



Comparing the Response of Growth and Physiologic Variables of Onion to Olive Mill Wastewater Application and Arbuscular Mycorrhizal Fungi Inoculation

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Received: 14 May 2022 / Accepted: 9 August 2022 / Published online: 30 August 2022
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Abstract

From plantations to farms, food safety is a major concern in food industry, particularly for perishable foods like onions. New environment friendly methods like the use of beneficial microbes and organic amendments became requirements to provide long-lasting alternatives. In this study, the impact of the direct use of olive mill wastewater (OMW) and native arbuscular mycorrhizal fungi (AMF) consortium on soil characteristics and onion plant (*Allium cepa*) growth and physiological responses as a mean to enhance soil quality and onion yield, was investigated. Two doses of OMW (4 and 8 L m⁻²) were directly applied once in a field experiment, one month after onion plantation. Soil characteristics and growth and physiological variables of onion were evaluated to assess the OMW impact and AMF on them. Among the measured variables, soil levels of phosphorus (Sp), leaf phosphorus (Lp), bulb phosphorus (Bp) and soil total organic carbon (TOC) were the manifestly improved traits with an increase of 108 to 409% for Sp, 60–102% for Lp, 39–74% for Bp and 50–139% for TOC. On the other hand, AMF colonization showed a decrease ranging from 23 to 50% under OMW treatments. In addition, the application of OMW and AMF improved the growth performances including the bulb weight, physiological (stomatal conductance and photosynthetic machinery) and biochemical (sugar, proteins and antioxidant enzymes activity) traits compared to the control. These findings highlight the importance of AMF and OMW, in improving onion agro-physiological, biochemical traits as well as soil characteristics under filed conditions.

Keywords Olive mill wastewater · Arbuscular mycorrhizal fungi · Crops · Onion · Phosphorus · Soil · Fertility · Organic amendment

Introduction

Olive oil extraction process produces wastes in large quantities. The liquid fraction is called olive mill wastewater (OMW). It is considered as a major environmental problem in the Mediterranean region where the production of this

kind of waste exceeds 10 million tons per year (D'Annibale et al. 2004). Currently, there are no proper methods for treating OMW since it is commonly discharged in inappropriate ways such as land spreading, which leads to disrupting soil chemical properties, biological activities and polluting soil surface (Karpouzaz et al. 2010; Souilem et al. 2017) and underground water (Doula et al. 2013). The main toxicity of this effluent resides in its high organic matter levels and toxic monophenolic compounds contents (Aktas et al. 2001). Its chemical oxygen demand (COD) is in the range of 40–210 g L⁻¹ and its biochemical oxygen demand (BOD₅) 10–150 g L⁻¹ (Feria 2000). Whereas OMW is known for being toxic and hazardous, its impact on soil chemical and biochemical parameters is not permanent. Soil electrical conductivity (EC), exchangeable K, soluble phenols and ammonium were observed to be affected for a short period, while arbuscular mycorrhizal fungal (AMF) root colonization was reduced but their arbuscules did increase (Di Bene

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et al. 2013). It can benefit soils by increasing their organic matter levels (Mekki et al. 2009), and in moderate levels, it has been shown that it may enhance soil microbiological flora (Mekki et al. 2009, 2013; Khalil et al. 2021). While being known that OMW could improve growth and yield of many important crops such as wheat, olive trees, tomato, strawberry, cucumber and pepper and many others (Barbera et al. 2013; Tubeileh and Abdeen 2017; Caruso et al. 2018; de los Santos et al. 2019; Khalil et al. 2021), it also has been shown to have a defensive impact in favor of some plant species against pathological agents (Yangui et al. 2008; de los Santos et al. 2019; Drais et al. 2021).

The majority of soils in the southern part of the Mediterranean region suffers from organic matter deficiency and water scarcity. In such context, re-cycling organic wastes, such as OMW, should represent a proper method to recover soil fertility and preserve water (Barbera et al. 2013; Tubeileh and Abdeen 2017). However, the research for a wise use of OMW as organic amendment requires the control of its hazards. Nevertheless, for this region, and considering the cultural, social and economic context where olive mills occur in, the costs and labor that could be spared for a proper disposing method are very limited even absent. Thus, in this study, the simplest way of using this organic waste is investigated: OMW direct application onto the soil especially after it was observed that OMW incubation in soil reduces its toxicity by more than half for raw OMW and more than third for the treated OMW (Mekki et al. 2008; Rajhi et al. 2021; Peña et al. 2022).

Besides OMW as organic amendment, beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) have emerged as a viable method for increasing agricultural production and soil quality, allowing the conventional farming system to better respond to long-term growth demand while providing adequate food today and in the future (Rouphael et al. 2020). Under arid and semi-arid conditions, these microorganisms can boost crop productivity and provide a defense system for them and boost agricultural production. AMF have been proven as an important plant growth-promoting fungi that can help plants development and productivity (Lahbouki et al. 2021; Anli et al. 2021). The direct influence of released molecules on plants or the indirect impact of affecting the environmental factors, such as the soil microbiota or soil pH-inducing mineral nutrients accessible for plant development, could be the case with AMF-mediated plant growth regulation (Begum et al. 2020; Rouphael et al. 2020). This type of multidimensional and multi-communication is accomplished through chemical signaling, in which fungi create substances that alter plant metabolism (Rouphael et al. 2020; Mitra et al. 2021). Several studies reported that AMF have the ability to boost plant and soil productivities as well as organic and mineral osmolytes such as sugar, protein, phosphorus (P) nitro-

gen (N), potassium (K), calcium (Ca) and magnesium (Mg) (Djouhou et al. 2019; Ben-Laouane et al. 2021). To perform this purpose, we suppose that OMW use in moderate doses could have beneficial effects on the soil and plants (onion in this case) rather than negative impact. In addition, AMF inoculum and two OMW doses (4 and 8 L m⁻²) were used in this study. A number of soil and plant variables were compared to a control to assess this one-time OMW application impact and AMF inoculation on both soil and plants.

