

Article

Role of Combined Inoculation with Arbuscular Mycorrhizal Fungi, as a Sustainable Tool, for Stimulating the Growth, Physiological Processes, and Flowering Performance of Lavender

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Abstract: Arbuscular mycorrhizal fungi (AMF) are essential soil microorganisms for terrestrial ecosystems and form beneficial symbioses with the root systems of most agricultural plants. The purpose of this paper was to examine the effect of the community of six AMF on the growth, physiological response, and flowering performance in organic potted lavender culture. The mixture of AMF containing *Rhizophagus irregularis*, *Claroideoglomus claroideum*, *Funneliformis mosseae*, *Funneliformis geosporum*, *Claroideoglomus etunicatum*, and *Glomus microaggregatum* was added in a pot with peat, volcanic rock, and coconut bark. We analyzed the fresh shoot biomass, root biomass, total plant biomass, leaf area, flowering performance, photosynthesis rate, and photosynthetic pigment content. Pearson's correlation coefficient was performed to get a better understanding of the relationships between the studied variables. The total plant biomass was more pronounced in plants with AMF-S_{20g} (212.01 g plant⁻¹) and AMF-S_{30g} (220.25 g plant⁻¹) than with AMF-S_{10g} (201.96 g plant⁻¹) or in untreated plants (180.87 g plant⁻¹). A statistically significant increase for Chl a, Chl b, and Car was found for AMF-S_{20g} and AMF-S_{30g}. Our findings suggest that the AMF mixture application in a growing substrate with peat, coconut bark, and volcanic rock improved plant growth, physiological processes, and ornamental value in mycorrhizal lavender plants. This environmentally friendly agricultural practice could be used for the sustainable production of lavender.

Keywords: arbuscular mycorrhizal fungi; environmentally friendly practices; organic growing substrate; lavender; plant physiology; sustainability of agricultural systems; symbiosis



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1. Introduction

Lavandula angustifolia (syn. *L. vera* or *L. officinalis*) is a member of the Lamiaceae family. This plant is an evergreen and perennial flowering shrub. Presently, lavender is cultivated worldwide in many countries [1]. This aromatic and medicinal plant, the “purple gold” of agricultural crops, lavender, is increasingly gaining importance among farmers and processors, especially from the cosmetics industry and consumers [2,3]. *Lavandula angustifolia* (Mill.), one of the best-known essential oil crops in the world [4], has great importance in the fragrance and pharmaceutical industries and landscaping [5].

The main species of lavender are *Lavandula angustifolia*, *Lavandula abrotanoides*, *Lavandula canariensis*, *Lavandula dentata*, *Lavandula lanata*, *Lavandula latifolia*, *Lavandula multifida*, *Lavandula pinnata*, *Lavandula stoechas*, *Lavandula viridis*, and *Lavandula x intermedia* [6].

The growing interest of producers for lavender cultivation is mainly the profitability of lavender plantations per unit area. Considering the multiple therapeutic, food, and cosmetic uses, this small fragrant shrub has become very popular among Romanian farmers.

Environmentally friendly practices are part of modern and sustainable agriculture. Nowadays, new strategies, practices, and technologies have been studied in order to improve the sustainability of agricultural systems. Agricultural practices that use chemical fertilizers and pesticides have a significant impact on the environment. The reduction of chemical inputs is an important objective in the application of agricultural technologies. The soil is a complex system that contains a multitude of beneficial organisms for plant production, and among these are arbuscular mycorrhizal fungi (AMF) [7]. Therefore, a potential tool to reduce the use of chemical inputs is to include arbuscular mycorrhizal fungi (AMF) products in agricultural practices. Golubkina et al. [8] affirmed that arbuscular mycorrhizal fungi (AMF) can be considered as an essential agricultural key to making agriculture more environmentally friendly. Wang and Qiu [9] noted that arbuscular mycorrhizas are a group of soil biota with six distinct morphological categories: arbuscular, arbutoid, ecto-, ericoid, monoptropoid, and orchid. From these mycorrhizas categories, arbuscular mycorrhizal fungi are the most widely spread plant symbionts [10]. Agricultural applications of arbuscular mycorrhizal fungi are sustainable alternatives for the replacement of chemical fertilizers. These natural compounds are considered biofertilizers, since they provide the plant host with water and nutrients, especially increasing phosphorus supply [11,12].

Mycorrhizal plants can enhance water use efficiency [12], plant growth, and biomass [13]. Birhane et al. [14] noted that AMF inoculation increased photosynthetic rate, while Sharif and Claassen [15] found a beneficial role for the accumulation of more dry matter in plants. Arbuscular mycorrhizal symbiosis promotes nutrient mobility in the soil [16].

There are several reports on the association of AMF with different plants, including lemon balm (*Melissa officinalis* L.), sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* Mill.) [17], *Albizia saman* [18], *Mentha arvensis* [19], *Salvia officinalis* [20], *Lavandula officinalis*, *Rosmarinus officinalis* [1], *Begonia semperflorens* [21], *Citrus tangerine* [22], and *Olea europea* L. [23].

The composition of the growth substrate has a significant influence on the agricultural performances of the potted ornamental plants [24]. Kotsiris et al. [25] reported that *Lavandula angustifolia*'s growth and physiology are affected by substrate type and depth, substrate type being less influential and having a differentiated response. Most experiments on the association of arbuscular mycorrhizal fungi with growth substrates and plants use commercial inoculum products [7,17,26,27]. Research studies on the application of AMF have taken into account different culture substrates. Additionally, many studies have focused on the effects of a single arbuscular mycorrhizal fungus applied in pot-grown plants with a peat-based substrate [28,29]. To the best of our knowledge, no studies on the effect of mixed AMF on a culture substrate composed of peat, volcanic rock, and coconut bark have been reported yet.

