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Original article

The effects of single species, dual species and indigenous mycorrhiza inoculation on citrus growth and nutrient uptake

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ABSTRACT

The objective of this study was to evaluate the effects of selected single arbuscular mycorrhiza (AM) species, dual species inoculations and indigenous mycorrhizae (IM) inoculations, using specific growing media (GM), on the growth and nutrient uptake of sour orange (*Citrus aurantium* L.) seedlings. Five mycorrhiza species and their dual species inoculations, one commercial and one indigenous (multiple species) mycorrhizae were investigated using two growing media, GM1 (andesitic tuff + peat (1:1, V:V)) and GM2 (andesitic tuff + peat + soil (4 + 5+1, V: V:V)), under controlled greenhouse conditions. The experiments were conducted over 12 months. Mycorrhizal inoculation increased certain plant growth parameters such as shoot height, diameter, and shoot and root dry matter. Generally, mycorrhiza species and their dual species inoculations exerted different responses on plant growth. In GM1, *Funneliformis mosseae*, *Rhizophagus clarus and* indigenous mycorrhiza (IM) species were the best inoculants to increase citrus growth, root colonization and nutrient uptake; however, in GM2, *Fu. mosseae* and IM were the best inocula. *Fu. mosseae* + *R. clarus* was the most efficient dual species inoculation in GM1, whereas in GM2 the most efficient combination was *Fu. mosseae* + *Fu. caledonium*. The beneficial effects of AM fungi and the dual species inoculation with IM was also significant. Citrus seedlings grew much better in GM1 than in GM2. Root colonization was also increased with dual species inoculation and IM. In addition, the plant phosphorus and zinc content increased with mycorrhizal inoculation. Our results reinforce the efficiency of IM and mycorrhizal inoculations as being as effective as exotic AM fungal isolates. Dual species inoculation and IM show promise in the production of healthy seedlings, using several growing media.

1. Introduction

Arbuscular mycorrhiza (AM) display symbiotic associations between higher plants and fungi, and nearly 90% of plant species exhibit a mycorrhizal association; the relationship between mycorrhizal fungal mycelia and plant roots is one of the most prominent of these associations. Mycorrhizal inoculation has many effects on horticultural plants including: increasing seedling survival rate, plant growth rate and the number of flowers produced [1]. Mycorrhiza have also been found to increase seedling quality and improve growth after transplanting from greenhouse to field conditions. Wu et al. [2] reported that AM fungi-inoculated citrus seedlings exhibited higher total root length, total root projected area, total root surface area and total root volume.

As citrus plants have a limited number of short root hairs, they are dependent upon mycorrhizal colonization in order to obtain sufficient nutrients and water [3]; hence, plant species such as citrus are obligatorily mycorrhizal dependent [4–8]. Wang et al. [9] showed that the colonization of *Glomus versiforme* significantly increased the plant height, stem diameter, leaf number and dry mass. Citrus seedling trifoliate orange/care graft inoculated with *Funneliformis mosseae* has been shown to significantly increase the plant height, stem diameter, leaf area and shoot length of test seedlings [10,11]. The same species of AM fungi, in different geographic locations, might vary in their ability to colonize roots and improve plant growth [12]. Brussaard et al. [13] reported that mycorrhizal diversity positively contributes to nutrient and possibly water utilization efficiency. AM species exhibit different responses to different citrus cultivars, and nutrient uptake is particularly dependent on slowly mobile phosphorus (P), zinc (Zn) and copper (Cu). Srivastava et al. [14] reported that mycorrhizal inoculated citrus seedlings were observed to be highly effective in low fertility, coarse-textured soils.

The Çukurova region is highly productive for citrus growth with nearly 80% of the Turkish citrus plantations located in the Eastern Mediterranean sector of Turkey. Since the soils of this region consist

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of high levels of clay and lime, the availability of nutrients is limited; therefore, farmers use heavy chemicals to ensure better growth. Consequently, the biological fertility of the soil is negatively affected. As citrus plants are mycorrhizal dependent [4], it is important to produce mycorrhiza-inoculated seedlings for newly established citrus orchards. As citrus plants exhibit different responses to different mycorrhiza species it is reasonable to use different mycorrhiza species, in several growing media, to identify an efficient inoculum and elucidate their interaction under semi-arid Mediterranean conditions. It is also logical to determine a suitable growing medium for healthy seedling production; in addition, the use of indigenous mycorrhiza (IM) under field conditions is vitally important if the aim of better management is to be achieved [3].

This study was based on the hypothesis that multiple species inoculation and dual species inoculation (using two species) would enhance sour orange growth and nutrient uptake. The objective of the study was to evaluate the effects of single mycorrhiza species, dual species inoculation and multiple species inoculation, using specific growing media (GM), on the growth and nutrient uptake of sour orange seedlings.

