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The Combined Effect of Mycorrhization and Olive Mill Wastewater on Biomass Productivity and Physiological Performance in Young Olive Plants

Abderrahmane Yaakoubi[1, 2*], Badia Aganchich [2], Raja Ben Laouane[2,3], Meddich Abdelilah[2]

[1] Department of Biology, Biochemistry and natural resources research team, Moulay Ismail University, Faculty of Science and Technology, BP 509, Boutalamine, Errachidia, Morocco

[2] Department of Biology, Biotechnology and Plant Physiology Unit, Cadi Ayyad University, Faculty of Science Semlalia, BP 2390, Marrakesh, Morocco

[3] Laboratory of Environment and Health, Department of Biology, Moulay Ismail University, Faculty of Sciences & Techniques, Errachidia, Morocco

* Corresponding author's e-mail: a.yaakoubi@umi.ac.ma

ABSTRACT

The study aims to determine the optimal dose of olive mill wastewater (OMW) for maximum mycorrhization and to assess the biomass, physiological, and biochemical tolerance of mycorrhizal olive plants to stress induced by this wastewater. The study involved injecting *Rhizophagus irregularis* into young olive trees (*Olea europaea* L.) under greenhouse conditions. At rates of 100, 150, and 200 m³/ha, OMW is applied to the soil surrounding each olive tree. For comparison, a non-inoculated group was added as a control. Dry matter, mycorrhization rate, leaf relative water content (RWC), foliar nutrients (N, P, and K), and a number of physiological and biochemical parameters were among the variables that were assessed. When arbuscular mycorrhizal fungi and olive mill wastewater were combined at a rate of 100 m³/ha, the results showed significant increases in dry matter (22.5%), Leaf relative water content (16.32%), potassium levels (38.46%), stomatal conductance (30.4%), and photosynthesis compared to the control. However, high olive mill wastewater doses (150 and 200 m³/ha) caused water stress: Compared to non-mycorrhizal plants (NM), the decrease in mycorrhizal plants (M) was less pronounced. Leaf relative water content dropped by 14.9% and 21.27% in mycorrhizal plants and by 22.22% and 28.88% in non-mycorrhizal plants. Under these high doses of Olive mill wastewater, the mycorrhization rate dropped significantly by as much as 30% and 45% respectively. All OMW treatments increased proline and sugar content as stress responses. Consequently, our study reveals that the optimal application of 100 m³/ha of OMW, combined with *Rhizophagus irregularis*, enhances the growth and performance physiology of young olive plants while increasing mycorrhization rates, stomatal conductance, and photosynthetic efficiency. This synergy between OMW and AMF also promotes better plant resilience to water stress by activating biochemical mechanisms such as increased proline and sugar levels. Additionally, AMF mitigates the negative effects of high OMW doses, highlighting its crucial role in managing the risks associated with wastewater application.

Keywords: olive mill, wastewater, arbuscular mycorrhizal fungi, olive plants, photosynthetic.

INTRODUCTION

The continuous three-phase method of extracting olive oil generates a significant volume of liquid waste known as olive mill wastewater (OMW). This by-product is created in the course of making olive oil. Roughly 30 million m³ of OMW are produced worldwide, while 580,000 m³ are produced in Morocco (Chatzistathis and al., 2017). This effluent's high concentration of organic matter and polyphenolic chemicals qualify it as hazardous waste (Hamimed et al., 2022). Despite its negative effects on the environment, there is a chance to recycle OMW highlights as an inexpensive biofertilizer because of their large volume and richness in organic matter and important soil nutrients like nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg) (Chaari et al., 2015; Magdich et al., 2016; Dakhli and Maalej, 2017). Furthermore, Mediterranean nations dealing with water scarcity and soil deterioration might use this effluent as an irrigation supply (Belaqziz et al., 2016; Ahmali et al., 2020). This suggests that OMW can be an effective tool for optimizing nutrient management in crops, promoting better growth and yield. The application of 50 and 100 m^3 ha^{-1} of olive mill effluent does not seem to provide any problems for environmental pollution, according to other authors (Mechri et al., 2011). In 60 days after treatment, the phenolic compounds from OMWs break down, unless an excessive amount (300 m^3/ha) is applied, which is not advised for plant nutrition (Sahraoui et al., 2012). In addition, OMW has been demonstrated to boost the growth and output of vital crops like wheat, olive trees, tomatoes, strawberries, cucumbers, and peppers (Tubeileh and Abdeen, 2017; Khalil et al., 2021). In order to restore soil fertility and preserve water, recycling organic wastes like OMW can be a helpful tactic (Barbera et al., 2013; Tubeileh and Abdeen, 2017). Furthermore, beneficial microbes such as arbuscular mycorrhizal fungi (AMF) may be able to help plants withstand high concentrations of olive mill effluent by improving their physiological function. Lahbouki et al. (2021) and Anli et al. (2022) have provided evidence of the significance of AMF for plant growth, demonstrating how these associations enhance nutrient uptake and increase tolerance to environmental stress, leading to increased growth and yields. Furthermore, through increasing vital nutrient levels, AMF has been demonstrated in several studies to increase soil and plant productivity (Djouhou et al. 2019; Ben-Laouane et al. 2020). If properly regulated and with the right application rates, the agricultural use of OMW with AMF presents a great option for valorizing these effluents. However, reluctance to use this technology has been brought on by worries about possible phytotoxic effects on crops. This study was carried out in a greenhouse to comprehensively analyze the influence of effluent application on plant and soil health in order to resolve these issues, which frequently result from divergent opinions on OMW use in agriculture. Clear statistics and useful recommendations for the ethical and advantageous use of OMW in agriculture are the main objectives. In this regard, we will investigate how OMW and mycorrhizal fungi interact to affect photosynthesis and root absorption in young olive plants (*Olea europaea* L., Moroccan picholine).

MATERIALS AND METHODS

OMW and AMF materials

Source and characterization of OMW and soil used

During the 2018–2019 olive harvest, OMW samples from an olive oil mill (three-phase system, Moroccan Picholine variety) in El Attaouia, Marrakech-Safi, Morocco, underwent 30 days of natural pretreatment in the storage basin. The Physicochemical properties of OMW are: pH: 5.04, EC: 19 mS/cm at 20°C, COD: 85 g/L, DBO5: 31.25 g/L, residual oil: 0.31, total phenols: 3.95 g of caffeic acid/L, N 0.39 g/L, P 0.58 g/L, K 5.55 g/L. The pH was measured using a HANNA HI 2209 pH meter. The electrical conductivity (EC) was determined with a HANNA EC 215 conductivity meter. The residual oil (RO) in the OMW dry matter was determined by hexane at a temperature of 40–60 °C using a Soxhlet apparatus. The COD was determined by the closed reflux dichromate colorimetric method. The BOD5 (AFNOR T90-103) was determined using a BOD meter. The phenolic compounds, expressed as caffeic acid equivalents, were determined by the Folin-Ciocalteu reagent method (Macheix, 1990). Kjeldahl N and total P, according to Afnor methods (1983). Potassium (K) was measured using the JENWAY PFP7, a flame photometer.

Soil samples of each treatment were collected at 10 to 30 cm from the Community Nursery on the Essaouira road, 10 km from Marrakech. The physicochemical characteristics of the soil at this site are as follows: pH: 8.72, EC (µS cm^{-1}: 330, OM %: 0.42, TOC %: 0.24 and Soil water retention capacity (SWRC) % : 20.05, N: 146 mg/kg (DM), P: 55 mg/kg (DM), K:155 mg/kg (DM). The pH and electrical conductivity (EC) were measured in a 1:2 (w:v) solution. Total organic carbon and organic matter were determined using the Anne titrimetric method (Nelson and Sommers, 1996). Available P was determined as described by Olsen et al. 1965. Available potassium (K) was mesured as described byKnudsen, 1982. Soil water retention capacity (SWRC) was determined as indicated by Klute, 1986.

