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Abstract

Green and blue molds are the most important postharvest diseases affecting citrus in storage. These diseases are commonly controlled with fungicides, but legislative restrictions, consumer concerns and the development of resistant strains of the pathogens have increasingly led to the search for alternative methods of control. A pomegranate peel extract (PGE) was very effective in controlling Valencia orange and clementine postharvest rot under commercial conditions. After cold storage and 7 days of shelf life, the incidence of decay on oranges sprayed before harvest with PGE at 12, 6, and 3 g/l was reduced by 78.9, 76.0, and 64.6%, respectively. Similarly, postharvest dipping treatments with PGE reduced rot by 90.2, 84.3, and 77.6%, respectively. Comparable levels of protection were also achieved on clementines. On both, oranges and clementines, the extract provided a significantly higher level of protection compared to Imazalil (IMZ), a fungicide commonly used for postharvest treatments. The high level of efficacy and the consistence of results on different fruit species (clementines and oranges) and with different application methods (pre- and postharvest) was evidence of reliability and flexibility. PGE also showed a strong antimicrobial activity against fungi and bacteria, suggesting its possible use as sanitizers to reduce the microbial contamination of recirculated water in packinghouses. The results of the present study encourage the integration of conventional chemical fungicides and sanitizers with PGE to control citrus postharvest rot.

KEYWORDS: Citrus rot; Blue mold; Green mold; Alternative control methods; Plant extract; *Penicillium digitatum; Penicillium italicum*; Pomegranate peel extract; Valencia orange; Clementine

INTRODUCTION

The genus *Citrus* comprises many different species of trees and shrubs and their production is distributed in over one hundred countries representing the widest source of fruit for human consumption (Siddiq, 2012). Among citrus species, oranges (*Citrus sinensis* (L.) Osbeck) and clementines (*Citrus clementina* hort. ex Tanaka) are characterized by increasing worldwide production and are the most cultivated in Italy. Oranges and clementines have a relatively long shelf life compared to other sub-tropical and tropical fruits even though postharvest decay can cause severe waste and quality deterioration. These losses can reach 30-50% of the total production after 30-60 days of storage (Strano et al., 2017). Green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum* are the most common and serious postharvest diseases affecting citrus production (Youssef et al., 2012). Several other fungal diseases can affect citrus fruit after harvest; however, their incidence is generally low and represent a serious problem only under particular environmental conditions (Timmer et al., 2000). They include Brown rot (*Phytophthora* spp.), Alternaria rot (*Alternaria* spp.), Sour rot [*Geotrichum candidum (syn. Dipodascus geotrichum*)], Stem-end rot (*Lasiodiplodia theobromae* and *Diaporthe citri*) and Whisker mold, a third *Penicillium* rot, caused by *Penicillium ulaiense*. Both *P. digitatum* and *P. italicum* are wound pathogens that infect the fruit through peel injuries produced in the field, the packinghouse, or during the fruit commercialization chain (Palou et al., 2014). Due to the high economic impact of green and blue molds on the citrus industry, the main goal of postharvest disease management programs in most production areas is their control. Traditionally, these diseases are controlled by the application of conventional fungicides in the packinghouse, before fruit storage and transportation (Kanetis et al., 2008). In particular, Imazalil (IMZ) is the most widely used fungicide (Altieri et al., 2013). However, increasing legislative restrictions, consumer concerns regarding chemical residues in the fruit and the development of resistant strains of the pathogens have increasingly led to the search for alternative methods of postharvest disease control (Palou et al., 2014; Erasmus et al., 2015; Wisniewski et al., 2016). In this context, several biological and physical control strategies that include the use of antagonistic microorganisms, natural fungicides, and resistance inducers have been evaluated under experimental conditions, but their application in the citrus industry is still very limited (El-Ghaouth et al., 2001; Janisiewicz and Korsten 2002; Talibi et al., 2014). Sodium bicarbonate and other generally recognized as safe (GRAS) salts stand out as alternatives that are more frequently used in the citrus industry and are particularly useful to complement the efficacy of conventional fungicide treatments (Smilanick et al., 2020). The main limitations of alternative control methods include inconsistent efficacy when used in practical commercial conditions, lack of curative effects, low persistence and risk of fruit injury (Palou et al., 2008, Youssef et al., 2012).

Plant extracts have been proposed as effective natural fungicides to control postharvest pathogens (Cabral et al., 2013; Gatto et al., 2011). In particular, extracts from pomegranate peel (*Punica granatum* L.) have emerged as promising antimicrobial substances against plant pathogens due to their high content of phenolic compounds such as punicalagin, ellagic acid, and gallic acid (Tehranifar et al., 2011; Elsherbiny et al., 2016). Tayel and co-workers (2009) evaluated the potential applications of pomegranate peel extracts for the control of citrus green mold in experimental conditions, while other studies showed a positive effect in extending the shelf life of fruit when incorporated into formulations as coatings (Rongai et al., 2018; Nair et al., 2018).