Materials and Methods

The Experiment Site

The experiment was carried out in an agricultural field in Tamesloht region, 15 Km Southwest of Marrakesh city, Morocco (31°54'18"N, 8°02'08"W). The climate is Mediterranean with 20.5°C annual average temperature and 281 mm annual precipitation. The experimental field is spreading over an area of 3 ha where organic agricultural practices were solely used, no herbicides and/or chemical additives. Weeds were handled manually. The analyzed physico-chemical characteristics of the field soil were sand: 67.04%; clay: 16.60%; loam: 16.36%; available phosphorus: 20.60 mg kg⁻¹; total organic matter (TOM): 14.10 mg kg⁻¹; total organic carbon (TOC): 8.20 mg kg⁻¹; electrical conductivity (EC): 0.14 mS cm⁻¹; and pH, 8.12.

OMW Source Material and Characteristics

OMW was obtained from a semi-modern three-phase olive mill in Souihla, a village 18 km West of Marrakesh city (Morocco), it was stored at 4°C until needed. OMW characteristics were: pH: 4.70; EC: 23.50 mS cm⁻¹; TOM: 12.70 mg kg⁻¹; TOC: 2.6 mg kg⁻¹; dry matter: 21.79 g L⁻¹; total suspended solid: 11.35 g L⁻¹; ash: 3.00 g L⁻¹; volatile matter: 8.38 g L⁻¹ and total phenol content: 0.22 g Gallic acid equivalent (GAE) L⁻¹.

Arbuscular Mycorrhizal Fungi

The arbuscular mycorrhizal consortium was collected from rhizospheric soil of Tafilalet palm grove located 500 km southeast of Marrakesh, Morocco (Meddich et al. 2015). Spore extraction was performed as described by Gerde-mann and Nicolson (1963) and Walker et al. (1982). Furthermore, collected spores were then added to distilled water (5 mL) and identified morphologically (shape, color, size, some characteristic structures, germination shield, suspensor and bulb, sporulous saccule), using a binocular microscope regarding the determination key of Perez and Schenck (1990), Morton and Benny (1990) and Mukerji

(1996), and the international database (<https://invam.wvu.edu>) following the classification of Redecker et al. (2013). The mycorrhizal consortium was composed of 15 species: *Acaulospora delicata*, *Acaulospora leavis*, *Acaulospora sp.*, *Claroideoglomerus claroideum*, *Glomus aggregatum*, *Glomus claroides*, *Glomus clarum*, *Glomus deserticola*, *Glomus heterosporum*, *Glomus macrocarpum*, *Glomus microcarpum*, *Glomus sp.*, *Glomus versiforme*, *Rhizophagus intraradices*, *Pacispora boliviana*. These species belong to four families (Acaulosporaceae, Claroideoglomeraceae, Glomaceae, and Pacisporaceae) which include five genera: *Acaulospora*, *Claroideoglomerus*, *Glomus*, *Rhizophagus*, and *Pacispora*. The genus *Glomus* has the highest percentage (60%) of species, followed by *Acaulospora* (20%), and the genera *Claroideoglomerus*, *Rhizophagus*, *Pacispora* with a low percentage (6.66%). This consortium was developed in corn roots for three months. Corn seeds were disinfected using sodium hypochlorite (5%) during 5 min and rinsed ten times using tap water, germinated inside vermiculite (sterilized for 3 h at 200 °C), and watered using sterile distilled water. A week after seeds germination, corn plants were sown in plastic pots (13 cm × 09 cm) filled with soil containing AMF. Plants were regularly watered with distilled water (30 mL) intake of the modified nutrient solution of Long Ashton (Plenchette et al. 1982). Three months later, the mycorrhizal corn roots were deposited and disinfected with sodium hypochlorite (10%) for 10 min (Strullu et al. 1986), washed three times for 10 min with sterile distilled water and cut into fragments (1–2 mm), and used in form of soil and mycorrhizal corn roots. The AMF consortium used contained 47 spores/100 g soil. AMF inoculation was done by supplying each plant with 10 g of AMF inoculum. Control plants were supplied the same amount of inoculum except it was sterilized (120 °C, 1 bar for 20 min).

Experimental Procedure

The experiment was carried out in a complete randomized block design with 4 treatments: 4 L m⁻² (T1) and 8 L m⁻² (T2) of OMW and AMF consortium (T3) and a control. OMW was applied to the soil once at the start of the experiment at an application rate of 4 and 8 L m⁻². After OMW application, the soil was plowed and overturned for OMW mixing. Six blocks of 1.2 m² (1.5 m × 0.8 m) per plot. Each block had two rows of 5 plants per row and 0.5 m distance in-between. Plots were distanced by 0.4 m with 1 m distance between rows. Each row was equipped with two drips irrigation system, which watered the plants by underground water every three days for 2 h (50 L m⁻²) during four months.

AMF Colonization

AMF roots infection was determined using the modified Phillips and Hayman method (Phillips and Hayman 1970). The method consists of treating roots with KOH (10%) for clearing and Trypan blue (0.05%) for coloring. Fine roots (1 cm) were examined under microscope (× 40). The frequency (F%) and intensity (I%) of AMF infection were estimated according to the following formula:

Mycorrhization frequency F%:

$$F\% = \frac{MF}{TF} \times 100 \quad (1)$$

where: MF: mycorrhized root fragments number, TF: total root fragments number.

Mycorrhization intensity I%:

$$I\% = \frac{(95 \times n_5 + 70 \times n_4 + 30 \times n_3 + 5 \times n_2 + n_1)}{TF} \quad (2)$$

It is measured by assigning a mycorrhization index from 0 to 5, 0: no colonization, 1: traces of colonization, 2 colonization is less than 10%, 3: 11 to 50%, 4: 51 to 90%, 5 more than 91%:

where n₅: the number of root fragments which represents the degree of mycorrhization matching index 5, so is n₄, n₃, n₂ and n₁.

