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The Impact of Mycorrhiza and Trichoderma Treatment on Malondialdehyde Levels and Antioxidant Activity in Common Beans under Drought Stress

Kuraklık Stresi Altındaki Fasulyelerde Mikoriza ve Trichoderma Tedavisinin Malondialdehit Düzeyleri ve Antioksidan Aktivitesi Üzerindeki Etkisi

Hilal Yılmaz[1]

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Abstract: As global temperatures rise and drought conditions become increasingly frequent, the need to develop sustainable agricultural practices has become paramount. Enhancing crop resilience to water scarcity is essential to secure food supplies for a growing global population. This study examined the effects of Arbuscular Mycorrhizal Fungi (AMF) and *Trichoderma harzianum* on the physiological responses and growth of common bean (*Phaseolus vulgaris*) under 100% and 50% irrigation regimes. Under a 50% irrigation regime, AMF and *Trichoderma harzianum* inoculation led to substantial increases in plant height (34.5%) and root length (16.79%), compared to the control. Additionally, significant enhancements were observed in chlorophyll a (175%), chlorophyll b (194%), and total chlorophyll (180%) content in plants subjected to *T. harzianum* inoculation under water deficit. The application of AMF resulted in an 18% increase in total carotenoid content, showing its efficacy in sustaining photosynthetic pigments. Furthermore, the study revealed that both treatments significantly reduced malondialdehyde (MDA) accumulation, with reductions of 46.3% compared to the control under drought conditions. Catalase (CAT), increased by 201% with *T. harzianum* application under full irrigation and by 217% with AMF under reduced irrigation, highlighting the role of these biostimulants in mitigating oxidative stress. Principal component analysis (PCA) further confirmed that these treatments effectively maintained cellular integrity and enhanced stress tolerance. These findings underscore the potential of AMF and *T. harzianum* as vital tools in enhancing crop resilience against drought, with significant implications for sustainable agriculture in arid and semi-arid regions.

Keywords: Drought tolerance, biostimulants, oxidative stress mitigation, AMF, global warming

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Öz: Küresel sıcaklıkların artması ve kuraklık koşullarının giderek daha sık hale gelmesiyle birlikte, sürdürülebilir tarım uygulamalarını geliştirme ihtiyacı her zamankinden daha önemLi hale gelmiştir. Su kıtlığına karşı bitki direncinin artırılması, artan dünya nüfusunun gıda tedarikini güvence altına almak için hayati bir öneme sahiptir. Bu çalışmada, Arbusküler Mikoriza Fungus (AMF) ve *Trichoderma harzianum*'un, fasulye (*Phaseolus vulgaris*) bitkisinin fizyolojik tepkileri ve büyümesi üzerindeki etkileri %100 ve %50 sulama rejimLeri altında incelenmiştir. %50 sulama rejimi altında, AMF ve *T. harzianum* inokülasyonu, kontrol grubuna kıyasla bitki boyunda %34.5, kök uzunluğunda ise %16.79 oranında önemLi artışlar sağlamıştır. Ayrıca, su kısıtı koşullarında *T. harzianum* uygulanan bitkilerde klorofil a (%175), klorofil b (%194) ve toplam klorofil (%180) içeriğinde belirgin artışlar gözlemLenmiştir. AMF uygulaması, toplam karotenoid içeriğinde %18'lik bir artış sağlayarak fotosentetik pigmentlerin sürdürülebilirliğini göstermiştir. Bunun yanı sıra, her iki uygulamanın da malondialdehit (MDA) birikimini önemLi ölçüde azalttığı, kuraklık koşullarında kontrol grubuna kıyasla %46.3 oranında azalma sağladığı tespit edilmiştir. Katalaz (CAT), tam sulama altında *T. harzianum* uygulamasıyla %201, azaltılmış sulama altında ise AMF ile %217 artış göstermiştir, bu da bu biyostimülanların oksidatif stresi hafifletmedeki rolünü vurgulamaktadır. Temel bileşen analizi (PCA), bu tedavilerin hücresel bütünlüğü etkili bir şekilde koruduğunu ve stres toleransını artırdığını doğrulamıştır. Bu bulgular, AMF ve *T. harzianum* nın, kuraklığa karşı bitki direncini artırmada hayati araçlar olarak potansiyelini, kurak ve yarı kurak bölgelerde sürdürülebilir tarım için önemLi sonuçlarla birlikte ortaya koymaktadır.

Anahtar Kelimeler: Kuraklığa tolerans, biyostimülanlar, oksidatif stres azaltma, AMF, küresel ısınma

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[1] Lecturer Dr. Hilal Yilmaz, Kocaeli University, Izmit Vocational School, Plant and Animal Production Program hilal.yilmaz@kocaeli.edu.tr (Corresponding author)

INTRODUCTION

Common beans (*Phaseolus vulgaris*), commonly called the "Poor Man's Meat" due to their high mineral, protein, and vitamin content, are a vital food source for over 300 million people worldwide (Blair et al., 2013; Yilmaz et al., 2023). According to the FAO, the global bean production area was 36.8 million hectares, with a production volume of 28.35 million tonnes in 2022 (FAO, 2024). A significant portion of this production occurs on lands with limited irrigation capabilities, leading to yield losses of up to 80% during unexpected drought periods (Rosales et al., 2012). Drought, resulting from extreme and rapid changes in climate conditions worldwide, is one of the most devastating abiotic stresses affecting plants during their growth and development stages. The frequency, severity, and duration of drought stress, along with the type of crop and agricultural region, amplify the extent of damage, posing a significant threat to global food security by reducing crop productivity (Khatun et al., 2021). In less developed and low-to-middle-income countries, more than 34% of plant and animal production losses are attributed to drought (FAO, 2021). The yield of major crops in drought-affected regions is projected to decrease by over 50% by the year 2050. (Malhi et al., 2021). Increasing droughts due to global climate change and conflicts from regional disputes led to 691 to 783 million people facing hunger worldwide in 2022 (WHO, 2023). The greatest challenge for agriculture in the coming years will be the sustainable production of sufficient food to meet the growing global demand (Agbodjato et al., 2022). Enhancing the drought tolerance of common bean is crucial for agricultural sustainability and food security (Yeken, 2023). To effectively address these challenges, it is paramount to develop plant varieties that are resistant to drought stress and to implement appropriate agricultural techniques. These strategies are essential for ensuring agricultural sustainability and securing global food supplies in the face of increasing environmental stressors.

