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Improving Morphological and Physiological Parameters of Rose Flowers by Biofertilizer Application in a Hydroponic System

Mina Taghizadeh[1]*, Mohammad Ali Arab[1], Mousa Solgi[1]

1 Department of Horticultural Sciences, Faculty of Agriculture and Environment Science, Arak University, Arak, Iran

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ABSTRACT

Yield quality and quantity in hydroponic greenhouses usually rely on optimum crop root health and effective nutrient mobility. This study evaluated the effects of applying arbuscular mycorrhizal fungi and biochar on different growth characteristics of commercial rose cultivars in a hydroponic cultivation system. In this experiment, *Rosa hybrida* cultivars were 'Angelina' and 'Dolce Vita'. Treatments included concentrations of 0, 1.5, 3 and 6 g L^{-1} biochar, and 0 and 7 g L^{-1} of arbuscular mycorrhizal fungi as organic biofertilizers for the growth of rose plants. Biofertilizer application at 3 g L^{-1} biochar and 7 g L^{-1} arbuscular mycorrhizal fungi optimally increased flowering shoot diameter, flower diameter, flowering shoot length, and leaf phosphorus content. The 'Dolce Vita' cultivar responded more favorably to higher concentrations of arbuscular mycorrhizal fungi and biochar than the 'Angelina' cultivar in the hydroponic culture medium. Stem diameter, stem length, and flower diameter in both cultivars improved in response to higher biochar concentrations. The increased flowering shoot length resulted from root colonization by arbuscular mycorrhizal fungi and an enhanced nutrient uptake, especially phosphorus. A higher nutrient supply increased cell division and shoot growth.

Abbreviations: Arbuscular Mycorrhizal Fungi (AMF), Dry weight (DW), Electrical conductivity (EC), Fresh weight (FW), Indole-3-acetic acid (IAA)

Introduction

A rise in demand for food is manageable through a developed farming system by merging controlled environmental cultivation. Hydroponics is a controlled environmental cultivation system that constitutes an essential branch of modern greenhouse industries in developed countries. Hydroponic greenhouse systems provide a controlled environment, parallel to a significant increase in plant growth and productivity, with better agricultural water management and continuous production (Jagnade et al., 2022). Yield production, quality, and quantity in a hydroponic greenhouse are essentially reliant on optimum crop root health and nutrient management, in addition to lighting, pH, EC supplies, decontamination, and appropriate pollination conditions (Mishra et al.,

*Corresponding author's email: m-taghizadeh@araku.ac.ir

2018).

Biofertilizers involve a group of bacteria or fungi that can consequently increase plant yields. Biofertilizers include bacterial, mycorrhizal, and algal types (Han et al., 2006). Arbuscular Mycorrhizal Fungi (AMF) are a group of biological factors that establish symbiosis with more than 33% of crops (Barea et al., 2005). AMF improves plant access to immobile nutrients, especially phosphorus, improved water relations, and higher pathogen resistance (Veresoglou et al., 2012; French, 2017; Li and Cai, 2021). AMF symbiosis offers a natural relationship between plant roots and fungi that can increase plant growth, reduce plant need for nutrients and water, increase plant viability and help growth, improve plant resistance to biological and environmental stress, increase rooting in cuttings, and promote fruit production (Garmendia et al., 2004; Augé et al., 2015). AMF are symbiotic and appear on roots, in the cortex, and around the epidermis of root cells. Improved root morphology may be due to alterations in hormonal balance encouraged by AMF symbiosis, which may affect the progress of the rooting system, favoring the development of lateral roots (Linderman, 1992). Zhang et al. (2015) indicated that AMF can expand root absorption by creating a hyphal network, which can then absorb phosphorus from soil pores and transfer it to host plants.

Previous studies on rose flowers inoculated with two cultivars of AMF, i.e., *Glomus mosseae* and *G. intraradices*, showed improvements in flower production. Also, root colonization percentage in rose flowers increased in inoculated cultivars. The plants inoculated with *G. mosseae* had early flowering, with an increase in flower counts of *R. hybrida* cultivars compared to non-inoculated cultivars. Flower yield in plants inoculated with *Glomus mosseae* increased 30-50% a month after application (Muller et al., 2001). The symbiotic effects of AMF were evaluated on water uptake efficiency and growth indices of osteospermum as an ornamental plant. The results showed that *Glomus mossea* CA and *Glomus mossea* st6 significantly improved growth indices in osteospermum compared to other fungi (Khandan Mir Koohi et al., 2016).

Biochar is charcoal prepared from plant biomass and agricultural waste that burn in the presence of low or no oxygen. Adding biochar to the soil can entail important benefits due to the several roles that carbon plays in the chemical, biological, and physical processes in soils (Wang et al., 2020). The beneficial effects of biochar application include increased organic matter, improved soil water retention, increased cation exchange capacity and interaction with the soil nutrient cycle by modifying the soil pH, and reduced nutrient leaching (Glaser et al., 2002). Biochar has physicochemical characteristics similar to coir and peat, with applications as an alternative growing medium (Pringle et al., 2009). Dunlop et al. (2015) used biochar as a medium for soilless, hydroponic vegetables. They suggested that biochar can be effective as a medium for hydroponic systems, improving growth in high-value food crops (Balestrini et al., 2015). Also, Dare et al. (2010) investigated applications of coconut biochar as substrate in closed hydroponics systems and suggested that biochar has a primary role in growth and yield quality. Altland and Locke produced biochar from tomato green waste and applied it in a hydroponic system for tomato production. Adding biochar to aquaponics improved microbial activity but lowered nutrient leaching (Copetta et al., 2011). Biochar can create a robust medium for microbes in problematic soils. Biochar pores may act as a shelter for microbes. The composition of biochar in soils can improve AM fungi efficiency (Mia et al., 2017).

