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Arbuscular Mycorrhizae Contribute to Growth, Nutrient Uptake, and Ornamental Characteristics of Statice (*Limonium sinuatum* [L.] Mill.) Subject to Appropriate Inoculum and Optimal Phosphorus

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Abstract: With the world's population and pollutants on the rise, it is crucial to find sustainable and environmentally friendly solutions that increase production efficiency. Organic horticulture is an effective strategy for creating a harmless and sustainable crop production system. Arbuscular mycorrhizal fungi (AMF) have been proposed as reliable biofertilizers for sustainable agriculture, and inoculum production is a rapidly expanding market. AMF can enhance plant nutrition and growth, but their efficacy varies depending on the plant species, inoculum type, and available P concentrations. This study evaluates the response of ornamental statice (*Limonium sinuatum* [L.] Mill.) to mycorrhizal inoculation (first factor) with *Glomus mosseae* (M1), *G. intraradices* (M2), or their mixture (M3), plus non-inoculation (M0), and varying available P concentrations (second factor) of 10 (control, P1), 20 (P2), and 40 (P3) mg kg^{-1} soil in greenhouse conditions in a factorial experiment based on randomized complete block design with three replications. Root colonization, growth parameters, some ornamental traits, and the absorption of P, N, K, Ca, Zn, and Fe were measured. Root colonization was estimated as 30–65% and was reduced approximately by 32.4% with increasing P concentration in the soil. The lowest colonization percentage was recorded in P3 (45.69, 39.31, and 30.18 for M1, M2, and M3, respectively). Statice plants were positively influenced by inoculation, especially with *G. mosseae* in moderately available P (P2), which was also confirmed by the results of the principal component analysis. Overall, inoculated plants exhibited better nutritional status, growth, and ornamental traits than non-inoculated plants. Furthermore, mycorrhization delayed the time to the flowering of statice by 12, 7, and 9 days in M1, M2, and M3, respectively, compared to non-mycorrhizal (M0) plants. In conclusion, mycorrhizal inoculation can improve the plant nutrition, growth, and ornamental value of statice by selecting appropriate inoculum and optimal P concentrations. The results of this study suggest that mycorrhizal inoculation can be effectively used in the future to increase the quantity and quality of statice production.

Keywords: AMF inoculation; cut flowers; phosphorus levels; colonization; fungal isolates

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1. Introduction

The cut flower industry is thriving in many countries due to the growing global demand for flowers [1]. *Limonium* is a genus in the family *Plumbaginaceae* that comprises approximately 15 to 20 cultivated species, including *L. sinuatum* (statice), hybrids of *L. bellidifolium*, and *L. latifolium*, *L. sinense*, and *L. perezii* [2]. The genus *Limonium* has therapeutic values such as antibacterial [3], antiviral [4], and anti-inflammatory activities [5].

Moreover, some of its species are used for culinary purposes, while others are employed as antioxidants in cosmetics and health products [6]. Different classes of metabolites, such as flavonoids, phenolic acids, anthocyanins, phytosterols, proanthocyanidins, saponins, hydrolysable tannins, and essential oils, have been identified in *Limonium* species [7]. Specifically, natural antioxidant compounds have been extracted from *L. sinuatum* flowers [8]. *Limonium* species are also suitable candidates for the phytoremediation and especially phytostabilization of lead and cadmium [9].

Statice has become increasingly important in the cut flower industry due to its unique and vibrant colors, attractive appearance as both fresh and dried flowers, and its ability to last for an extended period [10,11]. It is also commonly used as a bedding plant in landscapes. The quality of cut flowers, including statice, depends on the quality of the product. To increase crop yield and quality, fertilizers and other chemicals are commonly used. However, with the growing interest in organic horticulture [12,13], maintaining high crop quality with minimal chemical inputs can present challenges, especially for ornamental plants where organic fertilization schemes are not well developed [12,14]. Moreover, the repeated use of chemical fertilizers at supra-optimal rates in modern farming is costly and has the potential to harm the environment and soil health [15]. As a result, a more environmentally friendly and sustainable approach should be employed.

Phosphorus (P) is necessary for various biochemical and physiological processes in plants. Despite its widespread use in agriculture, P is a nonrenewable resource with a limited geographical distribution. As a result, P fertilizers should be applied sparingly, taking into account the actual crop needs [16]. Several studies have investigated the role of P in the growth and development of ornamental plants [12,16]. For example, Verlinden and McDonald (2007) showed that the maximum number of stems and total weight in statice (*L. sinuatum*) and celosia (*Celosia argentea*) plants were obtained with phosphorus application between 30 and 46 mg L^{-1} [12]. P shortage has been shown to diminish plant height and fresh weight in *Petunia*, *Impatiens walleriana*, *Salvia splendens*, *Euphorbia pulcherrima*, and *Pelargonium zonale* [16].

The use of microbial inoculants, also known as biofertilizers, in sustainable production enables plants to effectively absorb mineral elements such as nitrogen and phosphorus [17]. Biofertilizers, which are microorganisms such as bacteria, fungi, and algae, have been proposed as workable alternatives to conventional agricultural methods that are not only organic, eco-friendly, and cost-effective, but also preserve the soil's structure and biodiversity [18]. When applied to soil, seeds, or plants, biofertilizers promote plant growth by increasing nutrient availability to host plants. They boost the availability of nutrients by colonizing the rhizosphere and encouraging microbial activity, making elements more readily absorbed by plants [19].

Bashan and Holguin [20] have identified two categories of microorganisms that are commonly used as microbial inoculants (biofertilizers). The first category includes those with symbiotic systems such as *Rhizobium* spp., *Frankia* spp., and *Azolla* spp. The second category consists of those without symbiotic systems such as *Azotobacter* spp., *Azospirillum* spp., and blue-green algae [20]. Therefore, biofertilizers can be asymbiotic free nitrogen fixers (*Azotobacter*, *Azospirillum*, etc.), symbiotic nitrogen fixers (*Rhizobium* spp.), algae biofertilizers (blue-green algae or BGA in association with *Azolla*), phosphate-solubilizing bacteria, *mycorrhizae*, and organic fertilizers [21].

