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Physiological improvements of young olive tree (*Olea europaea* L. cv. Chetoui) under short term irrigation with treated wastewater

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ABSTRACT

The treated wastewater (TWW) reuse in crop irrigation is becoming a frequent practice in areas with limited water resources. This alternative water may be considered as nutrients source that can fill the water deficit. However, TWW contains high salts concentration and may negatively affect plant performances. The ability of short term irrigation with TWW in order to improve young olive (*Olea europaea* L. cv. Chetoui) performance was studied. One-year-old olive plants were subjected, for four months, to different concentrations (33, 50 and 100%) of TWW. As compared to the control olive plants, results showed that irrigation with TWW induced an enrichment of plant tissues with macro- and micronutrients. This enhancement significantly ($p \leq 0.05$) improved the photosynthesis activity, soluble sugars and thus plant growth. The irrigation with undiluted TWW was more efficient than diluted TWW in ameliorating the physiological performance of young olive plants. Therefore, TWW seems to be an interesting bio-fertilizer of young olive plant when applied for a short period.

1. Introduction

In the recent years, the irrigated areas expansion has caused a decrease in the availability of fresh water as irrigation is fundamental for the development of agriculture (García-Ruiz et al., 2011). Generally, agriculture is the major consumer of water as it accounts for around 70% of all global water use (Winpenny et al., 2010). Alternative sources of water like treated wastewater (TWW) could be a useful option to satisfy different agricultural needs (Uzen et al., 2016). Recently, TWW reuse in agriculture is gradually becoming a frequent practice in areas with limited sources of water (Petousi et al., 2015).

The potential benefits in using TWW for irrigation purposes include (i) reducing fresh water demand (ii) recycling nutrients and (iii) minimizing the discharge of pollutants into the waterways (Hanjraa et al., 2012). According to Martínez et al. (2013), TWW is a rich source of nutrients (nitrogen (N), phosphorus (P), potassium (K) and micronutrients), which reduce the need for chemical fertilizers resulting in net cost savings to farmers. Kiziloglu et al. (2008) showed that wastewater irrigation increases the availability of nitrogen, phosphorus and potassium as well as micro-elements to plants, which lead to increase the yields of cauliflower and red cabbage.

Many studies related to TWW application in agriculture were developed for several crops including eggplant, tomato (Cirelli et al., 2012), lemon (Pedrero et al., 2012), chickpea (Tak et al., 2013) and cotton (Uzen et al., 2016). These authors showed the benefits of TWW irrigation in increasing the biomass and the production, in improving the plant mineral status and in reducing the fertilizers application rates. Indeed, Tekaya et al. (2016) reported that TWW irrigation improved the growth and increased the photosynthetic capacity of olive tree (*Olea europaea* L.). However, some disadvantages like salinization of soil, sensitive plants damage and contamination by coliforms such as *Escherichia coli* that represent a public health, are still a concern (Cirelli et al., 2012; Bedbabis et al., 2015). Interestingly, it was suggested that good agricultural practices executed with planning and management may reduce these undesirable consequences (WHO, 2006).

In Tunisia, oleiculture is considered as one of the main economical and agricultural sectors. This species known for its tolerance to drought (Wang et al., 2018) and salinity (Chehab et al., 2018) constituted the most important crop. Recently, in water-scarce countries such as Tunisia, TWW was used as an additional water resource for plant irrigation. According to Hentati et al. (2014), in the Mediterranean Basin, Tunisia is considered as a country suffering of a high level of water scarcity. To a large extent, TWW may be considered as an important source of nutrients for olive crop production, which can reduce the

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Table 1
Physico-chemical characteristics of the soil used in the experiment.

Parameters	Value
Sand (%)	86
Silt (%)	12
Clay (%)	2
Organic matter (%)	1.2
pH	7.8
EC ($dS.m^{-1}$)	1.14

chemical fertilizer application. The response of young olive plants (*Olea europaea* L. cv. Chetoui) face to TWW irrigation remains unknown.

The objective of this study was to examine the comportment of young olive plants irrigated with different concentrations (33, 50 and 100%) of TWW. Besides, the optimal dilution of TWW was determined in order to beneficiate of the fertilizer value of this water. The parameters regarding the plant growth, biomass, mineral status and photosynthetic capacity were studied.