Origin of the AMF

An exogenous strain of AMF (*Rhizoglomus irregulare*, DAOM 197198) was used in our experiment, supplied by the Plant Biotechnology Institute of Montreal (Canada). The inoculum was enriched with propagules by co-culturing with *Zea mays* L. as the host plant under controlled conditions in a greenhouse. Maize roots containing hyphae, vesicles, and spores were harvested, cut into small pieces, and used as inoculum. Olive seedlings were inoculated by adding 5 g of inoculum (roots and substrate containing spores) to the root system of the olive seedlings.

Plant material and experimental design

Between 2018 and 2019, the study was carried out at the Faculty of Sciences Semlalia in Marrakech, Morocco. A semi-controlled greenhouse was utilized to house potted one-year-old olive trees (*Olea europaea L.*, Moroccan Picholine variety) in March 2018. A mixture of tourbe/sable/silty clay in a 1:1:2 (v/v/v) ratio was placed in each pot, containing about 4 kg of sterilized soil. Five grams of rhizophagus irregularis inoculum were inserted under the roots of half of the twenty plants. On March 15, 2019, three different doses of olive mill wastewater (100, 150, and 200 m^3/ha) were applied to 40 plants of uniform size, half of which are mycorrhized with AMF. Weekly manual irrigation using a watering can was done, supplying 25% of the SWRC (soil water retention capacity).

The OMW were mixed, filtered, and then manually sprayed into the soil of each pot using a sprayer. There have been tests conducted on three different OMW doses: S100 (100 m^3/ha), S150 (150 m^3/ha), and S200 (200 m^3/ha). Since we discussed the doses in m^3/ha, the OMWs amounts are determined based on the surface of the pot rather than its volume. For each pot, the corresponding additions were 700 ml, 1050 ml, and 1400 ml of OMW. Treatments were compared to a well water-irrigated control, with 5 repetitions per dose. Weekly schedules for irrigation were established.

Productivity and mycorrhization assessments

Dry weight

Five at hazard chosen plants were brought into the lab for measurements of their aboveground and belowground sections after the experiment. The dry weight of the aboveground components (leaves, branch, shoots and stem) was measured after drying at 70 °C for 72 hours.

For the belowground portion, roots were divided into structural roots (diameter > 2 mm) and absorbing roots (diameter ≤ 2 mm). The dry weight was also calculated after drying.

Analysis of foliar material

The analysis of foliar material was done on oven-dried leaves. In order to determine the levels of nitrogen (N), phosphorus (P), and potassium (K), a 1 g sample was broken down in a diacid mixture containing H_2SO_4 and HNO_3 (4:1). Nitrogen (N) was measured using the Kjeldahl method, potassium (K) by atomic emission spectrophotometry (JENWAY PFP7), and phosphorus (P) using a UV spectrophotometer (JENWAY 6405).

Mycorrhization assessments

At the end of the trial, roots from five seedlings per treatment were collected. One group received OMW at doses of 100, 150, and 200 m^3/ha, while the other was a control. Less than 0.5 mm small roots were cleaned and preserved in 70% ethanol. One-centimeter root fragments were prepared using the procedure outlined by Philips and Hayman (1970): they were water-washed, treated with 10% KOH at 90°C, and stained with 1.8% trypan blue in lactophenol for an hour at 90°C. Five root fragments from each treatment are mounted and crushed between slide and coverslip in glycerol and observed under a light microscope to estimate the percentage of colonization. Using the following formula, the colonization percentage was determined as the ratio of colonized points to total points:

$$\text{Mycorrhization (\%)} = \text{TNPN/MPC} \times 100 \quad (1)$$

where: TNPN – total number of points noted, MPC – mycorrhized point count

Physiological parameters

Leave relative water content (RWC)

RWC was measured using the third leaf from the top of each plant, with five replicates per treatment. Fresh weight (Fw) was recorded immediately after detachment by placing the leaves in distilled water in pre-weighed sealed tubes. After 48 hours in low light, turgid weight (Tw) was measured. The leaves were then oven-dried at 80°C for 48 hours to obtain their dry weight (Dw). RWC was determined in this way:

$$LRWC\ \% = [(Fw\text{-}Dw)/(Tw\text{-}Dw)] \times 100 \quad (2)$$

Stomatal conductance (gs)

At 28°C and roughly 60% relative humidity, stomatal conductance was measured using a leaf porometer (model LP1989, Decagon Device, Version 2012, Inc., Washington, USA). Measurements of conductance are given in mmol $H_2O\ m^{-2}\cdot s^{-1}$.

Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters (Fv/Fm and Fv/F0; where F0 is initial fluorescence, Fm is maximum fluorescence, and Fv = Fm – F0, representing variable fluorescence) were measured approximately every 40 days (specifically on day 0, as well as on days 40, 80, and 120, the final day of the experiment) using a portable fluorometer (Opti-sciences OSI 30p). Leaves were preconditioned in the dark for 20 minutes prior to measurement.

Biochemical parameters

Proline content and soluble sugars content

Proline content was measured using the method outlined by Khedr et al. (2003), while total soluble sugars were quantified using the phenol-sulfuric acid method described by DuBois et al. (1965).

Statistical analysis

Two plant categories, mycorrhized (M) plants and non-mycorrhized (NM) plants, each with five replicates and four treatments, were used in the experiment. (100, 150, and 200 m^3 OMW/ha for control (Sc). The data were subjected to analysis of variance (ANOVA) and Tukey's test using SPSS 26, with a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Characterization of the OMW used

The OMW used are characterized by an acidic pH (5.04), relatively low electrical conductivity (19 mS cm^{-1}). This effluent is characterized by average BOD5 and COD values, reaching 31.25 g/L and 85 g/L, respectively. These OMW are rich in minerals (0.39 g l^{-1} for NTK, 0.58 $g\cdot l^{-1}$ for PT, and 5.55 $g\cdot l^{-1}$ for K). They have a relatively low polyphenol content (3.95 $g\cdot l^{-1}$) and residual oil content (0.31%). These results indicate that the OMW used have lower values compared to fresh OMW, as they were taken from storage basins (30 days of retention) after the oil extraction process. Phenolic compounds degrade during storage, leading to reduced concentrations, which explains these findings. The low residual oil content is due to the centrifugation process the OMW undergo at the end of olive oil extraction. The BOD5 and COD values are within the lower limits of the ranges reported in the literature for OMW obtained in an oil press similar to the one used in this experiment: Proietti and Nasini (2004) indicate a range of 50-180 g/l for COD and 14–90 g/l for BOD5; finally, Khatib et al., 2009 reported values of 98 g/l for COD and 45 g/l for BOD5. The low values we found are due to the storage of OMW that can be considered an effective pre-treatment method. The pollutant load, assessed through COD, BOD, or total phenols, is moderate to low. In this context, the analyzed OMW showed a BOD/COD ratio higher than 0.33, indicating that the effluent is potentially easily biodegradable (Rajib et al., 2016). Therefore, the OMW used pose no phytotoxic risk, as they have undergone prior treatment in the storage basin

Effects of OMW application on soil pH, EC, OM and SRWC

All amended soils (S100, S150, S200) showed a slightly alkaline pH (8.01–7.70) after 120 days,

Table 1. Soil properties of the control and OMW-amended samples after 120 days of OMW agronomic application. Values are presented as the mean ± standard deviation

Parameters	Control (Sc)	Agronomic application of OMW (m³/ha)		
	Sc ± *SD*	S100 ± *SD*	S150 ± *SD*	S200 ± *SD*
pH	8.01 ± 0.5	7.89 ± 0.25	7.78 ± 0.25	7.70 ± 0.25
EC (μS cm^{-1}) OM (%)	430 ± 1.05 0.28 ± 0.05	645,65 ± 4.45 1.21 ± 0.05	782,43 ± 3.55 1.58 ± 0.05	938,73 ± 3.45 1.98 ± 0.05
TOC (%)	0.162 ± 0.01	0.701 ± 0.01	0.916 ± 0.01	1.148 ± 0.01
SRWC (%)	25.95 ± 0.12	28.55 ± 0.42	30.65 ± 0.55	34.45 ± 0.52

similar to the control soil (Table 1). A significant increase in soil electrical conductivity (EC) was observed with all higher doses and longer incubation times. For instance, EC rose from 430 µS cm^{-1} in the control soil to 938,73 µS·cm^{-1} in soil S200 after 120 days of incubation (Table 1). Organic matter rose from 0.28 % in the control soil to 1.98 % with 200 m^3·ha^{-1} of OMW after four months (Table 1). As shown in Table 1, the soil water retention capacity (SWRC) increased with the amount of OMW applied (from 25% (Control) to 30 (at 200m^3/ha).

