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The Effects of Mycorrhiza Application and Reduced Phosphorus Fertilization on Plant Growth, Nutrient Uptake, and Yield in Greenhouse Pepper Cultivation

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ABSTRACT

Following the Industrial Revolution and the resulting growth in the world's population, maximising agricultural production yields has become a fundamental objective. Consequently, the use of chemical fertilisers has become widespread. However, the long-term intensive use of these inputs has led to a decline in soil fertility and salinisation, as well as the deterioration of soil health. This has prompted the development of alternative approaches to sustainable agricultural practices. One such approach is the use of arbuscular mycorrhizal fungi (AMF), a type of biological fertiliser that establishes a symbiotic relationship with plant roots. This enhances plant growth, increases the root surface area, and improves nutrient uptake and resistance to abiotic stress conditions. In greenhouses in particular, eliminating beneficial microorganisms through the use of high inputs and soil disinfection processes results in the disappearance of mycorrhizal populations. This increases dependence on chemical fertilisers for greenhouse cultivation. In this study, two different AMFs (*Rhizophagus clarus* and *Funneliformis caledonius*) were applied in three different ways (once during seed sowing, once during seedling stage, and twice during seed sowing and seedling stage) to pepper cultivation in steam-sterilized soil where natural AMFs were eliminated. The effects of these applications on the development, yield, and nutrition of the pepper plant were investigated. In conclusion, the addition of AMF to the soil in greenhouses positively affected plant growth, nutrient uptake, and yield. Yield increased by 16% in single-application treatments compared to the control, while it increased by 29% in two-application treatments. Similar results were obtained for plant growth and nutrition. The use of mycorrhizae in greenhouse vegetable cultivation also guarantees less chemical use, a cleaner environment, and higher-quality products.

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1. Introduction

With the growth of the world population, the increase in food demand and the reduction of arable land due to urban transformation have made maximizing yield per unit area the primary goal of growers. Agronomists have also focused on practices and studies aimed at this goal. Greenhouse farming is one of the farming systems that yields the most per unit area, and the need for such intensive production systems has increased [1, 2]. In countries with suitable climates, particularly in the Mediterranean region, greenhouse farming has been adopted by producers rather than open field farming because heating costs are lower. Today, greenhouse farming areas and production are also showing an upward trend. However, traditional intensive fertilization strategies applied in these systems impair soil health and limit seasonal application rates to between 10% and 25%, particularly due to the low mobility of phosphorus (P) in the soil [3, 4]. In greenhouse agriculture, compared to open cultivation, the reasons for intensive fertilization in greenhouses include not leaving plant residues, high yields, long vegetation periods, and rapid organic matter loss observed in summer. Since greenhouses are fixed structures, excessive application of chemical fertilizers to the soil negatively affects soil health and also causes soil salinization. Furthermore, as greenhouses are closed environments, there is no washing away of these salts by rainfall.

In recent years, researchers have been working not only on applications aimed at increasing yield per unit area, but also—perhaps even disregarding yield—on ensuring that products are of high quality, have higher nutritional value, and contain as few chemical residues as possible. The growing awareness of consumers about their nutrition, the increasing demand for healthy and nutrient-rich products, and the questioning of residues harmful to health have led producers and researchers to adopt production methods that are chemical-free, environmentally friendly, do not harm the soil and plants, and do not pose a threat to human health. Chemical fertilizers were the first choice to meet the nutritional needs of plants in the soil, but the role of beneficial microorganisms that dissolve and mobilize nutrients has been neglected [5]. However, it is widely accepted that balanced fertilization and cultivation integrated with soil microorganisms are the key to high yields, agricultural sustainability, and high income [6]. Fertilization strategies planned in conjunction with soil microorganisms benefit producers and ensure that products are marketed in line with consumer demands. Mycorrhiza, one of these beneficial microorganisms, is a practice that has the potential to reduce chemical fertilizer use by increasing the uptake of nutrients by plants and has gained importance within the scope of sustainable agriculture approaches.

