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The Effects Of Amf Inoculation And Growing Medium Differences On The Development Of Red And Green Lettuce (Lactuca Sativa Var. Crispa)

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Abstract
This study aimed to investigate the effects of arbuscular mycorrhizal fungus (AMF) inoculation (ERS – Endo Roots Soluble, 4 g/plant) on plant growth parameters in green and red lettuce (Lactuca sativa L.) varieties with different pigment contents, and how these effects varied in different growing media (peat and natural soil). AMF inoculation was applied during seedling transplantation using the "sandwich method" Within the scope of the study, morphological and physiological parameters such as plant age and dry weight, root age and dry weight, root length, number of leaves, leaf diameter, plant height, and root infection rate were evaluated. The data obtained revealed that green lettuce plants grown in peat and inoculated with AMF achieved the highest values in all developmental parameters, and these differences were statistically significant (P<0.01). The findings indicate that plant pigment composition (chlorophyll, anthocyanin, etc.) and environmental characteristics play a decisive role in the effectiveness of the symbiotic relationship with AMF. The results highlight the importance of considering plant genotype and environmental interactions in microbial biofertilizer applications.
Keywords: *Arbuscular mycorrhizal fungi, lettuce, pigment, anthocyanin, chlorophyll, symbiosis, peat, soil, plant growth.*

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I. Introduction

The increasing adoption of microbial biofertilizers is pivotal for achieving sustainable agricultural productivity, reducing reliance on chemical fertilizers, and preserving soil health. Arbuscular mycorrhizal fungi establish symbiotic relationships with plant roots, thereby improving the uptake of essential mineral nutrients, particularly phosphorus, and positively influencing plant growth, resilience to stress, and soil microbial activity (Smith and Read, 2010; Hestrin et al., 2021).

The efficacy of mycorrhizal symbiosis is contingent upon a multitude of factors, including plant species, the composition of root exudates, the growing environment, and critically, the plant's physiological characteristics (Liu et al., 2019; Veresoglou and Rillig, 2012). In this regard, variations in plant pigment composition and morphology can impact the success of the symbiotic association with AMF. For instance, green lettuce is characterized by high chlorophyll content, whereas red lettuce possesses greater concentrations of anthocyanins and other phenolic compounds. Anthocyanins not only aid plants in managing oxidative stress but may also interfere with microbial signaling processes within the root milieu. Consequently, a comparative analysis of the physiological responses of lettuce varieties with differing pigment profiles to AMF symbiosis is crucial for refining microbial fertilization strategies (Chalker-Scott, 1999; Shtark et al., 2021).

Furthermore, the successful colonization by AMF is sensitive to environmental conditions. Environments rich in organic matter and possessing a low pH, such as peat, are conducive to AMF proliferation, whereas soils with high lime content and low organic matter may impede this symbiotic relationship (Li et al., 2018; van der Heijden et al., 2015). This study comparatively assessed the morphological and physiological impacts of AMF inoculation on green and red pigmented lettuce varieties across diverse growing conditions and discussed the potential influence of pigment structure on the symbiotic relationship in the context of existing literature.

II. Materials And Methods

Materials

Greenhouse, plant-beds, soil and peat

The experiment was conducted using beds (plant beds) in non-computer-controlled greenhouse conditions. The beds, made of wood, provide an environment that keeps the plants above ground level. The dimensions of the beds are 60x120x35 cm, as shown in Figure 1.

Figure 1. A plot where plants are grown for testing purposes.

Two different growing media were used in the experiment: soil and peat. The soil has a pH of 7.75, electrical conductivity (EC) of 130 mS/cm, lime content of 30%, available phosphorus (P) content of 9.6 ppm, and organic matter content of 1.45%. Peat, on the other hand, had a pH of 6.2, EC of 700 mS/cm, available phosphorus of 4.12 ppm, and organic matter content of 45.81%. Soil samples were collected from the surface (0–30 cm) of an agricultural field belonging to the Faculty of Agriculture at Selçuk University, sieved through a 4 mm mesh, and used without sterilization. Peat was purchased in commercially packaged form and used directly as a sterile substrate.

Lettuce Plant

The study used two different pigment structures: a curly-leaved lettuce (*Lactuca sativa* var. *crispa*) variety with high chlorophyll content and a red lettuce variety with high anthocyanin content. Each plant variety was prepared for the experiment by applying AMF at the seedling stage during the average 4 true leaf stage.