Despite the high acidity of OMW (pH = 5.04), its application only slightly reduced the soil's initial pH. Chaari et al., 2015 and Chartzoulakis et al., 2010 also observed no significant pH changes with increasing OMW doses. This is due to the soil's buffering capacity, which neutralizes OMW acidity by converting carbonates in the sur-face horizon into bicarbonates. A significant increase in soil electrical conductivity (EC) was observed with all higher doses and longer incubation times. This increase reflects the higher salt concentration in the soil solution (Sierra et al., 2007). These findings align with Mekki et al. (2013), who reported that OMW application increased soil EC proportionally with the OMW quantity. Similarly, Di Bene et al., 2013 found that OMW application led to higher soil salinity due to the salts present in OMW, with the highest dose nearly doubling soil salinity. Kokkora et al. (2015) also observed a gradual increase in EC with increasing amounts of treated OMW. The applica-tion of OMW, rich in organic matter expressed by COD (85 g·L^{-1}), increased soil organic matter significantly compared to the control soil (Sc), consistent with previous studies. This increase also improved soil structure due to interactions between organic molecules and soil components (Mechri et al., 2011; Abichou et al., 2014). Soils treated with OMW retained more water compared to the control. This improvement in soil water retention ca-pacity (SWRC) is likely due to the lipid compounds in OMW, which create a mulch layer that reduces water evaporation from the soil (Abichou et al., 2014; Ben Rouina et al., 2014).

Biomass productivity

Effects of OMW on the dry matter that the plant accumulates and synthesizes

Both the hypogeous and epigeous portions of the plant treated with S100 had the highest dry matter content (Table 2), with a more significant increase in the absorbing roots. Figure 1 shows that both mycorrhizal and non-mycorrhizal plants

Table 2. At the end of the trial, the dry matter (g) accumulated in epigeous and hypogeous portions of the young olive plant (*Olea europaea* L.) mycorrhized and non-mycorrhized under various treatments was measured.

Treatment	Epigeous portion		Hypogeous portion			
			Structural root		Absorbent root	
	M	NM	M	NM	M	NM
Sc	230.70b	220.70b	21.3bc	19.51b	15.26b	13.75b
S100	290.54a	280.54a	14.86a	14.68a	40.78a	32.46a
S150	210.15c	195.15c	19.80b	19.85b	20.23c	12.68c
S200	194.73d	184.73d	13.77c	14.33c	16.52d	10.59d

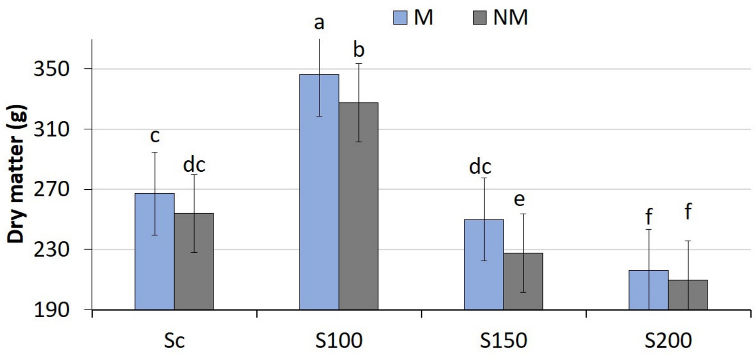

Figure 1. Dry matter content in young olive plant (*Olea europea L.*) in different treatments (Control (Sc), S100, S150 and S200) at the end of the trial. Values represent mean Standard Error (SE). Different letters represent significant difference at P ≤ 0.05

grown in soils treated with S100 had significantly higher dry biomass productivity compared to controls, with a greater increase in M plants. In contrast, plants treated with S150 and S200 showed a marked reduction in shoot and root productivity compared to controls. Indeed, after 120 days of incubation, the biomass productivity of young olive plants can be measured in terms of dry matter produced, which indicates the efficiency of the plant. In comparison to the control (254 g and 267 g), this parameter increased significantly in the S100 treatment (327 g and 346 g for non-mycorrhizal and mycorrhizal plants, respectively) (Fig. 1). Comparing the S150 and S200 treatments to the control, a decrease of 10% and 17%, respectively, was seen (Fig. 1).

These results can be attributed to the relatively high chloride content in the applied olive mill wastewater (OMW), which leads to high electrical conductivity at elevated doses. This can cause phytotoxic and nutritional disturbances in plant-soil interactions. Consequently, the application of OMW at high doses appears to negatively impact the biomass productivity of *Olea europaea* L. plants

Effect of OMW on foliar nutrient absorption

Table 3 showed a constant nitrogen content in the leaves, with no significant change in phosphorus. Potassium levels increased with the S100 application, reaching 1.57% dry matter for non-mycorrhizal plants and 1.66% for mycorrhizal plants.

In this experiment, leaf nitrogen levels were sufficient for growth, ranging from 1.1% to 2% dry matter (Magdich et al., 2015). The agronomic application of OMW did not yield significant differences between plants treated with various OMW doses and the control plants. Chartzoulakis et al. (2010) found that a high OMW dose (252 m³ ha^{-1}) did not affect nitrogen concentration in leaves after two years of application. In contrast, López-Escudero et al. (2007) reported an increase in leaf nitrogen content with raw OMW application, linking it to nitrogen availability in the soil solution. All phosphorus concentrations were within the normal to excess range (0.07–0.14% dry matter) (Panagiotopoulos et al., 2001), with no deficiencies observed. Magdich et al. (2015) also found no significant impact on phosphorus levels from the three OMW doses (50, 100, and 200 m³ ha^{-1}) tested over two campaigns. Leaf potassium (K) concentrations varied but remained above 0.7% dry matter, optimal for olive growth (Panagiotopoulos et al., 2001). Statistical analysis showed significant differences in K levels between the control group and those treated with 100 m³ ha^{-1} of OMW ($p < 0.05$), but not with 50 m³ ha^{-1} of OMW. In the olive grove, applying 100 m³ ha^{-1} of OMW could influence leaf potassium nutrition after 120 days, due to the increased K content in the soil, thereby enhancing its absorption by the plants (Magdich et al., 2013).

OMW effects on AMF colonization in the olive tree roots

Figure 2 shows that the mycorrhization rate is influenced by the doses of olive mill wastewater. Indeed, under the S100 dose, the mycorrhization rate remains similar to that of the control. This indicates that the 100 m³/ha dose promotes root mycorrhization in a way comparable to the control. However, beyond this dose (i.e., S150 and S200), the mycorrhization rate significantly decreased, with a reduction of up to 30% and 45%, respectively.

Ben Rouina et al. (2014) and Meddich et al. (2018) corroborate these findings, demonstrating that adding olive mill wastewater to soils at high doses (100 m³/ha) enhances fungal dominance. The high concentration of phenolic compounds in OMW (Table 1), which are recognized for their antimicrobial and phytotoxic qualities, may be

Table 3. Effect of OMW application on the leaf contents of nitrogen, phosphorus, and potassium in young olive plants non-mycorrhizal and mycorrhizal grown in pots after 120 days of incubation. Different letters indicate significant differences at $P \leq 0.05$.

