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Determination of different growth media and various mycorrhizae species on citrus growth and nutrient uptake

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ABSTRACT

The influence of mycorrhiza species and growing media (GM) on the growth and nutrient uptake of citrus seedlings (sour orange (*Citrus aurantium* L.)) was studied. The experiments were conducted over 10 months using: four growth media, eight mycorrhiza species, one cocktail of mycorrhizae spores and one indigenous mycorrhiza spore (collected from the citrus orchard rhizosphere). Four different growing media were tested under controlled greenhouse conditions: GM-A, andesitic tuff + peat (1:1, v/v); GM-B, andesitic tuff + compost (1:1, v/v); GM-C, andesitic tuff + peat + compost (2:1:1, v/v/v) and GM-D andesitic tuff + peat + soil (from the Balcalı region) (2:1:1, v/v/v).

After a 10 month growing period, the highest values of leaf number, height, shoots and root dry weight were found in GM-C, followed by GM-A and GM-D; whereas the lowest values were found using the GM-B treatment. The results reveal that mycorrhizal inoculation increased the shoot and root dry weight production, compared with the levels found in non-inoculated plants. It was observed that various mycorrhiza species exhibit different responses when varying the growth media; it was also observed that *G. clarium*, *G. margarita*, *Glomus mosseae*, *Dr. Kindom* (commercial inoculum) and indigenous mycorrhiza (IM) are efficient mycorrhizae species for seedling growth. Mycorrhiza-inoculated citrus seedlings contained a higher content of phosphorus (P), zinc (Zn) than non-inoculated plants.

1. Introduction

Over 80% of the Turkish citrus plantations are located in the Eastern Mediterranean part of Turkey (Ortas, 2012). Every year, nearly one million citrus seedlings are produced in the Çukurova region for newly established citrus orchards. The soils of this part of the Çukurova region consist of high levels of clay and lime, resulting in the availability of nutrients being limited, especially phosphorus (P), zinc (Zn) and iron (Fe). In order to obtain an optimum yield, farmers use more than plant needed chemical fertilizers which cause degradation of the soil and water pollution. Accompanying the use of heavy fertilizers is a decrease in soil quality, along with the biological fertility of the soil being negatively affected. Mycorrhizae are of natural origin, and play a role in providing essential elements and water for plants, in addition to contributing to soil formation (development). It has been indicated that an arbuscular mycorrhiza fungi (AMF) association significantly increased plant height, stem diameter, leaf number per plant, shoot and root biomass, total root length, total root projected area, total root surface area and total root volume (Wu and Zou, 2010; Wu et al., 2011a). Citrus plants have a coarse and sparse root system, devoid of root hairs, making an early season nutrient and water supply crucial. A well-developed AM may increase the percentage of seedling adaptation to the field by reducing the mortality ratio. AM infection is a common association between plant roots and microorganisms; it is responsible for increasing plant nutrient uptake, especially immobile P, Zn and copper (Cu), and partly ammonium-N in soils of low fertility (Marschner, 1993; Ortas, 2003; Ortas et al., 2002a,b; Tanaka and Yano, 2005). Mycorrhizal plants normally grow more rapidly and appear healthier than non-mycorrhizal plants, especially in soils of low fertility. However, a high concentration of P was found to decrease the concentration of Zn in the tissue of the leaves and mycorrhizal activity. In addition, high levels of organic matter (Menge et al., 1982) and ammonium-N (Chambers et al., 1980; Johnson, 1984) reduce the mycorrhizal colonization of plant roots, and reduce or eliminate growth-mediated responses. Citrus rootstocks are known to have a range of dependency on mycorrhizae (Menge et al., 1978a,b; Nemec, 1979). The work of Menge et al. (1978b) demonstrated the mycorrhizal dependency of several citrus cultivars in low P soil. Citrus plants are mycorrhizal dependent (Ortas, 2012; Ortas et al., 2002b), it is therefore reasonable to produce mycorrhiza-inoculated seedlings for newly establish citrus orchards.

It has been indicated that different mycorrhizae species have shown differing responses dependent on the plant species employed (Ortas, 2010). Wu and Zou (2012), conducted

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experiments with four inoculation methods, using *Glomus mosseae* on trifoliate orange seedlings, and the results show that the method of inoculation can result in significant differences to plant growth. Nemec (1992), reported that *G. intraradix* has shown different responses to different potting materials.

Moreover, Chang and Chien (1989) have tested different mycorrhizae species on *Citrus sinensis* and found that *G. epigaeum* proved to be one of the effective mycorrhizal fungi, both in terms of mycorrhizal infection and growth response. Viyanak and Bagyaraj (1990), tested 18 different mycorrhizae species on trifoliate orange (*Poncirus trifoliate* (L.)) and found that different species show differences in plant height, stem diameter, plant biomass, P, Zn and Cu content. Khalil et al. (2011) and Cardarelli et al. (2010) reported that AM inoculated seedlings tended to increase the levels of P, K, Mg and Zn; this is significant as P and Zn deficiency is predicted extensively in the Mediterranean area, especially in citrus orchards.

