Control of postharvest fungal rots on citrus fruit and sweet cherries using a pomegranate peel extract

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ABSTRACT

A pomegranate peel extract (PGE) was evaluated as a natural antifungal preparation for the control of postharvest rots. *In vitro* trials revealed a strong fungicidal activity against germination of conidia of *Botrytis cinerea*, *Penicillium digitatum* and *Penicillium expansum*. Almost complete inhibition of all fungal spore germination was achieved after 20 hours of incubation with PGE. PGE was very effective in inhibiting decay following artificial inoculations of lemons by *P. digitatum* and *P. italicum*, grapefruits by *P. italicum* and apples by *P. expansum*. At concentrations of 1.2 and 12 g/l complete inhibition of infection was achieved in the majority of host pathogen combinations. Furthermore, it was also effective in reducing natural rots under semi-commercial conditions on both sweet cherries and lemons: on cherries *Monilinia laxa* and *B. cinerea* rots were reduced by 61.0% (cv. Bigarreau Moreau) and 95.6% (cv. Giorgia), respectively, and on lemons 87.8% reduction of total rot was achieved. PGE treatment showed residual effect as it was effective in inhibiting infections made at 6, 12, and 24 h after the application of the extract in fruit wounds. Additionally, PGE exhibited curative activity and reduced the incidence of rots when it was applied 6 and 24 hours after inoculation. Considering that PGE was extracted and stabilized using safe chemicals (food grade ethanol and citric acid) and that it did not have any apparent phytotoxic effect on treated fruit, PGE proved to be effectively eco-friendly and safe control mean for postharvest rots of fruit.

Keywords: postharvest rots; natural fungicide; pomegranate peel extract; citrus fruits; sweet cherries; apples

Introduction

Fruit and vegetable are an important part of the human diet since they are a source of vitamins and minerals and contain important compounds such as antioxidants. The increased consumer awareness about the importance of a diet rich in these products for human health is increasing their consumption and the request for high quality and safe products free of pesticide residues, toxins and harmful microorganism. Fruit and vegetables, however, are highly perishable and losses caused mainly by fungal pathogens can amount up to 25 and 50% of the total production in industrialized and developing countries, respectively (Eckert and Ogawa, 1985; Spadaro *et al*., 2004). Furthermore, fungal proliferation may result in the contamination of products with mycotoxins (Wu *et al*., 2014). Currently, synthetic fungicides are a primarily mean to control postharvest decays, however, consumers concerns about chemical residues and the developments of resistant strains of the pathogens is increasingly stimulating the search for safer and more eco-friendly alternative control means (Bautista-Baños, 2014; Feliziani and Romanazzi, 2013; Mari *et al*., 2014.).

A number of alternatives to chemical fungicides have been proposed in the last 20-30 years for the control of post-harvest fungal diseases including the application of antagonistic microorganisms (Droby *et al*., 2009), natural antimicrobial substances (Ippolito and Nigro, 2003; Sharma *et al*., 2009) and sanitizing products (Mari *et al*., 2003). Particular interest has been shown in the powerful antimicrobial action of plant extracts which are considered relatively safe thanks to their natural origin, decomposability and low toxicity to the environment (Cabral *et al*., 2013; Gatto *et al*., 2011; 2013). In addition, their use fits in well with the concept of sustainable agriculture because it mostly exploits natural cycles with reduced environmental impact. Antimicrobial properties of plant extracts are generally related to different classes of compounds like phenols that occur in plants as preformed compounds and may act both on the pathogen and on the host by inducing resistance responses (Sanzani *et al*., 2011; 2012).

Promising results obtained with natural products to control postharvest rots suggest the possible development of natural antifungal compounds that would be as effective as synthetic fungicides (Schena *et al.*, 2007; Spadoni *et al.*, 2015). Approximately 10,000 secondary plant metabolites have been chemically defined for their antimicrobial activity, but the number of available plant active substances is considered much higher (Boulogne *et al.*, 2012). Natural compounds of plant origin with well documented antimicrobial activity include volatile organic compounds, isothiocyanates, trans-2-Hexal, carvacrol, thymol, citral, trans-cinnamaldehide and essential oils (Spadoni *et al*., 2015). However, the practical application of plant extracts to control postharvest rots represents the early stage of process geared to the development of commercially viable products. There are still major obstacles in large scale use of plant extracts for controlling postharvest

pathogens. These include the reduced and inconsistent efficacy as a result of fruit physiology and environment, low residual activity, lack of curative effect and limited range of activity against different fungal pathogens (Bautista-Baños *et al*., 2014).

Pomegranate has been long used in traditional medicine to treat a variety of human diseases (Nonaka *et al.,* 1990). In particular, fruit peel extracts have a free radical scavenger effect and potent antioxidant capacity due to the presence of a high concentration of various biologically active components (Akhtar *et al*., 2015; Fischer *et al*., 2011; Lee *et al*., 2010). Several reports indicates that extracts from pomegranate peel are effective as natural inhibitor for pathogenic bacteria and fungi (Akhtar *et al*., 2015; Jayaprakasha *et al*., 2006; Osorio *et al*., 2010; Tehranifar *et al*., 2011). However, only few studies have investigated its possible application to control plant pathogens (Tayel *et al*., 2011; 2009). In a recent study, the characterization of a concentrated pomegranate peel extract (PGE), containing a high concentration of phenols was reported and its possible use against fungal plant pathogens was suggested (Romeo *et al*., 2015). The aim of the present study was the evaluation of the efficacy of PGE in controlling different postharvest rots of citrus, apples and sweet cherries.

Material and Methods

Pomegranate extract

A concentrated extract of pomegranate peel (PGE) was obtained according to Romeo et al. (2015) from ripe pomegranate (*Punica granatum* L.) fruit cv. '*Mollar De Elche'*, harvested in Acireale (Italy). The concentrated solution containing 120 g/l of dry matter and 1% citric acid as antioxidant was stored at 5°C until use.

