

Incidence of dairy wastewater on morphological and physiological compartment of Chemlali and Chetoui olive

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ABSTRACT

In the present study the analysis of the physico-chemical parameters of the collected TWW shows the BOD, COD, COT, Cl⁻, NO₃, NO₂, suspended matter, organic matter, turbidity and conductivity were in accordance with the required Tunisian legislations. In the same occurrence, the ICP-MS and the UPLC-MS/MS analysis show that TWW were devoid of different toxic metals and antibiotics, respectively. We opted to reuse of the TWW in the irrigation of young olive trees of two varieties: *Olea europaea* L. cv. *Chetoui* and *Olea europaea* L. cv. *Chemlali* which receive 1 L/week of TWW during five months. Results show that dry roots weight and the content of chlorophyll a in 'Chetoui' variety increased significantly ($p < 0.05$) when compared to the control group. On the same way, significantly increase of leaf area, dry roots and leaves weights was observed in 'Chemlali' variety treated with TWW.

1. Introduction

Nowadays, many countries face significant problems of water scarcity and quality deterioration and according to Morrison et al. [21] these problems are expected to intensify in the coming decades. The Scarcity of the conventional water resources was the consequence of the increasing of the agricultural use and the human consumption. Moreover, climate change resulting to short duration of rainfall combined with increased evapotranspiration, is expected to lead to groundwater depletion [19]. In another side, the loading of industrial pollutants has potentially more significant impacts on water resources; therefore, the recycling is imperative as much water as possible to minimize his discharge into the environment and to make it reusable.

In arid and semi-arid regions, wastewater reclamation and reuse has become an important element in water resources planning [1]. In particular for agriculture practices, it is encouraged by governments and official entities worldwide [2]. Imperatively, for human health safety and environment protection, wastewater must undergo different treatments to ensure parameter concentrations,

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such as Biochemical Oxygen Demand (BOD₅) and heavy metals, according to the law [23].

In the dairy industry, the requirement of water is huge; it is used throughout all processing steps such as sanitization and cleaning. Furthermore, the liquid effluents are considered as sources of pollution in this industry. To remove pollutants, such as fats, organic matter and suspended solids, effluents must ensure primary then secondary biological treatments [4].

In Tunisia, the reuse of wastewater in agriculture is increasingly extended from 2013. This country contains 65 million olive trees, specially 'Chemlali' cultivar, which grown in south and center of Tunisia and contributes to 80% of oil production. Therefore, oleiculture deals the principal agricultural and economical strategic sectors [7,17]. The second principal oil production variety is 'Chetoui', grown in the north of Tunisia and provides 20% of the national production [3].

The present work was intended to evaluate the irrigation effects with treated wastewater (TWW), collected from dairy industry located in Mahdia city (center of Tunisia), on the 'Chemlali and 'Chetoui' olive vegetative growth. Then, the physiological compartment of these varieties, cultivated in pots under field conditions during a 5-months period, was studied. The results will be discussed in relationship with the physic-chemical parameters, UPLC-MS/MS and ICP-MS analysis.

2. Material and methods

2.1. Study site

The experiment was conducted from February 2016 to April 2016. It was carried out in pots with the TWW collected from dairy central VITALAIT, located in the Mahdia city (35°31N, 10°58E), center of Tunisia.

2.2. Water source

The TWW used in this study was collected from biological wastewater treatment plant (WWTP) of the dairy industry. The WWTP runs on average charge and generate 2000 m³/day. The collected treated wastewater samples were brought to the laboratory and immediately pH and EC (Electrical conductivity) of water samples were measured then this water was analyzed.

2.2.1. Water physic-chemical parameters analysis

In this study,² the BOD, COD, TOC, total suspended solids (TSSs), nitrates (NO₃), and absorbable organically bound halogens (AOX) were determined using a portable UV analyzer (Pastel UV, Secomam, Alès, France). Turbidity and conductivity were determined using AQUALITIC® (Dortmund, Germany); conductivity was measured using a conductimeter WTW 315i and the pH, using pH-meter WTW.

2.2.2. Inductively coupled plasma mass spectrometry analysis

Analyses of lead (Pb), manganese (Mn), zinc (Zn), nickel (Ni), copper (Cu), mercury (Hg), cadmium (Cd), cobalt (Co), iron(Fe), and chromium (Cr) were determined by a inductively coupled plasma mass spectrometer (model JY-2000; HORIBA Jobin Yvon, Switzerland).