Treatment	N (%)		P (%)		K (%)	
	M	NM	M	NM	M	NM
Sc	1.57b	1.52a	0.12c	0.098c	1.19c	0.97b
S100	1.55a	1.48a	0.17a	0.15a	1.66a	1.57a
S150	1.54c	1.50b	0.19b	0.13b	1.35b	1.05b
S200	1.61c	1.55b	0.15c	0.11c	1.21c	1.13c

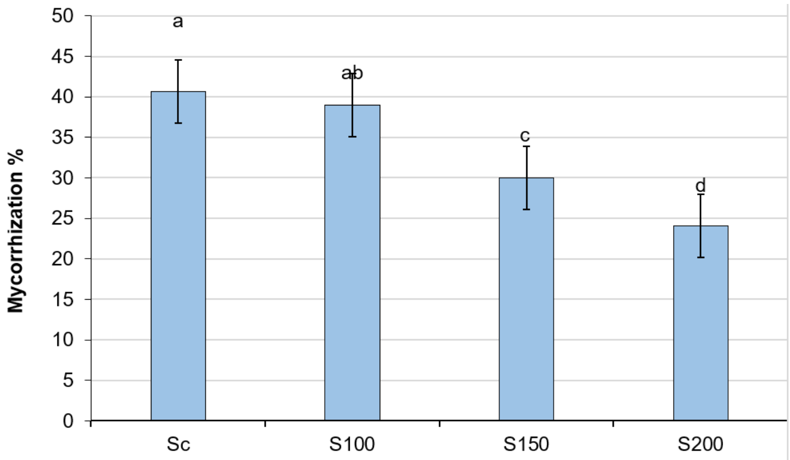

Figure 2. Mycorrhization (%) of young olive plant roots by AMF, grown in pots after 120 days of incubation, under three OMW treatments (S100, S150 and S200) and treatment with well water (Control: Sc). Different letters indicate significant differences at $P \leq 0.05$.

the cause of the decrease in AMF colonization in olive roots. The findings align with the findings of (Isidori et al., 2005; Martín et al., 2002), who similarly noted a decline in mycorrhization rates as a result of the olive mill wastewater's high phenolic content at 200 m³/ha. These results also align with Ben Rouina et al. (2014), who noted a high presence of microorganisms at 100 and 200 m³/ha doses, linked to the nutrients required for fungi and bacteria to demineralize organic matter. Additionally, they noticed a decrease in polyphenols at 200 m³/ha as a result of significant microbial colonization of the soil.

In addition, in control plants (Sc), AMF colonized only the tips of root fragments, not penetrating the central cylinder (Fig. 3A) Conversely, in roots treated with S100, AMF colonized even the central cylinder (Fig. 3B).

Changes in physiological parameters

Relative water content of leaves

Olive mill wastewater and mycorrhization influenced the relative water content of the leaves (Fig. 4). At the S100 dose, RWC increased significantly compared with the control, whose rate of increase was 16.32% and 4.76% for mycorrhizal plants and non-mycorrhizal respectively. The lowest RWC values were recorded only under high OMW doses (S150 and S200). Compared to non-mycorrhizal plants, the decrease in mycorrhizal plants was less pronounced. Relative leaf

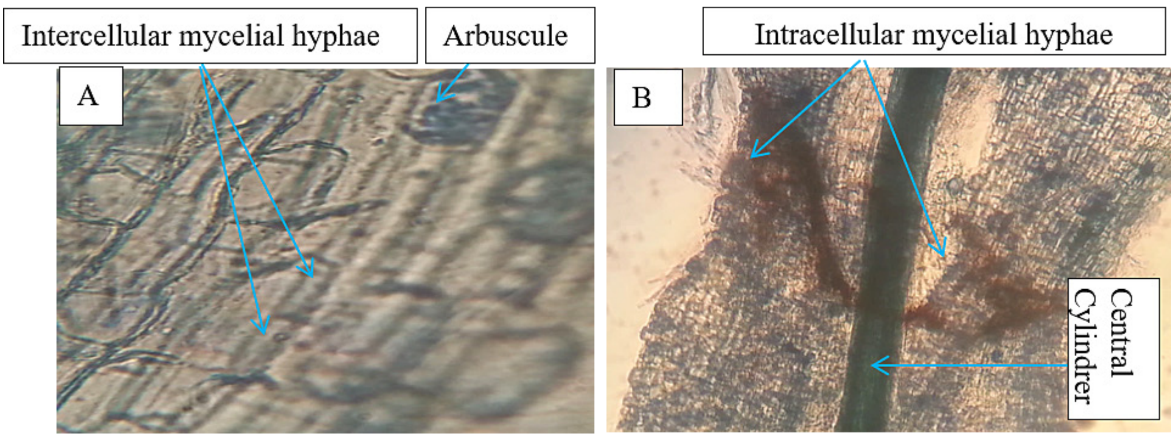

Figure 3. Microscopic observation of root fragments of young olive plants (*Olea europea L.)* inoculated with AMF: (A): untreated with OMW (control): Intercellular mycelial hyphae (Septate hyphae) which colonizes only the surface of the roots Gx 400; (B): Treated with OMW (100 m^3 ha^{-1} OMW): Intracellular mycelial hyphae which colonizes the central cylinder Gx100.

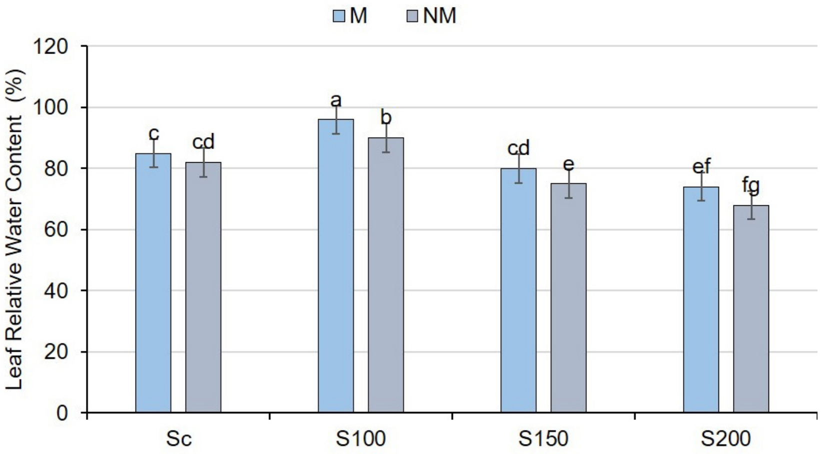

Figure 4. Changes of leaf relative water content after 120 days of incubation for mycorrhizal and non-mycorrhizal olive trees exposed to three OMW treatments (S100, S150 and S200) and control; different letters indicate significant differences at P ≤ 0.05

water content dropped by 14.9% and 21.27% in mycorrhizal plants and by 22.22% and 28.88% in non-mycorrhizal plants for the high doses (S150 and S200). Which shows that mycorrhization allows olive plants to better withstand high doses of concentrated OMW by reducing water stress.

These results are consistent with what Barros et al. (2018) and Aganchich et al. (2022) suggest, namely that mycorrhizal associations improve plant hydration levels by increasing the relative water content of the leaves. The more pronounced reduction in RWC observed in non-mycorrhizal plants could be attributed to the high salinity of olive mill wastewater, as reflected by its elevated electrical conductivity (Table 1). In fact, this olive mill wastewater contains a high concentration of dissolved salts, which can create an osmotic effect that makes water less accessible to the roots, thereby complicating water absorption. The presence of heavy metals in OMW can exacerbate this reduction. This decrease in stomatal conductance (Gs) is due to the fact that the application of high-concentration olive mill wastewater (OMW) to the soil caused leaf water stress and lower water use efficiency (Gullo et al., 2010).

