THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Ameni Ben Hassena et al, The Experiment, 2019, Vol 47 (4), 2683-2694

Effect of saline treated wastewater and arbuscular mycorrhizal fungi on soil properties and growth of potted young olive plants

Abstract:
The reuse of treated wastewater (TWW) in olive orchards may affect the soil quality and the growth of plants under long term irrigation. This investigation aims to study the impact of different arbuscular mycorrhizal fungal (AMF) inoculums (*Glomus deserticola*, *Gigaspora margarita* or a 1:1 mixture of *Glomus deserticola* and *Gigaspora margarita*) in improving the soil quality and the growth of potted young olive plants (*Olea europaea* L. cv. Chetoui) after one year of irrigation with saline TWW. Results showed that irrigation with this water caused an increase in electrical conductivity (EC) and an accumulation of Na^+ and Cl^- in the soil solution. As a result, young olive plants showed a reduction in shoots elongation and leaf areas, whereas the leaf thickness was increased. Mycorrhizal symbiosis played an important role in decreasing the EC and the concentration of Na^+ and Cl^- in soil irrigated with TWW. In addition, AMF inoculation mitigated the salt stress caused by saline TWW and improved all the growth parameters.

Keywords: Olive tree, Treated wastewater, Arbuscular mycorrhizal fungi, Salinity, Soil quality, Growth

1. Introduction:

In arid and semi-arid areas, the use of treated wastewater (TWW), for irrigation in orchards, nurseries, and landscapes is an important strategy to conserve fresh water (Valdés et al., 2015). The TWW contain high concentrations of nutrients (N, P and K), which provide a fertilizer value for soils and crops (Rusan et al., 2007; Pedrero et al., 2010). Nevertheless, depending on its source and the degree of treatment, TWW may contain excessive levels of salts and heavy metals, that may cause deleterious effect on soil properties and plant growth (Pedrero et al., 2013). According to many researchers, saline TWW application caused soil salinization due to high electrical conductivity (EC) and induced sodicity problems as a result of excessive amounts of exchangeable Na^+ (Toze, 2006; Elgallal et al., 2016; Acosta-Motos et al., 2017). Such effect may consequently change the osmotic pressure in the root zone and reduce the soil hydraulic conductivity, leading thus to decrease the water availability to crops (Al-Jasser, 2011; Muamar et al., 2014; Elgallal et al., 2016). As a result, harmful effects such as reduction in plant height, biomass and leaf areas were reported in several plants irrigated with saline TWW (Acosta-Motos et al., 2014; Gómez-Bellot et al., 2015a; Valdés et al., 2015).

As shown by several authors, application of arbuscular mycorrhizal fungi (AMF), as a biological process, could be a potential solution to salinity problems in agricultural lands (Zarei and Paymaneh, 2013; Gómez-Bellot et al., 2015b). In fact, AMF are known to play an important role in ameliorating the quality of degraded soil *via* improving the aggregation and the fertility of soil as well as storing the toxic compounds in the cell walls of fungus in roots (Göhre and Paszkowski, 2006; Aggarwal et al., 2011; Yan et al., 2012). Furthermore, AMF are able to enhance the uptake of water and nutrient and consequently the growth of plants. This increase is due to the extraradical mycelium of AMF, that form bridges between the roots of host plants and the soil (Smith and Read, 2008; Vicente-Sánchez et al., 2014; Gómez-Bellot et al., 2015a). It has also been showed that AMF colonization may improve the tolerance of host plants to abiotic stresses, particularly salinity, in several plants such as wheat (*Triticum aestivum* L.) (Abdel-Fattah and Asrar, 2012), rough lemon (*Citrus jambhiri* Lush.) (Zarei and Paymaneh, 2013) and fenugreek (*Trigonella foenum-graecum* L.) (Metwally and Abdelhameed, 2018).

Olive tree (*Olea europaea* L.) is one of the best adapted species to the Mediterranean climate, which is known as moderately tolerant to salinity (Rugini and Fedeli, 1990; Chartzoulakis, 2005). Recently, in Tunisia, olive irrigation is expanded in new orchards, whereas water is becoming more and more scarce (Bedbabis et al., 2015). Thus, TWW irrigation could be an alternative water resources in olive orchards. However, Tunisian TWW is characterized by a high level of salinity (Bedbabis et al., 2015), which may consequently cause soil degradation and growth disturbance to plants. Interestingly, AMF inoculation could be a sustainable solution to avoid salinity problems in TWW irrigated olive plants.

