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

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Keywords: *Prunus avium* L., verbascoside, flavonoids, antimicrobial, postharvest rot, cold storage

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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<sub>2</sub>, and sodium bicarbonate, NaHCO<sub>3</sub>), and their combination (i.e., extracts with added CaCl<sub>2</sub> or NaHCO<sub>3</sub>), 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<sub>2</sub> and NaHCO<sub>3</sub>; 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.

**Cover Letter to the Editor**

Dear Prof. Tonutti,

Please find enclosed the revised version of the manuscript entitled: **“Phenolic extracts from wild edible plants to control postharvest diseases of sweet cherry fruit”**, by Maria Antonia GATTO, Lucrezia SERGIO, Antonio IPPOLITO, and Donato DI VENERE.

In a separated file I provide a point-by-point reply to your comments, which have been used to further improve the manuscript. I hope it might be now suitable for publication in Postharvest Biology and Technology.

With my best regards

Donato DI VENERE

Point by point reply to Editor's comments

Dear Editor,  
thank you very much for your suggestions; they have been considered to improve the manuscript, as below reported.

Ms. Ref. No.: POSTEC-D-16-00071R1  
Title: Phenolic extracts from wild edible plants to control postharvest diseases of sweet cherry fruit  
Postharvest Biology and Technology

Dear Dr. Donato Di Venere,

The reviewers have commented on your revised paper, indicating that the manuscript improved. I also went through the manuscript and I ask you to consider the following issues:

Delete (or change) highlight #1 ( not informative of the main results),  
R. The highlight #1 has been deleted

and spell out the complete name of *O.crenata* and *S. minor* in the second and third highlight.

R. The complete plant names have been used

In the 4th highlight change "in phenolic extract treated sweet cherries" with "in sweet cherries treated with phenolic extracts".

R. The sentence has been corrected according to the suggestion

Lines 19-20: "the lowest and the highest" is too generic. Please specify.

R. To specify such issue, a new sentence reporting concentrations used has been introduced before the sentence indicated in the comment, i.e.: "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."

### Highlights

- Verbascoside proved to be the main phenolic compound in *Orobancha crenata* extract.
- *Sanguisorba minor* extract showed a complex HPLC phenolic pattern.
- Postharvest rot incidence was significantly reduced in sweet cherries treated with phenolic extracts.
- Phenolic extracts behaved as  $\text{CaCl}_2$  and  $\text{NaHCO}_3$  in controlling sweet cherry postharvest rot.



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

3

4 **Maria Antonia Gatto<sup>a</sup>, Lucrezia Sergio<sup>a</sup>, Antonio Ippolito<sup>b</sup>, Donato Di Venere<sup>a\*</sup>**

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13

14

15 **Abstract**

16 Postharvest treatments with extracts from two wild edible plants (*Orobancha crenata* and  
17 *Sanguisorba minor*), water solutions of two inorganic salts (calcium chloride, CaCl<sub>2</sub>, and  
18 sodium bicarbonate, NaHCO<sub>3</sub>), and their combination (i.e., extracts with added CaCl<sub>2</sub> or  
19 NaHCO<sub>3</sub>), were assayed to control sweet cherry postharvest diseases. Three extract  
20 concentrations for each plant were assayed, corresponding to 0.170, 0.340, and 0.510 g dry  
21 matter/mL and to 0.125, 0.250, and 0.500 g dry matter/mL for *S. minor* and *O. crenata*,  
22 respectively. At the lowest and the highest concentrations tested, *S. minor* extract was able  
23 to inhibit rot development in stored fruit by 79 and 89%, respectively, with an efficacy  
24 comparable to that of CaCl<sub>2</sub> and NaHCO<sub>3</sub>; for *O. crenata* extract such inhibition ranged  
25 between 64 and 76%, respectively. A dose effect was observed only for *O. crenata*.  
26 Moreover, the level of control was not improved by the combined application of plant  
27 extracts and salts. HPLC analysis of *O. crenata* extract showed verbascoside as the main  
28 phenolic compound, being about 95% of total phenolics; *S. minor* phenolic pattern appeared  
29 to be more complex, due to the presence of caffeic acid derivatives, quercetin-3-glucoside,  
30 kaempferol-3-glucoside and other quercetin, kaempferol, and luteolin derivatives, as well as  
31 many other unidentified compounds. Residues of phenolics resulting from plant extracts in  
32 treated sweet cherries after storage were below the analytical limit of detection. The study  
33 demonstrated that *S. minor* and *O. crenata* extracts might represent an alternative organic  
34 mean for controlling sweet cherry postharvest decay.

35

36 **Keywords:** *Prunus avium* L., verbascoside, flavonoids, antimicrobial, postharvest rot,  
37 cold storage.

38

39 **1. Introduction**

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

48 *Monilinia* spp. (brown rot), *Botrytis cinerea* Pers.:Fr. (gray mould), and, with a lower  
49 incidence, *Rhizopus stolonifer* (Ehrenb.) Vuill. (Rhizopus rot), *Alternaria alternata* (Fr.:Fr.)  
50 Keissl. (*Alternaria* rot), *Penicillium expansum* Link (blue mould), and *Cladosporium* spp.  
51 (*Cladosporium* rot) are the main postharvest sweet cherry fungal pathogens causing  
52 significant economic losses (Romanazzi et al., 2008). The control of such pathogens is  
53 performed by synthetic chemical fungicides (Förster et al., 2007), nevertheless their  
54 postharvest use on sweet cherry is not allowed in European Union. Since the use of  
55 fungicides has a significant impact on human health and environmental pollution, and  
56 contributes to select resistant strains of pathogens, alternative approaches are needed (Mari  
57 et al., 2010; Feliziani et al., 2013, 2015; Talibi et al., 2014; Romanazzi et al., 2016). They  
58 include biocontrol agents (Scheda et al., 2003; Oro et al., 2014), physical treatments (Nigro  
59 et al., 2000; Romanazzi et al., 2008; Xu and Du, 2012; Gatto et al., 2015), inorganic salts  
60 (Ippolito et al., 2005), and natural substances (Ippolito and Nigro, 2003; Serrano et al.,  
61 2005; Gatto et al., 2011; Romanazzi et al., 2013; Lachhab et al., 2014; Di Venere et al.,  
62 2016).