Rose is one of the most important cut flowers in the world, which has a unique position in world trade. As the queen of flowers, roses rank first among all flowers in the horticultural industry and appear as the national flower in 10 countries, i.e., Iran, USA, England, Italy, Romania, Iraq, Saudi Arabia, Morocco, Luxembourg, and Bulgaria (Bhattacharjee and Banerji, 2010).

The available literature on commercial products containing AMF and biochar centers around production enhancements in organic soil-based greenhouses, highlighting the various aspects of production (Mishra et al., 2018). However, studies on their usefulness in hydroponic greenhouses consider attempts to enhance pathogen control and more effective management of photo-assimilates. The two factors can enhance production efficiency and improve cost-effectiveness for growers (French, 2017).

Quantitative and qualitative studies on cut rose flowers are essential in each cultivation system, soil medium, or hydroponic condition to identify and promote different cultivation methods. Therefore, this study evaluated the effects of applying AMF and biochar in a hydroponic cultivation system aimed at commercial rose cultivars to increase their different growth characteristics.

Materials and Methods

Pot experiments were conducted under a commercial greenhouse in Khomein, Markazi

province, Iran, from October 2019 to 2020. The geographical coordinates of the mentioned greenhouse were 372994 405449° S 39, and the altitude was 1750 meters above sea level. The varieties cultivated in this experiment were *Rosa hybrida* cultivars (Angelina and Dolce vita) on Rosa canina rootstocks.

Biochar of sugar cane bagasse was produced in a pyrolator at 550 °C for 3 h. It was sprayed with water until the fire in the charcoal was quenched, sun-dried, sieved through a mesh with 150 m openings, and stored until use (Avisa company). The results of physical and chemical analyses of the biochar are presented in Table 1. Biochar was applied at concentrations of 0, 1.5, 3, and 6 grams per liter of cocopeat-perlite medium (biochar g L^{-1}). Arbuscular mycorrhizal fungi were applied at concentrations of 0 and 7 grams per liter of cocopeat-perlite medium (AMF g L^{-1}). Mycorrhizal fungi were purchased from Pishtaz Varian Company (Iran) and supplied under the commercial name "Mycoroot". Each 10 g of Mycoroot contained at least 100 active organisms of different species of mycorrhizal arbuscular fungi *Glomus etunicatum*, *Glomus intraradices*, and *Glomus mossea*. Each treatment consisted of three replications or three pots. Biochar and mycorrhizal fungi were mixed with an autoclaved substrate comprising cocopeat and perlite (70:30 v/v) to make them homogeneous. After two weeks of incubation, pots (7 L) were filled with the specific medium treatments. The rooted cuttings (20-30 cm) were carefully inserted into each pot in the hydroponic system, which consisted of the cocopeat-perlite mixture with AMF and biochar treatments. Plant roots were thoroughly washed under running tap water before transplanting. The nutrient solution for regular nutrient supply to the plant roots was prepared using the Hoagland and Arnon nutrient composition dissolved in tap water. The nutrient-irrigation system was automatically switched on to run for five minutes each hour to feed the culture medium. Fungicides were not used in the culture medium due to their negative effect on mycorrhizal fungus colonization. During the study cycle, daily average temperature and relative humidity were maintained at 20±5 °C and 85±5%, respectively.

Table 1. Some physiochemical properties of experimental sugarcane bagasse biochar.

Physiochemical characteristics	Macro elements	Micro elements	Other elements
pH: 7.55	Carbone (%): 69.65	Fe (mg kg^{-1}):1245	Cd (mg kg^{-1}): 0.35
EC (d S^{-1}): 0.84	N (%): 0.279	Zn (mg kg^{-1}): 45	Pb (mg kg^{-1}): 1.75
CEC: 36.3 (mol kg^{-1})	O (%): 19.51		Sulfate (mg kg^{-1}): 301
Density: 0.13 (g cm^{-2})	H (%): 3.38		Bicarbonate (mg kg^{-1}): 38
specific surface (m^2 kg^{-1}): 164	K (mg kg^{-1}): 2568		Stabilized carbon: 53.4
Ash (%): 5.6	P(mg kg^{-1}): 459		
Volatile compounds (%): 31.2	Ca (mg kg^{-1}): 1752		
Humidity (%): 9.8	Mg (mg kg^{-1}): 412		
O/C: 0.281			
C/N: 249.319			
O/H: 5.78			

Trait evaluation

During the growth period, traits such as shoot length, flowering shoot length, shoot stalk diameter (1 cm above the crown), the diameter of the flowering shoot, number of leaves in flowering stem, fresh and dry weight of flower and stem, vase life, flowering time (based on the number of days from the planting of cutting to the observation of the first flower bud), petal anthocyanin, leaf chlorophyll, ionic leakage, and the amounts of nitrogen, phosphorus, and potassium in leaves were evaluated and measured. The mentioned traits were evaluated as described below.