However, the concentration of active components and the efficacy of extracts can greatly vary depending on many factors including extraction method, fruit variety and maturity stage. Recently, an ethanolic extract obtained from the peel of pomegranate designated PGE, proved to be effective against different types of postharvest rot including *Botrytis cinerea* on table grapes and sweet cherries, *Monilinia* spp. on sweet cherries, *Colletotrichum* spp. on olives, *Penicillium expansum* on apples, and *P. digitatum* and *P. italicum* on citrus species (Romeo et al., 2015; Li Destri Nicosia et al., 2016). These studies have demonstrated that PGE has several important features that may enable its implementation in postharvest control strategies since it has curative and preventive activity, long persistence, a wide spectrum of activity and high efficacy. Furthermore, PGE has a direct fungicidal and bactericidal activity but also induces resistance in treated tissues (Pangallo et al 2016 and 2018; Belgacem et al., 2019).

In the present study, we tested the hypothesis that PGE can be used as an alternative control method against clementine and orange postharvest rots in practical commercial conditions either, by spraying the extract on the plant before harvest or by dipping fruit in an extract solution after harvest. The efficacy of PGE was compared to IMZ (chemical control) and two salts, sodium bicarbonate ($NaHCO_3$) and potassium bicarbonate ($KHCO_3$), which have been proposed as safe and effective alternative means of control (Smilanick et al., 1999; Palou et al., 2002; 2014; Youssef and Roberto, 2014). PGE proved very effective in controlling rot and showed a strong antimicrobial activity suggesting its possible use to integrate traditional chemical fungicides and sanitizers for the postharvest management of citrus fruits.

MATERIAL AND METHODS

Compounds

A concentrated stock solution of PGE containing 120 g/liter of dry matter was obtained according to Romeo et al. (2015) from ripe pomegranate (*Punica granatum* L.) fruit cv. "*Mollar De Elche*" harvested in Acireale (Italy). The stock solution was stored at 4°C and diluted with tap water to have solutions containing 12 (PGE 12), 6 (PGE 6), and 3 (PGE 3) g/liter of dry matter. $NaHCO_3$ and $KHCO_3$ were purchased from Sigma Aldrich S.r.l. (Milan, Italy) and dissolved in tap water to obtain 2% (W/V) solutions. A commercial formulation containing 500 g/liter of pure Imazalil (Deccozil® EC) was purchased from Decco (Italy) and used for dipping treatments at the recommended concentration of 0.1%. All solutions were prepared just before use.

Postharvest treatments

The experiment was conducted in April 2016 using Valencia oranges collected in a commercial orchard located in Calabria, southern Italy (GPS coordinates 38°13'06.24"N – 16°14'11.54"E). Oranges were dipped for 30 s in dump tanks containing water solutions of Imazalil (chemical control), PGE at the three different concentrations, 2% $NaHCO_3$, or 2% $KHCO_3$. Untreated oranges and oranges dipped in water were used as controls. All trials were conducted using potable tap water at a temperature of 5±1 °C. After dipping, all oranges were transferred into a commercial tunnel generating a flow of hot air at 40°C for 1 min in order to be dried and waxed.

Treated oranges were cold-stored for 7 days at 4±1°C and 95-98% RH and then subjected to 7 days of shelf life at 20±2°C. The incidence of rotten fruit was determined at the end of the cold storage and shelf life, respectively. For each treatment, 2400 fruit arranged in 12 plastic boxes (three replicates of 4 boxes) were visually inspected to determine the incidence of rot.

To evaluate the sanitizing activity of tested compounds, three subsamples (50 ml each) of solutions used for dipping treatments were collected and maintained at 5°C for approximately 3 h. After quick vortexing, 100 µl of water suspensions were diluted (1:10 and 1:100) and plated on Potato Dextrose Agar (PDA) amended with ampicillin and streptomycin sulphate (250 mg/liter each) or with cycloheximide (200 mg/liter) to detect fungi and bacteria, respectively. The plates (three per subsample) were incubated at 22°C for 3–4 days. The number of fungal and bacterial colonies (CFU) was recorded and converted into $\log_{10}$ CFU/ml.

