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Effects of long-term olive mill wastewater spreading on the physiological and biochemical responses of adult Chemlali olive trees (*Olea europaea* L.)

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ABSTRACT

Olive mill wastewater (OMW), a by-product from the olive oil extraction process, is annually produced in huge amounts in olive-growing areas and represents a significant environmental problem in Mediterranean areas. Whereas its high organic load and polyphenols concentrations are associated with troublesome environmental effects, its rich mineral and organic matter contents represent valuable nutrients. This study aimed to investigate adult 'Chemlali' olive trees subjected to OMW application at three doses (50, 100, and 200 m^3 ha^{-1} $year^{-1}$) after eight successive years in an olive field. The main focus of the study was on gas exchange characteristics, photosynthetic pigments, soluble sugars, total phenolic compounds, antioxidant activity, leaf mineral nutrient and yield of the trees. The findings showed significant raises of net photosynthesis (Pn) and stomatal conductance (Gs) in OMW treated olive trees by 50 and 100 m^3 ha^{-1}. The lowest Pn and Gs rates were recorded in treatment with the highest OMW dose (200 m^3 ha^{-1}). In addition, olive trees amended with 200 m^3 ha^{-1} exhibited a significant decrease of both chlorophyll (a + b) and carotenoid contents, while olive plants which received 50 and 100 m^3 ha^{-1} of OMW showed higher photosynthetic pigments contents compared to the control olive trees. However, there was no significant difference in carotenoid content. Soluble sugars, total phenol contents and antioxidant activity increment were noticed in leaf plants amended with a 200 m^3 ha^{-1} dose. The OMW spreading rates at 50 and 100 m^3 ha^{-1} significantly increased the mineral contents of leaves (N, P, K, Ca and Mg). Nevertheless, the highest OMW application was associated with a significant reduction of olive leaf nutrient concentrations. Olive yield showed a continuous improvement of up to 100 m^3 ha^{-1} $year^{-1}$ spread rate. The present study confirms that olive trees responses were dependent on OMW dose applied. Consequently, key physiological parameters of adult olive trees seemed to benefit from spreading OMW at moderate doses (50 and 100 m^3 ha^{-1} $year^{-1}$) while the application of a higher dose (200 m^3 ha^{-1} $year^{-1}$) seemed to disrupt them.

1. Introduction

Olive tree (*Olea europaea* L.) is the most important cultivated crop in the Mediterranean basin, which gives the olive oil sector a remarkable economical importance in this region (Killi and Kavdir, 2013). The olive oil production in these countries represents almost 98% of the total world's production. Consequently, about 30 million m^3 of olive mill wastewater (OMW) are annually produced in the region during a short duration, causing the most serious threats to the environment (Jarboui et al., 2010). Because Tunisia is considered one of the main olive oil producers in the world with a yearly average production of 260.000 tons in 2014/2015 crop season (COI, 2015), its oil mills discharge enormous quantities of this effluent every year. In the world, Tunisia is considered among the main olive oil producers with a yearly average production of 260.000 tons in 2014/2015 crop season (COI, 2015). The OMW chemical composition is variable and depends on the olive varieties, the harvesting period and the extraction techniques. It always includes, various simple and complex phenolic compounds, generating antimicro-

Abbreviations: OMW, olive mill wastewater; Pn, net photosynthesis; Gs, stomatal conductance; E, transpiration.

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bial and phytotoxic effects (Hachicha et al., 2009; Barbera et al., 2013). Moreover, it has a high fertilization value due to its richness in water and nutrient resources.

The fertilization effects of the OMW direct application were extensively investigated in agricultural soils (Sierra et al., 2007; Belaqziz et al., 2008). This practice was extended in the Mediterranean region particularly where olive grove soils presented very low organic matter levels and are exposed to progressive degradation processes (López-Piñeiro et al., 2007).

Indeed, Ammar et al. (2005) stated that OMW typically consisted of 83–94% water, 4–16% organic compounds, and 0.4–2.5% minerals. It was also reported to have high levels of potassium, nitrogen, phosphorus, calcium, magnesium, and iron (Sierra et al., 2007). Lesage-Meessen et al. (2001) and Quaratino et al. (2007) showed that its organic fraction included sugars, tannins, polyphenols, polyalcohols, pectins, lipids, and proteins.

Hence, OMW use was recommended for agricultural purposes because of its lower cost as a source of water and nutrients (Belaqziz et al., 2008; Sellami et al., 2008; Piotrowska et al., 2011; Barbera et al., 2013) particularly in arid regions, like Tunisia, suffering from serious water and soil organic matter deficiencies (Hachicha et al., 2009; Magdich et al., 2013). Indeed, the incorporation of this wastewater into the soil may constitute a valuable approach for C sequestration and runoff reduction responsible for soil loss (Lozano-García et al., 2011).