Flower vase life

The vase life was calculated by counting the days from the time of placing flowers in the tap water until the wilting of leaves and petals. Vase life finished with signs such as necrosis, wilting, petal abscission, bent neck, and curled petals, thus decreasing flower attraction and marketability (Mohammadi et al., 2011).

Total leaf chlorophyll

Acetone (80%) was used for leaf chlorophyll

extraction. Color absorbance of each solution was estimated by a spectrophotometer (Lambada 25, Perkin Elmer, USA) at 645 and 663 nm for total chlorophyll (mg g^{-1} FW) (Arnon, 1949).

$$Chla = 12.9A_{663} - 2.9A_{645}$$
$$Chlb = 12.9A_{645} - 4.68A_{663}$$
$$Total\ Chlorophyll\ (Tchl) = 20.2A_{645} + 8.02A_{663}$$

Where, Chla and Chlb are chlorophyll a and chlorophyll b. Letter A in the equation represents absorbance at specific wavelengths.

Petal anthocyanin

Petal anthocyanin content was measured according to Wagner (1979). Fresh petal tissue (0.1 g) was soaked in 10 mL acidified methanol [methanol: HCl 99:1 v/v], crushed and kept at 25 °C for 24 h in a dark environment. The extracts were then centrifuged at 3,000 g for 5 min at room temperature. The absorption rate of the supernatant was read by a spectrophotometer at 550 nm. The petal anthocyanin content was calculated by the following formula (Wagner, 1979).

$$A = \varepsilon bc$$

A is the absorption at a given wavelength, ε is the extinction coefficient equal to 33,000 (mol cm^{-2}), b is the cuvette width (cm), c is the anthocyanin content (mg g^{-1} FW).

Electrolyte leakage cell

To determine cell membrane stability, two petal samples were weighed, each including 0.2 g per replication, and were dipped in 10 mL of double-distilled water at 23 °C for 24 h in the dark. After a strong handshaking of the test tubes, electrical conductivity (EC1) of electrolytes was determined by a conductivity meter. The second electrical conductivity (EC2) was measured at 100 °C in a Bain-marie for 20 min. After reaching room temperature, the electrical conductivity of each solution (EC2) was measured with an EC-meter. Cell membrane leakage was determined according to Sullivan and Ross (1979) as follows:

$$EL(\%) = \frac{EC1}{EC2} \times 100$$

Leaf elements

Leaf samples in each treatment were ground and analyzed for nitrogen and potassium content by the micro-Kjeldahl method (Nelson and Sommers, 1973). Measurement of phosphorous content was done by hydrochloric acid digestion, followed by ammonium vanadomolybdate quantification using flame emission photometry, and the results appeared as percentages (Olsen, 1954).

Statistical analysis

This experiment was performed in a factorial arrangement as a completely randomized design. Three pots were in each replication, including six observations per treatment. Data analysis was done by SAS software (version 9.1). Mean values of three replicates were subjected to one-way ANOVA. Duncan's Multiple Range Test (DMRT) involved comparisons among mean values and determined the significance of statistical differences among the treatment groups ($p \leq 0.05$). Data were reported as mean values plus standard deviation ($X \pm SD$).

Results

Leaf count, shoot fresh weight, shoot dry weight, and leaf phosphorus content were positively influenced by arbuscular mycorrhizal inoculation compared to the non-mycorrhizal control group. Flower dry weight, neck diameter, flower width, flower stem length, and ionic leakage became significantly higher in response to 7 g L^{-1} arbuscular mycorrhizal inoculation. Arbuscular mycorrhizal application in the culture bed produced a statistically significant number of plants compared to the control. The number of leaves, flower neck diameter, flower stem diameter, and flower width were 19.1%, 11.1%, 18.46%, and 15.91% more than the control, respectively. Results in Table 2 indicated that the arbuscular mycorrhizal treatment significantly increased the shoot FW by 33%, shoot DW by 57.5%, flower DW by 57.5%, and P content by 6.9% compared to the control (Table 2).

Biochar 3 and 6 g L^{-1} caused the highest leaf count. The vase life of roses increased by applying 1 g L^{-1} and 3 g L^{-1} biochar, with no significant difference between them. The application of biochar accelerated the onset of flowering. When using 6 g L^{-1} biochar, flowering began one week earlier than the control. Leaf potassium and phosphorus content were significantly higher in response to the main effects of biochar, especially with 6 g L*-1* biochar application. Using biochar at 6% significantly accelerated the onset of flowering (4.5%), K content (13.3%), and P content (33.3%) in *Rosa hybrida*, compared to the control (biochar 0%). Significant increases in leaf count (21.3%) and vase life (10.71%) were observed when using biochar at 3%, compared to the control (biochar 0%) (Table 3).

Table 2. Effect of arbuscular mycorrhizal application on some morphological and physiological traits of roses under hydroponic cultivation.

