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BJ: designed the research; BJ, RA: conducted the research and data analyses

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ORIGINAL RESEARCH PAPER

Effect of mycorrhizal inoculation on development and flowering of *Tagetes patula* L. 'Yellow Boy' and *Salvia splendens* Buc'hoz ex Etl. 'Saluti Red'

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Abstract
The effect of mycorrhization on development and flowering of *Tagetes patula* L. 'Yellow Boy' and *Salvia splendens* Buc'hoz ex Etl. 'Saluti Red' was assessed. Control treatments were those in which plants were grown in peat substrate, as opposed to the treatments in which the substrate was supplemented with mycorrhizal fungi. Mycorrhiza-treated plants of *T. patula* 'Yellow Boy' produced more inflorescence buds. In *S. splendens* 'Saluti Red', thanks to symbiosis with fungi, more flowers per inflorescence developed. Mycorrhization had no effect on plant height in *T. patula* 'Yellow Boy' and *S. splendens* 'Saluti Red'. However, after its application plants tillered more intensively forming more primary lateral shoots. Mycorrhiza-treated cultivars of both species had a higher index of greenness.

Keywords
ornamental plants; mycorrhizal fungi; quality; flowering

Introduction

Mycorrhizae, which is a symbiosis of fungi and plants in nature, is a phenomenon that often occurs. Falkowski et al. [1] and Wang and Qiu [2] reported that 80% of plants take on a symbiotic relationship with fungi. However, mycorrhization is impossible in the cultivation of ornamental plants using sterile substrates [3].

Tagete patula and *Salvia splendens* belong to the group of the most valuable species whose cultivars are used in urban flowerbeds. They are produced from seedlings in greenhouses and plastic tunnels and sold in early spring.

The economic aspects of horticultural production force producers of ornamental plants to grow them in small-diameter pots with a limited volume of substrate, which therefore dries rapidly. With traditional watering methods, the substrate is seldom uniformly moistened, which in many cases leads to inferior plant quality. Moreover, for phytosanitary reasons plants are grown in sterile substrates in which plants, when grown in tunnels, use only the nutrients supplied by fertilization. Thus, traditionally fertilized plants are not uniformly provided with essential nutrients, which results in their inferior quality.

The quality of bed plants may be improved at the stage of production thanks to the application of mycorrhizal fungi. Mycorrhization of young plants may also contribute to their improved quality when planted on flowerbeds, as generally the quality of substrates varies and often it does not meet plant requirements.

The aim of this study was to assess development and flowering of *Tagetes patula* 'Yellow Boy' and *Salvia splendens* 'Saluti Red' after mycorrhization during young plant production.

Material and methods

Investigations were conducted in the growing seasons of 2013 and 2014. The effect of mycorrhization on development and flowering of *T. patula* L. 'Yellow Boy' and *S. splendens* Buc'hoz ex Etl. 'Saluti Red' was assessed.

Seeds were sown respectively on March 10, 2013 and March 14, 2014 in boxes filled with peat substrate mixed with sand. Seedlings were outplanted after 2 weeks to multitrays filled with peat substrate enriched with 1 g per L Universol compound fertilizer. After the next 2 weeks, young plants were planted in pots of 9 cm in diameter filled with peat substrate at pH 6.5 with an addition of 3 g per L Ocmocote (3–4M) slow-release fertilizer.

Control treatments were those in which plants were grown in peat substrate, as opposed to the treatments in which the substrate was supplemented with mycorrhizal fungi. Endorize-TA AMF (arbuscular mycorrhizal fungi) inoculum (Biorize Sarl, France), containing a mixture of endomycorrhizal fungi: *Glomus aggregatum*, *Funneliformis mosseae*, *Rhizophagus intraradices*, *R. clarus*, *Claroideoglomus etunicatu*, and *Gigaspora margarita*, was applied to the plants when their bulbs were being planted. The amount applied was 100 propagating units per plant.

The 2×2 experiment consisted of four treatments (Year of Study × Mycorrhization) within cultivars of the examined species. One treatment comprised 15 plants, five plants in three replications.

Plants grown in the greenhouse were regularly watered and fertilized every week with 0.2% solutions of brown Superba (NPK, 14:10:25 + Mg and S, 2.8:6) compound fertilizer.

Measurements were taken when *T. patula* plants developed three heads, while for *S. splendens* it was when flowers in the apical inflorescence were fully bloomed.

In *T. patula*, the following measurements were determined: plant height, number of primary lateral shoots, number of inflorescence buds, and head diameter. In *S. splendens*, the following traits were assessed: plant height and number of primary lateral shoots, number of flowers in the inflorescence, and inflorescence length. Moreover, the index of greenness (SPAD), correlated with chlorophyll content [4], was determined for *Salvia* and *Tagetes* using an N-Tester apparatus.

Root mycorrhizas were stained as described by Phillips and Hayman [5], and root colonization was expressed as the percentage of colonized root lengths versus observed root lengths. Soil hyphal length was determined according to the method described by Bethlenfalvay and Ames [6]. AMF colonization was determined in July 2013 and 2014.