Preparation of pathogen inocula

Fungal isolates were obtained from infected lemons (*Penicillium digitatum* and *Penicillium italicum*), apples (*Penicillium expansum*) and table grape berries (*Botrytis cinerea*). Conidia were directly collected from decayed fruit, serially diluted with sterile water and plated on potato dextrose agar (PDA, Sigma-Aldrich) in order to obtain single conidia colonies. Pure cultures were kept on PDA slants at 5°C for long term storage or grown at 22°C on PDA plates for 7-10 days to produce inocula. Conidia were collected with a spatula, suspended in sterile distilled water, filtered through a double layer of sterile muslin cloth (Artsana, Rome, Italy) and vortexed for 1 min to ensure uniform mixing. Concentration of the condia in the suspension was determined using a haemocytometer chamber (Brand Gmbh, Wertheim, Germany) and diluted to have stock solutions containing 10^7 conidia/ml.

In vitro antifungal activity of PGE

The inhibitory activity of PGE at different concentrations (0.12, 1.2, and 12 g/l), was evaluated using spore suspensions of *B. cinerea*, *P. digitatum*, and *P. expansum*. To assess the effect of PGE on the viability of the pathogens' conidia, 0.5 ml of conidial suspensions ($2x10^3$ conidia/ml) were transferred to 1.5 ml Eppendorf tubes containing 0.5 ml of PGE solutions with double concentrations of extract (2X) in order to obtain the above desired final concentrations (1X). Tubes containing 0.5 ml of citric acid 2% were used as a control. The obtained mixtures containing approximately 1000 conidia/ml were gently mixed and incubated at 22°C. After 20 h, tubes were vortexed and 100 µl of conidia suspensions were transferred and uniformly distributed in Petri dishes containing PDA amended with ampicillin and streptomycin sulphate (250 mg/l each). Dishes were incubated at 25°C and the number of colony forming units (CFU) was recorded after 3-4 days.

To evaluate the effect of PGE on spore germination and germ tube elongation, 12.5 µl of a conidia suspension (10^6 conidia/ml) were transferred to Eppendorf tubes and mixed with an equal volume of potato dextrose broth (PDB, Sigma-Aldrich) at strength of 24 g/l and 25 µl of 2X PGE solutions. In control samples pomegranate extract was replaced by citric acid at 1% final concentration. After 8 (*B. cinerea*) or 20 (*P. digitatum* and *P. expansum*) hours of incubation at 22°C tubes were vortexed and 2 µl of spore suspension were transferred to microscope slides and mixed with 2 µl of blue lactophenol to stop further spore germination. For each slide, three groups of 40 spores each were randomly selected and observed with a microscope set up for a 200× magnification to determine the percentage of germinated conidia and the average length of the germ tubes. Conidia were considered germinated when the length of the germ tube was at least equal to the length of the swollen conidia.

Trials on artificially inoculated fruit

Tests were performed on lemons (cv. *Femminello Siracusano*) with *P. digitatum* and *P. italicum*, grapefruits (cv. *Sunrise*) with *P. digitatum* and apples (cv. *Golden Delicious*) with *P. expansum*. All fruit were selected for uniformity in size and color. Fruit were surface sterilized by immersion in a 2% sodium hypochlorite solution for 1 min, washed twice with tap water, air-dried and fixed on polypropylene honeycomb panels using a double-sided tape. Fruit were kept 1–2 cm apart to avoid nesting and wounded with a nail to make 2 X 2 mm wounds. Lemons were wounded once in the equatorial zone while grapefruits and apples were wounded on three equidistant points per fruit. Wounds were treated by applying 10 µl of PGE at different concentrations (12, 1.2 or 0.12 g/l) and inoculated with 10 µl of a conidial suspension containing $3x10^4$ conidia/ml (*P. digitatum* and

P. italicum) or 10^5 conidia/ml (*P. expansum*). Mock wounds treated with 1% citric acid and inoculated with the pathogens served as controls.

Trials were made to evaluate the preventive and the curative effect of PGE. For the preventive effect, wounds were treated with PGE and then after 1 h (all fruit/pathogen combinations) ,12 and 24 h (only trials with lemons) were inoculated with the pathogens. To evaluate the curative effect, wounds were first inoculated with the pathogens and then treated with PGE after 6 or 24 h of incubation.

In all trials treated fruit were maintained at room temperature (22-24°C) in lidded plastic boxes containing wet paper to ensure high relative humidity (RH). The percentage of infected wounds and the diameter of lesions were evaluated daily starting from 4 days post inoculation.

Semi-commercial trials with sweet cherries

Trials were conducted twice using organic sweet cherries (cvs. *Bigarreau Moreau* and *Giorgia*) harvested in Apulia (Italy). Cherries of cv. *Bigarreau Moreau* were characterized by the presence of several cracking lesions due to recurrent raining during ripening and by the presence of rotted fruit (approximately 5%). Fruit of the cv. *Giorgia* did not show any apparent lesion of rots. Cherries were selected to have uniform fruit in size and ripening and fruits with apparent lesions and/or rot symptoms were discarded. Selected fruit were dipped for 2 min in a solution of pomegranate extract at different concentrations (12, 2.4 or 1.2 g/l), dried at room temperature for 2 hours on blotting paper, placed in plastic trays (approximately 100 fruit per tray), covered with plastic sheet, and stored at 1°C. Fruit dipped in 1% citric acid and untreated fruit were used as controls. Incidence and severity of rots were evaluated daily after 7 days of storage at 1°C and 3 days of shelf life at 20-22°C using an empirical scale with values ranging from 0 (healthy fruit) to 4 by step of 1 corresponding to 25, 26-50, 51-75 and 76-100% of rotted surface, respectively. Data were used to calculate the McKinney index (McKinney, 1923), expressing the weighted average of the disease severity as actual percentage in terms of the maximum disease severity. The index was calculated by the formula: $MI=[\Sigma(d\times f)/Tn\times D]100$, where d is the degree of disease severity assessed on the fruit and f its frequency, Tn is the total number of fruit examined (healthy and diseased) and D the highest degree of disease intensity occurring on the empirical scale.

