



Growth of mastic tree (*Pistacia lentiscus* var. *chia* Duham.) seedlings and the presence of arbuscular mycorrhizal fungi in V-shaped microcatchments

Ramazan KUZU¹ , Yasin KARAŞIN¹ , Meral ÖDEMiŞ² , Bülent TOPRAK^{1,*} 

¹İzmir Katip Çelebi University, Faculty of Forestry, Department of Forest Engineering, İzmir/TÜRKİYE

²Harran University, Faculty of Sciences and Letters, Department of Biology, Şanlıurfa/TÜRKİYE

*Corresponding author: bulent.toprak@ikc.edu.tr

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Abstract

Afforestation success in semi-arid Mediterranean regions is increasingly constrained by water scarcity and climate change. Conventional planting techniques often fail to provide sufficient soil moisture for optimal seedling establishment and growth, highlighting the need for effective water management strategies. Rainwater harvesting (RWH) techniques, combined with plant-supportive treatments, offer a promising approach to overcome these limitations. This study aimed to evaluate the effects of V-shaped microcatchment systems and plant-supportive applications, including polymer, osmoprotectant, and mycorrhiza, on the early growth performance of *Pistacia lentiscus* seedlings. The experiment was conducted under semi-arid Mediterranean conditions using a randomized complete block design. At the end of the fourth growing season, root collar diameter and shoot height were measured. The sturdiness index was calculated, and mycorrhizal spores were counted. The results revealed that the terrace control (C-A) consistently produced the lowest growth values, indicating the inadequacy of conventional methods under water-limited conditions. In contrast, all microcatchment treatments significantly improved seedling performance and mycorrhizal spore counts. Overall, microcatchment systems increased soil moisture availability and promoted significant improvements in both height and diameter growth compared to the control. In conclusion, water availability was identified as the primary limiting factor for afforestation success. The integration of V-shaped microcatchment systems with plant-supportive treatments provides an effective and sustainable strategy to enhance seedling establishment and growth under semi-arid conditions.

Keywords: *Pistacia lentiscus* var. *chia*, Rainwater harvesting, V-shaped microcatchments, Semi-arid afforestation

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1. Introduction

Paleoclimatic studies indicate that climate systems have always undergone continuous change. However, while these changes were historically driven by natural processes, they are now predominantly influenced by anthropogenic activities (Türkeş, 2013). In particular, energy production, industrialization, urban expansion, and improper land-use practices have led to a significant increase in greenhouse gas concentrations in the atmosphere, thereby accelerating global warming and rendering climate change a critical issue at both global and national scales (Desonie, 2008; Erdönmez et al.,

2023). Consequently, climate change manifests through a range of environmental impacts, including decreasing and irregular precipitation patterns, increased frequency of drought events, intensified forest fires, plant exposure to water stress, and progressive desertification (Öner et al., 2010; Örs et al., 2011).

One of the most critical consequences of climate change is the growing pressure on water resources. Declining and irregular precipitation, particularly in semi-arid and arid regions, significantly increases the risk of water scarcity and directly affects agricultural and forestry systems (Örs et al., 2011). Elevated temperatures



combined with reduced rainfall intensify plant water stress, adversely affecting plant growth and development and ultimately resulting in yield losses. Furthermore, the progressive transition of semi-arid regions into arid zones exacerbates plant survival challenges. Therefore, enhancing plant tolerance to water stress has become a central focus in recent research efforts.

Among the strategies developed to mitigate these challenges, water harvesting techniques have emerged as effective approaches for improving water availability. Water harvesting involves capturing and storing rainfall at the point of occurrence, thereby reducing surface runoff, minimizing soil erosion, and promoting water retention within the soil profile (Tari & Çakır, 2009; Örs et al., 2011). These techniques, which date back to ancient times, have regained considerable importance, particularly in arid and semi-arid environments (Oweis et al., 2001). Practices such as Negarim systems, semi-circular bunds, and contour-based structures effectively reduce runoff losses and support plant water requirements (Geremu et al., 2016). Nevertheless, these approaches alone are often insufficient, highlighting the need for complementary plant-based strategies.