Growing media also exert a significant effect on seedling growth. For the production of healthy and good quality mycorrhizae-inoculated seedlings, the selection of mycorrhizal species and growing media are very important. Furthermore, in order to achieve good growth of plants under nursery conditions, the nutrient status, organic matter content and water holding capacities are all important properties of the growing media. Ustuner et al. (2009), indicated that to derive maximal yields from organic-based farming, using AMF technology, the careful selection of an organic supplement and AMF is critical. Surprisingly, a combination of growing media is preferred to a single material (Bhagat et al., 2013). They also indicated that in the case of citrus plants to be ready for sale within one year, cocopeat and manure have the potential to improve the citrus seed germination and buddability. Schmitz et al. (2001), indicated that "soil + sand + decomposed Acacia bark" was the most appropriate substrate for the growth of *P. trifoliate*, due to its superior chemical and physical properties. However, as citrus plants respond differently to various mycorrhizae species, it is reasonable to test the effects of several mycorrhizae species and growing media on the growth and nutrient uptake of sour orange.

2. Materials and methods

2.1. Growth media and properties

One experiment was conducted, under greenhouse conditions, using the following four different substrates as a growth medium (GM):

1- GM-A: andesitic tuff + peat (1:1, v/v).
2- GM-B: andesitic tuff + compost (1:1, v/v).
3- GM-C: andesitic tuff + peat + compost (2:1:1, v/v/v).
4- GM-D: andesitic tuff + peat + soil (from the Balcalı region) (2:1:1, v/v/v).

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 7.7 and the 0.5 M $NaHCO_3$ (pH 8.5) extractable P was 87.6 kg ha^{-1} P. Compost had the following characteristics: pH of 7.7, 54% organic carbon, 1.13% N, 0.18% P and 0.92% K. Peat had the following characteristics: pH of 5.1, 55% organic carbon and 0.670 EC dS m^{-1}; andesitic tuff contained 4.3% K and 0.03% P. The physical and chemical characteristics of the growth medium (soil, peat and andesitic tuff) were measured according to Page et al. (1982) in the Rhizosphere Laboratory of Çukurova University, Adana, Turkey.

2.2. Growth material sterilization

Each GM was partially sterilized in an autoclave for 2 h at 120 °C. After sterilization, the GM was left to rest for three weeks before being used for soil microbial balance.

2.3. Mycorrhizal species

Several mycorrhizal species such as *G. mosseae* (Nicolson and Gerdemann) Rothamsted isolate, UK; *G. etunicatum* (Becker and Gerdemann) Nutri-Link isolate; USA; *G. clarium* (Nicolson and Schenk) Nutri-Link isolate, USA; *G. caledonium* (Nicolson and Gerdermann) Rothamsted isolate, UK; *G. intraradices* (Smith and Schenck) and *G. margarita* (Becker and Hall) USA; *G. macrocarpum* (Tulasne and Tulasne), USA; *G. fasccículatum* (Walker and Koske) USA; *Dr. Kindom* as a commercial inoculum (which was obtained from Japan), a cocktail (a mixture of five AM species) and a mixture of indigenous mycorrhiza (isolated from the Çukurova region, Turkey) were used. Maize plants were used as the trap culture plant for mycorrhizae spore propagation.

2.4. Production of seedlings

Before sowing the seeds, the perlite was washed 2–3 times with tap water, washed once with 0.01 M HCl, rinsed twice with ionized water and then autoclaved. Mycorrhizal and non-mycorrhizal seedlings were produced under glasshouse conditions. Sour orange (*Citrus aurantium* L.) seeds were surface sterilized with a sodium hypochlorite solution (5.25%) for 2 min, rinsed several times with deionized water and then with tap water. Seeds were sown in perlite for 4–5 weeks until the seedlings reached the three-leaf stage. Subsequently, two seedlings were transplanted to a 3 kg pot.

2.5. Experimental design and growth conditions

The inoculum was calculated based on the number of spores present in 10 g of inoculum, less than 1000 spores were situated approximately 50 mm below the seedlings. In non-mycorrhizal treatments, each seedling received the same amount of mycorrhizae-free substrate (autoclaved growth medium). The experiment was completely randomized with 3 replicates.

After transplanting the seedlings, the inoculated and non-inoculated plantlets were grown for 10 months in a controlled glasshouse with day-night temperatures of 27 ± 1 °C. Plantlets were maintained with a 16 h photoperiod using cool white fluorescent lamps of 350 μ m^{-2} s^{-1}. The relative humidity was 70–80% at night and 80–85% during the day period. Distilled water was added daily to maintain the moisture at 75% of field capacity. Plants were fertilized three times with Hoagland's solution including P (11 mg kg^{-1}) and Zn (1.1 mg kg^{-1}).