Semi-commercial trials with lemons

Lemons "*Femminello Siracusano*" uniform in size and color were washed with tap water using an experimental packing line (MAF RODA Italia S.r.l, FC, Italy), air-dried for 1 h and dipped for 2 min in pomegranate extracts diluted as already described for cherries. Fruits dipped in an Imazalil

solution (Deccozil 50, Decco, Italy) containing 1000 mg/l of active ingredient in 1% citric acid and untreated fruit were used as controls. Lemons (approximately 300 per each treatment) were placed in three plastic trays (100 fruit per tray) and cold-stored for 60 days at 12±1°C and 90-95% RH, followed by 7 days of shelf-life at 20±2°C. The percentage of decayed lemons was recorded at the end of the shelf-life. Fruit were considered decayed when infected by one or more pathogens including *P. digitatum, P. italicum* and minor fungal pathogens.

Experimental design and statistical analysis

In vivo experiments were made using three repetitions. In trials with artificially inoculated fruits, each repetition consisted of 10 (lemons) and 5 (grapefruits and apples) fruit kept in separate plastic boxes. In semi-commercials trials each repetition consisted of 300 lemons and 10 kg for sweet cherries (approximately 300 fruit). *In vitro* experiments were made using three repetitions consisting of 3 PDA plates (pathogen vitality) or microscope slides (conidia germination and germ tube length). The data were submitted to the analysis of variance (ANOVA) and means were compared using the Duncan's test ($P < 0.05$) to determine the significance of the treatments. Percentages were converted into Bliss angular values (arcsin √%) before analysis.

Results

In vitro antifungal activity of PGE

In vitro assays showed a significant antifungal activity of aqueous solutions of PGE. Viability of *B. cinerea* conidia incubated for 20 h was almost completely inhibited by PGE at 12 g/l and strongly reduced by 94.0 and 80.0% at 1.2 g/l and 0.2 g/l, respectively, as compared to the control (Fig. 1). A lower fungicidal effect was demonstrated for *P. digitatum* as CFUs were reduced by 80 and 64% at 12 and 1.2 g/l, respectively, but no significant effect was found at the lowest concentration (0.12 g/l) of the extract (Fig. 1). In *P. expansum* viability of conidia was markedly reduced (57.7%) only by PGE at 12 g/l (Fig. 1).

The germination of conidia in PDB containing 12 g/l of PGE was significantly inhibited as compared to the control by 100, 91.0 and 82.7% for *B. cinerea*, *P. digitatum* and *P. expansum*, respectively (Figs. 2 and 3). At 1.2 g/l PGE, the germination was reduced by 49.1, 82 and 25%, respectively. PGE at 0.12 g/l exhibited a slight inhibition effect. In general, the length of germ-tubes was less affected indicating that once germinated conidia grew almost normally (Figs. 2 and 3). However, significant reductions in germination and germtube length were evident for *P. digitatum* at 12, 1.2 and 0.12 g/l PGE.

Preventive effect of PGE on artificially inoculated fruit

The pomegranate extract proved to be very effective as preventive treatments in all tested host/pathogen combinations. The development of rots caused by *P. digitatum* and *P. italicum* on lemons and *P. digitatum* on grapefruits was completely inhibited by PGE applied at 12 g/l (Fig. 4), and fruits remained healthy up to the end of the experiment (data not shown). At the same concentration the incidence of *P. expansum* rot on apples was reduced by 80.0% (Fig. 4). Significant reductions of the incidence of rots were also obtained for lower concentrations of PGE (1.2 and 0.12 g/l). In particular, *P. italicum* was completely inhibited or was reduced by 80.1% on lemons treated with PGE at 1.2 and 0.12 g/l, respectively. On the same host, *P. digitatum* infection was reduced by 85.8% (1.2 g/l PGE) and 28.5% (0.12 g/l PGE), while on grapefruits the incidence of rots was reduced by 38.5 and 23% with PGE at 1.2 and 0.12 g/l, respectively. A lower efficacy was observed with PGE at 1.2 and 0.12 g/l against *P. expansum* on apples as it was reduced by only 31.1 and 6.7%, respectively (Fig. 4). Data about the severity of the disease (diameter of lesions) were almost always in agreement with those of the incidence of the disease (percentage of infected wounds).

In trials conducted on lemons with *P. digitatum* and *P. italicum* a high efficacy was obtained also when the pathogen was inoculated 12 and 24 hours after PGE application (Figs. 5A and 5B). In both inoculation times, both pathogens were completely inhibited by PGE at 12 and 1.2 g/l and a significant reduction of incidence and diameter of rots was evident with PGE at 0.12 g/l.

In all trials, fruit treated with PGE did not show any modification of color or morphology which may be indicative of phytotoxicity.

Curative effect of PGE on artificially inoculated fruit

Pomegranate extracts at 12 and 1.2 g/l significantly reduced both incidence and diameter of rots when applied 6 and 24 h after inoculation with the pathogens, while weaker and /or non-significant reduction of decay was observed with 0.12 g/l of PGE (Figs. 6A and 6B). On lemons, the application of PGE at 12 g/l 6 h after inoculation resulted in the reduction of infection of *P. italicum* and *P. digitatum* by 60 and 76%, while at 1.2 g/l the reduction was 45 and 46.7%, respectively. On grapefruit PGE at 12 or 1.2 g/l reduced the infection of *P. digitatum* by 68.9 % and 44.8%, respectively. On apples, *P. expansum* infections were reduced by 53.4 and 8.9% with PGE treatment at 12 and 1.2 g/l, respectively (Fig. 6A). The application of the extract 24 h after infection on the same host/pathogen combinations (*P.italicum* and *P. digitatum*/lemons, *P. digitatum*/grapefruits and *P. expansum*/apples) reduced the incidence of rots by 54.8, 35.6, 67.6 and 26.7% at 12 g/l and by 35.4, 29.6, 28.6 and 13.4 at 1.2 g/l, respectively (Fig. 6B).