63 There is an extensive literature on salt effectiveness against fungal pathogens, when tested  
64 either alone (Talibi et al., 2011; Youssef et al., 2014) or in combination with physical  
65 (Youssef et al., 2012; Fallanaj et al., 2013; Cefola et al., 2014) and biological treatments

66 (Ippolito et al., 2005; Lima et al., 2005). Salts have the advantage of being non-toxic,  
67 inexpensive, and usable with a minimal risk of injury for fruits. In particular, postharvest  
68 treatments with calcium chloride (CaCl<sub>2</sub>) and sodium bicarbonate (NaHCO<sub>3</sub>) have been  
69 proposed as effective alternative means to control postharvest rots of fruits and vegetables.  
70 Bicarbonate salts were tested against postharvest rots on papaya (Bautista-Baños et al.,  
71 2013), citrus (Smilanick et al., 2005; Youssef et al., 2012; 2012a), banana (Bazie et al.,  
72 2014), and sweet cherry (Ippolito et al., 2005; Karabulut et al., 2005). Moreover,  
73 postharvest calcium application proved to be effective against postharvest rots in citrus  
74 (Youssef et al., 2012a) and sweet cherry (Ippolito et al., 2005; Wang et al., 2014).

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

92 and brown rot on apricot and nectarine. Extract from *O. crenata* and *S. minor* proved to be  
93 effective *in vitro* against several other postharvest fungi (Gatto et al., 2013).

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

101 The objective of the present study was to find a new strategy for reducing postharvest  
102 diseases in sweet cherry, replacing or integrating the use of synthetic fungicides to ensure  
103 an acceptable level of disease control, associated with a low environmental impact. For this  
104 purpose, the phenolic composition of extracts from *O. crenata* and *S. minor* was  
105 characterized and their *in vivo* efficacy in controlling fungal postharvest diseases was  
106 evaluated. The combination of extracts with  $\text{CaCl}_2$  and  $\text{NaHCO}_3$  against sweet cherry  
107 postharvest rots and phenolic residues in treated fruit were also assayed.

108

## 109 **2. Materials and methods**

### 110 *2.1 Plant material*

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

114 *O. crenata* was collected in the field as weeds of cultivated fava bean near Bari (Italy), in  
115 springtime. *S. minor* was collected from Murgia hill area (Apulia region, Southern Italy) as  
116 well as cultivated in greenhouse starting from seed collected in the same environment. Only  
117 the edible part of the plants (i.e., leaves for *S. minor* and stems for *O. crenata*) was selected

118 and used for trials. A representative amount of fresh plant material (at least 2 kg per  
119 species) was dried in ventilated oven at 40 °C until constant weight (36-48 h) for dry matter  
120 evaluation; then, it was finely ground in a grinder, and stored vacuum sealed in a cold room  
121 until use.

122

### 123 *2.2 Chemical reagents*

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

132

### 133 *2.3 Preparation of plant extracts and salt solutions*

134 Plant extracts were prepared as described by Gatto et al. (2011). Briefly, for each species,  
135 an amount of dry matter corresponding to 50 g of fresh plant tissue (i.e., 6.25 g for *O.*  
136 *crenata* and 8.50 g for *S. minor*) was extracted twice with refluxing 80% aqueous methanol  
137 (1:5, w/v) for 1 h. After extraction, methanolic extracts were filtered through Whatman  
138 Grade 1 filter paper and evaporated to dryness under reduced pressure at 35 °C, using a  
139 rotary evaporator. The residue was dissolved in 50 mL of K-phosphate buffer, 0.1 M, pH  
140 5.5 to give a solution with conventional 1x concentration, corresponding to 0.125 and 0.170  
141 g of dry matter/mL of buffer for *O. crenata* and *S. minor*, respectively. This solution was  
142 centrifuged at 10,000 g, the supernatant filtered through sterile 0.22 µm pore size  
143 membrane filters (Millipore, Bedford, MA, USA), and then stored at -20 °C until use.

144 Moreover, some more concentrated extract solutions were prepared (i.e., up to the  
145 saturation limit), by dissolving the same amount of residue in proportionally smaller  
146 volumes of buffer. In particular, 2x and 4x, and 2x and 3x extract concentrations for *O.*  
147 *crenata* and *S. minor*, respectively, were prepared. Water solutions of CaCl<sub>2</sub> and NaHCO<sub>3</sub>  
148 (1% w/v) were prepared using commercial salts and HPLC grade deionized water. Plant  
149 extract saline solutions were prepared by adding suitable amounts of the two commercial  
150 salts (1% w/v) to 4x *O. crenata* and 3x *S. minor* extracts.

