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Inoculation of arbuscular mycorrhizal fungi and application of micronized calcite to olive plant: Effects on some biochemical constituents of olive fruit and oil

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ABSTRACT

Some physico-chemical properties of olive fruits (*Olea europaea* L., variety 'Sariulak') and their corresponding oils during 2007–2013 crop seasons were investigated following inoculation with arbuscular mycorrhizal fungi (AMF, *Rhizophagus intraradices*) and application of micronized calcite (MC) [as plant growth stimulating product] with special emphasis on individual phenolic compounds.

Olive fruits showed greater accumulation of hydroxytyrosol and p-hydroxybenzoic acid in plants on which AM was inoculated when compared to the values of uninoculated plants. AMF inoculation resulted in a reduction in vanillin, dimethyloleuropein and luteolin content of olive fruits. Secoiridoid aglycones in the olive oils from treated trees generally tend to decrease. There were differences in the levels of phenolics in fruits and their corresponding oils, such as the amount of hydroxytyrosol rose in AMF + MC applied fruits, nevertheless the amount of verbascoside, a secoiridoid conjugate of hydroxytyrosol, declined in the oil of AMF + MC treated plants.

After the application of AMF + MC, the oils did not show a significant change in the total phenolics content contrarily to olive fruits. These results highlight that phenolic compounds having hydrophilic structure were influenced by AMF + MC application more than those of lipophilic structure.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in the terrestrial ecosystem and they form symbiotic associations with the roots of the majority of plant species (Smith and Read, 1997). The most spectacular beneficial effects of AMF are, enhanced capture of mineral elements such as phosphorus, calcium, copper, sulphur, zinc and iron and increased resistance to different types of biotic and abiotic stress (Ghazi and Al-Karaki, 2006; Orlowska et al., 2011; Santander and Olave, 2012). The beneficial effects of arbuscular mycorrhizae in improving tolerance to environmental stress conditions such as water stress (Cruz et al., 2000), high soil salinity (Sheng et al., 2009), transplanting stress (Carretero et al., 2009; Dag et al., 2009), serpentine edaphic stress (Doubkova et al., 2012) in some plant species have been widely reported. There are several studies on the AMF inoculation to olive plant which however mostly focus on plant growth characteristics and nutrient uptake (Estaún et al., 2003; Caravaca et al., 2003; Castillo et al., 2006; Porras-Soriano et al., 2009; Kapulnik et al., 2010; Sidhoum and Fortas, 2013). The previous reports on relationship between AMF inoculation and secondary metabolites mostly focus on roots of the host plant and in shoot as Baslam et al. (2011, 2013) reported.

Micronized calcite is a natural leaf foliar which is produced by a patented technology known as "tribomechanical activation". Once this product as a powder is mixed with water and sprayed on a leaf surface, it starts working through directly penetrating the leaf pores and increases bioactivity in the leaf, creates a leaf microclimate suitable for photosynthesis, and decreases the plant's need for water, increases the plant's resistance to disease and pests and promote chlorophyll creation (Anonymous, 2014).

Exposition to high light levels results in quantitative changes of metals and metalloids in the skins of grape berries. Application of micronized calcite (a brownish, non-porous, non-swelling, non-abrasive, chemically inert fine-grained mineral sprayed as a suspension on leaf surface and forming a particles film that reduces light absorption by the berry) on the berry skin ionome of the red grape 'Aglianico' was reported to significantly affect macro element distribution (Sofo et al., 2013). The application of processed

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calcite particles reported shows that treated plants have nitrogen and cationic contents, in both apricot leaves and fruits, closer to the standard reference levels of this region compared to untreated plants (Martinez et al., 2010). The authors also reported that apricot fruits affected by processed calcite particles treatment showed higher total soluble solids and lower titratable acidity and a lower susceptibility to post-harvest physiological disorder or pathogen attacks.

Ecological culture practices have increased in many countries. Nowadays many discussions are on the extent to which ecological cultivation procedures affect value-giving secondary plant contents (Brandt and Mølgaard, 2001). Secondary metabolites apparently act as defence (against herbivores, microbes, viruses or competing plants) and signal compounds (to attract pollinating or seed dispersing animals), as well as protecting the plant from ultraviolet radiation and oxidants (Swain, 1977; Kutchan, 2001). Root colonization by AMF can alter plant biomass, nutrient status, carbon use and therefore may also alter the production and composition of secondary metabolites in plants (Strack and Fester, 2006; Strack et al., 2003; Baslam et al., 2011, 2013).

In the past decade there has been a surge in the number of *in vivo* and *in vitro* studies that has investigated the health promoting beneficial effects of olive oil phenolics (Tripoli et al., 2005; Bendini et al., 2007). Olive oil phenols have been demonstrated to have antioxidant effects on lipid and DNA oxidation (Fitó et al., 2000; Owen et al., 2000; Masella et al., 2004; Machowetz et al., 2007); quench free radicals (Massaro et al., 2002); inhibit platelet-induced aggregation (Beauchamp et al., 2005; Smith et al., 2005); show chemopreventive activity; alter the proportion of LDL and HDL cholesterols in humans (Covas et al., 2006), show anti-thrombotic and anti-inflammatory effects (Bogani et al., 2007). Oleuropein aglycone was found to dose-dependently inhibit growth in breast cancer cells (Menéndez et al., 2007). Visioli et al. (2000) concluded that h-tyrosol was effective in decreasing the oxidative stress associated with passive cigarette smoke. In addition to these bioactive properties, olive oil phenolics have been also found to contain antimicrobial properties *in vitro* (Medina et al., 2006; Bisignano et al., 1999).