Chemicals, standard solution and instrumentation are used in this analysis following the method described by Di Bella et al. [13].

2.3. Analytical study

An analytical study was established according to the method of Zouiten et al. [29] for the research of antibiotics in TWW using the ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

2.4. Plant material and growing conditions

Seven month-old and uniform olives transplants of *Olea europaea* L. cv. *Chemlali* and *Olea europaea* L. cv. *Chetoui* were tested. They are planted in February 2016 and were arranged in a randomized block design with two blocks. Each experimental block was divided in two treatments.

Plants are planted in pots containing a sandy clay soil. Olives were irrigated with TWW or TW using a manual irrigation. The irrigation was applied one time weekly and every plant receives one liter of water.

2.5. Growth and production parameters

- The vegetative growth of each variety was studied every two weeks during the growth phases and many parameters were followed
Branch elongation (cm): measured in three trees per treatment per variety. In each tree, this parameter was taken from two marked branches.
- Leaf area (cm²): determined using the method of Abajingin and Ajayi [30].

The dry and fresh weights were determined according to the method described by Kchaou et al. [31]. In fact, at the end of the experiment, each plant was separated into leaves and roots. Each part was weighted and then washed once with tap water and once with distilled water, dried at 70 °C for 72 h, and the dry weight of each plant part was determined.

Table 1

Physico-chemical characteristics of treated wastewater used in the experiment. TW = irrigated trees with tap water; TWW = irrigated trees with treated wastewater.

Parameter	Unit	TWW	Tunisian limits
pH	–	8.275 ± 0.1	6.50–8.50
EC	mS/m	409 ± 3.10	700
Salinity	‰	2.1 ± 0.02	–
Turbidity	NTU	39.18 ± 1.94	–
Kjeldahl nitrogen	mg/L	13.75 ± 1.10	1
Ammoniacal nitrogen	mg/L	0.808 ± 0.01	–
Cl ⁻	mg/L	379.5 ± 0.02	600
Nitrates	mg/L	0.6 ± 0.01	50
Nitrites	mg/L	0.038 ± 0.01	0.5
Oils and fats	mg/L	18.35 ± 2.34	10
Suspension Matter	mg/L	26.43 ± 1.51	30
COD	mg/L	52.30 ± 0.10	90
BOD ₅	mg/L	22.71 ± 0.03	30
detergents	mgABS/L	< 0.1	0.5

Data represents mean values ± standard deviation. EC: electrical conductivity, COD: chemical oxygen demand, BOD₅: five-day biochemical oxygen demand.

Production parameters were counted in three trees per variety per treatment. In each tree, we worked in two branches [28].

2.6. Biochemical analysis

The chlorophyll content was colorimetrically determined in fresh leaf samples from the two varieties in the end of the experiment as described by Herteman et al. [18].

A spectrophotometric quantification of polyphenols was made in leaves and roots of olive plants after three months of treatment according to the method described by Gbohaïda et al. [15].

2.7. Statistical analysis

Statistical analysis of the results was performed using SPSS standard version 13.0 software. standard deviations show the variability between samples taken individually and the significance of differences between means values was estimated using non parametric Kruskal- Wallis test. It was set at $P < 0.05$.

3. Results

3.1. Treated wastewater quality

The results of the analysis of the dairy central TWW used are reported in Table 1. The pH was 8.27 falling within the 6–9 range, appropriate for irrigation reuse [24]. Electrical conductivity bellowing the recommended Tunisian limits. Results show that the BOD and the COD were below the Tunisian norm for water reuse. On the other hand, the tested TWW contains considerable amount of nitrogen (13.75 mg/L) which is also present in the form of nitrate and in the form of ammonia (0.80 mg/L). The TWW was revealed overloaded by the mineral elements such as P, K, Na, Mg, and Ca compared to TW. Moreover, this water has levels of toxic and potentially toxic elements (Cd, Co, Ni, Pb, Mn, Zn, Cu, Fe and Cr) within the Tunisian limits (Table 2).

3.2. Plant growth and production

3.2.1. Branch elongation

The data presented in Table 3 revealed that the branch elongation of the two varieties varied considerably under the influence of water source that the longer branch is registered in plants irrigated with TW. In another side, ‘Chemlali’ TWW-irrigated olives had branches longer than branches of ‘Chetoui’ TWW-irrigated olives with an average of 16.19 cm.