Arbuscular mycorrhizal (AM) fungi are known potential biofertilizers that provide significant benefits to the host plant. Their broad host range makes them particularly useful in the inoculant sector [22]. Despite only about 240 species being documented based on morphology in the fungal phylum *Glomeromycota*, molecular research has shown that their diversity can be substantially higher [23]. Three families of arbuscular mycorrhizal fungi including *Gigasporaceae* (*Gigaspora* and *Scutellospora*), *Acaulosporaceae* (*Acaulospora* and *Entrophospora*), and *Glomaceae* (*Glomus* and *Sclerocystis*) have been identified [24,25]. The *Glomaceae* family is the oldest AMF family. The *Gigasporaceae* and *Acaulosporaceae* families

seem to have developed later and split off from one another some 250 million years ago, during the late Paleozoic period [26].

Arbuscular mycorrhizal fungi (AMF) can reach nutrients outside the rhizosphere by building a vast network of fine hyphae [27]. They have been introduced to cropping schemes to improve water and nutrient uptake [28–30], particularly for relatively immobile nutrients such as P [28,31,32]. In low P substrates, AMF improve plant P uptake [33–35]. Slow-moving nutrients such as phosphorus (P), zinc (Zn), and copper (Cu) in the soil that are normally inaccessible to plant roots due to their slow immobility become available to plants by mycorrhizal fungi [36].

The majority of AMF species may coexist with various plant species, and numerous AMF species can colonize a single plant. The reactions of the plants and their AMF, however, may vary depending on the conditions, indicating varying degrees of compatibility between particular AMF strains and plant species [37]. Overall, host plants exhibit varying responsiveness to (or dependence on) mycorrhizal colonization [38].

To promote environmentally friendly and sustainable agriculture, exploring the potential of AM fungi to enhance crop growth is important. However, there is currently a gap in knowledge regarding how AM can benefit ornamental plants compared to other horticultural products. Specifically, little information is available on how statice plants respond to mycorrhizal colonization at different P levels. To address this gap, the present study investigated the response of statice plants to mycorrhizal inoculation with two *Glomus* species under low, moderate, and high P concentrations in the soil substrate. The aim of the study was to address: (1) the level of mycorrhizal fungi colonization of the statice root system, (2) how the presence of phosphorus alters the mycorrhizal colonization of statice plants, and (3) the effect of mycorrhizal fungi on the ornamental characteristics, growth parameters, and elemental content of statice plants. Two hypotheses were considered in this study: (1) high phosphorus levels may reduce AMF efficiency, and (2) different AMF isolates may have different effects on statice plants.

2. Materials and Methods

2.1. Experimental Design

A factorial pot experiment was carried out in greenhouse conditions using a randomized complete block design with three replications. The study evaluated the factors of inoculation and P fertilization, with four levels of the inoculation factor and three levels of the P fertilization factor. The levels of the inoculation factor were non-mycorrhizal inoculation (M0), inoculation with *Glomus mosseae* (M1), *Glomus intraradices* (M2), and *G. mosseae* + *G. intraradices* (M3), while the levels of the P factor were 10 mg kg^{-1} (low P, P1), 20 mg kg^{-1} (moderate P, P2), and 40 mg kg^{-1} (high P, P3) mg P kg^{-1} soil. Each treatment in each replication consisted of 5 experimental units (plants), resulting in a total of 180 plants (15 plants in each treatment). Two experimental units of each replication were destructively used to estimate colonization, while the remaining three were used to evaluate vegetative and ornamental traits and measure elements.

The topsoil (0–20 cm) used in the experiment was collected from the research station of the Department of Horticultural Sciences, University of Tehran, Karaj, Iran (35°49′ N, 51°0′ E, and 1310 m asl). The basic soil properties are presented in Table 1. The growing medium was prepared by mixing the collected soil with fine–medium sand at a 2:1 (*v*:*v*) ratio and it was double autoclaved (121 °C for 50 min) before use. Phosphorus was added to the medium in the form of $Ca(H_2PO_4)_2$ and was allowed to equilibrate for 50 days at room temperature.

2.2. Mycorrhizal Inoculation and Plant Growth

The prepared substrate was packed into 4 L plastic pots, and 25 g of mycorrhizal inoculum was placed into a hole in each pot. The inoculum consisted of a mixture of spores, hyphae, colonized roots, and growth medium with 50 ± 10 active fungal structures per gram. Statice (*Limonium sinuatum* L.) plants were grown from seeds (Eurogarden, Barcelona,

Spain). At 6 weeks of age, they were selected based on uniformity in height, number of leaves, and root length, and one plant was transplanted into each 4 L pot. Non-inoculated pots received 25 g of double-autoclaved inoculum.

Table 1. Properties of the used soil, obtained from the Research Station of Department of Horticultural Sciences, University of Tehran, Karaj, Iran.

Trait		Value
Texture		Loamy clay
pH	[1:2.5 soil: water (*w*/*v*)]	7.63
N (%)		0.181
P (mg kg^{-1})	Sodium bicarbonate—extractable	9.89
K (mg kg^{-1})	Ammonium acetate—extractable	490

The mycorrhizal inoculum was propagated on sorghum roots according to the trap culture method [39] and was provided by the Biology Section of Soil Science of the University of Tehran. Briefly, 500 g of autoclaved substrate was placed in pots and covered with a thin coating. A layer of mycorrhizal inocula (50 g of soil sample) was then spread on top of the substrate in each pot. Each pot contained five 15-day-old sorghum plantlets that were without AMF. The trap cultures were kept in a greenhouse (20–24 °C; 55–60% relative humidity) for eight months. After seven months, the roots were examined to determine the level of AMF colonization, which could indicate the likelihood of infection.

Plants were grown in a greenhouse at mean day/night temperatures of 25/15 °C. Daytime temperatures ranged from 23 to 30 °C and air humidity from 60 to 75%. Pots were irrigated once or twice a week to 60–70% of pot capacity, depending on demand. Plants were fed with 300 mL of a solution lacking P once a week, which contained in mM: 2.75 N as $Ca(NO_3)_2$, KNO_3 and $(NH_4)_2SO_4$; 0.75 K as KNO_3 and K_2SO_4; 2 Ca as $Ca(NO_3)_2$ and $CaCl_2$; 1 Mg as $MgSO_4$; 1.25 S as $MgSO_4$, K_2SO_4, and $(NH_4)_2SO_4$; and in µM: 40 Fe as Fe-EDTA, 25 B as H_3BO_3, 1.5 Mn as $MnSO_4$, 1.5 Zn as $ZnSO_4$, 0.5 Cu as $CuSO_4$, and 0.1 Mo as $NaMoO_4$. Plants were harvested in full bloom.