Stomatal conductance (gs)

Figure 5(a, b) illustrates how both OMW and mycorrhization affected stomatal conductance (gs). Stomatal conductance (gs) was greater in mycorrhizal plants under the various OMW treatments than in non-mycorrhizal plants. At 40 days into the experiment, stomatal conductance (gs) underwent no significant changes under any of the treatments (Fig. 5(a,b). The stomatal conductance increased significantly, gradually, and proportionately with the increase in olive oil mill wastewater starting on day 80 of the experiment. When comparing the S100 dose to the other

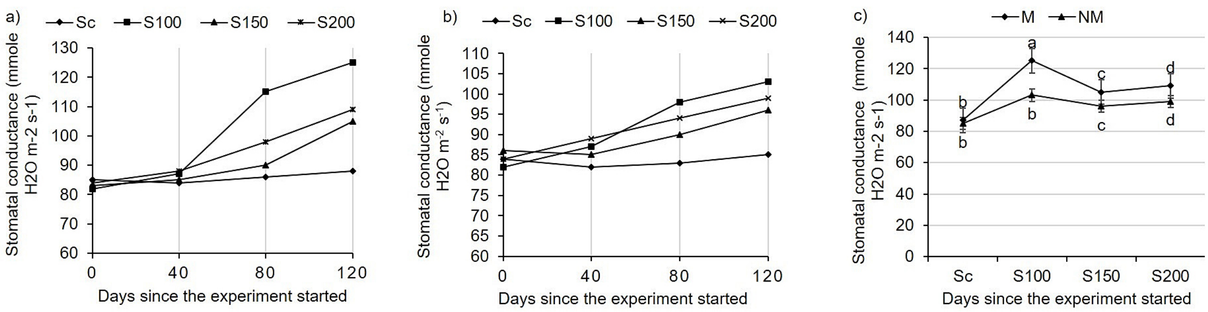

Figure 5. Stomatal conductance of young mycorrhizal olive plants (a) and non-mycorrhizal plants (b) treated with three OMW concentrations (S100, S150, S200) and a control (Sc). (c) Stomatal conductance at the end of the experiment (120 days) for mycorrhizal and non-mycorrhizal plants under the same treatments. Values are means ± SE from three replicates. Different letters indicate significant differences at P ≤ 0.05.

treatments, stomatal conductance showed a significant increase from the 80th day to the 120th day of the trial.

The control plants had the lowest stomatal conductance values (Fig. 5-c). When compared to the other treatments, the S100 treatment greatly increased gs (Fig. 5-c). The treated plants displayed higher gs than the control plants in every OMW treatment. In fact, the stomatal conductance of mycorrhized plants increased to 125 mmol H2O $m^{-2} \cdot s^{-1}$ at 100 m^3/ha, a 30.4 % improvement over the control, from 87 mmol H2O $m^{-2} \cdot s^{-1}$ (control). In contrast, stomatal conductance in plants that were not mycorrhized increased by only 17.47% when compared to the control. Nevertheless, this parameter was only marginally greater than in the control at higher doses (S150 and S200).

High potassium levels in the leaves are probably the cause of the increase in stomatal conductance (Table 3). This confirms the findings of (Gullo, 2010), who discovered that stomatal conductance (Gs) was significantly increased by elevated K content at 50 and 100 m^3/ha. K, which frequently accounts for up to 60% of the dry weight of leaves, is necessary for stomatal movement because it regulates opening and closing through variations in solute concentration.

This is consistent with research by Anli et al. (2022), which showed that plants treated with 40 and 80 m^3/ha of OMW had significantly higher photosynthetic quantum yield and stomatal conductance than the control groups. Furthermore, Boutaj et al. (2020) proposed a connection between the elevated CO_2 and the improved photosynthetic characteristics and stomatal conductance seen in plants irrigated with OMW. Our findings concur with those of Ouledali et al. (2019), who discovered that under OMW stress, mycorrhizal olive plants exhibited less disruption of stomatal function than non-inoculated plants. Similarly, compared to non-mycorrhized plants, mycorrhized plants showed a 30% increase in stomatal conductance, according to Anli et al. (2022). In addition, they saw that, in comparison to the controls, OMW treatments (40 and 80 m^3/ha) raised stomatal conductance and pigment levels. Moreover, Boutaj et al. (2020) connected increased photosynthetic activity and better stomatal conductance in plants receiving OMW irrigation. When olive plants are facing a water deficit, AMF inoculation increases both transpiration and stomatal conductance (Wu et al., 2017; Ouledali et al., 2019). This suggests that AMF can improve the plant's water status and increase net photosynthesis because more stomatal conductance allows more CO2 to diffuse into the mesophyll (Boldt et al., 2011). Zhu et al. (2018) also found that, in wheat leaves subjected to abiotic stress, arbuscular mycorrhizal symbiosis can modify stomatal morphology, impacting density, guard cell size, and pore size. This can improve stomatal conductance and water relations. Liu et al. (2018) discovered that under all circumstances, there was a strong correlation (0.81) between photosynthetic rate and stomatal conductance in potato plants.

Variation of chlorophyll fluorescence parameters

In young olive plants (*Olea europaea L.*), that were either mycorrhizal or non-mycorrhizal in relation to different concentrations of olive mill wastewater (S100, S150, and S200), the temporal variation of chlorophyll fluorescence parameters, specifically Fv/Fm and Fv/F0, is shown in Figure 6a (a,b), b (c,d). The variations in these fluorescence parameters were quite small over the course of the experiment. Notably, the final values recorded on the 120th day and the intermediate values seen on the 40th and 80th days for all OMW treatments did not drop below 0.8 for Fv/Fm and 3 for Fv/F0 (Fig. 6a (a,b), b (c,d)).

However, when exposed to higher concentrations of OMW (S150 and S200), both mycorrhizal and non-mycorrhizal olive plants showed a noticeable decline in these ratios beginning on the 80th day. In comparison to the values recorded on days 0 and 40, this decline was especially noticeable (Fig. 6a (a,b), b (c,d)).

The most noteworthy finding was that the leaves of non-mycorrhizal plants treated with the highest concentration of OMW (S200) had the lowest Fv/Fm ratio on day 120. This implies that elevated OMW concentrations have a negative impact on chlorophyll fluorescence, especially in olive plants that are not mycorrhizal. This emphasizes the significance of mycorrhizal associations in reducing stress responses to elevated OMW concentrations.

Following 120 days of treatment, the changes in chlorophyll fluorescence signals were assessed at different OMW doses, as illustrated in Figure 6 c (e). As compared to the control, the S100 treatment resulted in a noticeably higher PSII quantum yield (Fv/Fm). The Fv/Fm value for this particular dose was at an optimal level of about 0.85, indicating that *Olea europaea*'s photosynthetic process is operating effectively.