The rhizosphere is the habitat of a vast community of microorganisms found in the thin layer of soil surrounding plant roots, and most interactions between beneficial microorganisms and plant roots occur here [7, 8]. Over 80% of terrestrial plants form symbiosis with arbuscular mycorrhizal fungi (AMF) belonging to the Glomeromycota phylum [9]. Arbuscular mycorrhizal fungi (AMF) belonging to the Glomeromycota phylum establish symbiotic relationships with plant roots, increasing nutrient uptake through extensive hyphal networks. These networks facilitate the uptake of essential nutrients, particularly phosphorus, while host plants provide photosynthetic products to the mycorrhizae [10]. Plants can obtain more than 90% of the phosphorus and more than 50% of the nitrogen they need through these fungal hyphae [11, 12]. Due to these contributions to plant nutrition, the use of AMF can contribute to increasing yields in a more sustainable way without further compromising soil health [13, 14]. Mycorrhizal inoculation increases biomass, plant height, and yield by optimizing the plant's nutrient and water uptake [15-1]. Mycorrhizal fungi secrete a sticky glycoprotein called glomalin, which binds soil particles together and promotes the formation of water-resistant aggregates [3]. This process improves soil aeration and water retention capacity and prevents erosion [3-16]. Preserving soil structure and increasing productivity are of great importance, especially in areas where intensive production systems are applied.

In open-field crop production, even without external microbial inoculation, numerous beneficial microorganisms naturally present in the soil, including mycorrhiza, contribute to plant growth. However, in greenhouse agriculture, both harmful and beneficial microorganisms are destroyed during soil disinfection or solarization. Consequently, natural AMF is absent in greenhouse soil. It is a production system that requires intensive fertilization. Reducing chemical applications can be achieved by adding beneficial microorganisms to greenhouse soil. Such practices, which reduce intensive chemical fertilization in greenhouses, are necessary for obtaining healthier products.

Turkey is a Mediterranean country and, like other countries bordering the Mediterranean, intensive greenhouse agriculture is practiced. With the advantages of the Mediterranean climate, greenhouse areas are increasing day by day, supplying more greenhouse products to the market. As is well known, the majority of vegetable production takes place in greenhouses. We conducted research on peppers, one of the vegetable types produced. Peppers are the most widely produced greenhouse vegetable after tomatoes and cucumbers. This study aimed to determine the effects of arbuscular mycorrhizal fungi added to the environment on plant growth and development parameters, nutrient uptake, and yield factors in pepper plants grown in sterilized soil conditions under greenhouse conditions. Within this scope, the potential contributions of mycorrhiza application to the morphological characteristics, nutritional status, and productivity of pepper plants were examined in detail. Additionally, in chemical fertilization, phosphorus fertilizer use was reduced by 30% to achieve both fertilizer savings and more effective mycorrhiza activity.

2. Materials and Methods

The research was conducted in a 360m² plastic greenhouse (12x30m) belonging to the Department of Horticulture, Faculty of Agriculture, Çukurova University. The plant material used was the Balo F1 bell pepper variety. *Rhizophagus clarus* and *Funneliformis caledonius* were used as mycorrhizal fungus material. The research was conducted in plastic channels (240x30x20 cm). Eight plants were planted at 30 cm intervals in channels containing steam-sterilized soil. The experiment was set up in channels to provide a completely controlled environment for mycorrhiza. AMF was applied at seed sowing time (SS), seedling planting time (SP), and both seed sowing and seedling planting times (SS+SP). These applications were compared with control plants that did not receive AMF. In the two-season study, seeds were sown on December 23 for spring cultivation, and seedlings were planted in the greenhouse channels on March 3. For autumn cultivation, seeds were sown on August 23, and seedlings were planted in the greenhouse on October 16. Morgan and Lennard [17] were used for the nutrient solution to be given to the plants. In addition, in the autumn period, the phosphorus content in the nutrient solution was reduced by 30% approximately one month after planting.

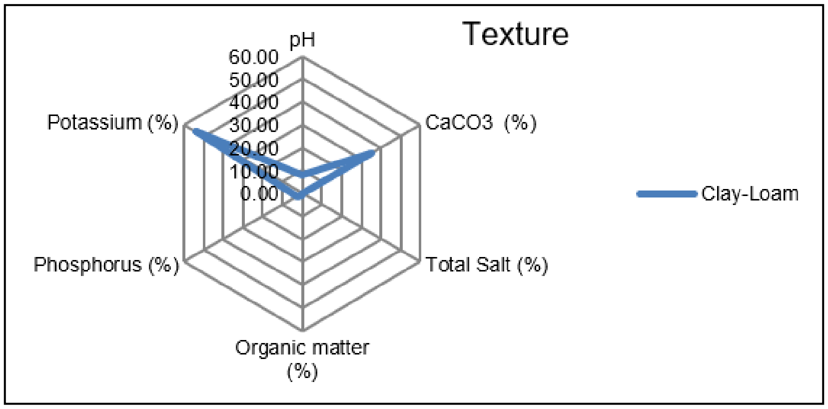


Figure 1: Soil analysis results of the soil used in the experiment.