Arbuscular mycorrhiza l (g L^{-1})	Leaf number	Neck diameter (mm)	Stem diameter (mm)	Flower width (mm)	Flower stem length (cm)	Shoot FW (g)	Shoot DW (g)	Flower DW (g)	Ionic leakage (Ms cm^{-1})	P (%)
0	8.9[b]	5.21[b]	5[b]	30.8[b]	39[b]	12.9[b]	4[b]	6.6[b]	53.4[b]	0.29[b]
7	10.6[a]	5.79[a]	5.6[a]	35.7[a]	46.2[a]	17.2[a]	6.3[a]	8.7[a]	61.9[a]	0.31[a]

Values followed in each column by the same letter were not significantly different at 5% level.

Table 3. Effect of biochar application on some morphological and physiological traits of roses under hydroponic cultivation.

Biochar(g L^{-1})	Leaf number	Vase life (day)	onset of flowering (day)	K (%)	P (%)
0	8.9[b]	8.4[b]	197.6[b]	2.4[b]	0.27[c]
1.5	9[b]	9.2[a]	193.5[ab]	2.63[a]	0.3[b]
3	10.8[a]	9.3[a]	191.4[a]	2.66[a]	0.3[b]
6	10.3[a]	8.5[b]	189.8[a]	2.72[a]	0.36[a]

Values followed in each column by the same letter were not significantly different at 5%.

Biochar treatments significantly increased the plant height by 16.6%, shoot dry weight by 21.0%, and root dry weight by 40.0% compared to the control level.

The biochar and arbuscular mycorrhizal fungi increased flower neck diameter, stem diameter, and shoot length compared to the control group. As such, the maximum flower neck diameter (6.3±1 mm), stem diameter (6.0±0.78 mm), and longest stem (49.0±6.9 cm) were observed in response to 3 g L^{-1} biochar and 7 g L^{-1} mycorrhiza. Also, flower width and phosphorus content were affected by the application of biochar and arbuscular mycorrhizal concentrations. In response to 6 g L^{-1} biochar and 7 g L^{-1} arbuscular mycorrhizal fungi, the phosphorus content (0.4±0.02%) and flower width (38.5±6.0 mm) were highest compared to other treatment groups. (Table 4 and Fig. 1).

Table 4. Comparison of the mean interaction of biochar and arbuscular mycorrhizal application on some morphological and physiological traits of roses under hydroponic cultivation.

Biochar (g L^{-1})	Arbuscular mycorrhizal (g L^{-1})	Neck diameter (mm)	Stem diameter (mm)	Flower width (mm)	Shoot length (cm)
0	0	5.05±0.27[h]	5.05±0.49[f]	29.6±2.7[f]	32.8±2.8[h]
0	7	5.07±0.4[g]	4.8±0.56[g]	31.2±4.9[e]	45.2±10.2[d]
1.5	0	5.3±0.59[d]	4.6±0.54[h]	28.0±2.3[h]	35.2±4.5[g]
1.5	7	6.1±1.0[b]	5.8±1.2[b]	36.5±7.3[d]	45.9±4.5[c]
3	0	5.22±0.41[f]	5.09±0.54[e]	36.8±4.2[b]	47.7±8.0[b]
3	7	6.3±0.87[a]	6.0±1[a]	36.7±3.8[c]	49.0±6.9[a]
6	0	5.25±0.59[e]	5.3±1.1[d]	28.5±4.8[g]	40.2±7.6[f]
6	7	5.6±0.84[c]	5.7±1.1[c]	38.5±6.0[a]	44.8±9.6[e]

Values followed in each column by the same letter were not significantly different at 5% level.

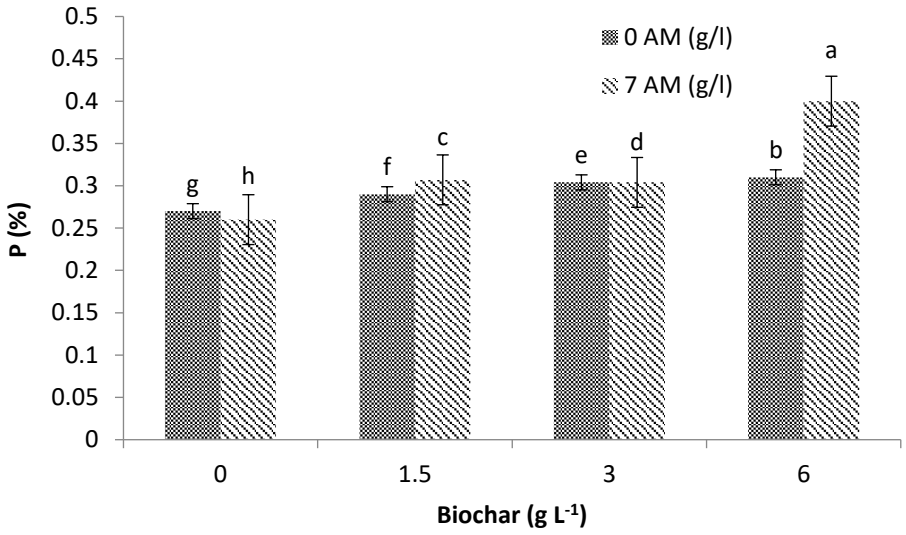

Fig. 1. Comparison of mean interactions between biochar and arbuscular mycorrhizal fungi on leaf phosphorus content of roses under hydroponic cultivation.