2. Materials and methods

All experiments were conducted under greenhouse conditions in the University of Çukurova, Soil Science and Plant Nutrition Department, Adana-Turkey.

2.1. Growing media

Two different growing media (GM) were used: growing medium 1 (GM1) consisted of andesitic tuff + peat (1:1 (V: V), and growing medium 2 (GM2) consisted of andesitic tuff + peat + soil (4 + 5+1) (V: V: V).

The physical and chemical characteristics of the growing medium (soil, peat and andesitic tuff) was measured according to Page et al. [15] in the Rhizosphere Laboratory of Çukurova University, Adana-Turkey. Growing media (soil material) was collected from surface horizons of the clay loam Menzilat soil series (0–20 cm) (Typic Xerofluvents) in the Çukurova Basin, which had a pH of 7.68 and the 0.5 M $NaHCO_3$ (pH = 8.5) extractable P was 87.6 kg ha^{-1} P. Light peat had the following characteristics: pH of 5.1, 55% organic carbon and 0.670 EC (electrical conductivity) dS m^{-1}. Andesitic tuff contained 4.3% K and 0.03% P.

The growing medium was sterilized for 2 h at 120 °C in an autoclave. After sterilization, the growing medium was left for three weeks prior to being used.

2.2. Production of seedlings

Mycorrhizal and non-mycorrhizal seedlings were produced under glasshouse conditions. Sour orange (*Citrus aurantium* L.) seeds were surface sterilized with a sodium hypochlorite solution (1% available chlorine) for 10 min, rinsed and then soaked in distilled water several times. Before sowing the seeds, perlite was washed once with 0.01 M HCl and rinsed twice with ionized water and then autoclaved. Seeds were sown for 5 weeks in perlite trays; when the seedlings grew to a 3-leaf stage they were transplanted into 3 L plastic pots.

2.3. Inoculum source

The experimental treatments consisted of five selected species of arbuscular mycorrhizal fungi: *Funneliformis mosseae* ((T.H. Nicolson & Gerd.) C. Walker & Schuessler (2010) [16]) Rothamsted isolate, UK; *Claroideoglomus etunicatum* ((W.N. Becker & Gerd.) C. Walker & Schuessler (2010) [16]) Nutri-Link isolate; USA; *Rhizophagus clarus* ((T.H. Nicolson & N.C. Schenck) C. Walker & Schuessler (2010) [16]) Nutri-Link isolate, USA; *Funneliformis caledonium* ((T.H. Nicolson & Gerd.) C. Walker & Schuessler (2010) [16]) Rothamsted isolate, UK; one commercial inoculum, *Dr. Kindom*, was provided from Japan as a commercial inoculum, and indigenous mycorrhizae (IM) (multiple species) (isolated from the Çukurova Region, Turkey, by trap culture). The inoculum of each AMF isolate was previously multiplied in the greenhouse using sudan grass [*Sorghum bicolor* (L.) Moench] as the host plant and grown in a mixture of andesitic tuff + soil + peat (6 + 3 + 1, V:V:V)). *Fu. mosseae* (1) was propagated using maize as the host plant and *Fu. mosseae* (2) was propagated using an onion host plant. The cocktail is a mixture of the five AMF species in equal portions. In the non-mycorrhizal treatments, each seedling received the same amount of mycorrhiza-free substrate (autoclaved growing medium).

For both growing media, 1000 spores per plant were used and placed 30 mm below the seedling roots. The control plants were transplanted into the growing medium without an inoculum. For the control plants, the same amount of mycorrhiza-free inoculum was used.

2.4. Experimental design

The experiment was completely randomized and employed three replicates. Once the seedlings were transplanted, the inoculated and non-inoculated plantlets were grown for 12 months in a controlled glasshouse with day–night temperatures of 27 ± 2 °C. Plantlets were maintained under a 16 h photoperiod, using cool white fluorescent lamps, at approximately 350 μmol m^{-2} s^{-1}. The relative humidity was 70–80% at night and 80–85% during the day. Distilled water was added daily to maintain the moisture at 75% of field capacity. Plants were fertilized monthly with Hoagland's solution including P (11 mg kg^{-1}) and Zn (1.1 mg kg^{-1}).

2.5. Biomass assessment and nutrient analysis

At the end of the growing season, shoot length (in cm), shoot diameter (in mm) and, shoot and root dry weight (in g $plant^{-1}$), were measured for each treatment. Dried material from each pot was ground with a Tema mill, 0.2 g of the ground plant material was then ashed at 550 °C followed by dissolution in 3.3% HCl. After digestion of the plant material, the concentration of P in this solution was determined calorimetrically according to Murphy et al. [17] using a flame photometer. An atomic absorption spectrophotometer (Perkin Elmer) was employed to determine the Zn content of the plant samples.

