Study on Some Chemical Composition of Five Olive Pollens

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Summary

The olive tree (*Olea europeae* L.) is grown in many parts of the world. Variations in the ability of pollen to germinate have been showed conspicuous difference among cultivars. However, olive pollen is the material that is commonly used for clinical and biological studies. The aim of this study was to evaluate the some chemical composition of pollen obtained from five olive cultivars. The content of moisture, ash, carbohydrate, protein, peroxidase, superoxide dismutase, catalase, ascorbate peroxidase and boron concentration were determined. The obtained results showed variability between cultivars, moisture from 5.92 to 6.30%, ash from 2.58 to 2.76%, protein from 2.34 to 3.66 $mg \cdot g^{-1}$ fresh weight (FW), ascorbate peroxidase from 62.95 to 182.69 $U \cdot min^{-1} \cdot g$ FW, peroxidase from 58.39 to 95.37 $U \cdot min^{-1} \cdot g$ FW, catalase from 12.48 to 25.57 U $U \cdot min^{-1} \cdot g$ FW, superoxide dismutase from 15.81 to 17.54 $U \cdot min^{-1} \cdot g$ FW, carbohydrate from 2 to 2.07 $mg \cdot g^{-1}$ dry weight (DW) and boron concentration from 1321 to 1453 $U \cdot min^{-1} \cdot g$ FW. Data obtained by studying chemical composition can play an important role in the quality of pollen grains.

Key words

chemical composition, enzyme activity, olive, pollen, protein

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Received: March 18, 2015 | Accepted: April 25, 2015

ACKNOWLEDGEMENTS
The authors would like to thank the Shiraz University, Shiraz, Iran.

Introduction

The olive tree was one of the earliest fruit crops to be domesticated, and has had major economic, social and culture importance in the Mediterranean Basin (Alché et al., 2007). Olive trees have abundant small, fragrant, cream coloured flowers. There are two kinds of olive flowers: the perfect flower, which contains both male and female reproductive organs, and the staminate flower, which contains only stamens. In olive trees and other hermaphrodite plants the presence of staminate or imperfect flowers is frequently observed (Reale et al., 2006). According to Cuevas et al. (2009) the most obvious advantage provided by staminate flower production is the increase in the number of pollen grains available to achieve fertilization. Chemical composition of pollen considerably varies between plant species. One of the first biochemical approaches applied for cultivar discrimination was the use of isoenzymes. For this purpose, pollen rather than leaves was the material of choice, particularly because of its higher degree of enzyme polymorphism (Pontikis et al., 1980). Pollen grains are well known to release proteins including enzymes on moistening, and it now seems likely that the bulk of the rapidly emitted material is derived from the wall sites, not from the vegetative cell itself. The hydrolytic enzymes are probably involved in germination, early pollen tube nutrition and the penetration of the stigma (Knox and Heslop-Harrison, 1971). Proteomics studies from Arabidopsis (Noir et al., 2005) and rice (Dai et al., 2006) have revealed that mature pollen pre synthesizes a complement of proteins required for pollen function, including those implicated in protein synthesis and carbohydrate/energy metabolism. Knox and Heslop-Harrison (1971) suggested that much of the rapidly released intine of the pollen grains is related to compatibility reactions. The early Egyptians and ancient Chinese used pollen as a rejuvenating medicinal agent. Pollen preparations are distributed worldwide for dietary purposes as diet supplement (Sani et al., 2013). In general, bee- collected pollens contain nutritionally essential substances like carbohydrates, proteins, amino acids, lipids, vitamins, mineral substances and trace elements but also significant amounts of polyphenol substances mainly flavonoids (Villanueva et al., 2002). The objective of this study was to investigate some chemical composition and nutritional value of pollen from five olive cultivars.

Materials and methods

Olive pollen grains were collected at the end of March from Olive Research Station, Iran from five olive cultivars: 'Amygdalolia', 'Konservolia', 'Dakal', 'Koroneiki', and 'Manzanillo'). The pollen was separated from flowers and stored in freezer until analysis. Four replications of each sample were made for each analysis. Moisture determination was made through gravimetric until constant weight, using vacuum oven at 70°C. Ash content was determined by gravimetric method after burning in oven at 550°C until constant weight. For determination of carbohydrate concentration samples were oven dried at 60°C for 48 h, and then ground into fine powder. Dry tissue samples were used for the soluble sugars assay. Phenol–sulfuric acid method was used to determine soluble sugars (Dubois et al., 1956).

The frozen samples were blended for 1 min in homogenizer (IKA A11B) containing liquid nitrogen, then crushed using liquid nitrogen in a mortar and homogenized in 2 ml of extraction buffer (0.607g TRIS, 0.05g polyvinylpyrolidine (PVP), the final volume was made up to 50 ml using distilled water, pH 8). The homogenate was centrifuged (13,000 g for 15 min at 4°C). The supernatant was collected, and subsequently used for assaying the protein content and the enzyme activity (Qaddoury et al., 2003).