The soil used in the experiment was sterilized with steam to eliminate harmful disease agents and any naturally occurring AMF present (Fig. **1**). The results of the analysis of the soil's physical and chemical properties are presented in Table **1**. The experiments were planned according to a split-plot design. The main plots contained AMF species (*Rhizophagus clarus, Funneliformis caledonius*, and Control), and the subplots contained application times (SS, SP, SS+SP). Mycorrhizal fungus applications were as follows: during the seed sowing period, 100 ml (1000 mycorrhiza spores) of inoculum was poured into the rows of plastic trays where seeds were sown, and 33 seeds were sown in each row (approximately 33.33 mycorrhiza spores/plant). To investigate the effects of mycorrhizal fungi on plant growth, 10 plants were harvested from each replicate at 21, 63, and 105 days after planting. Root length, root dry weight, shoot length, and green shoot dry weight were measured. In addition, plant nutrient contents in the leaves and mycorrhizal infection values in the roots were determined at the end of the experiment. Total yield values were calculated based on a total of 10 harvests in the spring and 6 harvests in the fall. Harvesting was done once a week during the warm season and once every two weeks during the cold season.

2.1. Plant Phosphorus, Potassium and Zinc Content (% and ppm)

Leaf samples were washed with distilled water and dried in an oven at 60°C. The dried samples were ground and then incinerated at 550°C for 6–7 hours. The resulting ash was dissolved in 3.3% (v/v) HCl and filtered. Potassium (K) and zinc (Zn) contents were determined in leaf samples. Potassium analyses were performed in emission mode, while Zn analyses were performed in absorption mode using an atomic absorption spectrophotometer [18].

2.2. Infection Rate in Roots (%)

On the final measurement date, plant roots were harvested, thoroughly washed to remove soil, and cleaned to determine mycorrhizal colonization. Root cleaning and trypan blue staining were performed according to the method described by Koske and Gemma [19]. The AM colonization percentage was calculated by counting 100 root segments, each 10 mm long, from roots inoculated with mycorrhiza under a stereo microscope at 20× magnification and expressed as a colonization percentage [20].

2.3. Statistical Analysis

Analysis of variance (ANOVA) was used to determine the effects of the treatments in the experiment, and the data were analyzed. The mean values of the treatments were compared using the Tukey method.

3. Findings

3.1. Root Length (cm)

Root length values for both periods are presented in Fig. (**2**). When root length values were examined, it was found that the difference in application times compared to the control was statistically significant on the second measurement date of the spring period, 63 days after planting. The application of mycorrhiza twice (during seed sowing and seedling planting periods) was significantly different from the control and resulted in longer roots. Mycorrhiza did not create a difference in root length on other measurement dates.

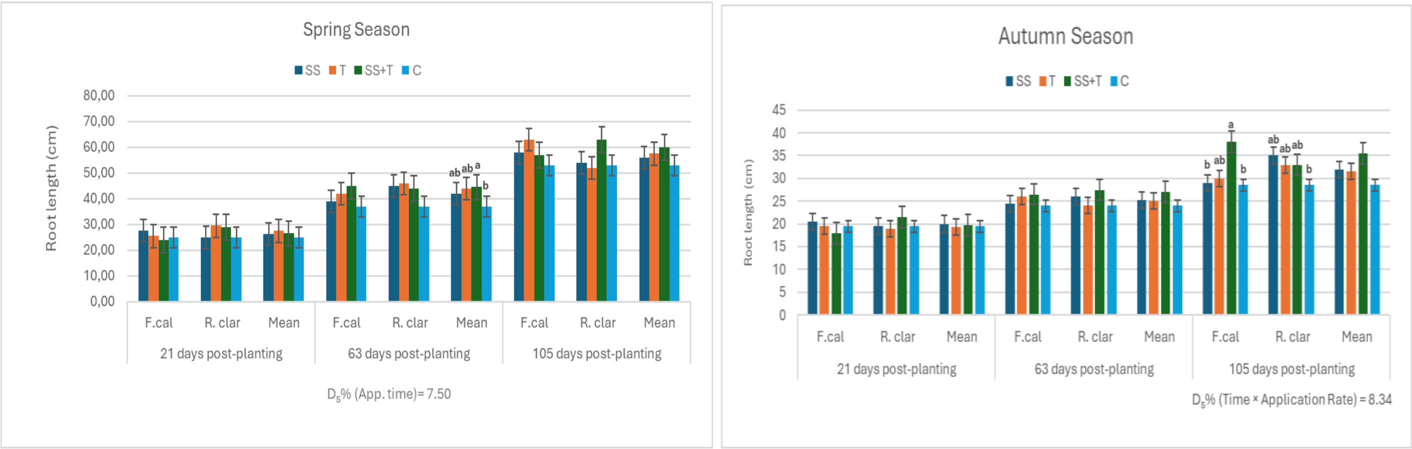


Figure 2: Effect of mycorrhiza applications on root length (cm) during spring and Autumn season.