2.6. Plant sampling

At the end of the growing season (10 months), shoot height (in cm), total number of leaves, shoot and root dry weight (in g $plant^{-1}$) were measured, using three samples from each treatment. At the harvest of each pot, total plant biomass (dry weight of shoot and root) and plant height were recorded. Dried material from each pot was grounded 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 colorimetrically (Murphy and Riley, 1962). Atomic absorption spectrophotometry was employed to determine the Zn content of the plant samples.

2.7. Root colonization

At harvest, the shoots were separated from the roots at 0.5 cm above the soil surface. 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, 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 by the method of Koske and Gemma (1989). Mycorrhizal fungus % root colonization was determined using the gridline-intersect method of Giovanetti and Mosse (1980).

2.8. Statistical analysis

The variation in plant growth and nutrient properties, attributed to the effects of the growth medium and mycorrhizal species, was assessed in an 11 inoculum × 4 growth medium factorial arrangement of the SAS analysis of variance procedure (SAS, 2009). The means were compared using the Duncan Multiple Range Test at $P < 0.05$.

3. Results and discussion

3.1. Plant height

Mycorrhiza-inoculated plants exhibited a significantly higher plant height than non-mycorrhizal plants. Seedling height differed significantly ($P < 0.0001$) among the growth media, with a maximum seedling height recorded in GM-C, at 44.9 cm, and the lowest in GM-B, at 31.8 cm. In general, the height of the seedlings was in the order GM-C > GM-A > GM-D > GM-B. Data presented in Table 1 show that mycorrhiza-inoculated seedlings were 2–4 times taller than non-inoculated ones. For the seedlings grown in GM-A (andesitic tuff + peat (1:1, v/v)), control plants were measured at a height of 14.5 cm however, *G. clarium*-inoculated seedlings were measured at a height of 53.7 cm. In GM-B (andesitic tuff + compost (1:1, v/v)), the height of the control plants was 21.7 cm and 40.0 cm for the *Dr. Kindom*-inoculated seedlings. In GM-C (peat + andesitic tuff + soil (2:1:1, v/v/v)), the control plants were 21.3 cm in height however, *G. margarita*-inoculated seedlings were the tallest at 55.8 cm; *G. clarium*-inoculated seedlings were 54.5 cm in height, not significantly different. In GM-D (andesitic tuff + peat + soil (from the Balcalı region) (2:1:1, v/v/v)), the height of the control plants was 21.0 cm, whereas *G. margarita*-inoculated seedlings were 43.7 cm in height. In general, the citrus seedlings inoculated by *G. margarita* and *G. clarium* were significantly higher than other mycorrhizal species, for the four growing media.

3.2. Number of leaves

Since there is a significant relationship between the number of leaves (leaf area and photosynthesis-capturing potential) and growth parameters, the number of leaves per seedling was counted, revealing that the highest number of leaves was obtained using GM-C (mean $X = 29.2$) and the lowest number was using GM-B ($X = 20.9$). The mean number of leaves was 29.2, 25.8, 24.0 and 20.9 leaves per pot^{-1}, with respect to GM-C> GM-A> GM-D> GM-B (Table 2). Seedlings grown in GM-A, GM-C and GM-D produced the highest number of leaves with the *G. clarium* inoculation however, seedlings grown in GM-B, GM-C and GM-D produced the highest number of leaves with the *Dr. Kindom* inoculation.

3.3. Dry matter production

The effect of different GM on seedling shoot dry weight (SDW) was significantly ($P < 0.0001$) different (Fig. 1). For seedlings grown in GM-A, the SDW ranged among the mycorrhizae inoculum from 0.55 g pot^{-1} (control) to 7.90 g pot^{-1} (*G. clarium*), with a mean value of $X = 4.92$ g pot^{-1}. When seedlings were grown in GM-C, the SDW mean value was $X = 7.10$ g pot^{-1}; the lowest SDW was determined from the control treatment, mean value as $X = 2.19$ g pot^{-1}, and the highest was determined from *G. clarium*-inoculated pots at 9.61 g pot^{-1}. In GM-D, control pots produced 1.33 g pot^{-1} and seedlings inoculated with *G. margarita* produced 7.62 g pot^{-1} SDW. Citrus seedlings grown in GM-B had the lowest SDW mean value at 3.69 g pot^{-1}. The highest means of SDW were obtained at 7.10, 4.92, 4.18 and 3.69 g pot^{-1}, with respect to GM-C> GM-A> GM-D> GM-B.