2.3. Evaluation of Vegetative and Ornamental Characteristics

Vegetative and ornamental characteristics were assessed using 3 plants randomly collected from each replication, resulting in a total of 9 plants for each treatment. The distance between the top inflorescence and stalk base was recorded as flowering stem length (cm). The fresh weights (g) of flowering stems, leaves, and roots were measured separately [9]. Dry weights were determined after drying in an oven (70 °C) for 48 h. The number of days from transplanting to 50% emergence of sepals was recorded as the number of days required for flowering. The leaf area (mm^2 $plant^{-1}$) was estimated using a leaf area meter (ΔT AREA METER MK2, Delta-T Devices, Cambridge, UK). The total root length (cm $plant^{-1}$) was estimated according to the gridline method of Tennant [40].

2.4. Assessment of Root Mycorrhizal Colonization

Root mycorrhizal colonization was assessed using 2 plants randomly collected from each replication, leading to a total of 6 plants for each treatment. Roots were completely rinsed, and then roots less than 2 mm in diameter were examined to determine root colonization percentage with trypan blue 0.05 in lactoglycerol [41]. Colonization was determined according to the gridline intersect method under a stereomicroscope with a magnification of 50× [42].

2.5. Measurement of Nutrients

Oven-dried roots and shoots were ground to determine mineral concentrations. Kjeldahl method [43], spectrophotometry [44], and flame photometer were used for N, P, and K, respectively, and an atomic absorption device (Shimadzu AA-670, Kyoto, Japan) was used for Ca, Fe, and Zn.

2.6. Statistical Analyses

Data were subjected to analysis of variance (two-way ANOVA), and the means were compared by Duncan's multiple range test ($p = 0.05$) using SAS software version 9.1 (SAS Institute, Cary, NC, USA). Principal component analysis (PCA) was conducted based on growth and ornamental characteristics, as well as shoot and root concentrations of P, N, Ca, Zn, and Fe. To understand the relationship between growth parameters and nutrient concentrations in shoots and roots, Pearson correlations were performed, using R Studio 2022 (version 4.2.1).

3. Results

3.1. Growth and Ornamental Traits

The interaction effect of mycorrhiza and phosphorus on growth indices was significant. Mycorrhiza had a positive effect on the fresh and dry weights of the flowering stems and the height of the statice flowering stems, particularly at lower P levels (Table 2). The highest flowering stem fresh weights were found with M1 (*Glomus mosseae*, 88.42 g) and M2 (*Glomus intraradices*, 84.90 g), but for root fresh weights, no significant differences were observed between different inocula. With the highest P level, there were no significant differences between mycorrhizal and non-mycorrhizal plants regarding growth parameters. However, root fresh and dry weights were reduced at high P levels regardless of mycorrhizal inoculation. P levels had no significant effects on leaf area, but it was increased by mycorrhization (Table 2). The largest leaf area was found with M1 (474,748 mm^2 $plant^{-1}$) and M2 (440,725 mm^2 $plant^{-1}$) at a moderate P level (Table 2). The number of flowering stems was not influenced by AM inoculation (Table 3). The highest number of flowering stems was observed at a moderate P level (20 mg kg^{-1} soil; Table 3).

Flowering was delayed with M1, M2, and M3 inoculation by 12, 7, and 9 days, respectively. However, neither inoculum nor P level had a significant effect on flowering time (Table 3). Mycorrhizal inoculation significantly increased root length with no significant difference between inocula, so the root length of the non-inoculated plants (M0) was 1.5 times shorter than that of plants inoculated with M1 (Table 3). The longest root length (9648 cm $plant^{-1}$) was found at a lower P level, while root length decreased drastically with the increasing P level (Table 3). The interaction effects of mycorrhizal inoculation and different P levels on root length were not significant.

3.2. Root Colonization

No colonization occurred in non-inoculated plants, while in inoculated plants, the colonization percentage was estimated to be between 30 and 65%. The root colonization was significantly reduced by approximately 32.4% with the increasing P concentration in the soil, resulting in the lowest colonization percentage being recorded in P3 (45.69, 39.31, and 30.18 for M1, M2, and M3, respectively). The three inocula had significant differences only in P2 (Figure 1).

3.3. Shoot and Root Nutrients

The shoot concentrations of P, N, Ca, Zn, and Fe were influenced by the interaction of P concentration and mycorrhizal inoculation (Table 4). AM inoculation enhanced the shoot concentrations of P, N, and Ca, with different inocula having different effects on nutrient concentration in the shoot. M1 was more effective than M2 and M3 in increasing shoot P and N, whereas M3 was better than M2 in enhancing shoot Ca. In non-inoculated plants grown in P1, the concentrations of P (3.48%), N (2.71%), and Ca (0.55%) were the lowest, and their concentrations were higher in inoculated plants with M1 at a moderate P level (6.08%, 3.57%, 0.80%, respectively). In P3, there were no significant differences between mycorrhizal and non-mycorrhizal plants regarding P, N, Zn, and Fe concentrations (Table 4). Mycorrhizal inoculation was not able to significantly affect shoot K concentration (Table 3). Mycorrhizal inoculation significantly increased root P concentration, but it reduced the N and had no significant effect on the Zn concentration (Table 4). These effects were different

depending on the given inoculum, with plants inoculated with M1 having higher P and N than those inoculated with M2 and M3 under P2. The highest Zn was measured in plants inoculated with M2 (48.93 mg kg^{-1}) and M3 (44.00 mg kg^{-1}) under P1. Overall, the highest P and N concentrations were recorded in M1-inoculated plants grown in P2. The lowest P concentration was recorded in non-mycorrhizal plants grown in P1. In addition, the lowest Zn and Fe concentrations were measured in non-mycorrhizal plants grown in P3 (Table 4). Data presented in Table 3 show that the effect of mycorrhiza on root Ca was not significant, but the P concentration affected the root Ca concentration significantly. Increasing the P concentration to 20 mg kg^{-1} soil increased the root Ca concentration. Neither the soil P concentrations nor the mycorrhizal inoculation was effective on the root K concentration of statice.

Table 2. Effect of AMF inoculation on some of growth and ornamental characteristics of statice at different phosphorus levels.