Stem diameter, length, and flower diameter in both cultivars were stimulated in response to higher biochar concentrations. These traits were higher than in the absence of biochar. The 'Dolce Vita' cultivar responded more evidently to the growth stimuli than the 'Angelina' cultivar when using higher biochar concentrations. The cultivar 'Dolce Vita' had the largest flower neck diameter in response to 1.5 g L^{-1} biochar (6.4±0.7 mm), and had the highest stem diameter (5.7±1.2 mm) in response to 6 g L^{-1} biochar. The largest flower was observed in the 'Dolce Vita' cultivar using 3 g L^{-1} biochar (39.6±2.3). The longest flowering stem was observed in the 'Angelina' cultivar using 3 g L^{-1} biochar (53.2±6.4). The anthocyanin content of petals in biochar-treated plants did not change much compared to the control and occasionally decreased in response to the biochar application. The highest anthocyanin (0.000038 mg eq. ASC g^{-1} DW) was obtained in the 'Angelina' cultivar at 1.5 g L^{-1} biochar application (Table 5 and Fig. 2).

Table 5. Comparison of mean interactions between biochar and cultivar on some morphological and physiological traits of roses under hydroponic cultivation.

Biochar (g L^{-1})	Cultivar	Neck diameter (mm)	Stem diameter (mm)	Flower width (mm)	Shoot length (cm)
0	Angelina	5.05±0.3^{g}	5.0±0.6^{e}	30.4±4.4^{f}	35.9±9.4^{h}
0	Dolce vita	5.08±0.3^{f}	4.9±0.3^{f}	30.5±3.6^{e}	42.1±9.3^{d}
1.5	Angelina	5.01±0.3^{h}	4.8±0.6^{g}	29.8±2.7^{g}	40.9±3.5^{e}
1.5	Dolce vita	6.4±0.7^{a}	5.7±1.2^{c}	34.7±8.8^{c}	40.2±9.2^{f}
3	Angelina	5.8±0.8^{b}	5.3±0.5^{d}	33.9±3^{d}	53.2±6.4^{a}
3	Dolce vita	5.6±0.8^{d}	5.7±1.2^{b}	39.6±2.3^{a}	43.5±4.7^{c}
6	Angelina	5.1±0.7^{e}	4.7±0.8^{h}	29.8±5.7^{h}	38.0±7^{g}
6	Dolce vita	5.8±0.5^{c}	6.3±0.6^{a}	37.7±6.6^{b}	47±8.4^{b}

Values followed in each column by the same letter were not significantly different at 5% level.

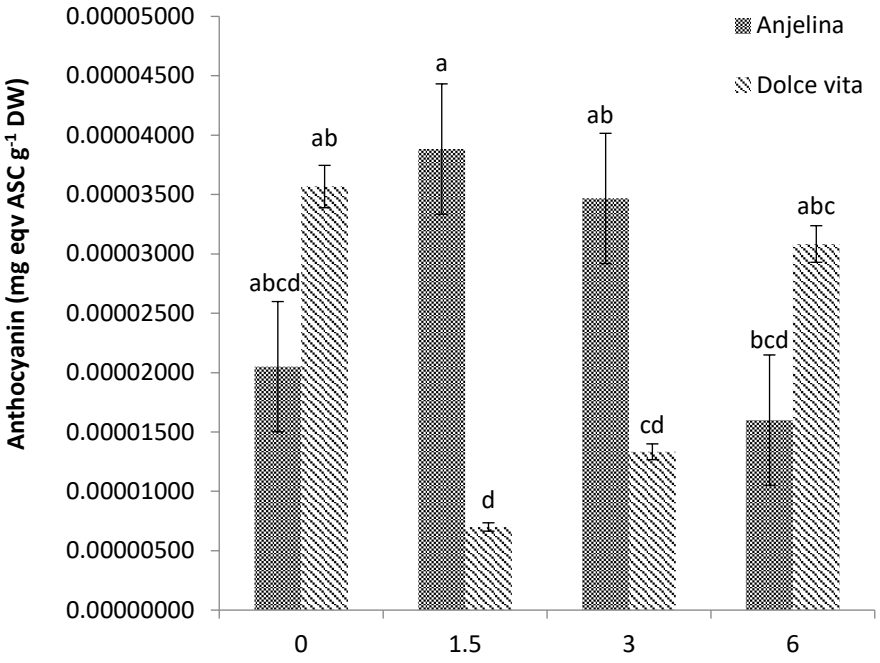

Fig. 2. Comparison of mean interactions between biochar and cultivar on petal anthocyanin content in roses under hydroponic cultivation.

Arbuscular mycorrhizal fungi (7 g L^{-1}) enhanced the stem diameter, flower width, and shoot length in 'Dolce Vita'. This treatment improved shoot fresh weight and total fresh weight in the 'Angelina' cultivar, compared to the other treatment groups.

The highest stem diameter (6.3±0.9 mm), flower width (39.2±5.6 mm), and shoot length (48.8±6.0 cm) were observed in the 'Dolce Vita' cultivar. The highest shoot dry weight (20.03±6.3 g) and total dry weight (35.2±11.7 g) were observed in the 'Angelina' cultivar in response to 7 g L^{-1} arbuscular mycorrhizal fungi (Table 6).

Table 6. Comparison of mean interactions between arbuscular mycorrhizal application and cultivar on some morphological and physiological traits of roses under hydroponic cultivation.