In this context, biological approaches that enhance plant water and nutrient acquisition have gained increasing attention. Mycorrhizal fungi establish symbiotic associations with plant roots, significantly improving the efficiency of water and nutrient uptake. In this mutualistic relationship, fungi facilitate the absorption of water and mineral nutrients from the soil, while plants supply the fungi with photosynthetically derived organic compounds (Smith & Read, 2008; Dighton, 2009). These associations enhance plant tolerance to drought stress and sustain plant growth even under limited water availability (Cooper, 1984; Perry et al., 1987).

Mycorrhizal fungi are classified into different types depending on plant species, soil characteristics, and environmental conditions. Among these, endomycorrhiza and ectomycorrhiza are particularly significant for agricultural and forestry applications (Erzurumlu & Kara, 2014; Yılmaz, 2019). In endomycorrhizal associations, fungi colonize the cortical tissues of plant roots and facilitate nutrient exchange through specialized structures known as arbuscules. In contrast, ectomycorrhizal fungi develop between root cells and form extensive external mycelial networks, substantially increasing the plant's capacity to absorb water and nutrients (Anonymous, 2014). This enhanced absorption capacity provides a critical

advantage for plant survival, particularly under arid and semi-arid conditions.

The benefits of mycorrhizal fungi extend beyond improved water and nutrient uptake. These fungi also promote plant growth, enhance root development, and increase resistance to plant pathogens (Erzurumlu & Kara, 2014; Altuntaş et al., 2015; Rafique & Ortas, 2018). Additionally, mycorrhizal associations contribute to soil structure improvement and play a vital role in maintaining ecosystem sustainability (Palta et al., 2010; Ataklı et al., 2021). Empirical studies conducted in arid and semi-arid regions have demonstrated that mycorrhizal inoculation significantly improves seedling establishment success and enhances plant resistance to water stress (Perry et al., 1987; Sanchez, 1994).

Within this framework, the mastic tree (*Pistacia lentiscus*), an economically and ecologically valuable species, holds particular importance. This species is naturally distributed only in the Çeşme Peninsula and Chios Island, making these regions key production centers. Mastic is widely utilized in various sectors, particularly in the food, cosmetic, and pharmaceutical industries, and constitutes a significant source of income for local communities. However, despite the ecological suitability of regions such as the Çeşme Peninsula, commercial production remains largely confined to Chios Island. This highlights the need to expand cultivation efforts through large-scale seedling production and plantation initiatives.

Given the increasing severity of climate change and associated water stress conditions, the successful establishment and growth of mastic seedlings in semi-arid environments remain a significant challenge. Therefore, integrating water harvesting techniques with mycorrhizal applications represents a promising approach to enhance plant survival and growth under drought conditions. Such integrated strategies may contribute significantly to sustainable forestry and agricultural practices, particularly in regions vulnerable to climate change.

2. Material and Method

2.1. Site description

The study was conducted in the Mediterranean Basin, where the adverse effects of climate change have recently become increasingly evident and are expected to intensify further. The research was carried out in three different sites located within the jurisdiction of the İzmir Regional Directorate of Forestry in Türkiye: one site in Çeşme (38°25'01"N, 26°31'16"E) and two sites in Urla (38°26'53"N, 26°30'13"E; 38°26'43"N, 26°30'12"E) (Figure 1).