In this context, the aim of this work was to study the impact of both saline TWW irrigation and inoculation with single (*Glomus deserticola* or *Gigaspora margarita*) or mixed AMF (a 1:1 mixture of *Glomus deserticola* and *Gigaspora margarita*) on the soil chemical properties and the growth of young olive plants (*Olea europaea* L. cv. Chetoui).

2. Material and Methods:

2.1. Water sources:

In the current study, two types of water were used for irrigation: (i) the tap water and (ii) the treated wastewater (TWW) collected from the wastewater treatment plant of Southern Sfax. The chemical characteristics of irrigation water are shown in Table 1.

2.2. Soil origin and preparation:

The soil used in this study was obtained from the first layer (0–20 cm) of the Taous experimental station of the Olive Tree Institute of Sfax, Tunisia. The soil was sieved, air dried and then autoclaved for 30 min at 121°C. Physical and chemical characteristics of soil are presented in Table 2.

2.3. Arbuscular mycorrhizal fungal inoculums:

In this investigation, three arbuscular mycorrhizal fungal (AMF) inoculums were used: (i) *Glomus deserticola* (M_1); (ii) *Gigaspora margarita* (M_2) and (iii) 1:1 mixture of *Glomus deserticola* and *Gigaspora margarita* (M_1+M_2). The inoculum is a commercial product obtained from Agrauxine-Biorize (Dijon, France) that contain spores and fragments of hyphae.

2.4. Plant material and experimental conditions:

The experiment was performed in a completely randomized design at the Olive Tree Institute of Sfax, Tunisia (34°43'N, 10°41'E). One-year-old olive plants (*Olea europaea* L. cv. Chetoui) were transplanted into 20 L pots filled with sterilized soil and were divided into mycorrhizal plants by adding 5 g of AMF inoculum (M_1; M_2 or M_1+M_2) below the roots of each plant and non mycorrhizal plants where 5 g of autoclaved M_1+M_2 inoculum were added. All pots were irrigated with tap water during two months of acclimation. Then, young olive plants were subjected from May 2015 to April 2016 to two type of water (tap water or TWW) according to the following treatments: (i) NM W_1 (non-mycorrhizal plants irrigated with tap water (control plants)); (ii) NM W_2 (non-mycorrhizal plants irrigated with TWW); (iii)

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M_1 W_2 (plants inoculated with *Glomus deserticola* and irrigated with TWW); (iv) M_2 W_2 (plants inoculated with *Gigaspora margarita* and irrigated with TWW); (v) M_1+M_2 W_2 (plants inoculated with *Glomus deserticola* and *Gigaspora margarita* and irrigated with TWW). Each of the five treatments was replicated seven times to give a total of 35 pots. During the experimental period, all pots were covered with plastic film and kept under ambient environmental conditions with natural sunlight and temperature. The air temperature ranged between 17±3 and 32±3°C and the relative humidity varied from 53 to 74%. All plants were irrigated twice a week to maintain soil moisture below the field capacity according to the method described by Acosta-Motos et al. (2015) and Acosta-Motos et al. (2017). Such technique of irrigation avoids the drainage of water and causes an increase in the salinity of soil due to the long period of experimentation (one year). It should be noted that drainage was applied only once during the experiment.

2.5. Mycorrhizal colonization:

At the beginning and the end of the experiment, samples of fresh roots were collected from each treatment and stained with 0.05% (w/v) trypan blue in lactophenol according to the method described by Phillips and Hayman (1970). The percentage of mycorrhizal colonization was calculated using the following formula (Hashem et al., 2016):

Mycorrhizal colonization (%) = (total number of infected segments/total number of observed segments) × 100

2.6. Soil analyses:

At the end of the experimental period, samples of soil were collected from the pots of all treatments, air dried and sieved at 2 mm to be used for analyses. The soil pH was determined in a soil water mixture (1:2.5 soil: distilled water) and measured with a pH meter (Istek pH 240L, Seoul, Korea). The soil electrical conductivity was measured on a saturated paste using a conductivity meter (WTW inoLab Cond 720, Weilheim, Germany) according to the method described by Pauwels et al. (1992). The exchangeable Na^+ in the soil was extracted with 1 M ammonium acetate solution at a ratio of 1:20 referring to the NF X 31-108 method described in AFNOR (2004) and then measured using a flame spectrophotometry (JENWAY PFP7, Milan, Italy). The determination of chloride content (Cl^-) in soil was carried out following the Mohr method as described by Pauwels et al. (1992).