In plants coping with drought stress, abscisic acid accumulates in the roots, while the flow of K+ ions from the leaves accelerates. This raises leaf temperature and speeds up transpiration, causing stomatal closure and reducing photosynthetic activity (Anjum et al., 2011; Farooq et al., 2012; Kaur and Asthir, 2017). Impaired water relations restrict shoot growth but enhance root growth (Claeys and Inzé, 2013). Drought stress impacts numerous cellular processes, including molecular and biochemical functions, signal perception, nutrient uptake, and photosynthesis (Wahab et al., 2022). It also affects hormone production, reducing growth and crop productivity (Prasad et al., 2011; Farooq et al., 2012). Drought stress induces cellular dehydration, leading to secondary stresses such as osmotic and oxidative stress (Yang et al., 2021). This condition results in increased production of reactive oxygen species (ROS) in cellular compartments like chloroplasts and mitochondria (Foyer and Hanke, 2022). Plants have developed various strategies to manage the increase in ROS, including ROS detoxification and the maintenance of cellular redox balance (Wahab et al., 2022). The detoxification mechanism involves an antioxidative defense system comprising both enzymatic and non-enzymatic components (Soares et al., 2019; Ilyas et al., 2021). Additionally, plants' adaptive strategies in response to drought stress include osmoregulation mechanisms, cellular water potential maintenance, and water use efficiency enhancement. These strategies are critical for improving plant survival and productivity (Gupta et al., 2020; Öztürk et al., 2021). Various approaches exist to combat drought, but recently, the use of biostimulants as a sustainable strategy has gained attention. Biostimulants have emerged as a significant solution in modern agriculture to enhance crop productivity under the pressures of increasing population and environmental degradation (Lephatsi et al., 2022). Among biostimulants, arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum*, which support plant-root symbiosis and optimize nutrient uptake, are particularly noteworthy. AMF is primarily used as a biofertilizer capable of forming symbiotic interactions with approximately 90% of crop plants (Ferlian et al., 2018; Yilmaz et al., 2022). By colonizing plant roots, AMF enhances water and nutrient availability, soil health, and productivity, thereby increasing plant resilience to stress conditions (Wu and Zou, 2017; Yilmaz et al., 2023). Numerous studies have demonstrated AMF's beneficial effects, including improved plant growth, reduced drought damage, and rapid recovery once stress is alleviated (Abdel-Salam et al., 2018; Zhang et al., 2019; Begum et al., 2019; Mathur et al., 2019; Sheteiwy et al., 2021; Eshaghi Gorgi et al., 2022). Trichoderma's efficacy against drought stress involves mechanisms such as morphological adaptations to avoid drought, physiological and biochemical changes to develop drought tolerance, and enhanced post-drought recovery (Shukla et al., 2012; Kaur and Kumar, 2020; Boorboori and Zhang, 2023). Various studies have shown that *Trichoderma harzianum* enhances plant growth by increasing nutrient access through root colonization and elevating enzyme levels that reduce reactive oxygen species (ROS), thereby conferring stress resistance (Shukla et al., 2012; Mona et al., 2017; Khoshmanzar et al., 2020).

This study aims to investigate the effects of AMF and *Trichoderma harzianum* inoculation on a dwarf common bean variety under 50% water deficit conditions, focusing on plant growth parameters, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, malondialdehyde (MDA), and the activities of ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) enzymes. This research is significant as it explores novel biostimulant applications to enhance drought tolerance in common beans, offering potential advancements in agricultural sustainability and resilience against environmental stressors.

MATERIAL AND METHOD

Plant Material

The dwarf common bean variety "Yunus 90," obtained from the Eskişehir Transitional Zone Agricultural Research Institute, was used as the plant material. The study was conducted in the research greenhouse of the Faculty of Agriculture at Bolu Abant İzzet Baysal University between April and June 2024.

AMF and Trichoderma Applications

For the mycorrhiza application, a powder form ERS (Endo Roots Soluble) package containing 9 different AMFs was obtained from Bioglobal®. The package contains 78.85 propagules gram^{-1} of mycorrhiza. The contents of the package include fungi: *Glomus intraradices* (25), *Glomus aggregatum* (24), *Glomus mosseae* (24), *Glomus clarum* (1), *Glomus monosporus* (1), *Glomus deserticola* (1), *Glomus brasilianum* (1), *Glomus etunicatum* (1), and *Gigaspora margarita* (1). The application was carried out as recommended by the company for 7500 seedlings/250 grams. Additionally, Trichoderma fungus, specifically *Trichoderma harzianum* Rifai KRL-AG2 strain T-22 Planter Box, was purchased from Bioglobal®. The powder mixture contains 4x10^{8} (400 million spores/g) fungi per gram. The application was performed as recommended by the company (7500 seedlings/50 grams). The AMF and *T. harzianum* were applied in powder form to the seedbed before planting. Before sowing, the seeds were surface-sterilized by immersing them in 1% sodium hypochlorite for 2 minutes and then rinsing them three times with sterile distilled water. The pots, each with a capacity of 1 kg, were filled with a mixture of 2/3 soil and 1/3 peat (Abant torf®). Three seeds were planted in each pot. Immediately after emergence, the seedlings were thinned to leave one plant per pot. The study was designed with 3 treatments (Control, AMF, *Trichoderma harzianum*) and 2 irrigation periods (100% Field Capacity (Full), 50% Field Capacity (Half). The experiment was conducted in a randomized plot design with three replications. To determine the field capacity, the soil was saturated with water and allowed to stand for 24 hours. The amount of water held by the soil particles against gravity was weighed to determine 100% field capacity (Özel et al., 2016). Depending on the air temperature, the pots were weighed every 2-3 days, and the amount of water lost was calculated and brought to the desired field capacity. The transition to 50% field capacity irrigation was gradually made after the common bean seedlings produced true leaves. The plants were harvested after a 5-week growing period. Leaf samples were immediately stored at -80°C until analysis.

