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Impact of inoculation with single and mixed species of arbuscular mycorrhizal fungi on the soil fertility and the nutrient uptake of young olive plants

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Abstract

The current study aimed to determine the effect of single and dual inoculation with arbuscular mycorrhizal fungi (AMF) on soil quality and mineral content of young olive plants. One-year-old self-rooted olive plants (*Olea europaea* L.) of the cultivar Chetoui were inoculated with different AMF: (i) *Glomus deserticola* (AMF_1); (ii) *Gigaspora margarita* (AMF_2) or (iii) a 1:1 mixture of *G. deserticola* and *G. margarita* (AMF_3). After one year of symbiosis, the obtained results showed that AMF played an important role in improving the fertility of the experimental soil by increasing the organic matter and the micro-nutrients contents (N_t, P and K^+), as compared to control soil. Such effect induced an improvement in marco- and micro-nutrient contents in leaves and roots of all inoculated olive plants. The beneficial effect of mycorrhizal association was more important under inoculation with mixed species of AMF.

1. INTRODUCTION

The olive tree (*Olea europaea* L.) is a long-living tree, which considered as one of the most cultivated crops due to its profound economic and environmental importance (Bizos et al., 2020). In the last few decades, olive orchards have widely expanded to many new regions, mainly in marginal and infertile soils characterized by low nutrient levels (Zipori et al., 2020). Thereby, new plantations require the supply of a high number of vigorous plants well adapted to different climatic conditions, as well as the use of chemical fertilizers. Recently, inoculation of potted plants during the nursery period with arbuscular mycorrhizal fungi (AMF) is a largely proposed strategy to reduce excessive chemical fertilization and to develop well-nourished plants in a short period (Jiménez-Moreno et al., 2018; Trejo et al., 2021).

AMF are ubiquitous plant symbionts, which associate with the majority of plant species (Brundrett and Tedersoo 2018; Delavaux et al., 2022). Several studies reported that AMF are an eco-friendly approach to improve the physico-chemical characteristics of the soil, such as the soil structure via the effect of glomalin, the microbial activities and the soil nutrients availability (Yang et al., 2017; Okonji et al., 2018; Fall et al., 2022). The enhancement of the soil fertility in inoculated soils was attributed to the role played by AMF in the decomposition and the mineralization of organic matter, as well as the mobilization of nutrients, including N and P for the benefit of host plants (Okonji et al., 2018; Frey, 2019; Wei et al., 2019; Fall et al., 2022). In addition, AMF play a key role in enhancing the uptake of water and nutrients and consequently the growth of plants. The extra-radical mycelium increases the root surface areas and penetrate in soil pores, which are inaccessible to root hairs, to acquire nutrients, particularly immobile elements like P, Fe^{2+} and Zn^{2+} (Evelin et al., 2019).

The aim of the current work was to study the effect of colonization with different AMF, such as *Glomus deserticola*, *Gigaspora margarita* and a mixed inoculum of *G. deserticola* and *G. margarita* on the soil property and mineral content of young olive plants.