Growth, Physiological and Biochemical Parameters

For this study, the following parameters were determined: Growth variables: shoot height (Sh), root length (RI), leaves number (Ln), shoot fresh weight (Sfw), root fresh weight (Rfw), bulb fresh weight (Bfw), shoot dry weight (Dws) and root dry weight (Dwr). For dry matter weight, shoots and roots were dried at 80 °C for 48 h. Physiological parameters: Chlorophyll *a* (Chla), chlorophyll *b* (Chlb), carotenoids (Crt), stomatal conductance (Sc) and photosynthetic quantum yield (PQY) were assessed. Biochemical parameters: leaf sugars (Ls), bulb sugars (Bs) leaf proteins (Lpr), bulb proteins (Bpr), leaf POX activity (Lpox), bulb POX activity (Bpox), leaf PPO activity (Lppo) and bulb PPO activity (Bppo) were evaluated.

Phosphorus Levels

After harvest, phosphorus (P) was measured using Olsen and Sommers method (Olsen and Sommers 1982), a colorimetric method in which a blue color resulting from the formation of complex of phosphoric acid and molybdcic acid is evaluated. Phosphorus in soil sample is extracted using sodium bicarbonate. The extract is filtrated and added to sodium molybdate and hydrazine sulfate, placed in a water bath to get a blue coloration proportional to the quantities

of phosphorus in the sample. Optical density (OD) was read with a spectrophotometer at 820 nm.

Stomatal Conductance

Stomatal conductance was measured during clear day, between 10 h and 12 h, using a portable porometer (Model Sc-1, Decagon Devices, Pullman, USA). Measurements were taken on the second youngest and healthy leaf of 6 different plants per treatment.

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured on healthy leaves using a portable fluorometer (Opti-sciences OSI 30p) after 30 min of darkness. The initial (F_0), maximal (F_m) and variable ($F_v = F_m - F_0$) fluorescence values were measured and PSII efficiency was expressed as F_v/F_m .

Photosynthetic Pigments

Photosynthetic pigments were extracted in acetone 90% from fresh leaves material. The extract was centrifuged at $10,000 \times g$ for 10 min. Then, the supernatant OD was measured at 663, 645 and 480 nm using a UV/visible spectrophotometer. Chlorophyll *a*, chlorophyll *b* and carotenoids were estimated using Arnon formulas (Arnon 1949).

Total Soluble Sugars Content

Total soluble sugars content was determined according to Dubois et al. (1956) method. The preserved alcoholic extract was added to phenol (5%) and concentrate sulphuric acid. After cooling, the OD was measured at 485 nm. The soluble sugars content was determined using a glucose standard curve and expressed as mg/g of leaves fresh weight and bulb fresh weight.

Total Soluble Protein Content and Antioxidant Enzymatic Activities

Fresh sample (0.1 g) was homogenized in a cold mortar with 4 mL of 0.1 M phosphate buffer (pH 7) containing 5% polyvinylpyrrolidone for enzymatic activity assay. The homogenized material was centrifuged for 15 min at 4°C at $18,000 \times g$ (Model MIKRO 220, R centrifuge, New York, NY, USA), and the antioxidant enzymatic activity was evaluated. The peroxidase (POX, EC 1.11.1.7) and polyphenol oxidase (PPO) activities were evaluated as described by Hori et al. (1997). In a 3 mL reaction mixture comprising 100 mM phosphate buffer (pH 7), 20 mM guaiacol, and 40 mM H_2O_2 , 0.1 mL extract was used to activate the chemical reaction for 3 min. A UV visible spectrophotometer was

used to measure the POX activity after 3 min at 470 nm. PPO activity was determined in a solution of 20 mM catechol in 0.1 M phosphate buffer (pH 7) to which 0.1 mL of extract was added. After 3 min of cooling at room temperature, the absorbance was measured at 420 nm. A modified procedure from Bradford was used to determine the total soluble protein content of the different samples (Bradford 1976).

Soil Chemical Analyses and Glomalin Contents

Soil samples of each treatment were collected at 0 to 40 cm before and after the experiment. The pH and electrical conductivity (EC) were measured in a 1:2 (w:v) solution. Total organic carbon and organic matter were measured using Anne titrimetric method (Nelson and Sommers 1996). Available P was determined as described before using the colorimetric method of Olsen et al. (1954).

Easily extractable glomalin-related soil protein (EE-GRSP) and total glomalin-related soil protein (T-GRSP) were determined using Wright and Upadhyaya (1998). EE-GRSP was extracted from soil samples by adding 20 mM sodium citrate (pH 7.0), followed by autoclaving the solution at 121°C for 30 min, while T-GRSP was extracted by autoclaving soil samples in 50 mM sodium citrate at pH 8.0 for 60 min. Both EE-GRSP and T-GRSP supernatants were stored at 4°C after centrifuging at $5000 \times g$ for 12 min, the two variables were then measured using Bradford method Bradford (1976).

Statistical Analysis

To test the significance of the difference between the treatments effects on the studied parameters, two-way analysis of variance (ANOVA) was used after validating normality and homoscedasticity with Shapiro-Wilk and Levene's tests respectively. Tukey's Honest Significant Difference (HSD) was performed as post-hoc test to distinguish relevant significance. Correlations were calculated using Pearson product-moment correlation coefficient for which *p*-values were approximated by using the *t* or *F* distributions. Values of *p* lower than 0.05 were considered significant. Principal Components Analysis (PCA) was also performed on all the variables to emphasize their variation and visualize how they evolve. Statistical analyses were performed using LibreOffice Calc 7.1.3.2 and R v4.0.3.

Results

Growth Parameters

Growth variables measurements are presented in Table 1. Overall, T2 had the highest values of all the parameters by 15, 17, 48, 121, 74, 114, 76 and 93% for Sh, Rl, Ln, Sfw, Rfw, Bfw, Dws and Dwr respectively compared to the control. It helped plants produce more biomass, retain more water, and produce bigger bulbs. T3 was second to T2 when it came to water holding, while for Dwr, T3 (110%) was better than T2 (93%) compared to the control. Root-to-shoot dry weight ratio (Dwr/Dws) was significantly higher in T3 by 49% in comparison to the control.

Physiological Variables

Stomatal conductance, photosynthetic pigments contents and photosynthetic quantum yield changing under the different treatments are represented in Table 2. In general, the treatments resulted in higher values of the noted variables compared to the control. T1 particularly gave higher carotenoids levels (149%), while the highest values for Chla and Chlb were recorded in plants treated with T1 by 53 and 40% and T3 by 53 and 48%, respectively compared to the control. As for stomatal conductance, the highest value was recorded in T3-treated plants with 30% improvement over the control.

