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Ecophysiologicals Responses of Olive Trees (*Olea europaea L.*) Hybrid Varieties in Soil Amended with Olive Mill Waste Waters

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Abstract

The main objective of this study was to evaluate the effects of the application of olive mill wastewaters (OMW) at a fixed dose (50 $m^3 ha^{-1}$) on the agronomic development and the eco-physiological responses of three olive trees (*Olea europaea L.*) hybrid varieties grown under Mediterranean arid climate (Tunisia, North of Africa). Results showed that the amendment of the sandy soil by OMW with the dose of 50 $m^3 ha^{-1}$ does not modify its granulometric particle size repartition. The soil water retention capacity (SWRC) was improved. A remarkable richness in soil organic matter (SOM) and a rise in the various mineral elements such as nitrogen, phosphorus, potassium and calcium were recorded. Moreover, the addition of OMW significantly improved the soil biological activity. In relation to the olive trees agronomic development and their eco-physiological responses, our results showed that the three varieties responded positively to the addition of OMW at the above mentioned dose (50 $m^3 ha^{-1}$). The comparison of the different parameters followed shows that the H3 hybrid variety (obtained from a self-crossing of *Chemlali Sfax* with *Chemlali Sfax*) was the most performing variety in terms of agronomic development and ecophysiological adaptation in response to the OMW addition.

Keywords: Olive mill waste waters; *Olea europaea L.*; Hybrid; Soil; Fertilization

Introduction

Olive growing and olive oil production are the most agricultural and economic activities for the Mediterranean countries [1]. In fact, more than 65 % of the total areas planted with olive trees are found in Mediterranean Europe and contribute to 72% of world olive production [2]. The world's olive-growing heritage covers 9.5 million hectares with more than 900 million olive trees, 95% of which are cultivated in the Mediterranean countries [3]. The main producing countries in the world are Spain, Italy, Tunisia and Greece followed to a lesser extent by Turkey, Morocco and Syria [3]. In Tunisia (North of Africa), the cultivation of the olive trees is becoming more and more spacious not only for its socio-economic importance and its adaptation to the arid climate distinguishing the region, but also for the benefits of olive oil, the main oil consumed in the region as well as in the Mediterranean countries [4]. Currently, Tunisia's olive-growing heritage is estimated at more than 88 million trees, occupying 1.8 million hectares of agricultural land and 79 % of arable land [5].

However, in addition to its main product which is olive oil, the olive oil industry generates massive amounts of bio-wastes, mainly olive mill wastewaters (OMW) and solid olive husk (SOH) [6]. Worldwide, more than 30 million tons of OMW were generated annually [7]. In Tunisia alone, olive oil extraction process generates an average annual production of 0.8 10^6 tons of OMW [8]. The chemical composition of olives, which is the raw material for olive oil extraction, is very variable and depends on diverse factors such as the olive variety, soil type and climatic conditions, but in general it consists of 18-28% oil, 40-50% vegetation water and stone and 30-35% of olive pulp [9]. The improper disposal of OMW causes serious environmental impacts to soil, water and air, and related to its high organic content made up largely of phenolic compounds [10]. The OMW phenolic compounds were most often reported to be responsible for its phytotoxicity and antibacterial properties [11-13]. Santi, *et al.* [14] have noted that while OMW represents a source of plant nutrients including organic matter and micronutrients such as N, P, K, Ca, Mg and Fe, its addition to soil can result in the accumulation of salts and phytotoxic compounds and can potentially contaminate aquifers.

Nowadays and in front of the absence of an alternative of depollution /valorization of these biowastes, the uncontrolled disposal of OMW represents a major social, economic, and environmental problem in Mediterranean olive oil producing countries [5]. In this way, many researchers concluded that OMW spreading to agricultural soils could be a successful approach of OMW valorization if spreading was done in controlled conditions and with convenient doses [4,6,13,15-19]. However, the wealth of OMW in water, organic and mineral matter, makes from this biowaste a low-cost soil fertilizer, as well as a source of irrigation water in Mediterranean countries suffering from water scarcity and soil degradation [8,17]. It is true that irrigation with vegetable waters has many beneficial effects on soil and plants, as was pointed out and demonstrated by several research studies [10,20-23]. However, there is still a lack of knowledge about the impact of the use of irrigation water in particular on the agronomic development, the biological and the physiological behavior of young olive trees (*Olea europaea L.*) grown in soil under arid climate soil, as is the case for the Tunisian olive grove.

The main objective of this work was based on previous works, the results of which led to the agronomic valorization of the OMW in ferti-irrigation of Tunisian soils under arid Mediterranean climate (Decree 1306 of February 26, 2013). More specifically, we will focus on the study of the effects of the OMW application at a fixed dose (50 m^3 ha^{-1}) on the granulometric, physicochemical and microbiological composition of a soil of the Sfax region (South of Tunisia) and more specifically on the agronomic development and the eco-physiological responses of three hybrid varieties of olive trees (*Olea europaea L.*) obtained by crossing different varieties cultivated in Tunisia.