2.6. Mycorrhizal colonization (%)

At harvest, the shoots were separated from the roots 0.5 cm above the soil surface, the roots were separated from the soil by washing with running tap water and distilled water. Prior to drying the roots at 70 °C for 48 h, small sub-samples were taken and preserved in a mixture of ethanol, glacial acetic acid and formalin, for the determination of mycorrhizal infection. Portions of preserved roots were stained using the method of Koske et al. [18]. Mycorrhizal fungus colonization was determined using the gridline-intersect method of Giovanetti et al. [19].

2.7. Statistical analysis

The variation in plant growth, root colonization and nutrient content, attributed to the effects of growing media and mycorrhizal

species, were assessed via the analysis of variance procedure using the SAS [20] 9.1 computer program. For all statistical analyses, using the analysis of variance ANOVA, the main significant interactive effects of mycorrhizal inoculation and growing medium were separated by the Duncan Multiple Range Test (DMRT) at $P < 0.05$.

3. Results

3.1. Plant length and shoot diameter

Single species inoculation and dual species inoculation exerted different responses on the growth of citrus seedlings. Generally, inoculated citrus plants grew better than non-mycorrhizal plants (control); in addition, inoculated plant leaves looked healthier than the non-inoculated control plants.

In general, the plant height increased significantly upon application of the mycorrhizal inoculation compared with control treatments. The plant height ranged from 78.82 cm to 115.46 cm in GM1 ($\overline{X} = 96.26$ cm; 60.4 cm–98.17 cm in GM2, $\overline{X} = 85.03$ cm). In GM1, the citrus seedling roots inoculated with *Fu. mosseae* (1) + *R. clarus*, resulted in a plant height of 115.46 cm. IM-inoculated plants reached a height of 101.46 cm and 98.17 cm for GM1 and GM2, respectively (Table 1). Furthermore, mycorrhizal interaction treatments generally resulted in significantly higher plant height than single mycorrhizal inoculation. Plants grown in GM1 exhibited a plant height of 84.24 cm in the control, compared with 115.46 cm in the *Fu. mosseae* (1) + *R. clarus* treatment. In GM2, the plant height was 86.37 cm in the control, compared with 98.17 cm in IM and 97.47 cm in the *Fu. mosseae* (1) + *Fu. caledonium* treatment.

The shoot diameter measured at harvest differed with mycorrhizal inoculation and ranged from 6.37 cm to 7.97 cm in GM1, with a mean of $\overline{X} = 7.28$ cm, and 5.33 cm–7.27 cm in GM2, with a mean of $\overline{X} = 6.45$ cm (Table 1). For both GM1 and GM2, seedlings inoculated with *R. clarus* exhibited the largest shoot diameters at 7.97 cm and 7.27 cm, respectively.

3.2. Dry matter production

Mycorrhiza-inoculated plants produced a significantly higher dry weight (shoot and root) than non-inoculated plants. In GM1, control plants produced 12.93 g pot^{-1} shoot dry weight (SDW), however, *R. clarus* gave 26.79 g pot^{-1}, *Fu. mosseae* (1) resulted in 24.41 g pot^{-1} and the *Fu. mosseae* (2) + *R. clarus* interaction produced 29.7 g pot^{-1} SDW. Furthermore, in GM2, the control plants produced 11.62 g pot^{-1} and the IM-inoculated plant produced significantly more at 26.30 g pot^{-1} SDW. In GM2, the *Fu. mosseae* (1) + *Fu. caledonium* interaction produced 24.2 g pot^{-1} SDW. Seedlings grown in GM1 produced an average of 22.97 g pot^{-1} SDW; however, those grown in GM2 produced an average of 19.47 g pot^{-1} SDW.

In GM1, the control seedlings produced 5.06 g pot^{-1} RDW; seedlings which received *Fu. mosseae* produced 11.73 g pot^{-1} RDW and *R. clarus*-inoculated seedlings produced 11.68 g pot^{-1} RDW. In GM2, the control seedlings produced 4.08 g pot^{-1} RDW with mycorrhizal-inoculated seedlings producing 14.81 g pot^{-1} RDW. For both GM media, *R. clarus*-inoculated seedlings generally produced a higher root dry weight than other mycorrhizal inocula. In addition, dual mycorrhizal inoculation exhibited a higher RDW than the single inoculum. Seedlings grown in GM1 produced an average of 10.36 g pot^{-1} RDW; however, those grown in GM2 produced an average of 10.50 g pot^{-1} RDW.

3.3. Root infection

In GM1 and GM2, mycorrhiza inoculation exerted a substantial impact upon root colonization. Statistically, the mycorrhiza species and their interaction for both growing media are significant. The

Table 1
The effect of single, dual, multiple (cocktail of five species, indigenous mycorrhiza) and a commercial arbuscular mycorrhizal product on shoot height and diameter of seedlings of sour orange on two growing media.