Semi-commercial trials with sweet cherries

Treatments with PGE under semi-commercial conditions markedly reduced the development of natural postharvest rot on sweet cherries cvs. *Bigarreau Moreau* and *Giorgia* (Fig. 7). On cv. *Bigarreau Moreau*, PGE treatment at 12 and 2.4 g/l reduced development of total natural rots by 51.6 and 28.0 %, respectively (Fig. 7). A higher level of efficacy was achieved with PGE at 12 and 2.4 g/l for the cv. *Giorgia* with total rots reduced by 94.9 and 37.9% as compared to untreated cherries, respectively (Fig. 7). On both cvs. PGE at 1.2 g/l exhibited slight reduction in total rots. Nevertheless, this effect was statistically significant as compared to sweet cherries dipped in a 1% solution of citric acid. Rots recorded in trials with cv. *Giorgia* were almost exclusively caused by *Monilinia laxa* (99%) and in small portion by *B. cinerea* (1%). On cv. *Bigarreau Moreau* there was a prevalence of *M. laxa* (85%) but a consistent presence of *B. cinerea* (12%) and *Penicillium* spp. (3%) was also observed.

Semi-commercial trials with lemons

A low incidence of natural rots, mainly caused by *P. digitatum* and *P. italicum* was observed on lemons after 60 days of storage at 6°C followed by 7 days of shelf life at 20°C (Fig. 8). However, the incidence of rots on fruit dipped in PGE solutions containing 12, 2.4 and 1.2 g/l of dry matter was reduced significantly by 86.6, 73.5 and 47.0%, respectively, as compared to the untreated lemons (Fig. 8). No significant differences were found between the highest PGE concentration (12 g/l) and the chemical control (Imazalil).

Discussion

In the present study the use of a pomegranate alcoholic extract (PGE) as a natural antifungal product to control postharvest decay was evaluated by means of *in vitro* and *in vivo* trials on both artificially infected fruit and also on naturally infected ones under semi-commercial conditions. Among plant extracts, pomegranate peel has gained attention as an alternative antifungal substance because of its antioxidant and antimicrobial capacity due to its relatively high phenolic content (Osorio *et al*., 2010; Quattrucci *et al*., 2013; Tehranifar *et al*., 2011). In particular, the PGE used in this study was chemically characterized revealing a high phenolic content with a prevalence of punicalagins, a group of ellagitannins known for their antifungal activity (Glazer *et al*., 2012; Romeo *et al*., 2015).

Results of the present study revealed several important PGE's features that may enable its practical applications for the control of postharvest rots. *In vitro* trials demonstrated a strong antimicrobial activity against conidia germination of major fungal postharvest pathogens. The fungicidal effect of PGE at 12 g/l in which conidia were incubated for 20 h was of particular interest

since complete killing was achieved against *B. cinerea* and strong effect was shown against *P. digitatum* and *P. expansum*. This result is consistent with previous reports on the *in vitro* antifungal effect of pomegranate peel extracts (Daham *et al*., 2010; Osorio *et al*., 2010; Glazer *et al*., 2012) and may be is important at a practical level. Indeed PGE could be used to reduce the concentration of fungal inocula on fruit surface in the packhouse pre-wash and/or hydrocooling phase.

In *in vivo* trials PGE proved to be very effective on the artificially inoculated fruits exhibiting complete inhibition of the development of most rots when applied at the concentrations of 12 or 1.2 g/l. Furthermore, it was also effective in semi-commercial conditions providing a reduction of natural rots by 61.05% (cv. *Bigarreau Moreau*) and 95.7% (cv. *Giorgia*) on sweet cherries and by 87.8% on lemons, at the concentration of 12 g/l. In the case of lemons, treatments were made using a pilot packing line with a layout similar to those of commercial fruit processing equipment. The efficacy demonstrated in semi-commercial conditions is remarkable considering that most fruit are washed as soon as they reach the packinghouse and that hydrocooling is one of the most common pre-refrigeration techniques to quick reduce fruit temperature, particularly for sweet cherries. Consequently, treatments in this phase may be easily implemented on a practical commercial scale. Nonetheless additional trials are still needed to confirm the efficacy under commercial conditions since many alternative control methods have proved effective in laboratory conditions but failed when tested under large scale trials (Bautista-Baños, 2014). Due to the high value of fruit and vegetables after harvest, the development of alternative control means while achieving high levels of control is still a challenge (Droby, 2001).

It is also important to emphasize that PGE was obtained using ethanol as solvent and 1% citric acid as antioxidant or acidifying agent since both chemicals are regarded as safe and widely accepted by the public opinion and authorities (Romeo *et al*., 2015). Indeed a high level of efficacy has been previously reported for another pomegranate peel extract but it was obtained using methanol as solvent (Tayel *et al*. 2009). In this regard, several studies showed high efficacy of methanol as an extraction solvent but its use on fruit commodities should be avoided taking into consideration health risks involved (Marjorie, 1999; Tehranifar *et al*., 2011).

Another important feature of PGE is its broad range of antifungal activity. Findings of this study demonstrated the efficacy of the extract in controlling some of the most important postharvest pathogens including *P. digitatum* and *P. italicum* on lemons, *P. digitatum* on grapefruits, *P. expansum* on apples, and *M. laxa* and *B. cinerea* on sweet cherries. Additionally, previous preliminary tests performed with the same extract were also effective against *B. cinerea* on artificially inoculated table grape berries (Romeo *et al*., 2015). A broad range of activity is extremely important since two or more fungal pathogens can simultaneously infect fruits after harvest. Besides it can accelerate the

long and costly process needed for the registration and commercialization of natural and cost-effective fungicides.