2.7. Growth measurements:

The shoots elongation represented the difference in the total shoots length of young olive plants between the beginning and the end of the experiment. The leaf thickness and the leaf area were measured at the end of experimental phase using a digital slide caliper and a leaf-area-meter AM 300 (ADC Bioscientific Ltd., UK), respectively. The measurements of leaf thickness and leaf area were taken on five leaves from the shoot median part of three young olive plants from each treatment (in total 15 leaves per treatment).

2.8. Statistical analyses:

Statistical analyses were carried out using SPSS 20.0 statistical software. A one-way analysis of variance (ANOVA) was used to compare the average value of all parameters using the Duncan test ($p \leq 0.05$). In this study, results were the averages of at least three replicates.

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3. Results and Discussion:

At the end of the experimental phase, analyses showed that irrigation with TWW had no effect on soil pH value, in comparison to control soil irrigated with tap water (Table 3). Many authors have studied the effect of TWW irrigation on soil pH (Rusan et al., 2007; Castro et al., 2011; Singh and Agrawal, 2012; Bedbabis et al., 2015) but different results were observed. Rusan et al. (2007) and Castro et al. (2011) reported that the pH of soil has not changed under TWW application. However, Singh and Agrawal (2012) observed a decline in pH values after two years of TWW irrigation. Such decrease was attributed to the nitrification of ammonium. Other authors found that the increase of soil pH under TWW could be due to the high amounts on exchangeable cations (Tarchouna et al., 2010; Bedbabis et al., 2015). In this study, inoculation with the different AMF inoculums declined slightly the soil pH value of treated wastewater irrigated soil, as compared to non-inoculated soil. No significant differences ($p>0.05$) were recorded between all mycorrhizal treatments in soil pH value. Similar results were observed by Hu et al. (2010) in sandy loam soil cultivated by wheat (*Triticum aestivum* L.) and inoculated by *Glomus caledonium*. The same authors explained the reduction in pH value of soil to the role played by AMF in enhancing the H^+ production or the exudation of organic acid by the plant roots. Such mechanism is effective in decreasing the soil pH in order to improve the availability of nutrients to plants (Li et al., 1991; Hu et al., 2010).

The values of soil EC, measured at the end of the experiment, has increased in all treatments and particularly in TWW irrigated soil (Table 3). This increase was due to (i) the absence of drainage in pots during a long period of irrigation (one year), (ii) the high level of salts in treated wastewater (5.84 dS m^{-1}). Similarly, Acosta-Motos et al. (2017) reported that irrigation with three wastewater treatments containing different salt concentrations (EC=2.97 dS m^{-1}; 4.38 dS m^{-1} and 6.96 dS m^{-1}), during 23 weeks, induced an increase in soil EC of potted Eugenia (*Eugenia myrtifolia* L.). In our study, the high EC in TWW irrigated soil (12.13 dS m^{-1}) is an indicator of high salts concentrations, which may alter the soil quality, induce osmotic stress and disturb the plant growth (Elgallal et al., 2016). The presence of AMF appeared to not affect the soil EC in all mycorrhizal treatments. The obtained findings were in accordance with the results of Zuccarini and Okurowska (2008) in soil inoculated with *Glomus intraradices* under saline conditions. Nevertheless, contradictory results were observed by Yan et al. (2012) in soil cultivated with cucumber (*Cucumis sativus* L.) and inoculated with different AMF. In the same study, the author explained the reduction in EC values of inoculated soil as a result of a high uptake of salt ions due to a larger root system of colonized plants.

In accordance with the results of soil EC, an accumulation of both Na^+ and Cl^- was observed among all treatments after one year of irrigation, particularly in TWW irrigated soils (Table 3). This result agrees with the findings of many authors who observed an accumulation of salt ions due to TWW application and attributed such result to the high concentrations of Na^+ and Cl^- in TWW (Castro et al., 2011; Bedbabis et al., 2015; Acosta-Motos et al., 2017). In this study, the concentrations of Na^+ and Cl^- in soil solution may cause soil sodification, which may consequently affect the infiltration rate of water and thus its availability to plants (Al-Jasser 2011; Muamar et al., 2014). Interestingly, the inoculation of soil with AMF had significantly ($p\leq0.05$) reduced the content of both Na^+ and Cl^-. In comparison to NM W_2, the reductions of Na^+ content in the soil were 15, 17 and 9% in M_1W_2, M_2W_2 and M_1+M_2 W_2