The purpose of this paper was to evaluate the effect of the community of six arbuscular mycorrhizal fungi using a commercial mycorrhizal inoculant on the growth, physiological response, and flowering performance of *Lavandula angustifolia* Mill. In the present paper, we investigated the response of lavender to inoculation with a mixture of arbuscular mycorrhizal fungi on a potted culture containing organic products: peat, volcanic rock, and coconut bark. In this experiment, we optimized the technology related to biofertilization and growing media formula for potted lavender production in order to provide environmentally friendly agricultural practices to aromatic and medicinal plant producers.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The experiment was established in an open field situated in the agricultural company LavandArt from Albertirsa, Hungary. The climate of this area is temperate continental characterized by a mean annual precipitation of about 500 mm or less and annual mean temperature is around 11 °C. This research was conducted during April–July 2018 as a pot experiment (one plant per pot). The plants were transported in the laboratories of the

University of Pitesti (Romania) in order to determine the effects of arbuscular mycorrhizal fungi on lavender potted culture and to provide environmentally friendly agricultural practices to plant producers. The plant material tested and evaluated in this study was represented by two cultivars of lavender, two of the most popular and important aromatic and medicinal plants *Lavandula angustifolia* and *Lavandula hidcote*. The culture substrate used for the cultivation of lavender in pots had the following composition: peat (70%), coconut bark (20%), and volcanic rock (10%). The volume of the pot was 3 L.

All components of growing media are organic, environmentally friendly. In combination with different organic or inorganic components of growing media, volcanic rock plays an essential role in improving the stability and permeability of the culture substrate. The volcanic rock is a very light and porous component, has a stable structure, and can provide excellent drainage. The coconut bark, known as coir, is a natural, biodegradable, and environmentally friendly source, with fibrous texture, which can enhance the aeration and water retention properties, increase plant water availability, and reduce the risk of hydrophobicity for faster growth of roots and the plant in general.

We used a commercial inoculum represented by Symbivit product (Symbiom Company, Lanškroun, Czech Republic) which contains a mixture of six arbuscular mycorrhizal fungi: *Rhizophagus irregularis* (BEG140), *Claroideoglomus claroideum* (BEG96), *Funneliformis mosseae* (BEG95), *Funneliformis geosporum* (BEG199), *Claroideoglomus etunicatum* (BEG92), and *Glomus microaggregatum* (BEG56). This commercial product of AMF inoculum contains propagules (spores, mycelium, and colonized root fragments) of a mixture of six arbuscular mycorrhizal fungi species on an inert carrier (mixture of zeolite and expanded clay). This mixture of AMF was used to inoculate the plants. The effects of fungal mycorrhiza inoculum applications were analyzed using four treatments designed as follows: (1) control (non-inoculated plants); (2) AMF-S_{10g} = arbuscular mycorrhizal fungi—Symbivit inoculum 10 g; (3) AMF-S_{20g} = arbuscular mycorrhizal fungi—Symbivit inoculum 20 g; (4) AMF-S_{30g} = arbuscular mycorrhizal fungi—Symbivit inoculum 30 g. One gram of inoculum contains 20 AMF propagules. For non-inoculated plants, seedlings were transplanted with no inoculation. Lavender young plants at the stage of 3–4 leaves and 4–5 cm height were transplanted into experimental pots in a formula of one plant per pot. Commercial mycorrhizal inoculant of Symbivit was placed through the lavender seedling roots at different points of each pot. The plants, the components of the growing media, and the Symbivit commercial inoculum product were provided by the company LavandArt (Hungary). During the experiment, there were no other fertilizer or pesticide applications. Pots were watered weekly.

2.2. Data and Measurements

The effects of the commercial inoculum containing a mixture of six arbuscular mycorrhizal fungi on growth were evaluated using the following parameters: fresh shoot biomass (g plant^{−1}); root biomass (g plant^{−1}), shoot/root biomass ratio, total plant biomass (g plant^{−1}), and leaf area (cm²). The flowering performance of lavender under the AMF inoculum application was analyzed by determining floral shoot length (cm) and the number of floral shoots for each treatment.

In order to evaluate the physiological response of the community of six arbuscular mycorrhizal fungi, several biochemical and physiological processes like photosynthesis and chlorophyll content were determined in leaves: photosynthesis rate (μmol CO₂ m^{−2} s^{−1}), chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid (Car) contents, and total photosynthetic pigments content (PPC).

Photosynthesis rate was measured in leaves and expressed in μmol CO₂ m^{−2} s^{−1}. The equipment used for photosynthesis was represented by S151 Infrared CO₂ portable plant analyzer (IRGA from Qubit Biology Inc., Ontario, Canada). During testing, photosynthetic photon flux density was 1300–1500 μmol m^{−2} s^{−1}, the air temperature was 25 ± 2 °C, and ambient relative humidity was between 55% and 60%.

Photosynthetic pigments were measured in leaves and expressed in mg g^{-1} fresh weight. The level of photosynthetic pigments was determined using a spectrophotometer (BOECO S-20VIS from Boeckel & Co., Hamburg, Germany) at absorbance wavelengths of 662 and 644 nm, while the concentration of carotenoids was similarly measured but at 470 nm. A solution of 95.5% (*v/v*) acetone was used as a blank. The amounts of chlorophyll a, chlorophyll b, and carotenoid pigments in the leaf tissue were calculated according to the Holm's equations (1954): $\text{Chl a} = 9.78 A_{662} - 0.99 A_{644}$; $\text{Chl b} = 21.4 A_{644} - 4.65 A_{662}$; $\text{Car} = 4.69 A_{440.5} - 0.267 (\text{Chl a} + \text{Chl b})$.