The results of the trials conducted on artificially inoculated fruit indicated that the inhibitory effect of PGE persists after its application on the infection sites since it was proved to be effective when applied 12 and 24 hours before pathogen inoculation. Studies to elucidate the mechanism by which PGE affect infection still need to be undertaken. However, PGE ingredients are likely to remain active after its dehydration and/or to induce mechanisms of resistance in the host tissues. This latter speculation is based on the widely reported role of phenolic compounds as plant defense response inducer (Rodov *et al*., 1994; Sanzani *et al*., 2009; 2010).

From a practical point of view, the ability of PGE to protect infection sites for several hours after its application may prove synergic with the observed curative activity. In this relation, PGE significantly reduced the incidence of rots on fruit treated 6 or 24 h after inoculation with the pathogen., i.e. a sufficient time for conidia to germinate and start the infection process. These results suggest the possible protection of fruit during all processing phases after harvest. A treatment just before or soon after harvest may be useful to reduce latent infections and protect fruit during the critical stages of harvesting and packaging in which fungal pathogens can penetrate through wounds and establish infections (Tayel *et al*., 2009; Ippolito and Nigro, 2000). Similarly, a dip treatment in the packing line may be useful to reduce the incidence of previously established infections and to protect fruit during the subsequent phases of cold storage and/or shelf-life. In agreement with above assumptions the high level of protection achieved in semi-commercial trials with sweet cherries and lemons suggest a good persistence of PGE's action that protected fruit during cold storage and subsequent shelf-life. On sweet cherries the higher level of protection achieved for the cv. *Giorgia*, as compared to the cv. *Bigarreau Moreau*, is likely to be the results of a higher preventive action rather than curative effect. Sweet cherries cv. *Bigarreau Moreau* used in this study derived from a lot characterized by the presence of cracking lesions and rotted fruit (approximately 5%) suggesting a high external contamination with pathogen inocula and a high incidence of ongoing latent asymptomatic infections. In any case, the persistence of PGE is a relevant feature since most natural products lack long-lasting effect on commodities during post-harvest phase and this feature is essential to prevent rotting until fruit marketing and consumption (Ben-Yehoshua, 2005; Zhang and Swingle, 2005).

In conclusion the present study provides evidence that PGE has a high potential to be implemented in postharvest control strategies as a natural safe and eco-friendly extract. PGE's evaluation in laboratory and on semi-commercial scales highlighted its important practical behavior featuring a fungicidal action, both curative and preventive activity, long persistence, wide spectrum

of activity and high efficacy on different hosts under different conditions. Furthermore, the absence of signs of possible phytotoxic effect during *in vivo* trials and the wide availability of the pomegranate peel as a waste product of the processing factories, may facilitate the development of PGE as a commercial formulation.

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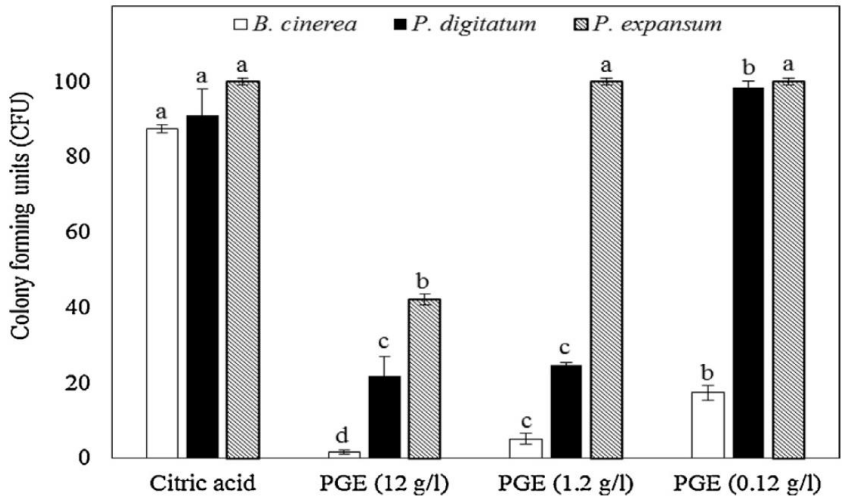

Fig. 1. Effect of pomegranate extract (PGE) on viability of conidia expressed as colony forming units (CFU) on potato dextrose agar (PDA) plates. Before plating conidia of *Botrytis cinerea*, *Penicillium digitatum* or *P. expansum* were incubated in PGE solutions at different concentrations (12, 1.2 or 0.12 g/l) for 20 h. Conidia maintained in a 1% solution of citric acid served as a control. Bars indicate standard errors of the means. For each pathogen, columns with different letters are statistically different according to the Duncan's test ($P \leq 0.05$).