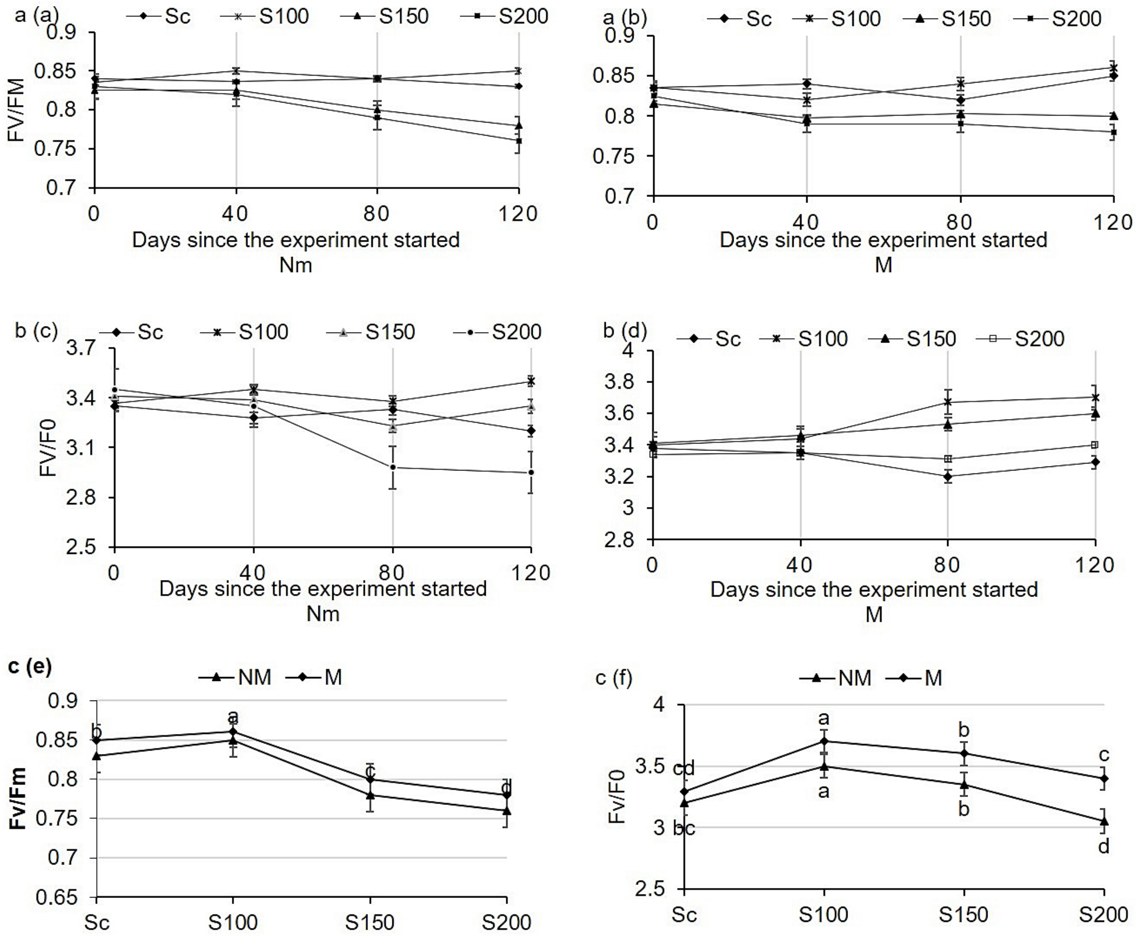

Figure 6. The changes over time in chlorophyll fluorescence parameters Fv/Fm (a (a, b)) and Fv/F0 (b (c, d)) were examined in young olive plants (*Olea europaea L.*), comparing non-mycorrhizal (NM) and mycorrhizal (M) plants under OMW treatment. c: Effect of OMW on chlorophyll fluorescence (Fv/Fm) (e) and PSII reaction center activity (Fv/F0)) (f) of young olive plants (*Olea europea L.*) after 120 days of incubation. Values are means ± SE of four replicates. Different letters indicate significant differences at P ≤ 0.05

According to findings from other studies, plants with a healthy photosynthetic process usually have a Fv/Fm value in the range of 0.80 to 0.86 (Mechri et al., 2011). When S150 and S200 were applied agronomically, PSII's photochemical efficiency (Fv/Fm) decreased, as evidenced by chlorophyll fluorescence. The decrease in Fv/Fm may be explained by an increase in protective non-radiative energy dissipation, which is connected to either the degradation of PSII centers or a controlled decline in photochemical activity and the rate of photosynthesis (Magdich et al., 2016; Tijani et al., 2019).

Furthermore, Figure 6 c (f) demonstrates that the PSII reaction center's activity (Fv/F0) follows a similar pattern to Fv/Fm, with the plant under more stress from the S200 treatment than from the S150 treatment. Increased stress is probably related to disruptions in the photosynthetic system, like damage to the donor side of the PSII thylakoid structure or problems with electron transport (Magdich et al., 2015, 2016; Jawahir et al., 2019; Tijani et al., 2019).

This supports the results of other researchers who found that applying 200 m^3 OMW ha^{-1} inhibited photosynthesis (Ayoub et al., 2014). This indicates changes in PSII photochemistry because the activity of the PSII reaction center (Fv/F0) appears to be more sensitive to OMW-induced stress than Fv/Fm. Damage to the thylakoid structure, which hinders electron transport in photosynthesis, may be one reason for the decline in net photosynthesis. This is indicated by the decline in the variable fluorescence to initial fluorescence ratio (Fv/F0). As a unique physiological process, photosynthetic activity affects the cell's overall metabolism. An increase in electron transport through PSII and an increase in the light-harvesting complex structure's capacity are linked to any molecular improvements in photosynthesis (Mohawesh et al., 2019).

Changes biochemical parameters

Contents of proline and soluble sugars

Mycorrhization and high doses of OMWW in particular had an impact on the changes in leaf proline content (Figure 7a). The proline content in both groups of plants (mycorrhizal and non-mycorrhizal) only showed a significant increase beyond the 100m³/ha dose. Indeed, the proline content showed a significant increase at S200, rising from 1.4 to 2.23 mmole proline/g dw for mycorrhizal plants and from 1.27 to 1.9 mmole proline/g dw for non-mycorrhizal plants. Proline accumulation was consistently higher in plants treated with S200 compared to control throughout the experiment, with 1.6-fold increase seen in mycorrhized plants and 1.5-fold increase in non-mycorrhized plants. Proline accumulation was significantly higher in mycorrhizal plants under S200 treatment compared to non-mycorrhizal plants.

Similarly, mycorrhization and all OMW treatments had an impact on the evolution of the soluble sugar concentration in leaves (Fig. 7b). Figure 4b shows that there were significant differences ($P \leq 0.05$) between the mycorrhization treatments and all other treatments. When olive plants were irrigated with well water (Sc) versus those treated with varying concentrations of OMW, the concentration of soluble sugars in the plants varied significantly ($P \leq 0.05$). The soluble sugar content increases noticeably with increasing OMW dose. Not only did mycorrhization improve the synthesis and accumulation of soluble sugars under the different OMW treatments (S100, S150, and S200), but it also did so in the well-water-irrigated control plants. The S200 treatment led to a higher sugar accumulation in both mycorrhizal and non-mycorrhizal plant groups than the well water treatment did. To be more precise, plants receiving the S200 treatment accumulated 1.5 times as much sugar as those receiving the S150 treatment.

The plant benefits from AMF because it helps it produce more proline and sugars to withstand the stress that high concentrations of olive mill wastewater cause. The high concentration of organic compounds and carbohydrates in OMW (Jawaher et al., 2019) likely contributes to the increase in proline and soluble sugars. These compounds also have a positive impact on leaf composition and soil quality. Our results are consistent with Jawaher et al., 2019, who reported a similar trend in total soluble sugars with varying OMW doses. Moreover, a variety of physiological growth and development processes may be involved in a plant's capacity to withstand harmful effects.

Our research suggests that Olea europaea L.'s ability to produce protective solutes like proline and soluble sugars is the main factor enabling it to withstand high concentrations of OMW (150 and 200 $m^3 \cdot ha^{-1}$) (Tajini et al., 2019). When heavy metals from OMW build up in the vegetative tissues of plants, these solutes help lessen their harmful effects (Tajini et al., 2019; Mekki et al., 2019). Plant osmotic potential has been found to be primarily influenced by total soluble sugars (Zelalem et al., 2015).

In order to cope with environmental stress, plants use a variety of sugar-based strategies (Zelalem et al., 2015; Tajini et al., 2019). Proline and sugars are thought to have multiple important roles in plant stress tolerance, according to Zelalem et al. (2015) and Tajini et al. (2019). It

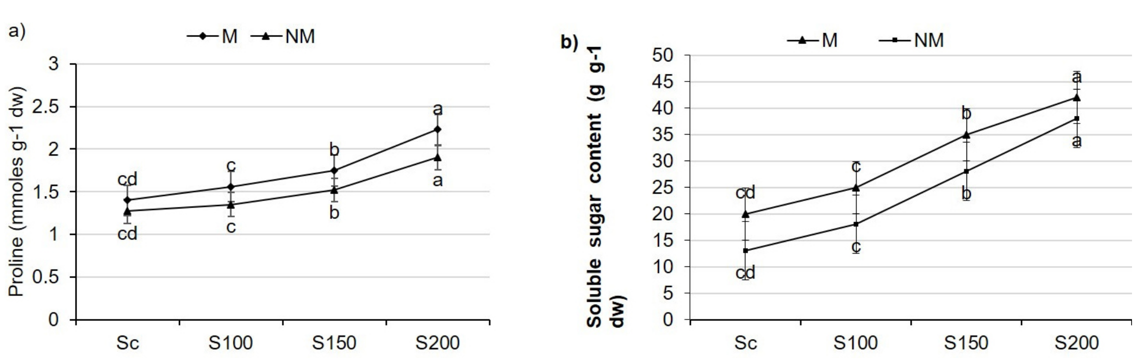

Figure 7. Evolution the proline (a) and soluble sugar content (b) after 120 days of incubation for mycorrhizal (M) and non-mycorrhizal (NM) olive trees exposed to three OMW treatments (S100, S150 and S200) and control (Sc). Different letters indicate significant differences at $P \leq 0.05$.

scavenges hydroxyl radicals, assists in protecting macromolecules during dehydration, and mediates osmotic adjustment. Proline and total soluble sugars are therefore employed as markers of stress in important vegetative organs. In response to different environmental stresses, these compounds are typically released and stored in the cells of these organs; they tend to accumulate more in the aerial parts of the plant than in the underground parts (Mekki et al., 2018; Tajini et al., 2019).