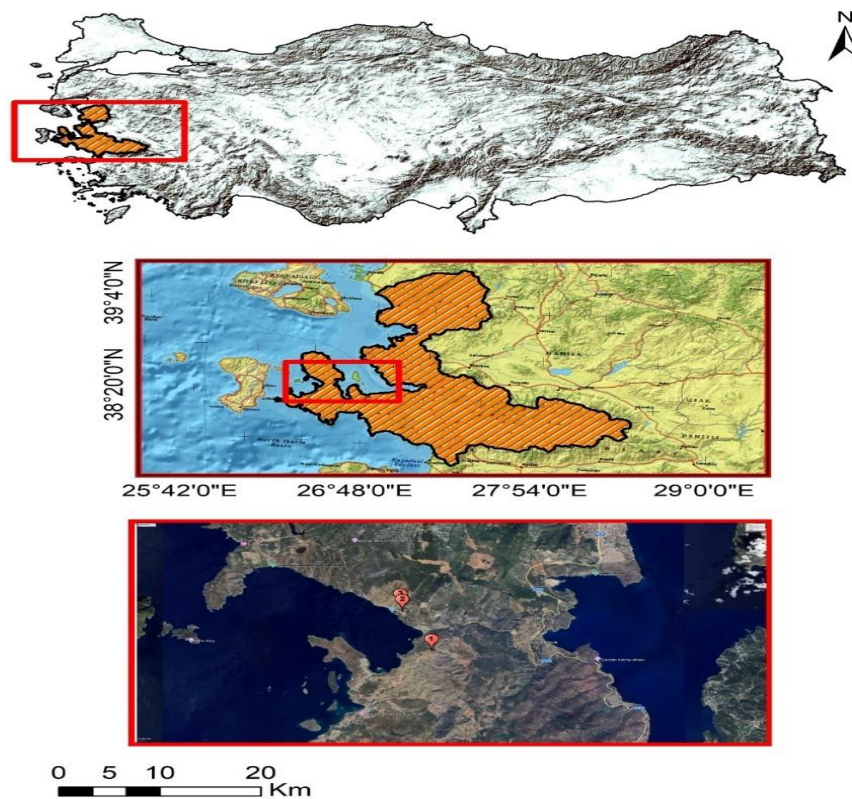


Figure 1. Location of the study sites

The treatments were applied over a total area of approximately 1.5 ha across the three sites. A randomized block design with three replicates (each site representing one “block”) was employed in the study. The dominant parent material across the sites is limestone. The study area is characterized by an average slope of 20% and a mean elevation of approximately 150 m above sea level.

2.2. Climatic conditions

Long-term meteorological records spanning more than 80 years were utilized to characterize the climate of the study area. Thornthwaite potential evapotranspiration analyses indicate a substantial water deficit during the growing season, particularly from April to October (Figure 2). Additionally, analysis of the regional precipitation regime shows that approximately 80% of the total annual rainfall occurs outside the main growing season, when soil moisture availability is relatively high.

Recent data derived from the Copernicus/ECMWF ERA5 dataset (Hersbach et al., 2020) indicate that precipitation patterns in the region have become increasingly variable, while temperature and precipitation anomalies have become more frequent. These climatic changes are likely to exacerbate water scarcity and increase the vulnerability of afforestation areas to the impacts of climate change.

2.3. Growth measurements and statistical analysis

At the end of the fourth growing season, root collar diameter and shoot height were measured. The sturdiness index (SI), calculated as the ratio of shoot height to root collar diameter ($SI = \text{Shoot Height} / \text{Root Collar Diameter}$), was determined for each seedling, and mycorrhizal spores were counted and mycorrhizal spores were counted. Data were analysed according to the randomised complete block design, with blocks representing site-level differences in climatic water deficit. Treatment effects on growth were evaluated by analysis of variance (ANOVA). Normality and homogeneity of variances were tested using the Shapiro–Wilk and Levene tests, respectively. When treatment effects were significant, means were compared using Tukey’s honestly significant difference (HSD) test at $\alpha = 0.05$. All analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

2.4. Plant species

Seedlings of *Pistacia lentiscus* var. *chia* obtained through air layering were used in the study (Figure 3). The mastic tree (*Pistacia lentiscus*) is one of the plant species native to the Mediterranean Basin and is naturally distributed particularly along the coastal regions of Greece, Türkiye, Italy, and Spain. This species is one of approximately 14 known species belonging to the genus *Pistacia* within the family Anacardiaceae.