The measurements were made in the flowering stage and were recorded when plants gained proper ornamental value. For each experimental pot plant, the root and shoot were separated, and the fresh weight was determined for each plant. Then the shoot/root biomass ratio was calculated.

2.3. Data Analysis

The experiment was carried out in a randomized complete block design (RCBD) including 4 different treatments with 5 replications per treatment for a total of 20 pots (1 plant per pot). The statistical analysis was computed using the analysis of variance in the IBM SPSS Statistics 26.0 software (SPSS Inc., Chicago, IL, USA) and means were compared using Duncan's multiple range tests at 95% confidence level ($p < 0.05$). The results of the current study are reported as mean $\pm$ standard deviation (SD). Additionally, the IBM SPSS Statistics 26.0 software was used to calculate Pearson's correlation.

3. Results and Discussion

3.1. Effects of the Community of Six Arbuscular Mycorrhizal Fungi on Plant Growth and Flowering Potential in Lavender

Enhancement of plant growth and flowering potential are very important biological processes for the ornamental and medicinal properties of plants, as well as for the improvement of commercial value. To study the effect of the combined inoculation with a mixture of AMF (*Rhizophagus irregularis* (BEG140), *Claroideoglomus claroideum* (BEG96), *Funneliformis mosseae* (BEG95), *Funneliformis geosporum* (BEG199), *Claroideoglomus etunicatum* (BEG92), and *Glomus microaggregatum* (BEG56)) on flowering potential in lavender, a series of analyzes and measurements were performed: floral shoot length and the number of floral shoots (Table 1).

Table 1. The effects of the community of six arbuscular mycorrhizal fungi on flowering potential and floral shoot length in lavender.

Treatments ¹	<i>Lavandula angustifolia</i>		<i>Lavandula hidcote</i>	
	Floral Shoot Length (cm)	Number of Floral Shoots	Floral Shoot Length (cm)	Number of Floral Shoots
AMF-S _{10g}	31.91 $\pm$ 0.19 c	64 $\pm$ 3.80 a	22.97 $\pm$ 0.34 c	70.2 $\pm$ 2.86 ab
AMF-S _{20g}	33.27 $\pm$ 0.16 b	65 $\pm$ 2.64 a	23.91 $\pm$ 0.43 b	72.8 $\pm$ 2.86 ab
AMF-S _{30g}	33.86 $\pm$ 0.48 a	66 $\pm$ 3.16 a	24.46 $\pm$ 0.13 a	73.6 $\pm$ 2.07 a
Control (non-inoculated)	29.46 $\pm$ 0.16 d	61 $\pm$ 4.12 a	21.42 $\pm$ 0.30 d	69.0 $\pm$ 3.53 b

¹ Data presented as mean $\pm$ standard deviation. Mean values followed by the same letter within columns are not significantly different according to Duncan's multiple range test ($p < 0.05$). Control: untreated lavender—non-inoculated plants; AMF-S_{10g} = arbuscular mycorrhizal fungi—Symbivit inoculum 10 g; AMF-S_{20g} = arbuscular mycorrhizal fungi—Symbivit inoculum 20 g; AMF-S_{30g} = arbuscular mycorrhizal fungi—Symbivit inoculum 30 g.

The application of the commercial inoculum (AMF-S_{30g}) with a mixture of AMF significantly enhanced floral shoot length in *Lavandula angustifolia* and *Lavandula hidcote* as compared to other treatments ($p < 0.05$). There were no statistical differences in the number of floral shoots between the plants treated with AMF mixture in different amounts of

inoculum and control plants (non-inoculated plants) for *Lavandula angustifolia*. Additionally, there was no significant interaction between AMF application with 10 g or 20 g of inoculum and non-AMF plants for *Lavandula hidcote*. However, in our study, we found significant differences between AMF-S_{30g} plants and non-AMF plants (Table 1). The ornamental value of plants can be improved using different combinations of AMF in potted plants. For instance, plant growth, number, and size of flowers, leaves, and flower color in potted carnation is increased by combining the mycorrhizal inoculation [29]. Asrar et al. [30] noted that AMF-inoculated plants of Kalanchoe (*Kalanchoe blossfeldiana* Poelln.) had a higher number of flowers and shoot dry matter compared to uninoculated plants.

Arbuscular mycorrhizal fungi, one of the prevalent soil microbes, can colonize most terrestrial plant species' roots. These symbiotic fungi have been reported to considerably offer various benefits to their host plants [31]. Studies related to the arbuscular mycorrhizal fungi application in order to improve the performance of agricultural technologies have been performed on different important ornamental and medicinal plants such as *Melissa officinalis* L., *Salvia officinalis* L., *Albizia*, *Mentha arvensis*, *Begonia semperflorens*, and *Pelargonium spp.* However, there are few studies regarding the effects of a mixture of six arbuscular mycorrhizal fungi in a pot with the following composition: peat (70%), coconut bark (20%), volcanic rock (10%).

Commercial inoculum of arbuscular mycorrhizal fungi applied in pots with peat-based substrate significantly increased shoot biomass and other biometric parameters for three ornamental plants (*Gazania rigens*, *Pelargonium peltatum*, and *Pelargonium zonale*). Additionally, the mycorrhizal colonization of plant roots intensified the flowering potential of *Gazania rigens* and *Pelargonium peltatum* [28]. Arbuscular mycorrhizal fungi colonization mostly improved the *Leymus chinensis* seedling dry and fresh weight under stress conditions [32].

The results for total plant biomass (g plant⁻¹), fresh shoot biomass (g plant⁻¹), root biomass (g plant⁻¹), and shoot/root biomass ratio in lavender non-inoculated or inoculated with a mixture of arbuscular mycorrhizal fungi are presented in Table 2.