Protein content was determined according to Bradford (1979). According to this method, 20 µl of extract was diluted in 80 µl extraction buffer. Then 5 ml of fresh Coomassie Brilliant Blue-G 250 reagent was added. The mixture was stirred for two minutes. Absorbance at 595 nm was recorded after five minutes and bovine serum albumin was used as the standard.

For assay of peroxidase (POD) activity, the oxidation of guaiacol was measured by the determination of the increase in absorbance value at 470 nm at 1 min interval, using spectrophotometer. The reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 5mM H_2O_2 and 13 mM guaiacol was prepared. The reaction was initiated by addition of 33µl of enzyme extract (Qiu et al., 2005).

The activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were determined by the methods described by Xu et al. (2011). The samples of ash were used for the subsequent determination of boron by using atomic absorption.

Data were subjected to analysis of variance (ANOVA). For comparison of means the LSD test was applied ($P<0.05$).

Results and discussion

There was no significant difference in the moisture and ash content in all of the cultivars (Fig. 1 and 2). Moisture content of collected pollen is a parameter of the quality, and regulations on the quality of pollen don't have clear recommendation for this determination (Melo and Almeida-Muradian, 2011; Basuny et al., 2013). The moisture content ranged from 5.92 to 6.30% respectively. The total protein content ranged from 2.34 to 3.66 $mg \cdot g^{-1}$ FW. The higher protein content was found in 'Dakal' and 'Koroneiki' (Fig. 3). Knox and Heslop-Harrison (1971) have shown that a large preparation of the total protein born by the pollen grain must be lodged in the wall sites. Since the function of proteins would seems to require that they be readily released on stigma, they are certain to be among the first to be emitted in contact with other biological surfaces. Proteins, unregulated during the germination, might be mainly responsible for fast pollen tube growth (Dai et al., 2007). In monocots, maize and rice, xylanase is the major protein component in the pollen coat and possibly facilitates pollen tube invasion by hydrolyzing the xylan on the stigma surface (Dai et al., 2006). The APX activity in cv. 'Amygdalolia' was significantly higher than in other cultivars (182.69 $U \cdot min^{-1} \cdot g$ FW) (Fig. 4). There was no significant difference of POD activity in 'Dakal', 'Koroneiki' and 'Manzanillo' cultivars. POD activity in 'Amygdalolia' and 'Konservolia' cultivars was the highest (95.37 and 93.33 $U \cdot min^{-1} \cdot g$ FW, respectively) (Fig. 5). CAT activity in 'Konservolia' was relatively higher (25.58 U $min^{-1} g\ fw^{-1}$) than in other cultivars but

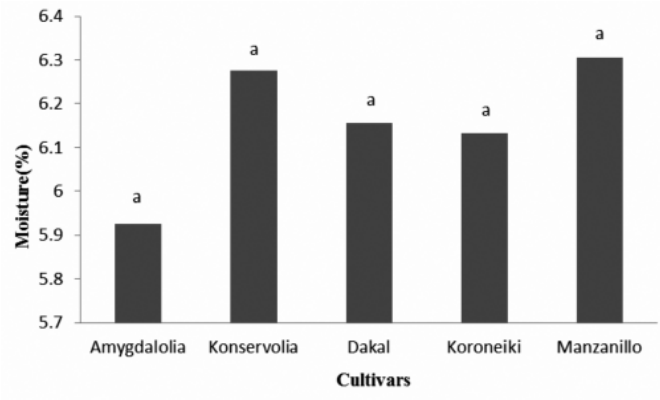

Figure 1.
Moisture content of five olive cultivars. The means followed by the same letters were not significantly different at P ≤ 0.05.

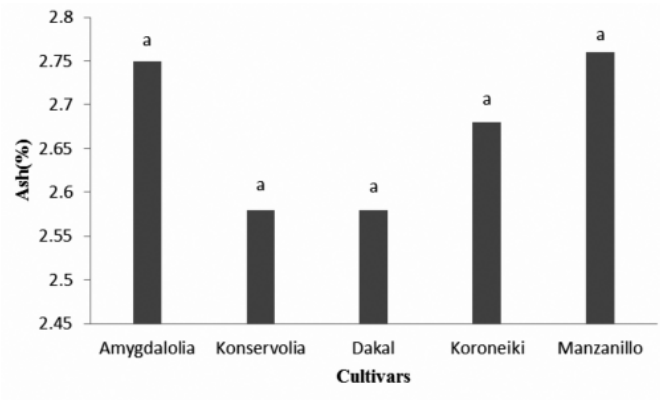

Figure 2.
Ash content of five olive cultivars. The means followed by the same letters were not significantly different at P ≤ 0.05.