P [1]	M [2]	Flowering Stem Fresh Weight (g)	Flowering Stem Dry Weight (g)	Flowering Stem Height (cm)	Leaf Area (mm^2 $plant^{-1}$)
P1	M0	42.75 ± 13.35 ^{d}	6.78 ± 2.05 ^{e}	52.00 ± 4.62 ^{f}	216,295 ± 30,778 ^{ef}
	M1	88.42 ± 10.19 ^{bc}	15.26 ± 1.62 ^{bc}	69.00 ± 6.66 ^{ef}	313,967 ± 64,359 $^{c–e}$
	M2	84.90 ± 15.08 ^{bc}	13.70 ± 2.92 ^{c}	76.33 ± 8.76 $^{c–e}$	360,591 ± 70,461 ^{bc}
	M3	45.52 ± 5.94 ^{d}	7.66 ± 0.99 ^{de}	88.33 ± 0.88 $^{b–e}$	328,889 ± 9964 ^{cd}
P2	M0	75.77 ± 10.29 ^{c}	12.92 ± 1.71 ^{cd}	74.67 ± 9.60 $^{d–f}$	203,345 ± 5875 ^{f}
	M1	121.92 ± 12.38 ^{a}	23.53 ± 2.49 ^{a}	113.00 ± 2.65 ^{a}	474,748 ± 14,030 ^{a}
	M2	124.22 ± 10.58 ^{a}	22.85 ± 2.40 ^{a}	107.67 ± 3.38 ^{ab}	440,725 ± 23,532 ^{ab}
	M3	112.83 ± 6.63 ^{ab}	20.98 ± 1.23 ^{ab}	77.33 ± 13.92 $^{c–e}$	374,688 ± 37,098 ^{bc}
P3	M0	103.02 ± 8.99 $^{a–c}$	16.61 ± 0.39 ^{bc}	94.67 ± 14.44 $^{a–d}$	240,351 ± 19,567 $^{d–f}$
	M1	105.58 ± 4.53 $^{a–c}$	18.22 ± 1.44 $^{a–c}$	82.67 ± 6.12 $^{c–e}$	338,014 ± 26,136 ^{cd}
	M2	92.06 ± 8.75 $^{a–c}$	14.94 ± 1.86 ^{c}	100.00 ± 5.77 $^{a–c}$	302,769 ± 13,271 $^{c–e}$
	M3	93.65 ± 4.24 $^{a–c}$	15.25 ± 0.22 ^{bc}	85.33 ± 8.45 $^{b–e}$	300,219 ± 39,104 $^{c–e}$
Sig. [3]	P	***	***	***	**
	M	**	***	**	***
	P × M	*	*	**	*
P	**M**	**Above-Ground Fresh Weight (g)**	**Above-Ground Dry Weight (g)**	**Root Fresh Weight (g)**	**Root Dry Weight (g)**
P1	M0	133.18 ± 4.85 ^{e}	12.80 ± 1.24 ^{de}	17.92 ± 0.35 ^{b}	1.71 ± 0.06 ^{c}
	M1	219.70 ± 42.45 ^{cd}	15.08 ± 3.75 $^{c–e}$	24.50 ± 0.19 ^{a}	2.49 ± 0.19 ^{a}
	M2	220.37 ± 23.11 ^{cd}	16.22 ± 1.27 $^{b–d}$	23.92 ± 0.53 ^{a}	2.21 ± 0.04 ^{b}
	M3	235.67 ± 7.71 $^{b–d}$	16.77 ± 0.99 $^{b–d}$	24.14 ± 0.61 ^{a}	2.33 ± 0.02 ^{ab}
P2	M0	127.36 ± 7.59 ^{e}	10.33 ± 0.96 ^{e}	12.84 ± 1.12 ^{de}	1.00 ± 0.17 ^{e}
	M1	356.87 ± 7.92 ^{a}	23.70 ± 0.49 ^{a}	14.29 ± 0.66 ^{cd}	1.41 ± 0.01 ^{d}
	M2	302.26 ± 16.94 ^{ab}	20.69 ± 0.81 ^{ab}	15.49 ± 0.74 ^{c}	1.49 ± 0.07 ^{cd}
	M3	259.02 ± 12.43 ^{bc}	18.31 ± 0.79 ^{bc}	14.21 ± 0.71 $^{c–e}$	1.33 ± 0.02 ^{d}
P3	M0	177.67 ± 25.19 ^{de}	14.94 ± 1.20 $^{c–e}$	12.21 ± 0.53 ^{e}	0.93 ± 0.14 ^{e}
	M1	288.01 ± 20.61 ^{bc}	20.02 ± 1.92 $^{a–c}$	12.83 ± 0.76 ^{de}	1.02 ± 0.15 ^{e}
	M2	246.89 ± 21.55 ^{bc}	17.17 ± 1.98 $^{b–d}$	12.45 ± 0.56 ^{de}	0.93 ± 0.10 ^{e}
	M3	270.87 ± 30.45 ^{bc}	18.59 ± 0.93 ^{bc}	12.73 ± 0.74 ^{de}	0.97 ± 0.10 ^{e}
Sig.	P	**	*	***	***
	M	***	***	***	***
	P × M	*	*	***	**

[1] Phosphorus treatments—P1, P2, P3: 10, 20, 40 mg kg^{-1} soil, respectively. [2] AM fungal inocula—M0: non-inoculated; M1: Iranian *Glomus mosseae*; M2: Iranian *G. intraradices*; M3: mixture of Iranian *G. mosseae* and Iranian *G. intraradices*. [3] *, ** and *** denote statistical significance from ANOVA at the 0.05, 0.01, and 0.001 levels, respectively. Data correspond to the means ± standard error of three independent replicates. Different letters in columns indicate significant differences between treatments within the same factor, Duncan's multiple range test (p = 0.05).

Table 3. Effects of the phosphorus (P), AMF inoculation (M), and their interaction (P × M) on the number of flowering stems per plant, days to flowering, root length, and K and Ca concentrations of statice.

Treatments		Number of Flowering Stems/Plant	Days to Flowering	Root Length (cm/plant)	Shoot K (%)	Root K (%)	Root Ca (%)
P [1]	P1	9.02 ± 0.39 [a]	122.17 ± 2.39 [a]	9648 ± 817.54 [a]	1.33 ± 0.04 [b]	0.69 ± 0.01 [a]	0.60 ± 0.02 [b]
	P2	11.65 ± 0.95 [a]	117.42 ± 2.10 [a]	5859 ± 498.61 [b]	1.51 ± 0.06 [a]	0.70 ± 0.01 [a]	0.67 ± 0.04 [a]
	P3	10.82 ± 0.74 [ab]	114.50 ± 3.60 [a]	4787 ± 347.66 [b]	1.45 ± 0.05 [a]	0.69 ± 0.01 [a]	0.65 ± 0.03 [ab]
M [2]	M0	9.80 ± 0.73 [a]	110.89 ± 3.64 [b]	5191 ± 433.26 [b]	1.45 ± 0.07 [a]	0.70 ± 0.01 [a]	0.64 ± 0.03 [a]
	M1	11.66 ± 0.96 [a]	123.33 ± 2.45 [a]	7698 ± 1112.30 [a]	1.43 ± 0.07 [a]	0.70 ± 0.01 [a]	0.66 0.05 [a]
	M2	11.06 ± 0.89 [a]	118.22 ± 3.16 [ab]	7322 ± 895.46 [a]	1.41 ± 0.07 [a]	0.69 ± 0.01 [a]	0.64 ± 0.03 [a]
	M3	9.46 ± 0.93 [a]	119.67 ± 2.81 [ab]	6847 ± 1180.00 [a]	1.42 ± 0.07 [a]	0.69 0.01 [a]	0.63 ± 0.03 [a]
Sig. [3]	P	ns	ns	***	***	ns	*
	M	ns	*	**	ns	ns	ns
	P × M	ns	ns	ns	ns	ns	ns