Treatment	Shoot height (cm)		Shoot diameter (cm)	
	GM1	GM2	GM1	GM2
Control	84.24 ± 33.71g–i	86.37 ± 7.17c–e	6.37 ± 0.49d	6.27 ± 0.23a–d
Funneliformis mosseae (1)	100.26 ± 5.81b–e	87.50 ± 6.71cd	7.10 ± 0.10b–d	6.63 ± 0.80a–d
Funneliformis mosseae (2)	91.48 ± 14.84d–g	90.97 ± 12.33a–d	7.23 ± 0.31a–d	6.23 ± 0.98a–d
Funneliformis caledonium	93.72 ± 44.99b–e	77.30 ± 19.75fe	7.50 ± 0.87a–d	5.83 ± 1.70b–d
Claroideoglomus etunicatum	78.82 ± 34.26i	88.80 ± 9.97b–d	7.60 ± 0.53a–c	6.17 ± 0.21a–d
Rhizophagus clarus	104.7 ± 9.45b	87.83 ± 4.66c–e	7.97 ± 0.85a	7.27 ± 0.46a
Indigenous mycorrhiza	101.46 ± 11.06b–d	98.17 ± 3.23a	7.30 ± 0.44a–d	7.13 ± 0.93ab
Dr. Kindom	100.44 ± 11.77b–d	86.77 ± 13.21cd	7.13 ± 0.90a–d	6.17 ± 0.93a–d
Fu. mosseae (1) + *Fu. caledonium*	91.10 ± 30.78f–h	91.43 ± 12.87a–d	7.40 ± 0.53a–d	7.27 ± 0.31a
Fu. mosseae (1) + *Fu. caledonium*	86.78 ± 27.56gh	97.47 ± 8.93ab	7.73 ± 0.25a–c	6.90 ± 0.75a–c
Fu. mosseae (1) + *Cl. etunicatum*	102.58 ± 10.43b–d	60.40 ± 22.48h	7.13 ± 0.12a–d	5.97 ± 1.29a–d
Fu. mosseae (1) + *R. clarus*	104.24 ± 5.25b	93.07 ± 5.32ab	7.10 ± 0.36b–d	6.90 ± 0.90a–c
Fu. mosseae (2) + *Fu. caledonium*	80.88 ± 37.27hi	86.57 ± 12.13dc	7.03 ± 0.06b–c	6.70 ± 0.61a–d
Fu. mosseae (2) + *Cl. etunicatum*	107.32 ± 10.07ab	74.90 ± 2.76fg	7.13 ± 0.15a–d	6.30 ± 0.52a–d
Fu. mosseae (2) + *R. clarus*	115.46 ± 6.91a	83.33 ± 10.02de	7.43 ± 0.49a–d	6.83 ± 0.29a–c
Fu. caledonium + *Cl. etunicatum*	97.74 ± 9.36b–e	82.33 ± 3.79de	6.90 ± 0.66cd	7.00 ± 0.00a–c
Fu. caledonium + *R. clarus*	95.54 ± 15.53f–h	87.67 ± 9.07de	7.40 ± 0.53a–d	6.00 ± 1.00a–d
Cl. etunicatum + *R. clarus*	89.90 ± 32.72bc	88.83 ± 4.04b–d	7.07 ± 0.12b–d	5.67 ± 0.58cd
Cocktail	102.3 ± 8.92	65.83 ± 12.79gh	7.77 ± 0.55ab	5.33 ± 1.15d
Mean	96.26	85.03	7.28	6.45
Pr > *F*	0.0001	0.0001	0.0001	0.0001

Mean of three replicates and ± is standard error.
Columns with different letters are significantly different ($P < 0.05$) according to Duncan's test.
Fu. mosseae – 1. propagated on maize host plant.
Fu. mosseae – 2. propagated on onion host plant.
Growing medium (GM1) consisted of andesitic tuff + peat (1:1 (V:V).
Growing medium (GM2) consisted of andesitic tuff + peat + soil (4 + 5 + 1) (V:V:V).

indigenous mycorrhiza colonized plant roots at a significant level; in GM1, % root colonization was 6 for the control, 50 for the IM inoculation and 53 for the *Fu. mosseae* (I) + *Fu. caledonium* interaction (Table 2). In GM2, % root colonization was 3 for the control, 53 for the IM and *Fu. mosseae* exhibited a root colonization of 50. In addition, the *Fu. mosseae* (I) + *Fu. caledonium* inoculated seedling roots displayed 57% root colonization.