Table 2. Total plant biomass (g plant⁻¹), fresh shoot biomass (g plant⁻¹), root biomass (g plant⁻¹), and shoot/root biomass ratio in lavender non-inoculated or inoculated with a mixture of six arbuscular mycorrhizal fungi: *Rhizophagus irregularis* (BEG140), *Claroideoglomus claroideum* (BEG96), *Funneliformis mosseae* (BEG95), *Funneliformis geosporum* (BEG199), *Claroideoglomus etunicatum* (BEG92), and *Glomus microaggregatum* (BEG56).

Cultivars	Treatments ¹	Fresh Shoot Biomass (g plant ⁻¹)	Root Biomass (g plant ⁻¹)	Total Plant Biomass (g plant ⁻¹)	Shoot/Root Biomass Ratio
<i>Lavandula angustifolia</i>	AMF-S _{10g}	140.78 ± 1.43 c	61.18 ± 1.97 c	201.96 ± 2.46 c	2.30 ± 0.03 c
	AMF-S _{20g}	146.06 ± 1.46 b	65.95 ± 1.52 b	212.01 ± 1.33 b	2.21 ± 0.06 b
	AMF-S _{30g}	149.61 ± 0.45 a	70.64 ± 1.40 a	220.25 ± 1.67 a	2.12 ± 0.04 a
	Control	128.62 ± 1.45 d	52.25 ± 0.90 d	180.87 ± 2.19 d	2.46 ± 0.07 d
	(non-inoculated)				
<i>Lavandula hidcote</i>	AMF-S _{10g}	64.10 ± 0.91 c	32.31 ± 0.66 c	96.41 ± 1.34 c	1.98 ± 0.03 b
	AMF-S _{20g}	74.35 ± 1.01 b	35.02 ± 1.01 b	109.37 ± 1.82 b	2.12 ± 0.04 a
	AMF-S _{30g}	81.07 ± 2.19 a	37.56 ± 1.06 a	118.63 ± 2.37 a	2.16 ± 0.08 a
	Control	60.44 ± 1.50 d	29.86 ± 1.41 d	90.30 ± 2.53 d	2.02 ± 0.08 b
	(non-inoculated)				

¹ Data presented as mean ± standard deviation. Mean values followed by the same letter within columns are not significantly different according to Duncan's multiple range test ($p < 0.05$). Control: untreated lavender—non-inoculated plants; AMF-S_{10g} = arbuscular mycorrhizal fungi—Symbivit inoculum 10 g; AMF-S_{20g} = arbuscular mycorrhizal fungi—Symbivit inoculum 20 g; AMF-S_{30g} = arbuscular mycorrhizal fungi—Symbivit inoculum 30 g.

Lavandula angustifolia and *Lavandula hidcote* plants treated with a mixture of six arbuscular mycorrhizal fungi at different concentrations (10 g, 20 g, or 30 g of inoculum) and non-inoculated plants were statistically different in fresh shoot biomass (g plant⁻¹).

The highest values for the fresh shoot biomass were recorded in inoculated plants with AMF-S_{30g} = arbuscular mycorrhizal fungi, while the lowest were observed in non-AMF plants. The influence of AMF on the root biomass (g plant^{−1}) of plants was significant in lavender treated with AMF-S_{10g}, AMF-S_{20g}, AMF-S_{30g} compared to non-treated plants.

The root biomass (g plant^{−1}) and total plant biomass (g plant^{−1}) were more pronounced in plants with AMF-S_{20g} and AMF-S_{30g} than with AMF-S_{10g} or in untreated plants. The results for total plant biomass (g plant^{−1}) ranged between 180.87 g plant^{−1} in non-inoculated plants and 220.25 g plant^{−1} in plants inoculated with AMF-S_{30g} for *Lavandula angustifolia* and from 90.30 plant^{−1} (control experimental variant, non-inoculated plants) to 118.63 plant^{−1} (AMF-S_{30g}). In general, results have suggested that AMF-S_{20g} and AMF-S_{30g} application has a more pronounced effect on growth processes. These findings are in line with the study of ALHadidi et al. [33] who concluded that inoculation of sweet potato seedlings with AMF is a functional association and improved length of roots and shoots, as well as the fresh weight of shoots and roots.

Additionally, shoot/root biomass ratio in lavender non-inoculated or inoculated with a mixture of arbuscular mycorrhizal fungi was calculated in order to appreciate the effect that AMF can have on plant growth processes. The greatest shoot to root ratio in *Lavandula angustifolia* was observed in the treatment without AMF, while the minimum values of this indicator were seen in the AMF-S_{30g} treatment. For *Lavandula hidcote* plants, the lowest shoot/root biomass ratio was found in the controls. There were no statistical differences in shoot/root biomass ratio between the plants treated with AMF-S_{20g} and AMF-S_{30g}. Additionally, there were no significant differences for this indicator between AMF-S_{10g} and non-inoculated plants for *Lavandula hidcote*.

AMF is considered one of the most important biological tools for enhancing plant growth and shoot biomass and maintaining a sustainable environment in agricultural production [34]. Additionally, Mitra et al. [35] noted that utilization of AMF is an eco-friendly approach and a useful component to achieve sustainable production in agriculture. According to Duc et al. [36], AMF mixture application improved plant height, fresh root, and shoot weight, leaf number, and leaf area of the medicinal plant, *Eclipta prostrata* (L.), compared with single AMF species application. Treatment with the mixture of six AMF significantly increased fresh shoot weight by 76% in relation to non-AM plants after 8 weeks of growth [36]. *Ajania* plants were transplanted into pots with and without mycorrhizal inoculum in order to evaluate the growth and flowering parameters as shoot length, number of shoots, number of inflorescences, or number of flowers. Vosnjak et al. [37] reported that *Ajania pacifica* inoculated plants developed more flowers inflorescences and shoots compared to uninoculated plants. The mycorrhizal (*Antirrhinum majus* L.) snapdragon plants had significantly higher shoots, root biomass, and flowering potential than those non-mycorrhizal plants [38].