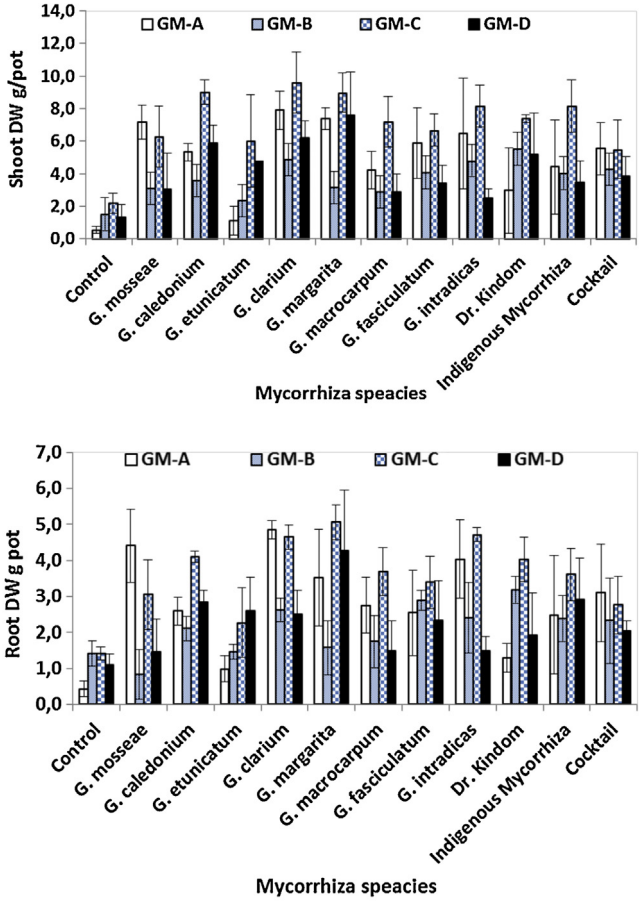

Fig. 1. Effect of mycorrhizal species and growth medias on shoot and root dry weight of sour orange seedlings.

The root dry weight (RDW) was also significantly different among the GM. In general, a similar trend to SDW was obtained for the RDW, with the order being GM-C > GM-A > GM-D > GM-B. As can been seen in Fig. 1, each growth medium resulted in a different response from the mycorrhizae species. GM-C produced the highest mean RDW, $X = 3.56$ g pot^{-1}, and GM-B produced the lowest RDW, at $X = 2.08$ g pot^{-1}. In GM-A, plants inoculated with *G. clarium* produced 4.86 g pot^{-1}, in GM-C, plants inoculated with *G. margarita* produced 5.06 g pot^{-1} and *G. clarium* resulted in an RDW of 4.66 g pot^{-1}. In general, *G. margarita*, *G. clarium* and *G. mosseae* were the most efficient species, producing a high RDW.

3.4. Root colonization and spore number

The root systems were widely colonized by mycorrhizae species. Data presented in Table 3 show that seedlings grown in GM-D produced the highest mean root colonization of $X = 45.6\%$. Plants grown

Table 1
Effect of mycorrhizal species and growth mediums on height of sour orange seedlings..

Mycorrhiza species	Shoot height (cm)			
	GM-A	GM-B	GM-C	GM-D
Control	14.5 ± 3.5c	21.7 ± 0.6a	21.3 ± 4.5d	21.0 ± 6.6b
G. mosseae	49.3 ± 5.9a	29.8 ± 3.7a	43.2 ± 3.0a–c	30.3 ± 15.0ab
G. caledonium	43.2 ± 1.1ba	30.0 ± 2.7a	50.8 ± 1.8a–c	40.3 ± 3.5ab
G. etunicatum	16.3 ± 6.7bc	28.0 ± 1.0a	42.2 ± 10.8a–c	36.5 ± 3.0ab
G. clarium	53.7 ± 3.1a	37.0 ± 6.2a	54.5 ± 4.8a–c	40.8 ± 8.3a
G. margarita	51.5 ± 3.1a	30.7 ± 6.2a	55.8 ± 3.6a	43.7 ± 14.0a
G. macrocarpum	37.8 ± 5.6a–c	28.0 ± 10.8a	43.2 ± 1.0a–c	27.0 ± 5.6ab
G. fasciculatum	44.7 ± 12.9a	34.5 ± 4.4a	50.0 ± 4.6a–c	30.0 ± 2.7ab
G. intradicas	43.0 ± 14.5ba	36.2 ± 11.7a	51.2 ± 3.7a–c	24.8 ± 2.8ab
Dr. Kindom	27.7 ± 16.7a–c	40.0 ± 8.7a	39.5 ± 7.1a–c	34.7 ± 15.4ab
Indigenous mycorrhiza	31.8 ± 14.3a–c	32.2 ± 8.1a	48.8 ± 6.8a–c	31.8 ± 4.1ab
Cocktail	45.2 ± 7.7a	33.7 ± 13.7a	35.5 ± 6.5cd	31.0 ± 5.6ab
Mean	38.2 ± 7.9	31.8 ± 6.5	44.9 ± 4.9	32.7 ± 7.2

Significance: growth mediums (GM), 0.0001; Mycor. Speci. (M), 0.0001; GM × M, 0.0025. Mean of three replicates and ±standard error. Columns with different letters are significantly different ($P < 0.05$), according to Duncan's test.

Table 2
Effect of mycorrhizal species and growth media on number of leaves of sour orange seedlings, at harvest.