3.4. Plant nutrient status

Statistically, the species of mycorrhiza and growing media significantly affected the plant P and Zn content (Table 3). Plant P percentage ranged from 0.06% to 0.21% with an average mean of 0.11% P for GM1; for GM2 treatments, the range was 0.7%–0.11%, with an average mean of 0.09% P. Generally, plants grown in GM1 exhibited a higher P % content than those grown in GM2 and the mycorrhiza interactions resulted in a higher P % content than the single mycorrhiza species. For example, the GM1 *Fu. caledonium* + *Cl. etunicatum* and *Fu. caledonium* + *R. clarus* interactions resulted in the highest P concentration at 0.21% P. However in GM2, the *Fu. caledonium* + *Cl. etunicatum* interaction resulted in 0.11% P (Table 4).

Plant Zn content was determined and revealed that statistically, inoculation with mycorrhiza significantly differentiated the plant Zn content. However, mycorrhizal species and interactions produced a Zn content which exceeded the critical level. IM-inoculated seedlings resulted in 32.4 Zn kg^{-1} DW, the *Fu. mosseae* (I) + *Cl. etunicatum* inoculum resulted in 38.8 mg Zn kg^{-1} DW, and the *Fu. mosseae* (I) + *R. clarus* interaction resulted in 39.5 mg Zn kg^{-1} DW in GM1. In GM2, *R. clarus* yielded 36.2 mg Zn kg^{-1} DW and the *Fu. mosseae* (1) + *Fu. caledonium* inoculum gave 35.5 mg Zn kg^{-1} DW. Citrus seedlings grown in GM1 contained an average mean of 29.1 mg Zn kg^{-1} DW and in GM2 the average mean was 30.3 mg Zn kg^{-1} DW.

4. Discussion

The results show that mycorrhizal inoculation significantly increased citrus seedling growth and provided a significant growth response in the pot media. As nursery companies produce citrus seedlings under greenhouse conditions, employing several growing media, one of the priorities was to use different growing media with several inocula to obtain better inoculation and seedling health. In the present work, GM1 resulted in a higher plant height, shoot diameter and growth than that found in GM2. As GM1 contains higher organic matter than in GM2, plants grown in GM1 showed higher responses than in GM2. In a similar experiment [21] reported that growth media has a significant effect on citrus growth. Mycorrhiza inoculation and mycorrhizal interactions in particular, exert a significant impact upon growth; in addition, the isolated IM spores significantly affected growth and nutrient levels. Moreover, in most cases there are differences, among the mycorrhizal species, in their contribution to plant growth and nutrient uptake. It may also be helpful to use the dual species inoculation of several species to allow a better prediction of the inocula effect. In the present work, several mycorrhiza species were used, however, it seems that mycorrhizal dual species inoculation is also important (Tables 1–3). Albrechtova et al. [22] showed that dual mycorrhizal inoculation increased onion yield by more than 50%. In the present work, dual species inoculation and IM resulted in a higher P % content than using a single inocula. As IM includes several mycorrhiza species, the effect may be higher than a single mycorrhiza inoculum. Furthermore, IM results in a higher root colonization than using a single species inocula. Williams et al. [23] reported that plants treated with indigenous AMF exhibited significantly greater survival than those treated with commercial AMF.

Wu et al. [11] showed that arbuscular mycorrhizal fungi inoculation could increase certain plant growth parameters, such as plant

Table 2
The effect of single, dual, multiple (cocktail of five species, indigenous mycorrhiza) and a commercial arbuscular mycorrhizal product on shoot and root dry weight of seedlings of sour orange on two growing media.