Physical Analyses, Chlorophyll, and Carotenoid Contents

As a physical analysis, the stem and root lengths (cm) of each plant in the experiment were measured. Total chlorophyll, total carotenoid, chlorophyll a, and b were determined using the Arnon method (Arnon, 1949). 0.1 gram leaf sample was homogenized with 80% acetone. The absorbance of the final mixture was determined at wavelengths of 663, 645, and 470 nm using a UV-visible spectrophotometer. The concentration of chlorophyll (a, b, total) was expressed as mg/g fresh weight. The determination of chlorophyll a, b, and total was carried out using the formula by Arnon:

$$\text{Chlorophyll a (mg g}^{-1}\text{ F.W)} = (12.7\ A663\ 2.69\ A645) \times V / 1000 \times g \qquad (1)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ F.W)} = (22.9\ A645 - 4.68\ A663) \times V / 1000 \times g \qquad (2)$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ F.W)} = (20.2\ A645 + 8.02\ A663) \times V / 1000 \times g \qquad (3)$$

100 mg leaf sample was homogenized with 80% (v/v) acetone and filtered using filter paper. In the obtained extract, absorbance values were measured at 470 nm with a spectrophotometer to determine the carotenoid content.

$$\text{Carotenoid (mg g}^{1}\text{)} = [((1000 \times A470) - (2.27 \times Cla) - (81.4 \times Clb)) / 227] \times V / g \qquad (4)$$

Here, V represents the volume of the extract, g represents the sample volume (mg), Cla represents chlorophyll-a, Clb represents chlorophyll-b, and A represents the absorbance at specific wavelengths.

Malondialdehyde (MDA) Analysis

Lipid peroxidation levels were determined by measuring the malondialdehyde (MDA) content, a product of lipid peroxidation. 500 mg plant sample was homogenized with 10 mL of 0.1% trichloroacetic acid (TCA). The mixture was centrifuged at 15,000 RPM. From the supernatant, 1 mL was taken, and 4 mL of the reaction mixture (20% TCA, 0.5% 2-thiobarbituric acid) was added. The mixture was incubated in a water bath at 95°C for 30 minutes. The rapidly cooled samples' absorbance was measured at 532 and 600 nm wavelengths (Sairam and Saxena, 2000; Canal et al., 2023).

Ascorbate Peroxidase (APX), Catalase (CAT), and Superoxide Dismutase (SOD) Analysis

The activity of ascorbate peroxidase (APX) was determined by measuring the change in absorbance at 290 nm. 200 mg sample was homogenized with 2 mL of extraction mixture (0.1 M sodium phosphate, 0.5 mM sodium EDTA, and 1 mM ascorbic acid). The mixture was then centrifuged at 15.000 RPM. To 2.8 mL of reaction mixture (50 mM sodium phosphate (pH:7), 0.5 mM ascorbic acid, 0.1 mM EDTA), 0.1 mL of sample extract was added. After adding 0.1 mL of 0.1 mM H_2O_2, the mixture was incubated for 60 minutes. The activity was calculated against an ascorbic acid standard, prepared by diluting 100 µM ascorbic acid (Yilmaz and Kulaz, 2019). Superoxide dismutase (SOD) enzyme activity was analyzed by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), according to the method proposed by Beauchamp and Fridovich (1971). 200 mg sample was homogenized with 2 mL of extraction mixture (0.1 M sodium phosphate and 0.5 mM sodium EDTA). The mixture was then centrifuged at 15.000 RPM. For SOD analysis, 0.1 mL of supernatant was taken, and 2.9 mL of reaction mixture (11.33 mM methionine, 75 µM nitroblue tetrazolium, 0.1 mM EDTA, 50 mM sodium phosphate (pH: 7.8), 50 mM sodium carbonate) and 0.1 mL of 2 mM riboflavin were added and vortexed. The tubes were then placed under light (75 mol m^{-2} s^{-1} (40 W)) for 15 minutes to start the reaction. Readings were taken at 560 nm. For catalase (CAT) analysis (EC 1.11.1.6), a reaction solution containing 0.036% hydrogen peroxide and 50 mM sodium phosphate (pH: 7) was prepared. 3 mL of the prepared reaction mixture was placed in a quartz cuvette and inserted into the spectrophotometer. Then, 100 microliters of the supernatant obtained from the SOD analysis extraction were added. Absorbance values were taken at 240 nm with a UV-visible spectrophotometer at 0 and 60 seconds (Beers and Sizer, 1952).

Statistical Analysis

The study was conducted in randomized plot designs consisting of three biological and three technical replicates for each treatment. The effects of water restriction AMF and Trichoderma inoculation were determined by performing a one-way analysis of variance (ANOVA). Differences between control and AMF and *T. harzianum* treatments were evaluated using LSD test. Correlation analysis was utilized to eliminate the intrinsic correlations of yield parameters and enzymes, and correlations were conducted only between yield characteristics and enzymes/chlorophyll for water restriction levels. Pearson's coefficient was used in the correlation analyses, and the data were visualized using the 'corplot' R package (Wei et al., 2017). The relationship between water-restricted AMF and *T. harzianum* applications and the examined characteristics was determined using principal component analysis (PCA) with the 'ggplot2' R package (Wickham, 2016).