3.2. Root Dry Weight (g)

Fig. (**3**) shows that AMF application had a positive and significant effect on the root dry weight of plants. In the spring period, AMF applications increased root dry weight at a statistically significant level on all measurement dates.

In the autumn period, an interaction was found between application methods and mycorrhizal fungus types at a 1% error level in the first measurement. The best application was found to be the two-time application of *Rhizophagus clarus*. At the final measurement date, the twice application of mycorrhiza was significantly different from the control, while the single applications were in the intermediate group. *Rhizophagus clarus* increased root dry weight more than the other fungus.

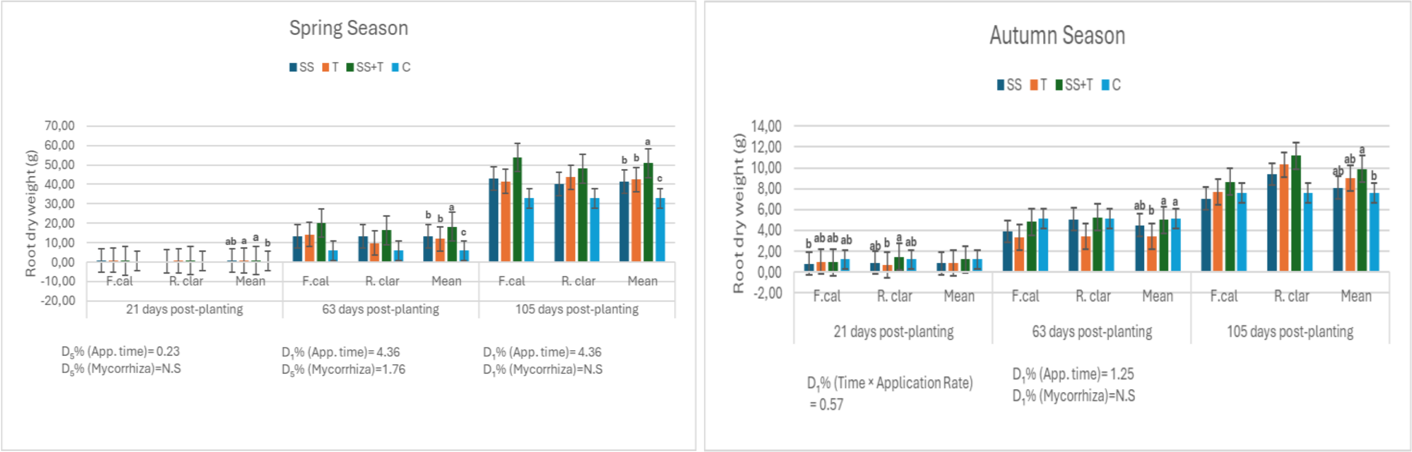


Figure 3: Effect of mycorrhiza applications on root dry weight (g) during Spring and Autumn seasons.

3.3. Total Plant Dry Weight (g)

When examining the effects of AMF applications on green part dry weight (Fig. **4**), no statistically significant difference was found between applications on the first measurement date and the last measurement date in the spring period. However, on the second measurement date, the difference between mycorrhizal species was insignificant, but the difference between application times was significant. Accordingly, it was determined that applying mycorrhiza twice and applying it during the seed sowing period created a significant difference compared to the control.

In the autumn period, at the first measurement date, the application of mycorrhiza during the seedling planting period was found to be statistically significant compared to the control, while the application of mycorrhiza twice and during the seed sowing period was statistically intermediate. No statistically significant differences were observed between the treatments in the subsequent two measurements.