Arbuscular mycorrhizal (g L^{-1})	Cultivar	Stem diameter (mm)	Flower width (mm)	Shoot length (cm)	Shoot FW (g)	Total FW (g)
0	Angelina	4.9±0.7^{c}	29.5±3.9^{d}	40.4±9.9^{c}	12.0±5.0^{b}	25.5±8.3^{b}
0	Dolce vita	5.07±0.77^{b}	32.05±5.8^{c}	37.6±6.0^{d}	13.7±5.5^{b}	33.2±6.4^{ab}
7	Angelina	4.9±0.7^{d}	32.3±4.5^{b}	43.6±8.9^{b}	20.3±7.6^{a}	35.2±11.7^{a}
7	Dolce vita	6.3±0.9^{a}	39.2±5.6^{a}	48.8±6.0^{a}	14.03±6.3^{b}	30.3±10.9^{ab}

Values followed in each column by the same letter were not significantly different at 5% level.

The highest stem diameter (6.817 mm) was obtained in the 'Dolce Vita' cultivar in response to 7 g L^{-1} biochar and 1.5 g L^{-1} arbuscular mycorrhizal fungi. Maximum flower width (42.72 mm) was observed in the 'Dolce Vita' cultivar in response to 7 g L^{-1} arbuscular mycorrhizal fungi and 6 g L^{-1} biochar. In general, the 'Dolce Vita' cultivar responded more significantly than the 'Angelina' cultivar to higher amounts of arbuscular mycorrhizal fungi and biochar in hydroponic conditions, thus stimulating vegetative growth to a greater extent (Table 7).

Table 7. Comparison of the mean interaction of cultivar, biochar and Arbuscular mycorrhizal application on some morphological traits of roses under hydroponic cultivation.

Arbuscular mycorrhizal (g L^{-1})	Biochar (g L^{-1})	Cultivar	Stem diameter (mm)	Flower width (mm)
0	0	Angelina	5.317^{g}	31.33^{j}
0	0	Dolce vita	4.783l	28.02^{n}
0	1.5	Angelina	4.783l	29.2^{m}
0	1.5	Dolce vita	4.6^{n}	26.93^{o}
0	3	Angelina	5.367^{e}	33.17^{g}
0	3	Dolce vita	4.817^{k}	40.58^{c}
0	6	Angelina	4.5^{o}	24.48^{p}
0	6	Dolce vita	6.117^{d}	32.68^{i}
7	0	Angelina	4.683^{m}	29.52l
7	0	Dolce vita	5.067^{h}	33.07^{h}
7	1.5	Angelina	4.85^{j}	30.55^{k}
7	1.5	Dolce vita	6.817^{a}	42.48^{b}
7	3	Angelina	5.333^{f}	34.77^{f}
7	3	Dolce vita	6.683^{b}	38.7^{d}
7	6	Angelina	4.9^{i}	34.43^{f}
7	6	Dolce vita	6.65^{c}	42.72^{a}

Values followed in each column by the same letter were not significantly different at 5% level

Discussion

According to the results, the biochar and arbuscular mycorrhizal application on rose plants caused a significant increase in shoot diameter, flower diameter, shoot length, and phosphorus content in plants. Cell walls consist of cellulose, hemicellulose, lignin, polysaccharides, and a robust fibrous network that may increase the constituents by accelerating photosynthesis and the movement and transport of metabolites. There are vascular bundles and sclerenchyma cells in the stem structure, with a unique arrangement and density, which seem to increase the stem diameter by transporting the metabolites in the vessels and cause accumulation in the sclerenchyma cells (Gislerd et al., 2003). Therefore, applying biochar in the rose culture medium increases the amount and transport of nutrients to the plant and, as a result, increases photosynthesis. This increase in photosynthesis probably causes an increase in the growth indices such as stem diameter. According to the results of this study, the presence of arbuscular mycorrhizal in the root medium increased the diameter of the flowering shoot, which is probably due to the increase in the root surface of the rose and, consequently, the increase in the absorption of nutrients, thus facilitating the transport of materials into plant vessels. A study by Martins et al. (1996) found that inoculating sweet chestnut roots with mycorrhizal fungus increased the growth rate (height, shoot diameter, root length, total plant length, fresh weight, and dry weight), which is consistent with the results of the present study. Since mycorrhiza fungus increases the absorption of water and nutrients by increasing the root area, these conditions can cause more photosynthesis and better growth in plant organs (Osonubi, 1994). Plant growth and metabolic activity take shape by photosynthetic capacity and nutrient utilization capacity. Adding biochar and the presence of AMF can modify the metabolic environment of plants by affecting the levels of available nutrients in the soil, thus increasing the ability of plants to absorb and assimilate CO_2 from the atmosphere. Former studies indicated that AMF improved antioxidant enzyme activity, net photosynthetic rate, and above-ground biomass. One possible mechanism relates to the ability of AMF hyphae to increase water and nutrient uptake by plants. Adding biochar increased peroxidase activity, the photosynthetic rate, above-ground biomass, plant height, and leaf area. With the addition of biochar and AMF inoculation under 60% FWC, maize growth indicators improved in parameters such

as peroxidase activity, chlorophyll content, photosynthetic rate, plant height, leaf area, and shoot biomass.

