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Kaolin and salicylic acid foliar application modulate yield, quality and phytochemical composition of olive pulp and oil from rainfed trees

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ABSTRACT

Olive orchards, rainfed managed, are threatened by the current and predicted adverse environmental conditions, which change the yield and quality of olive products, largely known for its benefits to human health. To mitigate these problems, it is highly recommended to perform some adjustments in agronomic practices, such as the use of foliar sprays that cloud help the trees to cope with climate change. During two consecutive years, olive trees were pre-harvest sprayed with kaolin (KL) and salicylic acid (SA) to attenuate the adverse effects of summer stress. Olive yield was increased by 97% and 72% with KL and SA, respectively. Phenolics and antioxidant capacity of both olives and olive oil increased and decreased in the first and second year, respectively, in a closely association with the prevailing climatic conditions. The foliar sprays did not significantly affect the oil quality indices, free acidity, peroxide value and K_{232} coefficient and decreased the K_{270} coefficient. This study strongly suggests that the applied products might be effective in mitigating the adverse environmental conditions, without substantial changes in fruit and olive oil quality.

1. Introduction

Olive tree (*Olea europaea* L.) is one of the oldest and emblematic cash crops of the Mediterranean Basin, being cultivated mostly under rainfed production systems. Although the species can tolerate harsh conditions, the sector is under threat by the current adverse environmental circumstances and even more by the future scenarios of climate change (IPCC, 2013). Agricultural yield losses due to environmental stresses are well documented and many studies have shown that is crucial to increase the efforts in adapting measures to help the plants to cope with such adverse conditions (Wang and Frei, 2011). The limited water resources associated with the rugged topography of traditional olive growing areas hinders the implementation of irrigation systems, and/or make it economically unsustainable. Alternatively, the exogenous application of kaolin (KL) and salicylic acid (SA) can be adequate short-term solutions to attenuate the adverse effects of summer associated stresses. KL is a white mineral clay that avoid the accumulation of heat load through the reflection of sunlight, reducing the risk of leaf and fruit damage from high temperatures and solar injury (Glenn and Puterka, 2004) and SA is a signaling phytohormone with diverse regulatory roles in plant metabolism and abiotic stress tolerance (Khan et al., 2015). However, the influence of these substances on crop quality have received less attention than the influence on yield, possibly because they are more difficult to detect and sometimes are not consensual. The application of SA increased the yield of olive (Abd El-Razek et al., 2013; Khalil et al., 2012), peach (El-Shazly et al., 2013) and strawberry (Jamali et al., 2011; Kazemi, 2013) crops. In peach trees, the fruit quality was negatively affected, with lower soluble solids and anthocyanins accumulation and higher acidity (El-Shazly et al., 2013), whereas the quality of strawberry fruits was improved, with higher accumulation of total phenols, both flavonoids and non-flavonoids, soluble solids and vitamin C (Kazemi, 2013). With KL application, an increase in yield of olive (Saour and Makee, 2003), grapevine (Correia et al., 2014), mango (Chamchaiyaporn et al., 2013) and apple (Glenn et al., 2003) crops was reported. In olive trees, KL affected positively the olive oil quality and composition, in terms of total phenols,

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Abbreviations: SA, Salicylic Acid; KL, Kaolin; C, Control; Csb, warm and temperate climate with dry and warm summer; MI, Maturation Index; TP, Total phenols; o-DP, *ortho*-diphenols; Fl, Flavonoids; FA, Free Acidity; PV, Peroxide Value; K_{232}, specific extinction at wavelength 232 nm; K_{270}, specific extinction at wavelength 270 nm; ΔK, Variation of the specific extinction

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pigments, oil content, fatty acids, free acidity, peroxide value and ultraviolet (UV) absorption coefficients (Khaleghi et al., 2015; Saour and Makee, 2003). In grapevines, KL contributed to the increase of secondary metabolites in fruits, as total phenols, flavonoids, anthocyanins and vitamin C, as well as antioxidant capacity (Dinis et al., 2016).

The beneficial effects of olives and olive oil in health can be attributed to the antioxidant properties associated to the phenolic composition (Ghanbari et al., 2012; Silva et al., 2006; Sousa et al., 2014a). Phenolic compounds in olives comprise 1–3% of the fresh pulp weight, standing out the phenolic acids, phenolic alcohols, flavonoids, and the secoiridoids, which include the predominant phenolic compound found in fresh olive, oleuropein (Charoenprasert and Mitchell, 2012; Silva et al., 2006). In extra virgin olive oil, the concentration of phenols varies from 50 to 800 mg kg^{-1} oil (Charoenprasert and Mitchell, 2012). Reactive oxygen species (ROS), formed as a result of oxidative stress, are known to be responsible for the development of some diseases, targeting lipids, proteins and deoxyribonucleic acid (DNA) in living organisms (Ghanbari et al., 2012). Phenolic compounds in olives can restrict the deleterious effects of ROS, either by their free radical scavenging ability (by donating a hydrogen atom to the ROS, reducing and stabilizing it), or by chelating transition metals, suppressing the oxidative reaction in which them are involved (Charoenprasert and Mitchell, 2012). These phytochemicals also affect the sensorial and aromatic characteristics of both olive fruits and oil and the chemical stability of olive oil (Ghanbari et al., 2012; Servili et al., 2009). However, the composition and concentration of phenolic compounds is the result of a complex interaction of various pre-harvest factors, such as cultivars, environmental conditions, ripening stage and agronomic practices (Barros et al., 2013; Brahmi et al., 2013; Damak et al., 2008; Dinis et al., 2016; Ghanbari et al., 2012; Gómez-Rico et al., 2006; Jemai et al., 2009; Kazemi, 2013; Machado et al., 2013; Silva et al., 2006; Soufi et al., 2014; Sousa et al., 2014a, 2015; Sousa et al., 2014b; Talhaoui et al., 2015, 2016; Vinha et al., 2005).