3.2.2. Leaf area

The data presented in Table 3 revealed a significant variability in ‘Chetoui’ plants in terms of leaf area under the influence of the two water treatments (TW and TWW). Leaf area of ‘Chetoui’ plants receiving TW was higher compared to those receiving TWW (2.35 cm²). It can be observed in the same Figure that it was a significant increase of leaf area in ‘Chemlali’ plants irrigated with TWW as compared to those irrigated with TW. Statistically, the two varieties ‘Chetoui’ and ‘Chemlali’ showed the same response to the TW treatment. But results indicated that, compared with ‘Chetoui’ plants, ‘Chemlali’ plants presented significantly the highest leaf area as response to TWW treatment.

Table 2
Metals concentrations (ppm) in tap water and treated wastewater tested in the experiment.

Parameter	TW	TWW	Tunisian limits
Na	353.13 ± 7.02	1325.21 ± 23.82	–
Mg	14.99 ± 1.04	20.56 ± 0.325	–
P	0.18 ± 0.03	115.65 ± 0.162	–
K	4.50 ± 0.005	35.00 ± 0.318	–
Ca	15.35 ± 0.23	46.25 ± 0.169	–
Mn	< 0.011	< 0.011	0.5
Fe	0.01 ± 0.002	< 0.013	5
Cu	< 0.010	< 0.010	0.5
Zn	0.037 ± 0.004	< 0.012	5
V	< 0.010	< 0.010	–
Cr	< 0.008	< 0.008	0.5
Co	< 0.008	< 0.008	0.1
Ni	< 0.012	< 0.012	0.2
As	< 0.010	< 0.010	–
Se	< 0.014	< 0.014	–
Cd	< 0.009	< 0.009	0.01
Hg	< 0.010	< 0.010	0.001
Pb	< 0.012	< 0.012	1

Data represents mean values ± standard deviation.

Table 3
Branch elongation and leaf area of olive trees grown under TW and TWW irrigation.

	Branch elongation (cm)		Leaf area (cm ²)	
	Chemlali	Chetoui	Chemlali	Chetoui
TW	19.73 ± 0.12 ^{*,**}	14.40 ± 0.60 ^{*,**}	2.37 ± 0.14 [*]	2.35 ± 0.07 [*]
TWW	16.19 ± 0.22 ^{*,**}	2.86 ± 0.15 ^{*,**}	2.83 ± 0.10 ^{*,**}	2.13 ± 0.12 ^{*,**}

Values represent average and standard deviation. Significance of the differences between means values was estimated using non parametric Kruskal-Wallis test.

* Above two treatments in the same variety indicates that means are significantly different ($p < 0.05$).

** Above the same treatment in two varieties indicates that means are significantly different ($p < 0.05$).

3.2.3. Flowering parameters

Flowering parameters showed a water source dependence ($p < 0.05$). Increases in these parameters were observed in the two studied cultivars passing from TW to TWW treatment (Table 4).

3.3. Plant physiological analysis

3.3.1. Dry leaves weight

Concerning dry leaves weight (Table 5) it is evident that the two varieties did not follow the same trend. In 'Chetoui' variety, this parameter was not significantly influenced by the two types of irrigation water. But the 'Chemlali' plants irrigated with TWW, showed a significant higher dry leaves weight compared with the others irrigated with TW. Also, the TWW treatment enhances significantly 'Chetoui' more than 'Chemlali' dry leaves weight.

3.3.2. Dry roots weight

The results obtained in Table 5 indicate that 'Chetoui' and 'Chemlali' samples irrigated with TWW showed a significant enhancement of dry roots weight compared with the TW-irrigated 'Chetoui' and 'Chemlali' plants reaching values of 44.21 and 42.55 g

Table 4
Effect of water irrigation source on production parameters of 'Chemlali' and 'Chetoui' samples.

	Chemlali			Chetoui		
	Flowers clusters number	Flowers buds number	Flowers number	Flowers clusters number	Flowers buds number	Flowers number
TW	25 ± 3a	250 ± 13a	221 ± 10a	1 ± 1a	12 ± 3a	6 ± 2a
TWW	43 ± 5b	475 ± 23b	446 ± 36b	5 ± 2b	58 ± 7b	31 ± 4b

Data represents mean values ± standard deviation. Vertically, values with the same letter are not significantly different at 5% probably level according to the Kruskal-Wallis test.