One of the crucial features in the marketability of cut flowers is the height of the flowering stem. Thus, any factor that increases this trait can effectively enhance crop production and sales. In the present study, using 3-6 g L^{-1} biochar increased the length of the flowering stem, which can be attributed to increased absorption of nutrients by plant roots (Houben et al., 2013; Jeffrey et al., 2010). Akhtar et al. (2015) studied the effects of biochar on wheat growth, reporting that biochar improved stem length, number of tillers, number of panicles, panicle length, number of spikes per panicle, and grain yield. The results obtained by Karami et al. (2011) and Lehman and Joseph (2009) are consistent with the results of this study. Karami et al. (2011) indicated that biochar has a high capacity to keep nutrients in the soil and has the soil capability to retain nutrients and exchangeable cations in a form accessible to the plant. Lehman and Joseph (2009) also suggested the increase in the maize height due to the application of biochar, which is similar to the results of the present experiment.

In this study, biochar and mycorrhiza did not increase the shoot length of rose flowers during their vegetative growth but increased this trait during the reproductive phase. Therefore, the development and propagation of mycorrhizal fungi in the culture medium occurs over time. During vegetative growth, mycorrhiza was insufficient to increase the longitudinal shoot growth. However, with fungal propagation over time, its effect became evident in shoot length during rose flowering. Research by Li et al. (2018) in China showed that inoculating *Elsholtzia splendens* roots with arbuscular mycorrhiza caused a significant increase in the germination rate and plumule length of hypocotyl. A considerable increase in stem length corresponded to higher rose stem length during flowering.

Sharaf-Eldin et al. (2008) observed the effect of biofertilizers on increasing the length of saffron plants (Gamalero et al., 2004; Mahfouz et al., 2007). Also, similar studies showed a significant increase in the height and fresh weight of plant organs in the plants treated with organic fertilizers. According to Antolin et al. (2005), the increased plant growth may be due to the increased mineralization of nutrients with organic fertilizers. According to previous research, using mycorrhiza increased broad bean plant height in fungal treatments compared to the control (Ahibor and Hirata, 1994; Abdel Fattah et al., 2002). Researchers reported that the mycorrhizal fungus increased the length and diameter of the plant stem by enhancing root area and volume, which absorb water and nutrients from larger soil capacities. This occurrence is due to the spread of the mycelium of mycorrhizal fungi associated with the internal tissues of the root in the soil around roots and the formation of an additional absorption system complementary to the root system of the plant, which makes it possible to utilize more soil capacity and nutrients. Eventually, it will increase the growth of different plant parts (Cakir, 2004). The results of the present study are consistent with the above studies. Thus, the increased length of the flowering shoot probably resulted from increased root colonization and higher nutrient uptake, especially phosphorus. Increased amounts of nutrients improve cell division and longitudinal shoot growth. Li and Cai (2021) stated that dual biochar and AMF increased the phosphorus content in maize under drought stress. Also, Hashem et al. (2019) reported that the combined application of biochar and AMF increased the photosynthetic rate in chickpeas under drought stress. Soil microorganisms play a significant role in plant phosphorus uptake by stimulating root growth or phosphorus distribution between inorganic and organic materials (Van Der Heijden et al., 1998).

Biochar increases root colonization and plant growth through soil improvement. Plant growth-promoting bacteria dissolve nutrients in the soil and increase the uptake of the elements by the plant (Afridi et al., 2019). Previous studies have shown that mycorrhizal propagation in the root medium depends on the type of biochar and soil properties (Solaiman et al., 2019; Dobo et al., 2018; Rafique et al., 2019). The porous structure of biochar can be a potential shelter for bacteria, hypomycorrhiza, and its spores, which increase the phosphorus uptake level (Solaiman et al., 2010). This finding is consistent with previous results on increased phosphorus levels. There are numerous studies on biochar and its positive effects on increasing nitrogen, phosphorus, and potassium contents (Nigussie et al., 2012; Njoku et al., 2016; Foster et al., 2016; Dume et al., 2016). While increasing the cation exchange capacity, biochar gives plants better access to nutrients (Pühringer, 2016). Adding biochar to the culture medium can increase nutrient uptake and accelerate the uptake of elements by plant roots, thus making growth faster than the control group.

Masto et al. (2013) showed an increase in soil pH, electrical conductivity, organic matter, phosphorus, and potassium in response to biochar application. The increase in these

elements was attributed to slightly higher soil pH, the calcareous effect of biochar on increasing the phosphorus uptake, and the direct addition of potassium by biochar. Previous studies reported biochar efficiency in the soil, with its role in increasing the micronutrients and macronutrients, especially phosphorus and potassium, thus improving physical properties such as soil water availability (Duku et al., 2011). Major et al. (2010) showed a 140% increase in crop yield and nutrient uptake, e.g., calcium, magnesium, potassium, copper, and manganese, compared to the control. Other studies indicated increases in potassium, phosphorus, and magnesium due to the presence of biochar (Lehmann et al., 2006; Rajkovich et al., 2012).