Treatment	Shoot		Root	
	Dry weight (DW)			
	GM1	GM2	GM1	GM2
Control	12.9 ± 10.72b	11.6 ± 2.99b	5.06 ± 2.01b	4.08 ± 0.55b
Funneliformis mosseae (1)	24.4 ± 3.02a	22.4 ± 2.47ab	11.73 ± 3.27a	9.89 ± 4.11ab
Funneliformis mosseae (2)	21.8 ± 6.76a	18.3 ± 6.24ab	9.48 ± 2.57a	6.52 ± 3.14ab
Funneliformis caledonium	23.1 ± 13.36a	15.2 ± 3.44ab	11.41 ± 6.68a	9.97 ± 7.06ab
Claroideoglomus etunicatum	19.1 ± 12.80a	19.2 ± 3.60ab	7.09 ± 5.01a	7.30 ± 1.68ab
Rhizophagus clarus	26.8 ± 4.92a	18.5 ± 2.52ab	11.68 ± 2.37a	11.43 ± 0.89ab
Indigenous mycorrhiza	23.1 ± 7.34a	26.3 ± 1.77a	9.15 ± 2.95a	14.81 ± 1.76ab
Dr. Kindom	22.6 ± 7.61a	16.1 ± 6.14ab	10.26 ± 5.75a	7.16 ± 3.16ab
Fu. mosseae (1) + *Fu. caledonium*	23.2 ± 9.35a	24.2 ± 1.39a	11.42 ± 5.33a	12.56 ± 2.10ab
Fu. mosseae (1) + *Fu. caledonium*	21.1 ± 11.19a	22.9 ± 2.51ab	10.07 ± 6.98a	14.41 ± 2.92ab
Fu. mosseae (1) + *Cl. etunicatum*	25.2 ± 5.08a	18.0 ± 5.05ab	12.94 ± 3.80a	4.64 ± 2.80b
Fu. mosseae (1) + *R. clarus*	25.7 ± 2.42a	19.7 ± 0.53ab	11.33 ± 1.55a	15.20 ± 3.66ab
Fu. mosseae (2) + *Fu. caledonium*	19.6 ± 12.83a	23.1 ± 1.85ab	8.10 ± 4.79a	10.97 ± 4.49ab
Fu. mosseae (2) + *Cl. etunicatum*	26.2 ± 5.57a	16.0 ± 0.69ab	9.69 ± 2.81a	7.73 ± 0.67ab
Fu. mosseae (2) + *R. clarus*	29.7 ± 2.70a	20.3 ± 1.07ab	13.32 ± 3.11a	13.38 ± 1.42ab
Fu. caledonium + *Cl. etunicatum*	21.5 ± 4.31a	19.9 ± 0.67ab	10.02 ± 3.13a	14.37 ± 0.66ab
Fu. caledonium + *R. clarus*	23.9 ± 7.85a	21.6 ± 3.17ab	10.95 ± 3.80a	11.04 ± 3.23ab
Cl. etunicatum + *R. clarus*	20.4 ± 11.10a	23.0 ± 1.39ab	9.48 ± 8.25a	16.91 ± 7.49a
Cocktail	26.2 ± 2.92a	13.6 ± 2.34b	12.67 ± 2.12a	6.09 ± 1.02b
Mean	22.97	19.47	10.31	10.45
Pr > *F*	0.0	0.0105	0.02	0.0006

Mean of three replicates and ± is standard error.
Columns with different letters are significantly different ($P < 0.05$) according to Duncan's test.
Fu. mosseae – 1. propagated on maize host plant.
Fu. mosseae – 2. propagated on onion host plant.
Growing medium (GM1) consisted of andesitic tuff + peat (1:1 (V: V).
Growing medium (GM2) consisted of andesitic tuff + peat + soil (4 + 5 + 1) (V: V: V).

Table 3
The effect of single, dual, multiple (cocktail of five species, indigenous mycorrhiza) and a commercial arbuscular mycorrhizal inoculation on root colonization of seedlings of sour orange on two growing media.

Treatment	Root colonization (%)			
	GM1		GM2	
Control	6 ± 0.0	d	3 ± 2.8	d
Funneliformis mosseae (1)	20 ± 10.0	cc	50 ± 10.0	ab
Funneliformis mosseae (2)	30 ± 20.0	a–d	37 ± 28.7	a–c
Funneliformis caledonium	30 ± 10.0	a–d	43 ± 20.6	a–c
Claroideoglomus etunicatum	30 ± 10.0	a–d	27 ± 5.8	b–d
Rhizophagus clarus	20 ± 0.0	cd	30 ± 10.0	cd
Indigenous mycorrhiza	50 ± 0.0	a	53 ± 11.6	a
Dr. Kindom	30 ± 0.0	a–d	30 ± 20.0	bc
Fu. mosseae (1) + *Fu. caledonium*	43 ± 25.2	ac	57 ± 15.3	a
Fu. mosseae (1) + *Fu. caledonium*	23 ± 5.8	b–d	30 ± 26.6	bc
Fu. mosseae (1) + *Cl. etunicatum*	53 ± 15.3	a	30 ± 17.3	bc
Fu. mosseae (1) + *R. clarus*	30 ± 10.0	a–d	46 ± 5.8	a–c
Fu. mosseae (2) + *Fu. caledonium*	40 ± 20.0	a–c	37 ± 15.3	a–c
Fu. mosseae (2) + *Cl. etunicatum*	53 ± 30.6	a	40 ± 10.0	a–c
Fu. mosseae (2) + *R. clarus*	20 ± 10.0	cd	37 ± 11.5	a–c
Fu. caledonium + *Cl. etunicatum*	37 ± 28.8	a–c	37 ± 15.3	a–c
Fu. caledonium + *R. clarus*	47 ± 15.3	ab	37 ± 5.8	a–c
Cl. etunicatum + *R. clarus*	43 ± 11.6	a–c	50 ± 10.0	ab
Cocktail	40 ± 20.0	a–c	30 ± 10.0	bc
Mean	33.95		35.47	
Pr > *F*	0.0384		0.0452	