151

#### 152 2.4 *Experimental design*

153 Trials were performed in two consecutive years (2007 and 2008) in a packinghouse (Fra.Va.  
154 srl) located in Rutigliano (Bari, Italy). Sweet cherry fruit were processed few hours after  
155 harvesting. Fruit were subjected to hydrocooling, selected for uniform size, stage of  
156 ripening, and absence of visible defects and injuries, and placed in plastic trays (25 fruit per  
157 tray). Then they were sprayed with *O. crenata* extracts (1x, 2x, and 4x concentration) or *S.*  
158 *minor* extracts (1x, 2x, and 3x concentration), just as they are or added with salts (1% w/v).  
159 The concentration of extracts and inorganic salts and their combination were chosen on the  
160 basis of preliminary tests (data not shown), whereas the maximum extract concentrations  
161 used depended on the limit of solubility. Treatments were performed by spraying fruit with  
162 15 mL of extract solution per tray. Treated fruit were allowed to dry at room temperature,  
163 put in plastic bags (RH = 95-98%) and stored at 0±1 °C for 14 days followed by 3 days of  
164 shelf life at 20±1 °C in the first year, whereas at 0±1 °C for 21 days followed by 7 days of  
165 shelf life at 20±1 °C in the second year. Temperature and relative humidity inside the cold  
166 room were monitored and data processed by computerized systems. Fruit treated with the  
167 same buffer solution used to prepare plant extracts (0.1 M K-phosphate, pH 5.5) served as a  
168 control. A completely randomised experimental design with 10 replications of 25 fruit each  
169 was used.

170

## 171 2.5 Assay of rot incidence

172 Sweet cherries used for trials were not artificially inoculated in order to test the activity of  
173 the extracts on latent, quiescent, and incipient infections (natural infections) . Rot incidence  
174 was assessed daily and expressed as the percentage of rotted fruit with respect to the total  
175 number of fruit (25) in each tray (Fig. 2):

$$176 \text{ Rot incidence (\%)} = (n. \text{ rotted fruit}/25) \times 100.$$

177 Furthermore, the efficacy of each treatment was calculated as the percentage of rot  
178 inhibition compared to the control using the mean value of the ten replications:

$$179 \text{ Rot inhibition (\%)} = 100 - [(n. \text{ rotted fruit treated sample}/n. \text{ rotted fruit control}) \times 100].$$

180 In both years, rot inhibition values were assessed when rot incidence in the control was  
181 around 50% (Table 3).

182

## 183 2.6 Evaluation of phenolic residues in fruit and washing water

184 In the second year, the concentration of residual phenolics resulting from *O. crenata* extract  
185 present in treated sweet cherry tissue as well as in washing water, was evaluated on lots of  
186 fruit prepared *ad hoc* for such trials. Fruit were selected as previously described, placed in  
187 plastic trays (10 fruit per tray), and treated as above with *O. crenata* extract at the highest  
188 (4x) concentration. The analysis of phenolic compounds in fruit after treatment before and  
189 after storage, in treated fruit after washing, as well as in washing water, was performed in  
190 triplicate. Thirty grams of sweet cherry fresh tissue, taken from 10 fruit of each replication,  
191 were homogenized and refluxed (twice for 1 h) with methanol. After filtration through  
192 Whatman Grade 1 filter paper, methanolic extracts were concentrated under reduced  
193 pressure at 35 °C and the residue suspended in 50% (v/v) aqueous methanol, filtered  
194 through a 45 µm pore size membrane filter, and used for HPLC analysis. As the washing  
195 water analysis, 30 fruit, three for each tray, were weighed, placed in a flask, covered with



196 about 500 mL of distilled water, and shaken at 60 rpm for 30 min. The washing water was  
197 recovered and evaporated to dryness under reduced pressure at 35 °C, using a rotary  
198 evaporator. The residue was dissolved in 10 mL of 50% (v/v) aqueous methanol, filtered as  
199 reported above, and used for HPLC analysis.

200

### 201 *2.7 Determination of total phenolic content*

202 Total phenolic content (TPC) in *O. crenata* and *S. minor* extracts was determined  
203 spectrophotometrically using the Folin-Ciocalteu method. Briefly, an aliquot of each diluted  
204 extract was mixed with 0.5 mL of Folin-Ciocalteu reagent; after 3 min, 1 mL of sodium  
205 carbonate solution (20% w/v) was added to the reaction mixture. The solution was  
206 incubated at 40 °C for 20 min and cooled under running tap water; the absorbance was then  
207 measured at 750 nm using a Cary 50 UV-vis spectrophotometer (Varian Inc., Palo Alto, CA,  
208 USA). Caffeic acid was used as reference standard to estimate TPC in the sample. TPC was  
209 expressed as mg of caffeic acid equivalent (CAE) per gram of dry matter (DM).

210

### 211 *2.8 HPLC analysis of phenolic compounds*

212 The HPLC analysis of phenolic compounds present in *O. crenata* and *S. minor* extracts, in  
213 both treated and untreated fruit, as well as in washing water, was performed using an  
214 Agilent 1100 Series liquid chromatograph (Agilent Technologies Inc., Santa Clara, CA,  
215 USA) equipped with binary gradient pump (Agilent P/N G1312A) and spectrophotometric  
216 photodiode array detector (DAD) (Agilent P/N G1328A). The Agilent ChemStation (Rev.  
217 A.06.03) software was used for spectra and data processing. An analytical Phenomenex  
218 (Torrance, California, USA) Luna C18 5 µm (250 mm×4.6 mm) column at 35 °C in  
219 thermostatic oven (Agilent P/N G1316A) was used for peak separation. A binary gradient  
220 elution at a flow rate of 1 mL min<sup>-1</sup> with methanol (solvent A) and 5% (v/v) acetic acid in

221 deionized water (solvent B) was used. The elution profile was as already reported (Gatto et  
222 al., 2011).