Arbuscular Mycorrhizal Fungal (AMF) Spores and Plant Inoculation

Endo Roots Soluble (ERS) brand was used as commercial mycorrhizal inoculum. The product package contains $1x10^4$ spores of the arbuscular mycorrhizal species *Glomus* spp. (*G. intraradices, G. aggregatum, G. mossea, G. clarum, G. monosporus, G. deserticola, G. brasillanum, G. etunicatum*) and *Gigaspora margarita* (*Gig. margarita*) per $50g^{-1}$.

Commercial mycorrhizal inoculum, the Endo Roots Soluble brand, was applied under the roots using the "sandwich method" during seedling planting at a rate of 4 g per plant. In this method, a layer of peat/soil was first placed around the roots, followed by 4 g of commercial mycorrhizal fertilizer (approximately 800 spores), and then another layer of growing medium (peat/soil) was placed on top to ensure homogeneous distribution of the spores.

III. Methods

Soil Analysis Methods

Mechanical analysis: The sand, silt, and clay fractions of the soil were determined using the Bouyoucos (1995) hydrometer method.

Soil reaction (pH): 1:2,5 ratio soil:pure water mixture was measured using a glass electrode pH meter according to Jackson (1962).

Electrical conductivity ($ECx10^6$ µmhos cm^{-1}): It was done using an electrical conductivity device in a 1:5 soil:pure water mixture, according to U.S. Salinity Lab. Staff, (1954).

Organic matter (%): It was performed according to the Smith and Weldon (1941) method.

Lime ($CaCO_3$%): It was performed volumetrically using a Scheibler Calcimeter, as described by Kacar (1995).

Available phosphorus (ppm): It was performed using Olsen's NaHCO3 method, modified by Bayraklı (1987).

Peat Analysis Methods

pH: Peat samples were diluted at a ratio of 1:2.5 (soil:water) as recommended by the International Society of Soil Science for pH determination and measured with a glass electrode pH meter (Sağlam, 2008).

Electrical conductivity (µmhos cm^{-1}): To measure the electrical conductivity of peat samples, saturated mud was prepared, and measurements were taken using an EC meter (Şişaneci and Direnç, 2008).

Organic matter (%): The organic matter content of peat was determined using the Walkley-Black method (Greweling and Peech, 1960).

Available phosphorus (ppm): Phosphorus determinations were performed according to the Olsen Method (Sağlam, 2008).

Plant measurements
Root length: To determine the root length, a transparent plastic shallow container was placed on the bottom of a (30x40 cm) grid of 1x1 cm squares. Plant roots, cut to a length of a few centimeters and weighed to 1 g, were randomly placed on the grid, and the intersection points of the roots and lines were counted using a hand counter in both the transverse and longitudinal directions (Tennat, 1975).
Plant height: The length between the soil surface and the tip of each plant was measured using a ruler with mm divisions, and the plant height was determined in cm.
Plant fresh weight: The fresh weights of harvested lettuce leaves were determined by weighing them on a precision scale (± 0.1 g).
Plant dry weight: Lettuce leaves from the test plots were placed on filter paper and left in an oven set at 70°C until they reached a constant weight, then weighed on a sensitive scale (± 0.1 g) to determine their dry weight.
Plant fresh root weight: The fresh weights of harvested lettuce roots were determined by weighing them on a precision balance (± 0.1 g).
Plant dry root weight: The lettuce roots, whose wet weights were determined, were placed in paper bags and kept in an oven set at 70°C until they reached a constant weight. Their dry weights were then determined by measuring them on a sensitive scale (± 0.1 g).

Some Mycorrhizal Analyses in Soil and Plants
AMF Sports Count in Soil: The wet sieving method was used to count AMF spores in the test soil. According to the method, the soil-water mixture was poured onto a metal sieve (38 µm), and the material remaining on the sieve was transferred to Petri dishes. The spores were counted under a stereo microscope at 40–90x magnification (number of spores/10 g of soil) (Gerdeman and Nicolson 1963).
Painting the roots with Trypan blue: In the greenhouse study, arbuscular mycorrhizal spore inoculation was performed, and plant root samples harvested were stained using Koske and Gemma's (1989) Trypan Blue method and examined under a microscope to determine the percentage of mycorrhizal infection. Images of infections in the roots of green lettuce (Figure 2) and red lettuce (Figure 3) grown in peat medium under a light microscope (x40).

Figure 2. Vesicles on the roots of green lettuce.