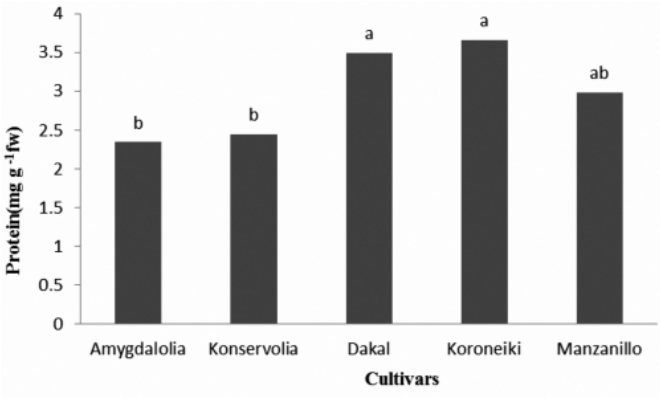

Figure 3.
Protein content of five olive cultivars. The means followed by the same letters were not significantly different at P ≤ 0.05.

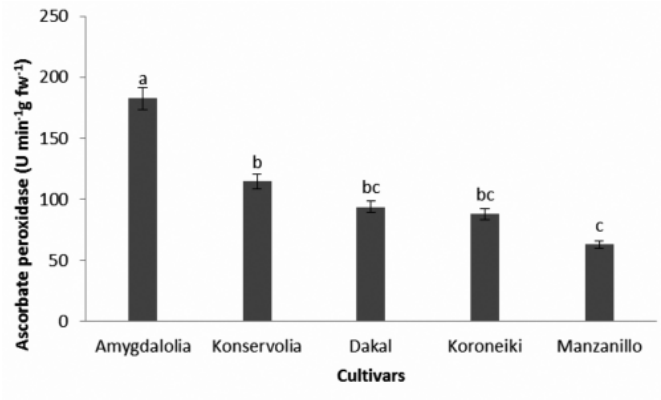

Figure 4.
Ascorbate peroxidase activity of five olive cultivars. The means followed by the same letters were not significantly different at P ≤ 0.05.

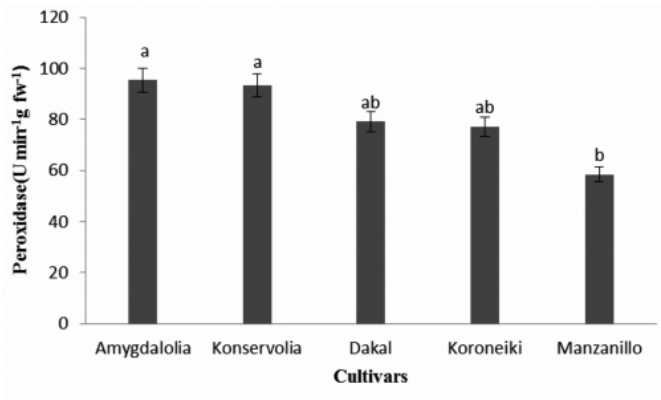

Figure 5.
Peroxidase activity of five olive cultivars. The means followed by the same letters were not significantly different at P ≤ 0.05.

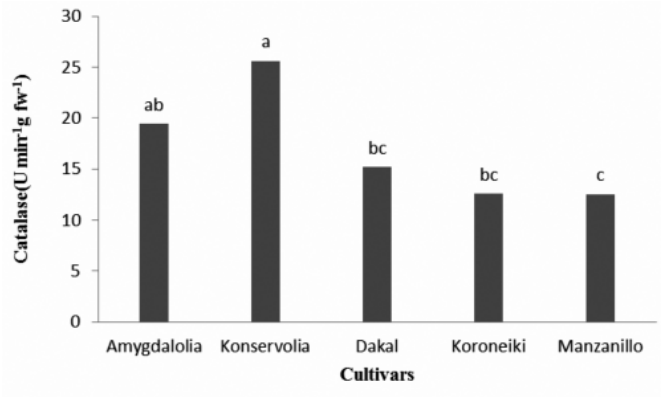

Figure 6. Catalase activity of five olive cultivars. The means followed by the same letters were not significantly different at P ≤ 0.05.