For these reasons, at present more attention is given to better managing the OMW spreading practice to improve production at the lowest cost possible, while maintaining the product quality and preserving the environment as required in terms of sustainable development. Nevertheless, OMW application seems to modify soil/plant relationships. These interactions appear to depend on trees species and the OMW doses (El Hassani et al., 2010). Indeed, Montemurro et al. (2011) emphasized that specific species showed different sensitivities to OMW. Some other studies focused on the effects of OMW on olive trees physiological and biochemical characteristics. In this context, Mechri et al. (2011) studied the effect of only one OMW application on olive trees without exceeding 150 m^3 ha^{-1}. Likewise, Chartzoulakis et al. (2010) investigated the OMW application effects on olive trees after 3 consecutive years of OMW use at increasing rate from 252 m^3 ha^{-1} (one year), to 420 m^3 ha^{-1} (for two years). Unfortunately, they did not assess its biochemical impact on the plants.

However, despite the wealth of information on OMW impact on plants including olive trees, there is a dearth of information on long-term effects of OMW spreading and the optimum doses of this effluent that would enhance the physiological and biochemical parameters of the adult olive tree.

Hence, this paper purports to investigate the long-term effect of spreading OMW on olive tree plantations and the appropriate quantities of this wastewater that would have a positive effect on the physiological parameters such as net photosynthesis, stomatal conductance and transpiration and biochemical properties such as photosynthetic pigments, soluble sugars, total phenols, antioxidant activity and leaf mineral nutrient of these plants.

2. Materials and methods

2.1. Field investigation

In the present study, the experiments were carried out in an olive-tree (*Olea europaea*, L., var. 'Chemlali') plantation at the Taous experimental station of the Olive Tree Institute of Sfax, in central Tunisia (34°43′ N, 10°41′ E). This area of the country has a typical Mediterranean climate, with an average annual rainfall of about 200 mm. The experimental orchard was characterized by a sandy soil (86.6% sand, 13.2% silt and 0.2% clay).

Table 1
Physico-chemical characterization of the raw OMW effluent spread (average of 8 years).

Parameters	Values
Water content (%)	87.60 ± 0.41
Dry matter (%)	12.40 ± 0.20
pH	4.37 ± 0.44
Electric conductivity (mS cm^{-1})	12.89 ± 0.55
Chemical oxygen demand (g L^{-1})	120.00 ± 0.32
Biochemical oxygen demand (g L^{-1})	44.60 ± 1.54
Total phenols (g L^{-1})	1.40 ± 0.42
Organic matter (g L^{-1})	53.00 ± 1.56
Nitrogen (g L^{-1})	1.60 ± 0.20
Phosphorus (g L^{-1})	0.60 ± 0.13
Potassium (g L^{-1})	6.20 ± 0.22
Calcium (g L^{-1})	0.78 ± 0.15
Magnesium (g L^{-1})	0.58 ± 0.14
Sodium (g L^{-1})	1.37 ± 0.11
Chloride (g L^{-1})	0.98 ± 0.16

Values represent means of three replications ± SE.

The field was divided into four plots among which three were treated once a year each February (from 2004 to 2012). The first plot received 50 m^3 ha^{-1}, the second 100 m^3 ha^{-1} and the third 200 m^3 ha^{-1}. The fourth was not submitted to OMW treatment and served as a control. Over the eight-year period, the same raw OMW volume was spread on the surface of the specific plots respecting the defined doses. Each of the four plots covered an area of 1 ha and contained 16 eighty-year old trees, with an inter-tree spacing of 24 m × 24 m. The raw OMW was transported to the field in a tank driven by a tractor, and was spread on the soil uniformly in the corresponding plot at the controlled volumes, at about 4 m of the olives trees to prevent the foliage contact. A homogenization of arable-level was achieved by superficial tillage after each OMW spreading.

2.2. OMW used for experimentation

Fresh OMW collected from a local oil mill in Sfax, Tunisia, close the plantation, was used for field application. This mill processed olives using a three-phase system. The effluent physico-chemical characteristics are presented in Table 1; this revealed an acidic pH and a high salinity as well as an important organic load.

The pH and electrical conductivity were determined according to Sierra et al. (2001) standard method. The dry weight and moisture content were determined by weighing a sample of the effluent before and after drying overnight at 105 °C. Chemical oxygen demand was determined according to the Knechtel method (1978). Biochemical oxygen demand was measured by the manometric method with a respirometer BSB-Controller, Model 620 T. WTW. Organic matter content was determined after heating the sample at 550 °C in a laboratory furnace for 4 h and the phenol content was measured by the Folin-Ciocalteau method (Box, 1983). Total nitrogen was determined by the Kjeldahl method, P was measured calorimetrically, K and Na by flame photometry and Ca and Mg by atomic absorption spectrophotometry (Hitachi U-2000).

2.3. Physiological parameters

At the end of the experiment, leaf gas exchange parameters (net photosynthesis, stomatal conductance and transpiration rates) were carried out using a portable gas exchange system (Li-CorInc 6200). All measurements were conducted on the fully expanded leaves from the median part of the shoot from six plants per treatment on clear days between 0900 and 1200 h during spring period,