RESULTS AND DISCUSSION

Growth Parameters

The study reveals that common bean subjected to a 50% irrigation regime exhibited significant reductions in both stem and root lengths compared to those under a 100% irrigation regime. Inoculation with AMF (Arbuscular Mycorrhizal Fungi) and *Trichoderma harzianum* significantly influenced plant height and root length in both irrigation treatments ($p \leq 0.05$, $p \leq 0.01$) (Figure 1; Table 1). Under 100% irrigation, plant height increased by 19.4%, from 21.50 cm to 25.67 cm, while under 50% irrigation, plant height rose by 34.5%, from 14.00 cm to 18.83 cm, compared to the control. Similarly, root length increased by 15.13%, from 46.33 cm to 53.34 cm, under 100% irrigation, and by 16.79%, from 39.67 cm to 46.33 cm, under 50% irrigation. Regarding the effects of inoculation, no significant difference in plant height and root length was observed between AMF and *T. harzianum* treatments under 100% irrigation. Under 50% irrigation, a statistically significant difference in plant height was observed between AMF and *T. harzianum* inoculations, whereas no significant difference was found in root length.

Table 1. Statistically significant differences of full irrigation, half irrigation regimes, mycorrhiza, and *T. harzianum* treatments.

Çizelge 1. Tam sulama, yarım sulama rejimleri, mikoriza ve T. harzianum tedavilerinin istatistiksel olarak anlamlı farklılıkları.

Trait	$F_{Half\ Irrigation}$	$F_{Full\ Irrigation}$	$F_{Irrigation*Treatment}$
Plant Height	28.30**	14.60**	0.28^{ns}
Root Lenght	11.26**	9.21*	1.49^{ns}
Chlorophyll a	64.77**	6.39*	13.48*
Chlorophyll b	51.39**	2.03^{ns}	13.45*
Total Chlorophyll	60.87**	4.97^{ns}	13.28*
Total Carotenoid	3.14^{ns}	56.11**	7.24*
MDA	16.62**	2.81^{ns}	2.71^{ns}
SOD	5.12*	13.71**	4.25*
CAT	25.11**	7.57*	10.20*
APX	20.19**	12.76**	0.89^{ns}

İndicate significant differences according to LSD test; ns: non-significant, * *($p \leq 0.05$)*, ** *($p \leq 0.01$)*

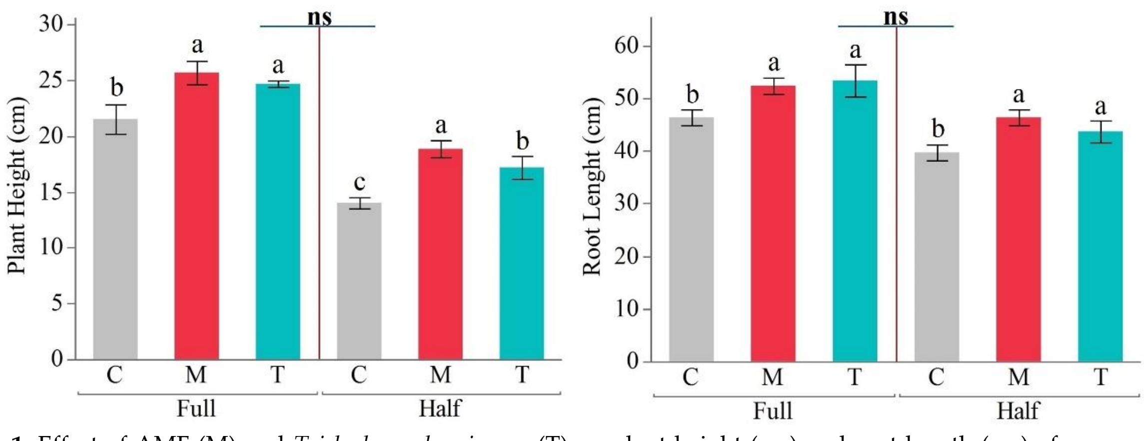

Figure 1. Effect of AMF (M) and *Trichoderma harzianum* (T) on plant height (cm) and root length (cm) of common bean under different irrigation regimes (100%-50%) conditions (C: Control, Different letters indicate significant differences according to LSD test, ns: not significant)

Şekil 1. Farkı sulama rejimi (%100-%50) koşullarında AMF (M) ve Trichoderma harzianum'un (T) fasulyenin bitki boyu (cm) ve kök uzunluğu (cm) üzerine etkisi (C: Kontrol, Farklı harfler LSD testine göre önemLi farklılıkları göstermektedir, ns: önemsiz)

The findings of this study are consistent with previous research indicating that water stress significantly impedes plant growth, affecting both stem and root development (Li et al., 2019; Pavithra and Yapa 2018; Begum et al., 2019; Mona et al., 2017; Bashyal et al., 2021). The enhanced growth observed in plants treated with AMF and *T. harzianum* can be attributed to the improved water and nutrient uptake facilitated by these microorganisms, as supported by similar studies (Poveda et al., 2019; Nanjundappa et al., 2019). Specifically, AMF have been shown to enhance root hydraulic conductivity and improve drought tolerance by maintaining higher water potential and photosynthetic rates (Abdalla et al., 2023; Abdalla and Ahmet, 2021). Similarly, Trichoderma spp. are known for their role in enhancing plant growth under stress conditions through mechanisms such as increased root growth, improved nutrient uptake, and induced systemic resistance (Gupta and Bar, 2020; Azad and Kaminskyj 2016). The significant increase in plant height and root length under 50% irrigation with microbial inoculation highlights the potential of AMF and *T. harzianum* as biostimulants to mitigate the adverse effects of drought stress. This is particularly important for sustainable agriculture in arid and semi-arid regions where water scarcity is a major challenge (Meddich et al., 2022).

Chlorophyll-a, Chlorophyll-b, Chlorophyll a/b, Total Chlorophyll and Total Carotenoid

In this study, under the 100% irrigation regime, chlorophyll a values ranged from 0.0027 to 0.0052 mg g^{-1} F.W., chlorophyll b values ranged from 0.0014 to 0.0023 mg g^{-1} F.W., total chlorophyll content ranged from 0.0042 to 0.0075 mg g^{-1} F.W., and total carotenoid content ranged from 275.88 to 495.35 mg g^{-1} F.W. (Figure 2; Table 1). Under the 50% irrigation regime, chlorophyll a values ranged from 0.004 to 0.011 mg g^{-1} F.W., chlorophyll b values ranged from 0.0017 to 0.0050 mg g^{-1} F.W., total chlorophyll content ranged from 0.0057 to 0.016 mg g^{-1} F.W., and total carotenoid content ranged from 344.32 to 426.84 mg g^{-1} F.W.