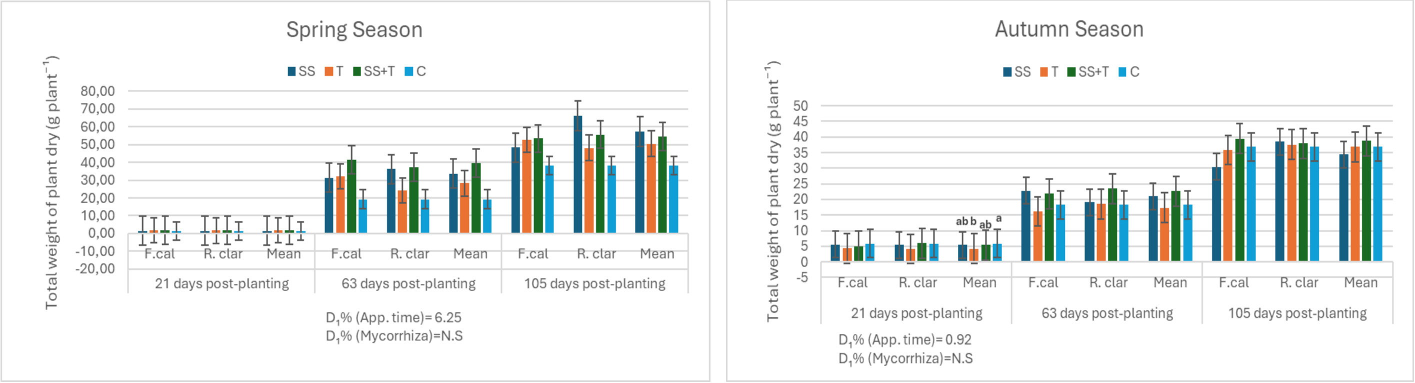
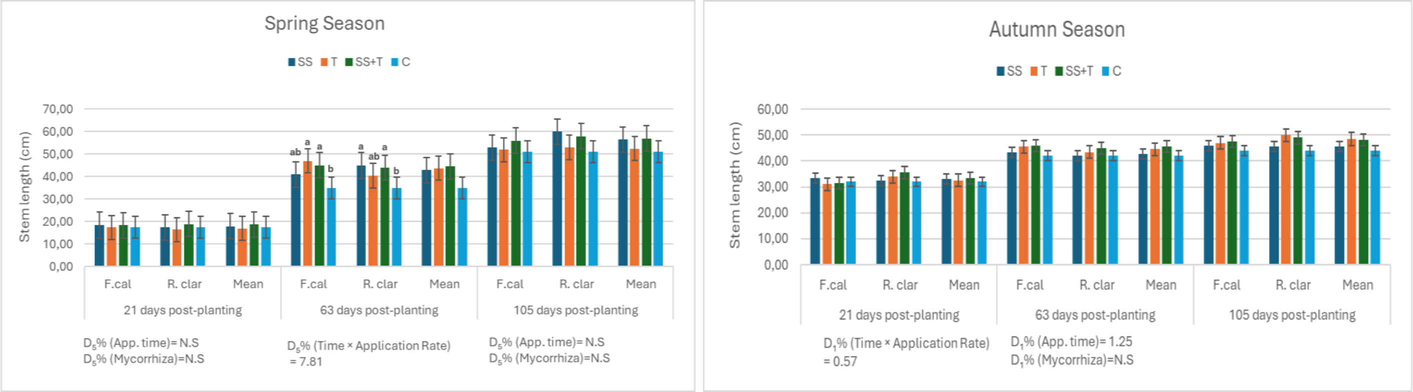


Figure 4: Effect of mycorrhiza applications on green biomass dry weight (g) during spring and autumn.

3.4. Stem Length (cm)

As shown in Fig. (**5**), the first measurement taken during the spring period revealed no significant difference between the treatments. However, on the second measurement date, there was an interaction between the treatments at a 5% error level. The most effective treatments were found to be: application of *Funneliformis caledonius* during the seedling planting period; application of *Funneliformis caledonius* during the seed sowing and seedling planting periods; and application of *Rhizophagus clarus* during the seed sowing and seedling planting periods. Applications of *Funneliformis caledonius* during seed sowing and of *Rhizophagus clarus* during seedling planting were intermediate, while the control was at the end. According to the statistical analysis results, the effect of the applications was not significant in the final measurement period.



D_5% (App. time)= N.S
D_5% (Mycorrhiza)=N.S

D_5% (Time × Application Rate) = 7.81

D_5% (App. time)= N.S
D_5% (Mycorrhiza)=N.S

D_1% (Time × Application Rate) = 0.57

D_1% (App. time)= 1.25
D_1% (Mycorrhiza)=N.S

Figure 5: Effect of mycorrhiza applications on stem length (cm) during Spring and Autumn Season.

3.5. Plant Phosphorus, Potassium and Zinc Content (% and ppm)

Examining the effects of mycorrhizal types on the nutrient content of pepper plants (Table **1**) revealed that applying arbuscular mycorrhizal fungi (AMF) twice during the spring increased phosphorus content by 4% compared to the control. Examining potassium content revealed that two applications differed significantly from the others in terms of timing. Similarly, two applications also significantly increased zinc content. This application provided a 22% increase compared to the control, while single applications provided an average increase of 11%. The effect of AMF applications in the autumn was not clearly observed. However, in terms of zinc content only, the two-time application was found to differ significantly from the control.

