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Changes in key photosynthetic parameters of olive trees following soil tillage and wastewater irrigation, modified olive oil quality

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ABSTRACT

The use of wastewater (WW) provides a reliable source of water and nutrients for crop production. Soil tillage is one of the key soil management practices in agricultural land use. In the present study, we studied the effects of these two agricultural practices on key physiological parameters of olive trees and on olive oil quality. The experiment was carried out on mature olive trees (*Olea europaea* L. cv. *Chemlali*). Two tillage practices treatments (Trees grown in Tilled soil: TTS; and Trees grown in No-tilled soil: TNTS) were combined with two irrigation treatments (Trees irrigated with WW: IT; and Trees grown under rainfed condition: TRC) during two successive years (2013 and 2014). Statistical analyses demonstrated that the factor "soil tillage" was more efficient in improving photosynthetic parameters of olive trees than WW irrigation. The combination of soil tillage with WW irrigation improved significantly leaf chlorophyll fluorescence parameters, stomatal conductance, transpiration and chlorophyll content, which closely associated with an increase of photosynthetic rate, and a decrease of stress metabolites (leaf phenolic compounds and carbohydrates). On the other hand, a decrease of key photosynthetic parameters and an accumulation of stress metabolites in leaves were observed when combining rainfed condition and No-tilled soil. WW irrigation affected negatively the oxidative stability and nutritional value of olive oil, especially by decreasing the contents of antioxidant compounds (total phenols and pigments) and the levels of MUFA, C18:1, C18:3 and elevating SFA and PUFA contents. However, soil tillage combined with TRC improved oil stability to oxidation and its nutritional value by increasing the contents of antioxidant compounds and the levels of MUFA and C18:1, as well as C18:1/C18:2 and MUFA/PUFA ratios.

The results of this study prove that, under water scarcity conditions, wastewater irrigation in oleiculture should be combined with soil tillage and recommended only in the '*Off*'-year to support vegetative growth and improve physiological performance of olive trees. In the '*On*'-year, tilling the soil under rainfed conditions proved to be the most favorable cropping strategy for enhancing both physiological performance of olive trees and olive oil quality. These findings must be taken into consideration when developing strategies for oil quality enhancement, mainly in an exporting country like Tunisia.

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1. Introduction

In Tunisia, oleiculture represents one of the principal economical and agricultural strategic sectors. In fact, about 65 million olive trees are spread over 1.6 million hectares (Hannachi et al., 2007). The main olive cultivar is *Chemlali*, grown in northern and central Tunisia, and contributes to 80% of Tunisia's oil production (Baccouri et al., 2007). Olive oil biochemical composition depends, not only to olive cultivar, but also to pedoclimatic conditions, processing steps and also agronomic practices (Salvador et al., 2003; Torres and Maestri, 2006; Guerfel et al., 2010). In the present study, particular attention has been paid to two agronomic practices: irrigation and soil tillage.

Traditionally, in Tunisia, olive trees have been cultivated under rainfed conditions. However, this country is facing more and more serious water deficiency problems, particularly in arid and semi-arid regions. One way to deal with this situation is to reuse wastewater in agriculture. In Tunisia, WW reuse in agriculture is regulated by the 1989 decree (Decree No. 89-1047). This practice is increasingly extended and by 2013, wastewater treatment sta-

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tions were of 110 generating approximately 230 million m^3 $year^{-1}$ of Treated WW among which 26% were recycled and supplied for irrigation of 8100 ha of agricultural lands (ONAS, 2013).

In general, wastewater (WW) proved beneficial in improving the plant growth characteristics due to its considerable content of essential nutrients (Tak et al., 2013). In the last years, there has been a growing interest in studying the effects of WW irrigation on olive oil quality and composition (Bedbabis et al., 2009, 2015), and few studies have reported that irrigation with WW can affect negatively olive oil composition (Bedbabis et al., 2015).

Soil tillage is one of the key soil management practices in agricultural land use. Tunisia's climate is characterized by a low rainfall, irregularly distributed through the year and occurring generally in spring and autumn. These conditions lead to minimum water storage from rainfall events and an increase in soil erosion processes (López-Bermudez and Albaladejo, 1990). Soil management practices are essential for reducing soil compaction, increasing water availability and reducing surface runoff (López-Garrido et al., 2014). The effects of soil tillage on soil characteristics such as infiltration rate, aggregate-stability, soil and water conservation, affect directly the soil productivity and sustainability (Busari et al., 2015). However, literature reported controversial effects with soil tillage systems on soil properties and water availability (Gharaibeh and Eltaif, 2014; Busari et al., 2015). Yield consequences of soil tillage have been also investigated in many crops (Botta et al., 2006; Mirleau-Thebaud et al., 2011). However, only few studies have been carried out in olive trees, and none of them include the olive oil biochemical analyses, neither physiological parameters in olive leaves (Gómez et al., 1999; Ferraj et al., 2011).