In common beans subjected to 100% irrigation, significant differences were observed in all parameters except for chlorophyll b (chlorophyll a, total chlorophyll, total carotenoid) at $p \leq 0.05$ *and* $p \leq 0.01$ (Table 1). The application of AMF significantly increased the chlorophyll a, total chlorophyll, and carotenoid contents of common bean compared to the control group, with increments of 92.6% for chlorophyll a, 78.6% for total chlorophyll content, and 79.2% for total carotenoid content. Although the increase for chlorophyll b was 64.3%, it was not statistically significant. Under 50% irrigation, common beans exhibited statistically significant differences at $p \leq 0.05$ in all parameters except for total carotenoid content (chlorophyll a, chlorophyll b, and total chlorophyll). In common beans subjected to 50% water restriction, *T. harzianum* inoculation significantly increased the contents of chlorophyll a, chlorophyll b, and total chlorophyll compared to the control group, with increments of 175% for chlorophyll a, 194% for chlorophyll b, and 180% for total chlorophyll. The most significant increase in total carotenoid content compared to the control was observed with AMF application (18%); however, no statistical difference was found among the treatments.

The results of this study highlight the significant impact of irrigation regimes on chlorophyll and carotenoid content in common bean, providing insights that align with and expand upon existing literature. Moderate to severe drought conditions reduce leaf number and area, diminishing the levels of key photosynthetic pigments, such as chlorophylls and carotenoids. This reduction ultimately disrupts plants' photosynthetic efficiency (Zhang et al., 2018; Mashabela et al., 2023; Spinoso-Castillo et al., 2023; El-Sawah et al., 2023). Chlorophyll degradation is enhanced by the increased expression of chlorophyll-degrading enzymes, while the biosynthesis of chlorophyll is reduced due to the downregulation of its associated enzymes, leading to a decrease in chlorophyll content (Ilyas et al., 2021; Saxena et al., 2022). Nevertheless, inoculating plants with Arbuscular Mycorrhizal Fungi (AMF) under drought conditions has been shown to mitigate these effects by enhancing chlorophyll levels (Shankar et al., 2024). In a study involving *Cicer arietinum*, it was observed that drought stress reduced chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll content by 54.61%, 46.81%, and 39.84%, respectively. However, AMF inoculation significantly alleviated these reductions, resulting in increases of 60.08%, 40.87%, and 45.87% in Chl a, Chl b, and total chlorophyll content, respectively (Hashem et al., 2019). Similar observations were reported in *Zea mays, Triticum aestivum, Nicotiana tabacum, and Rosa damascena Mill.* (Hu et al., 2020; Begum et al., 2019; Mathur et al., 2019; Begum et al., 2020; Abdel-Salam et al., 2018).

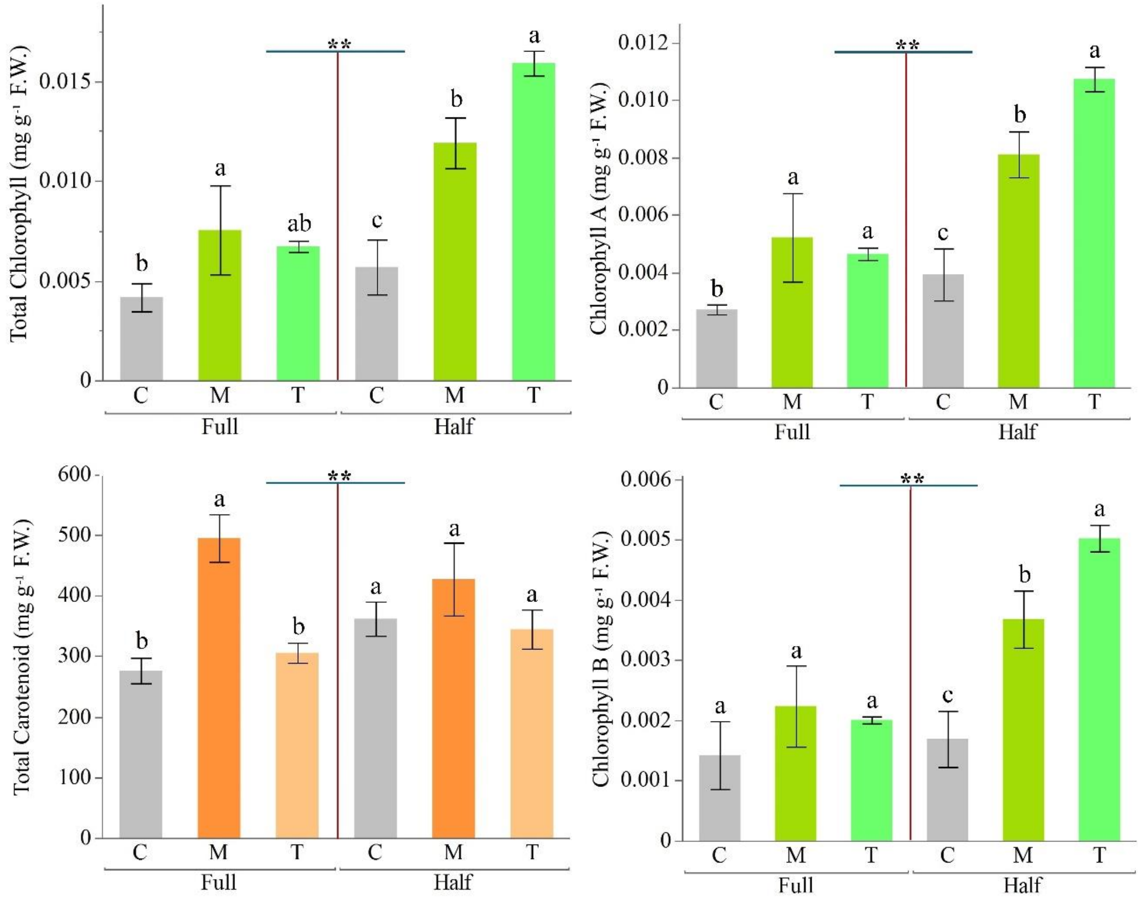

Figure 2. Effect of AMF (M) and *Trichoderma harzianum* (T) on chlorophyll a, b, total chlorophyll, and total carotenoid content of common bean under different irrigation regimes (100%-50%) conditions. (C: Control, Different letters top of the bars indicate significant differences according to LSD test, ** ($p \leq 0.01$).