AMF colonization greatly improved the leaf area of lavender especially when a mixture of arbuscular mycorrhizal fungi with 30 g of inoculum treatment has been applied (Table 3). Only very small differences were obtained for the AMF-inoculated plants under the 20 g and 30 g inoculum. However, there were significant differences between the leaf area of AMF lavender plants and non-inoculated plants ($p < 0.05$). The lowest values of the leaf area were observed in uninoculated plants. The above-described results are in agreement with published studies on *Eclipta prostrata* [36]. Additionally, seedlings inoculated of Black locust (*Robinia pseudoacacia* L.) with AMF had the largest leaf area and highest biomass, compared with non-mycorrhizal plants [39].

Table 3. Leaf area (cm²) in mycorrhizal and non-mycorrhizal lavender with a mixture of six arbuscular mycorrhizal fungi: *Rhizophagus irregularis* (BEG140), *Claroideoglomus claroideum* (BEG96), *Funneliformis mosseae* (BEG95), *Funneliformis geosporum* (BEG199), *Claroideoglomus etunicatum* (BEG92), and *Glomus microaggregatum* (BEG56).

Treatments ¹	<i>Lavandula angustifolia</i>	<i>Lavandula Hidcote</i>
	Leaf Area (cm ²)	Leaf Area (cm ²)
AMF-S _{10g}	3.92 ± 0.19 c	3.06 ± 0.11 c
AMF-S _{20g}	4.61 ± 0.17 b	3.36 ± 0.11 b
AMF-S _{30g}	4.95 ± 0.23 a	3.58 ± 0.10 a
Control (non-inoculated)	3.76 ± 0.19 c	2.89 ± 0.11 d

¹ Data presented as mean ± standard deviation. Mean values followed by the same letter within columns are not significantly different according to Duncan's multiple range test ($p < 0.05$). Control: untreated lavender—non-inoculated plants; AMF-S_{10g} = arbuscular mycorrhizal fungi—Symbivit inoculum 10 g; AMF-S_{20g} = arbuscular mycorrhizal fungi—Symbivit inoculum 20 g; AMF-S_{30g} = arbuscular mycorrhizal fungi—Symbivit inoculum 30 g.

Seedling development is one of the most important and sensitive stages during plant growth and development. In this study, lavender seedlings inoculated with a mixture of six arbuscular mycorrhizal fungi had higher fresh shoot biomass (g plant⁻¹) and root biomass (g plant⁻¹). A very important aspect in the production of ornamental, aromatic, and medicinal plants is controlled and induced vegetative and floral growth by horticultural practices. Results indicated that AMF mixture application in a growing media substrate with peat, coconut bark, and volcanic rock, improved growth plant and flowering performance in inoculated plants compared with non-inoculated plants.

Table 4 presents the Pearson correlation coefficient which expresses the relationship between the amount of inoculum from the community of six arbuscular mycorrhizal fungi and plant growth and flowering parameters in mycorrhizal lavender plants. The Pearson correlation coefficient was considered significant at the 0.05 and 0.01 levels (from −1 to 1). Pearson's correlation was used to better understand the relationships between the studied variables. The mixture of six arbuscular mycorrhizal fungi showed significant positive correlations with all plant growth and flowering parameters. However, the amount of AMF inoculum is very well correlated with root biomass, total plant biomass, and floral shoot length in mycorrhizal *Lavandula angustifolia* plants (Pearson correlation coefficients = 0.967 **, 0.967 **, and 0.951 **, respectively). The relationship between AMF associations and the number of floral shoots indicates that the correlation is significant at the 0.05 level (Pearson correlation coefficient = 0.492 *). The mixture of six arbuscular mycorrhizal fungi associations was less correlated with the number of floral shoots.

Table 4. Pearson correlation coefficient between the amount of inoculum from the community of six arbuscular mycorrhizal fungi and plant growth and flowering parameters in mycorrhizal lavender plants.

Pearson Correlation	Fresh Shoot Biomass (g plant ⁻¹)	Root Biomass (g plant ⁻¹)	Total Plant Biomass (g plant ⁻¹)	Shoot/Root Biomass Ratio	Floral Shoot Length (cm)	Number of Floral Shoots	Leaf Area (cm ²)
<i>Lavandula angustifolia</i>	0.950 **	0.967 **	0.967 **	0.915 **	0.951 **	0.492 *	0.916 **
<i>Lavandula hidcote</i>	0.973 **	0.949 **	0.978 **	0.647 **	0.947 **	0.576 **	0.927 **

* indicates that correlation is significant at the 0.05 level. ** indicates that correlation is significant at the 0.01 level.

3.2. Physiological Response as the Effect of the Community of Six Arbuscular Mycorrhizal Fungi Application

Physiological response as an effect of the community of six arbuscular mycorrhizal fungi application was investigated in terms of photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in leaves, chlorophyll a (mg g^{-1}), chlorophyll b (mg g^{-1}), carotenoids (mg g^{-1}), and total photosynthetic pigment content (mg g^{-1}) in leaves of mycorrhizal and non-mycorrhizal lavender plants. The photosynthesis process is essential for the development of plants and can directly influence productivity and biomass.