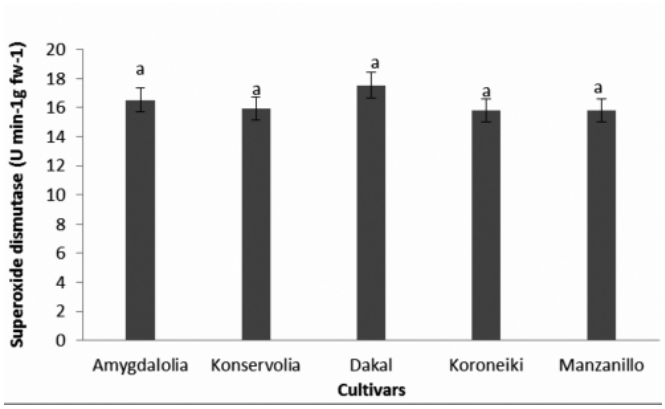

Figure 7.
Superoxide dismutase activity of five olive cultivars. The means followed by the same letters were not significantly different at $P \leq 0.05$.

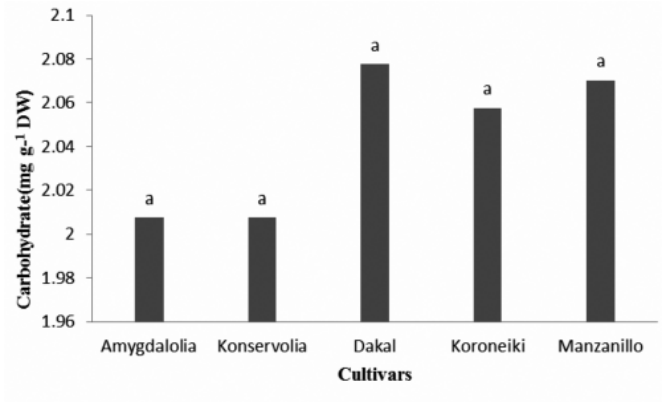

Figure 8.
Carbohydrate content of five olive cultivars. The means followed by the same letters were not significantly different at $P \leq 0.05$.

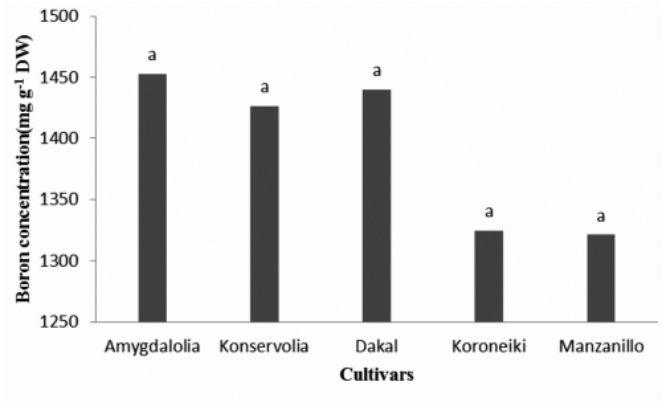

Figure 9.
Boron concentration of five olive cultivars. The means followed by the same letters were not significantly different at $P \leq 0.05$.

there was no significant difference between 'Konservolia' and 'Amygdalolia' (Fig. 6). The SOD activity showed no significant difference in all of the cultivars (Fig. 7). Antioxidant enzymes, such as SOD, POD, CAT and APX may lead to adjustment to environmental stress by reducing damage of reactive oxygen species. Sometimes, antioxidant enzymes are also called protective enzymes in plant (Elbaz et al., 2010; Sun et al., 2010; Zeng et al., 2010). APX activity in pollen grains is high, i.e. ten- fold higher than that of catalase, which is really modest, similar to what was found in *Dasypyrum villosum* seedlings. This seems to support that in pollen grains APX rather than CAT is the key enzyme for removing H_2O_2 (Degara et al., 1991). It is well known that proteins and enzymes are released by pollen grains and that they are involved in both cell recognition mechanisms and pollen tube growth (Degara et al., 1993). It can be presumed that during the interaction between the growing male gametophyte and the stylar transmitting tissue hydrogen peroxide may be generated, and consequently, APX should be required to remove the toxic H_2O_2 and thus to maintain pollen tube growth (Degara et al., 1993). Several isoenzymes of SOD and CAT have been reported in mature pollen from certain imbred line of maize. The presence of SOD in olive pollen grain suggested the existence of a protection mechanism against oxidative stress phenomena at least during the late stages of pollen development (Acevedo and Scandalios, 1990). The presence of such a complex antioxidant system in the olive pollen and the occurrence of inter cultivar differences points out to a putative involvement of the system in the compatibility behavior of species (Acevedo and Scandalios, 1990). There was no significant difference in the carbohydrate and boron content in all of the cultivars (Fig. 8 and 9). Since boron plays an important physiological role within the pollen grain, it was to be expected that boron levels would be high. The higher boron content in the membranes of plant tissue and its role in cell elongation make it an essential element for the germination process in pollen grains (Marguard, 1992).

Conclusion

Chemical analysis has revealed the presence of a wide range of biochemically important substance in olive pollens. These enzymes are likely to be involved in pollen germination, pollen tube growth and penetration of the stigma.

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