Materials and methods

Biological Material

OMW sampling and characterization: The OMW used in this study was obtained from a continuous extraction factory located at Sfax governorate, Tunisia (North Africa). Physicochemical and microbiological analyzes were done on OMW fresh samples in the laboratory. For the determination of the pH and the EC, a standard method was used [24]. Suspended matter was determined by simple filtration. Dry matter (DM) was evaluated by drying a fresh sample of OMW at 105 °C. Organic matter (OM) and mineral matter (MM) were estimated by the difference between DM and the residue after calcinations at 550 °C for 4h. Kjeldahl total nitrogen was assessed according to standard method [25]. The chemical oxygen demand (COD) was determined by application of a standard method [26]. Five-day biochemical oxygen demand (BOD_5) was estimated by the manometric method [27]. The determination of the mineral elements (K, Ca and Na) was carried out by flame photometer from the ashes obtained by the OMW incineration according to [28]. The OMW residual oil content (ROC) was determined according to a standard method [29]. OMW total phenolic compounds were determined by using the Folin-Ciocalteau method [30].

Hybrid Seedlings of Olive Trees: In this work, we worked on three olive trees hybrids (H1, H2, H3) resulting from the crossings between three olive trees varieties (*Chemlali, Chemchali* and *Coratina*). Hybridization consists in crossing two parent varieties in order to obtain hybrids having characteristics better than those of the parent varieties. The three hybrids come from the following crosses:

- Hybrid H1: *Chemlali Sfax* with *Chemchali Gafsa,*
- Hybrid H2: *Chemlali Sfax* with *Coratina,*
- Hybrid H3: *Chemlali Sfax* with *Chemlali Sfax.*

Soil Origin Description and Sampling: Soils samples were collected in February 2018, from the experimental station "Taous" of the Olive Tree Institute of Sfax, Tunisia (North latitude 34° 3', East longitude 10° 20'). Soil samples intended for granulometric and physicochemical analysis, were sieved to 2 mm and dried in ambient air and then stored in plastic bags. A quantity of soils necessary to determine the water content, the mineral nitrogen and the microbiological analyzes were stored at 4 °C.

Experimental Set-Up

The soil collected from an uncultivated plot of the experimental station "Taous" was well homogenized and then distributed in plastic pots. The pots used have a diameter of 30 cm, a depth of 30 cm and a volume capacity of the order of 25 kg of soil. 24 pots were used, with 8 pots for each hybrid variety (H1, H2 and H3). For each hybrid variety, 4 planted pots were irrigated with OMW at a fixed dose (50 m^3 ha^{-1}) and 4 control pots were planted and irrigated with tap water. The amount of OMW added to the soil planted by the different hybrids was calculated on the basis of the pot volumic capacity, which corresponds to 350 ml pot^{-1}. OMW addition was done on February 26, 2018. Each row of pots irrigated by the OMW was compared with a control row irrigated with tap water. After olive trees planting, the irrigation of the various plants with tap water was carried out manually using a watering can with a periodicity of 15 days and at 25% of the soil water retention capacity (SWRC) to avoid soil leaching.

Soil Sampling and Characterization

The sampling of soils was started 30 days after the OMW spreading. Then, soil sampling was done monthly up to 150 days, according to Öhlinger's method [31]. All control and OMW amended soils samples were air dried, sieved at 2 mm and analyzed for granulometric and physicochemical properties. Soil texture was determined using the pipette method [32]. SWRC was evaluated gravimetrically by saturating the soil overnight. The pH and EC were measured using a pH meter and an EC meter, respectively. DM, OM, and MM were determined according to Sierra, *et al.* [24] method. The various soils nitrogen forms as total nitrogen (TN), ammoniacal nitrogen (NH_4-N), and nitrate nitrogen (NO_3-N) were expected according to the Kjeldahl method [25]. Soil respirometric activities were assessed according to [33].

Plants Analyzes

For all the olive plants studied, the following set of parameters were studied

Morphological and Agronomic Parameters: Branch counting was started at planting. During the study period, the number and height of twigs for each plant studied were measured once for every two weeks. Trunk circumference was calculated for all plants every 2 weeks. The evolution of the leaves number was carried out similar to the others morphological and agronomic parameters every two weeks. Measurement of leaf area for each plant was determined according to [34]. The number of inflorescence was evaluated by counting the flowers during the period of their appearance. Only the flowers that give olives (fruits) were then followed for fruit biochemical analyzes.

Biochemical Parameters: For all the olive plants studied, 03 leaves of each plant (12 leaves of each row of hybrids) were taken on July 20, 2018. The yield of fresh material was evaluated directly by simply weighing a fixed number of sheets using a precision balance. The dry matter yield was determined by drying the fresh leaves at 70°C for a few days until all the water contained in the leaves evaporates. For chlorophyll pigments, the chlorophyll a (chl a), b (chl b) and carotenoid (carot) were evaluated according to [35]. For the leaves soluble sugars and starch contents, the method of [36] was used. The organic nitrogen and protein contents in the aerial parts as well as the root parts were determined according to [8].

Statistical Analyses

All the parameters studied were carried out using SPSS software (Statistical Package for the Social Sciences, version 20). Results are expressed in mean standard deviation, using analysis of variance ANOVA.