The results for photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in lavender non-inoculated or inoculated with a mixture of AMF are shown in Figure 1. In our study, we found a beneficial effect of arbuscular mycorrhizal fungi application on the photosynthesis rate of lavender. The effect of the community of six arbuscular mycorrhizal fungi on photosynthesis was more pronounced in inoculated *Lavandula angustifolia* plants treated with 20 g plant^{-1} and 30 g plant^{-1} than in the untreated plants or the plants treated with 30 g plant^{-1} inoculum. However, the statistical difference of photosynthesis rate in *Lavandula angustifolia* plants was not significant between each of the samples treated with 20 g plant^{-1} and 30 g plant^{-1} inoculum, while we found significant differences between AMF plants and non-AMF plants. Regarding *Lavandula hidcote*, the community of six arbuscular mycorrhizal fungi treatments induced a statistically significant increase in the photosynthesis rate compared to the controls. At the same time, no significant differences were observed between the mycorrhizal plant variants for *Lavandula hidcote* ($p < 0.05$). Arbuscular mycorrhizal fungi protect host plants from environmental stress by numerous physiological mechanisms such as better photosynthesis rate or total photosynthetic pigment content [40].

According to Lin et al. [32], the photosynthesis process of *Leymus chinensis* seedlings could be improved by AFM application under salt-alkali stress and nitrogen deposition.

The findings for chlorophyll a (mg g^{-1}), chlorophyll b (mg g^{-1}), carotenoids (mg g^{-1}), and total photosynthetic pigment content (mg g^{-1}) in lavender non-inoculated or inoculated with a mixture of AMF are presented in Figure 2.

The photosynthetic pigments of the plants, chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) contents, in each AMF treatment were determined with a spectrophotometer and it was performed to understand the photosynthetic capacity of inoculated plants. It is very well known that chlorophyll content is essential for plant photosynthesis [41].

The community of six arbuscular mycorrhizal fungi increased the content of Chl a, Chl b, and Car in the leaves for both cultivars. A statistically significant increase for Chl a, Chl b, and Car was found for AMF-S_{20g} and AMF-S_{30g} compared to AMF-S_{10g} or non-mycorrhizal lavender plants. However, no significant differences were observed in carotenoid pigments between the AMF-S_{10g} mycorrhizal and non-mycorrhizal *Lavandula angustifolia* plants ($p < 0.05$). The potential of total photosynthetic pigment content (mg g^{-1}) for AMF associations was highest when plants were treated with AMF-S_{20g} and AMF-S_{30g}. In the case of photosynthetic pigments, we did not find any statistically significant difference between the plants treated with $20 \text{ g AMF inoculum}$ and plants treated with $30 \text{ g AMF inoculum}$. The same trend was also observed when the total photosynthetic pigment content was statistically analyzed ($p < 0.05$). Our findings showed that AMF application had a beneficial effect on chlorophyll content. The results of the study also revealed that AMF symbiosis stimulated the content of carotenoid pigments. Similar results were found in *Antirrhinum majus*, *Leymus chinensis*, and *Robinia pseudoacacia* L. plants. Seedlings inoculated of Black locust (*Robinia pseudoacacia* L.) with AMF had significantly greater, chlorophyll content and photosynthetic rate compared with non-mycorrhizal seedlings [39]. Asrar et al. [30] found that the content of photosynthetic pigments in leaves (chlorophyll a, chlorophyll b, and carotenoid pigments) of mycorrhizal Kalanchoe plants significantly were higher than those in uninoculated plants. The snapdragon (*Antirrhinum majus* L.) AMF-inoculated plants had a significantly higher content of photosynthetic pigments in leaves compared with non-mycorrhizal snapdragon plants [38]. Arbuscular mycorrhizal fungi

colonization increased the *Leymus chinensis* chlorophyll content [32]. Hashem et al. [42], in a pot experiment with *Cucumis stivus* L. found that AMF colonization supported and increased the pigment synthesis of chlorophyll and carotenoids.