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treatments, respectively. In the same treatments, the Cl^- was declined by 10, 18 and 21%. Thus, we suggest that the mycorrhizal young olive plants may have taken up more Na^+ and Cl^- and immobilized them in the root system. In fact, according to Göhre and Paszkowski (2006) and Porcel et al. (2012), AMF are known to play an important role in the sequestration of toxic compounds in the fungus and therefore provided an additional detoxification mechanism for host plants and contributed in ameliorating the quality of disturbed soils.

In this study, all non-inoculated young olive plants did not show mycorrhizal colonization in roots. However, all inoculated olive plants responded positively to the inoculation with the different AMF inoculums used. At the beginning of the experiment, the mycorrhizal colonization rate was about 68, 67 and 79% in plants inoculated by M_1, M_2 and M_1+M_2, respectively. Our results were in accordance with Ortas and Ustuner (2014) who reported that inoculation with a mixture of inoculums results in a higher root colonization. At the end of the experiment, our results showed that this parameter was slightly reduced in all mycorrhizal treatments. This result can be explained by the high amounts of Na^+ and Cl^-present in TWW (Table 1). Similar observations were noted by Gómez-Bellot et al. (2015b) who reported that treated wastewater irrigation reduced the mycorrhizal colonization in roots of laurustinus (*Viburnum tinus* L.) plants due to the salinity of TWW. Moreover, several authors indicated that mycorrhizal colonization decreased with increased salinity level (Hashem et al., 2016; Elhindi et al., 2017).

In response to TWW irrigation, shoots elongation, leaf area and leaf thickness of young olive plants were negatively affected, in comparison to control ones. The obtained findings were in accordance with the results of Valdés et al. (2015) who observed a decrease in the height and the leaves area of geranium (*Pelargonium* × *hortorum* L.H.) plants exposed to four levels of saline TWW irrigation (EC=5; 5.5; 6 and 6.5 dS m^{-1}) during three months. In our study, such responses could be due to the excessive amounts of toxic salts (Na^+ and Cl^-) present in TWW, which disturbed the uptake of water and nutrient from the soil and consequently inhibited the growth of young olive plants.

Interestingly, mycorrhization significantly ($p \leq 0.05$) enhanced all growth parameters of young olive plants under TWW application, as compared to non-colonized plants. For instance, in M_1+M_2 W_2 treated plants, the increase of shoots elongation was 44% in comparison to NM W_2 treated plants. This enhancement showed the beneficial effect of mycorrhizal symbiosis in mitigating the deleterious effect caused by the high salinity level of TWW on the growth of young olive plants. Such effect was also reported by Abdel-Fattah and Asrar (2012), Elhindi et al. (2017) and Metwally and Abdelhameed (2018), under saline conditions. Referring to these authors, the improvement of growth in young olive plants may be attributed to an increase in the uptake of water and nutrients through the hyphal network of AMF.

3. Conclusions:

The present work showed that application of saline TWW during one year negatively affected the chemical properties of soil, the root colonization as well as the growth of potted young olive plants cv. Chetoui. Interestingly, inoculation with AMF improved the soil quality *via* reducing the EC and the concentration of Na^+ and Cl^- in TWW irrigated soil. Furthermore, AMF symbiosis alleviated the negative effect of TWW by enhancing the shoots elongation and the leaf area of young olive plants. In this context,

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AMF colonization could be a potential solution to reduce the risk of salts accumulation in soil irrigated with saline TWW, which may consequently ameliorate the growth and development of olive plants.

Table 1. Chemical characteristics of tap water and treated wastewater used in the experiment.

Characteristics	W_1	W_2
pH	7.74±0.34	7.95±0.27
EC (dS m^{-1})	2.28±0.06	5.84±0.40
NO_3 (mg L^{-1})	1.49±0.25	14.88±1.24
NH_4 (mg L^{-1})	2.02±0.07	66.50±2.37
P_{tot} (mg L^{-1})	0.01±0.00	4.23±0.29
K (mg L^{-1})	10.00±1.00	66.67±2.89
Na (mg L^{-1})	370.00±14.14	730.00±10.00
Cl (mg L^{-1})	592.07±10.64	1465.39±16.38
Ca (mg L^{-1})	130.00±5.00	266.67±5.77
Mg (mg L^{-1})	64.33±6.03	105±9.54
Zn (mg L^{-1})	<0.01	1.02±0.18
Fe (mg L^{-1})	0.08±0.00	0.98±0.10
Cu (mg L^{-1})	0.02±0.00	0.10±0.01

Data represents mean values ± SD. W_1: tap water; W_2: treated wastewater.