Due to the importance of olive cultivation in the social and economic life of Tunisia, and to the significance of olive oil as a vegetable oil with considerable nutritional values, it is very important to suggest the most suitable agronomical practices for olive tree cultivation and for oil quality, with regard to the climatic conditions. In this context, the present work aimed to study the effects of WW irrigation combined with soil tillage on some physiological parameters in olive trees c.v. *Chemlali* as well as the effects of these two agricultural practices on oil quality and fatty acid composition. Existing data on the effects of these two agricultural practices on olive tree physiology and virgin olive oil quality are few and sometimes contradictory. Therefore, we studied the changes of net photosynthesis rate, stomatal conductance, transpiration, chlorophyll fluorescence and chlorophyll content after WW applications and soil tillage, and their consequences on the concentrations of stress metabolites, such as phenolic compounds and carbohydrates, in olive leaves. We proposed in the next step to examine the effects of WW irrigation and soil tillage on the antioxidant profile of olive oil (including the contents in total phenols, α-tocopherol and pigments, as well as oxidative stability), and on fatty acid composition. To our knowledge, this is the first paper that elucidated the response of all these physiological and biochemical parameters to the combined use of soil tillage and irrigation with wastewater. The results were then discussed in order to determine the most appropriate strategies for enhancing physiological performance of olive trees and for the production of best quality olive oil, depending on the climatic conditions and the alternate bearing of olive trees.

2. Materiel and methods

2.1. Field site and experimental material

The present study was carried out in Mourdine, Provence of Sousse, Tunisia (North latitude 35° 46′ 31″, East longitude 10° 33′ 51″), in 2013 and 2014, using an orchard of mature olive trees *(Olea europaea* L. cv. *Chemlali)* spaced 12m × 12 m apart (69 trees per ha). The climate of this region is typical Mediterranean with a mean annual rainfall of 350 mm concentrated mainly from autumn to spring (from September to Mid-May) and an average annual temperature of 18–20 °C. A composite sample soil (0–30 cm depth) taken from the soil of the experimental farm was analyzed. Physico-Chemical properties of the soil are provided as Supplementary material.

The experimental olive trees were at similar growth stage and having a trunk diameter of 0.66 m ± 0.048 and a canopy volume of 137 m^3 ± 0.08. A randomized block design with three blocks of 0.5 ha each and four treatments were used:

*T1: Block of 36 olive trees irrigated with wastewater and grown in No-tilled soil (Zero tillage, no soil surface disturbance was involved).

*T2: Block of 36 olive trees irrigated with wastewater and grown in a tilled soil

*T3: Block of 36 olive trees grown under rainfed condition and a tilled soil.

*TC: Block of 36 olive trees grown under rainfed condition and No-tilled soil (Zero tillage, no soil surface disturbance was involved).

Thus, in the present study, two soil management practices (Trees grown in Tilled soil: TTS; and Trees grown in No-tilled soil: TNTS) were combined with two irrigation treatments (Trees irrigated with WW: IT; and Trees grown under rainfed condition: TRC).

2.2. Water irrigation and soil management

For IT treatment, the irrigation was applied during two successive growing seasons (2013 and 2014), at two dates per season (in total: four applications in this experiment), during the most sensitive fruit growth stages: at 40 DAFB (day after full bloom, beginning of June) and at 100 DAFB (Beginning of August). At these two dates, water irrigation was applied by submersion with a dose of 35 mm (Total annual water irrigation was about 70 mm = 10 m^3 $tree^{-1}$). The amount of water supplied to olive trees was estimated according to the Penman–Monteith–FAO equation (Doorenbos and Pruitt, 1977) as described by Ben Ahmed et al. (2007). The characteristics of WW were provided as Supplementary material.

For TTS treatment, the soil was mechanically tilled, during two successive years (2013 and 2014) at a depth of 0.1–0.15 m periodically perpendicular to the slope using a cultivator with dovetail with internal separation of 2.2 m. In the first year (no-crop year/"*Off*-year"), the land is plowed 4 times: before blooming on February 2013 (to increase the ability of soil to hold winter rain), then on June 2013 (10 days after the first irrigation with WW), on August 2013 (10 days after the second irrigation with WW) and finally on mid October 2013. In the second growing season, (cropping year/"*On*-year"), the land is plowed also 4 times: on February 2014, then on June 2014 (10 days after the third irrigation with WW), on August 2014 (10 days after the 4th irrigation with WW) and finally on mid October 2014. TNTS treatment was characterized by the absence of tillage (only direct drilling), spraying also the plot with pre-emergence herbicides and leaving the crop residues on the surface.

2.3. Physiological and biochemical analyses of olive tree leaves

2.3.1. Measurement of photosynthesis and chlorophyll fluorescence parameters

On 15 June 2014, net photosynthesis (*Pn*), stomatal conductance (*gs*) and transpiration (*Tr*) were measured in the field by using a portable CI-301 PS (CID Incorporation, USA). Net photosynthesis rate (in μmol CO_2 m^{-2} s^{-1}), was measured at fixed CO_2 concentration (CO_2 at 400 μmol mol^{-1}), air temperature (25 °C),

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