Preharvest treatments

Trials were carried out in commercial orchards of Valencia oranges (GPS coordinates 38°13’06.24”N 16°14’11.54”E) and clementines (GPS coordinates 38°15'56.5"N 16°11'08.3"E) in April 2016 and in November 2017, respectively. In both years, treatments were carried out according to a completely randomized block design with 3 replicates, each consisting of 5 plants. The plants were selected according to the uniformity of size and age, quantity of fruit and absence of disease symptoms or damage. The selected plants were uniformly sprayed with a backpack atomizer using approximately 10 liters of solution containing PGE at the three different concentrations, 2% salt solutions ($NaHCO_3$ and $KHCO_3$), or potable tap water (control). After 1 day, the treated fruit were harvested, placed in plastic boxes and stored for 1 week at 4±1°C and 95-98% RH. The fruit from untreated plants were stored without any additional treatment (field control), or were dipped in a solution of IMZ (chemical control) as described above for postharvest treatments. For all the fruit, cold storage was followed by 7 days of shelf life at 20±2°C. The incidence of rotted fruit was determined at the end of the cold storage and shelf life respectively. For each treatment, 2400 oranges or 6000 clementines arranged in 12 plastic boxes (three replicates of 4 boxes) were visually inspected to determine the incidence of rot.

The epiphytic population of bacteria and fungi was evaluated on both Valencia oranges and clementines before and at the end of the cold storage. Five disks of flavedo and albedo were cut from each fruit from lots of 10 fruit per treatment, replicated three times, using a sterile cork borer 10 mm in diameter. The disks collected from each fruit were transferred into 200 ml sterile cups containing 100 ml of sterile water and shaken on a rotary shaker at 200 rpm for 30 min. After serial dilutions, 100 µl of each dilution were plated on PDA amended with ampicillin and streptomycin sulphate or with cycloheximide and analyzed as described above to determine fungi and bacteria. The number of fungal and bacterial colonies (CFU) was converted into CFU/cm^2 of fruit surface.

Statistical analyses. Each dataset was preliminary tested to determine if assumptions of one-way ANOVA analysis were met. In particular, normality of distribution and homogeneity of variances were assessed by Shapiro-Wilk test ($p>0.05$) and Levene's test ($p>0.05$), respectively. Percentages were converted into angular values (arcsine √%) before analyses. The concentration of bacteria and fungi in water solutions used for dipping treatments of Valencia oranges were $\log_{10}$ transformed to improve homogeneity of variances. Data were submitted to the analysis of variance (ANOVA) and mean values were compared using Tukey's test ($P<0.05$) to determine the significance of the treatments. The % reduction was calculated as comparisons to the controls.

RESULTS

Evaluation of natural decays

In all trials, oranges and clementines were considered decayed as soon as clear symptoms of rot softening appeared on the peel. After mycelial development and sporulation, the rot was found to be mainly caused by *P. digitatum* (around 80%) and to a lesser extent (around 20%) by *P. italicum*. Around 10% of the fruit (oranges and clementines) were infected by both pathogens. Gray mold infections caused by *Botrytis cinerea* were exclusively found on the clementines and represented around 5% of the detected rots.

Postharvest treatments

At the end of the cold storage, 13.3% of the oranges dipped in tap water (control) were rotten. This incidence was reduced by 97.5, 71.3, and 58.2% on fruit treated with PGE at 12, 6, and 3 g/l respectively (Fig. 1). Compared to untreated oranges, significant reductions ($P \leq 0.05$) in rot was only achieved with PGE 12 and PGE 6, while other treatments did not have a significant impact on the incidence of rot (Fig. 1). After 7 days of shelf life, the incidence of decay reached 75.8% on oranges dipped in water (control) and PGE confirmed to be the most effective treatment reducing rots by 78.9, 76.0, and 64.6% with the three tested concentrations. Lower, but still significant reductions in rots were achieved with IMZ (49.6%) and $NaHCO_3$ (48.8%). Compared to untreated oranges, significant reductions in rot were achieved with PGE 12, PGE 6 and PGE 3 (Fig. 1).

The analysis of the aqueous solutions used for dipping treatments revealed a strong antimicrobial activity of PGE against bacteria ($F = 96.215$; $df = 6, 56$; $p < 0.001$) and fungi ($F = 128.49$; $df = 6, 56$; $p < 0.001$). After dipping treatments, the concentration of bacteria reached 4.0×10^5 CFU/ml in tap water and was reduced by 98.5, 98.4, and 78.0% with PGE 12, PGE 6, and PGE 3, respectively. The total fungal contamination reached 23,000 CFU/ml in tap water and was reduced by 86.7, 85.3, and 58.8% in water containing PGE 12, PGE 6, and PGE 3. Both, bacterial and fungal populations were not significantly affected by KHCO3, NaHCO3, and IMZ.