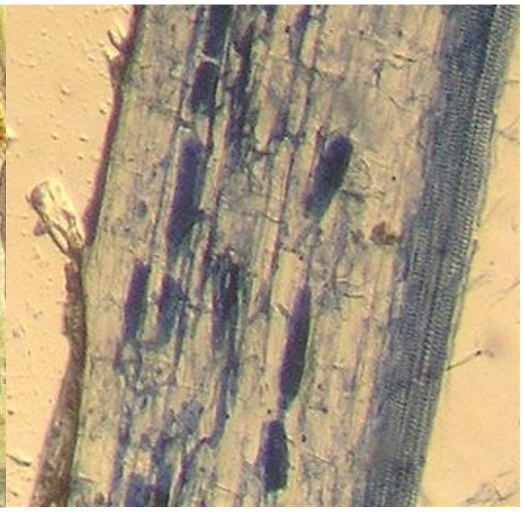

Figure 3. Arbuscules on the roots of red lettuce.

Experimental Design

The study was set up in a randomized block design with four replicates. Each experimental unit contained one plant.

The factors and their levels are as follows:

Factors	Levels
Plant variety	Green lettuce, red lettuce
Plant growing medium	Soil, peat
AMF inoculation	Inoculated, non-inoculated

Factors	Levels
In total: 2 (plant var.) x	2 (media) x 2 (AMF) x 4 (replicates)= 32(evaluated based on 32 pots)

Statistical Analysis

All data was subjected to one-way and multiple variance analysis (ANOVA) using SPSS 25.0 software. The significance of differences was compared using the Tukey HSD test at a level of 1% (P<0.01).

IV. Results And Discussion

The results obtained from the study on the effect of arbuscular mycorrhizal fungus (AMF) spore inoculation on the growth parameters of two lettuce (*Lactuca sativa* L.) varieties grown in different environments are presented in Table 5.

Table 5. The Effect of Arbuscular Mycorrhizal Fungus (AMF) Spore Inoculation on the Development Parameters of Two Lettuce Varieties Grown in Different Media.

M	App.	P.V.	P.F.W.	P.D. W	P.H.	R.F.W.	R.D. W	R.L.	N.L.	L.D.	R.I.R
Soil	-AMF	G.L.	3,44B	0,57B	9,57BC	0,48C	0,29D	7,63C	10,33B	3,07C	0,00D
		R.L.	3,62B	0,38B	7,93C	0,73C	0,19D	7,30C	10,00B	3,23C	0,00D
	+AMF	G.L.	6,66B	0,73B	10,80AC	1,69C	0,46D	6,50C	10,33B	4,90BC	73,27B
		R.L.	7,78B	0,49B	8,10C	1,83C	0,39D	9,93BC	12,00B	4,27BC	37,00C
Peat	-AMF	G.L.	23,16AB	2,50B	13,57AC	10,82AB	2,70AB	17,10AB	11,66B	6,57AB	0,00D
		R.L.	24,96AB	2,04B	12,33AC	9,60AB	2,00BC	18,23AB	17,00AB	6,90AB	0,00D
	+AMF	G.L.	45,65A	5,43A	16,17A	13,19A	3,28A	23,33A	23,00A	8,37A	92,67A
		R.L.	20,53AB	2,22B	14,13AB	6,57B	0,99CD	17,80AB	12,00B	7,00AB	34,61C

Letters have been assigned according to the significance level P<0.01

P.F.W.: Plant Fresh Weight (g)
P.D.W.: Plant Dry Weight (g)
P.H.: Plant Height (cm)
R.F.W.: Root Fresh Weight (g)
R.D.W.: Root Dry Weight (g)
R.L.: Root Length (m/g root)
N.L.: Number of Leaves (number/plant)
L.D.: Leaf Diameter(cm)
R.I.R.: Root Infection Rate (%)
M.: Media
App.: Application
G.L.: Green lettuce
R.L.: Red lettuce
P.V.: Plant variety

This investigation examined the impact of applying arbuscular mycorrhizal fungus spores to red and green lettuce seedlings in peat and soil substrates on various plant growth parameters. The findings, detailed in Table 5, indicate that the most substantial increases in plant fresh weight (45.65), plant dry weight (5.43 g), plant height (16.17 cm), plant root fresh weight (13.19 g), plant root dry weight (3.28 g), plant root length (23.33 m/g root), plant leaf number (23.00 leaves/plant g), plant leaf diameter (8.37 cm), and plant root infection rate (92.67%) were obtained from green lettuce grown in peat and inoculated with AMF spores. The differences between the obtained values were found to be statistically significant (P<0.01). Thus, AMF spore inoculation in the peat medium significantly increased plant growth factors compared to other media, plants, and treatments in the experiment. This significant effect is consistent with the existing literature on the supportive effects of AMF on plant growth.