223

### 224 2.9 Statistical analysis

225 Data were subjected to analysis of variance (ANOVA) using Statistica Software (version  
226 6.0; StatSoft Inc., Tulsa, OK, USA). Duncan's Multiple Range Test (DMRT;  $P \leq 0.05$ ) was  
227 used to compare the mean values. Statistical differences ( $P \leq 0.05$ ) were evaluated by two-  
228 tailed Student's t-test. The error on the percentage of rot incidence inhibition (Table 3) was  
229 calculated using the law of error propagation (Cowan, 1998).

230

## 231 3. Results and discussion

### 232 3.1 Phenolic content and composition of plant extracts

233 Phenolic concentrations of plant extracts used in the trials of the first year are shown in  
234 Table 1. In *O. crenata*, TPC increased almost linearly with the extract concentration,  
235 whereas, in 3x *S. minor* extract, TPC was only slightly above the previous dilution (2x).  
236 Phenolic concentration was not significantly influenced by salts, except for 3x *S. minor*  
237 solution containing  $\text{NaHCO}_3$ , in which a strong reduction (more than 60%) compared to the  
238 corresponding solution without  $\text{NaHCO}_3$  was found (Table 1). Such result might be  
239 explained by the possible presence in this solution of components particularly sensitive to  
240 alkaline pH induced by  $\text{NaHCO}_3$  (pH= 9.5). Phenolic concentrations of plant extracts used  
241 in the trials of the second year were not significantly different from those of the first year  
242 (data not shown).

243 Figure 1 shows HPLC phenolic patterns of the two plant extracts; caffeic acid derivatives  
244 and flavonoids were identified by a combined analysis of retention times and UV spectra of  
245 the different peaks. According to Gatto et al. (2011), caffeic acid derivatives represented the  
246 main class of phenols in *O. crenata*, whereas flavonoids were the most abundant in *S.*

247 *minor*. In *O. crenata*, verbascoside was about 95% of TPC; moreover, isoverbascoside and  
248 another unidentified caffeic acid derivative were also detected (Fig. 1a). The HPLC analysis  
249 of *S. minor* extract showed a rich phenolic pattern. In particular, caffeic acid derivatives,  
250 quercetin-3-glucoside, kaempferol-3-glucoside, and other quercetin, kaempferol, and  
251 luteolin derivatives, besides many other unidentified compounds, were found (Fig. 1b).

252

### 253 3.2 Residues of phenolics from extracts in sweet cherry tissue

254 In order to establish the potential impact of treatments on product safety, the presence of  
255 phenolic compounds resulting from extracts in the sweet cherry tissue was assessed. For  
256 this purpose, HPLC analyses were carried out on fruit before and after treatments, and on  
257 treated fruit after washing at the beginning and at the end of storage, as well as on washing  
258 water.

259 The HPLC analysis of sweet cherry tissues showed the presence of some constitutive  
260 cinnamic acid derivatives, flavonoids, and anthocyanins; none of the main phenolic  
261 compounds present in the extracts was found in the sweet cherry HPLC phenolic pattern  
262 (data not shown). HPLC analyses of fruit, just after spraying with extracts at the major  
263 concentrations and left to dry for about 30 min, showed the presence of very low amounts  
264 of residual compounds ascribable to extracts. Whereas, the analyses performed after storage  
265 on fruit treated with extracts at the major concentrations and then washed, did not allow to  
266 detect any of the main phenolic compounds resulting from extracts. Actually, taking *O.*  
267 *crenata* extract as reference, 100 g of sweet cherry fresh tissue (corresponding to about 8-10  
268 berries) retained about 2 mg of verbascoside (i.e., the main phenolic compound detected in  
269 this extract) (Table 2), being such amount less than 0.5% of the average content of 100 g of  
270 fresh edible tissue of broomrape, corresponding to about 10 shoots (i.e., an average eaten  
271 portion) (Gatto et al., 2011). Moreover, HPLC analyses performed on washed fruit allowed  
272 to detect verbascoside below the instrumental limit of quantification (LOQ) before storage,

273 instead it resulted absent or below the limit of detection (LOD) after storage (Table 2). On  
274 the other hand, the analysis of the washing water showed that about 90% of verbascoside  
275 retained by fruit had been removed by washing and was found in the water (Table 2), thus  
276 demonstrating the low absorption of this compound into the fruit tissue.

277 All experimental evidences suggest that residues of phenolics resulting from extracts found  
278 in treated sweet cherries after storage represent a very negligible amount (below LOQ or  
279 LOD), especially if compared to the amount that can be ingested by consuming a normal  
280 portion of the considered edible species. For these reasons, residues present in one portion  
281 of treated cherries are not of any concern from a toxicological point of view. On the  
282 contrary, the possible presence of such compounds on treated fruit might rather provide a  
283 beneficial effect by improving the product healthy properties, due to the high antioxidant  
284 capacity of phenolic compounds present in the edible herbs (Di Venere et al., 2009).  
285 Organoleptic tests performed to assess changes in the fruit taste caused by treatments with  
286 extracts did not reveal significant differences between treated and control fruit (data not  
287 shown).

288

### 289 *3.3 Inhibitory effect of plant extracts and salts on postharvest development of fungal* 290 *diseases*

291 Naturally infected sweet cherries utilized in the trials were mainly attacked by *Monilinia*  
292 spp., *B. cinerea*, and *R. stolonifer*, and in a minor extent by *A. alternata*, *P. expansum*, and  
293 *Cladosporium* spp. Due to the different climatic conditions occurred in the two years,  
294 different protocols of storage and shelf life duration were applied. Actually, during the fruit  
295 ripening season (from April to June), a greater amount of rain (about double) was recorded  
296 in the production area in the first year rather than in the second. The higher disease pressure  
297 caused by the abundant rainfall in the first year can explain the shorter storage and shelf life  
298 time withstood by fruit in trials of this year.