Table 1: The nutrient content of the plants in both periods was determined.

Treatments	*F. caledonius*			*R. clarus*			Mean		
	P (%)	K (%)	Zn (ppm)	P (%)	K (%)	Zn (ppm)	P (%)	K (%)	Zn (ppm)
Spring Season									
SS	0.32	3.63	30.40	0.33	3.42	35.40	0.33 ab	3.53 b	32.90 ab
T	0.33	3.50	31.75	0.34	3.74	35.80	0.34 ab	3.62 b	33.78 ab
SS+T	0.36	3.31	36.80	0.36	3.44	38.50	0.36 a	3.73 b	37.65 a
C	0.31	3.37	29.40	0.31	3.37	29.40	0.31 b	3.37 b	29.40 b
Ort.	0.33	3.70	32.09	0.34	3.74	34.78	0.34	3.72	33.09
Autumn Season									
SS	0.29	3.85	37.60	0.28	3.63	39.10	0.29	3.74	38.35 ab
T	0.27	3.84	45.70	0.26	3.24	40.83	0.27	3.54	43.27 ab
SS+T	0.28	3.62	50.60	0.29	3.87	47.60	0.29	3.75	49.10 a
C	0.27	3.46	36.50	0.27	3.46	36.50	0.27	3.46	36.50 b
Ort.	0.28	3.69	42.60	0.28	3.55	41.01	0.28	3.62	41.80

D_5% (App.time) = 0,04 (P), D_5% (App.time) = 0,31 (K), D_5% (App.time) = 6,65 (Zn)

3.6. Root İnfection Rate (%)

Statistical analysis results indicate that both mycorrhizal species and application timing significantly affect infection rates (Fig. **6**). As expected, the highest infection rate was observed with two applications of mycorrhiza, which was statistically significantly different from single applications. The low infection rate in the control group is thought to be due to irrigation. Among AMF species, *Rhizophagus clarus* infected roots better than other fungi. In the fall season, no difference was found between mycorrhizal species, while in terms of application timing, two applications of mycorrhiza achieved the highest value, and single applications were statistically within the same group.

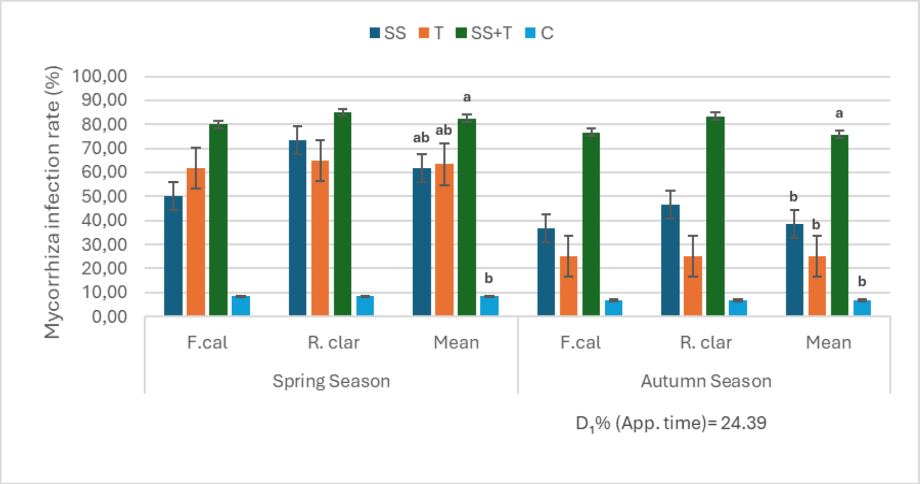


Figure 6: Infection rate (%) in the roots of the test plants in both periods.

3.7. Yield (g/plant)

The effect of mycorrhizal fungus applications on total yield is presented in Fig. (**7**). The effect of application times on yield was found to be statistically significant during the spring season, when climatic conditions were favorable for peppers. The highest yield was found with two applications of AMF species, which differed significantly from the control. Single applications were in the intermediate group. When examining yield results in the fall period, yields were lower compared to the spring period due to the lower number of harvests. Although there were differences between applications, these were not statistically significant.