Mycorrhiza species	Number of leaves			
	GM-A	GM-B	GM-C	GM-D
Control	14.7 ± 0.6c	16.7 ± 5.1b	20.3 ± 1.5d	14.7 ± 5.5d
G. mosseae	31.0 ± 1.0ba	22.7 ± 4.7ba	25.3 ± 3.1bc	22.7 ± 13.0a–d
G. caledonium	28.0 ± 3.0ba	19.0 ± 2.6ba	30.0 ± 2.6ba	28.3 ± 3.2ba
G. etunicatum	14.3 ± 4.6c	16.7 ± 2.5b	26.3 ± 5.5bc	26.3 ± 5.0a–c
G. clarium	33.0 ± 1.0a	22.3 ± 1.5ba	29.3 ± 3.2a	32.7 ± 4.1a
G. margarita	28.0 ± 1.7ba	18.3 ± 0.6ba	31.7 ± 2.1a	29.3 ± 5.7a
G. macrocarpum	27.0 ± 3.6ba	23.0 ± 2.0ba	26.0 ± 0.0bc	18.3 ± 2.3b–d
G. fasciculatum	25.3 ± 3.1ba	22.0 ± 3.0ba	32.3 ± 2.5a	23.3 ± 6.5a–c
G. intradicas	30.7 ± 8.7ba	24.0 ± 6.6ba	30.0 ± 2.6ba	20.0 ± 3.6dc
Dr. Kindom	22.7 ± 3.5bc	24.3 ± 5.5a	35.3 ± 5.7a	29.7 ± 2.3a
Indigenous mycorrhiza	27.0 ± 12.5ba	19.7 ± 5.9ba	30.0 ± 3.5ba	26.3 ± 5.2a–c
Cocktail	28.0 ± 1.7ba	22.3 ± 7.4ba	33.7 ± 8.6c	24.0 ± 1.7a–c
Mean	25.8 ± 3.8	20.9 ± 3.9	29.2 ± 3.4	24.6 ± 4.8

Significance: growth mediums (GM), 0.0001; Mycor. Speci. (M), 0.0001; GM × M, 0.0025. Mean of three replicates and ±standard error. Columns with different letters are significantly different ($P < 0.05$), according to Duncan's test.

in GM-B had the lowest colonization at $X = 18.6\%$. Inoculated plants had a higher colonization percentage than the control seedlings. Root colonization also differed significantly among the inoculum species, with the maximum percentage of infection by *G. clarium* and *G. margarita* grown in GM-D (65%). *G. mosseae*-inoculated seedlings reached 40% and indigenous AM (37%) in GM-A medium; the cocktail value was 27% in GM-B. *G. intraradices* and *Dr. Kindom* followed the order: GM-D > GM-C > GM-A > GM-B.

The number of mycorrhizae spores, present in the rhizosphere of the seedlings, were determined at harvest; the mean spore numbers were 14.9, 11.2, 9.4 and 5.3 spore/10 g medium with respect to GM-C > GM-D > GM-B > GM-A (Table 4). In GM-C, the cocktail

Table 3
Effect of four growth media and several mycorrhizal species on sour orange seedlings root colonization.

Mycorrhiza species	Root colonization (%)			
	GM-A	GM-B	GM-C	GM-D
Control	10.0 ± 1.0b	3.3 ± 0.6b	6.7 ± 1.2d	6.7 ± 0.6c
G. mosseae	40.0 ± 2.0a	16.7 ± 0.6ba	10.0 ± 1.0dc	30.0 ± 0.0bc
G. caledonium	20.0 ± 2.0ba	23.3 ± 2.1a	20.0 ± 1.0a–c	40.0 ± 0.0ba
G. etunicatum	26.7 ± 3.1ba	23.3 ± 0.6a	36.7 ± 1.5ba	56.7 ± 1.5ba
G. clarium	16.7 ± 1.2ba	23.3 ± 0.6a	16.7 ± 0.6b–d	66.7 ± 1.5a
G. margarita	10.0 ± 1.0b	20.0 ± 0.0ba	20.0 ± 0.0a–c	63.3 ± 2.1a
G. macrocarpum	16.7 ± 0.6ba	20.0 ± 1.0ba	20.0 ± 1.0a–c	43.3 ± 1.5ba
G. fasciculatum	23.3 ± 1.2ba	20.0 ± 1.0ba	30.0 ± 1.0bac	46.7 ± 1.5ba
G. intradicas	3.33 ± 0.6b	16.7 ± 1.5ba	40.0 ± 2.7a	60.0 ± 1.7ba
Dr. Kindom	20.0 ± 2.0ba	10.0 ± 1.0ba	40.0 ± 1.7a	46.7 ± 2.3ba
Indigenous mycorrhiza	36.7 ± 0.6a	20.0 ± 1.0ba	26.7 ± 0.6a–c	50.0 ± 1.7ba
Cocktail	23.3 ± 0.6ba	26.7 ± 1.5a	33.3 ± 0.6ba	36.7 ± 3.8a–c
Mean	20.1 ± 1.7	18.6 ± 1.6	25.0 ± 3.4	45.6 ± 5.4

Significance: growth mediums (GM), 0.0001; Mycor. Speci. (M), 0.0014; GM × M, 0.0347. Mean of three replicates and ±standard error. Columns with different letters are significantly different ($P < 0.05$), according to Duncan's test.