Results and Discussion

Characteristics of OMW

Parameters	Values± SD
pH (25°C)	3.74 ± 0.02
EC (mS cm^{-1})	9.7 ± 0.05
Water content (%)	94.6 ± 1
Suspended matter (g L^{-1})	30.08 ± 1
Dry matter (g L^{-1})	54 ± 1
Organic matter (g L^{-1})	35.3 ± 1
Mineral matter (g L^{-1})	18.7 ± 0.5
TOC (g L^{-1})	20.52 ± 0.5
COD (g L^{-1})	78 ± 1
BOD5 (g L^{-1})	19± 0.5
BOD5 /COD	0.24± 0.01
Nitrogen (g L^{-1})	0.52± 0.02
Carbon/ Nitrogen	39.46 ± 0.5
K (g L^{-1})	6.5± 0.1
P (g L^{-1})	0.52 ± 0.01
Na (g L^{-1})	0.8 ± 0.02
Cl (g L^{-1})	0.72 ± 0.02
Ca (g L^{-1})	0.68 ± 0.02
Mg (g L^{-1})	0.58 ± 0.02
ROC (%)	1.21± 0.05
Phenolic compounds (g L^{-1})	2.1 ± 0.1
Total microflora (103 CFU. ml^{-1})	233 ± 5
Fungi (102 CFU. ml^{-1})	122 ± 2

SD: Standard deviation (P≤0.05)

Table 1: OMW characteristics (average values of three replications ± SD)

OMW are the liquid by-products of the olive industry. OMW used in the present work comes from a three phase's continuous extraction system. The main physico-chemical and microbiological parameters of the OMW used are summarized in Table 1. As shown in such table, these effluents are characterized by their acidity (pH = 3.74). This acidity is mainly due to their richness in organic acids [23]. In addition to their acidity, OMW are known by their high salinity which results in a high value of their electrical conductivity (EC = 9.7 mS $cm^{-1)}$. The EC value reflects the high OMW salts content. Our results are in agreement with the previous results mentioned [17, 37]. The studied OMW are moderately rich in organic compounds, which can be explained by their relatively high COD (78 g L^{-1}) and their relatively low

BOD_5 (19 g L^{-1}). On the other hand, these effluents showed high potassium content (6.5 g L^{-1}) as well as relatively high concentrations of sodium, calcium and phosphorus (Table 1). In fact, the OMW mineral content depends closely on the olives variety, the degree of olives maturity, the conditions of conservation and the olive oil extraction system [38].

Organic matter (OM) accounts for more than 60% of dry matter (DM) in OMW. The OMW contents in DM and OM are respectively of the order of 54 g L^{-1} and 35.3 g L^{-1}. Such values are in line with those cited by others studies done on Tunisian OMW for more than 20 years [39]. In addition, OMW contains large amounts of suspended matter (SM) (30.08 g L^{-1}) and high phenolic compounds levels, which gives them antimicrobial potency [40]. The rate of OMW residual oil content (ROC = 1.21%) is relatively high [29]. It is well known that OMW can no longer be a medium for microorganism's culture [24,41]. Microbiological analyzes showed that only a few microorganisms are able to grow in OMW. They are mainly fungi (yeasts and molds), which due to their acidophilic and halophilic characteristics, can adapt to the high acidity and salinity of OMW [37,41].

OMW Effects on Control Soil Characteristics

All the granulometric and physicochemical characteristics of the control soil used are summarized in Table 2. The analysis of the values shows that the texture of the studied soil of the "Taous" station is of a sandy nature (sand = 86.7%), with a relatively alkaline pH (8.6). The soil organic matter content is very low (SOM = 0.45%), which confirms previous works that proves that Tunisian soils are very poor in organic matter, whose content not exceeding 2% [42]. The soil electrical conductivity (SEC) is of the order of 324 µS cm^{-1}, reflecting a deficiency of mineral elements. The soil total nitrogen is of the order of 0.17 g kg^{-1}, which means the poverty of the soil studied in nitrogen. In addition, the control soil shows a low cationic exchange capacity (CEC = 3.18 meq100 g^{-1}) which can be explained by the low soil content of humic compounds [43].The effects of OMW added at the above mentioned dose (50 m^3 ha^{-1}) on the soil granulometric, physicochemical and microbiological characteristics have been studied. Results showed that the addition of OMW does not illustrate any significant effects on the particle size distribution of the soil studied (Table 2). In fact, the grain size of the soil remains predominantly "sandy". However, the addition of OMW influences the percentage distribution of the various fractions analyzed (sand, silt and clay).

Thus, the silty and clay fractions showed a small increase, but which remain insignificant, not modifying the sandy granulometric nature of the control soil. These results are in agreement with those demonstrated by [44] who demonstrate that the effect of OMW on the structural and textural properties of sandy soil is closely related to the amount of OMW added. In relation to the soil physicochemical characteristics, the monitoring of the evolution of the pH of the OMW amended soil (S_{50}) during the incubation time proves that the pH values remain in the zone of neutrality and decrease slightly compared to the control soil (S_C). The decrease in pH values is much more noticeable after 70 days of OMW addition. However, after 150 days of incubation, this decrease in pH is moderated by the buffering effect of the soil and the recorded values are aligned with the pH of the control soil (Table 2). Our results are in agreement with previous results, which prove that the pH of the OMW amended soils still remains close to neutrality thanks to the buffer effect of the soil [45,46].