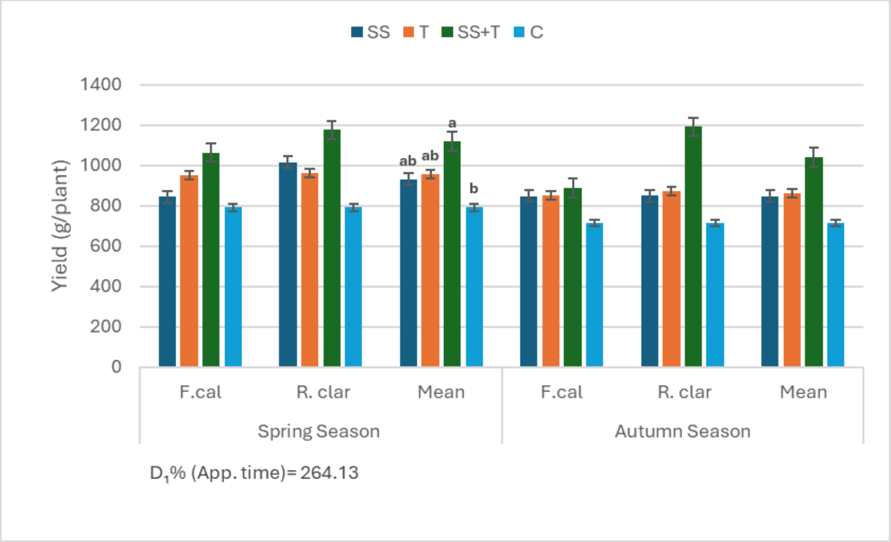


Figure 7: Effect of mycorrhiza applications on yield (g/plant) during spring and fall seasons.

4. Discussion

The positive effect of the three application methods and the timing of the two types of AMF on plant growth and yield in greenhouse pepper cultivation was evident. This effect was particularly noticeable in spring, when conditions were more favourable for pepper cultivation. In autumn, the results were less pronounced due to the cold weather. However, the differences between the applications in both periods were consistent. The most effective method was two applications of mycorrhiza at the time of sowing seeds and planting seedlings. Total yield in the spring increased by 29% compared to the control group with two applications of mycorrhizae, and by an average of 16% with one application. In autumn, two applications increased yield by 31% compared to the control group, while one application increased yield by an average of 17%. Reducing phosphorus application in the autumn period led to more effective mycorrhizal activity. Based on this, phosphorus can be reduced when adding mycorrhizae to soil suitable for peppers under warmer conditions. This is important both economically, due to reduced inputs, and in terms of reducing chemical use. As the growing season for plants is longer in greenhouses and phosphorus is a macronutrient, the amount of fertiliser applied is higher. Reducing this amount by 30% would also be economically beneficial. Similar results have been obtained in studies where the nutrient solution was reduced by this percentage. Dasgan *et al*. [21] applied nutrient solutions to tomatoes with reductions of 40%, 60% and 80%. In this study, a nutrient solution containing 100% of the required nutrients was used as a control and the highest yield was obtained from the solution with a 60% reduction. Similarly, Dasgan *et al*. [22] used nutrient solutions containing 20% and 40% reduced nutrients in their study on squash. They obtained the highest yield in terms of the effect of mycorrhiza use on yield from the 20% reduced nutrient solution.

Dry root weight was found to be higher than in control plants, partly due to root length and mainly due to higher root density. Root density increased with mycorrhizal root colonisation. Regarding the results on root length, we believe that the limited growth environment prevented the roots from growing deeper, given that the experiment was conducted in channels. Furthermore, as nutrients were supplied to the plants in solution at regular intervals, it can be concluded that the plants did not increase their root length in order to search for water or nutrients. However, the channels were used to determine the mycorrhizal infection rate in the roots and to ensure complete control of the water and nutrient content of the soil in which the roots were located. Nevertheless, the effect of the mycorrhizae was evident in the parameter of root dry weight. During the spring period, two applications of mycorrhizae at the final measurement date increased root dry weight by 35% compared to the control group, whereas one application increased it by around 23%. Similar results were reported by Charron *et al*. [23] in two studies conducted on onions. Many Allium species responded to AMF inoculation by promoting growth. Onion (*Allium cepa*) plants grown in mineral potting soil and inoculated with AMF had higher biomass than uninoculated plants and reached marketable size (diameter >25 mm) 2–3 weeks earlier. Karagiannidis *et al*. [24] reported increased fresh and dry weight and plant height with AMF application in eggplant and tomato.