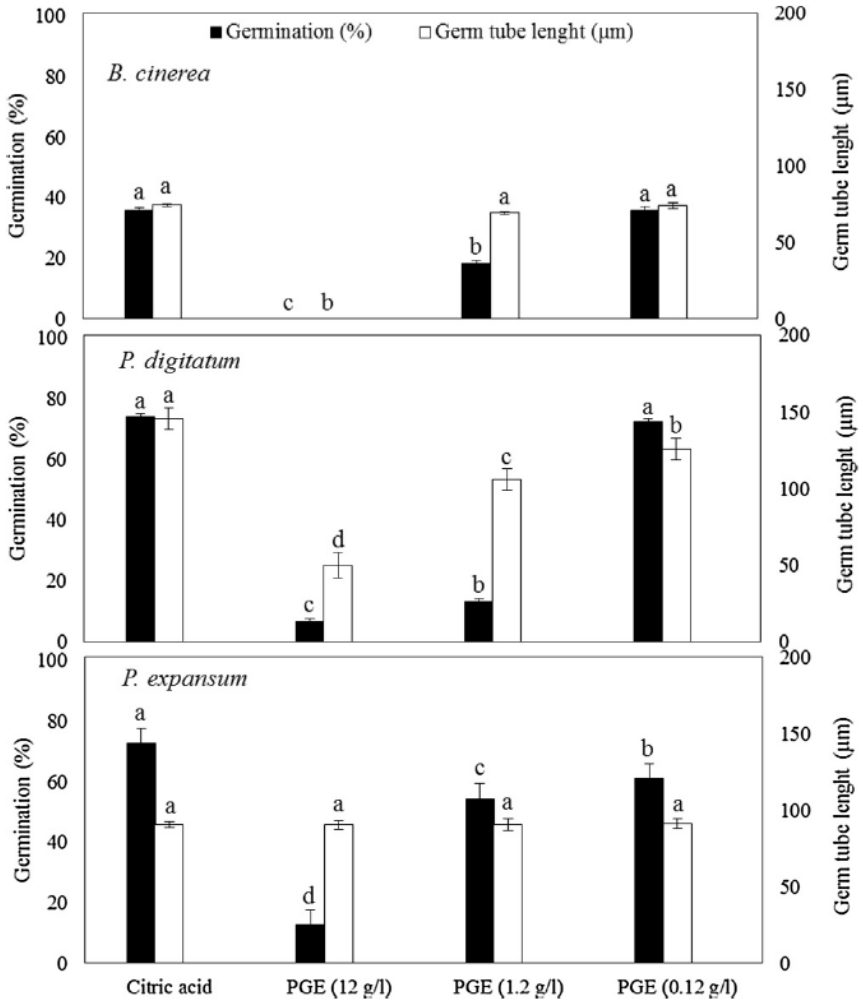

Fig. 2. Effect of pomegranate extract (PGE) on germination and germ tube elongation of conidia incubated for 8 h (*B. cinerea*) or 20 h (*P. digitatum* and *P. expansum*) at 22 °C in potato dextrose broth (PDB) containing PGE at different concentrations (12, 1.2 or 0.12 g/l). Conidia incubated in PDB containing citric acidat 1% served as a control. Bars indicate standard errors of the means. For each analyzed parameter (germination and germ tube length) columns with different letters are statistically different according to the Duncan's test ($P \leq 0.05$).

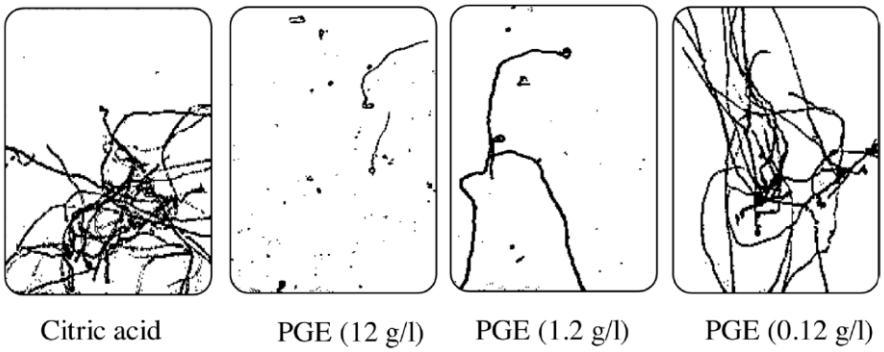

Fig. 3. Germination of conidia of *P. digitatum* incubated for 20 h at 22 °C in potato dextrose broth (PDB) containing pomegranate extract (PGE) at different concentrations (12, 1.2 or 0.12 g/l) or 1% citric acid (control).

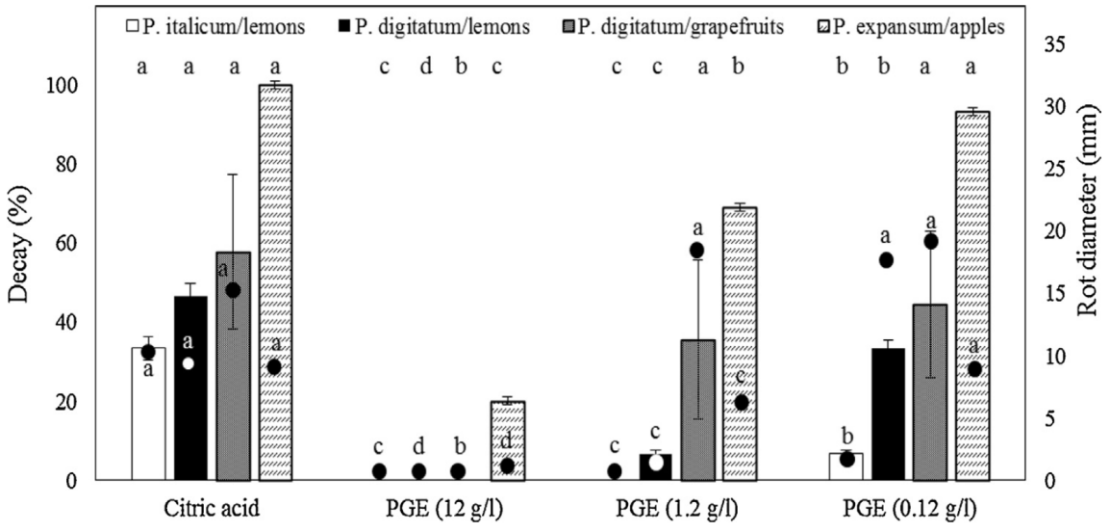

Fig. 4. Preventive effect of pomegranate extract (PGE) on incidence of decay (columns) and rot diameter (dots) of lemons, grapefruits and apples. Fruit were treated with PGE at different concentrations (12,1.2 or 0.12 g/l) and inoculated 1 h later with *Penicillium italicum* or *P. digitatum* (lemons), *P. digitatum* (grapefruits) or *P. expansum* (apples). Mock treated fruit with a solution of 1% citric acid and inoculated with the pathogens, served as controls. Bars indicate standard errors of the means. For each host/pathogen combination, columns and dots with different letters are statistically different according to the Duncan's test ($P \leq 0.05$). Letters on the top of the figure refer to the columns.