Soil characteristics	S_C ± SD	S_{50} ± SD
Sand (%)	86.7 ± 1	83.2 ± 1
Clay (%)	11.4 ± 0.5	13.6 ± 0.5
Silt (%)	1.9 ± 0.1	3.2 ± 0.1
pH (25°C)	8.6 ± 0.2	8.25 ± 0.2
EC (µS cm^{-1})	324 ± 2	570 ± 5
SWRC (%)	31.28 ± 0.5	42.7 ± 0.5
CEC (meq% DM)	3.18 ± 0.2	5.1 ± 0.2
OM (g Kg^{-1} DM)	4.5 ± 0.05	8.2 ± 0.05
TOC (g Kg^{-1} DM)	2.6 ± 0.02	4.73 ± 0.02
TN (g Kg^{-1} DM)	0.17 ± 0.01	0.29 ± 0.01
Organic Nitrogen	0.15 ± 0.01	0.21 ± 0.01
Mineral Nitrogen	0.02 ± 0.001	0.08 ± 0.01
C/N ratio	15.3 ± 2	16.31± 2
P (g Kg^{-1} DM)	0.068 ± 0.01	0.083 ± 0.01
K (g Kg^{-1} DM)	0.53 ± 0.05	1.19 ± 0.05
Na (g Kg^{-1} DM)	0.33 ± 0.02	0.58 ± 0.02
Ca (g Kg^{-1} DM)	0.27 ± 0.02	0.39 ± 0.02

SD: Standard deviation (P≤0.05)

Table 2: OMW Effects on the soil control granulometric and physicochemical characteristics after 150 incubation days (average values of three replications ± SD)

The measurement of the soil electrical conductivity shows an increase in the OMW amended soil EC. This elevation can be explained mainly by the OMW high salinity and their richness in mineral elements (9.7 mS cm^{-1}). However, the decrease in OMW amended soils EC observed after 150 days of incubation can be explained by the salts infiltration as well as the capacity of soil to neutralize salts. These results are consistent with previous findings [7,47]. The measurement of the soil water retention capacity (SWRC) of the different soils studied, as a function of the incubation time, shows that the addition of OMW increases the SWRC in comparison with that of the control soil. This increase becomes clearly perceptible after two months of OMW incubation. Indeed, the richness of OMW in suspended and colloidal materials increases the adsorption capacity of the soil aggregates which reduces the infiltration of water (Table 2). These results confirmed the findings of [48,49] who demonstrates that the microporosity of soil aggregates increases significantly after the addition of OMW to the soil.

The soil cationic exchange capacity (CEC) reflects its fertility by indicating the nutrient retention capacity. The results clearly showed that the addition of OMW has a significant positive effect on this soil characteristic. This CEC remains important over the incubation time compared to that of the control soil (Table 2). In this context, [44] demonstrated that the addition of OMW improves the soil cation exchange and its adsorption capacity. Soil organic matter (SOM) is composed of living organisms, plant and animal residues and decomposing products. Results showed that the addition of OMW increases in a significant manner the SOM. Still in comparison with the control soil, the application of OMW increases the soil total organic carbon (Table 2). These results are consistent with several previous findings [50-52].

The soil total nitrogen (STN) is composed of the sum of its organic and inorganic forms. The effects of the OMW addition on soil nitrogen content in its various forms are summarized in Table 2. Data showed that the major fraction of the soil nitrogen is in the organic form. The mineral fraction is low for all soils with a significant difference between the control soil and the amended soils. After 150 days of application of OMW, we observed an increase in mineral nitrogen levels in the amended soils compared to the control soil. This can be explained by the proliferation of nitrifying microorganisms in the amended soils, which are involved in the mineralization of OMW organic nitrogen [51, 52]. OMW are rich in mineral elements mainly in potassium element (6.5 g L^{-1}). The application of OMW modifies the mineral content of the control soil in a noticeable way (Table 2). The decrease in calcium (Ca) and sodium (Na) contents after 150 days of OMW addition can be explained by the infiltration of salts at depth following successive irrigations with water [24,46,52,53].

Concerning the effects of OMW on soil microbiological activity, the monitoring of soil respirometric activity provides an overall idea of the capacity of the native soil microflora in the added organic matter mineralization [5,8]. The respirometric technique also makes it possible to evaluate the degree of recalcitrance of the exogenous organic matter brought to the soil as well as the biodegradability potential of this OM by the autochthonous microflora of the soil [33]. It is in this context that we focused the evaluation of the cumulative respiratory activity of the amended soils (S_{50}) in comparison with the control soil (S_C), 90 days after the OMW addition and during 28 days of incubation. Results showed that the organic matter mineralization kinetics in terms of cumulative C-CO_2 is proportional to the OMW added and the incubation time (Figure 1). On the other hand, the monitoring of the specific respiratory activity seems essential to carefully evaluate the evolution of microbial respiration in relation to the soil organic matter nature and content.

This is why we must always think in terms of specific breathing (C-CO_2 / TOC), since it allows us to evaluate the capacity of the soil microflora to biodegrade the added organic matter as well as to evaluate the degree of biodegradability of this organic matter. The soils amended with OMW showed a greater respiratory activity in comparison with the control soil. However, the specific respiration (C-CO_2 / TOC) of the different soils in relation to the content of organic matter shows a remarkable decrease in the C-CO / TOC ratio for the OMW amended soil (S_{50}) compared to the control soil (S_c) (Figure 1). These findings are consistent with many previous studies, which show that OMW spreading enriches soils with organic matter, which enhances soil respiration activity accompanied by decreased specific respiration [13,54].