Table 5
Chemlali and Chetoui physiological parameters contents produced under the two irrigation treatments.

Treatment	Variety	Dry leaves weight (g)	Dry roots weight (g)	Chlorophyll a (mg/kg)	Chlorophyll b (mg/kg)	Total chlorophylls (mg/kg)	Carotenoids (mg/kg)	L-polyphenols (mg/kg)	R-polyphenols (mg/kg)
TW	Chemlali	33.72 ± 0.55 ^{***}	35.18 ± 0.35 ^{***}	440.74 ± 10.25 ^{***}	148.66 ± 0.50 ^{***}	648.82 ± 0.78 ^{***}	40.01 ± 1.26 ^{***}	23,654.18 ± 125.05 ^{***}	5053.09 ± 58.20 ^{***}
	Chetoui	43.93 ± 0.34 ^{***}	41.69 ± 0.54 ^{***}	92.27 ± 1 ^{***}	138.62 ± 0.52 ^{***}	455.33 ± 6.65 ^{***}	175.78 ± 5.35 ^{***}	40,065.18 ± 11.93 ^{***}	8157.17 ± 56.81 ^{***}
TWW	Chemlali	35.31 ± 0.38 ^{***}	42.55 ± 0.47 ^{***}	422.33 ± 11.59	147.23 ± 1.07	635.94 ± 5.11 [*]	38.33 ± 0.44	22,249.04 ± 97.93 [*]	5674.19 ± 104.92 ^{**}
	Chetoui	44.51 ± 0.56 ^{***}	44.21 ± 0.41 ^{***}	223.98 ± 7.65 [*]	122.36 ± 0.60 ^{***}	379.91 ± 7.57 ^{***}	173.54 ± 3.96 ^{***}	37,303.49 ± 280.18 ^{***}	9586.99 ± 631.17 ^{***}

Values represent average and standard deviation. Significance of the differences between means values was estimated using non parametric Kruskal-Wallis test.

* Above two treatments in the same variety indicates that means are significantly different (p < 0.05).

** Above the same treatment in two varieties indicates that means are significantly different (p < 0.05).

respectively. Contrary to TW, TWW increases significantly 'Chetoui' more than 'Chemlali' dry roots weight.

3.3.3. Chlorophylls and carotenoids contents determination

Chlorophyll a, chlorophyll b and total chlorophylls contents of olive leaves are showed in [Table 5](#).

The chlorophyll a amount of 'Chetoui' samples seems to vary with treatment. Indeed, TWW irrigated-plants had significantly ($p < 0.05$) the highest content (223.98 mg/kg). Moreover, we observed that there are no significant differences between irrigation treatments for chlorophyll a content in leaves 'Chemlali' cultivar. TWW treatment didn't vary significantly chlorophyll a values between 'Chetoui' and 'Chemlali' samples.

'Chetoui' plants receiving TWW had less chlorophyll b content than others irrigated with TW () but in 'Chemlali' olives, this value didn't varied remarkably passing from a treatment to another. Statistically, 'Chemlali' cultivar showed the highest amounts in response to TW and TWW compared with 'Chetoui' cultivar.

The total chlorophylls contents of olive leaves are presented in [Table 5](#), these results indicate that leaves of 'Chetoui' receiving TW registered significantly a higher content than those receiving TWW. The quantification of total chlorophylls in 'Chemlali' leaves showed that plants irrigated with TW also had the highest content compared with plants irrigated with TWW and amounted to the value of 648.82 mg/kg. In response to TWW, 'Chemlali' plants marked significantly high total chlorophylls content compared to 'Chetoui' olives, but, the same results were obtained in response to TW.

The two tested varieties showed a no significant variation between the tap water and the treated wastewater treatments in carotenoids leaves content ([Table 5](#)). Statistically, the highest carotenoids content was observed in 'Chetoui' samples in response to TW and TWW treatments compared to 'Chemlali' plants with values of 175.78 mg/kg and 173.54 mg/kg respectively.

3.3.4. Changes in polyphenols content of olive leaf and root extracts following irrigation with TW and TWW

The data presented in [Table 5](#) indicates that leaf polyphenols (L-polyphenols) in TW-irrigated 'Chetoui' and TW-irrigated 'Chemlali' registered the highest contents. 'Chetoui' cultivar was characterized by the significant highest L-polyphenols content in response to TW and TWW compared with 'Chemlali' cultivar.