*Şekil 2. AMF (M) ve Trichoderma harzianum'un (T) farklı sulama rejimLeri altında (%100-%50) fasulyenin klorofil a, b, toplam klorofil ve toplam karotenoid içeriğine etkisi. (C: Kontrol, Çubukların üstündeki farklı harfler LSD testine göre önemLi farklılıkları göstermektedir,,** ($p \leq 0.01$).*

In the study by Shukla et al. (2015), seed biopriming with drought-tolerant isolates of *T. harzianum* significantly increased chlorophyll a and b content in *Triticum aestivum* under drought stress. This enhancement in chlorophyll levels contributed to maintaining the plants' photosynthetic capacity, thereby improving their overall drought tolerance. Similar results have been observed in studies involving *Oryza sativa*, *Zea mays*, and *Glycine max*, where *T. harzianum* application enhanced chlorophyll content and improved drought tolerance (Pandey et al., 2016; Musaddaq et al., 2021; Nahrawy et al., 2020). These findings highlight the potential of AMF and *T. harzianum* to protect and maintain chlorophyll levels in plants under drought-stress conditions. The use of AMF and *T. harzianum* plays a critical role in maintaining chlorophyll and carotenoid levels under drought conditions by enhancing antioxidant activity and reducing oxidative stress. This physiological response not only preserves photosynthetic efficiency but also supports the overall metabolic stability of plants, allowing them to better withstand water-limited environments. The ability of these biostimulants to modulate stress-related metabolic pathways suggests their potential as effective tools for improving crop resilience to drought.

Malondialdehyde (MDA) Levels and Antioxidant Enzymes (SOD, CAT, APX) Activity

In this study, MDA accumulation in common bean under the 100% irrigation regime ranged from 13.08 to 16.52 nmol g^{-1} F.W., with the highest levels observed in the control group (Figure 3; Table 1). Although MDA levels were 26.3% higher in the control plants compared to those treated with *T. harzianum*, the difference was not statistically significant. Under the 50% irrigation regime, MDA levels ranged from 16.34 to 23.91 nmol g^{-1}

F.W. Significant differences ($p \leq 0.01$) were observed between the control and both *T. harzianum* and AMF treatments, with no statistical difference between *T. harzianum* and AMF themselves. Notably, MDA accumulation in the control plants was 46.3% higher compared to those treated with AMF. These results indicate that *T. harzianum* and AMF are highly effective in reducing oxidative damage in common bean under drought stress. By clearly demonstrating the ability of these biostimulants to mitigate oxidative stress, the study underscores their potential to enhance plant resilience under challenging environmental conditions. The significant reduction in MDA accumulation, particularly under the 50% irrigation regime, aligns with findings from other studies on different crops. For example, in *Triticum aestivum* (wheat), *T. harzianum* similarly reduced MDA levels, indicating decreased lipid peroxidation and enhanced stress tolerance (Shukla et al., 2015; Singh et al., 2020). Additionally, *Oryza sativa* (rice) studies have shown that AMF inoculation leads to lower MDA accumulation, further supporting the role of these biostimulants in protecting plant cell membranes under stress conditions (Pandey et al., 2016).

These consistent findings across different crops suggest that the application of *T. harzianum* and AMF can be a broadly effective strategy to enhance plant resilience to drought-induced oxidative stress. These biostimulants' ability to reduce lipid peroxidation and protect cell integrity under limited water availability highlights their potential for improving crop performance in arid and semi-arid regions.