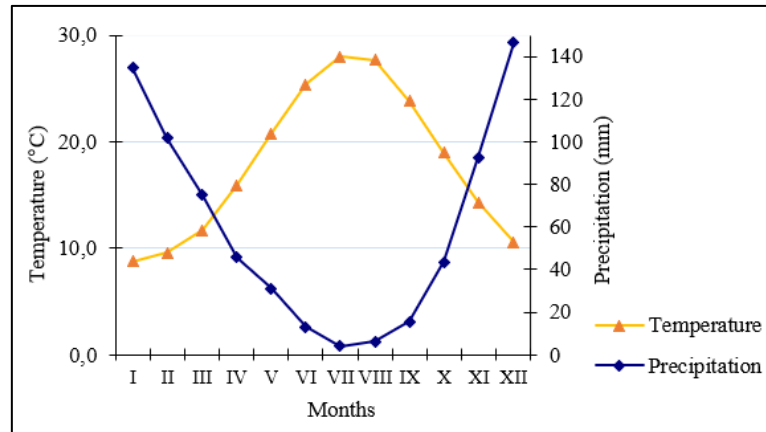


Figure 2. Diagram illustrating seasonal temperature and precipitation patterns at the study sites



Figure 3. Mastic seedling in the experimental area

2.5. Experimental design

The experiment was established using a randomized complete block design (RCBD) with three replications across three afforestation sites characterized by differing levels of seasonal water deficit (Koyuncu, 2024; Figure 4). Following the removal of vegetation, V-shaped microcatchments were constructed with their arms aligned parallel to contour lines. Conventional terracing was included as a reference treatment representing standard afforestation practices.

Across each block, six experimental units were established, four of which consisted of V-shaped microcatchments. Within these units, four treatments were applied: mycorrhiza (M), polymer (P), osmoprotectant (O), and polymer + osmoprotectant (P+O). In addition, an untreated microcatchment control (C-V) and a terraced control (C-A) were included. The C-A treatment represented conventional afforestation practice and received no plant-supportive treatments, as the primary objective was to compare V-shaped microcatchments against traditional terracing rather than to assess the interaction between catchment type and soil amendments. In total, 18 experimental units

were established across the three sites, comprising 360 seedlings.

A commercial mycorrhizal mixture (RhizoMyx[®], Novozymes) containing arbuscular mycorrhizal fungi and growth-enhancing components (i.e., humic acids, amino acids, vitamins, and organic extracts; Table 1) was used for mycorrhizal treatments. For application, 1 g of the mycorrhizal mixture was dissolved in 500 mL of water and applied to each seedling at the time of planting. For osmoprotectant treatments, a commercial product containing glycine betaine as the active ingredient was used. A 0.5% glycine betaine solution was applied as a foliar spray four times during the growing season (June, July, August, and September) using a backpack sprayer. For polymer treatments, a straw-based superabsorbent polymer (Natural Aquatic[®]) was applied at planting. The polymer, produced through the modification and acrylic polymerization of straw, has the capacity to absorb 200–300 times its volume in water and nutrients. Each seedling received 50 g of polymer, which was placed directly into the planting hole.