Table 2. Physico-chemical characteristics of soil at the beginning of the experiment.

Parameter	Value
Sand (%)	75.88
Silt (%)	16.29
Clay (%)	7.83
pH	8.10±0.02
EC (dS m^{-1})	0.63±0.01
OM (%)	0.92±0.08
Na (mg kg^{-1})	52.00±6.29
Cl (mg kg^{-1})	59.80±2.25

Data represents mean values ± SD. EC: electrical conductivity; OM: organic matter.

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Table 3. Chemical characteristics of soil at the end of the experiment.

	pH	**EC (dS m^{-1})**	**Na$^+$ (mg kg^{-1})**	**Cl$^-$(mg kg^{-1})**
NM W$_1$	8.12±0.12^a	4.29±0.78^b	406.67±41.63^c	288.35±36.34^d
NM W$_2$	8.23±0.12^a	12.13±0.2^a	900.00±86.60^a	488.66±27.71^a
M$_1$ W$_2$	7.74±0.04^b	12.06±0.13^a	766.67±28.87^b	438.44±17.49^b
M$_2$ W$_2$	7.59±0.17^b	11.53±0.44^a	750.00±50.00^b	402.39±23.25^{bc}
M$_1$+M$_2$ W$_2$	7.77±0.24^b	11.39±0.44^a	816.67±57.74^{ab}	385.85±21.15^c

Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences between treatments ($p \leq 0.05$). EC: electrical conductivity; NM: non mycorrhizal plants; M$_1$: *Glomus deserticola*; M$_2$: *Gigaspora margarita*; M$_1$+M$_2$: mixture of *Glomus deserticola* and *Gigaspora margarita*; W$_1$: tap water; W$_2$: treated wastewater.

Table 4. Mycorrhizal colonization rate in roots of young olive plants inoculated with different arbuscular mycorrhizal fungal inoculums at the beginning (I) and the end of the experiment (II).

	M$_1$	**M$_2$**	**M$_1$+M$_2$**
% colonization (I)	67.78±5.09^b	66.67±6.67^b	78.89±3.85^a
% colonization (II)	50.00±5.77^c	52.22±5.09^c	62.22±3.85^b

Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences between arbuscular mycorrhizal fungal inoculums by Duncan test ($p \leq 0.05$). M$_1$: *Glomus deserticola*; M$_2$: *Gigaspora margarita*; M$_1$+M$_2$: mixture of *Glomus deserticola* and *Gigaspora margarita*.

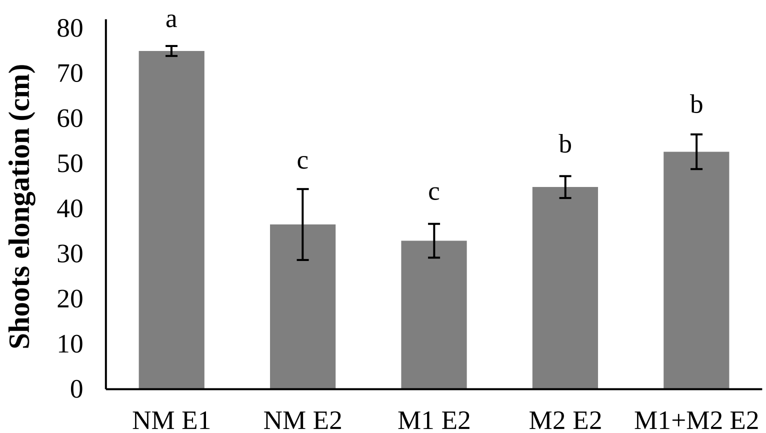

Figure 1. Effects of treated wastewater irrigation and arbuscular mycorrhizal fungal inoculation on shoots elongation of young olive plants. NM: non mycorrhizal plants; M$_1$: plants inoculated with *Glomus deserticola*; M$_2$: plants inoculated with *Gigaspora margarita*; M$_1$+M$_2$: plants inoculated with a mixture of *Glomus deserticola* and *Gigaspora margarita*; W$_1$: tap water; W$_2$: treated wastewater. Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences between treatments ($p \leq 0.05$).