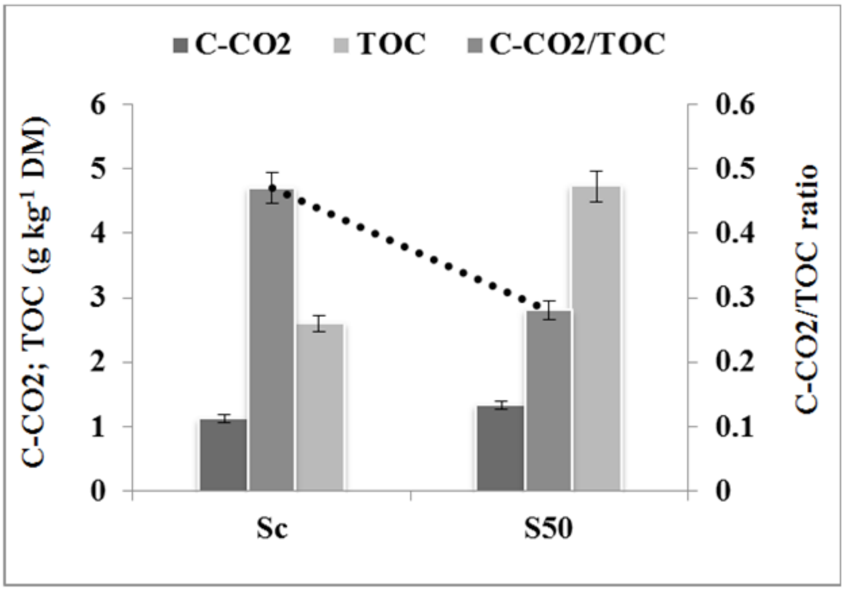

Figure 1: Respirometric activities (as cumulative C–CO_2) and specific respiration (as C–CO_2/TOC) in OMW treated soil (S_{50}) in comparison with the soil control (S_c)

Olive Trees Hybrids: Selection Criteria and Agronomic Characteristics

In Tunisia, the variety of olive tree *Chemlali* is the most commonly cultivated variety. It is present in almost 85% of Tunisian olive plantations and accounts for almost 80% of the national production of olive oil [55]. However, this variety has a disadvantage that manifests essentially in the acid composition of its olive oil. Indeed in olive oil "*Chemlali*" the percentage of oleic acid is < 65% while the percentage of palmitic acid is > 15% (and can reach 27% whereas it must be < 15%) [56]. It is for this reason that the researchers of the Olive Tree Institute (Sfax, Tunisia) have resorted to the hybridization of this variety in order to improve the acidic ratio (oleic acid / palmitic acid) and that the objective is to better valorize the *Chemlali* olive oil [55]. Hybridization consists in crossing two olive trees parents in order to obtain hybrids with characteristics better than those of both parents. The varieties tested in this work are hybrids from the following crosses: the hybrid H1 (*Chemlali Sfax* with *Chemchali Gafsa*), the hybrid H2 (*Chemlali Sfax* with *Coratina*) and the hybrid H3 (*Chemlali Sfax* with *Chemlali Sfax*).

Effects of OMW on the Morphological and Agronomics Parameters of the Hybrids Olive Trees

The effects of OMW on some morphological and agronomic parameters as plant height growth, trunks circumferences, twig growths, leaves number and surfaces evolution are studied along the study period (Figure 2). The follow-up of plant height growth by measuring the shoot elongation of the various plants cultivated in the presence of OMW in comparison with those cultivated in control soil was carried out for 150 days. Results showed that the height of the various plants was not significantly influenced by the OMW addition (Figure 2). However, the comparison of the height growth of the different varieties shows that the H3 hybrid variety appears the most distinguished growth essentially in the presence of OMW ($H3_{50}$) and this in comparison with the same variety grown in control soil ($H3_c$) and with the other two hybrid varieties (H1 and H2). The trunk circumference constitutes an important morphological parameter in the development and growth of the olive plants [57]. The monitoring of this parameter was performed in parallel with the follow-up of height elongation for 150 days.

Results showed that the different hybrid varieties respond differently with respect to growth in their trunks circumferences (Figure 2). Thus for H1 and H3, the plants grown in OMW amended soil ($H1_{50}$ and $H3_{50}$) showed a greater circumferential growth than plants grown in control soil ($H1_c$ and $H3_c$), while the H2c shows a higher growth than the $H2_{50}$. However, the H3 hybrids showed the highest values in trunks circumference, which confirms their highest heights growth (Figure 2). The twigs are large branches of the trunk that give the general shape to the olive tree and their number varies according to the size class of the olive tree [58]. The monitoring of the twigs number clearly shows that the addition of OMW stimulates plant branching for the different varieties studied (Figure 2). Also and as revealed in the others parameters, the variety H3 present the highest twigs number compared to the other two varieties H1 and H2. The study of the leaves number evolution of the various hybrids shows that the plants cultivated in the presence of OMW present the most important leaves numbers and this is valuable for the three varieties studied ($H1_{50}$, $H2_{50}$ and $H3_{50}$) in comparison with the same plants grown in control soils (Figure 2).