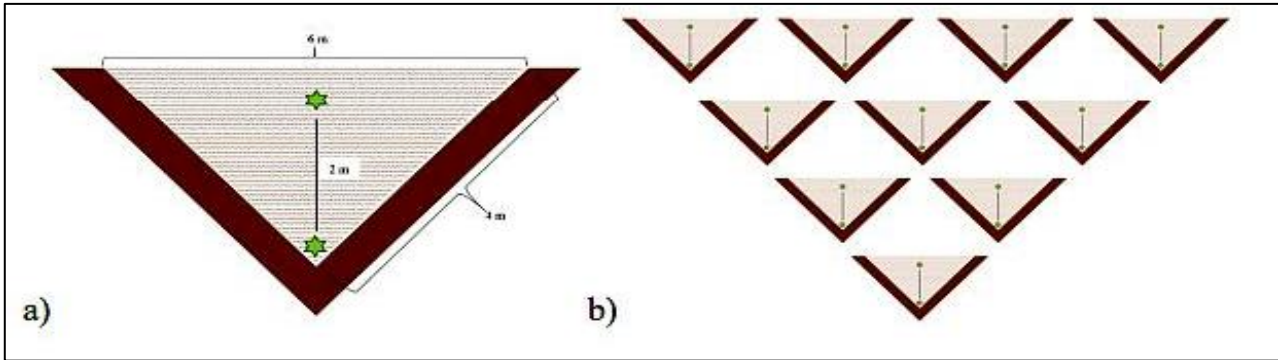


Figure 4. V-shaped microcatchment (a) and the appearance of V-shaped microcatchments in the experimental units (b)

Table 1. Composition of mycorrhizal mixture [RhizoMyx[®](Novozymes)]

Arbuscular Mycorrhiza	(propagule g ⁻¹)	Inert Ingredients	%
<i>Rhizophagus irregularis</i>	25	Humic acids	28.70
<i>Funneliformis mosseae</i>	24	Cold-water kelp extracts	18.00
<i>Glomus aggregatum</i>	24	Ascorbic acid	12.00
<i>Rhizophagus clarus</i>	1	Amino acids	6.00
<i>Glomus monosporum</i>	1	Myo-inositol	2.50
<i>Glomus deserticola</i>	1	Surfactant	2.50
<i>Glomus brasilianum</i>	1	Thiamine	1.75
<i>Glomus etunicatum</i>	1	Alpha-tocopherol	1.00
<i>Gigaspora margarita</i>	1		

2.6. Determination of mycorrhizal spore density in soil

Mycorrhizal spore density in soil was determined using the wet sieving method (Gerdemann & Nicolson, 1963). For this purpose, 10 g of soil sample collected from each sampling area was mixed with an adequate amount of water and allowed to stand for 1–2 minutes. The soil–water suspension was then thoroughly homogenized, left to settle again for 1–2 minutes, and subsequently passed through a series of sieves with mesh sizes of 750, 250, 125, and 50 μm . This procedure was repeated until the filtrate became clear.

The material retained on the sieves was transferred into 100 mL centrifuge tubes and subjected to centrifugation. After centrifugation, the supernatant was discarded, and a 60% glucose solution was added to the remaining sediment, followed by a second centrifugation step. The sugar solution was then decanted, and distilled water was added to the samples. The resulting samples were examined under a light microscope at 40 \times magnification, and mycorrhizal spores were counted (Gerdemann & Nicolson, 1963). Spore density was calculated as the number of spores per unit weight of soil using the following equation (Mahulette et al., 2022).

3. Results

3.1. Effects of treatments on root collar diameter

Root collar diameter (RCD) values for 2025 are presented in Figure 5. A statistically significant difference was detected among treatments ($p=0.0165$). While no significant differences were found among the V-shaped microcatchment treatments (C-V, M, P, O, and P+O), their mean RCD was approximately 60.4% higher than that of the C-A treatment.