2. Materials and methods

2.1. Plant material and treatment conditions

The trials were carried out at the Olive Tree Institute of Sfax, Tunisia (34°43′N, 10°41′E). Uniform one-year-old olive plants (*O. europaea* L. cv. Chetoui) were transplanted into five-liter pots (23 cm diameter, 17.6 cm deep) filled with 5 kg soil collected from the experimental site of the Olive Tree Institute of Sfax, Tunisia. The characteristics of the experimental soil were summarized in Table 1. The pots were kept under ambient environmental conditions with natural sunlight and temperature and they were covered with plastic film to prevent rain from affecting the experiment. The mean minimum air temperature ranged between 16 and 20 °C and the mean maximum temperature ranged between 26.5 and 31.5 °C. The photosynthetic active radiations ranged between 921 ± 20 and 1450 ± 30 μmol $m^{-2}s^{-1}$. The relative humidity varied from 47 ± 3 and 58 ± 2.5%. All olive plants were irrigated during two months with tap water (acclimation period) and then they were subjected to the following treatments during four months (15th Febrary-15th June 2012).

(i) Cp: control olive plants irrigated with tap water;
(ii) T_1: olive plants irrigated with TWW diluted two times with tap water (33% TWW);
(iii) T_2: olive plants irrigated with TWW diluted one time with tap water (50% TWW);
(iv) T_3: olive plants irrigated with undiluted TWW (100% TWW).

Treatments were arranged in a completely randomized design. The number of young olive plants for each treatment was five ($n = 5$). The tap water used was the normal drinking water supplied by Tunisian National Water Distribution Utility. The used TWW was collected from the wastewater treatment plant of Sfax-South (34°43′N, 10°41′E). This water comes from domestic and industrial sources and is treated at the secondary level using biological processes. Table 3 summarized the mineral analysis of the tap water as well as the TWW used for the treatments. Over the experimental phase, all plants were irrigated twice a week and the volume of water used to irrigate olive plants was equal to that lost by transpiration. This volume was calculated every month as the difference between the weight of plant early in the morning and the weight of plant late in the evening (Table 2).

At the end of the experimental period (the 121th day), plants were harvested and divided into leaves and roots. Olive plants tissues were then washed with distilled water and dried on a filter paper. Samples of leaves and roots were either used immediately for analyses or stored at −80 °C. Other samples of plant tissues were oven-dried at 70 °C to a constant weight.

Table 2
Monthly amounts of water used for the irrigation of young olive plants during the experimental period (15th Febrary-15th June).

	15 February-15 March	15 March-15 April	15 April-15 May	15 May-15 Juin
Water (ml)	280	425	545	700

2.2. Determination of macro- and micronutrients contents

Samples of dried leaves and roots (0.5 g) were ground to a fine powder and placed for 3 h in an oven at 250 °C. Samples were then digested with 5 ml of 1 M HNO_3 and the obtained solutions were next adjusted to 50 ml using distilled water. The concentrations of Na^+, K^+ and Ca^{2+} were determined by flame spectrophotometry (Jenway PFP7, Milan, Italy). Micronutrients (Cu^{2+}, Zn^{2+} and Fe^{2+}) and Mg^{2+} contents were measured using an Analytik Jena ZEEnit 700 atomic absorption spectrophotometer (Analytik Jena AG, Germany). Phosphorus (P) was measured by a vanado-molybdate colorimetric procedure with a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) (Pauwels et al., 1992).

2.3. Plant growth and biomass measurements

After drying the plants tissues until a constant weight, the leaf and root dry weights were obtained. Plant height represented the difference in the length of the main stem between the beginning and the end of treatment period. Leaf area was measured at the end of the experimental period by a leaf-area-meter AM 300 (ADC Bioscientific Ltd., UK).

2.4. Gas exchange measurements

At the end of the experimental period (the 121th day), net photosynthesis (Pn), stomatal conductance (Gs) and transpiration rate (E) were measured for each plant on six well-exposed and fully expanded leaves from the median part of the shoot (in total 30 leaves per treatment). Water use efficiency (WUE) was determined as Pn/E ratio. Measurements were taken using a portable infrared gas analyzer (CID 301 PS, USA) on a sunny day from 9:00 to 11:00 A.M. The leaf average air temperature was 28 ± 2 °C and the Photosynthetic Active Radiation was 1100 μmol m^{-2} s^{-1} (Zouari et al., 2016).

2.5. Total chlorophyll content

The total chlorophyll content was measured according to the method described by Lichtenthaler and Buschmann (2001). Thus, 0.2 g of fresh olive leaves was homogenized using a pestle and mortar in 5 ml of pure acetone and made up to 20 ml with pure acetone after filtration. The absorbance was measured using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 661.6 and 644.8 nm.

2.6. Soluble sugars and starch contents

Soluble sugars were measured following the method of Robyt and White (1987). Samples of fresh olive leaves (0.5 g) were boiled for 30 min in 80% methanol at 70 °C. Then, 1 ml of the obtained extract was blended with 1 ml of phenol and 5 ml of concentrated sulfuric acid. After agitation and cooling, the absorbance was read using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 640 nm with methanol as a blank. The soluble sugars concentration was determined using glucose solutions to develop a standard curve.