Biochemical Variables

Leaf sugar, bulb sugar, leaf proteins and bulb proteins contents and leaf POX, bulb POX, leaf PPO and bulb PPO activities are represented in Table 3. The applied treatments showed higher values of the leaf and bulb sugar contents by 167 and 190%, 192 and 199% and 163 and 179% for T1, T2 and T3 respectively compared to the control. Proteins levels were significantly higher in T2 (116 and 158% for leaf and bulb respectively) than the rest of the treatments. POX was at its highest under T1 (48 and 47% for leaf and bulb respectively), T2 (46 and 39% for leaf and bulb respectively) and T3 (52% for bulb), while PPO was at its highest under T3 (68 and 44% for leaf and bulb respectively) compared to the control.

AMF Colonization

AMF infection percentages are shown in Fig. 1. Evidently, T3 had the highest values of the studied variables since it consisted in inoculating plants with an AMF consortium, while the percentages in the other treatment came from the indigenous AMF present in the field soil. The I% was particularly and significantly lower in T2 than in the control.

Table 1 Growth variables under the different treatments

Variables/ Treatments	Shoot height (cm)	Root length (cm)	Leaves number	Shoot fresh weight (g per plant)	Root fresh weight (g per plant)	Bulb fresh weight (g per plant)	Shoot dry weight (g)	Root dry weight (g)	DWr/DWs
Control	43.42 ± 1.8 b	14.55 ± 0.88 b	10.33 ± 0.52 c	39.22 ± 3.7 d	3.42 ± 0.4 b	25.83 ± 1.35 c	4.54 ± 0.39 c	0.30 ± 0.04 c	0.07 ± 0.01 b
T1	48.87 ± 0.85 a	15.83 ± 0.75 ab	14.17 ± 0.41 b	70.17 ± 4.09 b	4.80 ± 0.57 a	45.92 ± 2.12 b	6.25 ± 0.42 b	0.41 ± 0.04 b	0.07 ± 0.00 b
T2	50.00 ± 0.89 a	17.03 ± 0.84 a	15.33 ± 0.52 a	86.50 ± 5.29 a	5.93 ± 0.97 a	55.25 ± 1.92 a	7.99 ± 0.57 a	0.58 ± 0.05 a	0.07 ± 0.07 b
T3	48.17 ± 1.94 a	14.92 ± 1.11 b	14.50 ± 0.55 b	48.45 ± 2.59 c	5.15 ± 1.19 a	48.30 ± 8.51 ab	6.42 ± 0.29 b	0.63 ± 0.08 a	0.10 ± 0.01 a

Values are means ± Standard Deviation ($n = 6$). Letters represent the significance of the difference according to Tukey HSD test. Different letters mean significant difference between the treatments Control control treatment, T1 treatment with olive mill waste water at 4 L m⁻², T2 treatment with olive mill waste water at 8 L m⁻², T3 treatment with AMF consortium

Table 2 Physiological variables under the different treatments

Variables/ Treatments	Chlorophyll <i>a</i> (mg/g FW)	Chlorophyll <i>b</i> (mg/g FW)	Carotenoids (mg/g FW)	Stomatal conductance (mmol m ⁻² s ⁻¹)	Photosynthetic quantum yield (Fv/Fm)
Control	10.37 ± 0.9 b	11.54 ± 0.53 c	44.02 ± 1.64 c	108.43 ± 6.16 b	0.76 ± 0.01 b
T1	15.94 ± 0.59 a	16.19 ± 0.54 a	109.44 ± 6.82 a	139.57 ± 1.62 a	0.79 ± 0.01 a
T2	14.84 ± 0.63 a	14.23 ± 0.73 b	76.13 ± 6.36 b	140.80 ± 4.33 a	0.81 ± 0.02 a
T3	15.82 ± 0.92 a	17.06 ± 0.31 a	81.22 ± 4.93 b	133.10 ± 1.21 a	0.81 ± 0.01 a

Values are means ± Standard Deviation ($n=6$). Letters represent the significance of the difference according to Tukey HSD test. Different letters mean significant difference between the treatments

Control control treatment, *T1* treatment with olive mill waste water at 4L m⁻², *T2* treatment with olive mill waste water at 8L m⁻², *T3* treatment with AMF consortium

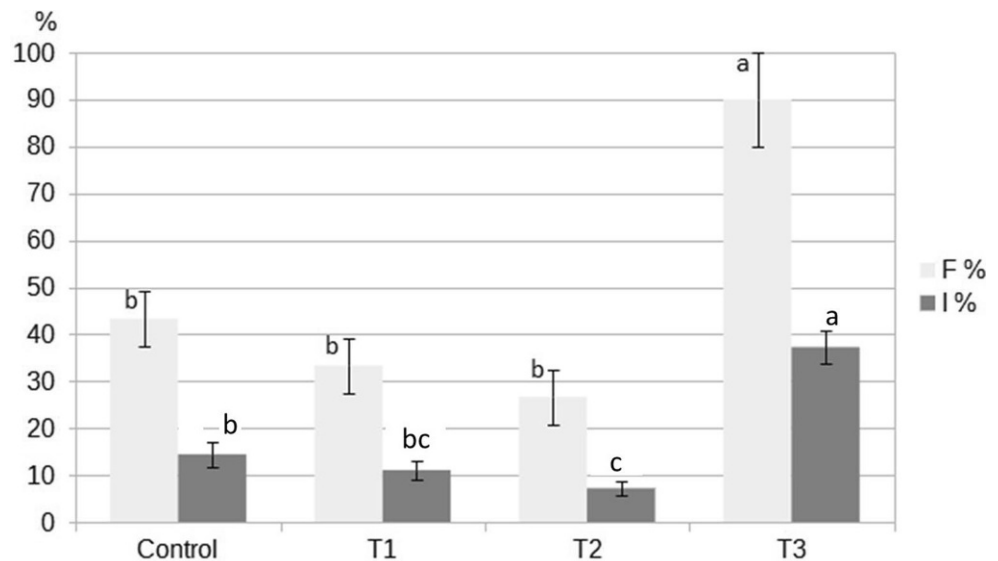
Table 3 Biochemical variables under the different treatments