Mean of three replicates and ± is standard error.
Columns with different letters are significantly different ($P < 0.05$) according to Duncan's test.
Fu. mosseae – 1. propagated on maize host plant.
Fu. mosseae – 2. propagated on onion host plant.
Growing medium (GM1) consisted of andesitic tuff + peat (1:1 (V: V).
Growing medium (GM2) consisted of andesitic tuff + peat + soil (4 + 5 + 1) (V:V:V).

height, stem diameter, leaf area, shoot dry weight, root dry weight and plant dry weight, compared with the control. Tong et al. [24] investigated the effect of several mycorrhizal inocula on seedling growth and found that AM fungi effectively colonized seedling roots and that shoot and root growth, especially fibrous root growth, was significantly improved when compared with the control.

Our results show that dual inoculations, when compared with single inoculation, increased plant growth and nutrient uptake. It was determined that different single mycorrhiza species show different responses in GM1 and GM2. In GM1, the *R. clarus* and *Fu. mosseae* (1) treatment, and IM were the best mycorrhiza species in terms of SDW and RDW; whereas, *Fu. mosseae* (1) and IM were the most efficient inocula in GM2. Graham et al. [25] reported that mycorrhiza have varying degrees of influence on lemons in different growing media and with different P doses. In addition, inoculation of the soil with cultured spores of AM isolates of *Fu. mosseae* or a mixture of isolates of *Fu. mosseae* and *R. clarus*, markedly improved P and Zn uptake, and the dry matter accumulation of citrus plants. Similarly, Wu, et al. [26] indicated that *Fu. mosseae* inoculation improved the growth performance of citrus plantlets and stimulated the accumulation of N, P, Ca, Cu, Zn and Mn in leaves and roots, compared with the non-inoculated treatment. In general, citrus fruits show an optimum % P between 0.12 and 0.16%, which is under the critical level. Generally in GM1, the IM *Fu. caledonium* and *R. clarus* inoculated plants contained the highest P level. In GM2, *Fu. mosseae* (2) and *Fu. caledonium* inoculated plants exhibited the highest P content. However, in the present work, in general, the plant P % was less than the critical level. Previously, Ortas, et al. [27] obtained similar results with sour orange seedlings under the same soil and climate conditions.

The results show that mycorrhiza inoculated plants have a high P concentration, which is in harmony with results obtained by other researchers [4,21,27–30]. In the present work, *R. clarus* and interactions with *R. clarus* were also efficient. Ortas, et al. [27] previously used five different single AM species under greenhouse conditions, in two different experiments, and found that *R. clarus* gave the best improvement in growth. Similarly, Schmitz, et al. [31] reported that, using three growing media, *R. clarus* exhibited a higher relative growth with *Poncirus trifoliata*.

Table 4
The effect of single, dual, multiple (cocktail of five species, indigenous mycorrhiza) and a commercial arbuscular mycorrhizal product on P and Zn concentration of seedlings of sour orange on two growing media.