Preharvest treatments

At the end of cold storage, the incidence of rot on oranges treated before harvest was quite low since 6.5% of the oranges sprayed with tap water (control) showed rot symptoms. However, a significant reduction in rot (92.3%) was achieved on fruit treated with PGE 12 (Fig. 3). At the end of the shelf life, the incidence of decay reached 81.4% on fruit used as control and was greatly reduced (90.2, 84.3, and 77.6%) by PGE at the three tested concentrations (Fig. 3). After 7 days of shelf life, PGE was significantly ($P \leq 0.05$) more effective than IMZ and salts at all tested concentrations.

Overall, a higher incidence of decay was assessed on clementines, compared to oranges (Fig. 4). Notwithstanding the high disease incidence, PGE at the three tested concentrations was very effective and at the end of cold storage reduced rot by 98.1, 94.1, and 91.3%, respectively. At the end of the shelf life, all clementines of control samples (fruit untreated or sprayed with water) were rotten while, PGE 12, PGE 6 and PGE 3 reduced decay by 87.1, 83.7, and 68.9%, respectively. A significant level of protection ($P \leq 0.05$) was also achieved with IMZ, $KHCO_3$ and $NaHCO_3$ at the end of both cold storage and shelf life (Fig. 4). However, these latter treatments were significantly less effective than PGE 12, PGE 6, and PGE 3 at the end of shelf life, and significantly less effective than PGE 12, at the end of cold storage.

The analysis of the epiphytic population of bacteria and fungi revealed a significant impact of treatments and produced similar results on Valencia oranges and on clementines, before and after cold storage (Table 1). Compared to fruit treated with tap water, PGE 12 reduced bacterial CFU between 94.3 and 97.7%. Significant reduction ($P \leq 0.05$) was also achieved with PGE 6, PGE 3 and, to a lesser extent, with IMZ. A lower impact on bacteria was determined for $NaHCO_3$ and $KHCO_3$ (Table 1). A trend similar to that described for bacteria was also revealed for fungi, although the percentage of reductions was lower overall (Table 1).

DISCUSSION

Experiments conducted in large-scale commercial conditions showed that PGE is highly effective in controlling postharvest rot of orange and clementines when applied both before and after harvest. Although the efficacy of PGE was concentration dependent, the extract significantly reduced rot at all tested concentrations and at the end of shelf life was always more effective compared to tested salts ($NaHCO_3$ and $KHCO_3$) and fungicide (IMZ). This higher efficacy compared to IMZ is outstanding considering that this fungicide is currently regarded as the most effective fungicide to control postharvest citrus rot despite the well documented presence of fungicide-resistant isolates of *P. digitatum* and *P. italicum* (Kinay et al., 2007; Pérez et al., 2011; Erasmus et al., 2015). Furthermore, although experiments were not properly repeated, the consistency of results on different fruit types (clementines and oranges) and with different application methods (preharvest spray and postharvest dipping), is indirect evidence of reliability. Other alternative control methods, such as those based on microbial antagonists, may produce inconsistent results because they are greatly influenced by external factors including environmental conditions and host species (Ippolito et al., 2005). The salts tested in the present study demonstrated good efficacy against green and blue molds on citrus but provided a significantly lower level of protection when compared to PGE. If confirmed, our results suggest the potential use of PGE as an alternative to fungicides, while salts, in agreement with previous investigations, may be more appropriate in integrated control strategies providing the combination of more control measures (Kanetis et al., 2008; Usall et al., 2008; Youssef and Roberto, 2014; Wisniewski et al., 2016). The achievement of very high levels of protection is very important for harvested commodities considering their high value and the consequent economic impact of losses due to postharvest rot (Droby et al., 2001).

Positive results with both pre- and postharvest application of PGE also indicate a high level of flexibility and the chance to modulate treatments according to need. Field treatments may be useful for citrus fruit that are packed and marketed without specific treatments such as washing and waxing. In Italy, this is commonly the case with early varieties of clementines since they are quickly introduced into the distribution chain and fruit are frequently sold with some leaves, testifying to the freshness of the product. Similar situations may occur in other countries and for other fruit species such as table grapes and strawberries that cannot be subjected to dip treatments (Nigro et al., 2006). Furthermore, field treatments may be strategic to protect fruit from wound infections occurring during harvesting and pre-packaging phases and may be useful in less technologically advanced countries since they can be performed with the simple equipment commonly used for conventional fungicides sprays. On the other hand, postharvest dipping treatments can be easily and cheaply integrated into common citrus packinghouse operations by adding the active ingredient to water used to wash fruit and/or reduce temperature (hydro-cooling).