Table 4
Effect of mycorrhizal species and growth media on number of AM species spore at rhizosphere of sour orange seedlings.

Mycorrhiza species	Number of spores (10 g soil)			
	GM-A	GM-B	GM-C	GM-D
Control	1.3 ± 0.6B	9.0 ± 2.6ba	9.0 ± 1.7d	9.0 ± 1.0b
G. mosseae	4.7 ± 3.2B	4.0 ± 2.6b	14.0 ± 11.4b–d	6.7 ± 5.0b
G. caledonium	6.0 ± 4.6B	6.3 ± 4.5ba	15.0 ± 7.8a–c	11.3 ± 4.2b
G. etunicatum	8.3 ± 4.9B	9.0 ± 0.0ba	14.0 ± 4.6b–d	6.7 ± 3.8b
G. clarium	20.7 ± 18.6A	11.0 ± 2.0ba	9.3 ± 4.9d	11.7 ± 4.7b
G. margarita	3.7 ± 0.6B	9.0 ± 6.2ba	8.0 ± 6.1d	12.7 ± 0.6ba
G. macrocarpum	4.7 ± 3.8B	10.3 ± 1.2ba	11.7 ± 3.2dc	9.0 ± 2.7b
G. fasciculatum	1.3 ± 0.6b	14.7 ± 5.7a	6.0 ± 4.4d	20.3 ± 8.1a
G. intradicas	3.3 ± 2.3b	6.0 ± 4.0b	22.7 ± 5.5ba	10.7 ± 5.0b
Dr. Kindom	5.0 ± 1.7b	12.0 ± 3.5ba	22.7 ± 4.7ba	13.3 ± 4.9ba
Indigenous mycorrhiza	3.3 ± 1.5b	11.3 ± 9.3ba	21.3 ± 8.5a–c	12.3 ± 7.2ba
Cocktail	1.7 ± 1.2b	9.7 ± 8.9ba	24.7 ± 4.9a	10.3 ± 4.1b
Mean	5.3 ± 3.6	9.4 ± 4.2	14.9 ± 5.7	11.2 ± 4.3

Significance: growth mediums (GM), 0.0001; Mycor. Speci. (M), 0.0816; GM × M, 0.0005. Mean of three replicates and ±standard error. Columns with different letters are significantly different ($P < 0.05$), according to Duncan's test.

and indigenous mycorrhizal inoculated seedlings produced more spores than inoculations with other mycorrhizae species.

3.5. Plant nutrient status

In terms of plant P content, control seedlings contained less % P than the mycorrhizal inoculated seedlings, in all four growth media. The highest mean of P, $X = 0.15\%$, was measured for GM-A, with the lowest mean of % P being $X = 0.11$ for the GM-B treatment (Table 5). It was also found that different mycorrhizae species exhibited a varying response to different growth media. Although mycorrhizal inoculation exerted a statistically significant effect on citrus P concentration, the plant P content was less than the critical level of %P > 0.20. It was found that in GM-A, GM-B, GM-C and GM-D respectively, seedlings inoculated with *G. caledonium* contained 0.20% P, the value in *G. etinicatinium* was 0.13% P, *G. etinicatinium* had 0.18% P, and *G. mosseae* and *G. fasciculatum* contained 0.15% P (Table 5).

The Zn concentration in the leaves of the plants was determined. It was found that seedlings grown in GM-A and GM-D contained a mean of 20.9 mg Zn kg^{-1} and 20.4 mg Zn kg^{-1} respectively, which is over the critical level of 20 mg Zn kg^{-1}. Seedlings inoculated with *G. caledonium* contained a mean of $X = 25.8$ mg Zn kg^{-1} in GM-A, the cocktail content was 23.0 mg Zn kg^{-1} in GM-B, the *G. macrocarpum* value was 22.7 mg Zn kg^{-1} in GM-C and the indigenous species contained 24.3 mg Zn kg^{-1} (Table 6).

4. Discussion

Growth medium plays a significant role in seedling growth as the plantlets require moisture, air and nutrients. Due to the fact that citrus seedling root systems are so shallow, the physical and chemical characteristics of GM play a very important role in growth. Also, as citrus plants are highly dependent upon mycorrhizae, it is extremely important to find an improved harmony between the growth medium composition and mycorrhiza colonization. We have been working over a prolonged period to produce mycorrhizae-inoculated citrus seedlings and have investigated a combination of growth media and mycorrhizae species. The nutrient content of growing media is a key driver impacting upon mycorrhizae colonization.