The results were processed statistically applying a two-factor analysis with Statistica ver. 8.0 software. Uniform groups were determined using the Newman–Keuls test.

Results

AMF colonization at the end of experiment, judged by the percentage of root lengths colonized by hyphae, was 34.2% and 33.7% in *Tagetes patula* 'Yellow Boy', 30.4% and 32.2% in *Salvia splendens* 'Saluti Red' for plants cultivated in both years.

The conducted analyses showed no effect of mycorrhization on plant height in *T. patula* and *S. splendens* in either of the 2 years of the study. Control plants had a height of 14.9–15.3 cm in *Tagetes* and 28.9–29.4 cm in *Salvia*. Mycorrhized plants had a height of 14.8–15.6 cm in *Tagetes* and 29.9–31.9 cm in *Salvia*. However, when comparing the number of primary lateral shoots in the cultivars of both species, it was found that it depended significantly only on mycorrhization (Tab. 1 and Tab. 2). The

cultivars of *T. patula* and *S. splendens* grown in symbiosis with fungi formed significantly greater numbers of primary lateral shoots in comparison to the control in both years of the study.

In *Tagetes patula* 'Yellow Boy', a significantly greater number of inflorescence buds was recorded in plants treated with mycorrhizal fungi in both years of the study, while head diameter was comparable in the control (3.4–3.8) and in plants growing in symbiosis with fungi (3.6–3.8) (Tab. 1). Mycorrhiza-treated plants formed 36% and 73.7% more buds and inflorescences in 2013 and 2014, respectively. In *Salvia splendens* 'Saluti Red', no significant effect of mycorrhization was observed on the formation of longer inflorescences (control plants: 14.6–15.2; mycorrhized plants: 14.9–15.9); however, plants growing in symbiosis with fungi developed significantly greater numbers of flowers in the inflorescence, by 27.8% and 27.4% in 2013 and 2014, respectively (Tab. 2).

A comparison of the index of greenness showed a significantly higher value in mycorrhiza-treated *Tagetes patula* 'Yellow Boy' and *Salvia splendens* 'Saluti Red' in both years of the study (Tab. 1, Tab. 2).

Tab. 1 Effect of mycorrhization on development and flowering of *Tagetes patula* 'Yellow Boy'.

Mycorrhization	Year		Mean
	2013	2014	
Number of primary lateral shoots			
-	10.80 [a]	11.20 [a]	11.00 [a]
+	18.70 [b]	20.10 [b]	19.40 [b]
Mean	14.75 [a]	15.65 [a]	
Number of inflorescence buds			
-	14.70 [a]	15.20 [a]	14.95 [a]
+	20.00 [b]	26.40 [c]	23.20 [b]
Mean	17.35 [a]	20.80 [a]	
Leaf greenness index (SPAD)			
-	38.00 [a]	40.50 [a]	39.25 [a]
+	50.80 [b]	48.20 [b]	49.50 [b]
Mean	44.40 [a]	44.35 [a]	

Means followed by the same letter do not differ significantly at α = 0.05.

Tab. 2 Effect of mycorrhization on development and flowering of *Salvia splendens* 'Saluti Red'.

Mycorrhization	Year		Mean
	2013	2014	
Number of primary lateral shoots			
-	5.40 [a]	5.50 [a]	5.45 [a]
+	11.40 [b]	11.40 [b]	11.40 [b]
Mean	8.40 [a]	8.45 [a]	
Number of flowers in inflorescence			
-	32.80 [a]	33.60 [a]	33.20 [a]
+	41.90 [b]	42.80 [b]	42.35 [b]
Mean	37.35 [a]	38.20 [a]	
Leaf greenness index (SPAD)			
-	45.50 [a]	46.50 [a]	46.00 [a]
+	50.40 [b]	52.20 [b]	51.30 [b]
Mean	47.95 [a]	49.35 [a]	

Means followed by the same letter do not differ significantly at α = 0.05.

Discussion

In the study, AMF colonization was 30.4–34.2% in the tested species. Nowak [7] reported that the roots of the inoculated plants of *Salvia splendens* Sello 'Torreador' were colonized by AM fungi at 40%. The addition of Cd and Pb to the growth substrate decreased root colonization.

In the present study, mycorrhization had no effect on plant height in *Tagetes patula* 'Yellow Boy' and *Salvia splendens* 'Saluti Red'. However, after its application plants tillered more intensively forming more primary lateral shoots. Abundant tillering of plants after mycorrhization is probably caused by better assimilation of nutrients, as confirmed by the research of Schmidt and Şumălan [8]. It is worth noting that very good tillering is one of the criteria in the selection of annual species for urban