When examining the environmental impact on the study's outcomes, it is widely understood that the symbiotic relationship established by arbuscular mycorrhizal fungi within plant roots enhances the absorption of water and mineral nutrients, particularly phosphorus (Smith and Read, 2010; Johnson et al., 2015). Although the soil environment in this study presented a challenge with relatively low available phosphorus (9.6 ppm), high pH, and elevated lime content, the peat environment, characterized by lower pH and richer organic matter, proved more conducive. While the higher electrical conductivity of peat might typically induce salinity stress, previous research indicates that AMF inoculation can bolster plant resilience under such conditions (Augé, 2001; Ruiz-Lozano et al., 2012).

The elevated rate of arbuscular mycorrhizal fungus root colonization observed in green lettuce grown in peat suggests superior fungal establishment in this substrate. This enhanced colonization likely explains the significant improvements in plant growth parameters, attributed to the positive impact of peat's low bulk density on root structure and function. Specifically, increases in root age and dry weight indicate an augmented capacity for water and nutrient uptake, while the elongation of roots facilitates nutrient acquisition from a broader soil volume (Smith and Read, 2010).

The heightened presence of arbuscular mycorrhizal fungus within the roots of green lettuce cultivated in peat suggests more effective fungal establishment in this medium. This enhanced colonization is likely responsible for the significant improvements observed in plant growth metrics, which are attributed to the beneficial influence of peat's low bulk density on root structure and function. Increases in root maturity and dry mass indicate a greater capacity for water and nutrient absorption, while the elongation of roots facilitates nutrient acquisition from a wider soil volume.

Increases in leaf count and diameter contributed to a rise in the plant's photosynthetic capability and, consequently, biomass accumulation. Conversely, the impact of AMF spore inoculation on red lettuce and in soil conditions was less pronounced than in peat. This disparity may be linked to the soil environment's elevated pH, high calcium carbonate content, and comparatively low organic matter, factors that can impede the development and efficacy of AMF spores. Existing literature frequently highlights that high pH and calcium carbonate levels can negatively affect mycorrhizal fungal colonization, thus underscoring the critical role of environmental conditions in determining mycorrhizal activity (Chen et al., 2016; Li et al., 2018).

From a plant physiological standpoint, arbuscular mycorrhizal fungi are understood to enhance photosynthesis and carbon assimilation by augmenting the uptake of essential nutrients like phosphorus and nitrogen, as well as certain micronutrients, by the roots (Smith and Smith, 2011; Hestrin et al., 2021). In this investigation, green lettuce exhibited elevated chlorophyll levels, which correlated with more active photosynthesis, thereby bolstering AMF symbiosis and increasing the potential carbon supply to the fungi. Conversely, the presence of anthocyanins and phenolic compounds in red lettuce may impede AMF symbiosis by attenuating photosynthetic activity and filtering light absorption, which could account for the superior growth and dry weight observed in green lettuce within this study (Albert et al., 2014; Zhang et al., 2022).

The red and green color differences in lettuce are mainly due to the presence of different pigments. Red lettuces are characterized by high anthocyanin and other phenolic compound content, while green lettuces mainly contain chlorophyll. These pigment differences not only affect visual characteristics but also directly influence the plant's physiological processes, stress tolerance, and the development of mycorrhizal symbiosis. Color pigments can shape the AMF relationship in different ways by creating differences in the plant's metabolic activity, oxidative stress tolerance, and signaling pathways. Indeed, in the study, the highest AMF spore infection rate was 92.67% in green lettuce grown in peat, followed by 73.27% in green lettuce grown in soil. As can be seen here, lettuce pigmentation was a more decisive factor in root infection rates than environmental differences (peat/soil). This is because the anthocyanins present in high concentrations in red lettuce have strong antioxidant properties and protect plants against oxidative stress (Chalker-Scott, 1999; Gould, 2004).