2. MATERIAL AND METHODS

2.1. Plant material, mycorrhizal inoculation and experimental conditions

A pot experiment was performed in a completely randomized design from March 2015 to April 2016 at the Olive Tree Institute at Sfax, Tunisia (34°43'N, 10°41'E). During the experimental period, the average values of air temperature and relative humidity were around 24°C and 63%, respectively. Uniform one-year-old self-rooted olive plants (*Olea europaea* L.) of the cultivar Chetoui were selected and transplanted into 20 L pots filled with sterilized soil collected from the experimental site of the Olive Tree Institute of Sfax, Tunisia. The used soil was sandy loam with the following characteristics (Ben Hassena et al., 2022): sand 75.88%; silt 16.29%; clay 7.83%; organic matter 0.92%; total N 135.33 mg kg^{-1}; P 10.40 mg kg^{-1}; K^{+} 89.33 mg kg^{-1}; Zn^{2+} 46.12 mg kg^{-1}; Fe^{2+} 1013.25 mg kg^{-1}; Cu^{2+} 0.12 mg kg^{-1}. At the time of transplanting, the young olive plants were inoculated either with a single or a mixture of arbuscular mycorrhizal fungi (AMF) species *(G. deserticola* (AMF1); *G. margarita* (AMF2) or a 1:1 mixture of *G. deserticola* and *G. margarita* (AMF3)). The used inoculum is a mixture of spores and fragments of hyphae provided by Agrauxine-Biorize (Dijon, France). For each potted olive plant, 5 g of inoculum were placed directly below the roots. For control plants, 5 g of autoclaved inoculum (mixture of *G. deserticola* and *G. margarita*) were added (AMF−). Each treatment had seven replicates. All plants were irrigated by tap water twice a week below the weight at field capacity as described by Ben Hassena et al. (2021). The chemical properties of the tap water were as follows: NH_4^{+} 2.02 mg/L; NO_3^{-} 1.49 mg/L; P 0.01 mg L^{-1}; K^{+} 10.00 mg L^{-1}; Zn^{2+} <0.01 mg L^{-1}; Fe^{2+} 0.008 mg L^{-1}; Cu^{2+} 0.02 mg L^{-1}. At the end of the experimental period, soil samples were collected from each treatment, air-dried and sieved to be used for soil analyses. The plants were harvested and divided into leaves and roots and washed with distilled water. The samples were either used immediately, stored at −80°C or oven-dried at 70°C to a constant mass.

2.2. Mycorrhizal colonization rate

The mycorrhizal colonization rate was determined at the end of the experiment. Samples of fresh olive roots were selected and cut into 1 cm root segments. The roots were then stained with 0.05% (w/v) trypan blue according to the method of Phillips and Hayman (1970) and examined with a Lieder microscope. The mycorrhizal colonization (mycelium, vesicles or arbuscules) within olive roots was calculated using the following formula (Hashem et al., 2016):

Mycorrhizal colonization rate (%) =

(Total number of infected segments/Total number of observed segments) × 100

2.3. Soil analyses

The soil organic matter (OM) was determined by the total organic carbon as described by Walkley and Black (1934). The total nitrogen (Nt) was estimated according to the Kjeldhal procedure (Pauwels et al., 1992). The phosphorus (P) was assessed by a vanado-molybdate colorimetric procedure with a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) as described by Pauwels et al. (1992). The exchangeable potassium (K^{+}) was extracted with 1 M ammonium acetate solution according to NF X 31-108 method (AFNOR, 2004) and then measured using a flame spectrophotometer (JENWAY PFP7, Milan, Italy). The Zn^{2+}, Fe^{2+} and Cu^{2+} were extracted with an aqua regia acid digestion (1:1 v/v HNO_3/HCl) (McGrath and Cunliffe, 1985) and measured using an atomic absorption spectrophotometer (PerkinElmer, Wellesley, MA, USA).

2.4. Plant mineral analyses

The plant mineral analyses were performed in samples of dried leaves and roots, ground to fine powder as described by Pauwels et al. (1992). The Nt content was measured according to the Kjeldhal method. The P content was measured by a vanado-molybdate colorimetric method using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The analyses of K^{+}, Zn^{2+}, Fe^{2+} and Cu^{2+} were carried out after dry ashing of samples in an oven at 250°C and a digestion of these ashes with 1 M nitric acid solution. The content of K^{+} was determined by a flame spectrophotometer (JENWAY PFP7, Milan, Italy). The content of Zn^{2+}, Fe^{2+} and Cu^{2+} were measured using an atomic absorption spectrophotometer (PerkinElmer, Wellesley, MA, USA).

2.5. Statistical analyses

Statistical analyses were carried out using SPSS 20.0 statistical software. The average value of all measured parameters (at least three replicates) were compared by one-way analysis of variance (ANOVA) using the Duncan test ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Mycorrhizal colonization rate

In the current study, the AMF structures such as hyphae and vesicles were observed in all inoculated olive plants, while no fungal infection was noted in control olive plants (Fig.1). All inoculum and olive plants formed a symbiotic relationship that result in high percentage of colonized roots. The root mycorrhizal colonization varied between 69 and 81% and the highest rate was noted under plants inoculated with *G. deserticola* and *G. margarita* (Fig.2). Similarly, Seifi et al. (2014) reported a high percentage of root colonization in olive plantlets using *Glomus mosseae* and *Glomus intraradices*, which prove that olive tree is a highly mycotrophic plant. In addition, Hart et al. (2015) noted that dual inoculation led to the highest root

