dis. 91, 907. https://doi.org/10.1094/PDIS-91-7-0907B.

Eddya, M., Tbib, B., EL-Hami, K., 2020. A comparison of chitosan properties after extraction from shrimp shells by diluted and concentrated acids. Heliyon 6, e03486. https://doi.org/10.1016/j.heliyon.2020.e03486.

El boumlasy, S., La Spada, F., Pane, A., Licciardello, A., Debdoubi, A., Tuccitto, N., Cacciola, S.O., 2022. A super absorbent polymer containing copper to control *Plenodomus tracheiphilus* the causative agent of mal secco disease of lemon. Front. Microbiol 13. https://doi.org/10.3389/fmicb.2022.987056.

El boumlasy, S., La Spada, F., Tuccitto, N., Marletta, G., Mínguez, C.L., Meca, G., Rovetto, E.I., Pane, A., Debdoubi, A., Cacciola, S.O., 2021. Inhibitory activity of shrimp waste extracts on fungal and oomycete plant pathogens. Plants 10, 2452. https://doi.org/10.3390/plants10112452.

Erwin, D.C., Ribeiro, O.K., 1996. Phytophthora diseases worldwide. American Phytopathological Society (APS Press), St. Paul, Minnesota, USA. https://doi.org/10.1046/j.1365-3059.1998.0179a.x.

Farina, P., Conti, B., 2024. Liabilities of essential oils as insect repellents. Curr. Opin. Environ. Sci. Heal 40, 100564. https://doi.org/10.1016/J.COESH.2024.100564.

Farina, P., Tognocchi, M., Conte, G., Casarosa, L., Trusendi, F., Conti, B., 2025. Benefits of essential oil-enriched chitosan on beef: from appearance and odour improvement to orotection against blowfly oviposition. Foods 14, 897. https://doi.org/10.3390/FOODS14050897/S1.

Fernández-Fernández, M., Naves, P., Witzell, J., Musolin, D.L., Selikhovkin, A.V., Paraschiv, M., Chira, D., Martínez-álvarez, P., Martín-García, J., Jordán, Muñoz-Adalia, E., Altunisik, A., Cocuzza, G.E.M., Di Silvestro, S., Zamora, C., Diez, J.J., 2019. Pine pitch canker and insects: relationships and implications for disease spread in Europe. 2019 10, 627. https://doi.org/10.3390/F10080627.

González-Reza, R.M., Hernández-Sánchez, H., Quintanar-Guerrero, D., Alamilla-Beltrán, L., Cruz-Narváez, Y., Zambrano-Zaragoza, M.L., 2021. Synthesis, controlled release, and stability on storage of chitosan-thyme essential oil nanocapsules for food applications. Gels 7, 212. https://doi.org/10.3390/GELS7040212.

Hou, T., Sana, S.S., Li, H., Xing, Y., Nanda, A., Netala, V.R., Zhang, Z., 2022. Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: a review. Food Biosci. 47, 101716. https://doi.org/10.1016/J.FBIO.2022.101716.

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. Polym. (Basel) 16, 884. https://doi.org/10.3390/POLYM16070884/S1.

Ismail, M., Zhang, J., 2004. Post-harvest citrus diseases and their control. Outlooks Pest Manag 15, 29–35. https://doi.org/10.1564/15feb12.

Khodadadi, F., González, J.B., Martin, P.L., Giroux, E., Bilodeau, G.J., Peter, K.A., Doyle, V.P., Aćimović, S.G., 2020. Identification and characterization of *Colletotrichum* species causing apple bitter rot in New York and description of