plantings. The effectiveness of mycorrhization in improving tillering intensity was shown in *Tagetes patula* by Schmidt and Şumălan [8]. In turn, Nowak [9] showed that in *Callistephus chinensis* 'Miledy' mycorrhization, which reduced substrate pH and soluble mineral contents, had an adverse effect on plant quality since the plants had lower fresh weight, produced fewer shoots, and were lower in comparison to the control. In her earlier study, Nowak [7] showed that mycorrhization inhibits growth of *Salvia splendens* Sello 'Torreador' and increases accumulation of cadmium and lead in shoots. This allows us to state that not improving height is a positive feature. Uniformity is a huge advantage in potted plants. However, these are only preliminary studies on mycorrhization of annual plants. In practice, growth retardants are most commonly applied in order to improve their quality. As a result of their application, producers expect plants to be lower, more compact, and well tillered. However, growth inhibition is frequently accompanied also by a reduction of the number of lateral shoots, which is an adverse phenomenon [10]. Studies are also conducted to assess the effect of cytokinin growth regulators to improve the quality of ornamental plants [11,12]. However, in view of the ecological considerations producers should apply natural methods facilitating growth regulation in ornamental plants. According to Lovato et al. [13] and Janowska et al. [14], mycorrhization considerably improves flowering and quality of plants, enhances stress resistance, provides better plant nutrition, and has a profound effect on physiological changes occurring in plants [15–17]. It can be supposed that mycorrhized plants assimilated phosphorous more effectively, which is important for abundant flowering. It is worth pointing out that the content of phosphorous in the composition of brown Superba fertilizer for top-dressing in this research was 2.5 times less than that of potassium.

Our study demonstrated that mycorrhiza-treated plants of *Tagetes patula* 'Yellow Boy' produced more inflorescence buds. In *Salvia splendens* 'Saluti Red', thanks to symbiosis with fungi more flowers developed per inflorescence. It can be said that mycorrhizal fungi form a bridge between plants and soil. They provide better assimilation of nutrients. Plants give them carbohydrates which they are not able to synthesize. An advantageous effect of mycorrhization on flowering in *Tagetes patula* was reported by Schmidt and Şumălan [8]. The authors showed that by growing this species in symbiosis with mycorrhizal fungi, the number of inflorescence buds increased significantly. In turn, Janowska et al. [14] reported an advantageous effect of mycorrhization on flowering of *Zantedeschia albomaculata*. Those authors found that flowering intensity in *Zantedeschia* with colored inflorescence spathes is dependent on the cultivar and size of rhizomes. However, even planting very large rhizomes does not guarantee abundant yielding, which was confirmed by a study conducted on the cultivar 'Albomaculata', in which the yield of flowers increased only after mycorrhization. An additional advantage resulting from symbiosis with fungi was connected with better quality of flowers manifested in peduncle length. Moreover, their investigations indicated that mycorrhization of *Zantedeschia* may replace the commonly applied gibberellic acid since endomycorrhizal fungi produce growth regulators, including also gibberellins [18].

Mycorrhiza-treated plants in the examined cultivars of both species had a higher index of greenness. Studies conducted worldwide show that mycorrhization in numerous plant species has an advantageous effect on chlorophyll content [19–21]. An elevated chlorophyll content suggests a more intensive course of photosynthesis, which was confirmed by Gernns et al. [22]. In turn, as it was reported by Dixon et al. [23], different fungal species have a varied effect on the intensity of this process.

Conclusions

- Mycorrhiza-treated plants of *Tagete patula* 'Yellow Boy' produced more inflorescence buds. In *Salvia splendens* 'Saluti Red', thanks to symbiosis with fungi more flowers per inflorescence developed.
- Mycorrhization had no effect on plant height in *Tagetes patula* 'Yellow Boy' and *Salvia splendens* 'Saluti Red'. However, after its application plants tillered more intensively, forming more primary lateral shoots.

- Mycorrhiza-treated plants in the examined cultivars of both species had a higher index of greenness.

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Wpływ mikoryzacji na rozwój i kwitnienie aksamitki rozpierzchłej (*Tagetes patula* L.) 'Yellow Boy' i szałwii błyszczącej (*Salvia splendens* Buc'hoz ex Etl.) 'Saluti Red'

Streszczenie

Badania przeprowadzono w sezonie wegetacyjnym 2013 i 2014. Oceniano wpływ mikoryzacji na wzrost i kwitnienie *Tagetes patula* L. 'Yellow Boy' i *Salvia splendens* Buc'hoz ex Etl. 'Saluti Red'. Wyodrębniono kombinacje kontrolne, w których rośliny uprawiano w substracie torfowym oraz kombinacje, w których do podłoża dodano grzyby mikoryzowe. Mikoryzowane rośliny *Tagete patula* 'Yellow Boy' tworzyły więcej pąków kwiatostanowych. U *Salvia splendens* 'Saluti Red' dzięki symbiozie z grzybami w kwiatostanach rozwijało się więcej kwiatów. Mikoryzacja nie miała wpływu na wysokość roślin *Tagetes patula* 'Yellow Boy' i *Salvia splendens* 'Saluti Red'. Jednak po jej zastosowaniu rośliny krzewiły się intensywniej, tworząc więcej pędów bocznych I rzędu. Mikoryzowane rośliny odmian obu gatunków miały wyższy indeks zazielenienia liści.