The analysis of fungal and bacterial populations associated with oranges and clementines treated before harvest revealed a strong antimicrobial activity against fungi and bacteria whose populations were sharply reduced on both clementines and oranges, before and after cold storage. Since microorganisms naturally occurring on the carposphere may play an important role in protecting fruits, the broad-range antimicrobial activity of PGE may represent a negative feature (Chalutz and Wilson, 1990). However, the persistent efficacy of PGE after 7 days of cold storage and 7 days of shelf life indicates that the reduction of microbial populations does not represent a relevant issue from a practical point of view. In this context, the capability of PGE to induce resistance in treated citrus tissue (Pangallo et al., 2016; Belgacem et al., 2019) is likely to play a fundamental role in determining a high level of efficacy over time. This feature of PGE may be strategic to protect washed citrus fruit since water may flush microorganisms and reduce the consistency of the carposphere microbiota.

Furthermore, in the case of postharvest dipping treatments, the activity of PGE against water-borne microorganisms may be useful to reduce the inoculum of postharvest plant pathogens and foodborne bacteria in recirculated water and avoid the use of chlorine or other sanitizers commonly used in citrus packinghouses (Belgacem et al., 2020).

The overall results of this study demonstrated that PGE represents a new valid alternative to control postharvest citrus rot. It is important to point out that all trials were carried out on a large scale since many other alternative control methods had proved effective under laboratory conditions but failed when tested under commercial conditions (Bautista-Baños, 2014). According to our results, the potential replacement or integration of IMZ treatments with PGE may lead to significant reductions in losses, lower environmental impact and improved quality and safety of products because of the lack of chemical residues (Romeo et al., 2015). In this context, the potential market of PGE is not restricted to organic productions since it may also be useful for conventional and/or integrated farming systems. Although specific investigations need to be performed to confirm safety of PGE for humans and environment, the growing interest toward the use of pomegranate peel extracts to protect and improve the quality of foods as well as the increasing interest in applications in human medicine are encouraging (Akhtar et al., 2015; Sorrenti et al., 2019). The final considerations, together with the absence of signs of possible phytotoxic effects (Li Destri Nicosia et al., 2016;

Pangallo et al., 2018) and the wide availability of pomegranate peel as a byproduct of processing factories, are elements that may facilitate the future use of PGE as a natural fungicide.

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Table 1. Epiphytic populations of bacteria and fungi on Valencia oranges and clementines before and after cold storage. Analyzed fruit were treated before harvest with PGE at three different concentrations (12, 6 and 3 g/l), $NaHCO_3$, $KHCO_3$ or Imazalil (IMZ). Untreated fruit and fruit sprayed with tap water were used as controls.

	Before storage				After storage			
	Fungi		Bacteria		Fungi		Bacteria	
	CFU/cm²*	PR (%)**	CFU/cm²*	PR (%)**	CFU/cm²*	PR (%)**	CFU/cm²*	PR (%)**
Valencia oranges								
Water	18900	0.0 a	56140	0.0 a	17260	0.0 a	52410	0.0 a
Untreated	12500	33.9 ab	52460	6.6 a	12780	26.0 ab	51470	1.8 a
IMZ	6237	67.0 bc	22120	60.6 b	6080	64.8 c	24950	52.4 b
$KHCO_3$	7540	60.1 bc	47270	15.8 a	7310	57.6 bc	46460	11.4 a
$NaHCO_3$	7170	62.1 bc	45190	19.5 a	7400	57.1 bc	45760	12.7 a
PGE 12	1980	89.5 c	2500	95.5 c	2920	83.1 c	2970	94.3 c
PGE 6	2970	84.3 bc	6510	88.4 bc	3910	77.3 c	7500	85.7 bc
PGE 3	5420	71.3 bc	14900	73.5 bc	5700	67.0 c	13540	74.2 bc
*ANOVA****	*F=7.963; p<0.001*		*F=34.98; p<0.001*		*F=16.219; p<0.001*		*F=29.155; p<0.001*	
Clementines								
Water	11100	0.0 ab	58550	0.0 a	13680	0.0 a	59070	0.0 a
Untreated	11600	-4.5 a	58976	-0.7 a	10420	23.8 b	58740	0.6 a
IMZ	4950	55.4 cd	17970	69.3 cd	5142	62.4 cd	17400	70.5 bcd
$KHCO_3$	6690	39.7 bc	40000	31.7 abc	6690	51.1 c	33730	42.9 b
$NaHCO_3$	5944	46.5 cd	27360	53.3 bc	6510	52.4 c	26750	54.7 b
PGE 12	1604	85.5 d	1360	97.7 d	1650	87.9 e	1270	97.9 d
PGE 6	2830	74.5 cd	1690	97.1 d	2870	79.0 de	1550	97.4 d
PGE 3	4570	58.8 cd	2260	96.1 d	4620	66.2 cd	2170	96.3 cd
*ANOVA****	*F=10.005; p<0.001*		*F=44.558; p<0.001*		*F=11.472; p<0.001*		*F=10.005; p<0.001*	