In the present work, differences in the mean values of plant height, leaf numbers, SDW, RDW, root colonization, P and Zn content, among the different types of growing media, were statistically significant (Tables 5 and 6). In general, there is a close relationship and harmony between the parameters of plant growth. The greatest shoot and root DW, plant height and number of leaves were recorded with GM-C. Since GM-C contained a higher level of organic matter, seedlings exhibited better vegetative and root growth than when grown in other growth media. Similarly, Bhagat et al. (2013) indicated that citrus plants grown in a media of soil + farmyard manure + cocopeat (2:1:1) displayed the greatest seedling height, stem diameter, number of leaves, leaf area and root-shoot ratio.

Table 5
Effect of mycorrhizal species and growth media on leaf P concentration of sour orange seedlings.

Mycorrhiza species	Leaves %P			
	GM-A	GM-B	GM-C	GM-D
Control	0.11 ± 0.1b	0.08 ± 0.0c	0.07 ± 0.0d	0.06 ± 0.0c
G. mosseae	0.15 ± 0.0ba	0.11 ± 0.0b	0.15 ± 0.0ba	0.15 ± 0.0a
G. caledonium	0.20 ± 0.0a	0.12 ± 0.0ba	0.14 ± 0.0bc	0.13 ± 0.0ba
G. etunicatum	0.14 ± 0.1ba	0.13 ± 0.0a	0.18 ± 0.0a	0.13 ± 0.0ba
G. clarium	0.15 ± 0.0a	0.11 ± 0.0b	0.14 ± 0.0bc	0.13 ± 0.0ba
G. margarita	0.16 ± 0.0a	0.11 ± 0.0b	0.14 ± 0.0bc	0.12 ± 0.0ba
G. macrocarpum	0.19 ± 0.0a	0.12 ± 0.0ba	0.14 ± 0.0bc	0.15 ± 0.0a
G. fasciculatum	0.14 ± 0.0ba	0.11 ± 0.0b	0.13 ± 0.0bc	0.12 ± 0.0ba
G. intradicas	0.13 ± 0.0ba	0.12 ± 0.0ba	0.13 ± 0.0bc	0.15 ± 0.0a
Dr. Kindom	0.14 ± 0.1ba	0.10 ± 0.0bc	0.13 ± 0.0bc	0.11 ± 0.0b
Indigenous mycorrhiza	0.14 ± 0.0ba	0.11 ± 0.0b	0.13 ± 0.0bc	0.13 ± 0.0ba
Cocktail	0.19 ± 0.0a	0.12 ± 0.0ba	0.12 ± 0.0c	0.11 ± 0.0b
Mean	0.15 ± 0.0	0.11 ± 0.0	0.13 ± 0.0	0.12 ± 0.1

Significance: growth mediums (GM), 0.0001; Mycor. Speci. (M), 0.0001; GM × M, 0.4225. Mean of three replicates and ±standard error. Columns with different letters are significantly different ($P < 0.05$), according to Duncan's test.

Table 6
Effect of mycorrhizal species and growth media on leaf Zn concentration of sour orange seedlings.

Mycorrhiza species	Leaves Zn (mg Zn/kg DW)			
	GM-A	GM-B	GM-C	GM-D
Control	19.2 ± 0.15a–c	16.5 ± 4.1ba	21.7 ± 4.4ba	15.7 ± 1.3c
G. mosseae	20.1 ± 2.3a–c	22.5 ± 8.7a	19.5 ± 0.8a–c	19.3 ± 7.8a–c
G. caledonium	25.8 ± 1.3a	22.0 ± 4.7ba	21.2 ± 1.7ba	20.1 ± 2.4a–c
G. etunicatum	21.2 ± 2.8c	15.3 ± 1.7b	14.9 ± 05ed	17.9 ± 1.0bc
G. clarium	19.0 ± 2.1a–c	20.5 ± 3.8ba	18.8 ± 1.9a–d	21.9 ± 6.0ba
G. margarita	19.5 ± 1.8a–c	17.8 ± 1.4ba	16.9 ± 2.1b–e	19.6 ± 3.3a–c
G. macrocarpum	24.0 ± 2.8ba	19.1 ± 4.5ba	22.7 ± 4.5a	22.5 ± 2.6ba
G. fasciculatum	21.4 ± 1.8a–c	18.3 ± 1.7ba	19.9 ± 3.2a–c	20.8 ± 0.6a–c
G. intradicas	17.8 ± 4.1bc	19.1 ± 3.8ba	15.0 ± 1.6c–e	20.2 ± 2.3a–c
Dr. Kindom	20.4 ± 3.8a–c	22.7 ± 4.1a	14.4 ± 0.9e	21.8 ± 1.2ba
Indigenous mycorrhiza	18.4 ± 0.7a–c	20.8 ± 4.2ba	15.8 ± 1.3c–e	24.3 ± 1.9a
Cocktail	22.4 ± 2.8ba	23.0 ± 3.4a	18.5 ± 6.4a–e	20.4 ± 2.3a–c
Mean	20.9 ± 2.2	19.8 ± 3.8	18.3 ± 2.4	20.4 ± 2.7

Significance: growth mediums (GM), 0.0803; Mycor. Speci. (M), 0.0077; GM × M, 0.0329. Mean of three replicates and ±standard error. Columns with different letters are significantly different ($P < 0.05$), according to Duncan's test.