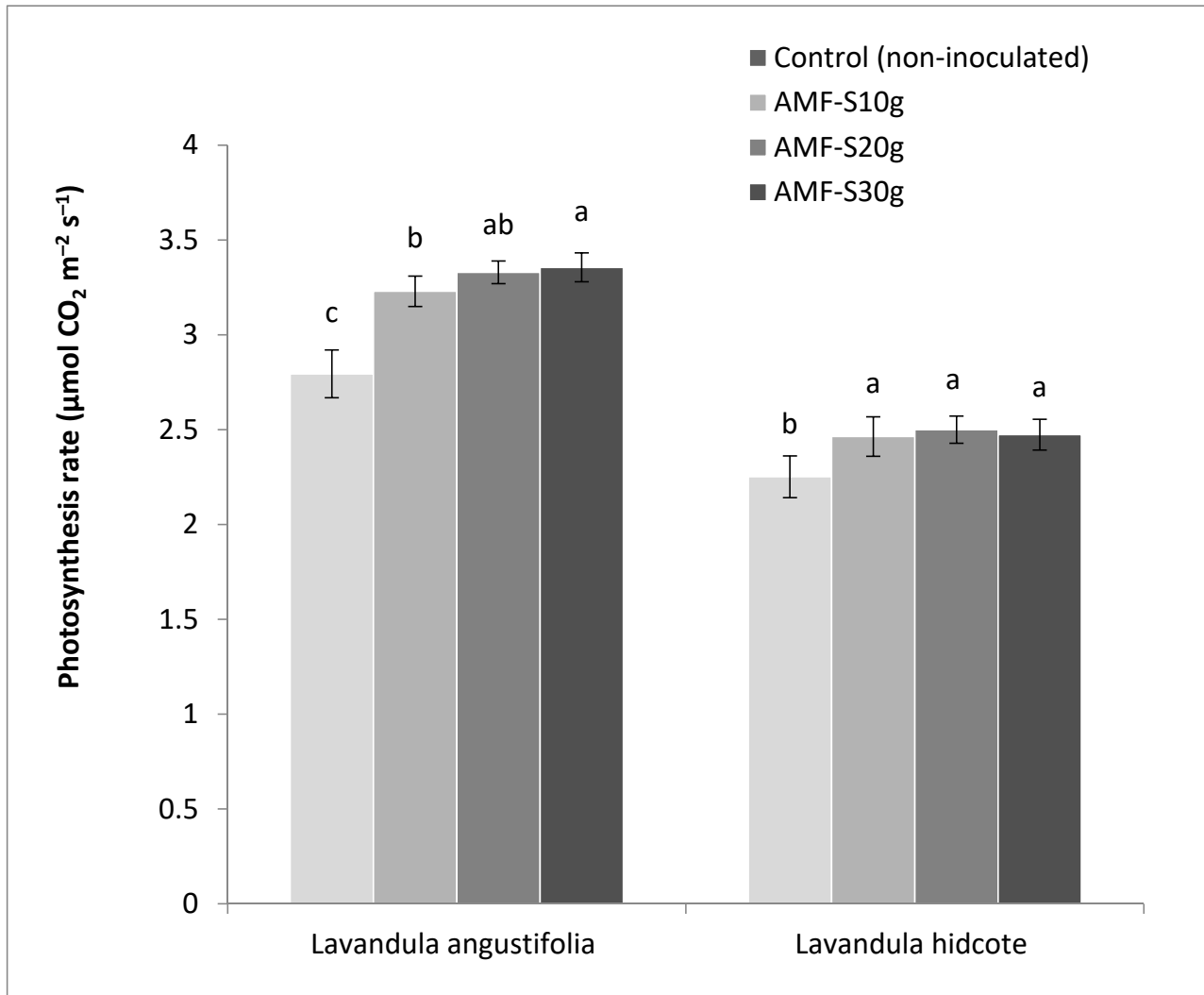


Figure 1. Photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in mycorrhizal and non-mycorrhizal lavender with a mixture of six arbuscular mycorrhizal fungi: *Rhizophagus irregularis* (BEG140), *Claroideoglomus claroideum* (BEG96), *Funneliformis mosseae* (BEG95), *Funneliformis geosporum* (BEG199), *Claroideoglomus etunicatum* (BEG92), and *Glomus microaggregatum* (BEG56). Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($p < 0.05$). Vertical bars indicate the standard deviation of the mean value. Control: untreated lavender—non-inoculated plants; AMF-S_{10g} = arbuscular mycorrhizal fungi—Symbivit inoculum 10 g; AMF-S_{20g} = arbuscular mycorrhizal fungi—Symbivit inoculum 20 g; AMF-S_{30g} = arbuscular mycorrhizal fungi—Symbivit inoculum 30 g.

