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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 h of incubation with PGE. PGE was very effective in inhibiting decay following artificial inoculations of lemons by *P. digitatum* and *Penicillium* 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 h 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.

1. 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 and Gullino, 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 micro-organisms (Droby et al., 2009), natural antimicrobial substances (Ippolito and Nigro, 2000; 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). 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

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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., 2010, 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-cinnamaldehyde 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, 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 (Al-Zoreki 2009; 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.

2. Material and methods

2.1. 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.

2.2. 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 conidia in the suspension was determined using a haemocytometer chamber (Brand Gmbh, Wertheim, Germany) and diluted to have stock solutions containing 10^7 conidia/ml.

2.3. 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 (2×103 conidia/ml) were transferred to 1.5 ml Eppendorf tubes containing 0.5 ml of PGE solutions with double concentrations of extract ($2\times$) in order to obtain the above desired final concentrations ($1\times$). 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/land 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.

2.4. 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.

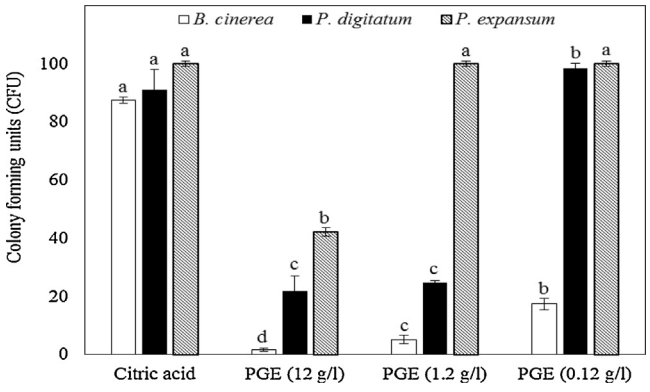

Fig. 1. Effect of pomegranate extract (PGE) on viability of conidia expressed as colony forming units (CFU) on potato dextrose agar (PDA) plates. Before plating conidia of *Botrytis cinerea*,*Penicillium digitatum* or *P. expansum* were incubated in PGE solutions at different concentrations (12, 1.2 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 ($P \leq 0.05$).