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Phenolic extracts from wild edible plants to control postharvest diseases of sweet cherry fruit

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ABSTRACT

Postharvest treatments with extracts from two wild edible plants (*Orobanche crenata* and *Sanguisorba minor*), water solutions of two inorganic salts (calcium chloride, $CaCl_2$, and sodium bicarbonate, $NaHCO_3$), and their combination (i.e., extracts with added $CaCl_2$ or $NaHCO_3$), were assayed to control sweet cherry postharvest diseases. Three extract concentrations for each plant were assayed, corresponding to 0.170, 0.340, and 0.510 g dry matter/mL and to 0.125, 0.250, and 0.500 g dry matter/mL for *S. minor* and *O. crenata*, respectively. At the lowest and the highest concentrations tested, *S. minor* extract was able to inhibit rot development in stored fruit by 79 and 89%, respectively, with an efficacy comparable to that of $CaCl_2$ and $NaHCO_3$; for *O. crenata* extract such inhibition ranged between 64 and 76%, respectively. A dose effect was observed only for *O. crenata*. Moreover, the level of control was not improved by the combined application of plant extracts and salts. HPLC analysis of *O. crenata* extract showed verbascoside as the main phenolic compound, being about 95% of total phenolics; *S. minor* phenolic pattern appeared to be more complex, due to the presence of caffeic acid derivatives, quercetin-3-glucoside, kaempferol-3-glucoside and other quercetin, kaempferol, and luteolin derivatives, as well as many other unidentified compounds. Residues of phenolics resulting from plant extracts in treated sweet cherries after storage were below the analytical limit of detection. The study demonstrated that *S. minor* and *O. crenata* extracts might represent an alternative organic mean for controlling sweet cherry postharvest decay.

1. Introduction

Sweet cherry (*Prunus avium* L.) cv. Ferrovia is greatly appreciated by consumers for its nutritional and organoleptic features. It is principally cultivated in the province of Bari (Apulia region, Southern Italy) which, with about 33,500 tons, represents 33% of the total Italian sweet cherry production (ISMEA, 2012). The preservation of quality during postharvest storage is crucial for its competitiveness, since it allows extension of marketing and maintenance of high selling price. However, factors including water loss, softening, peduncle discolouration and dehydration, and postharvest rots, cause a rapid product decay reducing greatly the postharvest life (Wang et al., 2014).

Monilinia spp. (brown rot), *Botrytis cinerea* Pers.:Fr. (gray mould), and, with a lower incidence, *Rhizopus stolonifer* (Ehrenb.) Vuill. (Rhizopus rot), *Alternaria alternata* (Fr.:Fr.) Keissl. (Alternaria rot), *Penicillium expansum* Link (blue mould), and *Cladosporium* spp. (Cladosporium rot) are the main postharvest sweet cherry fungal pathogens causing significant economic losses (Romanazzi et al., 2008). The control of such pathogens is performed by synthetic chemical fungicides (Förster et al., 2007), nevertheless their postharvest use on sweet cherry is not allowed in European Union. Since the use of fungicides has a significant impact on human health and environmental pollution, and contributes to select resistant strains of pathogens, alternative approaches are needed (Mari et al., 2010; Feliziani et al., 2013, 2015; Talibi et al., 2014; Romanazzi et al., 2016). They include biocontrol agents (Schena et al., 2003; Oro et al., 2014), physical treatments (Nigro et al., 2000; Romanazzi et al., 2008; Xu and Du, 2012; Gatto et al., 2015), inorganic salts (Ippolito et al., 2005), and natural substances (Ippolito and Nigro, 2003; Serrano et al., 2005; Gatto et al., 2011; Romanazzi et al., 2013; Lachhab et al., 2014; Di Venere et al., 2016).

There is an extensive literature on salt effectiveness against fungal pathogens, when tested either alone (Talibi et al., 2011; Youssef et al., 2014) or in combination with physical (Youssef et al., 2012a,b; Fallanaj et al., 2013; Cefola et al., 2014) and biological treatments (Ippolito et al., 2005; Lima et al., 2005). Salts have the advantage of being non-toxic, inexpensive, and usable with a

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minimal risk of injury for fruits. In particular, postharvest treatments with calcium chloride ($CaCl_2$) and sodium bicarbonate ($NaHCO_3$) have been proposed as effective alternative means to control postharvest rots of fruits and vegetables. Bicarbonate salts were tested against postharvest rots on papaya (Bautista-Baños et al., 2013), citrus (Smilanick et al., 2005; Youssef et al., 2012a,b), banana (Bazie et al., 2014), and sweet cherry (Ippolito et al., 2005; Karabulut et al., 2005). Moreover, postharvest calcium application proved to be effective against postharvest rots in citrus (Youssef et al., 2012a) and sweet cherry (Ippolito et al., 2005; Wang et al., 2014).

In the recent years, there was also an increasing interest in the possible use of natural compounds, and in particular of plant extracts, to prevent microbial growth in foodstuffs (Rauha et al., 2000; Gatto et al., 2011; Bazie et al., 2014). The biocide activity of plant extracts can be ascribed to the presence of different phenolic compounds or derivatives (Rauha et al., 2000; Gatto et al., 2011, 2013; Di Venere et al., 2016). Parvu et al. (2015) showed the efficacy of phenolic extracts from *Hedera helix* (ivi) against important phytopathogenic fungi (*Aspergillus niger*, *B. cinerea*, *Fusarium oxysporum*, and others). Pomegranate extracts showed a strong fungicidal activity against *B. cinerea*, *Penicillium digitatum*, and *P. expansu*m (Li Destri Nicosia et al., 2016). Extracts from *Cistus populifolius* and *C. ladanife*r were effective against citrus sour rot caused by *Geotrichum citri-aurantii* (Karim et al., 2015). Extracts from some extremophile plants from Argentine Puna (i.e., *Chuquiraga atacamensis, Parastrephia phyliciformis*, and *P. lepidophylla*) proved to possess strong activity in controlling citrus postharvest pathogens, such as *P. digitatum* and *G. citri-aurantii* (Sayago et al., 2012; Palavecino Ruiz et al., 2016). Gatto et al. (2011) investigated the antifungal activity of extracts from nine wild edible herbaceous species, among which, those of *Orobanche crenata* Forsk. and *Sanguisorba minor* Scop. s.l. proved to be very effective in reducing both *in vitro Monilinia laxa* conidia germination and brown rot on apricot and nectarine. Extract from *O. crenata* and *S. minor* proved to be effective *in vitro* against several other postharvest fungi (Gatto et al., 2013).