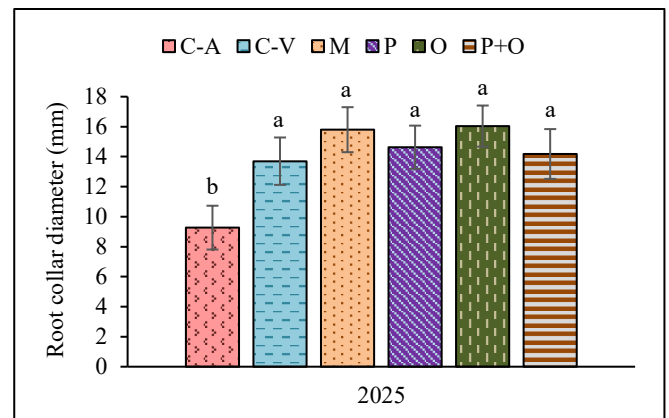


Figure 5. Effects of different treatments on root collar diameter (RCD) of *Pistacia lentiscus* seedlings in 2025

3.2. Effects of treatments on seedling height

Seedling height values for 2025 are presented in Figure 6. A statistically significant difference was detected among treatments ($p=0.0004$). While no significant differences were found among the V-shaped microcatchment treatments (C-V, M, P, O, and P+O), their mean seedling height was approximately 77.9% higher than that of the C-A treatment.

3.3. Effects of treatments on seedling sturdiness index

Seedling sturdiness index (SI) values for 2025 are presented in Figure 7. A statistically significant difference was detected among treatments ($p<0.0001$). The P treatment exhibited approximately 41% higher values than the C-V and P+O treatments, and about 115% higher than the C-A treatment. The mean SI values of the M, P+O, O, and C-V treatments were approximately 66.4% higher than that of the C-A treatment. In addition, the highest SI value was observed in the P treatment, whereas the lowest value was recorded in the C-A treatment. However, no statistically significant differences were found between the P treatment and the M and O treatments.

3.4. Effects of treatments on mycorrhizal spore density

Mycorrhizal spore counts for June and September are presented in Figure 8. A statistically significant difference was detected among treatments in both sampling periods ($p<0.0001$). In June, in terms of mycorrhizal spore density, the M treatment exhibited the highest value among all treatments. The M treatment was approximately 16.3 times higher than C-A, and 157.9%, 58.1%, 145.0%, and 133.3% higher than C-V, P, O, and P+O, respectively. The P treatment was approximately 10.3 times higher than C-A, and 63.2%, 55.0%, and 47.6% higher than C-V, O, and P+O, respectively. In addition, the mean value of the P+O, O, and C-V treatments was approximately 6.7 times (about 566.7%) higher than that of the C-A treatment.

In September, the M treatment exhibited the highest mycorrhizal spore density among all treatments, being 288.9% higher than C-A, 52.2% higher than C-V, 34.6% higher than P, 66.7% higher than O, and 45.8% higher than P+O. The P treatment also showed higher values than C-A, C-V, and O, being 188.9% higher than C-A, 13.0% higher than C-V, and 23.8% higher than O. In addition, the P+O, C-V, and O treatments were approximately 2.52 times (about 151.9%) higher than the C-A treatment.

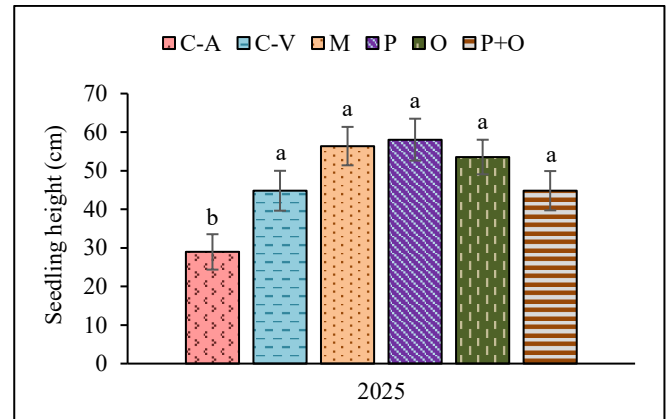


Figure 6. Effects of different treatments on seedling height of *Pistacia lentiscus* seedlings in 2025