In this study, an increase in flower diameter by 24.3% occurred in response to 3 g L-*1* biochar. Biochar efficiency also depends on factors such as biochar quality, soil materials, and nutrient concentrations (Wang et al., 2012; Khorram et al., 2016; Cheng et al., 2017; Yousaf et al., 2018). One of the reasons for the increase in flower diameter can be the presence of sufficient nutrients in plants, so a higher amount of nutrients absorbed by plants can enhance plant growth. Adding mycorrhiza to the culture medium increased the flower width compared to the corresponding control. At the cellular level, phytohormones such as auxin and cytokinin can accelerate cellular division and expansion (Stals and Inzé, 2001). It has been reported in previous research that the AMF colonization of the roots of tomato seedlings modulated endogenous hormone levels, which further affected other physiological and metabolic processes. AMF inoculation can adjust plant growth regulation and cause hormonal balance in plants. AMF inoculation significantly increased the IAA and cytokinin concentrations in roots (Wang et al., 2021). As mentioned in other studies, the positive effects of mycorrhiza on the diameter and length of rose shoots were determined at flowering. Thus, an increase in flower diameter occurred as expected in the literature (Osonubi, 1994; Gislerd et al., 2003). The microporous structure of biochar and its content of readily oxidizable organic carbon provide a satisfactory environment for the development of microorganisms. Biochar can alter the soil environment by improving soil porosity and cation exchange capacity, thus enhancing root growth space and inducing root extension (Li and Cai, 2021). Also, biochar contains several nutrients for plants. The hyphae of AMF are thinner than root hairs, so they can spread into the soil and access essential nutrients in micropores that plant roots cannot reach. Then, the AMF transfers those nutrients to their host in exchange for carbohydrates. Therefore, mycorrhizal symbiosis has a significant impact on the growth and adaptability of plants. Soil microorganisms can increase phosphorus content in plants by improving root growth (hormonal stimuli), thus expanding phosphorus access in the soil, facilitating organic phosphorus mineralization, and contributing to inorganic phosphorus dissolution (Richardson, 2001).

As an essential pigment in petals, anthocyanin is affected by various environmental factors, such as nutrients, temperature, plant access to water, and period light and intensity. The results of this study showed that using biochar alone at specific concentrations did not show a significant effect on increasing the amount of anthocyanin in petals. However, the biochar-AMF combination increased the anthocyanin content in 'Dolce Vita' and 'Angelina.' Thus, different rose cultivars respond differently to biochar application due to genetic differences. Similar results indicated that the application of biochar significantly improved chlorophyll content while decreasing anthocyanin and lycopene in *Spinacia oleracea* L. (Danish et al., 2019). Genesio et al. (2015) and Schmidt et al. (2014) showed that wood-based biochar did not affect fruit total acidity, sugar content, or total anthocyanins in table grapes because the biochar amendment could not change soil quality and provide more transformable nutrients for plants.

The mycorrhiza treatment in the present study caused a significant increase in stem fresh weight, stem dry weight, and total dry weight compared to the control. However, no significant effect was observed in increasing the flower fresh weight, flower dry weight, and total fresh weight. Increases in shoot dry weight, shoot fresh weight, and total dry weight were 57.5%, 33.4%, and 31.8%, respectively, compared to the treatment group without mycorrhiza inoculation. Tasang and Maum (1999) reported that inoculating *Strophostyles helvala* with a strain of mycorrhiza (*G. mosseae*) caused a significantly higher shoot dry weight, root dry weight, and chlorophyll content than non-mycorrhizal plants. In pepper (*Piper nigrum* L.) inoculated with *G. intraradices*, chlorophyll a and b significantly increased compared to non-mycorrhizal plants (Demir, 2004). Ansari Jovini et al. (2011) reported increased amounts of dry matter in aerial and underground organs after inoculation with mycorrhizal fungus compared to the non-inoculation case. The observation was more likely due to increased water and nutrient uptake, better nutrient transport in plant organs, and increased plant photosynthesis (Ansari Jovini et al., 2011). Martins et al. (1996) experimented

with mycorrhiza in a sweet chestnut culture medium and increased the fresh and dry weight of plants. Plant species may respond differently to different isolates of arbuscular mycorrhizal fungi. In an experiment by Yooyongwech et al. (2014), an inoculated treatment with arbuscular mycorrhiza caused a significant increase in the fresh and dry weight of sweet potato tubers. In addition to easy access to water in fine soil pores that are far from root access, an increased root network and plant uptake level by their hyphae can enhance soil water uptake by the symbiotic plant. By improving growth conditions and facilitating more nutrient absorption, AMF can increase dry matter production in symbiotic plants (Ghollarata and Raiesi, 2007). Therefore, an increase in shoot fresh and dry weights occurs because of an increase in water uptake and nutrient uptake. This increase in uptake enhanced the shoot fresh and dry weights in rose plants.

Conclusion

Biochar increased the soil pH and the alkaline level of the culture medium. This factor may prevent the plant from absorbing some micronutrients, but using mycorrhizae can control this limiting effect on nutrient uptake through the development of root colonization. In contrast, biochar provides a suitable medium for fungal growth and other soil microorganisms, thus increasing the activity of these beneficial organisms. AMF colonization can be strongly affected by pH, although the effects on various fungal isolates may differ. The decrease in soil pH probably contributed to more root colonization and AMF biomass (Van Aarle et al., 2002). Accordingly, using these treatments on plants can have a synergistic effect on increasing desirable traits in plants. In this experiment, the best biofertilizer treatment was 3 g L^{-1} biochar and 7 g L^{-1} arbuscular mycorrhizal fungi that significantly increased the flowering shoot diameter, flower diameter, flowering shoot length, and leaf phosphorus content.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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