C. noveboracense sp. nov. Sci. Rep. 10, 1–19. https://doi.org/10.1038/S41598-020-66761-9;SUBJMETA.
Konstantinou, S., Karaoglanidis, G.S., Bardas, G.A., Minas, I.S., Doukas, E., Markoglou, A. N., 2011. Postharvest fruit rots of apple in Greece: pathogen incidence and relationships between fruit quality parameters, cultivar susceptibility, and patulin production. Plant Dis. 95, 666–672. https://doi.org/10.1094/PDIS-11-10-0856.
La Spada, F., Aloi, F., Coniglione, M., Pane, A., Cacciola, S.O., 2021. Natural biostimulants elicit plant immune system in an integrated management strategy of the postharvest green mold of orange fruits incited by *Penicillium digitatum*. Front. Plant Sci. 12, 684722. https://doi.org/10.3389/fpls.2021.684722.
La Spada, F., Bua, C., Pane, A., Tuccitto, N., Riolo, M., Cacciola, S.O., 2024. Exploring eco-friendly solutions for Phytophthora disease management: harnessing the anti-oomycete potential of a fermented lemon waste formulation. J. Agric. Food Res. 17, 101227. https://doi.org/10.1016/J.JAFR.2024.101227.
La Spada, F., Stracquadanio, C., Riolo, M., Pane, A., Cacciola, S.O., 2020. *Trichoderma* counteracts the challenge of *Phytophthora nicotianae* infections on tomato by modulating plant defense mechanisms and the expression of crinkler, necrosis-inducing *Phytophthora* protein 1, and cellulose-binding elicitor lect. Front. Plant Sci. 11, 1653. https://doi.org/10.3389/fpls.2020.583539.
Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z., Barka, E.A., 2022. Biological control of plant pathogens: a global perspective. Microorganisms 10, 596. https://doi.org/10.3390/MICROORGANISMS10030596.
Landi, L., D'Ortenzio, A.L., Makau, S.M., De Miccolis Angelini, R.M., Romanazzi, G., 2025. Validation of *Monilinia fructicola* putative effector genes in different host peach (*Prunus persica*) cultivars and defense response investigation. J. Fungi 11, 39. https://doi.org/10.3390/JOF11010039/S1.
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/BIBTEX.
Lee, Y.-S., Kim, J., Shin, S.-C., Lee, S.-G., Park, I.-K., 2008. Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi. Flavour Fragr. J. 23, 23–28. https://doi.org/10.1002/ffj.1850.
Luciano-Rosario, D., Keller, N.P., Jurick, W.M., 2020. *Penicillium expansum*: biology, omics, and management tools for a global postharvest pathogen causing blue mould of pome fruit. Mol. Plant Pathol. 21, 1391–1404. https://doi.org/10.1111/MPP.12990.
Luo, Y., Hou, L., Förster, H., Pryor, B., Adaskaveg, J.E., 2017. Identification of *Alternaria* species causing heart rot of pomegranates in California. Plant Dis. 101, 421–427. https://doi.org/10.1094/PDIS-08-16-1176-RE.
Ma, M., Liu, Y., Zhang, S., Yuan, Y., 2024. Edible coating for fresh-cut fruit and vegetable preservation: biomaterials, functional ingredients, and joint non-thermal technology. Foods 13, 3937. https://doi.org/10.3390/FOODS13233937.
Mahmutović-Dizdarević, I., Bektaš, N., Gazić, Š., Jerković-Mujkić, A., Hukić, M., Avdić, M., 2023. *In vitro* evaluation of selected essential oils as possible antifungal and antibiofilm agents. Acta Microbiol. Bulg. 39, 317–323. https://doi.org/10.59393/AMB23390313.
Maswanganye, L.T.C., Pillai, S.K., Sivakumar, D., 2025. Chitosan coating loaded with spearmint essential oil nanoemulsion for antifungal protection in soft citrus (*Citrus reticulata*) fruits. Coatings 15, 105. https://doi.org/10.3390/COATINGS15010105/S1.
Migheli, Q., Cacciola, S.O., Balmas, V., Pane, A., Ezra, D., Di San Lio, G.M., 2009. Mal secco disease caused by *Phoma tracheiphila*: A potential threat to lemon production worldwide. Plant Dis. 93, 852–867. https://doi.org/10.1094/PDIS-93-9-0852.
Mohamed, A.A., Ahmed, M.A., Korayem, A.S., Abu-Hussien, S.H., Rashidy, W.B., 2025. Antifungal, toxicological, and colorimetric properties of *Origanum vulgare*, *Moringa oleifera*, and *Cinnamomum verum* essential oils mixture against Egyptian Prince Yusuf Palace deteriorative fungi. BMC Biotechnol. 25, 1–15. https://doi.org/10.1186/S12896-024-00940-8/FIGURES/10.