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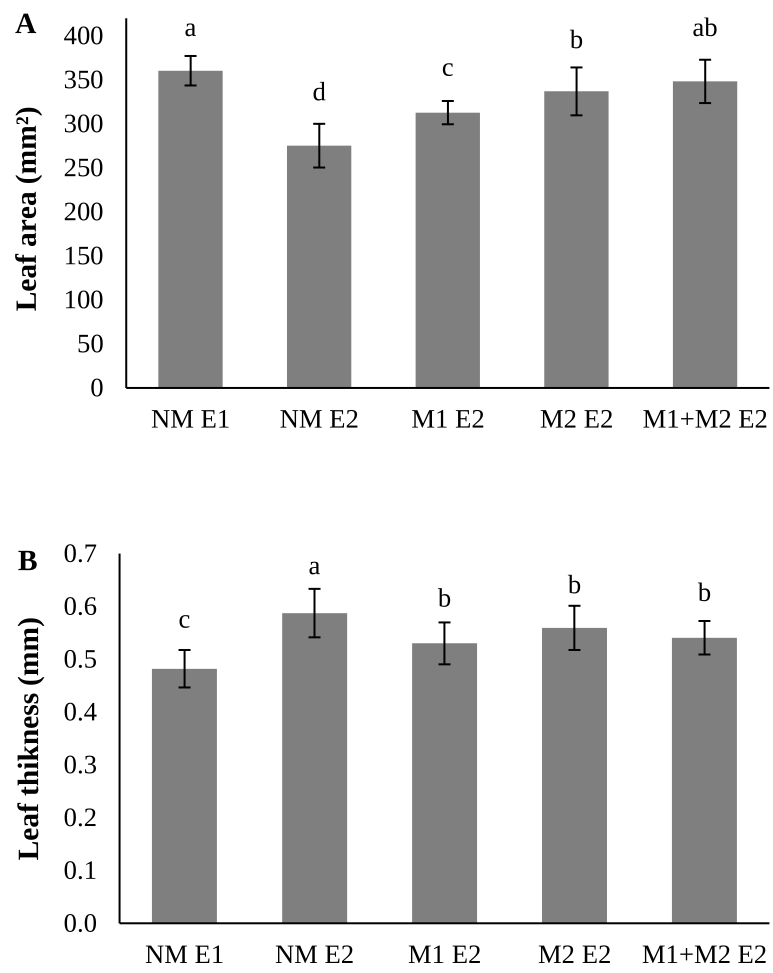

Figure 2. Effects of treated wastewater irrigation and arbuscular mycorrhizal fungal inoculation on leaf area (A) and leaf thickness (B) of young olive plants. NM: non mycorrhizal plants; M_1: plants inoculated with *Glomus deserticola*; M_2: plants inoculated with *Gigaspora margarita*; M_1+M_2: plants inoculated with a mixture of *Glomus deserticola* and *Gigaspora margarita*; W_1: tap water; W_2: treated wastewater. Values represent the means of 15 replications per treatment ± SD. Different letters indicate significant differences between treatments ($p \leq 0.05$).

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Acknowledgments:
This research was financially supported by the Tunisian Ministry of Higher Education and Scientific Research (LR16IO01, Ressources génétiques de l'olivier: Caractérisation, valorisation et protection phytosanitaire) and the Tunisian Ministry of Agriculture. The authors wish to thank Dr. Kamel Gargouri from the Olive Tree Institute of Sfax for his help and allowing the access to his respective laboratory (LR16IO02, Durabilité de l'oléiculture et de l'arboriculture en region semi-aride et aride: Amélioration de la productivité de l'olivier et de la qualité du produit).