299 The efficacy of plant extracts, salt solutions, and their combination in reducing the  
300 incidence of rotted fruit is shown in Fig. 2. In spite of the different environmental  
301 conditions, a good and comparable efficacy of all treatments was observed in both years. In  
302 particular, the two salts showed similar efficiency, with an average percentage of inhibition  
303 of about 80 and 87% for  $\text{CaCl}_2$  and  $\text{NaHCO}_3$ , respectively, as compared to the control  
304 (Table 3). These results confirmed the effectiveness of both salts in counteracting the  
305 development of postharvest fungal diseases on sweet cherry (Karabulut et al., 2005; Ippolito  
306 et al., 2005). Regarding the extracts, *S. minor* extract reached an inhibitory capacity  
307 comparable to that of salts already at the lowest concentration (1x), while for *O. crenata*  
308 such result was obtained at higher concentrations (4x in the first year, 2x and 4x in the  
309 second year). An increase in extract concentration produced an increase in the percentage of  
310 inhibition, from 64 to 76% and from 79 to 89% for *O. crenata* and *S. minor*, respectively  
311 (Table 3). The efficacy of extracts in inhibiting fruit rotting is shown in Fig. 3.  
312 These results confirmed the strong efficacy of *S. minor* extract already reported by Gatto et  
313 al. (2011). Moreover, it should be considered that these results refer to crude extracts,  
314 which could contain substances (e.g., sugars) able to counteract the inhibitory effect of  
315 active compounds present in the extract. It is possible that purified extracts and/or single  
316 active principles might provide better results. The combined use of extracts and salts did  
317 not show any additive/synergistic effect. Indeed, the observed inhibitory effect was not  
318 significantly different from the one obtained with extracts or salts applied alone (Fig. 2).  
319 The presence of salts in plant extracts could modify the phenolic compounds activity; for  
320 example, the alkaline pH of  $\text{NaHCO}_3$  solution (pH=9.5) might alter compound stability,  
321 while  $\text{Ca}^{2+}$  ions present in  $\text{CaCl}_2$  solution might interfere with the complex inhibition  
322 mechanism either reducing the effectiveness of phenols or stabilizing the fungal  
323 cytoplasmic membrane (Pitt and Ugande, 1984).

324 In *O. crenata* extract, the inhibition efficacy was probably due to the presence of  
325 verbascoside (acteoside), the main phenolic compound of the extract (Fig. 1a), which  
326 showed specific activity against several postharvest fungi (Gatto et al., 2013). Actually, in  
327 addition to the well known antioxidant, anti-inflammatory, and antiviral properties,  
328 verbascoside has also antimicrobial activity, until now little documented (Avila et al., 1999;  
329 Gatto et al., 2011; 2013). On the other hand, *S. minor* extract was found to contain caffeic  
330 acid derivatives and flavonoids derived from quercetin, kaempferol, and luteolin. In  
331 particular, according to Gatto et al. (2011), quercetin-3-glucoside and kaempferol-3-  
332 glucoside were found the most abundant flavonoids (Fig. 1b). In *S. minor* fresh tissues, the  
333 presence of gallic acid, ellagic acid, quercetin, kaempferol derivatives, and coumarins has  
334 been reported (Ayoub, 2003). Conceivably, the strong activity of *S. minor* extract against  
335 diseases could be explained by a possible synergistic effect of the complex phenolic pattern.  
336 In this regard, the cytotoxic and antimicrobial activities of quercetin-3-glucoside were  
337 reported (Razavi et al., 2009), whereas other authors showed the antibacterial activity of  
338 quercetin-3-glucoside and kaempferol-3-glucoside, demonstrating their synergistic efficacy  
339 when present together in the same extract (Akroum et al., 2009).

340 The mechanisms of action of such phenolic compounds in inhibiting pathogen/disease  
341 development is not fully understood. Cushnie and Lamb (2005) reported that different  
342 flavonoids inhibit some important cellular functions (i.e. nucleic acid synthesis, cytoplasmic  
343 membrane functionality, energy metabolism), being the most common cause of their  
344 antimicrobial activity. Palavecino Ruiz et al. (2016) demonstrated that the main inhibitory  
345 effect of ethanolic extracts of *Parastrephia lepidophylla*, an Argentine spread plant species,  
346 against *P. digitatum* would be to alter mechanisms leading to the swelling of conidia and to  
347 the subsequent germ tube elongation. Microscopic observations of important postharvest  
348 fungal pathogens incubated with *S. minor* and *O. crenata* extracts showed malformations of  
349 the germ tube, disorganization of the cell wall, and leakage of cytoplasmic material from

350 the hyphae (Gatto et al., 2011). Furthermore, the antifungal activity of verbascoside and its  
351 isomer might be due to their molecular structure, since the presence of two hydroxyl groups  
352 on aromatic rings might be responsible for their toxicity to microorganisms (Di Venere et  
353 al., 2016). Besides the direct action on pathogen, another mechanism of antimicrobial  
354 activity potentially exerted on fruit by phenolic exogenous treatments might be the  
355 induction of resistance to pathogen growth or the change of pathogen secondary metabolism  
356 (Sanzani et al., 2009; 2010). Such hypothesis, already proved as effective for NaHCO<sub>3</sub> and  
357 other salts against green mould on citrus fruit (Youssef et al., 2014), might be an exciting  
358 topic for further investigation on the mechanism of action of phenolic extracts against sweet  
359 cherry postharvest rotting.