Munhuweyi, K., Caleb, O.J., Lennox, C.L., van Reenen, A.J., Opara, U.L., 2017. *In vitro* and *in vivo* antifungal activity of chitosan-essential oils against pomegranate fruit pathogens. Postharvest Biol. Technol. 129, 9–22. https://doi.org/10.1016/J.POSTHARVBIO.2017.03.002.
National Institute of Standards and Technology, 2014. NIST/EPA/NIH Mass Spectral Library, NIST Standard Reference Database Number 69. The NIST Mass Spectrometry Data Center, Gaithersburg, MD, USA.
Niu, A., Wu, H., Ma, F., Tan, S., Wang, G., Qiu, W., 2022. The antifungal activity of cinnamaldehyde in vapor phase against *Aspergillus niger* isolated from spoiled paddy. LWT 159, 113181. https://doi.org/10.1016/J.LWT.2022.113181.
Ojagh, S.M., Rezaei, M., Razavi, S.H., Hosseini, S.M.H., 2010. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. Food Chem. 122, 161–166. https://doi.org/10.1016/J.FOODCHEM.2010.02.033.
Oliveira Lino, L., Pacheco, I., Mercier, V., Faoro, F., Bassi, D., Bornard, I., Quilot-Turion, B., 2016. Brown rot strikes *Prunus* fruit: an ancient fight almost always lost. J. Agric. Food Chem. 64, 4029–4047. https://doi.org/10.1021/ACS.JAFC.6B00104.
Parichanon, P., Farina, P., Vicente, I., Cesarini, M., Hotaj, E., Sarrocco, S., Pellegrini, E., Conti, B., 2024. Chitosan/mandarin essential oil-based films on citrus fruits for the control of the medfly attack and to prevent the occurrence of grey and blue mould in post-harvest. J. Stored Prod. Res. 108, 102380. https://doi.org/10.1016/J.JSPR.2024.102380.
Parlascino, R., Conti Taguali, S., Riolo, M., Salvatore, M., Bakonyi, J., Ezra, D., Benigno, A., La Spada, F., Pane, A., Cacciola, S.O., 2025. Unveiling the invisible threat: cutting-edge methods for detecting fungal and oomycete plant pathogens and mycotoxins. Physiol. Mol. Plant Pathol. 140, 102893. https://doi.org/10.1016/J.PMPP.2025.102893.
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/CITE/REFWORKS.
Puglisi, I., De Patrizio, A., Schena, L., Jung, T., Evoli, M., Pane, A., Van Hoa, N., Van Tri, M., Wright, S., Ramstedt, M., Olsson, C., Faedda, R., Magnano di San Lio, G., Cacciola, S.O., 2017. Two previously unknown *Phytophthora* species associated with brown rot of pomelo (*Citrus grandis*) fruits in Vietnam. PLoS One 12, e0172085. https://doi.org/10.1371/journal.pone.0172085.
Quesada, J., Sendra, E., Navarro, C., Sayas-Barberá, E., 2016. Antimicrobial active packaging including chitosan films with *Thymus vulgaris* L. essential oil for ready-to-eat meat. Foods 5, 57. https://doi.org/10.3390/FOODS5030057.
Riolo, M., Aloi, F., Faedda, R., Cacciola, S.O., Pane, A., 2020. First report of postharvest fruit rot caused by *Fusarium sacchari* on lady finger banana in Italy. Plant Dis. 104. https://doi.org/10.1094/PDIS-01-20-0143-PDN.
Riolo, M., Aloi, F., La Spada, F., Sciandrello, S., Moricca, S., Santilli, E., Pane, A., Cacciola, S.O., 2020. Diversity of *Phytophthora* communities across different types of mediterranean vegetation in a nature reserve area. Forests 11, 1–21. https://doi.org/10.3390/F11080853.
Riolo, M., Aloi, F., Pane, A., Cara, M., Cacciola, S.O., 2021. Twig and shoot dieback of citrus, a new disease caused by *Colletotrichum* species. Cells 10. https://doi.org/10.3390/cells10020449.
Riolo, M., Luz, C., Bua, C., Barreca, S., Tambè, M.C., Calpe, J., Masi, M., Cimmino, A., Tirrò, G., Meca, G., Ezra, D., Cacciola, S.O., 2025. Qualitative and relative abundance analysis of *Alternaria alternata* metabolites in pomegranate (*Punica granatum* L.) cultivars affected by Heart Rot using UHPLC-Q-TOF-MS. Food Control 172, 111204. https://doi.org/10.1016/J.FOODCONT.2025.111204.
Riolo, M., Luz, C., Santilli, E., Meca, G., Cacciola, S.O., 2023a. Antifungal activity of selected lactic acid bacteria from olive drupes. Food Biosci. 52, 102422. https://doi.org/10.1016/J.FBIO.2023.102422.