Root polyphenols (R-polyphenols) were quantified after each treatment and results were shown in [Table 5](#). Significant differences ($p < 0.05$) were encountered for the root 'Chetoui' and 'Chemlali' polyphenols between the two treatments. These two varieties registered the highest contents in response to TWW irrigation (9586.99 and 5674.19 mg/kg respectively). After irrigation with TW or with TWW, the greater value of R-polyphenols was obtained by 'Chetoui' samples.

4. Discussion

Tunisia suffers a very severe fresh water shortage as other Arab countries. The reuse of the non-conventional water seems to be a reliable solution. In the present work, the evaluation of water type tested on olive morphology indicated that it was no positive effect of TWW on 'Chetoui' branch elongation, leaf area, and dry leaves weight. These results indicate a high correlation between growth parameters which may results from the anatomical relationship between different olive tree parts characterizing olive species [20]. On the another hand, this data is in accordance with results obtained by Trad et al. [28] who reported that dry leaves weight of 'Chetoui' cultivar was not significantly influenced by the irrigation with TWW and this parameter is independent of irrigation water source.

Leaf area in 'Chemlali' cultivar irrigated with TWW increases compared with those receiving TW. The results obtained confirm the finding of Charfi et al. [11] who indicated that leaf area of olives irrigated with treated wastewater was higher than those of the control. Thus, the enhancement of leaf area and dry leaves weight can be explained by the TWW wealth containing a significant quantity of nutrients as well as essential elements (N, P, K...) compared to TW [16].

Our data indicates that the flowering was better when 'Chetoui' and 'Chemlali' plants are irrigated with TWW compared with those receiving TW, this result may be correlated with the height nutritive value of this water. The decreasing of branch elongation under TWW treatment and the increasing of flowering parameters may have resulted from competition for assimilates between branch elongation and fructification, our results are in accordance with data obtained by Masmoudi-charfi and Ben Mechlia [20] indicating that in 'Chetoui' cultivar, development of olives from flowering to harvest may affect branch elongation.

In 'Chemlali' variety, the positive effect of wastewater on production parameters has been also reported by Bedbabis et al. [8], it was reflected by a significant yield increasing. Therefore, this water was considered as fertigation stimulator. This data is not in line with results obtained by Alghobar et al. [2], in fact, they founded that irrigation with sewage treated wastewater didn't improve production characters of rice crop.

Under this industrial treated wastewater usage, a significant increase was seen in dry roots weight of 'Chetoui' and 'Chemlali' samples ([Table 5](#)) over that of the control (TW). This increase in 'Chemlali' plants explains the highest root density reaching a value of 0.27 ([Fig. 1](#)), this root density may provide an excellent detection of nutrients especially immobile accumulated near the soil surface. In another side, many investigators have reported a substantial increase in biomass production upon recycled effluent application on several species [32] as well as on olive [11], this finding may not be observed in 'Chetoui' olives with the enhancement of dry roots weight can be explained by pedo-climatic stresses since this cultivar is widespread in the north of Tunisia [9]. The wastewater tested didn't present excessive salts concentrations because at height levels, salts have an adverse effect and they reduce dry biomass, this data was founded by Alghobar and Suresha [2] in rice plants irrigated with an industrial treated wastewater.

Concerning chlorophyll a and b contents, the two tested varieties registered different amounts between treatments. In fact, chlorophyll content is usually affected by various environmental factors such as water [12,33]. Also, this variation can be explained