O. crenata, belonging to the Orobanchaceae family, is the most important parasite of faba bean (*Vicia faba* L.) in the Mediterranean basin and North and East Africa. It has some interest as edible plant because of the high content in antioxidant phenolics of its tender shoots. *S. minor* belongs to the Rosaceae family and is native of Europe, Middle East, and Northern Africa. It is used as an ingredient in salads and dressings, having a flavour described as "light cucumber". Typically, only the youngest leaves are used, since their degree of bitterness increases with the developmental stage of the plant (Gatto et al., 2013).

The objective of the present study was to find a new strategy for reducing postharvest diseases in sweet cherry, replacing or integrating the use of synthetic fungicides to ensure an acceptable level of disease control, associated with a low environmental impact. For this purpose, the phenolic composition of extracts from *O. crenata* and *S. minor* was characterized and their *in vivo* efficacy in controlling fungal postharvest diseases was evaluated. The combination of extracts with $CaCl_2$ and $NaHCO_3$ against sweet cherry postharvest rots and phenolic residues in treated fruit were also assayed.

2. Materials and methods

2.1. Plant material

Sweet cherry fruit (cv. Ferrovia) were collected in a farm located in Rutigliano (Bari, Italy); plants had been grown according to the best agronomical practices and fruit free of defects were picked from commercial lots.

O. crenata was collected in the field as weeds of cultivated fava bean near Bari (Italy), in springtime. *S. minor* was collected from Murgia hill area (Apulia region, Southern Italy) as well as cultivated in greenhouse starting from seed collected in the same environment. Only the edible part of the plants (i.e., leaves for *S. minor* and stems for *O. crenata*) was selected and used for trials. A representative amount of fresh plant material (at least 2 kg per species) was dried in ventilated oven at 40 °C until constant weight (36–48 h) for dry matter (DM) evaluation; then, it was finely ground in a grinder, and stored vacuum sealed in a cold room until use.

2.2. Chemical reagents

High performance liquid chromatography (HPLC) grade water was obtained by a Milli-Q system (Millipore, Bedford, MA, USA). Sodium bicarbonate, calcium chloride, methanol (HPLC grade), caffeic acid, sodium carbonate, and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Sternheim, Germany). As HPLC standards, verbascoside and isoverbascoside were from Phytolab GmbH & Co.KG (Vestenbergsgreuth, Germany), whereas luteolin-7-glucoside, quercetin-3-glucoside, and kaempferol-3-glucoside were from Extrasynthèse (Genay, France). They had a declared purity >95% (by HPLC assay). All other reagents were of analytical grade.

2.3. Preparation of plant extracts and salt solutions

Plant extracts were prepared as described by Gatto et al. (2011). Briefly, for each species, an amount of dry matter corresponding to 50 g of fresh plant tissue (i.e., 6.25 g for *O. crenata* and 8.50 g for *S. minor*) was extracted twice with refluxing 80% aqueous methanol (1:5, w/v) for 1 h. After extraction, methanolic extracts were filtered through Whatman Grade 1 filter paper and evaporated to dryness under reduced pressure at 35 °C, using a rotary evaporator. The residue was dissolved in 50 mL of K-phosphate buffer, 0.1 M, pH 5.5 to give a solution with conventional 1× concentration, corresponding to 0.125 and 0.170 g of dry matter/mL of buffer for *O. crenata* and *S. minor*, respectively. This solution was centrifuged at 10,000*g*, the supernatant filtered through sterile 0.22 μm pore size membrane filters (Millipore, Bedford, MA, USA), and then stored at −20 °C until use. Moreover, some more concentrated extract solutions were prepared (i.e., up to the saturation limit), by dissolving the same amount of residue in proportionally smaller volumes of buffer. In particular, 2× and 4×, and 2× and 3× extract concentrations for *O. crenata* and *S. minor*, respectively, were prepared. Water solutions of $CaCl_2$ and $NaHCO_3$ (1% w/v) were prepared using commercial salts and HPLC grade deionized water. Plant extract saline solutions were prepared by adding suitable amounts of the two commercial salts (1% w/v) to 4× *O. crenata* and 3× *S. minor* extracts.

2.4. Experimental design

Trials were performed in two consecutive years (2007 and 2008) in a packinghouse (Fra.Va. srl) located in Rutigliano (Bari, Italy). Sweet cherry fruit were processed few hours after harvesting. Fruit were subjected to hydrocooling, selected for uniform size, stage of ripening, and absence of visible defects and injuries, and placed in plastic trays (25 fruit per tray). Then they were sprayed with *O. crenata* extracts (1×, 2×, and 4× concentration) or *S. minor* extracts (1×, 2×, and 3× concentration), just as they are or added with salts (1% w/v). The concentration of extracts and inorganic salts and their combination were chosen on the basis of preliminary tests (data not shown), whereas the maximum extract concentrations used depended on the limit of solubility.