Treatment	P concentration (%)				Zn concetration (mg kg DW)			
	GM1		GM2		GM1		GM2	
Control	0.06 ± 0.00	gh	0.07 ± 0.01	cd	27.4 ± 2.6	b–e	27.8 ± 2.6	c–e
Funneliformis mosseae (1)	0.07 ± 0.01	f–h	0.09 ± 0.01	a–d	28.1 ± 5.5	b–e	29.8 ± 1.1	a–d
Funneliformis mosseae (2)	0.06 ± 0.01	h	0.1 ± 0.01	a–c	20.5 ± 2.2	fe	31.2 ± 7.9	a–c
Funneliformis caledonium	0.08 ± 0.00	f–h	0.11 ± 0.01	ba	18.4 ± 1.5	f	32.6 ± 7.7	a–c
Claroideoglomus etunicatum	0.06 ± 0.01	gh	0.08 ± 0.01	b–d	27.3 ± 8.8	b–e	28.3 ± 6.9	b–e
Rhizophagus clarus	0.09 ± 0.01	d–e	0.09 ± 0.02	a–c	31.4 ± 1.8	ab	36.2 ± 2.9	a
Indigenous mycorrhiza	0.1 ± 0.01	d–e	0.08 ± 0.00	b–d	32.2 ± 2.9	ab	35.1 ± 0.9	ab
Dr. Kindom	0.08 ± 0.01	f–g	0.07 ± 0.03	dc	33.4 ± 5.6	ab	27.7 ± 3.1	c–e
Fu. mosseae (1) + *Fu. caledonium*	0.08 ± 0.01	f–g	0.09 ± 0.00	a–d	31.6 ± 3.4	ab	35.5 ± 3.4	a
Fu. mosseae (1) + *Fu. caledonium*	0.08 ± 0.00	f–g	0.09 ± 0.01	a–d	29.6 ± 5.9	b–d	32.6 ± 0.3	a–c
Fu. mosseae (1) + *Cl. etunicatum*	0.11 ± 0.00	d–e	0.07 ± 0.04	d	38.8 ± 1.3	a	32.2 ± 0.7	a–c
Fu. mosseae (1) + *R. clarus*	0.1 ± 0.00	d–e	0.1 ± 0.01	a–c	39.2 ± 0.8	a	30.2 ± 5.7	a–c
Fu. mosseae (2) + *Fu. caledonium*	0.11 ± 0.04	d	0.09 ± 0.00	a–d	30.4 ± 9.8	bc	22.2 ± 1.8	fe
Fu. mosseae (2) + *Cl. etunicatum*	0.18 ± 0.01	bc	0.07 ± 0.01	d	22.3 ± 2.5	d–e	18.3 ± 4.7	f
Fu. mosseae (2) + *R. clarus*	0.16 ± 0.01	c	0.1 ± 0.02	a–c	22.5 ± 4.6	c–e	22.8 ± 6.2	d–e
Fu. caledonium + *Cl. etunicatum*	0.21 ± 0.01	ab	0.11 ± 0.01	a	32.6 ± 4.6	ab	33.6 ± 5.1	a–c
Fu. caledonium + *R. clarus*	0.21 ± 0.03	a	0.1 ± 0.00	ba	30.1 ± 5.1	b–d	32.2 ± 2.2	a–c
Cl. etunicatum + *R. clarus*	0.16 ± 0.02	c	0.1 ± 0.02	ba	28.8 ± 3.8	b–d	34.1 ± 2.9	a–c
Cocktail	0.17 ± 0.01	c	0.07 ± 0.03	dc	28.6 ± 6.3	b–d	32.5 ± 2.3	a–c
Mean	0.11		0.09		29.1		30.3	
Pr > *F*	0.0001		0.0252		0.0002		0.0003	

Mean of three replicates and ± is standard error.
Columns with different letters are significantly different ($P < 0.05$) according to Duncan's test.
Fu. mosseae – 1. propagated on maize host plant.
Fu. mosseae – 2. propagated on onion host plant.
Growing medium (GM1) consisted of andesitic tuff + peat (1:1 (V:V).
Growing medium (GM2) consisted of andesitic tuff + peat + soil (4 + 5 + 1) (V:V:V).

The plant Zn content was limited in the plant leaves, in control treatments for both growing media, at less than 20 mg Zn kg^{-1}. However, mycorrhiza inoculation led to a Zn content of the plants which was over the critical level of >20 mg Zn kg^{-1}; as soil in the area is Zn deficient, this discovery is very important. Also, the Zn content of the plants was found to be similar to the levels measured in our previous studies showing that inoculated citrus plants have a higher Zn content that non-inoculated plants [4,27]. Generally, it was observed that dual species inoculation resulted in a greater response, compared to using a single mycorrhiza species, and also showed different responses to differing environmental conditions. Previously, Wu, et al. [32] reported that compared with a sole AMF inoculation, additional putrescine and spermine markedly increased the total dry weight of Trifoliate Orange, and elevated the leaf P and K content and root P, Mg, Fe and Zn content. In the present work, a dual species inoculation such as *Fu. caledonium* + *Cl. etunicatum* and *Fu. caledonium* + *R. clarus* also increased the plant P content in GM1.

Mycorrhiza inoculated plants grew better and had a higher P and Zn content, which may be due to the presence and function of the AM, established on the roots at multiple sites per plant. Khalil et al. [33] reported that inoculating the seedlings with AM tended to increase the levels of P and Zn. Mycorrhizal inoculation may have some other benefit, apart from nutrient uptake, such as water uptake and disease control. In addition, IM inoculation significantly contributed to plant growth and nutrient uptake. Since IM include environmentally adapted species, they may play a significant role in citrus growth. Previously [3], reported that IM made a significant contribution to the growth of citrus seedlings. In the present work, the interaction of IM inoculation with exotic inocula appears to be efficient and may be used in further experiments.

5. Conclusions

Under greenhouse conditions, the efficiency of selected mycorrhizal species and growing media for citrus seedling production in the Mediterranean region was investigated. It was found that plants grown in GM1 grew much better than in GM2 and that IM have a significant impact on citrus seedling growth in GM2. *R. clarus* has a significant effect on the zinc concentration of the plant. The differential growth and survival responses caused by the IM and dual species inoculation treatments have potentially important implications for citrus seedling production. It is suggested that mycorrhizal colonization is an important factor prior to sowing and transplanting the citrus seedlings into field conditions. Further work needs to be performed to determine the effect of mycorrhizal inoculation and their dual species inoculation under field conditions.

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