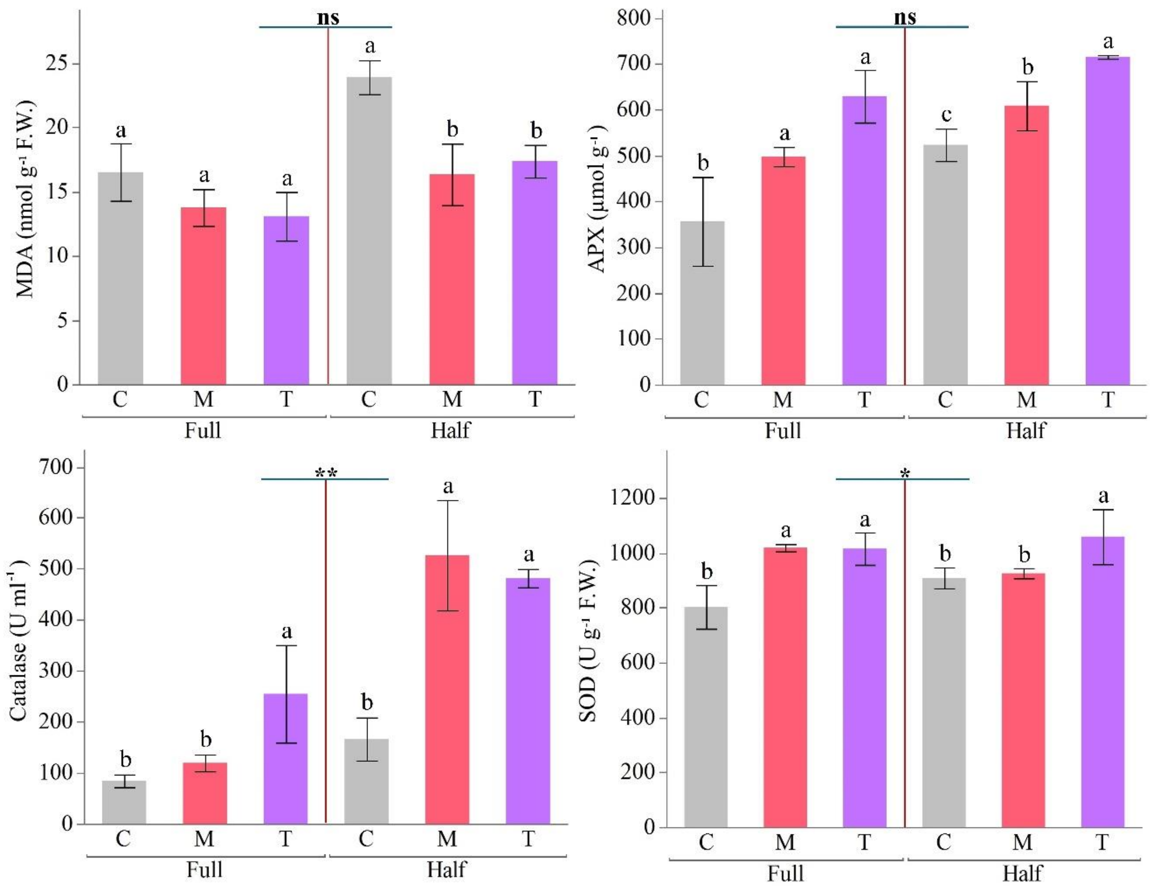

Figure 3. Effect of AMF (M) and *Trichoderma harzianum* (T) treatments on malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities in common bean under different irrigation regimes (100%-50%) conditions (C: Control, Different letters top of the bars indicate significant differences according to LSD test, ns: non-significant, * ($p \leq 0.05$), ** ($p \leq 0.01$)).

*Şekil 3. AMF (M) ve Trichoderma harzianum (T) uygulamalarının farklı sulama rejimLeri altında (%100-%50) fasulyede malondialdehit (MDA) düzeyleri, süperoksit dismutaz (SOD), katalaz (CAT) ve askorbat peroksidaz (APX) aktiviteleri üzerine etkisi (C: Kontrol, Çubukların üstündeki farklı harfler LSD testine göre anlamLı farklılıkları göstermektedir, ns: anlamsız, * (p ≤ 0.05), ** (p ≤ 0.01)).*

In this study, the activities of antioxidant enzymes in common bean grown under a 100% irrigation regime were assessed. The ascorbate peroxidase (APX) activity ranged from 356.66 to 629.60 μmol g^{-1}, catalase (CAT) activity ranged from 84.62 to 254.94 U mL^{-1}, and superoxide dismutase (SOD) activity ranged from 802.97 to 1018.09 U mL^{-1} F.W. The application of *T. harzianum* led to the most significant increases in enzyme activities compared to the control plants, with APX, CAT, and SOD activities increasing by 76.5%, 201%, and 26.8%, respectively. Statistically significant differences ($p \leq 0.05$, $p \leq 0.01$) were observed between the control and the treatments (AMF and *T. harzianum*) across all analyses. However, aside from CAT, the differences in SOD and APX activities between AMF and *T. harzianum* were not statistically significant.

Under the 50% irrigation regime, APX activity ranged from 523.38 to 715.30 μmol g^{-1}, CAT activity ranged from 166.09 to 526.51 U mL^{-1}, and SOD activity ranged from 908.41 to 1058.40 U mL^{-1} F.W. The highest increase in APX activity was observed with *T. harzianum* inoculation, showing a 36.7% increase compared to the control plants. Significant differences ($p \leq 0.01$) were found among all treatments. In terms of CAT activity, AMF inoculation led to the highest increase (217%) compared to the control, though no significant difference was found between the AMF and *T. harzianum* treatments. Regarding SOD activity, no significant difference was detected between the control and AMF treatments, with *T. harzianum* leading to the highest increase (16.5%) compared to the control. Statistical analysis between the 100% and 50% irrigation groups revealed no significant differences in MDA and APX activities, while significant differences were observed in SOD ($p \leq 0.05$) and CAT ($p \leq 0.01$) activities. This analysis highlights the role of *T. harzianum* and AMF in modulating antioxidant enzyme activities, which are crucial for mitigating oxidative stress in common bean under varying irrigation conditions. The findings suggest that these biostimulants enhance the plant's defense mechanisms, particularly in response to drought-induced oxidative stress, with differential effects depending on the specific enzyme and irrigation regime.

The observed increases in ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) activities align with previous research, indicating that these biostimulants enhance the antioxidant defense system of plants. Specifically, *T. harzianum* has been shown to upregulate genes encoding antioxidant enzymes, thereby increasing their activity and reducing reactive oxygen species (ROS) levels (Yan et al., 2021; Zehra et al., 2017). Similarly, AMF has been found to boost antioxidant enzyme activities in various crops, contributing to improved stress tolerance and reduced oxidative damage (Begum et al., 2019; He et al., 2020; Hashem et al., 2019). The substantial increase in CAT activity, particularly under AMF treatment, underscores its critical role in detoxifying hydrogen peroxide and preserving membrane integrity under stress conditions. This finding is consistent with other studies that have identified CAT as a key enzyme in mitigating oxidative damage during drought stress, likely due to its high runover rates, which make it highly efficient at neutralizing hydrogen peroxide (Sofo et al., 2015; Hussain et al., 2019). The differential effects of *T. harzianum* and AMF on specific antioxidant enzymes depending on the irrigation regime highlight the potential of these biostimulants to target specific stress-related pathways, optimizing the plant's overall defense strategy. The broader implications of these findings suggest that *T. harzianum* and AMF could be valuable tools in sustainable agriculture, particularly in regions where water availability is limited. By enhancing stress tolerance, these biostimulants can improve crop resilience, leading to better growth and yield outcomes under challenging environmental conditions (Duc et al., 2018; Sun and Shahrajabian, 2023; Fazeli-Nasab et al., 2022).