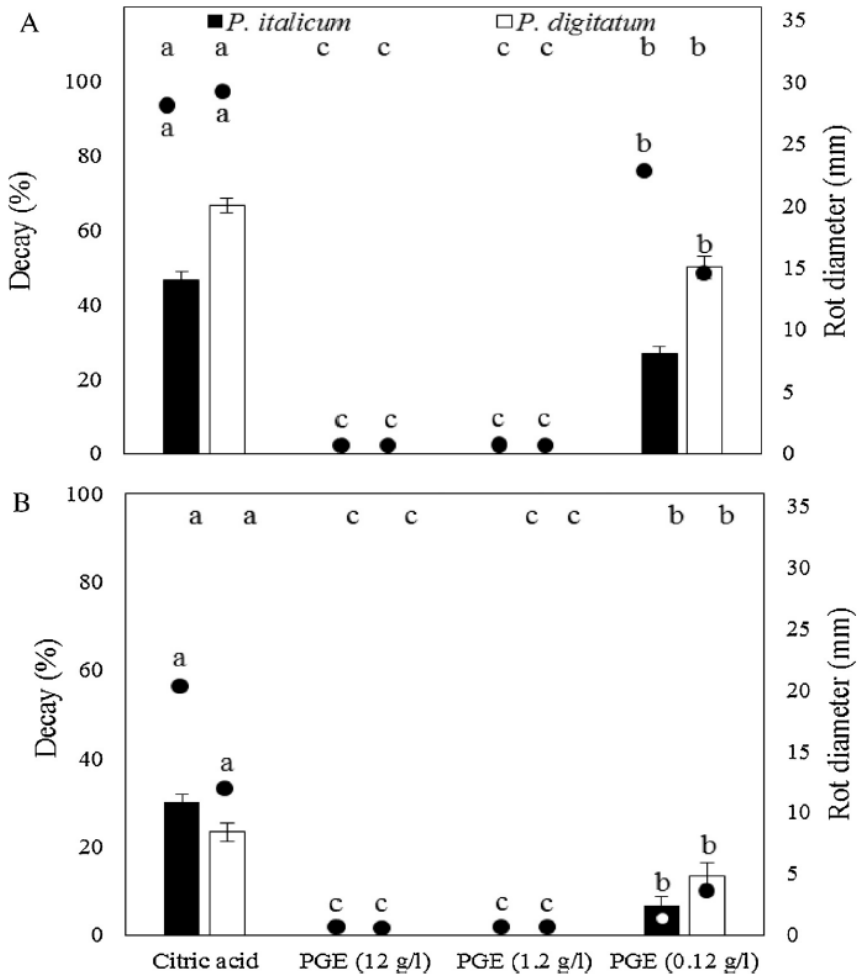

Fig. 5. Preventive effect of pomegranate extract (PGE) on incidence of decay (columns) and rot diameter (dots). Lemons were treated with PGE at different concentrations (12, 1.2 or 0.12 g/l) and inoculated 12 (A) and 24 (B) hours later with Penicillium italicum or P. digitatum. Mock fruit treated with a solution of 1% citric acid and inoculated with the pathogens served as controls. Bars indicate standard errors of the means. For each pathogen, columns and dots with different letters are statistically different according to the Duncan's test ($P \leq 0.05$). Letters on the top of the figures refer to the columns.