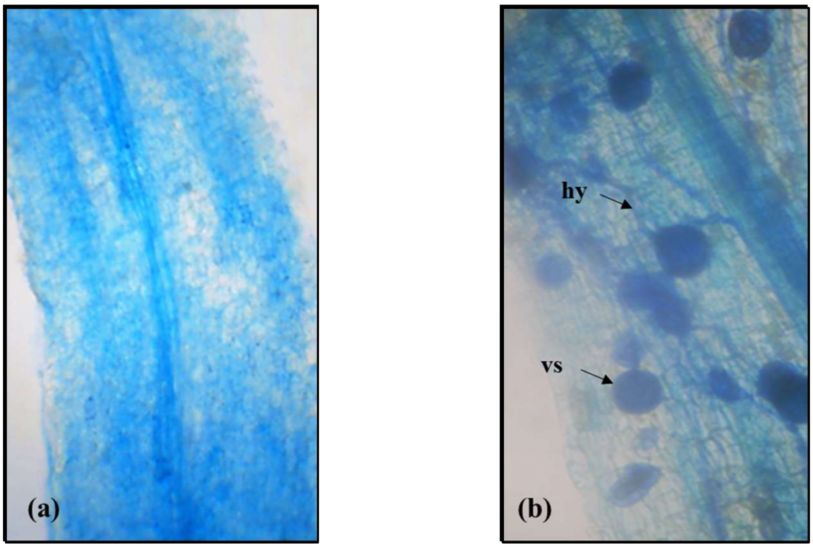

Fig. 1. Microscopic observation of root of young olive plants: (a) uninoculated plants; (b) AMF inoculated plants showing fungal structures (hyphae (hy); vesicles (vs)) (G: ×250).

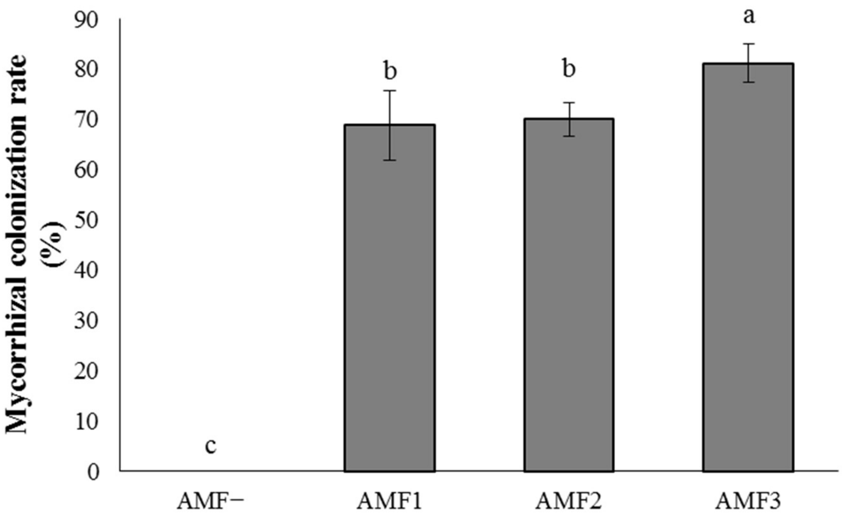

Fig. 2. Mycorrhizal colonization rate in roots of young olive plants inoculated with different arbuscular mycorrhizal fungal inoculums at the end of the experiment. AMF⁻: uninoculated plants; AMF_1: plants inoculated with *Glomus deserticola*; AMF_2: plants inoculated with *Gigaspora margarita*; AMF_3: plants inoculated with a mixture of *Glomus deserticola* and *Gigaspora margarita*. Values represent the means of 3 replications per treatment ± SD. Means followed by the same letter do not differ significantly by Duncan test ($p \leq 0.05$).

colonization rate in tomato plants (*Solanum lycopersicon* L.), as compared to single inoculation. Thus, our result may suggest the presence of a synergistic interaction between the two used AMF species.