*CFU/cm² = Populations of bacteria and fungi expressed as colony forming units (CFU) per square centimeter (cm²) of fruit surface

**PR = Percentage reduction of bacterial and fungal populations in terms of percentage of detected CFU compared to the water control. In each column, different letters indicate significantly different values according to Tukey's test (P≤0.05).

***ANOVA = F and p values determined per group of data according to analysis of variance (ANOVA)

CAPTIONS TO FIGURES

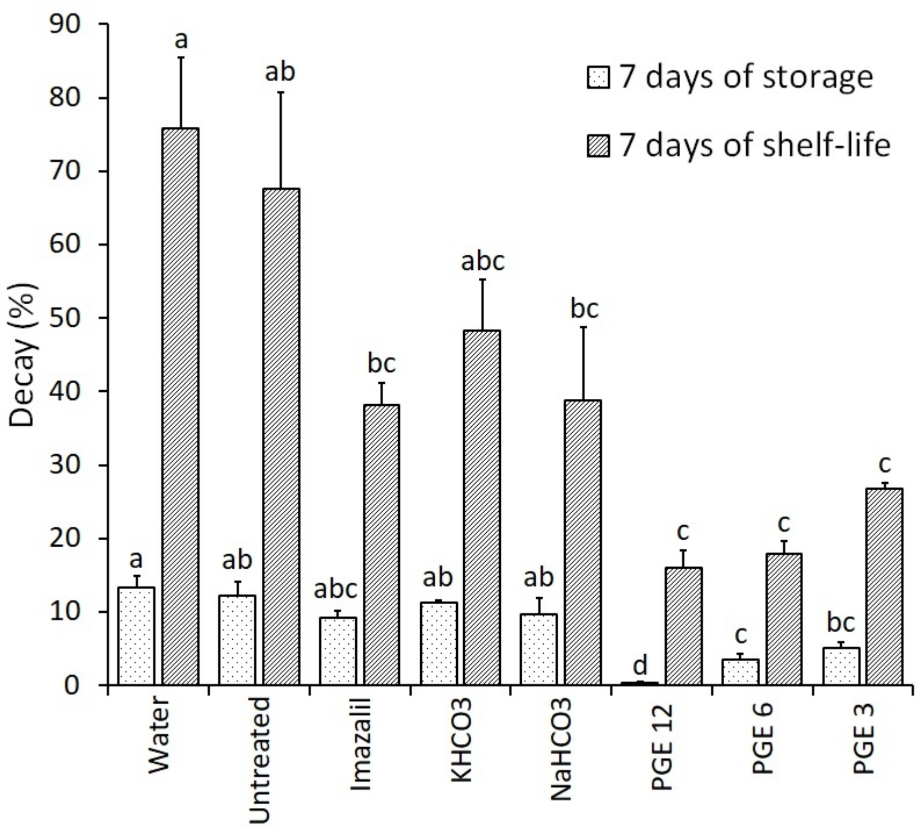
Fig. 1 - Incidence of decay after 7 days of cold storage and 7 days of shelf life on Valencia oranges treated by dipping before storage. Treatments comprised PGE at the three different concentrations (12, 6, and 3 g/l), 2% $NaHCO_3$, 2% $KHCO_3$, and 0.1% Imazalil (chemical control). Untreated oranges and oranges dipped in tap water were used as controls. Data were transformed (arcsine √%) for statistical analysis and back transformed for presentation. Bars indicate standard errors of the means. For each assessment time different letters indicate significantly different values according to Tukey's test ($P \leq 0.05$).

Fig. 2 - Concentration of bacteria and fungi in water solutions used for dipping treatments of Valencia oranges (Fig. 1). Treatments comprised PGE at the three different concentrations (12, 6, and 3 g/l), 2% $NaHCO_3$, 2% $KHCO_3$, and 0.1% Imazalil (chemical control). Untreated oranges and oranges dipped in tap water were used as controls. Bars indicate standard errors of the means. For each group of microorganism (fungi and bacteria), different letters indicate significantly different values according to Tukey's test ($P \leq 0.05$).