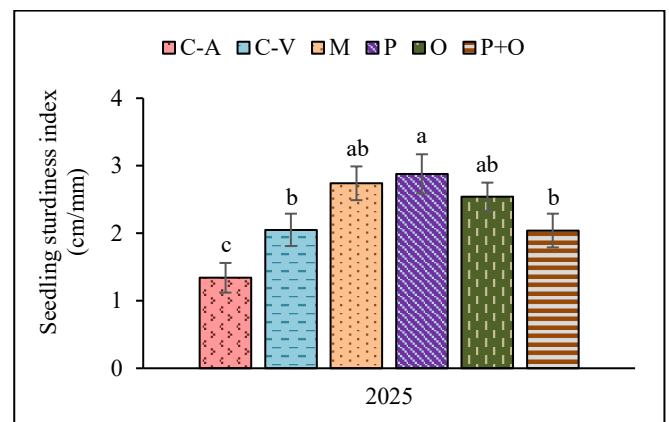


Figure 7. Effects of different treatments on seedling sturdiness index of *Pistacia lentiscus* seedlings in 2025

4. Discussion

The findings of this study clearly demonstrate that V-shaped microcatchment techniques, combined with plant-supportive treatments, play a decisive role in improving seedling performance under semi-arid Mediterranean conditions. The consistently lower values observed in all growth parameters under C-A treatment indicate that conventional afforestation practices are insufficient to sustain optimal seedling development in water-limited environments. Similar limitations of traditional methods under drought conditions have been reported in previous studies, where low soil moisture availability resulted in reduced growth and survival rates (Omer & Ahmed, 2021).

In contrast, all microcatchment-based treatments, including C-V treatment, exhibited superior growth performance compared to the terrace control. This highlights the effectiveness of V-shaped microcatchment systems in enhancing soil moisture availability within the root zone. Microcatchment structures reduce surface runoff and promote water infiltration, thereby increasing plant-available water.

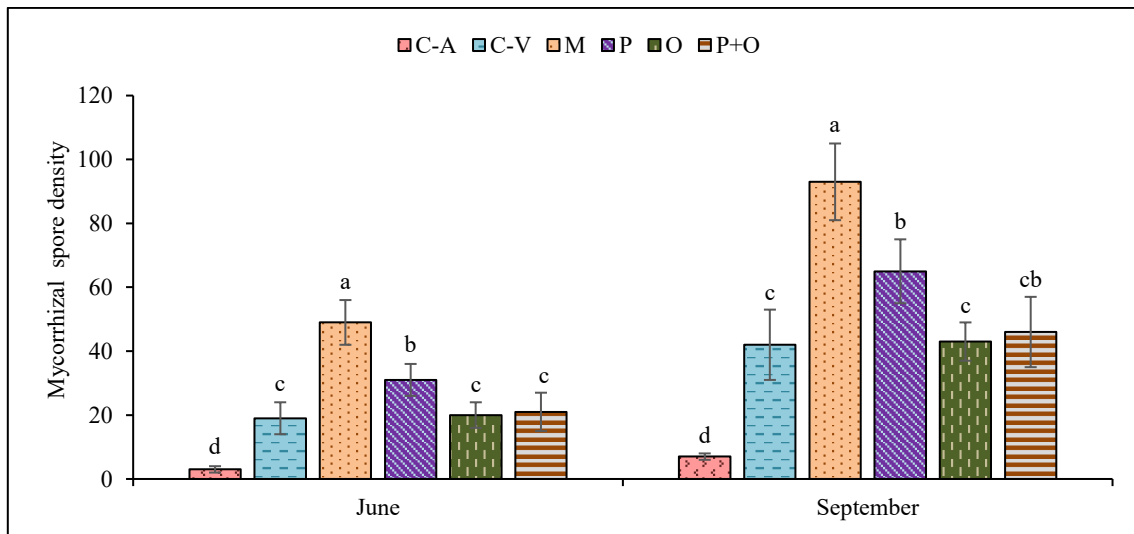


Figure 8. Effects of different treatments on mycorrhizal spore density in June and September

These findings are consistent with previous studies demonstrating that microcatchment techniques significantly improve soil moisture and seedling growth (Karaşin et al., 2024; Ma et al., 2024; Aydın et al., 2025). Moreover, studies conducted in different ecosystems have shown that rainwater harvesting systems can substantially enhance plant height and root collar diameter (Singh et al., 2013; Feng et al., 2024).