Variables/ Treatments	Leaf sugar content (mg/g FW)	Bulb sugar content (mg/g FW)	Leaf proteins content (mg/g FW)	Bulb pro- teins con- tent (mg/g FW)	Leaf POX activity ($\mu\text{mol mg}^{-1}$ protein min ⁻¹)	Bulb POX activity ($\mu\text{mol mg}^{-1}$ protein min ⁻¹)	Leaf PPO activity ($\mu\text{mol mg}^{-1}$ protein min ⁻¹)	Bulb PPO activity ($\mu\text{mol mg}^{-1}$ protein min ⁻¹)
Control	40.75 ± 5.67 b	108.93 ± 10.67 b	3.67 ± 0.27 c	5.74 ± 0.35 d	0.46 ± 0.03 b	0.31 ± 0.02 b	0.51 ± 0.04 c	0.31 ± 0.02 c
T1	108.65 ± 2.13 a	316.35 ± 6.39 a	5.75 ± 0.49 b	12.77 ± 0.49 b	0.68 ± 0.04 a	0.46 ± 0.06 a	0.71 ± 0.03 b	0.43 ± 0.00 a
T2	118.91 ± 16.64 a	325.80 ± 14.79 a	7.93 ± 0.35 a	14.78 ± 0.79 a	0.67 ± 0.03 a	0.44 ± 0.04 a	0.65 ± 0.06 b	0.37 ± 0.02 b
T3	107.07 ± 10.9 a	304.15 ± 11.91 a	5.33 ± 0.46 b	10.52 ± 1.19 c	0.58 ± 0.06 a	0.48 ± 0.04 a	0.86 ± 0.05 a	0.44 ± 0.03 a

Values are means ± Standard Deviation ($n=6$). Letters represent the significance of the difference according to Tukey HSD test. Different letters mean significant difference between the treatments

POX peroxidase, *PPO* polyphenol oxidase, *Control* control treatment, *T1* treatment with olive mill waste water at 4L m⁻², *T2* treatment with olive mill waste water at 8L m⁻², *T3* treatment with AMF consortium

Fig. 1 AMF colonization variables. Letters represent Tukey HSD significance. Data are mean ± Standard Deviation ($n=6$). Following Tukey's test, values with the same letter are not statistically different at $p<0.05$. (*F%* colonization frequency, *I%* colonization intensity, *Control* control treatment, *T1* treatment with olive mill wastewater at 4L m⁻², *T2* treatment with olive mill wastewater at 8L m⁻², *T3* treatment with AMF consortium)



Plant Phosphorus Content

P plant levels under the applied treatments are shown in Fig. 2. All the treatments gave significantly higher levels of P than the control. In the other hand, T3 was not significantly different from T1 and T2, while these two differed significantly, with T2 giving the highest leaf and bulb P levels (102 and 74% respectively) than in the control. A strong correlation was also noted between Lp and I%

for T1 and T3 ($r^2=0.964$ and $r^2=0.936$ respectively with $p<0.01$ for both), and between Lp and F% for T2 ($r^2=0.989$ with $p<0.001$).

Soil Properties and Glomalin Content

Soil chemical analyses results and glomalin content are represented in Table 4. All treatments significantly increased the studied soil chemical variables, except pH, which was

Fig. 2 Plant and bulb P levels under the different treatments. Letters represent Tukey HSD significance. Data are mean \pm Standard Deviation ($n=6$). Following Tukey's test, values with the same letter are not statistically different at $p<0.05$. (Control control treatment, T1 treatment with olive mill wastewater at 4L m^{-2} , T2 treatment with olive mill wastewater at 8L m^{-2} , T3 treatment with AMF consortium)