CONCLUSIONS

In conclusion, the study achieved its primary goal of determining the optimal dose of olive mill wastewater that maximizes mycorrhization while enhancing the physiological and biochemical tolerance of young olive plants. The S100 dose (100 m³/ha of OMW) emerged as the optimal point where the biomass productivity and photosynthetic activity were maximized, while also promoting the health of arbuscular mycorrhizal fungi. Beyond this dose, OMW began to negatively impact the symbiotic relationship, highlighting the critical need for careful management of wastewater application. This study uncovered that the combination of OMW and AMF not only improves nutrient uptake and foliar nutrition but also significantly boosts the plants' tolerance to environmental stress. Mycorrhized olive seedlings demonstrated greater resilience to OMW-induced stress than non-mycorrhized seedlings, which had not been previously reported in earlier studies. A novel finding of this research is the increased chlorophyll fluorescence and stomatal conductance at the S100 dose, which improves photosynthesis. While previous studies explored the effects of OMW or AMF separately, this study is one of the first to clearly demonstrate their synergistic role in enhancing photosynthesis and stress resilience in olive plants. The study also confirmed that at higher OMW concentrations (150–200 m³/ha), olive plants become stressed, but they respond by producing biochemical compounds such as proline and sugars. These compounds help the plant adapt to stress and develop more absorbent roots. This physiological adaptation had not been well-documented in response to OMW stress, making it a valuable contribution to understanding plant resilience mechanisms. The study filled an important knowledge gap by identifying the precise OMW dose (100 m³/ha) where the benefits of mycorrhization and wastewater integration are maximized without detrimental effects. Prior research focused on either the negative impacts of OMW or the positive effects of AMF in isolation, but this study demonstrated how integrating both approaches can strike a balance between enhancing plant growth and avoiding stress-induced damage. This research opens up the prospect of using OMW, a waste byproduct, in agricultural practices to enhance sustainable olive farming while mitigating environmental pollution. The combination of AMF and moderate OMW doses can improve plant resilience, reduce the need for synthetic fertilizers, and promote sustainable waste management. Additionally, future research can explore the long-term effects of these findings on olive oil production quality and further refine the dose-response relationship between OMW and mycorrhization for broader agricultural applications.

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REFERENCES

1. Abichou M., Gargouri K., Rhouma A., Khatteli H. 2014. Long term effect of olive mill waste water spreading on microbial population and the natural floristic composition of a sandy soil. Agricultural Science, Engineering and Technology Research. 1, 2, 16–25.
2. Afnor, Recueil de normes françaises : eau, méthodes d'essai, 2e édition, Paris, 1983.
3. Aganchich B., Wahbi S., Yaakoubi A., El-Aououad H., Bota, J. 2022. Effect of arbuscular mycorrhizal fungi inoculation on growth and physiology performance of olive tree under regulated deficit irrigation and partial rootzone drying. South African Journal of Botany 148, 1–10
4. Ahmali A., Mandi L., Loutfi K., El Ghadraoui A., El Mansour T.E., El Kerroumi A., Hejjaj A., Del Bubba M., Ouazzani N. 2020. Agro-physiological responses of Koroneiki olive trees (*Olea europaea* L.) irrigated by crude and treated mixture of olive mill and urban wastewaters. Scientia Horticulturae, 263, 109101.
5. Anli M., Fakhech A., Boutasknit A. et al. 2022. Comparing the Response of Growth and Physiologic

Variables of Onion to Olive Mill Wastewater Application and Arbuscular Mycorrhizal Fungi Inoculation. Gesunde Pflanzen 75, 655–666 (2023). https://doi.org/10.1007/s10343-022-00731-1.

6. Barbera C., Maucieri C., Cavallaro V., Ioppolo A., Spagna V. 2013. Effects of spreading olive mill wastewater on soil properties and crops, a review. Agric. Water Manag, 119, 43–53.
7. Barros V., Frosi G., Santos M., Ramos D.G., Falcao H.M., Santos M.G. 2018. Arbuscular mycorrhizal fungi improve photosynthetic energy use efficiency and decrease foliar construction cost under recurrent water deficit in woody evergreen species. Plant Physiol. Biochem, 127, 469–477. https://doi.org/10.1016/j.plaphy.2018.04.016.
8. Belaqziz M., El-Abbassi A., El Khadir L., Evita A., Charis M.G. 2016. Agronomic application of olive mill wastewater: Effects on maize production and soil properties. Journal of Environmental Management., 172, 158–165. DOI: 10.1016/j.jenvman.2016.02.006
9. Ben-Laouane R., Baslam M., Ait-El-Mokhtar M., Anli M., Boutasknit A., Toubali S., Mitsui T., Wahbi S., Meddich A. 2020. Potential of native arbuscular mycorrhizal fungi, rhizobia, and/or green compost as alfalfa (*Medicago sativa*) enhancers under salinity. Microorganisms, 8, 11, 1695. https://doi.org/10.3390/microorganisms8111695.
10. Ben Rouina B., Bedbabis S., Boukhris M., Ferrara G. 2014. Effect of irrigation with treated wastewater on soil chemical properties and infiltration rate. Journal of Environmental Management.. 133, 45–50.
11. Boldt K., Pors Y., Haupt B., Bitterlich M., Kuhn C., Grimm B., Franken P. 2011. Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza. Journal of Plant Physiology. 168, 1256–1263. https://doi.org/10.1016/j.jplph.2011.01.026.
12. Boutaj H., Boutasknit A., Elhaissoufi W., Anli M., Meddich A. 2020. Olive mill wastewater spreading improves growth, physiological and biochemical traits of *Phaseolus vulgaris*. Desalination and Water Treatment, 185, 87–98. https://doi.org/10.5004/dwt.2020.25423
13. Chaari L., Elloumi N., Mmseddi S., Gargougri K., Ben rouina B., Mechichi T., Kallel M. 2015. Changes in soil macronutrients after a long-term application of olive mill wastewater. Journal of Applied Chemistry and Environmental Nanotechnology, 4, 1–13.
14. Chartzoulakis K., Psarras G., Moutsopoulou M., Stefanoudaki E. 2010. Application of olive mill wastewater to a Cretan olive orchard: effects on soil properties, plant performance and the environment. Agricultural Ecosystems and Environment. 138: 293–298.
15. Chatzistathis, T., Koutsos, T., 2017. Olive mill wastewater as a source of organic matter, water and nutrients for restoration of degraded soils and for crops managed with sustainable systems. Agric. Water Manage. 190, 55–64.
16. Dakhli R., Maalej E.M. 2017. Olive mill wastewater spreading in southern Tunisia: effects on abarley crop: (*Hordeum Vulgare*. L). Journal of Agriculture and Environment for International Development, 111, 87–103.
17. Di Bene C., Pellegrino E., Debolini M. 2013. Short- and long-term effects of olive mill wastewater land spreading on soil chemical and biological properties. Soil Biology and Biochemistry, 56, 21–33.
18. Djouhou F.M.C., Nwaga D., Fokou E. 2019. Comparative effect of arbuscular mycorrhizal fungi and biostimulants on the antioxidant and nutritional potential of *Moringa oleifera*. Nutrition and Food Science International Journal, 9, 1–6.
19. https://doi.org/10.19080/NFSIJ.2019.09.555758
20. Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A., Smith F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3), 350–356. https://doi.org/10.1021/ac60111a017
21. Gullo G., Branca V., Cannavò S., Dattola A., Zappia R. 2010. Irrigation Effects with Different Mixtures of Olive Mill Wastewater (OMW) and Clean Water on the Growth and quality of Young Olive Plants cv. 'Ottobratica', in a Nursery. Terrestrial and aquatic environmental toxicology 4 (special issue 1), Global Science Books, 84-90
22. Hamimed S., Chamekh A., Slimi H., Chatti A. 2022. How olive mill wastewater could turn into valuable bionanoparticles in improving germination and soil bacteria. Industrial Crops and Products, 188, 115682.
23. Isidori M., Lavogna M., Nardelli A., Parella A. 2005. Model study on the effect of 15 phenolic olive mill wastewater constituents on seed germination and *Vibrio fischeri* metabolism. Journal of Agricultural and Food Chemistry, 53, 8414-8417.
24. Jawaher S.G., Bargougui L., Chaieb M., 2019. Study of the phytotoxic potential of olive mill wastewaters on a leguminous plant (*Vicia faba* L). Water Science and Technology, 80, 1295–1303.
25. Khalil J., Habib H., Alabboud M., Mohammed S. 2021. Olive mill wastewater effects on durum wheat crop attributes and soil microbial activities: a pilot study in Syria. Energy Ecology and Environment, 6, 469–477. https://doi.org/10.1007/s40974-021-00209-2
26. Klute A. 1986. Water Retention. In Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods. American Society of Agronomy.
27. Knudsen D., Peterson G.A., Pratt P.F. 1982. Lithium, sodium, and potassium. In: Methods of soil analysis. Part 2. Chemical and microbiological properties, 225–246.