Indeed, the comparison between the varieties shows that the hybrids H2 and H3 have comparable leaves numbers and that are larger than of the variety H1. Likewise, the follow-up of the leaves surfaces evolution gives results which confirm those of the leaves numbers evolution. Thus, the varieties $H3_{50}$ and $H2_{50}$ showed the most developed leaves surfaces in comparison with the same plants cultivated in control soil ($H3_c$, $H2_c$) and with the H1 variety grown in the presence or absence of OMW (Figure 2). Such results confirmed that the shape and the leaves areas depended closely on the olive tree variety and not on the soil type [58].

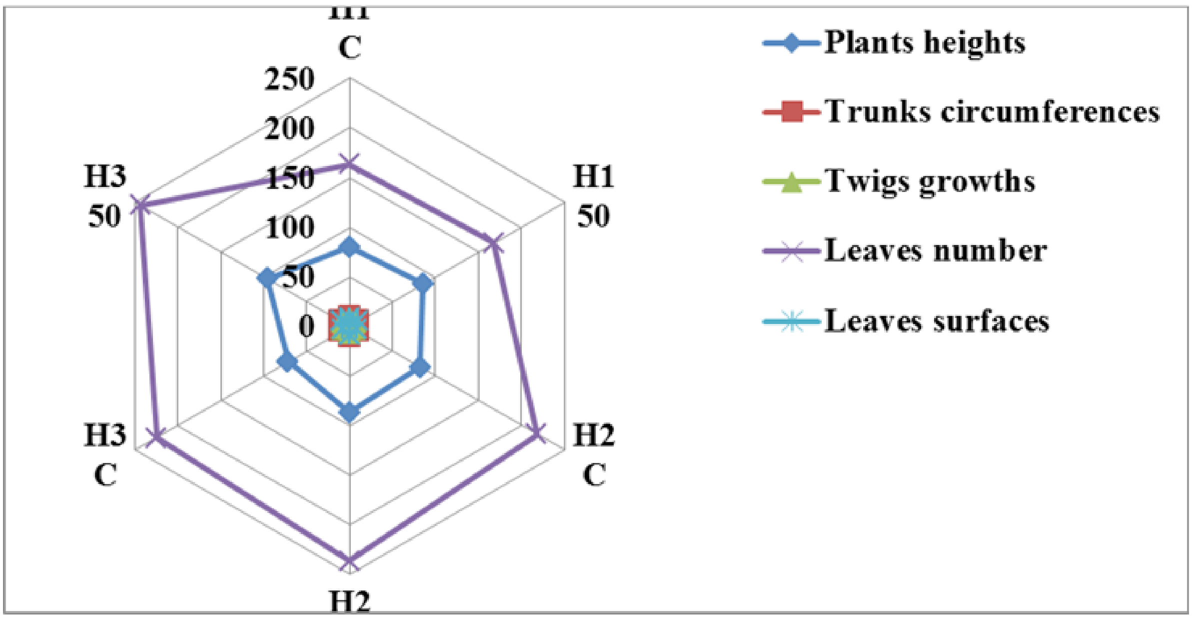

Figure 2: Evolution of the morphological and agronomics parameters of the hybrids olive trees (as: plants heights (cm), trunks circumferences (mm), twigs growths, leaves number and leaves surfaces (cm^2)) in OMW treated soil (S_{50}) in comparison with the soil control (S_c) and after 150 incubation days

Effects of OMW on the Morphological and Agronomics Parameters of the Hybrids Olive Trees

The effects of OMW on some biochemicals parameters such as the analysis of mineral elements in the leaves, the quality of the olives, the olives chlorophyll and ß-carotene content, the olives reducing sugar and starch content and also the organic nitrogen and protein content were studied at the end of the growing season. The analysis of the phosphorus (P), potassium (K), calcium (Ca) and sodium (Na) elements in the leaves of the various plants studied was carried out after 150 days of culture. Results clearly showed the influence of OMW on the leaves content in these elements (Figure 3). Thus, in all the hybrid varieties studied, we found that the plants grown in the presence of OMW have the highest levels mineral elements contents, compared to the same plants grown in control soils. Moreover and as noted previously for the other parameters mentioned above, the leaves of the H3 variety have the highest levels of different mineral elements studied (Figure 3). These results can be explained by the significant contribution of OMW mineral elements to the soil and plants metabolism variability [4,8].

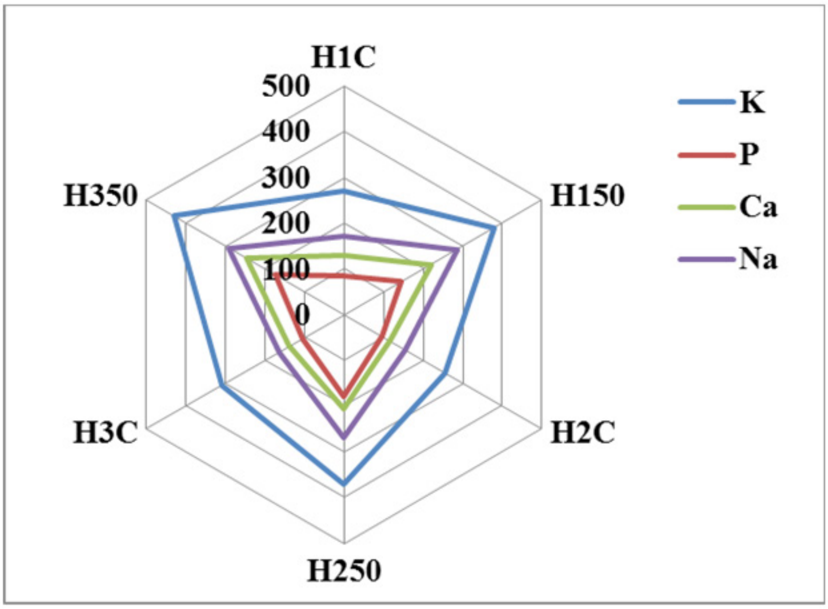

Figure 3: Evolution of the main minerals elements amounts (K (ppm); P (ppm); Ca (ppm) and Na (ppm)) in the hybrids olive trees leaves after 150 incubation days