In the view of the above literature reports, there are not any study carried out on joint the use of AMF and micronized calcite on olive plant. Thus, in this study the effects of AMF inoculation and calcite application (reduced incident light by means of micronized calcite) to olive tree were investigated for the first time in terms of some qualitative and quantitative characteristics of olive fruit and their corresponding oils including individual phenolic compounds.

2. Materials and methods

2.1. Chemicals and reagents

n-Hexane, cyclohexane, potassium hydroxide, acetic acid, chloroform, Na thiosulphate, potassium iodide, folin ciocalteu's reagent, DPPH, indole-3-butyric acid, ammonium, nitrate and phosphate were obtained from Sigma–Aldrich (Steinheim, Germany). Methanol and phophoric acid were from Merck (Darmstadt, Germany). Standard of hydroxytyrosol (h-tyrosol) and tyrosol were obtained from Extrasynthése (Genay, France) and the remaining standards (except secoiridoids, acetates of h-tyrosol and tyrosol and lignans) were purchased from Fluka (Steinheim, Germany). Ultrapure water (MilliQ system, Millipore, Bedford, MA, USA) was used throughout the experiments.

2.2. Olive origin

Young olive plants produced from cuttings of selected material supplied by East Mediterranean Olive Association. Olive cuttings were prepared at East Mediterranean Olive Association olive propagation facilities in Seyhan location of the city of Adana at September 2007 as in length 30 cm and diameter 20–25 mm applied 2000 ppm indole-3-butyric acid solution in bottom 3 cm length. Cuttings rooted in greenhouse in perlite media, under mist propagation unit and then transferred into 12 cm × 25 cm polyethylene bags fulfilled by 1:1 sterile perlite and peat material as plant growth media. Rooted cuttings were grown for one season under the shed by 50% light transmitted.

The experiment took place during the crop season 2007–2008 on "Sariulak" olive cultivar grown in the experimental farm in Seyhan, in south Turkiye (Latitude: 36″58′ N; Longitude: 35″16′ E; Altitude: 20 m above sea level). The site is characterized by a typically Mediterranean climate with a mean annual rainfall of 647 mm (almost half of this amount takes place from autumn to spring and the remaining part in winter); an average annual relative humidity of 65.93%. The warmer month is August and the coldest is January with a mean annual temperature varied from 9.3 °C to 28.8 °C.

2.3. Mycorrhizal inoculation

Two years old young plants in pots were inoculated with a mycorrhizal fungus at the second week of August 2008 and planted in field at first week of March 2009 between 3 m × 6 m of distances in Sarıçam town of Adana. Mycorrhizal inoculum was prepared according to methodology (MycoSym, International AG, Basel), with *Rhizophagus intraradices* spores and hyphea, together mycorrhized root fragments used an inert carrier. Active fungal component has a minimum 200 infective mycorrhizal propagules mL^{-1} of which minimum 50 living spores mL^{-1}. This mycorrhizal preparation contained ammonium-N <0.01%, nitrate-N <0.01%, phosphate-P <0.01%, and potassium <0.01%.

2.4. Micronized calcite applications

Micronized calcite as plant growth stimulating product [HerbaGreen (HG), 100% natural product containing CaO, MgO, Fe_2O_3, SiO_2] was sprayed on leaves four times each year. The main components of micronized calcite (MC) applications are $CaCO_3$ (carbonate calcium) 40%, SiO_2 (silisium dioxide) 4%, MgO (magnesium oxide) 1%, Fe_2O_3 (iron) 1% (pH 8–10 warranted by producer company) were pulverized onto the plant with the concentration of 0.5%500 g MC stirred in 100 L water (according to the specifications of the company) and afterwards shaken vigorously before application. Pulverizations were commenced with four replications in each year after plantation. The first application was on the middle of May that just after the blooming fruit size was 2–3 mm and repeated 15–20 days intervals depending on weather conditions, and finished on the middle of July, and while no mycorrhiza inoculation and micronized calcite applications were performed to those belonging to control group.

All the plants were drip irrigated; the first irrigation was in the middle of July and repeated 15 days intervals, and intervals in July and August were decreased to 10 and 5 days, respectively, depending on weather conditions. Nutritive elements as 18%N, 18%P, 18%K were applied as 75 g $plant^{-1}$ in four parts which the first was at the middle of April, second was at the middle of May, third was at the middle of June, and the last one was applied at the beginning of August without using any supplementary nutritional matter.

Investigations on the additive effects of *R. intraradices* and plant growth stimulating product on fruit quality stimulation trials was planned as three replicated casual parcels in orchard. Effects were determined by fruit quality measurements of sample fruits harvested at 10 September 2012. After harvest, the fruits were then stored in a fridge (2 ± 0.5 °C) for 15 days.