[1] Phosphorus treatments—P1, P2, P3: 10, 20, 40 mg kg^{-1} soil, respectively. [2] AM fungal inocula—M0: non-inoculated; M1: Iranian *Glomus mosseae*; M2: Iranian *G. intraradices*; M3: a mixture of Iranian *G. mosseae* and Iranian *G. intraradices*. [3] *, **, ***, and ns denote statistical significance from ANOVA at the 0.05, 0.01, and 0.001 levels, and the absence of significance, respectively. Data correspond to the means ± standard error of three independent replicates. Different letters in columns indicate significant differences between treatments within the same factor, Duncan's multiple range test ($p = 0.05$).

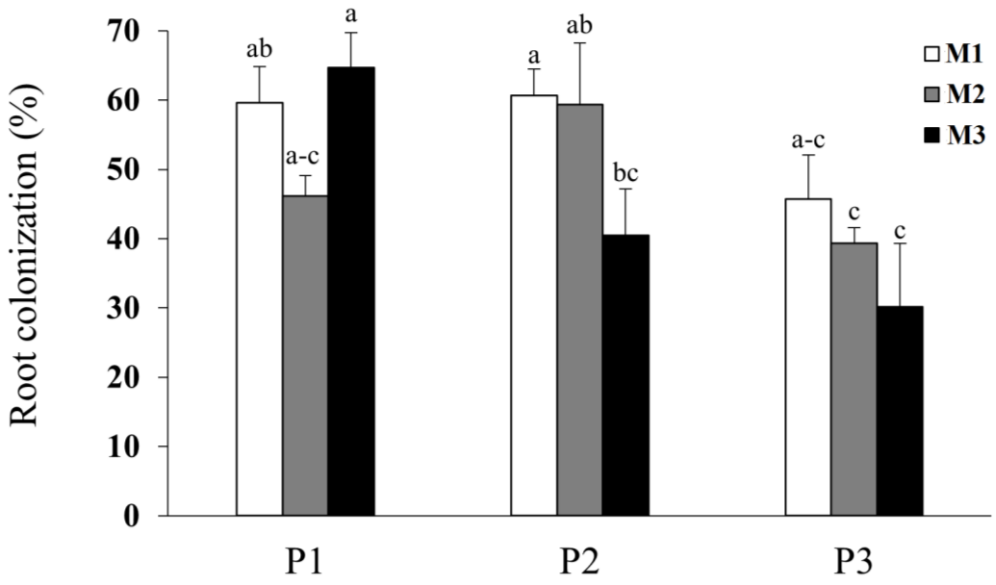

Figure 1. Effect of AMF inoculation (M1: Iranian *Glomus mosseae*; M2: Iranian *G. intraradices*; M3: a mixture of Iranian *G. mosseae* and Iranian *G. intraradices*) on root colonization of statice at different levels of phosphorus (P1, P2, P3: 10, 20, 40 mg P kg^{-1} soil). Values are means and vertical bars are standard errors. Different letters indicate significant differences between treatments, Duncan's multiple range test ($p = 0.05$). No colonization was observed in non-inoculated plants.

3.4. Principal Component Analysis and Correlation

Principal component analysis was performed considering growth, ornamental characteristics, and nutrient concentration in the shoot and root systems (Figure 2). According to the PCA, the first two components accounted for 75.09% of the variation (PC1 55.41% and PC2 19.68%). The analysis revealed that P2M1 and P2M2 (mycorrhizal plants at moderate P levels) were located on the positive side of the PC1 in the upper right quadrant, resulting in plants with higher leaf area, above-ground fresh weight, above-ground dry weight, shoot P, and shoot N. There was a strong and positive correlation among these traits (Figures 2 and 3). AMF plants grown at 10 mg kg^{-1} P (P1M1, P1M2, and P1M3) were

grouped close to the root fresh weight, root dry weight, root Zn, shoot Zn, and root Fe, which were located in the upper left quadrant of the biplot. As shown in Figure 2, there was also a positive correlation between flowering stem height, flowering stem fresh weight, flowering stem dry weight, and root P, which was related to the mycorrhizal plants at 40 mg kg^{-1} P (P3M1, P3M2, and P3M3), although these associations were further away from those of the AMF plants grown at 20 mg kg^{-1} P and the aforementioned variables (Figures 2 and 3). Non-mycorrhizal treatments (P1M0 and P2M0, located in the lower left quadrant) had the least contributions to dimensions 1 and 2, and those grown at the highest level of P (P3M0) had no part in any of the two components (Figure 2).

Table 4. Effect of AMF inoculation on the nutrient concentration of statice at different phosphorus levels.