3.2. Soil quality

The introduction of different AMF inoculums into the experimental soil was shown to induce changes in the soil chemical properties (Table 1). The obtained results showed that the soil organic matter (OM) was slightly increased when the soil was inoculated with AMF, particularly under dual inoculation. The increase was about 17.65, 5.88 and 35.29% in AMF1, AMF2 and AMF3 treatments, respectively, in comparison to the control treatment. Similar results were reported by Okonji et al. (2018) in AMF inoculated soil and cultivated during one year with different varieties of rice. The contribution of AMF on improving the soil organic matter was discussed in many studies (Okonji et al., 2018; Frey, 2019; Wei et al., 2019; Fall et al., 2022). According to these authors, AMF may increase the OM content of the soil through releasing a range of exudate compounds like glucose and organic acids. Furthermore, AMF play an important role in organic decomposition by stimulating the degradative enzymes and regulating the soil microbial biomass.

In addition, inoculation with AMF, especially with AMF3 (dual inoculation), induced a slight amelioration in the content of Nt, P and K+ compared to uninoculated soil. In AMF3 treatment, the increase of Nt, P and K+ was 24.07, 36.69 and 3.74%, respectively, as compared to AMF− treatment. The obtained results were in accordance with the findings of Okonji et al. (2018) that noted a remarkable increase in macro-nutrients in soil inoculated with AMF. Recently, Fall et al. (2022) reported that mycorrhizal association may play a key role in improving the availability of macro-nutrients in the soil, particularly phosphorus and nitrogen. Indeed, AMF are able to hydrolyze the organic P into inorganic form and to solubilize N for the benefit of host plants.

For micro-nutrients, the soil inoculation with AMF increased the Zn2+ content, while a reduction in Fe2+ and Cu2+ contents was observed in comparison to the control soil (except for Fe2+ in AMF2 treatment and Cu2+ in AMF1 treatment). It is known that mycorrhizal association enhance the uptake of low mobile micronutrients like Fe2+ and Cu2+ (Fall et al., 2022), which may induce a reduction in their contents in the soil.

Table1. Chemical characteristics of used soil at the end of the experiment.

	OM %	**N_t** mg kg^{-1}	**P** mg kg^{-1}	**K^+** mg kg^{-1}	**Zn^{2+}** mg kg^{-1}	**Fe^{2+}** mg kg^{-1}	**Cu^{2+}** mg kg^{-1}
AMF$^-$	0.78 ±0.08^b	126.00 ±12.12^b	13.17 ±0.74^b	142.67 ±2.31^c	10.48 ±0.34^b	1127.83 ±35.58^{ab}	0.20 ±0.03^{ab}
AMF$_1$	0.92 ±0.08^{ab}	144.67 ±8.08^{ab}	17.55 ±1.20^a	145.33 ±1.15^{bc}	15.53 ±4.72^{ab}	1118.25 ±89.42^{ab}	0.24 ±0.04^a
AMF$_2$	0.83 ±0.14^b	133.00 ±7.00^{ab}	16.19 ±1.76^{ab}	152.67 ±1.15^a	20.31 ±3.11^a	1290.17 ±157.90^a	0.17 ±0.02^{bc}
AMF$_3$	1.06 ±0.08^a	156.33 ±17.62^a	18.00 ±2.69^a	148.00 ±2.00^b	20.87 ±2.62^a	1083.42 ±61.85^b	0.14 ±0.04^c

AMF$^-$: uninoculated plants; AMF$_1$: plants inoculated with *Glomus deserticola*; AMF$_2$: plants inoculated with *Gigaspora margarita*; AMF$_3$: plants inoculated with a mixture of *Glomus deserticola* and *Gigaspora margarita*. ^{a,b,c} Different lower case letters in the same line indicate significant differences between the different treatments (p≤0.05).

3.3. Plant mineral content

Results describing the macro- and micro-nutrients contents in leaves and roots of young olive plants were presented in Table 2. According to the obtained results, the observed changes in the soil chemical properties affected the mineral content of young olive plants. Inoculation with different AMF increased significantly the Nt, P and K+ contents in the tissues of young olive plants, compared to control treatment (except for P in roots of AMF1 treated plants and K+ in leaves of AMF2 treated plants). For instance, compared to AMF− treated plants (control treatment), the increase of Nt, P and K+ contents in the leaves of AMF3 treated plants reached 16.75, 93.75 and 35%, respectively. Likewise, the increase of these macro-nutrients in the roots of young olive plants was about 30.69, 50.0 and 125.0%, respectively in the same treatments. In addition, a slight accumulation of micro-nutrients including Zn2+,

Table 2. Mineral contents in leaves and roots of young olive plants irrigated with treated wastewater and inoculated with different arbuscular mycorrhizal fungi.