In this work, the lowest growth performance was recorded with GM-B, which is a mix of andesitic tuff + compost (1:1, v/v). Due to the fact that the other GM contained andesitic material, it is possible that compost may exert a negative effect.

The results show that mycorrhizae inoculation increased plant shoot and root DW, which is in accordance with the findings of Graham and Timmer (1984). Mycorrhizal inoculation exerted a significant growth response, to varying degrees, in all the growing media employed in this study. The possible cause of this varied growth performance could be due to differences in the organic components, especially peat, and this aspect may need further study. From the current work, GM-C is the optimum growth media for citrus seedling quality. Previously Bhagat et al. (2013) reported that growth media have significantly different effects on citrus seedling growth. Schmitz et al. (2001), used three different growing media, which indicated that *G. clarium* displayed a higher relative growth on *Poncirus trifoliata*. Large inter-species differences exist between AM fungi on the plant growth response and in their ability to supply less mobile nutrients (Munkvold et al., 2004). Similarly, (Nemec, 1992) used four different growth media with a *G. intradicas* inoculation and found that mycorrhizae increased sour orange shoot and root growth. Chang and Chien (1989), used 11 different mycorrhizae species on *Citrus sienensis* and found that *G. epigaeum* mycorrhiza was the most effective species; the results show the same parallel with our prior work. Graham and Timmer (1984), reported that mycorrhizae exerted an influence, to varying degrees, on lemons in different growth media. In all growth media used in this study, the control plants displayed the lowest level of root colonization. In general, root colonization is low due to the rough and woody root system of citrus plants. It should be noted that precisely assessing the level of root colonization was very difficult. Compared with other treatments, root colonization was higher with seedlings grown in GM-D. Spore numbers were higher in GM-C compared with other treatments. Since citrus is a wood plant and lives longer under field conditions, the spore number is very important for the sustainability of mycorrhizae diversity.

In the current work, mycorrhiza-inoculated seedlings increased plant P and Zn content in all growth media. In general, the growing media which included peat, such as GM-A, GM-C and GM-D, exhibited more effect on plant growth, and P and Zn content; this may be related to the physical (retain more water) and chemical properties of the growth medium. Further research should consider the effects of other physical and chemical characteristics of growing media, such as total porosity and water retention at low tensions of moisture. Also different growth medium contain different organic source it may be worth to test the effect of different levels of organic matter and peat on mycorrhizal colonization.

Mycorrhizae-inoculated plants reached a greater P and Zn content, compared with non-inoculated seedlings. Results of the P concentrations show some harmony with results obtained by other research (Gnekow and Marschner, 1989; Menge et al., 1978b,c; Vinayak and Bagyaraj, 1990). In the present work, *G. mosseae*-inoculated seedlings had a high P content in GM-A, GM-C and GM-D, but also *G. etunicatum* and *G. macrocarpum*, respectively in GM-C and GM-D. Previously, Wu et al. (2011b) reported that *G. mosseae*-inoculated citrus plantlets contained high N, P, Ca, Cu, Zn, and Mn levels in the leaves and roots, compared with the non-inoculated treatment. In addition, the results of (Wu and Zou, 2009), show that the sole inoculation of AMF significantly increased the leaf P, K, Ca, Mg, Fe, Cu and Mn content of the citrus seedlings, compared with the non-AMF control. Generally, *G. macrocarpum*, *G. caledonium*, *G. mosseae* and indigenous mycorrhiza-inoculated plants exhibited a high Zn content. These results agree with those by Ortas et al. (2002b), who found that inoculated citrus plants have high levels of P and Zn.

Although the P and Zn values of the seedlings are below the critical level, mycorrhiza and non-mycorrhiza inoculated plants did not show any deficiency symptoms; however, the universal critical level of P and Zn content for citrus seedlings needs further investigation.

5. Conclusions

A variety of citrus seedlings were inoculated with different mycorrhizae spores, using different growth media, and then tested after a period of 10 months. In all the growth media studied, increased plant growth and nutrient uptake were observed for seedlings grown after inoculation with *G. clarium* and *G. margarita*, which are efficient species for the production of sour orange seedlings. Also *G. mosseae*, *G. intraradices*, Cocktail and indigenous mycorrhiza are effective inoculums.

GM-C consisting of peat + andesitic tuff + soil, with a ratio of 2:1:1 respectively, was found to be the most suitable media, followed by GM-A and GM-D. GM-B exerted the least effect on plant growth. Overall, the results indicate that mycorrhiza spores, in combination with a growth media containing peat, for example GM-C, can be used as a suitable environment for citrus seedling growth. Mycorrhizae inoculation may effectively be used to increase seedling P and Zn content. Further work needs to be

performed to test the effect of different levels of organic matter and peat on mycorrhizal colonization.

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