The "olive" fruit is a fleshy mesocarp drupe, rich in lipids, its shape is ovoid or ellipsoid and its dimensions are very variable according to the varieties [57]. The study of the effect of OMW on the fruit quality of different hybrids studied showed firstly that only the hybrids H1 and H3 which gave fruits, whereas for the variety H2, we did not obtain the fruit in the presence or absence of OMW (Figure 4). For the H1 and H3 varieties, the results showed that the plants grown in the presence of OMW have slightly higher fruit weights than plants grown in control soils. On the other hand, the fruits of the H1 hybrids have larger masses than those of the H3 hybrids. In contrast, the core / fruit ratios are higher in H3 than in H1. Thus, although OMW are known by their contribution to fertilizing elements that are useful for plants and their fruits, they depend closely on the variety, which explains the differences observed [4,29]. Chlorophyll is the main assimilating pigment of photosynthetic plants. This pigment, located in the chloroplasts of plant cells, intervenes in photosynthesis to intercept light energy, the first step in the conversion of this energy into chemical energy [35].

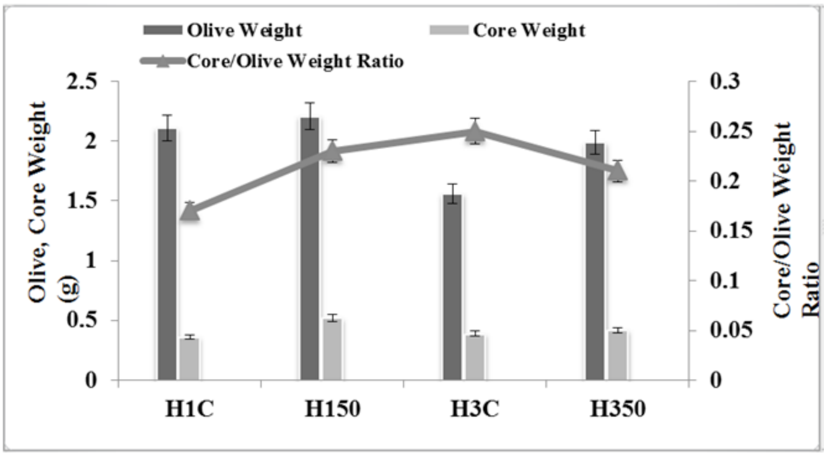

Figure 4: Effects of OMW on the fruit quality (olive weight (g); core weight (g); core/olive weight ratio) in the hybrids olive trees H1 and H3

The Figure 5 illustrates the incidence of OMW addition on the content of hybrids olives leaves in these photosynthetic pigments. Results showed that the levels of chlorophyll a, chlorophyll b, chlorophyll (a + b) and β-carotenoids of the different hybrids H1, H2 and H3 are comparable either in the presence or absence of the OMW, which shows that the OMW have not influenced the content of the leaves in these compounds. Moreover, the variety H1 shows the highest values in these pigments regardless of the state of the soil (amended or not). The recorded results are concordant and are found in the same intervals as those made on olive plants grown in sandy soil under arid conditions [59]. The fresh olive leaves are characterized by a dry matter around 50% [56]. The chemical composition of the leaves varies according to many factors: variety, climatic conditions, time of harvest and age of plantations [60]. The leaves are particularly rich in carbohydrates. Organic matter consists of proteins, lipids, phenolic monomers and polymers (such as tannins) and mainly polysaccharides (such as cellulose, hemicellulose, starch).

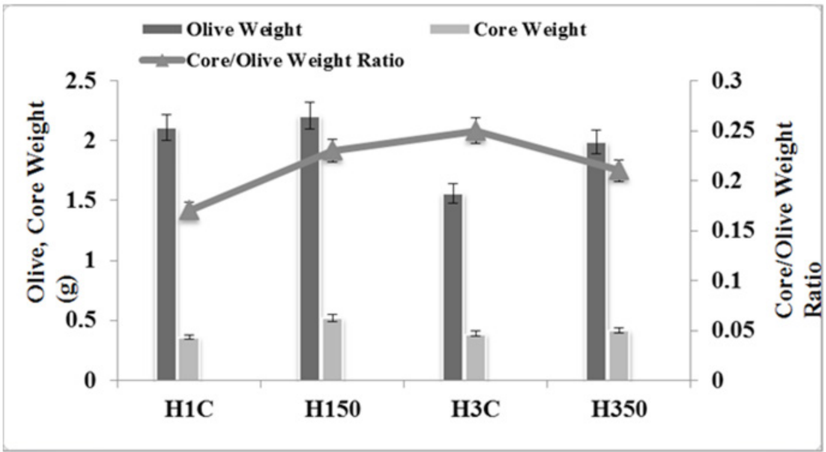

Figure 5: Evolution of the chlorophyll pigments amounts (Chl a; Chl b; Chl (a+b) and β carotene) in the hybrids olive trees leaves after 150 incubation days