Principal Component Analysis (PCA) Interrelations Among the Studied Characteristics

Principal Component Analysis (PCA) was employed to reduce the dimensionality of the dataset, allowing for the identification of key variables and the underlying structure within the data (Demirel et al., 2021; Türkoğlu et al., 2023). The PCA biplot analysis (Figure 4) reveals the distinct impact of AMF (M) and *Trichoderma harzianum* (T) treatments on various physiological and biochemical parameters in common bean, particularly under different irrigation regimes. The two principal components (PC1 and PC2) account for a combined 77.8% of the total variance, with PC1 explaining 48.8% and PC2 explaining 29%. The analysis demonstrates that both AMF and *T. harzianum* treatments are strongly associated with increased activities of key antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), as well as enhanced chlorophyll content (Chl a, Chl b, total chlorophyll). These findings indicate that these treatments effectively mitigate oxidative stress and maintain cellular integrity, particularly under drought conditions represented by the 50% irrigation regime. Conversely, the control (C) group, especially under limited water supply, shows a strong association with higher malondialdehyde (MDA) levels, indicating increased oxidative damage and reduced stress tolerance.

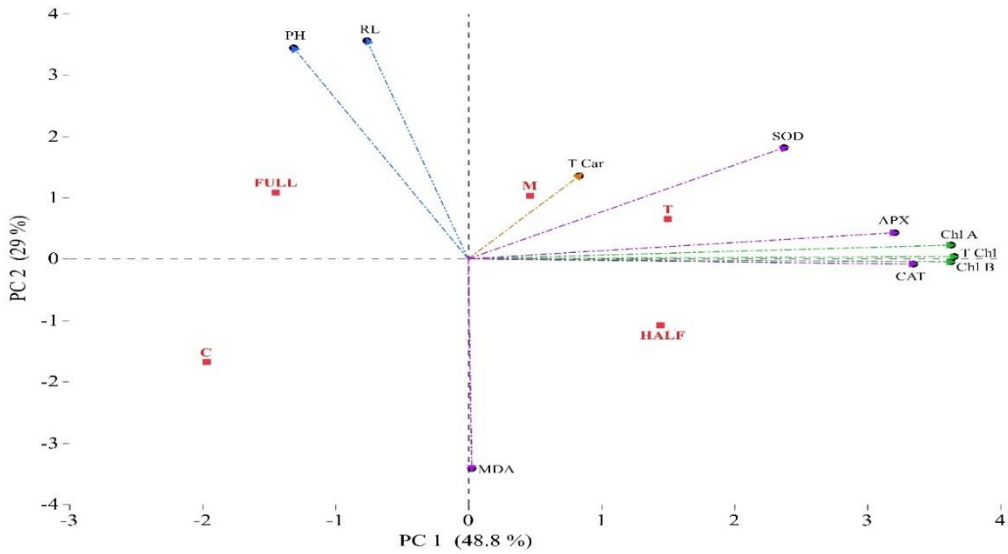

Figure 4. The biplot from PCA analysis illustrates the distribution of the AMF (M) and *Trichoderma harzianum* (T) treatments. The variables included in the analysis are Full: 100% irrigation regime, Half: 50% irrigation regime, C: Control, PH: Plant Height, RL: Root Length, Chl a: Chlorophyll a, Chl b: Chlorophyll b, T Chl: Total Chlorophyll, T Car: Total Carotenoid, SOD: Superoxide Dismutase, APX: Ascorbate Peroxidase, CAT: Catalase, MDA: Malondialdehyde.
Şekil 4. PCA analizinden elde edilen biplot, AMF (M) ve Trichoderma harzianum (T) uygulamalarının dağılımını göstermektedir. Analizde yer alan değişkenler şunlardır: Tam: %100 sulama rejimi, Yarım: %50 sulama rejimi, C: Kontrol, PH: Bitki Boyu, RL: Kök Uzunluğu, Chl a: Klorofil a, Chl b: Klorofil b, T Chl: Toplam Klorofil, T Car: Toplam Karotenoid, SOD: Süperoksit Dismutaz, APX: Askorbat Peroksidaz, CAT: Katalaz, MDA: Malondialdehit.

The biplot further illustrates that *T. harzianum* and AMF treatments support better maintenance of photosynthetic pigments and antioxidant defenses, which are crucial for sustaining plant health under stress. The clustering of plant height (PH) and root length (RL) with the 100% irrigation regime (Full) underscores the importance of adequate water availability for optimal growth, yet the effectiveness of biostimulants in enhancing stress tolerance is evident from their proximity to antioxidant markers even under reduced irrigation. The inverse relationship between MDA and antioxidant parameters, as shown by their opposing positions in the biplot, highlights the role of these treatments in lowering oxidative damage. This comprehensive analysis reinforces the potential of AMF and *T. harzianum* as vital components in improving plant resilience to environmental stressors, particularly in water-limited conditions.

CONCLUSION

This study provides compelling evidence of the effectiveness of Arbuscular Mycorrhizal Fungi (AMF) and *Trichoderma harzianum* in improving the drought tolerance of common beans under varying irrigation conditions. The significant increases in plant growth parameters, chlorophyll and carotenoid content, and antioxidant enzyme activities observed in treated plants highlight the potential of these biostimulants to mitigate the adverse effects of drought stress. The reduction in malondialdehyde (MDA) levels and the substantial enhancement of catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) activities indicate that these treatments are effective in reducing oxidative damage and maintaining cellular stability under water-deficit conditions. These findings suggest that integrating AMF and *T. harzianum* into agricultural practices could be a strategic approach to enhancing crop resilience in regions facing water scarcity. Further research should focus on exploring the molecular mechanisms underlying these responses and assessing the long-term benefits of these biostimulants across different crops and environmental conditions.

CONFLICT OF INTEREST

The author declares no conflicts of interest concerning this article's research, authorship, and/or publication.

DECLARATION OF AUTHOR CONTRIBUTION

The author solely contributed to the conception, design, execution, analysis, and interpretation of the study, as well as the writing and revision of the manuscript.

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