References:

1. Abdel-Fattah GM, Asrar AWA. Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticumaestivum* L.) plants grown in saline soil. Acta Physiol Plant 2012; 34: 267–277.
2. Acosta-Motos JR, Diaz-Vivancos P, Álvarez S, Fernández-García N, Sanchez-Blanco MJ, Hernández JA. Physiological and biochemical mechanisms of the ornamental *Eugenia myrtifolia* L. plants for coping with NaCl stress and recovery. Planta 2015; 242: 829–846.
3. Acosta-Motos JR, Hernández JA, Álvarez S, Barba-Espín G, Sánchez-Blanco MJ. The long-term resistance mechanisms, critical irrigation threshold and relief capacity shown by *Eugenia myrtifolia* plants in response to saline reclaimed water. Plant Physiol Bioch 2017; 111: 244–256.
4. Acosta-Motos JR, Álvarez S, Barba-Espín G, Hernández JA, Sánchez-Blanco MJ. Salts and nutrients present in regenerated waters induce changes in water relations, antioxidative metabolism, ion accumulation and restricted ion uptake in *Myrtus communis* L. plants. Plant Physiol Biochem 2014; 85: 41–50.
5. AFNOR. Evaluation de la qualité des sols. vol. 1. Paris: AFNOR Editions; 2004. pp.461.
6. Aggarwal A, Kadian N, Tanwar A, Yadav A, Gupta KK. Role of arbuscular mycorrhizal fungi (AMF) in global sustainable development. J Appl Nat Sci2011; 3: 340–351.
7. Al-Jasser AO. Saudi wastewater reuse standards for agricultural irrigation: Riyadh treatment plants effluent compliance. J K S U E S2011; 23: 1–8.
8. Bedbabis S, Trigui D, Ben Ahmed C, Clodoveo ML, Camposeo S, Vivaldi GA, et al. Long-terms effects of irrigation with treated municipal wastewater on soil, yield and olive oil quality. Agric Water Manag 2015; 160: 14–21.
9. Castro E, Mañas MP, Heras JDL. Effects of wastewater irrigation on soil properties and turfgrass growth. Water Sci Technol 2011; 63: 1678–1688.
10. Chartzoulakis KS. Salinity and olive: Growth, salt tolerance, photosynthesis and yield. Agr Water Manag 2005; 78: 108–121.
11. Elgallal M, Fletcher L, Evans B. Assessment of potential risks associated with chemicals in wastewater used for irrigation in arid and semiarid zones: A review. Agric Water Manag 2016; 177: 419–431.
12. Elhindi KM, El-Din AS, Elgorban AM. The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.). Saudi J Biol Sci 2017; 24: 170–179.

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13. FAO. Users manual for irrigation with treated wastewater. Cairo: FAO Regional Office for the Near East; 2003. pp.41.
14. Göhre V, Paszkowski U. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 2006; 223: 1115–1122.
15. Gómez-Bellot MJ, Nortes PA, Ortuño MF, Romero C, Fernandez-Garcia N, Sánchez-Blanco MJ. Influence of arbuscular mycorrhizal fungi and treated wastewater on water relations and leaf structure alterations of *Viburnum tinus* L. plants during both saline and recovery periods. J Plant Physiol2015a; 188: 96–105.
16. Gómez-Bellot MJ, Ortuño MF, Nortes PA, Vicente-Sánchez J, Martín FF, Bañón S, et al. Protective effects of *Glomus iranicum* var. *tenuihypharum* on soil and *Viburnum tinus* plants irrigated with treated wastewater under field conditions. Mycorrhiza 2015b; 25: 399–409.
17. Hashem A, Abd_Allah EF, Alqarawi AA, Wirth S, Egamberdieva D. Comparing symbiotic performance and physiological responses of two soybean cultivars to arbuscular mycorrhizal fungi under salt stress. Saudi J Biol Sc 2016; 26: 38–48.
18. Hu J, Lin X, Wang J, Cui X, Dai J, Chu H, et al. Arbuscular mycorrhizal fungus enhances P acquisition of wheat (*Triticum aestivum* L.) in a sandy loam soil with long-term inorganic fertilization regime. Appl Microbiol Biotechnol 2010; 88: 781–787.
19. Li XL, George E, Marschner H. Phosphorus depletion and pH decrease at the root–soil and hyphae–soil interfaces of VA mycorrhizal white clover fertilized with ammonium. New Phytol 1991; 119, 397–404.
20. Metwally RA, Abdelhameed RE. Synergistic effect of arbuscular mycorrhizal fungi on growth and physiology of salt-stressed *Trigonella foenum-graecum* plants. Biocatal Agric Biotechnol 2018; 16: 538–544.
21. Muamar AJ, Tijane MH, Shawqi EA, El Housni A, Zouahri A, Bouksaim M. Assessment of the impact of wastewater use on soil properties. J Mater Environ Sci 2014; 5: 961–966.
22. Ortas I, Ustuner O. The effects of single species, dual species and indigenous mycorrhiza inoculation on citrus growth and nutrient uptake. Eur J Soil Biol 2014; 63: 64–69.
23. Pauwels JM, Van Ranst E, Verloo M, MvondoZe A. Manuel de Laboratoire de Pédologie – methods d'analyses de sols et de plantes ; equipment et gestion des stocks de verrerie et de produits chimiques. Publications Agricoles nr. 28, Bruxelles, Belgium; 1992.
24. Pedrero F, Mounzer O, Alarcón JJ, Bayona JM, Nicolás E. The viability of irrigating mandarin trees with saline reclaimed water in a semi-arid Mediterranean region: A preliminary assessment. Irrigation Sci 2013; 31: 759–768.
25. Pedrero F, Kalavrouziotis I, Alarcón JJ, Koukoulakis P, Asano T. Use of treated municipal wastewater in irrigated agriculture—Review of some practices in Spain and Greece. Agric Water Manag2010; 97: 1233–1241.
26. Phillips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. T Brit MycolSoc1970; 55:158–161.
27. Porcel R, Aroca R, Ruiz-Lozano JM. Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agron Sustain Dev2012; 32: 181–200.