Riolo, M., Pane, A., Santilli, E., Cacciola, S.O., Moricca, S., 2023b. Susceptibility of Italian olive cultivars to various *Colletotrichum* species associated with fruit anthracnose. Plant Pathol. 72, 255–267. https://doi.org/10.1111/ppa.13652.
Riolo, M., Villena, A.M., Calpe, J., Luz, C., Meca, G., Tuccitto, N., Cacciola, S.O., 2024. A circular economy approach: a new formulation based on a lemon peel medium activated with lactobacilli for sustainable control of post-harvest fungal rots in fresh citrus fruit. Biol. Control 189, 105443. https://doi.org/10.1016/J.BIOCONTROL.2024.105443.
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, 412244. https://doi.org/10.3389/FMICB.2018.02745/BIBTEX.
Rovetto, E.I., Garbelotto, M., Moricca, S., Amato, M., Spada, F.La, Cacciola, S.O., 2024a. A portable fluorescence-based recombinase polymerase amplification assay for the detection of mal secco disease by *Plenodomus tracheiphilus*. Crop Prot. 184, 106825. https://doi.org/10.1016/j.cropro.2024.106825.
Rovetto, E.I., La Spada, F., Aloi, F., Riolo, M., Pane, A., Garbelotto, M., Cacciola, S.O., 2024b. Green solutions and new technologies for sustainable management of fungus and oomycete diseases in the citrus fruit supply chain. J. Plant Pathol. 2024, 1–27. https://doi.org/10.1007/S42161-023-01543-6.
Rovetto, E.I., La Spada, F., El boumlasy, S., Conti Taguali, S., Riolo, M., Pane, A., Cacciola, S.O., 2024c. Biological control of green mold in simulated post-harvest chain of citrus Fruit: efficacy of *Candida oleophila* strain O and molecular insight into elicitation of host immune system. Biol. Control 193, 105531. https://doi.org/10.1016/J.BIOCONTROL.2024.105531.
Sangsuwan, J., Pongsapakworawat, T., Bangmo, P., Sutthasupa, S., 2016. Effect of chitosan beads incorporated with lavender or red thyme essential oils in inhibiting *Botrytis cinerea* and their application in strawberry packaging system. LWT 74, 14–20. https://doi.org/10.1016/J.LWT.2016.07.021.
Santilli, E., Riolo, M., La Spada, F., Pane, A., Cacciola, S.O., 2020. First report of root rot caused by *Phytophthora bilorbang* on *Olea europaea* in Italy. Plants 9, 826. https://doi.org/10.3390/plants9070826.
Servili, A., Feliziani, E., Romanazzi, G., 2017. Exposure to volatiles of essential oils alone or under hypobaric treatment to control postharvest gray mold of table grapes. Postharvest Biol. Technol. 133, 36–40. https://doi.org/10.1016/J.POSTHARVBIO.2017.06.007.
Shah, Y.A., Bhatia, S., Al-Harrasi, A., Tarahi, M., Khan, M.R., 2025. Chitosan-orange (*Citrus sinensis*) essential oil coatings with combined treatment of KMnO4 to enhance the shelf-life and storage quality of ercolini pears. Food Sci. Nutr. 13, e70045. https://doi.org/10.1002/FSN3.70045.
Shao, X., Cao, B., Xu, F., Xie, S., Yu, D., Wang, H., 2015. Effect of postharvest application of chitosan combined with clove oil against citrus green mold. Postharvest Biol. Technol. 99, 37–43. https://doi.org/10.1016/j.postharvbio.2014.07.014.
Silva, N.C., Madureira, A.R., Pintado, M., Moreira, P.R., 2024. Chitosan coatings reinforced with cellulose crystals and oregano essential oil as antimicrobial protection against the microbiological contamination of stone sculptures. Cellulose 31, 9825–9845. https://doi.org/10.1007/S10570-024-06149-4/TABLES/4.
Soppelsa, S., Van Hemelrijck, W., Bylemans, D., Andreotti, C., 2023. Essential oils and chitosan applications to protect apples against postharvest diseases and to extend shelf life. Agronomy 13, 822. https://doi.org/10.3390/AGRONOMY13030822/S1.
Tančinová, D., Mašková, Z., Mendelová, A., Foltinová, D., Barboráková, Z., Medo, J., 2022. Antifungal activities of essential oils in vapor phase against *Botrytis cinerea* and their potential to control postharvest strawberry gray mold. Foods 11, 2945. https://doi.org/10.3390/FOODS11192945/S1.