		Shoot				
P [1]	**M** [2]	**P (%)**	**N (%)**	**Ca (%)**	**Zn (mg kg^{-1})**	**Fe (mg kg^{-1})**
P1	M0	3.48 ± 0.07 ^{f}	2.71 ± 0.06 ^{d}	0.55 ± 0.12 ^{e}	27.52 ± 2.29 ^{ab}	213.67 ± 18.67 ^{a}
	M1	5.80 ± 0.07 ^{b}	3.15 ± 0.11 ^{b}	0.65 ± 0.08 ^{d}	19.25 ± 1.39 ^{cd}	165.67 ± 13.57 ^{bc}
	M2	5.63 ± 0.08 $^{b–d}$	3.03 ± 0.14 ^{bc}	0.67 ± 0.12 ^{cd}	29.33 ± 1.35 ^{a}	198.33 ± 30.33 ^{ab}
	M3	5.67 ± 0.07 ^{bc}	3.03 ± 0.10 ^{bc}	0.79 ± 0.07 ^{ab}	25.07 ± 3.16 $^{a–c}$	180.33 ± 23.38 ^{bc}
P2	M0	5.04 ± 0.04 ^{e}	2.75 ± 0.06 ^{cd}	0.71 ± 0.7 $^{b–d}$	25.65 ± 2.10 $^{a–c}$	195.00 ± 24.33 $^{a–c}$
	M1	6.08 ± 0.03 ^{a}	3.57 ± 0.11 ^{a}	0.80 ± 0.04 ^{ab}	14.35 ± 0.75 ^{d}	162.00 ± 24.79 ^{c}
	M2	5.60 ± 0.13 $^{b–d}$	2.96 ± 0.15 $^{b–d}$	0.76 ± 0.5 ^{bc}	18.51 ± 1.08 ^{cd}	188.67 ± 17.70 $^{a–c}$
	M3	5.54 ± 0.10 $^{b–d}$	3.08 ± 0.12 ^{b}	0.87 ± 0.02 ^{a}	20.91 ± 1.71 $^{b–d}$	192.00 ± 43.84 $^{a–c}$
P3	M0	5.38 ± 0.07 ^{cd}	3.24 ± 0.06 ^{b}	0.75 ± 0.07 $^{b–d}$	18.29 ± 1.45 ^{cd}	163.33 ± 22.60 ^{c}
	M1	5.52 ± 0.07 $^{b–d}$	3.27 ± 0.13 ^{b}	0.71 ± 0.07 $^{b–d}$	23.68 ± 3.39 $^{a–c}$	184.67 ± 27.57 $^{a–c}$
	M2	5.33 ± 0.03 ^{d}	3.13 ± 0.13 ^{b}	0.67 ± 0.07 ^{cd}	24.43 ± 3.65 $^{a–c}$	182.33 ± 17.84 $^{a–c}$
	M3	5.45 ± 0.03 ^{cd}	2.99 ± 0.06 $^{b–d}$	0.77 ± 0.09 ^{b}	24.00 ± 2.33 $^{a–c}$	192.67 ± 28.06 $^{a–c}$
Sig. [3]	P	***	*	**	**	ns
	M	***	***	ns	*	ns
	P × M	***	**	*	*	*
		Root				
P	**M**	**P (%)**	**N (%)**		**Zn (mg kg^{-1})**	**Fe (mg kg^{-1})**
P1	M0	3.42 ± 0.06 ^{f}	2.44 ± 0.11 ^{b}		43.73 ± 5.27 ^{ab}	1206 ± 22.42 ^{a}
	M1	4.05 ± 0.16 ^{d}	2.05 ± 0.04 ^{fg}		41.93 ± 3.19 ^{bc}	836 ± 105.01 $^{b–d}$
	M2	4.17 ± 0.03 ^{d}	1.92 ± 0.9 ^{h}		48.93 ± 4.60 ^{a}	1092 ± 132.82 $^{a–c}$
	M3	4.20 ± 0.07 ^{d}	2.09 ± 0.09 ^{ef}		44.00 ± 3.92 ^{ab}	932 ± 111.20 $^{a–d}$
P2	M0	3.76 ± 0.15 ^{e}	2.54 ± 0.09 ^{b}		39.67 ± 4.93 $^{b–d}$	1176 ± 254.32 ^{ab}
	M1	5.84 ± 0.04 ^{a}	2.12 ± 0.11 ^{de}		34.27 ± 5.32 ^{ed}	698 ± 61.20 ^{d}
	M2	5.48 ± 0.10 ^{b}	2.10 ± 0.10 $^{d–f}$		34.33 ± 5.02 ^{de}	921 ± 156.10 $^{a–d}$
	M3	5.29 ± 0.04 ^{bc}	2.09 ± 0.07 ^{ef}		36.80 ± 3.44 $^{c–e}$	801 ± 65.12 ^{cd}
P3	M0	5.14 ± 0.03 ^{c}	2.79 ± 0.09 ^{a}		31.53 ± 3.60 ^{e}	675 ± 61.48 ^{d}
	M1	5.42 ± 0.05 ^{b}	2.01 ± 0.11 ^{g}		36.67 ± 4.36 $^{c–e}$	936 ± 194.30 $^{a–d}$
	M2	5.23 ± 0.04 ^{bc}	2.16 ± 0.08 ^{d}		35.67 ± 4.16 ^{de}	996 ± 187.62 $^{a–d}$
	M3	5.12 ± 0.04 ^{c}	2.06 ± 0.09 $^{e–g}$		31.87 ± 4.78 ^{e}	791 ± 36.67 ^{cd}
Sig.	P	***	***		***	ns
	M	***	***		ns	ns
	P × M	***	***		*	*

[1] Phosphorus treatments—P1, P2, P3: 10, 20, 40 mg kg^{-1} soil, respectively. [2] AM fungal inocula—M0: non-inoculated; M1: Iranian *Glomus mosseae*; M2: Iranian *G. intraradices*; M3: mixture of Iranian *G. mosseae* and Iranian *G. intraradices*. [3] *, **, ***, and ns denote statistical significance from ANOVA at the 0.05, 0.01, and 0.001 levels, and the absence of significance, respectively. Data correspond to the means ± standard error of three independent replicates. Different letters in columns indicate significant differences between treatments within the same factor, Duncan's multiple range test ($p = 0.05$).

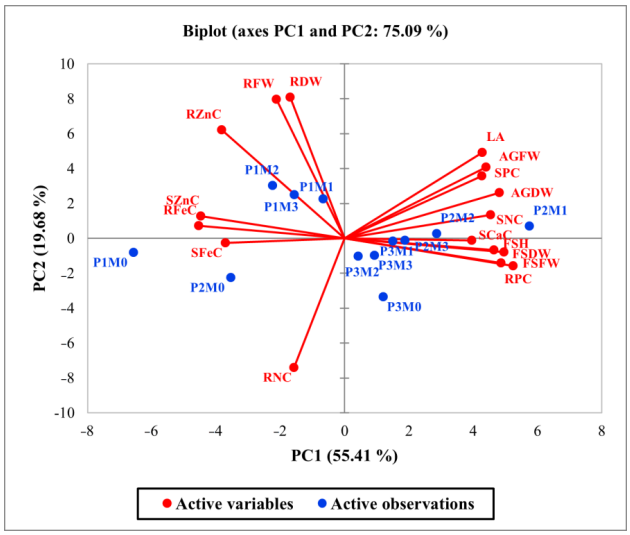

Figure 2. Principal component analysis of the AMF inoculation/no inoculation under different levels of phosphorus. The length of the arrow indicates how each trait is loaded onto the principal component analysis (PCA) axes. FSFW: Flowering stem fresh weight, FSDW: Flowering stem dry weight, FSH: Flowering stem height, LA: Leaf area, AGFW: Above-ground fresh weight, AGDW: Above-ground dry weight, RFW: Root fresh weight, RDW: Root dry weight, SPC: Shoot P concentration, SNC: Shoot N concentration, SCaC: Shoot Ca concentration, SZnC: Shoot Zn concentration, SFeC: Shoot Fe concentration, RPC: Root P concentration, RNC: Root N concentration, RZnC: Root Zn concentration, RFeC: Root Fe concentration.