The concentration of phenolic compounds in plant root cells may influence the plant's response to mycorrhizal symbiosis, with some research indicating that high levels of these compounds can impede mycorrhizal fungus infections, potentially by directly inhibiting fungal growth or interfering with signaling pathways on the root surface (Sikes et al., 2009). In contrast, green lettuce, characterized by lower phenolic and anthocyanin content but higher chlorophyll concentrations, exhibits a greater photosynthetic capacity. This enhanced carbon fixation ability allows green lettuce to supply more nutrients to the mycorrhizal fungus, a critical factor for fungal growth and function, as carbon availability can limit mycorrhizal symbiosis (Hestrin et al., 2021). Consequently, more effective colonization by arbuscular mycorrhizal fungi in green lettuce could lead to improved plant growth and associated development metrics (Smith and Read, 2010).

The formation of symbiosis is also influenced by the metabolic processes of root pigments and their impact on root secretions. Anthocyanins and phenolic compounds may either promote or inhibit AMF spores by altering the composition of root exudates (Liu et al., 2019). The study indicated that the substantial phenolic content in red lettuce might have partially hindered the attachment and proliferation of arbuscular mycorrhizal spores on the root surface. Evidently, disparities in the pigment profiles of different lettuce varieties have led to variations in metabolic and physiological activities that directly influence AMF colonization and, consequently, plant development. The reduced phenolic content and enhanced carbon sequestration capacity of green lettuce have amplified the effects of AMF inoculation in peat. These findings underscore the importance of considering plant color diversity in the context of mycorrhizal symbiosis and biological fertilizer applications (Bais et al., 2006; Hestrin et al., 2021).

The most favorable outcomes for root length, root age, and root dry weight were observed in the combination of peat, AMF, and green lettuce. This enhancement is attributed to the beneficial influence of AMF on root architecture, specifically by promoting lateral branching at the root tip and regulating root development via plant hormones (particularly auxin and gibberellin) (López-Ráez et al., 2010). The enhanced responsiveness of green lettuce to these effects can be ascribed to its limited phenolic inhibition and abundant carbon source. Furthermore, strigolactones, secreted by the plant during AMF symbiosis, also stimulate root branching, thereby facilitating more extensive mycorrhizal colonization. The peat medium contributes to improved plant root spread due to its low bulk density and superior water-retention capacity (López-Ráez et al., 2010; van der Heijden et al., 2015).

Arbuscular mycorrhizal fungi serve as a crucial feeding mechanism in symbiotic associations. Enhanced photosynthetic capacity, linked to leaf development, allows plants to produce greater amounts of carbon, which fuels AMF (Smith and Read, 2010). This symbiotic exchange appears to be more efficient in green lettuce, whereas the light-absorbing properties of pigments in red lettuce may impede this process. Although flavonoids and anthocyanins contribute to stress defense, their accumulation could potentially limit plant growth (Winkel-Shirley, 2002).

The highest root infection rates were documented in green lettuce cultivated in peat moss, indicating a highly compatible root environment with arbuscular mycorrhizal fungi (Liu et al., 2019). The reduced presence of phenolic compounds in green lettuce roots, which can inhibit AMF colonization, allowed for effective fungal integration. Conversely, flavonoids secreted by red lettuce roots may impede the colonization of certain AMF species by disrupting the uptake of essential signaling molecules for symbiosis, a hindrance less prevalent in green lettuce, thereby potentially facilitating symbiotic communication (Shtark et al., 2021). Furthermore, the peat substrate's organic matter likely enhanced mycorrhizal development by supplying crucial energy and a substrate for the fungus (Veresoglou and Rillig, 2012).

V. Conclusion

Based on the study's findings regarding the impact of arbuscular mycorrhizal fungus spore inoculation on lettuce growth in various environments, the applications proved most effective in peat-based media and with green lettuce varieties. The research indicated that plant mycorrhizal dependency varies by genotype, even within the same lettuce type. Green lettuce exhibited a more favorable response to AMF spore inoculation compared to red lettuce, showing enhanced growth. Red lettuce's growth appeared less dependent on AMF spores, with environmental factors, particularly peat media without AMF inoculation, playing a more significant role. For greenhouse or small-scale garden cultivation, AMF spore inoculation is advisable for green lettuce. In larger, commercial operations, its recommendation for green lettuce should consider the product-to-price ratio. AMF spore inoculation is not deemed necessary for red lettuce, regardless of cultivation scale; instead, efforts to increase organic matter are suggested. This study highlights the necessity of considering plant variety, pigment composition, and environmental conditions collectively for effective mycorrhizal biological fertilizer applications. Optimizing these factors is crucial for boosting plant production productivity. Further research comparing different lettuce varieties, soil types, and AMF species is recommended to refine application strategies.

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