360

#### 361 **4. Conclusions**

362 To our knowledge, this is the first time that extracts from *O. crenata* and *S. minor* were  
363 tested to prevent postharvest rotting of sweet cherry fruit, proving to have a high antifungal  
364 efficacy. Since such extracts were obtained from edible plants and used at concentrations  
365 comparable to those present in food preparations, it could be reasonable to believe in their  
366 safety for humans and environment. Nevertheless, in order to be proposed as organic  
367 alternative to synthetic chemical fungicides, deeper investigations about the absence of  
368 whatever form of toxicity are hoped. If this is the case, besides being added to water in  
369 hydrocooling (Karabulut et al., 2005), extracts might be applied as edible coatings (Karaca  
370 et al., 2014), thus improving sweet cherry fruit in nutraceutical compounds, due to the  
371 antioxidant properties of phenolics present in *O. crenata* and *S. minor*.

372 In the future, the postharvest protective efficacy of the tested plant extracts on other  
373 produce will be tested to extend the knowledge about their ability to preserve postharvest  
374 quality and safety. It might be useful to isolate, purify, and identify the most active  
375 antifungal compounds to reveal the effective component/s of extracts.

376

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382

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Table 1 Phenolic concentration of extracts from *Orobancha crenata* and *Sanguisorba minor* at different concentrations (1x, 2x, etc.), alone or in combination with CaCl<sub>2</sub> or NaHCO<sub>3</sub>, evaluated by Folin-Ciocalteu method. Values are the mean  $\pm$  SD of three replications. For each plant species, different letters indicate significantly different values among the different extracts according to DMRT ( $P \leq 0.05$ ).

Treatment	Phenolic concentration (mg L <sup>-1</sup> CAE <sup>†</sup> )
<i>O. crenata</i> 1x	2915 $\pm$ 145 c
<i>O. crenata</i> 2x	5780 $\pm$ 272 b
<i>O. crenata</i> 4x	11859 $\pm$ 425 a
<i>O. crenata</i> 4x + CaCl <sub>2</sub>	12340 $\pm$ 510 a
<i>O. crenata</i> 4x + NaHCO <sub>3</sub>	11460 $\pm$ 486 a
<i>S. minor</i> 1x	3655 $\pm$ 251 c
<i>S. minor</i> 2x	7220 $\pm$ 397 b
<i>S. minor</i> 3x	8140 $\pm$ 426 a
<i>S. minor</i> 3x + CaCl <sub>2</sub>	8260 $\pm$ 517 a
<i>S. minor</i> 3x + NaHCO <sub>3</sub>	3980 $\pm$ 291 c

<sup>†</sup> CAE = caffeic acid equivalent

Table 2 Residual verbascoside content in sweet cherries treated with *Orobancha crenata* extract at 4x concentration. Values are the mean  $\pm$  SD of three replications.

Treatment	Verbascoside (mg 100 g <sup>-1</sup> f.w. <sup>(a)</sup> )
<i>O. crenata</i> 4x <sup>(1)</sup>	2.1 $\pm$ 0.2
<i>O. crenata</i> 4x + washing before storage <sup>(2)</sup>	n.q. (< LOQ) <sup>(b)</sup>
<i>O. crenata</i> 4x + washing + storage <sup>(3)</sup>	n.d. (< LOD) <sup>(c)</sup>
washing water	1.8 $\pm$ 0.2

<sup>(1)</sup> fruit just after treatment; <sup>(2)</sup> washed fruit before storage; <sup>(3)</sup> washed and stored fruit

<sup>(a)</sup> stoned sweet cherry fresh weight

<sup>(b)</sup> n.q. = not quantified; LOQ = limit of quantification

<sup>(c)</sup> n.d. = not detectable; LOD = limit of detection



Table 3 Inhibition of postharvest rot incidence on sweet cherries (percentage compared to the control) produced by treatments with *Orobancha crenata* or *Sanguisorba minor* extracts at different concentrations (1x, 2x, etc.), 1% (w/v) water solutions of CaCl<sub>2</sub> or NaHCO<sub>3</sub>, and plant extracts in combination with salts. Buffer treated fruit were used as a control. Inhibition values of different treatments were evaluated when rot incidence in the control fruit was around 50%. Values were calculated using the mean of ten replications. Within each column, different letters indicate significantly different values among treatments, according to DMRT ( $P \leq 0.05$ ).

Treatment	Inhibition (%)	
	First year	Second year
Control	0 a	0 a
1% CaCl <sub>2</sub>	77 ± 18 bcd	83 ± 9 bc
1% NaHCO <sub>3</sub>	86 ± 15 d	87 ± 7 c
<i>O. crenata</i> 1x	64 ± 20 b	68 ± 39 b
<i>O. crenata</i> 2x	66 ± 21 b	70 ± 14 bc
<i>O. crenata</i> 4x	75 ± 12 bcd	76 ± 9 bc
<i>O. crenata</i> 4x + CaCl <sub>2</sub>	84 ± 7 d	80 ± 14 bc
<i>O. crenata</i> 4x + NaHCO <sub>3</sub>	82 ± 14 cd	83 ± 28 bc
<i>S. minor</i> 1x	79 ± 17 cd	80 ± 18 bc
<i>S. minor</i> 2x	82 ± 14 cd	83 ± 9 bc
<i>S. minor</i> 3x	89 ± 7 d	88 ± 11 c
<i>S. minor</i> 3x + CaCl <sub>2</sub>	67 ± 20 bcd	69 ± 13 bc
<i>S. minor</i> 3x + NaHCO <sub>3</sub>	73 ± 11 bcd	74 ± 13 bc