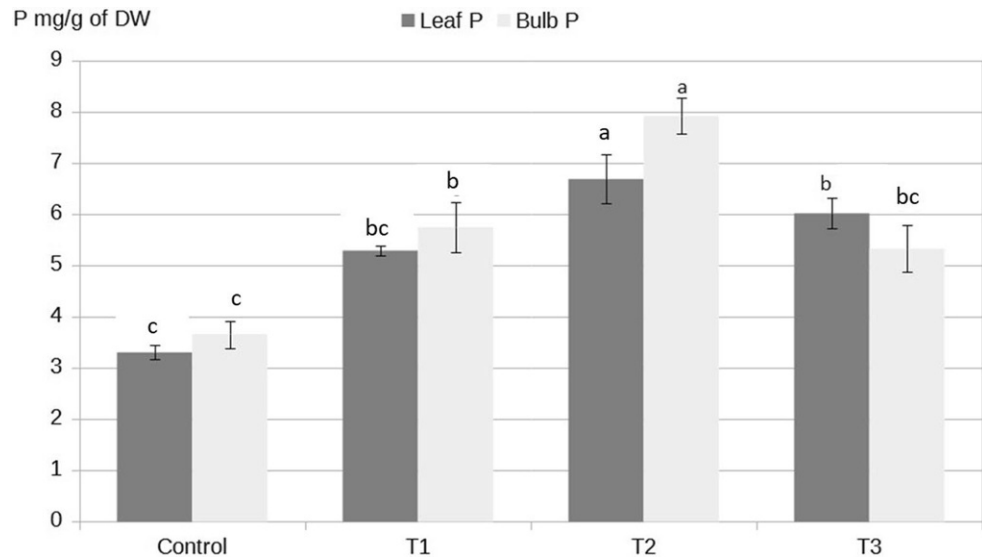


Table 4 Soil chemical properties and glomalin contents

Variables/ Treatments	pH	EC (mS/cm)	TOC (mg/g)	TOM (mg/g)	Glomalin (T- GRSP) (mg/g Dry soil)	Glomalin (EE- GRSP) (mg/g Dry soil)	P (mg/g of Dry soil)
Control	7.88 \pm 0.03 b	1.05 \pm 0.06 d	10.64 \pm 1.77 c	18.34 \pm 3.06 c	1.43 \pm 0.11 b	1.97 \pm 0.15 b	35.71 \pm 1.45 d
T1	7.83 \pm 0.06 b	1.78 \pm 0.05 b	15.95 \pm 1.77 b	27.51 \pm 3.06 b	7.79 \pm 0.06 a	1.46 \pm 0.16 c	181.83 \pm 23.94 a
T2	8.12 \pm 0.07 a	2.06 \pm 0.06 a	25.41 \pm 2.71 a	43.81 \pm 4.67 a	1.17 \pm 0.07 c	2.42 \pm 0.02 a	132.20 \pm 7.13 b
T3	7.73 \pm 0.04 b	1.63 \pm 0.04 c	18.32 \pm 1.02 b	31.58 \pm 1.76 b	1.01 \pm 0.05 c	1.92 \pm 0.07 b	74.43 \pm 7.68 c

Values are means \pm Standard Deviation ($n=6$). Letters represent the significance of the difference according to Tukey HSD test. Different letters mean significant difference between the treatments

EC electrical conductivity, TOC Total organic carbon, TOM total organic matter, T-GRSP total glomalin-related soil protein, EE-GRSP easily extractable glomalin-related soil protein, P soil available phosphorus, Control control treatment, T1 treatment with olive mill waste water at 4L m^{-2} , T2 treatment with olive mill waste water at 8L m^{-2} , T3 treatment with AMF consortium

only significantly increased under T2 treatment. T2 also gave the highest values of EC, TOC and TOM, while T1 gave the highest values of T-GRSP (443%) glomalin and soil P (409%) levels. A very strong positive correlation was noted between soil P (Sp) and F% for the control and T1 ($r^2=1.000$ and $r^2=0.850$ with $p<0.001$ and $p<0.05$ respectively), while T2 had a strong negative one between the same variables ($r^2=-0.928$ with $p<0.01$). Also, Sp and I% correlated strongly and positively for the control and T3 ($r^2=1.000$ and $r^2=0.999$ respectively with $p<0.001$ for both). A moderately positive correlation was noted between TOC and F% for the control, T1 and T3 ($r^2=0.865$, $r^2=0.866$ and $r^2=0.866$ respectively with $p<0.05$ for all of them). Between TOC and I%, a strong positive correlation was found for T2 and a moderate one for T3 ($r^2=0.964$ and $r^2=0.874$ with $p<0.001$ and $p<0.05$ respectively).

Principal Component Analysis

PCA explained more than 77% of the variance in the first two dimensions (Fig. 3). The evolution of the variables

and the effects of the treatments on them is represented in Fig. 3. PCA plot consolidates most of what the ANOVA and Pearson's correlation concluded. The control group is distinctively and significantly isolated from all the other treatments which means that they had significant impact on the studied variables. T1 and T2 are not far apart, they are both driven by primarily the same dimension (Dim 2). T3 is clearly having a distinctive evolution with its main variables that it had a drive on being Dwr/Dws followed by I% and F%, while T1 affected mainly T-Glomalin content and soil P levels.

Discussion

For growth variables, OMW application at 8L m^{-2} (T2) helped plants produce more biomass, retain more water, and produce bigger bulbs. OMW application at 4L m^{-2} (T1) was second to T2 when it came to water holding, while for bulb mass, AMF treatment (T3) was better than T2. The same pattern was noted in many other studies on different