We hypothesized that foliar sprays KL and SA might improve the olive trees yield without substantial changes in olives and olive oil quality. Hence, the aim of this work was to assess the influence of KL and SA in yield and in olive fruits and oil phenolics concentration and antioxidant capacity, as well in oil quality indices. The effects of harvest date and differences among years on those variables were also considered.

2. Material and methods

2.1. Field trial and sampling

The experiment took place in Bragança, Portugal, at Pinheiro Manso farm (41° 48´ N, 6° 44′ W), during two consecutive growing seasons (2015 and 2016), on a 5-years-old rainfed olive orchard (cv. "Cobrançosa") planted at 7 × 6 m. The climate is of Mediterranean type with some Atlantic influence. Under the Koppen-Geiger climate classification, Bragança had a warm and temperate climate with dry and warm summer (Csb) and rainy winters (IPMA, 2017). The average air temperature and monthly precipitation recorded during the experimental period are shown in Fig. 1. Selected physico-chemical characteristics of the olive grove soil (0–20 cm depth) at the beginning of the experiment are presented in Table 1. After drying (40 °C) and sieving (2 mm mesh), soil samples were subjected to several analytical determinations: 1) clay, silt and sand, by the Robinson pipette method; 2) pH (H_2O, KCl); 3) organic carbon (C), determined by the Walkley-Black method (easily oxidizable C), and by incineration (total organic C); 4) extractable B by the hot water extraction method and determined by azometine-H colorimetric procedure; 5) extractable P and K, by using ammonium lactate solution at pH 3.7; 6) exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}), determined by ammonium acetate, pH 7.0; and 7) exchangeable acidity extracted by 1 M KCl. Methods 1–3 and 6-7 are fully described in Van Reeuwijk (2002), 4 in Keren, 1996 and 5 in Houba et al. (1997).

The experiment comprises three treatments: control (C) trees, sprayed with distilled water; kaolin (KL) trees, sprayed with an aqueous solution of kaolin (Surround® WP, Engelhard Corporation, Iselin, NJ), at the manufacturer recommended dosage 5% (w/v); and salicylic acid (SA) trees, sprayed with an aqueous solution of 100 μM SA, selected based on results of preliminary research. The treatments were made in the absence of wind in the morning of 30th June 2015 and 23th June 2016. A second application in the same days was done for KL trees, in order to ensure the adhesion uniformity of kaolin clay particles. The kaolin treatment was repeated in 27th August 2016 after a heavy rain event. All spray applications were supplemented with 0.1% (v/v) Tween 20 and conducted according to good efficacy practice standard operating procedures adjusted for agricultural experiments. Each treatment included three replicates, completely randomized, with three trees of similar canopy size per plot. Each treatment was separated by a buffer line of trees and all trees were managed without irrigation and cared with the same fertilization, pruning, weed control and pest management practices, as applied by local commercial farmers.

Olive fruits were handpicked from the selected olive trees. In 2015 two harvests were performed in order to evaluate the maturation index and the evolution of the chemical composition of fruits, from an earlier harvest (9th November) (H1 2015) to the traditional harvest for olive oil extraction at the study site (30th November) (H2 2015). In 2016 only the last sampling (9th December) (H1 2016) was performed. Additionally, in both years, the overall production per tree was evaluated. In the H2 2015 and H1 2016 were also collected fruits for olive oil extraction.

2.2. Maturation index

The maturation index (MI) was determined according to the method proposed by Hermoso et al. (1991) and varied between 0 and 7. Olive fruits were classified into the following categories: 0 – olives with intense green epidermis; 1 – olives with yellowish green epidermis; 2 – olives with red spots or areas in less than half of the fruit; 3 – olives with red or light violet epidermis over more than half of the fruit; 4 – olives with black epidermis and totally white pulp; 5 – olives with black epidermis and less than half purple pulp; 6 – olives with black epidermis and more than half purple pulp; 7 – olives with black epidermis and totally purple pulp. With a to h being the number of fruits in each category, the MI was calculated as follows:

$$MI = (a \times 0 + b \times 1 + c \times 2 + d \times 3 + e \times 4 + f \times 5 + g \times 6 + \mathrm{h} \times 7)/100 \tag{1}$$

2.3. Olive oil extraction

Olive oil extraction was extracted within 24 h of the olive harvest. A bench hammer mill that reproduces industrial oil extraction was used for the extraction of olive oil from two kg of healthy fruits, without any kind of infection or physical damage. Then, the paste was slowly malaxed at about 25 °C for 40 min, centrifuged in a two-phase decanter at 3500 × for 10 min, and the oil collected was placed in dark glass bottles and kept at 4 °C for latter analysis.

2.4. Extraction of polyphenolic compounds from olive fruits and olive oil

The fruits and olive oil extraction was adapted from a procedure described by Sousa et al. (2014b). Freeze-dried olive pulp (300 mg) was grind and homogenized with 6 ml of MeOH/H_2O (70:30, v/v) for 30 min at room temperature. Then, the samples were centrifuged at 2800g for 10 min and the supernatant was removed and reserved in a flask after filtration. This procedure was repeated three times. To