Fig. 1

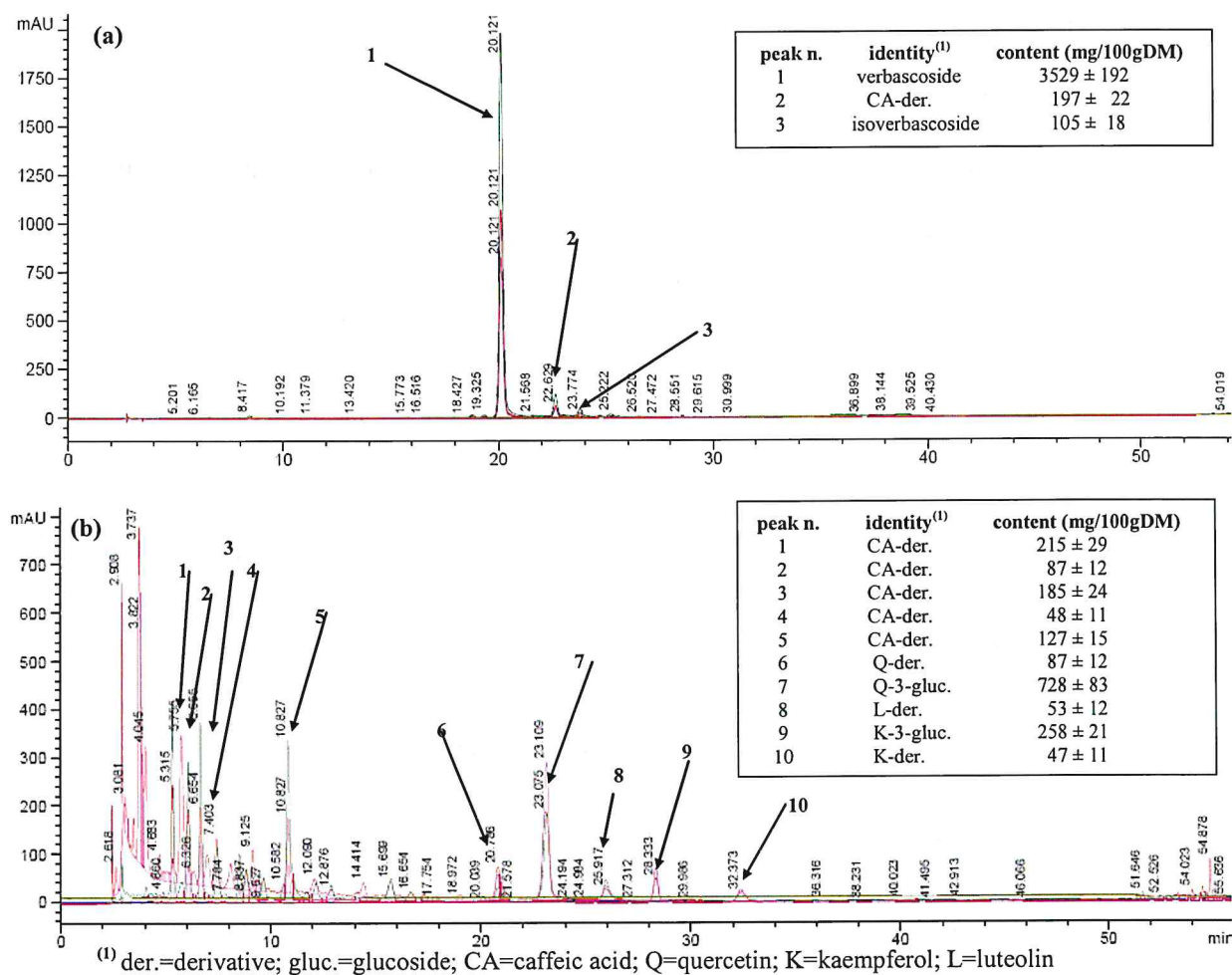


Fig. 1 HPLC chromatograms of *Orobanchaceae* (a) and *Sanguisorba minor* (b) phenolic extracts. The content of the main identified phenolic compounds was reported.