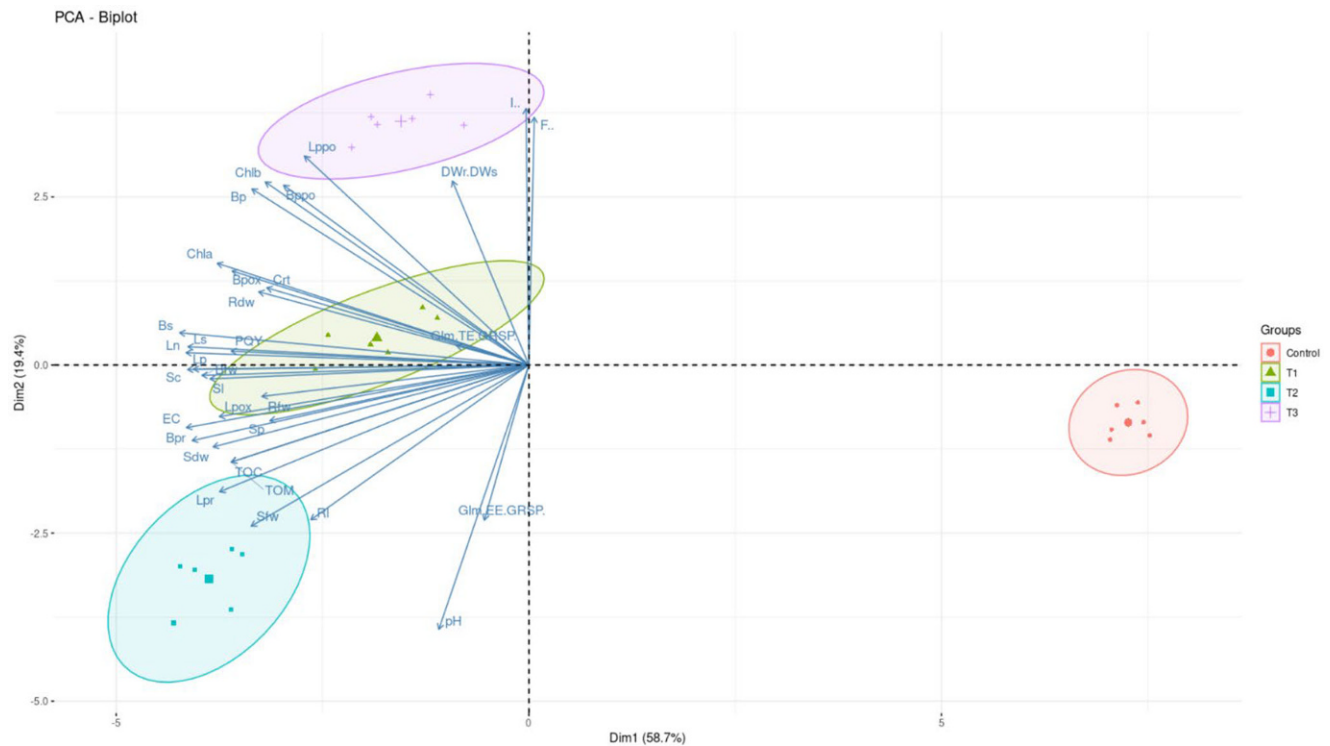


Fig. 3 Principal component analysis of the evaluated parameters according to the different applied treatments. (*Bfw* bulb fresh weight, *Bp* and *Lp* bulb and leaf phosphorus, *Bpox* and *Lpox* bulb and leaf peroxidase activity, *Bppo* and *Lppo* bulb and leaf polyphenol oxidase activity, *Crt* carotenoids, *EC* electrical conductivity, *Glm*, *EE-GRSP* Easily extractable glomalin-related soil protein, *Cs* stomatal conductance, *Chla* Chlorophyll *a*, *Chlb* Chlorophyll *b*, *F* Mycorrhization frequency, *I* Mycorrhization intensity, *Ln* number of leaves, *PQY* photosynthetic quantum yield, *Bpr* and *Lpr* bulb and leaf proteins, *Rl* root length, *Sh* shoot height, *Sfw* shoot fresh weight, *Rfw* root fresh weight, *Dws* shoot dry weight, *Dwr* root dry weight, *Sp* soil available phosphorus, *TOC* soil total organic carbon, *TOM* soil total organic matter, *Glm*, *T-GRSP* total glomalin-related soil protein, *Bs* and *Ls* bulb and leaf soluble sugar, *Control* control treatment, *T1* treatment with olive mill wastewater at 4 L m^{-2} , *T2* treatment with olive mill wastewater at 8 L m^{-2} , *T3* treatment with AMF consortium)

crops (Barbera et al. 2013; Mekki et al. 2013). When coupled with foliar application, OMW increased seed weight by 28% while root application alone resulted in an increase of 16% (Hanifi and El Hadrami 2008). However, it should be noted that it is not always the case since 10 times diluted OMW inhibited plant growth by 16 to 42.5% (Mekki et al. 2006). OMW also significantly increased shield medick dry biomass by 19.3%, and broad bean grain yield by 25% (Caruso et al. 2018). In addition, previous studies showed that native or exogenous AMF inoculation increased plants growth principally leaf area, root length, and shoot and root biomass of alfalfa (Ben-Laouane et al. 2020) and tomato (Ait Rahou et al. 2021). AMF plant growth promoting effect could be explained by the increase of mineral uptake, namely N and P nutrition and water uptake by hyphae and root length (Symanczik et al. 2020). *Dwr/Dws* ratio is an indicator of the resource availability and stress status for the plants. Roots are directly influenced by low soil availability of either water or nutrients, which leads to greater *Dwr/Dws* ratios. T3 was the treatment to show the significantly higher ratio, even higher than the control, which

means that higher infection percentages do not translate into better overall stress-free status.

On the other hand, stomatal conductance, photosynthetic pigments, leaf and bulb sugar contents and photosynthetic quantum yield were higher under all treatments in comparison with the controls. T1 particularly gave higher carotenoids levels, while T2 gave lesser values of *Chlb* than the other two treatments. Tubeileh and Abdeen (2017) reported different behavior where OMW repressed stomatal conductance while another study found that, chlorophyll *a*/chlorophyll *b* ratio was not significantly different from the control (Mekki et al. 2013). The first major finding from this experiment was that OMW was a good factor for onion growth modification. The rise in CO_2 could be linked to the increase in stomatal conductance and photosynthetic features in plants irrigated with OMW (Boutaj et al. 2020). Photosynthetic activity is a unique physiological process that affects the cell's overall metabolism. Any enhancement in photosynthesis at the molecular level is linked to high electron transport through PSII and/or PSII and light harvesting complex structure capacity (Mohawesh et al. 2019). Indeed, increased growth in the presence of OMW

could lead to increased CO₂ translocation in the leaf area and, as a result, increased CO₂ incorporation. In addition, AMF have the capacity to increase water and nutrients uptake and also improve the regulation of physiological and biochemical traits of host plants (Begum et al. 2020; Symanczik et al. 2020). AMF's influence on physiology and biochemistry was reported in many mycorrhizal plants growing under different environments (Ben-Laouane et al. 2020; Ait-El-Mokhtar et al. 2020; Anli et al. 2022). Anli et al. (2020) and Boutasknit et al. (2021) reported that AMF-inoculated date palm and garlic plants showed an increased photosynthetic performance by improving stomatal conductance and the efficiency of PSII and by regulating the energy flow between photochemical and non-photochemical reactions. The improvement in net assimilation rates through protection of photosystem II photochemical machinery and the increase in stomatal conductance could induce the improved growth performances in AMF-inoculated plants (Chang et al. 2018).

For biochemical variables, OMW helped onion plants produce significantly more sugar as well as protein contents, especially under the higher dose (T2). POX activity was not significantly different between the treatments. However, PPO activity was significantly higher in the leaves of AMF treated plants, while not significantly different from T1 at the bulb level. Furthermore, T1 and T3 PPO values were significantly higher than T2 and control at leaf and bulb levels. This result could be explained by the ideal environment provided to plants by T1 and T2 treatments. As for the improvement of organic osmolytes such as sugars and proteins, this could be the result of good exchanges between the soil amended with OMW and the plant, which results in a better absorption of mineral elements from the soil to the plant (Mechri et al. 2011). Furthermore, Chang et al. (2018) suggested that higher organic osmolytes accumulation could be attributed to improved mineral nutrients uptake like K, Ca, and Mg as a result of AMF application. This could also be explained by the buildup of glycine betaine and proline in mycorrhizal plants, which protects PSII pigment-protein complexes and CO₂ binding enzymes like RuBisCO and Rubisco activase and thereby promotes organic osmolytes production (Talaat and Shawky 2014), without discounting their role in osmotic adjustment mechanisms. Additionally, plants treated with AMF or OMW showed better performances through the enhancement of metabolites production, such as sugars and proteins, which serve as organic osmolytes, as well as inorganic osmolytes, particularly nitrogen, phosphorus, and potassium and antioxidant enzymes activity (Mechri et al. 2011; Toubali et al. 2020; Mitra et al. 2021).