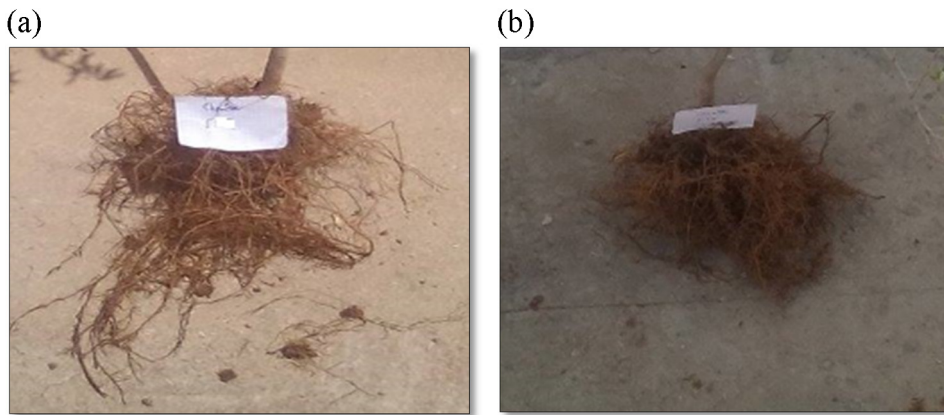


Fig. 1. Root density of ‘Chemlali’ plants irrigated with TW (a) and TWW (b). TW = irrigated trees with tap water; TWW = irrigated trees with treated wastewater.

by genetic factors influencing chlorophyll content, this finding was founded by Bojovic and Markovic [10] on wheat leaf.

‘Chetoui’ cultivars, irrigated with TWW, showed a significant increase of chlorophyll a content compared with those receiving TW treatment to reach a value similar to ‘Chemlali’ cultivar. These findings are in agreement with Herteman et al. [18] who reported that the application of recycled water enhances chlorophyll pigment concentration in mangroves leaves. Wastewater is not only a valuable source of water but also a rich source of nutrients; the concentration and the uptake of these nutrients by plants through irrigation with recycled water were the major factors in enhanced chlorophyll content [6]. The TWW tested in this paper was rich on Mg, known as necessary for the synthesis of chlorophyll [25].

Plant secondary metabolites, especially phenols, confer protection against a wide range of environmental stresses [27]. The significant increase of polyphenols, in ‘Chetoui’ and ‘Chemlali’ roots receiving TWW, may be due to a water stress condition which can lead to the elevation of reactive oxygen species, therefore, higher contents of antioxidants is required to compensate stress condition and increase the tolerance [27]. The literature reported that this enhancement may be due to the metals chelating activity of polyphenols [5]. In contrast, leaves polyphenols contents of the two tested varieties irrigated with TWW were lower than those registered by plants treated with TW. Paiva and Dixon [22] attributed this difference to a nutritional stresses like low nitrogen concentration inducing flavonoids and isoflavonoids synthesis, or in our case, the wastewater tested is rich with nitrogen.

The wastewater has a considerable content of nutrients, therefore, his application improves the plant growth characteristics and increase mineral elements concentration in olive leaves [26]. But, more efforts must be made to explore varietal changes in response to wastewater irrigation in olive cultivars [23].

5. Conclusion

Data resulting from this work improve the fundamental knowledge about the growth and biochemical composition of olive plants in response to TWW irrigation and such a determination is very important to explore varietal changes. In fact, we suggest that olive plants response was not only dependent on the water resource but also on varietal changes.

In Tunisia and in terms of economical weight, oleiculture is one of the principal sectors of agriculture and ‘Chemlali’ variety accounts for 80% of oil production. Under the light of our results, dairy industrial wastewater tested improves biomass production of this variety. Also, there are no significant differences in terms of chlorophyll a and b contents between ‘Chemlali’ receiving TW and ‘Chemlali’ receiving TWW plants. So, this water may be an alternative water source in irrigation of olive orchards under water scarcity situation, especially after showing in this paper that this water does not contain antibiotics. In addition, these findings must be accomplished with an analytical study of olive oil quality. More studies are needed for a clear understanding of the impact of irrigation with this treated wastewater on soil properties and his microbial community.

The present experimental work improves the fundamental knowledge about the growth and biochemical composition of olive plants in response to TWW irrigation and such a determination is very important to explore varietal changes. In Tunisia and in terms of economical weight, oleiculture is one of the principal sectors of agriculture and ‘Chemlali’ variety accounts for 80% of oil production. Under the light of our results, dairy industrial wastewater tested improves biomass production of this variety marked by a dry roots weight of 42.55 g and a dry leaves weight reaching a value of 35.16 g. Also, there are no significant differences in terms of chlorophyll a and b contents between ‘Chemlali’ receiving TW and ‘Chemlali’ receiving TWW plants. So, this water may be an alternative water source in irrigation of olive orchards under water scarcity situation, especially after showing in this paper that this water does not contain antibiotics. In addition, these findings must be accomplished with an analytical study of olive oil quality. More studies are needed for a clear understanding of the impact of irrigation with this treated wastewater on soil properties and his microbial community.

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