In tomatoes (*S. lycopersicum*), an increase in yield and fruit number was determined after inoculation with AMF [25]. Conversa *et al*. [26] found that the fastest increase in the plant leaf area index in tomatoes inoculated with mycorrhiza became apparent from the second month onwards, resulting in mycorrhizal plants showing better growth and development compared to the control. The highest biomass and, consequently, the highest yield were obtained in mycorrhiza-inoculated plants. AMF applications resulted in good root development, followed by good green shoot development, leading to increased yield. Douds and Reider [27] in peppers, Abdelhafez and Abdel-Monsief [28] in melons and cucumbers, and Ortaş [29] in peppers, tomatoes, and eggplants have reported that mycorrhizal fungus applications increased yield. Field applications of a mixture of 20 native AMF strains increased tomato yield by up to 26% and carrot yield by up to 300% [30]. The growth-promoting effect of AMF may be based on its significant contribution to the nutrient supply of host plants [31].

Nutrient uptake increased in both periods with mycorrhizal fungus applications. AMF plays important roles in plant nutrition [32]. In the plant-mycorrhiza association, AMF's hyphae, which can expand the surface area (up to 40 times), produce enzymes, and secrete organic matter, assist in nutrient uptake [33]. They contribute particularly to the uptake of the macronutrients P and then N. They also improve the uptake of micronutrients Zn, Cu, and Fe, which move slowly in the soil. AMF colonization increases plant uptake of both macronutrients and micronutrients when low levels of P and N fertilization are applied [34, 35]. Other studies have also supported the positive effect of AMF on nutrient uptake. Mycorrhizal pepper plants showed increases in chlorophyll index and leaf N, P, Fe, and Zn content compared to uninoculated plants [36]. Tanwar *et al*. [37] reported that the combined application of AMF (*G. mosseae and Acaulospora laevis*) and plant growth-promoting bacteria (*Pseudomonas fluorescens*) could reduce phosphorus fertilization by 50% in pepper cultivation. The researchers suggested that the application of mycorrhizae and bacteria together with 50% reduced P fertilizer doses during seedling planting could increase plant growth and yield performance and could be a sustainable alternative to high P fertilizer in pepper cultivation. Furthermore, in potato (*Solanum tuberosum*) plants, following inoculation with AMF under low soil P concentrations, increased plant growth, higher root/stem ratio, higher phosphorus use efficiency, and lower leaf/tuber ratio were observed compared to non-mycorrhizal plants [38]. Conversa *et al*. [26] demonstrated the applicability of mycorrhiza as a biological fertilizer for tomatoes. They noted that it could be an innovative, sustainable practice that increases crop profitability for growers while reducing the need for P fertilization.

In a study conducted on leeks, inoculation with AMF increased P and Zn uptake and growth of mycorrhiza compared to uninoculated seedlings [39]. Smith and Smith [40] investigated the effects of inoculating vegetables with AMF in terms of soil nutrient concentrations. Ultimately, in general, increased soil P concentrations reduced AMF mycorrhizal growth. When phosphate availability was high, AMF did not function effectively, and growth retardation was even observed in non-host species or host species [41].

Antunes *et al*. [42] emphasized in their study that soil fertility should be considered a strong determinant of the symbiotic function of AMF. They found that AMF not only promotes P supply for the host plant but also increases N uptake in vegetable crops. AMF also provides micronutrients (especially Zn) to the inoculated plant. For example, inoculating leek seedlings with mycorrhiza increased both phosphorus and zinc supply while also enhancing plant growth [39]. Ortas [29] noted that mycorrhizal inoculation increased phosphorus and zinc concentrations in pepper plants, particularly in soils deficient in phosphorus and zinc.

In today's conditions, utilizing natural resources is important for the environment. Our research confirms that using a natural resource such as mycorrhizal fungi in pepper production enables the cultivation of healthier plants and avoids excessive fertilization. In particular, reintroducing AMF into greenhouse soil, which had been depleted of beneficial microorganisms through solarization or disinfection, has positively impacted plant growth, nutrient uptake, and yield.

5. Conclusion

AMF can be used as a biofertilizer to increase yield and quality in vegetable production. Especially in greenhouse vegetable cultivation, seedling companies can perform mycorrhizal inoculation and market the seedlings as mycorrhizal seedlings. Alternatively, producers can apply commercially available organic mycorrhizae during the seedling planting stage. In this case, producers can see all the positive results that mycorrhiza provides in terms of plant growth and yield. According to these research results, it is possible to put the contributions of mycorrhiza to plants into practice. It can even be applied with reduced phosphorus. In subsequent studies, we may recommend testing the phosphorus reduction rate in other vegetable types and researching reductions in certain proportions for nutrients other than phosphorus.

Conflict of Interest

The authors declared that they have no conflict of interest.

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