Regarding AMF colonization percentages, T3 had the highest values, while T2 particularly showed significant lower I% than the control. It was reported that OMW re-

press these variables whereas encouraging more arbuscules formation (Di Bene et al. 2013). Leaf P levels were significantly higher than the control's in all the treatments, where T1 and T2 differed significantly, while T3 was not significantly different from both of them. T1 was the treatment which supplied soil with most P, while T2 gave the highest leaf P levels. Boutaj et al. (2020) noted that a significant increase in P was one of the most important effects of OMW application on soil composition. This improvement could be explained by the richness of OMW in OM and mineral elements that are involved in the robustness of plants and their development. The strong correlation between Lp and I% for T1 and T3 and between Lp and F% showed that more available P in soil does not translate to more P in plants because of its complexation with other mineral nutrients like Ca and Fe (Audette et al. 2020).

Soil chemical traits were significantly increased under the applied treatments, except pH which was only significantly increased by T2. Other case studies reported no significant impact of OMW on soil pH (Mekki et al. 2013) or its stabilization in higher values than control pH level (Sempiterno and Dias 2004). T2 also gave the highest values of EC, TOC and TOM in line with the findings of Mekki et al. (2013). The strongest and positive correlation noted between Sp and F% for the control is a natural occurring one especially in poor quality soils, while the same correlation recorded for T1 is the unusual one, especially, when T1 had the highest value of soil P. AMF are known to be sensitive to high concentrations of phosphorus in the soil. When the level of soil available phosphorus is high, the colonization percentage decreases (Anli et al. 2020), which is illustrated by the strong negative correlation between the same variables and T2. Soil structure is one of the most important components of soils (Gałazka et al. 2020), and it is intimately linked to soil water status, gas exchange, and nutrient exchange (Begum et al. 2020). By improving soil structure and aggregation, AMF could improve soil physical characteristics and then plant growth. AMF fit into the gaps between microaggregates and produce stable macroporous aggregates that allow air and water entry while avoiding soil erosion due to the breadth and thickness of their mycelial network (Šarapatka et al. 2019; Gałazka et al. 2020). Furthermore, AMF glomalin, which is secreted into the soil, may play a key role in this process. Glomalin is an hydrophobic and thermo-tolerant glycoprotein that is considerably more resistant to biological breakdown when it attaches to clay particles, ensuring excellent structural stability of soils (Yang et al. 2017; Gałazka et al. 2020). Several studies have found a link between the glomalin generated by AMF hyphae and the stability of soil aggregates (Ji et al. 2019; Ren et al. 2019). Higher hyphal length and spore density, on the other hand, can aid in the entanglement of microaggregates into macroaggregates (Ji

et al. 2019; Gałazka et al. 2020). Gałazka et al. (2020) speculate that mycorrhizal-mediated soil aggregation may also provide physical protection for organic carbon from microbial decomposition. As a result, AMF and their host plant (roots) are regularly discovered to be a critical force in promoting soil aggregation. However, understanding the properties of roots and mycorrhizal fungus could be quite useful in the field. This knowledge, according to Rillig et al. (2015), might lead to creative initiatives like seed combinations (or fungal inoculums) ideal for degraded land regeneration, which would optimize the coverage of soil aggregation properties within the available group of plant species, as well as set priorities for conservation efforts by predicting which ecosystems are most likely to be degraded due to invasive species and impending global changes. However, OMW are known to be rich in OM and mineral nutrients, which contribute to the soil fertility and quality (Sciubba et al. 2020). OM is a biological component that promotes soil physical, chemical and biological characteristics and consequently boosts the plant growth performances and development when OMW was applied (Bargougui et al. 2019). Because of its ability to influence plant growth both indirectly and directly, soil organic matter is one of the most essential elements and structure of soil (Badawi 2020; Karbout et al. 2021). Organic matter serves as a reservoir for plant nutrients such as N, P, K, Ca, S, and micronutrients, preventing nutrient loss and improving soil cation exchange capacity under dry lands conditions (Bendaly Labaied et al. 2020). Organic fertilizer application increased soil aggregation, reduced soil pH, improved organic matter in the soil and plant mineral nutrients uptake, especially N, P K, and Fe under arid and semi-arid regions (Badawi 2020; Karbout et al. 2021).

Conclusion

In this study, we demonstrated the beneficial effect of AMF and OMW on improving onion growth at the physiological and biochemical levels under field conditions. In summary, our results indicated that the application of OWM and/or AMF brings significant improvement of soil physico-chemical properties (pH, EC, organic carbon, organic matter and available phosphorus contents) and glomalin contents. OMW and AMF provide minerals needed by the plants. Thus, an increase in bulb and shoot growth, stomatal conductance, photosynthetic pigment content, chlorophyll fluorescence, yield and a remarkable improvement in sugar and protein content were observed. These data showed that the used OMW and AMF were a good choice for onion growth and yield under field conditions. Our results also suggest that OMW did not have significant negative or stressful effects on soil and onion plant. Therefore, the direct use

of OMW may be a promising tool to both recycle a toxic organic waste with less effort and cost and improve soil fertility and water levels. In addition, it can be concluded that the application of OMW and/or AMF is an effective and very encouraging method to increase agriculture sustainability and reduce environmentally hazardous chemicals use.

Acknowledgements This work was supported by the Socially Responsible Projects, Cadi Ayyad University UCAM/RSU 2018 Marrakech, Morocco and Mixed laboratory Morocco-Tunisia, laboratory of Plant Physiology and Biotechnology and Climate Change (LR11ES09).

Conflict of interest M. Anli, A. Fakhech, A. Boutasknit, M. Ait-El-Mokhtar, R. Ben-Laoane, Y. Ait-Rahou and A. Meddich declare that they have no competing interests.

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