Tripathi, A.K., Upadhyay, S., Bhuiyan, M., Bhattacharya, P.R., 2009. A review on prospects of essential oils as biopesticide in insect-pest management. J. Pharmacogn. Phyther 1, 52–063.

Trkulja, V., Čojić, B., Trkulja, N., Tomić, A., Matić, S., Ikanović, J., Popović Milovanović, T., 2024. *Colletotrichum* species associated with apple bitter rot and Glomerella leaf spot: a comprehensive overview. J. Fungi 10, 660. https://doi.org/10.3390/JOF10090660.

Xie, L., Yang, Q., Wu, Y., Xiao, J., Qu, H., Jiang, Y., Li, T., 2023. Fumonisin B1 biosynthesis is associated with oxidative stress and plays an important role in *Fusarium proliferatum* infection on banana fruit. J. Agric. Food Chem. 71, 5372–5381. https://doi.org/10.1021/ACS.JAFC.3C00179.

Yousef, S.A.M., Ali, A.M., Elsherbiny, E.A., Atwa, A.A., 2024. Morphological, genetic and pathogenic variability among *Botrytis cinerea* species complex causing gray mold of strawberry. Physiol. Mol. Plant Pathol. 134, 102395. https://doi.org/10.1016/J.PMPP.2024.102395.

Zargar, V., Asghari, M., Dashti, A., 2015. A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications. ChemBioEng Rev. 2, 204–226. https://doi.org/10.1002/CBEN.201400025.

Zhang, W., Goksen, G., Zhou, Y., Yang, J., Khan, M.R., Ahmad, N., Fei, T., 2023. Application of a chitosan–cinnamon essential oil composite coating in inhibiting postharvest apple diseases. Foods 12, 3518. https://doi.org/10.3390/FOODS12183518.

Zheng, K., Li, B., Liu, Y., Wu, D., Bai, Y., Xiang, Q., 2023. Effect of chitosan coating incorporated with oregano essential oil on microbial inactivation and quality properties of refrigerated chicken breasts. LWT 176, 114547. https://doi.org/10.1016/J.LWT.2023.114547.