Fig. 2

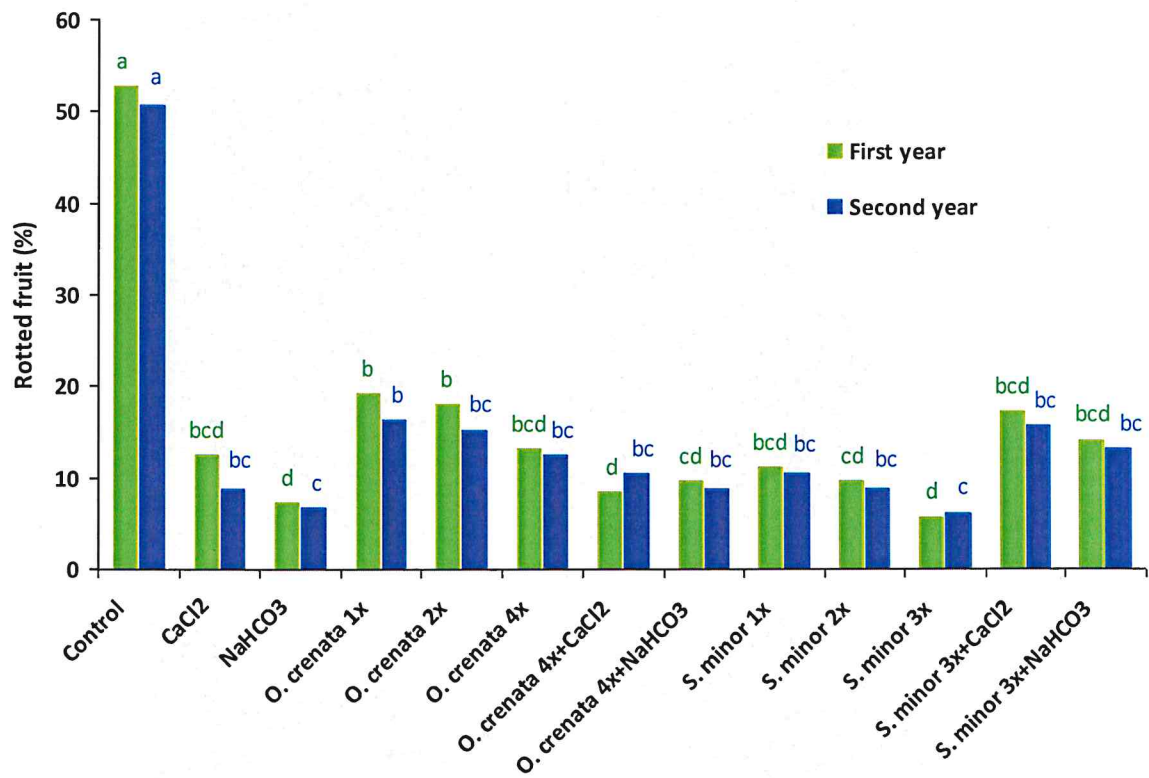


Fig. 2 - Rot incidence in sweet cherry fruit treated with extract of *Orobancha crenata* or *Sanguisorba minor* at different concentrations (1x, 2x, etc.), 1% (w/v) water solutions of CaCl<sub>2</sub> or NaHCO<sub>3</sub>, and their combinations. Each bar represents the mean of ten replications. Within each year, values with the same letters are not significantly different according to DMRT ( $P \leq 0.05$ ).

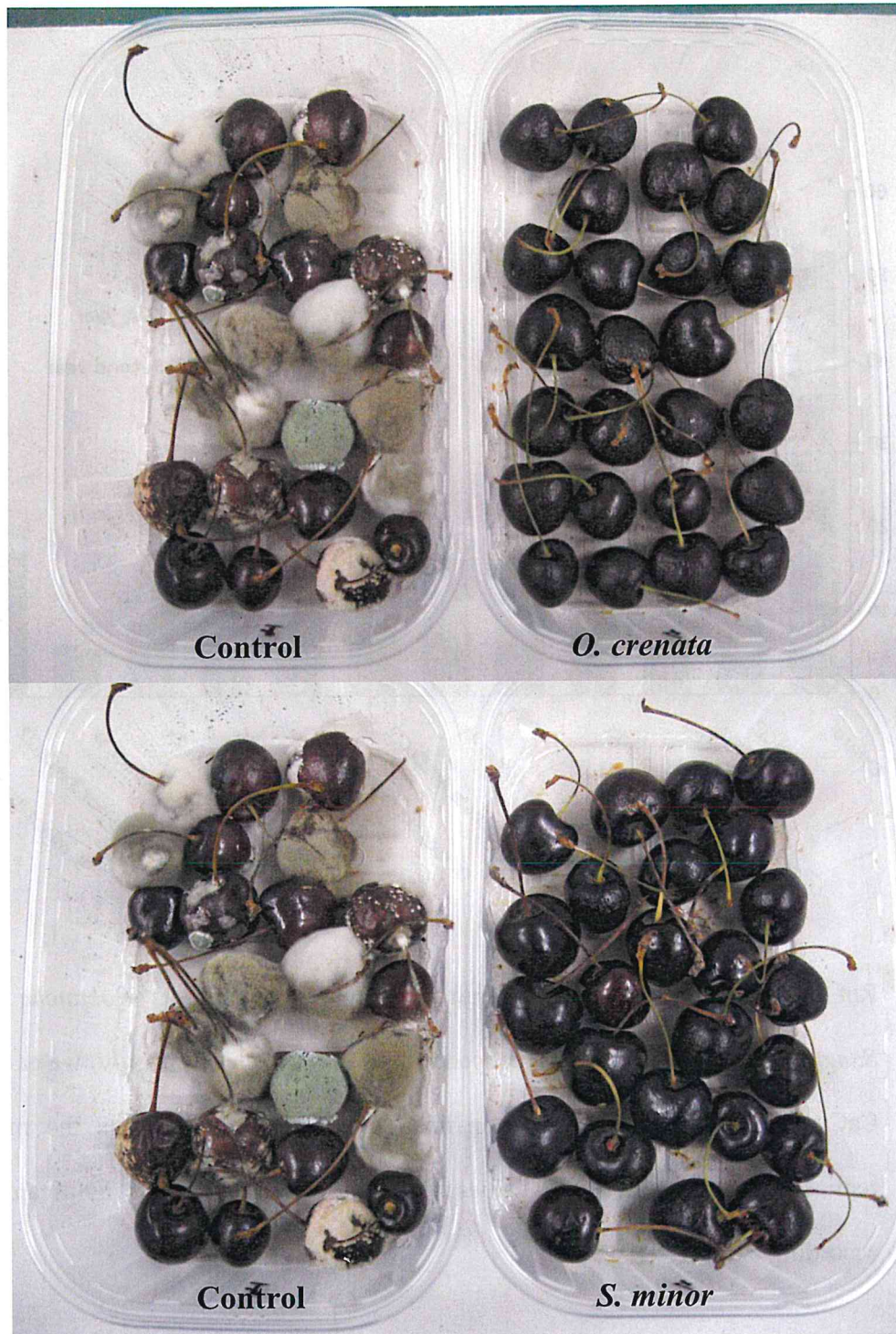


Fig. 3 Sweet cherries treated with *Orobancha crenata* or *Sanguisorba minor* extracts at 4x and 3x concentration, respectively, after 21 days of storage at  $0\pm 1$  °C and 7 days of shelf life at  $20\pm 1$  °C.