The increased growth observed in microcatchment treatments is also supported by the findings of Siyum et al. (2019), who reported that moisture harvesting structures significantly improved seedling height, root collar diameter, and crown width compared to control treatments. Similarly, in a study conducted under Mediterranean conditions, crescent-shaped rainwater harvesting structures significantly enhanced the growth performance of *Pistacia lentiscus* seedlings (Kamer et al., 2026).

When the treatments are evaluated, the relatively higher seedling height and sturdiness index observed under the P treatment indicate the important role of water-retentive materials in mitigating water stress under semi-arid conditions. Superabsorbent polymers enhance soil water-holding capacity and provide a gradual water supply to plants during dry periods, thereby supporting growth performance. These findings are consistent with previous studies showing that polymer applications improve plant growth and drought tolerance (Ünlü et al., 2025). Furthermore, when combined with microcatchment systems, these materials contribute to prolonged moisture retention in the root zone, indirectly supporting plant development (Karaşin et al., 2026).

Similarly, the relatively greater root collar diameter observed under the O treatment indicates that physiological stress mitigation mechanisms may

influence plant development under drought conditions. Osmoprotectants help maintain cellular osmotic balance and improve plant tolerance to water stress, allowing growth processes to continue more steadily (Ünlü et al., 2025). In this context, the integration of structural water management approaches with physiological support may create a complementary effect in enhancing plant growth performance.

The positive effects of M treatment applications are also supported by previous research. Mycorrhizal fungi enhance water and nutrient uptake by increasing the effective root surface area, thereby improving plant performance under drought stress. In this context, the findings of this study are consistent with those of Ma et al. (2024), who reported that rainwater harvesting systems improve plant hydraulic conductivity and root-related traits.

The absence of significant differences among microcatchment treatments, despite their clear superiority over C-A treatment, suggests that water availability is the primary limiting factor in this ecosystem. Once sufficient soil moisture is provided through microcatchment systems, additional treatments such as polymer, osmoprotectant, and mycorrhiza contribute to growth through complementary mechanisms.

Overall, the results indicate that rainwater harvesting techniques, particularly V-shaped microcatchment systems, play a critical role in enhancing afforestation success under semi-arid conditions. The integration of these systems with plant-supportive applications such as polymers, osmoprotectants, and mycorrhiza offers an effective and sustainable strategy for improving seedling establishment and growth in water-limited environments. These findings emphasize the

importance of integrated water management approaches for afforestation under increasing climate change-induced drought conditions.

5. Conclusion

The findings of this study clearly show that V-shaped microcatchment techniques play a significant role in improving the growth performance of *Pistacia lentiscus* seedlings under semi-arid Mediterranean conditions. The low growth values observed in C-A highlight the limitations of conventional afforestation practices in water-limited environments. In contrast, C-V proved to be highly effective in enhancing soil moisture availability, leading to noticeable improvements in both seedling height and root collar diameter.

Overall, the results clearly indicate that water availability is the main limiting factor for afforestation success in semi-arid environments. The study clearly demonstrated the positive effects of the microcatchment when combined with supportive treatments such as polymers, osmoprotectants, and mycorrhiza.

In conclusion, the combined use of V-shaped microcatchment systems with plant-supportive treatments offers an effective and practical approach to improving seedling performance under water-limited conditions. These findings contribute to the development of more sustainable afforestation strategies, particularly in regions increasingly affected by climate change.

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Conflict of interest

The authors declare no conflict of interest.

Ethical Approval

This article does not require ethics committee approval.

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