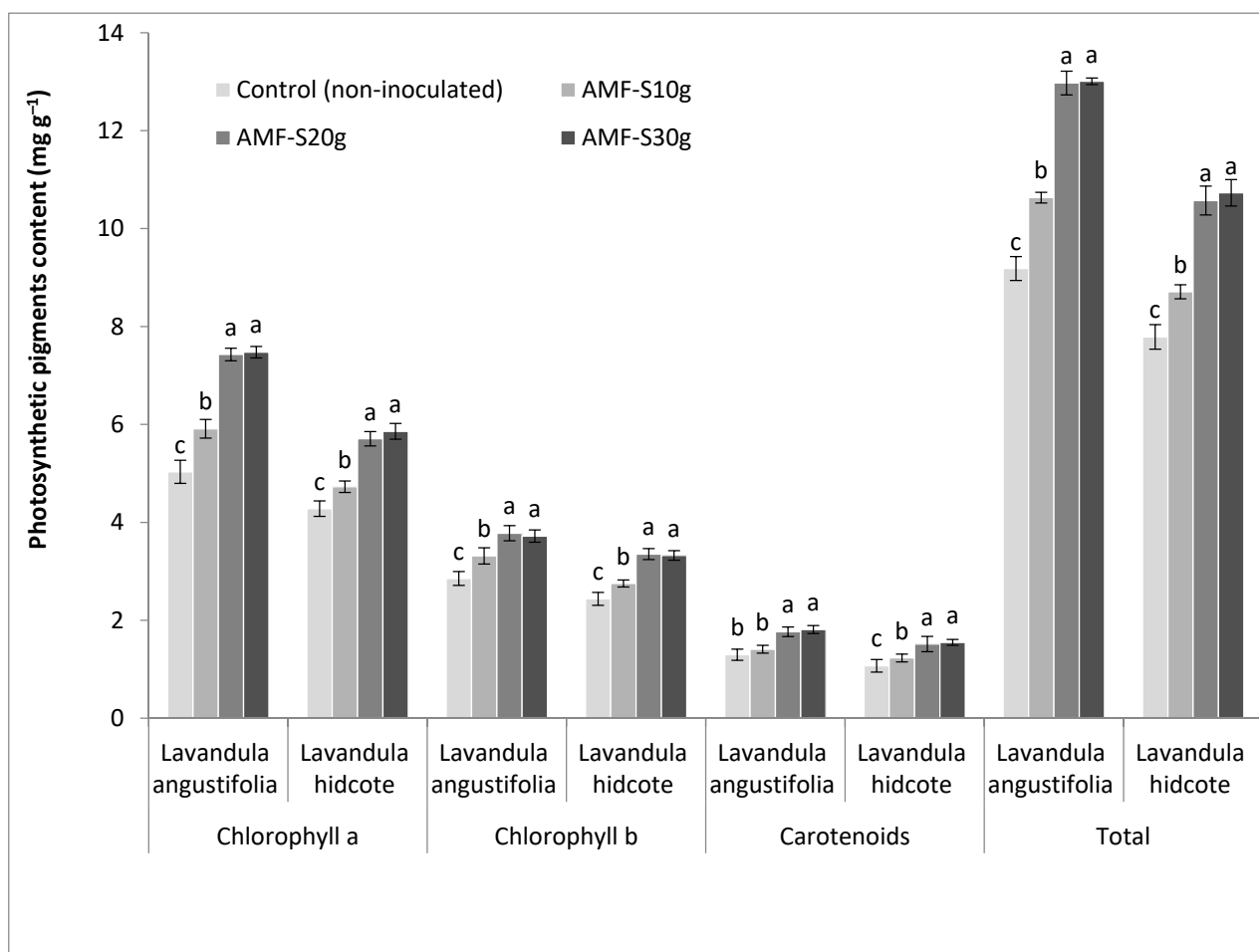


Figure 2. Chlorophyll a (mg g^{-1}), chlorophyll b (mg g^{-1}), carotenoids (mg g^{-1}), and total photosynthetic pigments content (mg g^{-1}) in lavender non-inoculated or inoculated with a mixture of six arbuscular mycorrhizal fungi: *Rhizophagus irregularis* (BEG140), *Claroideoglomus claroideum* (BEG96), *Funneliformis mosseae* (BEG95), *Funneliformis geosporum* (BEG199), *Claroideoglomus etunicatum* (BEG92), and *Glomus microaggregatum* (BEG56). Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($p < 0.05$). Vertical bars indicate the standard deviation of the mean value. Control: untreated lavender—non-inoculated plants; AMF-S_{10g} = arbuscular mycorrhizal fungi—Symbivit inoculum 10 g; AMF-S_{20g} = arbuscular mycorrhizal fungi—Symbivit inoculum 20 g; AMF-S_{30g} = arbuscular mycorrhizal fungi—Symbivit inoculum 30 g.

Chen et al. [43], in an experiment with combined inoculation with multiple arbuscular mycorrhizal fungi reported that distant combination AMF species may have a better effect than a single or closely related AMF spp. Authors noted that mixture of *Claroideoglomus* sp., *Funneliformis* sp., *Diversispora* sp., *Glomus* sp., and *Rhizophagus* sp inoculated in cucumber seedlings had a better effect on growth, chlorophyll content, or photosynthetic rate compared with non-inoculated plants, plants inoculated with the combination of *Glomus intraradices*, *G. microageregatum* BEG, and *G. Claroideum* BEG 210, or seedlings inoculated with a single AMF specie (*Funneliformis mosseae*). In this regard, the effects of arbuscular mycorrhizal symbiosis vary with the host plants and fungal species involved in the association [43]. Therefore, identification of the appropriate combinations of AMF is very important to have the best beneficial effect for plant growth and development.

Pearson's correlation was applied to examine the relationships between the amount of inoculum from the community of six arbuscular mycorrhizal fungi and physiological parameters in mycorrhizal lavender plants (Table 5). The Pearson correlation coefficient was considered significant at the 0.01 levels (from -1 to 1). The amount of AMF inoculum

showed significant positive correlations with chlorophyll a content and total photosynthetic pigment (Pearson correlation coefficients = 0.942 ** and 0.945 **, respectively) in mycorrhizal *Lavandula angustifolia* plants. A little less positive correlation was observed between AMF mixture application and photosynthesis rate ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \text{s}^{-1}$). Additionally, the mixture of six arbuscular mycorrhizal fungi showed significant positive correlations with all physiological parameters in mycorrhizal *Lavandula hidcote* plants (Pearson correlation coefficients ranged from 0.606 ** and 0.948 **). As in the case of mycorrhizal *Lavandula angustifolia* plants, AMF associations were less correlated with photosynthesis rate (Pearson correlation coefficient = 0.606 **). These findings demonstrated the positive effect of the community of six arbuscular mycorrhizal fungi application on the physiological processes of the plant.

Table 5. Pearson correlation coefficient between the amount of inoculum from the community of six arbuscular mycorrhizal fungi and physiological parameters in mycorrhizal lavender plants.

Pearson Correlation	Photosynthesis Rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Carotenoids (mg g^{-1})	Total Photosynthetic Pigments (mg g^{-1})
<i>Lavandula angustifolia</i>	0.832 **	0.942 **	0.870 **	0.894 **	0.945 **
<i>Lavandula hidcote</i>	0.606 **	0.948 **	0.912 **	0.861 **	0.945 **

** indicates correlation is significant at the 0.01 level.

4. Conclusions

Arbuscular mycorrhizal fungi are essential soil microorganisms for terrestrial ecosystems and form beneficial symbioses with root systems of inoculated lavender plants. Plant growth substrates with a mixture of six arbuscular mycorrhizal play an important role in the growth and development processes of plants. The improvement of plant growth and flowering characteristics is justified by the positive effect of the arbuscular mycorrhizal fungi symbiosis on the total photosynthetic pigments content and the process of photosynthesis. The mixture of AMF containing *Rhizophagus irregularis*, *Claroideoglomus claroideum*, *Funnelformis mosseae*, *Funnelformis geosporum*, *Claroideoglomus etunicatum*, and *Glomus microaggregatum* applied at different concentrations and added in a pot with peat, volcanic rock, and coconut bark enhanced the plant growth, physiological processes, and ornamental value in mycorrhizal lavender plants.

In brief, the results of our experiment show that the root biomass and total plant biomass were more pronounced in plants with AMF-S_{20g} and AMF-S_{30g} than with AMF-S_{10g} or in untreated plants. AMF colonization greatly improved the leaf area of lavender especially when a mixture of arbuscular mycorrhizal fungi with 30 g of inoculum treatment has been applied. Only small differences were obtained for the AMF-inoculated plants under the 20 g and 30 g inoculum for most studied parameters.

This research indicated that the potential of total photosynthetic pigment content for AMF associations were highest when plants were treated with AMF-S_{20g} and AMF-S_{30g}.

Our findings suggest that AMF mixture application in a growing substrate with peat, coconut bark, and volcanic rock could be a sustainable option to increase sustainability in the agricultural production of ornamental and medicinal plants.

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