The Figure 6 summarizes the contents of the different leaves of the hybrids studied in reducing sugars, starch, organic nitrogen and proteins. As shown in that figure, plants grown in OMW amended soils have significantly higher values for these mentioned compounds. In fact, the richness of OMW essentially in carbohydrates enriches the soil in these compounds which has positive effects on the composition of the plants leaves. As shown in such Figure 6, and given the contribution of OMW in nitrogenous organic matter, the plants grown in the presence of OMW showed higher levels of organic nitrogen than those of control plants. Besides, knowing that the organic nitrogen reveals the protein content (proteins (mg g^{-1}) = 6.5 x [Organic nitrogen]), our results clearly showed the improvement in the protein content of the different hybrids grown in OMW amended soils. In this context, [21] showed that OMW bring a surplus of nitrogen to the soil which stimulates the nitrogen assimilation of plants. In addition, [22] reported that protein levels in spearmint plants treated with OMW were higher than in untreated control plants.

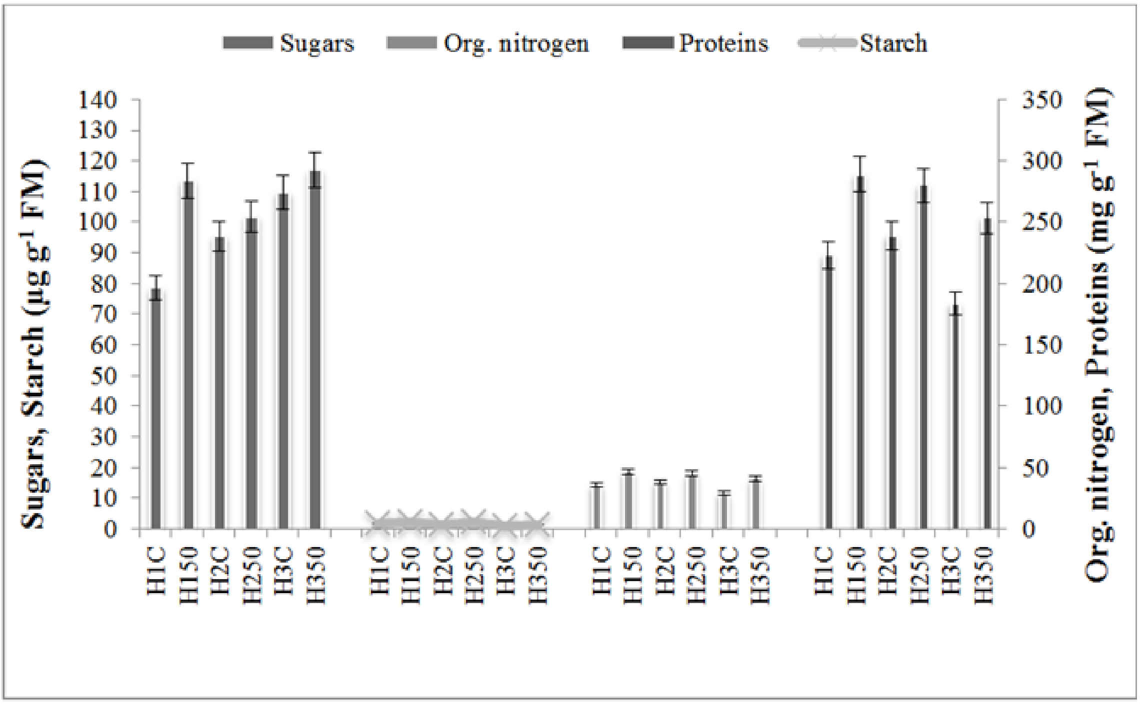

Figure 6: Evolution of some biochemical parameters (sugars; starch, organic nitrogen, proteins) in the hybrids olive trees leaves after 150 incubation days

Conclusion

In this research work, we are interested in the OMW agronomic valorization in the soils and the olive trees (*Olea europaea L.*) fertigation. Our results showed that the application of OMW with the dose of 50 m^3 ha^{-1} does not change in a remarkable manner the grain size nature of the control soil which remains sandy. However, the OMW addition improves the soil water retention capacity. A remarkable richness in the soil organic matter content and a rise in the concentrations of the various mineral elements have been recorded. Results showed also a significant improvement of the soil biological activity which is strongly correlated with soil microflora enrichment. Monitoring the evolution of the different morphological, agronomic and biochemical parameters of the olive trees hybrid plants shows that the three varieties studied respond positively to the OMW addition. Indeed, no inhibitory effect was detected by comparing the development of plants grown on unamended soils to those grown on OMW amended soils. Besides, the comparison of the different parameters followed between the varieties shows that the hybrid variety H3 is the most efficient. Following the results obtained, we can conclude that the application of OMW at a rate of 50 m^3 ha^{-1} is a beneficial solution for the OMW valorization and also for the fertilization of the soil and olive trees under Mediterranean arid climate.

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