28. Lahbouki S., Anli M., El Gabardi S et al. 2021. Evaluation of arbuscular mycorrhizal fungi and vermicompost supplementation on growth, phenolic content and antioxidant activity of prickly pear cactus (*Opuntia ficus-indica*). Plant Biosystems. http://doi.org/10.1080/11263504.2021.1947408
29. Liu C., Ravnskov S., Li, F., Rubæk G.H., Andersen M.N. 2018. Arbuscular mycorrhizal fungi alleviate abiotic stresses in potato plants caused by low phosphorus and deficit irrigation/partial rootzone drying. Journal of Agricultural Science. 156, 46–58.
30. https://doi.org/10.1017/S0021859618000023.
31. López-Escudero F.J., Blanco-López M.Á., Rincón C.D.R. and Reig J.M.C. 2007. Response of olive cultivars to stem puncture inoculation with a defoliating pathotype of Verticillium dahliae, Horticultural Science , 2, 294-298.
32. Macheix J.J., Fleuriet A., Billo J.A. 1990. Fruit Phenolics.CRC Press Inc, Boca Raton, FL, 378.
33. Magdich S., Ben Ahmed C., Boukhris M., Ben Rouina B., Ammar E. 2015. Olive mill wastewater spreading effects on productivity and oil quality of adult chemlali olive (*Olea europaea* L.) in the south of Tunisia. International Journal of Agronomy and Agricultural Research, 6, 56–67.
34. Magdich S., Abid W., Boukhris M., Ben Rouina B., Ammar E. 2016. Effects of long-term olive mill wastewater spreading on the physiological and biochemical responses of adult Chemlali olive trees (*Olea europaea* L.). Ecological Engineering. 97, 122–129.
35. Martín J., Sampedro I., García-Romera J.M., García-Garrido J.A., Ocampo J.A. 2002. Arbuscular mycorrhizal colonization and growth of soybean (*Glycine max*) and lettuce (*Lactuca sativa*) and phytotoxic effects of olive mill residues. Soil Biology and Biochemistry, 34, 1769-1775.
36. Mechri B., Cheheb H., Boussadia O., Attia F., Mariem F.B., Braham M., Hammami M., 2011. Effects of agronomic application of olive mill wastewater in a field of olive trees on carbohydrate profiles, chlorophyll a fluorescence and mineral nutrient content. Environmental and Experimental Botany,71, 184–191.
37. Meddich A., Oihabi A., Jaiti F., Bourzik W., Hafidi M., 2015a. Role of arbuscular mycorrhizal fungi on vascular wilt and drought tolerance in date palm (*Phoenix dactylifera* L.). Canadian Journal of Botany, 93, 369-377.
38. Meddich A., Ait El Mokhtar M., Wahbi S., Boumezzough A. 2017. Evaluation of mycorrhizogenic potential in relation to physico-chemical soil parameters in Moroccan palm groves (Marrakech and Tafilalet), Les oasis en Afrique du Nord: Dynamiques territoriales et durabilité des systèmes de production agricole. Cahiers Agricultures, 26, 45012 (in French)
39. Mekki A., Dhouib A., Feki F., Sayadi, S. 2013. Review : effects of olive mill wastewater application on soil properties and plants growth. Int. Journal of Recycling of Organic Waste in Agriculture, 2, 15.
40. Mekki A., Aloui A., Guergueb Z., Braham M. 2018. Agronomic valorization of olive mill wastewaters: Effects on *Medicago sativa* growth and soil characteristics. Clean, Soil, Air, Water, 100, 1–9.
41. Mekki A., Chaouch A., Bargougui L., Chaieb M., Amar F.B. 2019. Ecophysiologicals Responses of Olive Trees (*Olea europaea* L.) Hybrid Varieties in Soil Amended with Olive Mill Waste Waters. Journal of Water Science and Engineering, 5, 1–10
42. Nelson D.W., Sommers L.E. 1996. Total carbon, organic carbon, and organic matter. In: Methods of soil analysis, part 3: Chemical Methods, Soil Scien, 961–1010
43. Olsen S.R., Dean L.A. 1965. Phosphorus in: Methods of soil analysis. American Society of Agronomy, 9, 920–926.
44. Ouledali S., Ennajeh M., Ferrandino A., Khemira H., Schubert A., Secchi F. 2019. Influence of arbuscular mycorrhizal fungi inoculation on the control of stomata functioning by abscisic acid (ABA) in drought-stressed olive plants. South African Journal of Botany, 121, 152–158.
45. Ouzounidou G., Asfi M. 2012. Determination of olive mill wastewater toxic effects on three mint species grown in hydroponic culture. Journal of Plant Nutrition, 35, 5, 726–738.
46. Panagiotopoulos L. 2001. Mineral nutrition-fertilization of olive trees. Agriculture and Cattle-Raising, 3, 36–44 (in Greek)
47. Phillips J.M., Hayman D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55, 158–161.https://doi.org/10.1016/S0007-1536(70)80110-3
48. Rajib, M.A., Merwade., V. 2016. Improving soil moisture accounting and streamflow prediction 21 in SWAT by incorporating a modified time-dependent SCS CN method. Hydrol. Process. 22 30, 603-624. DOI: 10.1002/hyp.10639.
49. Sahroui H., Jrad A., Mellouli H.J. 2012. Épandage des margines sur les sols agricoles : impacts environnementaux microbiologiques. South African Journal of Science, 8, 97 – 106.
50. Tajini,F., Ouerghi A., Hosni K. 2019. Effect of irrigation with olive-mill waste-water on physiological and biochemical parameters as well as heavy-metal accumulation in common bean (*Phaseolus vulgaris* L.). Journal of New Sciences in Agriculture and Biotechnology, 66, 6, 4193–4203.
51. Tubeileh A., Abdeen M. 2017. Effect of one-Time olive-mill waste water application on yield

and water relations of olive trees. Acta Horticulturae, 1150, 303–306. https://doi.org/10.17660/ActaHortic.2017.1150.42

52. Wu Q.S., Zou Y.N. 2017. Arbuscular mycorrhizal fungi and tolerance of drought stress in plants. Arbuscular Mycorrhizas and Stress Tolerance of Plants. Springer, Singapore, pp. 25–41.
53. Zelalem G., Azamal H., Masresha F., Gietahun Y. 2015. Growth, Water Status, Physiological, Biochemical and Yield Response of Stay Green Sorghum (*Sorghum bicolor* L.) Varieties-A Field Trial Under Drought-Prone Area in Amhara Regional State, Ethiopian Journal of Agricultural Sciences, 14,188-202.
54. Zhu X., Cao Q., Sun L., Yang X., Yang W., Zhang H., 2018. Stomatal Conductance and Morphology of Arbuscular Mycorrhizal Wheat Plants Response to Elevated CO2 and NaCl Stress. Frontiers in Plant Science, 9, 1363. https://doi.org/10.3389/fpls.2018.01363.