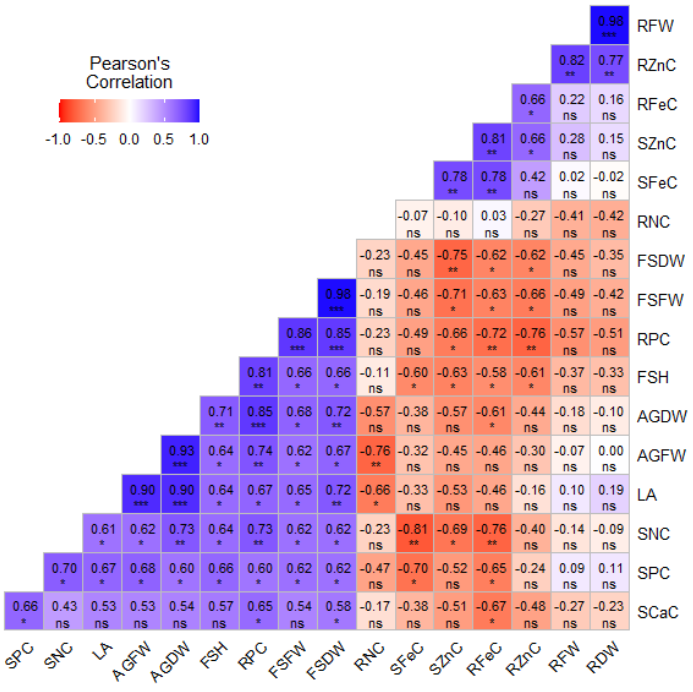

Figure 3. Correlation between ornamental characteristics, vegetative traits, and content of elements in statice plants. FSFW: Flowering stem fresh weight, FSDW: Flowering stem dry weight, FSH: Flowering stem Height, LA: Leaf area, AGFW: Above-ground fresh weight, AGDW: Above-ground dry weight, RFW: Root fresh weight, RDW: Root dry weight, SPC: Shoot P concentration, SNC: Shoot N concentration, SCaC: Shoot Ca concentration, SZnC: Shoot Zn concentration, SFeC: Shoot Fe concentration, RPC: Root P concentration, RNC: Root N concentration, RZnC: Root Zn concentration, RFeC: Root Fe concentration. *, **, ***, and ns denote statistical significance at the 0.05, 0.01, and 0.001 levels, and the absence of significance, respectively.

4. Discussion

4.1. Growth and Ornamental Parameters

Promoted growth and development in mycorrhizal compared to non-mycorrhizal plants has been reported in many plant species [31,32,45–47]. As an energy transporter, P plays a vital role in photosynthesis [48]. Therefore, an increase in P content, resulting from AM inoculation, can increase the photosynthetic rate [49,50]. Furthermore, these fungi can act as a metabolic sink, thereby transferring photosynthetic products to the roots of their host [51–53]. Additionally, they can impact their growth by increasing leaf area through morphologic compatibilities [54]. In this study, the mycorrhizal inoculation increased statice biomass significantly (Table 2), consistent with several other studies that have reported an increase in the shoot and root dry weights of statice [32,46,55]. Feng et al. reported that mycorrhizal maize plants grew better in both low and high soil P [56]. Consequently, they produced higher biomass compared to the control plants. Studies have shown that mycorrhizal fungi can affect the allocation and translocation of substances between roots and shoots. Therefore, the growth rate and weight of the aerial parts increase as a result of higher absorption and translocation of nutrients [57–59]. In addition, this might be due to the increased absorbing surface of the root [46,60].

Increased hormone levels, especially cytokinin, which are observed in mycorrhizal symbiosis, can raise the photosynthetic rate [61,62]. This is caused by affecting stomata, changing the translocation of ions, and regulating chlorophyll levels [52]. However, the similar biomass production seen between mycorrhizal and non-mycorrhizal plants in some treatments of the current study is also in agreement with other reports [63,64].

The enhanced leaf area of mycorrhizal statice plants (Table 2) could be due to promoted growth and development resulting from enhanced P absorption [65], consistent with results of Sohn et al. [66], Prasad et al. [67], and Liang et al. [68]. In addition, Nunes et al. [32] and Adeyemi et al. [69] demonstrated that mycorrhization significantly increased the leaf area of *Anthurium andraeanum* and *Glycine max*.

The increased root length of the mycorrhizal inoculated plants (Table 3), which is also confirmed by other studies [67,70], might have been due to the higher content of elements (Table 4). The increased root length by AM inoculation was attributed to the enhanced element uptake [66]. In the present study, the highest root length was observed in the lowest P concentration (Table 3), which might be due to the increased production of hairy roots and root branches to supply the plant's P requirement [71]. AM fungi can also increase root branches by promoting the production of phytohormones [51,72], thereby increasing total root length.

Mycorrhizal inoculation significantly increased the number of days required for the flowering of statice (Table 3), possibly due to the promotion of vegetative growth. Gaur and Adholeya [73] also observed mycorrhizal *Petunia hybrida* and *Tagetes erecta* plants flowering 6 and 14 days later than their non-mycorrhizal counterpart plants. Delayed flowering might be a positive trait from an economic point of view to manage product supply in demand.

Fresh and dry weights and the number of flowering stems were significantly greater in mycorrhizal plants than in non-mycorrhizal plants (Tables 2 and 3), consistent with the results reported by other authors [47,66]. Their study shows that mycorrhizal inoculation can considerably improve the vegetative and generative growth of ornamental plants.