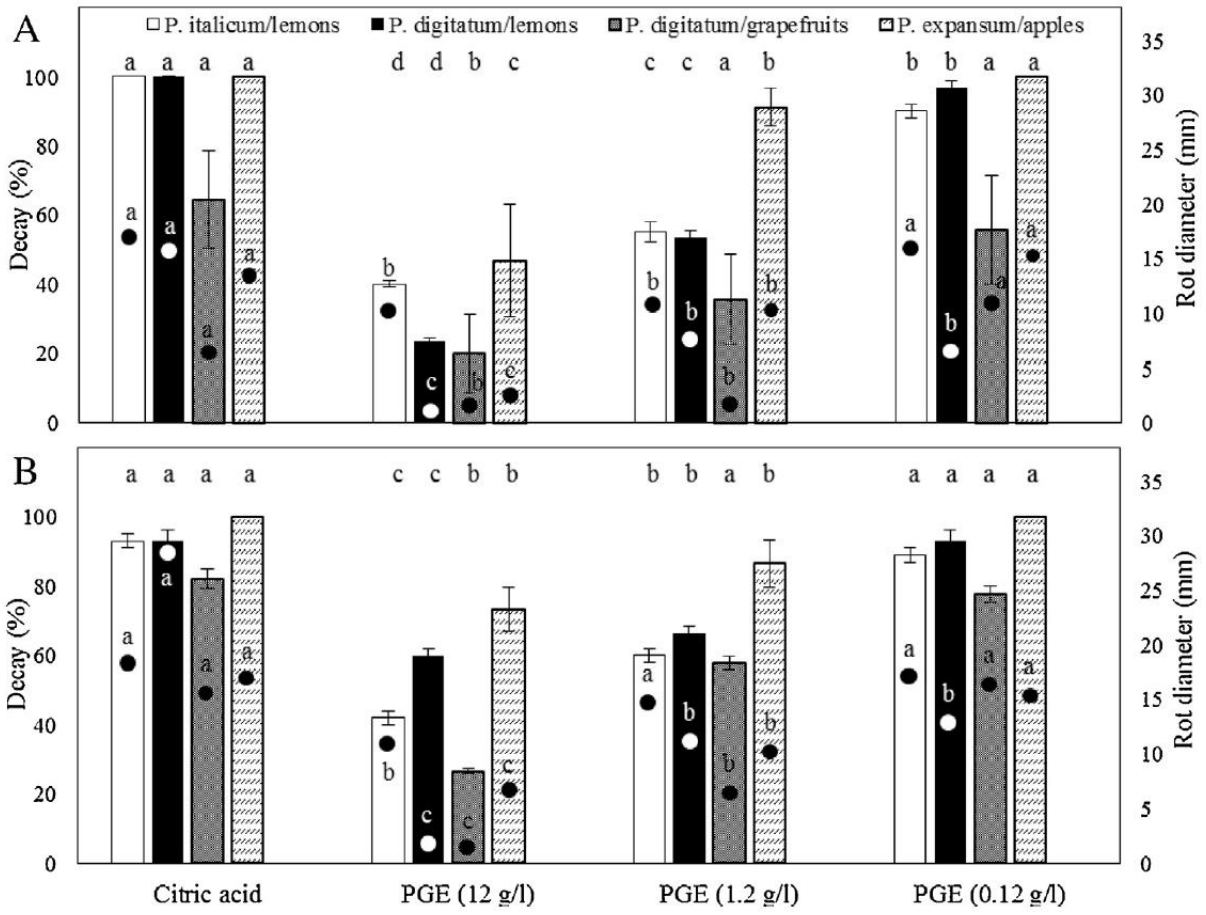

Fig. 6. Curative effect of pomegranate extract (PGE) on incidence of decay (columns) and rot diameter (dots) of lemons, grapefruits and cherries. Artificially inoculated fruit with *Penicillium italicum* or *P. digitatum* (lemons), *P. digitatum* (grapefruits) or *P. expansum* (apples) were treated with PGE at different concentrations (12, 1.2 or 0.12 g/l) after 6 (A) and 24 (B) hours. Mock fruit treated with a solution of 1% citric acid and inoculated with the pathogen served as controls. Bars indicate standard errors of the means. For each host/pathogen combination, columns and dots with different letters are statistically different according to the Duncan's test ($P \leq 0.05$). Letters on the top of the figures refer to the columns.

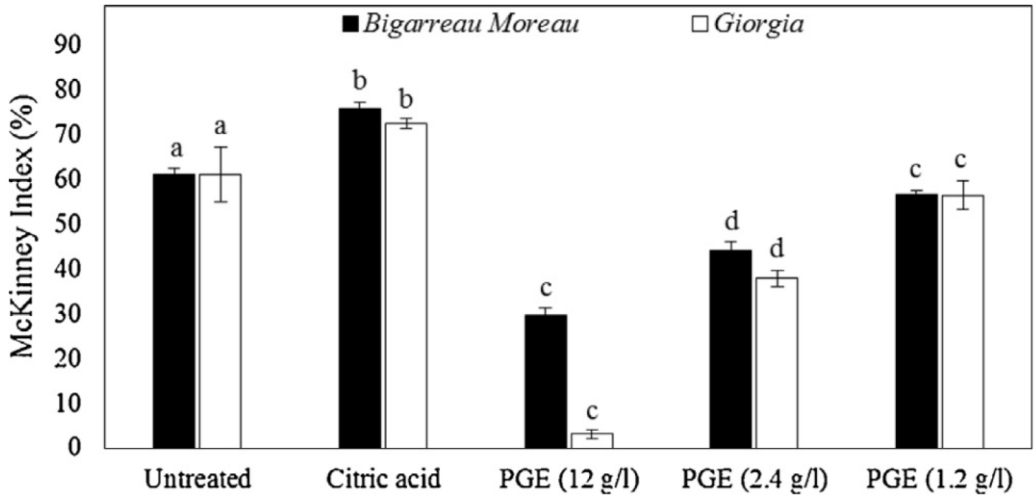

Fig. 7. Effect of pomegranate extract (PGE) on incidence and severity (McKinney index) of natural rots developed on sweet cherries cvs. Bigarreau Moreau and Giorgia after 7 days of cold storage at 1 °C and 3 days of shelf-life at 20–22 _C. Fruit dipped in a 1% solution of citric acid and untreated cherries served as controls. Bars indicate standard errors of the means. For each cultivar, columns and dots with different letters are statistically different according to the Duncan's test ($P \leq 0.05$).

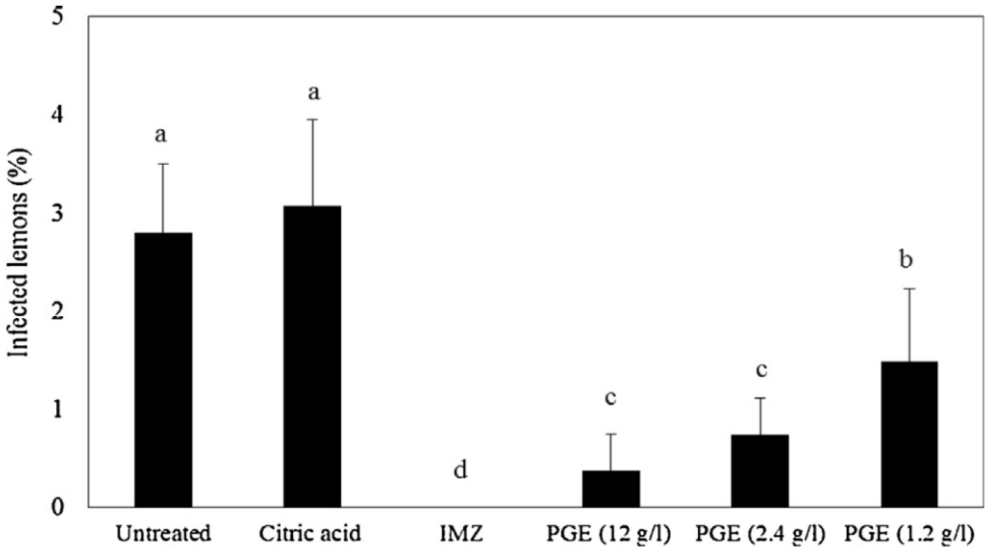

Fig. 8. Incidence of natural rots on lemon dipped in a solution containing Imazalil (chemical control) or PGE at different concentrations (12, 1.2 or 0.12 g/l) after 60 days of cold storage at 6 ± 1 °C and 7 days of shelf-life at 20 ± 2 °C. Fruit dipped in a 1% solution of citric acid and untreated lemons served as controls. Bars indicate standard errors of the means. Columns with different letters are statistically different according to the Duncan's test ($P \leq 0.05$).