Fig. 3 - Incidence of decay after 7 days of cold storage and 7 days of shelf life on Valencia oranges treated before harvest. Treatments comprised PGE at the three different concentrations (12, 6, and 3 g/l), 2% $NaHCO_3$, 2% $KHCO_3$, and 0.1% Imazalil (fungicide control). Untreated oranges and oranges sprayed with tap water were used as controls. Imazalil was applied after harvest by dipping. Data were transformed (arcsine √%) for statistical analysis and back transformed for presentation. Bars indicate standard errors of the means. For each assessment time, different letters indicate significantly different values according to Tukey's test ($P \leq 0.05$).

Fig. 4 - Incidence of decay after 7 days of cold storage and 7 days of shelf life on clementines treated before harvest. Treatments comprised PGE at the three different concentrations (12, 6, and 3 g/l), 2% $NaHCO_3$, 2% $KHCO_3$, and 0.1% Imazalil (chemical control). Untreated clementines and clementines sprayed with tap water were used as controls. Imazalil was applied after harvest by dipping. Data were transformed (arcsine √%) for

statistical analysis and back transformed for presentation. Bars indicate standard errors of the means. For each assessment time, different letters indicate significantly different values according to Tukey's test ($P \leq 0.05$).



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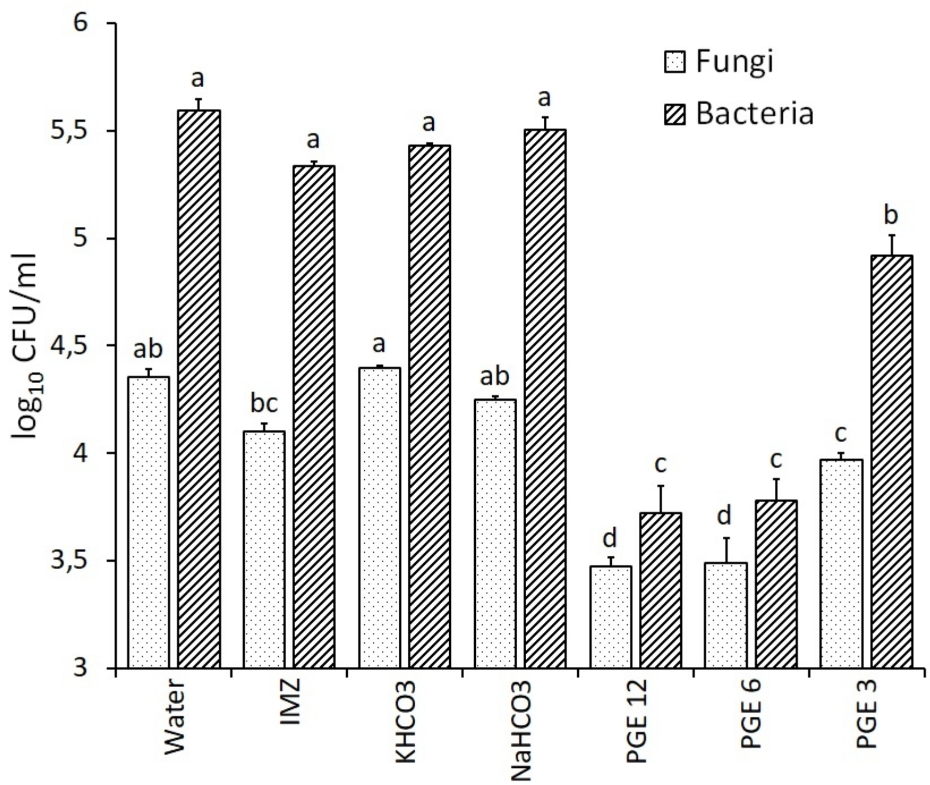