In our study, the inoculation of plants with *Glomus mosseae* had better results than with the mixed inoculum. Long et al. [74] observed that the mycorrhizal inoculation of *Zinnia elegans* resulted in an increased shoot biomass and number of flowers. They found *Glomus mosseae* to act better than the blended inocula. Gaur and Adholeya [73] found that AMF-inoculated *Callistephus chinensis* plants had higher concentrations of P in their shoots and produced 39% more flowers compared to non-inoculated plants. Aboul-Nasr [75] also found that *Glomus etunicatum* had positive effects on the number of flowers of *Tagetes erecta* and *Zinnia elegans*.

In studies on lavender (*Lavandula angustifolia*), Popescu and Popescu [76] observed that plants inoculated with AMF had considerably more flowers than non-inoculated plants. The increase in flower number was attributed to improved water absorption and the better nutritional status of inoculated plants. Additionally, the increased number of flowering stems of statice plants in our study might have been due to the promoted photosynthetic rate [72,77] and the production of phytohormones [78,79].

The longest-flowering stems were observed in mycorrhizal plants (Table 2), in agreement with other reports [45,46,67,75]. In a study on *Chrysanthemum indicum*, Prasad et al. [67] demonstrated that the highest stem length was recorded in mycorrhizal plants that were grown in moderate soil P concentrations, which also confirms our results (Table 2). Aboul-Nasr [75] observed that mycorrhizal *Tagetes erecta* and *Zinnia elegans* plants had significantly longer stems than non-mycorrhizal plants. They attributed this increased height to a higher photosynthetic rate [80] and enhanced nutrient uptake [46]. Plants that had the highest P and N contents in our study had the highest stem heights. Similarly, Liu et al. [46] found that mycorrhizal inoculated *Glycyrrhiza uralensis* had longer plant heights, which they attributed to enhanced element absorption. Rousseau and Reid [49] also found that mycorrhization enhanced the photosynthetic rate due to increased P concentration. The increased photosynthetic rate can result in promoted growth and higher height. In our study, inoculated statice plants with higher P concentrations also showed higher stem lengths.

4.2. Root Colonization

Reduced root colonization was observed in soils with high P (Figure 1). This phenomenon has also been reported in many studies [31,67,70]. The suppression of hyphal growth and spore production resulting from high P concentrations may be one of the major reasons for the reduced root colonization [67]. Mosse [81] also found that increasing the P concentration beyond a certain level in the soil inhibited colonization and prevented arbuscule formation.

4.3. Root and Shoot Nutrient Contents

Mycorrhizal statice plants had longer roots, which is very important for the better absorption of P [82]. The enhanced absorption of P can be attributed to an increased solubilization of P by the mycorrhizal root secretions, as well as the AMF-mediated expansion of soil zone under exploration by roots [28,83]. Moreover, special hyphal traits enable mycorrhizal roots to absorb more P per unit of area and weight [28,84]. Enhanced phosphorus absorption due to mycorrhizal inoculation is a widely recognized phenomenon [29,34,45,66,67,73,85]. Mycorrhizal associations use P sources more effectively, thereby increasing the efficiency of applied P fertilizers [86,87]. Nevertheless, there was no significant difference between the shoot P concentration of the mycorrhizal and non-mycorrhizal plants growing in P3, which is in agreement with the results of Watts-Williams and Cavagnaro [34]. Overall, the highest P concentration was recorded in the mycorrhizal plants growing in P2, which is consistent with the results of Prasad et al. [67]. High P concentrations can be harmful to mycorrhizal inoculation and may limit P absorption [88]. This could explain why the P concentration of the statice plants inoculated with M1 and grown in P3 was lower than that of those grown in P2. Using 33P, Smith et al. [89] demonstrated that up to 100% of P in *Linum usitatissimum*, *Medicago truncatula*, and *Lycopersicon esculentum* can be supplied through the mycorrhizal path, highlighting the significant role AMF play in the absorption of other nutrients [90].

The results of the study showed that mycorrhizal inoculation increased N, Ca, and Zn, but had no effect on K. These findings are in agreement with those of Hart and Forsythe [64]. Turjaman et al. [45] reported that the inoculation of plants with *Glomus clarum* and *Gigaspora decipiens* increased N by 70–153%. Measuring the direct hyphal absorption and transfer of 15N, Ames et al. [91] found that 25% of total plant N was derived from hyphal 15N. Studies have shown that AMF have a significant role in improving the N nutrition of plants by absorbing and transferring NO_3^- and NH_4^+ as well as amino acids [86,92,93]. Furthermore,

these fungi can indirectly affect the bioavailability of N by increasing P absorption [86]. The greater tendency of these fungi to transfer nutrients to aerial organs may account for the decreased root N in the mycorrhizal plants. AMF can expand soil area under root exploration through their extraradical hyphae [46,84,94]. In addition, the hyphae are so thin that they can penetrate tiny pores. The increased absorption of macro- and micro-elements by AMF has been described by many researchers [29,85,86], resulting in the improved nutrition of colonized plants through nutrient acquisition via the mycorrhizal path and/or indirect effects on root physiology and morphology [90]. Marschner and Dell [95] have suggested that mycorrhizal infection may affect the nutrition of host plants directly by increasing plant growth through nutrient attainment or indirectly by altering transpiration rate. The extraradical network of mycorrhizal hyphae facilitates the nutrient attainment and transfer of many ions, especially P, N, Ca, and Zn, into roots. It has been demonstrated that up to 25% of the Zn and N of a plant can be supplied by AMF extraradical hyphae [95]. The increased absorption of Zn [66,85,90], Ca [66], and N [45,73] has also been reported as a result of mycorrhization. Moreover, mycorrhizal roots absorb nutrients in a unit of area faster than non-mycorrhizal roots [28,84]. Consequently, the roots of a mycorrhizal plant can absorb more water and nutrients [28,31], resulting in an elevated concentration of nutrients in plant tissue. It is well known that mycorrhizal fungi can absorb nutrients (e.g., Zn) and transfer them into the host plant, thus improving the plant nutritional status [31,90]. By using 65Zn, Jansa et al. [85] revealed the transfer of a considerable amount of Zn by AMF.

5. Conclusions

The study suggests that mycorrhizal inoculation, combined with an optimal P concentration, has the potential to enhance the nutrition, growth, and ornamental characteristics of statice. The strongest flowering stems (higher biomass and height) were obtained by inoculation with *Glomus mosseae* and in moderate P concentrations (20 mg kg^{-1} of soil). Moreover, flower number and time to flowering were significantly affected by mycorrhizal inoculation. The highest number of flowering stems and delayed flowering were observed in mycorrhizal plants. This delay in flowering could be advantageous for market management. Therefore, the use of an appropriate inoculum and an optimal P concentration can be beneficial for the production of statice.

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