		N_t % DW	P % DW	K^+ % DW	Zn^{2+} ppm	Fe^{2+} ppm	Cu^{2+} ppm
AMF^-	**Leaves**	$1.97±0.02^b$	$0.32±0.02^c$	$0.80±0.03^c$	$43.57±5.46^b$	$37.53±2.56^a$	$0.37±0.06^a$
	Roots	$1.01±0.09^b$	$0.24±0.01^c$	$0.20±0.02^c$	$149.80±17.64^a$	$39.40±6.13^a$	$2.93±0.25^c$
AMF_1	**Leaves**	$2.23±0.07^a$	$0.35±0.02^b$	$1.33±0.03^a$	$52.90±4.47^{ab}$	$41.67±6.03^a$	$0.37±0.12^a$
	Roots	$1.33±0.01^a$	$0.24±0.01^c$	$0.43±0.03^a$	$164.20±11.61^a$	$44.40±4.86^a$	$3.30±0.10^{ab}$
AMF_2	**Leaves**	$2.22±0.13^a$	$0.37±0.02^b$	$0.83±0.11^c$	$42.17±7.47^b$	$44.80±6.41^a$	$0.47±0.12^a$
	Roots	$1.34±0.06^a$	$0.26±0.01^b$	$0.36±0.04^b$	$156.30±9.40^a$	$41.33±1.53^a$	$3.00±0.17^{bc}$
AMF_3	**Leaves**	$2.30±0.07^a$	$0.62±0.02^a$	$1.08±0.03^b$	$63.47±5.06^a$	$47.10±4.59^a$	$0.50±0.10^a$
	Roots	$1.32±0.02^a$	$0.36±0.00^a$	$0.45±0.05^a$	$165.50±29.43^a$	$48.07±2.10^a$	$3.40±0.17^a$

AMF^-: uninoculated plants; AMF_1: plants inoculated with *Glomus deserticola*; AMF_2: plants inoculated with *Gigaspora margarita*; AMF_3: plants inoculated with a mixture of *Glomus deserticola* and *Gigaspora margarita*. [a,b,c] Different lower case letters in the same line indicate significant differences between the different treatments ($p≤0.05$).

Fe2+ and Cu2+ was reported in leaves and roots of all inoculated olive plants, compared to control ones, but differences were not significant. Such nutrients improvement may allow host plants to become more vigorous and able to tolerate various environmental stresses (Boutaj et al., 2020). Similar observations were reported by Huang et al. (2020) who noted an enrichment of the root walnut plants with marco-nutrients, particularly N. Furthermore, Chen et al. (2017) found that AMF colonization greatly increased the uptake of marco- and micro-nutrients in cucumber seedlings and concluded that multiple inoculation procured a better effect in improving the mineral status and growth of inoculated plants than single inoculation. It was widely reported that mycorrhizal symbiosis induces changes in the root morphology of host plants, which help them to explore a large volume of soil for a better acquisition of nutrients (Evelin et al., 2019; Huang et al., 2020). Moreover, AMF forms a fine extra-radical mycelium network that act as a bridge and enhances the uptake of nutrients, especially immobile elements such as P and Zn2+ to host plants (Li et al., 2019; Huang et al., 2020).

4. CONCLUSION

The inoculation of young olive plants either with a single (*Glomus deserticola* (AMF_1) or *Gigaspora margarita* (AMF_2)) or a mixture of arbuscular mycorrhizal fungi (AMF) species (a 1:1 mixture of *G. deserticola* and *G. margarita* (AMF_3)) during one year improved the soil quality and the mineral contents of leaves and roots of inoculated plants. The beneficial role of mycorrhizal colonization was particularly observed under dual inoculation treatment, which may suggest the presence of a synergistic interaction between *G. deserticola* and *G. margarita*.

Therefore, olive inoculation with AMF during nursery establishment seems to be a viable solution to reduce the use of chemical fertilizers and to improve the growth and the productivity of this species.

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