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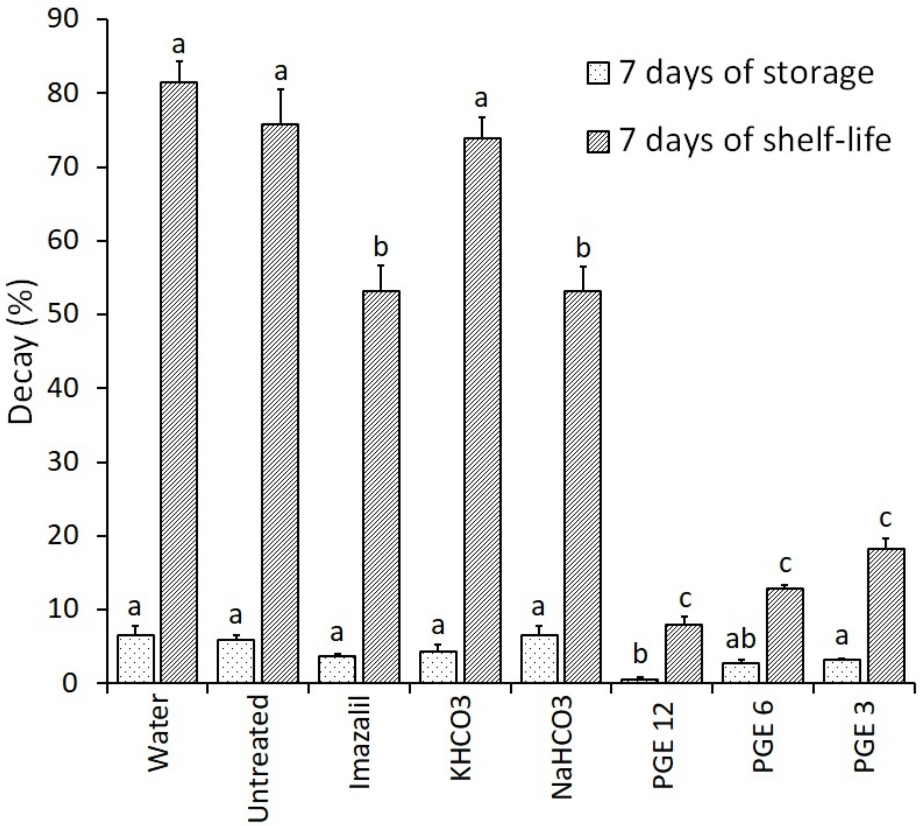

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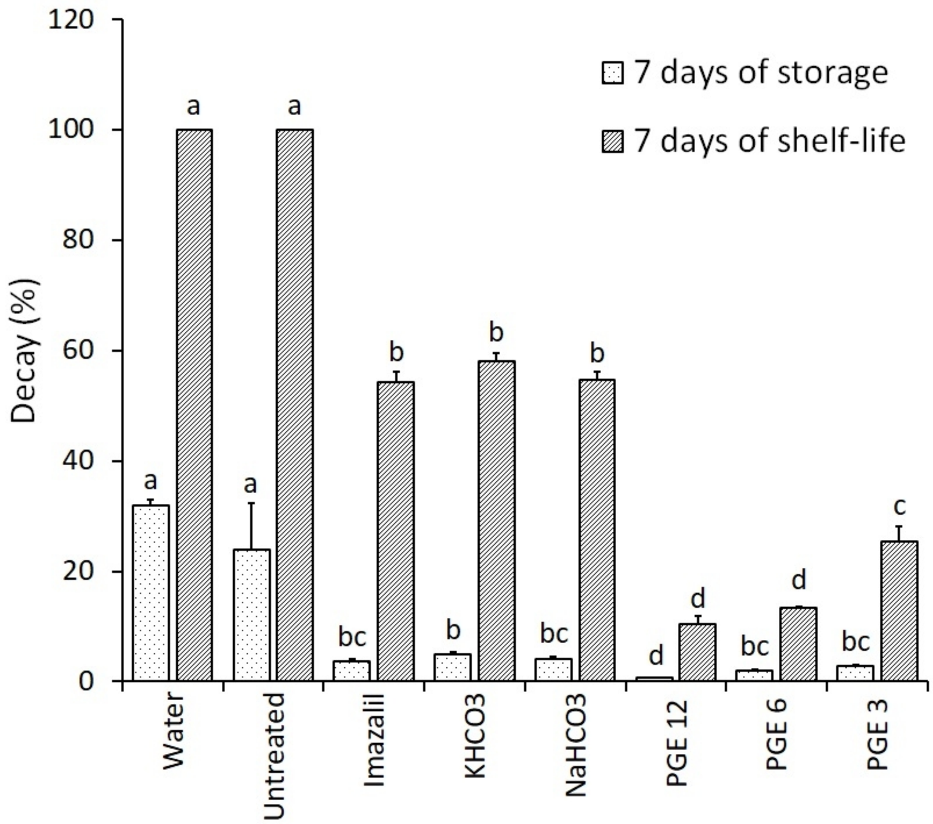

Fig. 4 - Incidence of decay after 7 days of cold storage and 7 days of shelf life on clementines treated before harvest. Treatments comprised PGE at the three different concentrations (12, 6, and 3 g/l), 2% $NaHCO_3$, 2% $KHCO_3$, and 0.1% Imazalil (chemical control). Untreated clementines and clementines sprayed with tap water were used as controls. Imazalil was applied after harvest by dipping. Data were transformed (arcsine √%) for statistical analysis and back transformed for presentation. Bars indicate standard errors of the means. For each assessment time, different letters indicate significantly different values according to Tukey's test ($P \leq 0.05$).

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