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28. Rugini E and Fedeli E. Olive (*Olea europaea* L.) as an Oilseed Crop. In: Bajaj YPS, editor. Legumes and Oilseed Crops I. Berlin, Heidelberg: Springer; 1990. Pp. 593–641.
29. Rusan MJM, Hinnawi S, Rousan L. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. Desalination 2007; 215: 143–152.
30. Singh A, Agrawal M. Effects of Waste Water Irrigation on Physical and Biochemical Characteristics of Soil and Metal Partitioning in Beta vulgaris L. Agric Res 2012; 1: 379–391.
31. Smith SE, Read DJ. Mycorrhizal Symbiosis. Cambridge, UK: Academic Press; 2008.
32. Tarchouna LG, Merdy P, Raynaud M, Pfeifer HR, Lucas Y. Effects of long-term irrigation with treated wastewater. Part I: Evolution of soil physico-chemical properties. Appl Geochem 2010; 25: 1703–1710.
33. Toze S. Reuse of effluent water—benefits and risks. Agric Water Manag 2006; 80: 147–159.
34. Valdés R, Ochoa J, Franco JA, Sánchez-Blanco MJ, Bañón S. Saline irrigation scheduling for potted geranium based on soil electrical conductivity and moisture sensors. Agr Water Manag 2015; 149: 123–130.
35. Vicente-Sánchez J, Nicolás E, Pedrero F, Alarcón JJ, Maestre-Valero JF, Fernández F. Arbuscular mycorrhizal symbiosis alleviates detrimental effects of saline reclaimed water in lettuce plants. Mycorrhiza 2014; 24: 339–348.
36. Yan LI, Ying-Long CHEN, Min LI, Xian-Gui LIN, Run-Jin LIU. Effects of arbuscular mycorrhizal fungi communities on soil quality and the growth of cucumber seedlings in a greenhouse soil of continuously planting cucumber. Pedosphere 2012; 22: 79–87.
37. Zarei M, Paymaneh Z. Effect of salinity and arbuscular mycorrhizal fungi on growth and some physiological parameters of *Citrus jambheri*. Arch Agron Soil Sc 2014; 60: 993–1004.
38. Zuccarini P, Okurowska P. Effects of mycorrhizal colonization and fertilization on growth and photosynthesis of sweet basil under salt stress. J Plant Nut 2008; 31: 497–513.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

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Authors & Affiliations

Ameni Ben Hassena[1,2,*], Mohamed Zouari[3], Nabil Soua[3], Wahid Khabou[1], Nacim Zouari[4,5]

[1] Laboratory LR16IO01, Olive Tree Institute of Sfax, University of Sfax, Sfax, Tunisia
[2] Faculty of Sciences of Gabes, University of Gabes, Gabes, Tunisia
[3] Laboratory LR16IO02, Olive Tree Institute of Sfax, University of Sfax, Sfax, Tunisia
[4] Laboratory of Enzyme Engineering and Microbiology, Engineering National School of Sfax, University of Sfax, Sfax, Tunisia
[5] High Institute of Applied Biology of Medenine, University of Gabes, Medenine, Tunisia

Corresponding author:
Ameni Ben Hassena, Olive Tree Institute of Sfax,
Sfax, Tunisia.