



Physiological and biochemical ameliorative effects of Biochar, *Trichoderma harzianum*, and combined applications in *Capsicum annuum* L. under water stress

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Abstract

In this study, the ameliorative effect of biochar (B), *Trichoderma harzianum* (TH) and their combined application (BTH) on plants of *Capsicum annuum* L. exposed to varying degrees of water deficit was investigated. In control plants exposed to full irrigation (100%) and water restrictions of 75% and 50%, drought stress caused a 20–40% reduction in macro- and micronutrient uptake and a significant increase in oxidative stress indicators (H_2O_2 and MDA). Under severe water limitation (50%), BTH application increased CAT, POD and SOD activities by more than 40% compared to the control (C50) and reduced H_2O_2 accumulation by about 25%. However, under moderate or no stress conditions, the SOD activity in the BTH groups was not higher than that of the biochar alone. A significant increase in proline and sucrose accumulation was also observed. At the hormonal level, BTH treatments increased the concentrations of growth-promoting hormones (IAA and GA) by over 1500% and 2900%, respectively, while ABA concentrations decreased by 99.5%. Significant increases of up to 50% were also observed in defense hormones such as SA and jasmonic acid. Principal component analysis (PCA) showed a clear separation of BTH-treated plants from other groups on the basis of growth promotion, antioxidant defense activation, hormonal regulation and osmotic adjustment. Taken together, these results indicate that the combined application of biochar and *Trichoderma harzianum* significantly improves the physiological and biochemical resistance of *Capsicum annuum* under water stress conditions and thus represents a promising strategy for sustainable agricultural production.

Keywords Oxidative stress · Hormonal balance · Antioxidant activity · Osmotic regulation · Sustainable agriculture

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Introduction

Climate change has emerged as a critical threat to agricultural productivity by disrupting the environmental conditions essential for crop development. Variations in temperature, altered precipitation regimes, and an increased frequency of extreme weather events have collectively reduced yield stability in many agricultural regions (Gangopadhyay et al. 2019; Parker et al. 2019). In many areas facing these challenges, farming systems remain highly exposed, as they rely heavily on natural climatic rhythms. This dependency increases their sensitivity not only to long-term climate shifts but also to sudden environmental disruptions (Desfitri et al. 2023; Gangopadhyay et al. 2019). Climate risks, ranging from temperature anomalies and soil moisture deficits to extreme weather events, have led to significant yield losses in agricultural production. Among the most pressing constraints affecting crop yield and sustainability is the growing scarcity of freshwater resources, which poses serious consequences for food availability, rural economies, and socio-economic resilience. Central to this issue is the heightened irregularity of precipitation regimes coupled with the progression of climate-related disturbances. These dynamics have markedly diminished green water reserves—namely, soil-stored rainwater—upon which an estimated majority of the world's agricultural output still relies (He and Rosa 2023). In addition, fluctuations in water availability have led to both rain-fed and irrigated agricultural land being exposed to acute water stress, creating hotspots where agricultural productivity is increasingly at risk (Gaviria et al. 2022). The progressive depletion of available freshwater supplies not only hampers crop productivity but also introduces substantial challenges to irrigation strategies and integrated water governance, ultimately threatening the long-term economic viability of agricultural systems (Waqas 2024). The decline in green water not only limits the water supply to crops, but also impairs critical physiological processes that are important for plant growth, resulting in significant yield losses and threatening global food security (He and Rosa 2023; Liu et al. 2022). Geospatial analytical approaches, including indicators like the Green Water Scarcity Index, have gained prominence for evaluating regional imbalances in water availability and guiding the formulation of context-specific resource allocation policies (Saicharan and Rangaswamy 2023). The collective synthesis of these studies underscores the urgent need to implement innovative water conservation practices and adaptive management strategies in agriculture. Increasing soil moisture retention and improving cultivation techniques, combined with the introduction of advanced assessment tools, could mitigate the negative impacts of green water scarcity.

Trichoderma spp. have been extensively studied for their dual functional capabilities in suppressing plant diseases and enhancing vegetative growth, positioning them as eco-compatible substitutes for conventional agrochemicals such as synthetic fertilizers and pesticides (Joo and Hussein 2022; Luo et al. 2023). These fungi are distinguished by their metabolic plasticity, producing diverse bioactive compounds—such as volatile organic molecules, hydrolytic enzymes, and plant hormone analogs—which collectively contribute to regulating developmental processes and strengthening the plant's endogenous defense systems (Joo and Hussein 2022; Luo et al. 2023; Martínez-Medina et al. 2014). These compounds facilitate nutrient solubilization and uptake, stimulate cell division and elongation, and trigger systemic resistance in host plants, ultimately leading to improved crop yields and stress tolerance (Martínez-Medina et al. 2014; Ortuño et al. 2016). Due to their multifaceted functional attributes, *Trichoderma* strains are recognized as promising biostimulants capable of enhancing plant resilience against water-limiting conditions (Li et al. 2022; Racić et al. 2018). Their modulatory effect on below-ground morphology is further supported by their regulatory impact on the biosynthesis of key plant hormones—including abscisic acid (ABA), gibberellins, and auxins—that are intricately involved in drought-related signal transduction and physiological adjustment processes (Racić et al. 2018). These hormones regulate stomatal conductance and improve water retention at both cellular and systemic levels, which increases the plant's overall resilience under stress (Racić et al. 2018). In addition to its effects on root development and hormonal balance, *Trichoderma* also strengthens the plant's intrinsic antioxidant defense systems. Numerous investigations have demonstrated that the application of *Trichoderma* species enhances the functional activity of key antioxidant enzymes, including superoxide dismutase and catalase, which play a critical role in mitigating the detrimental effects of reactive oxygen species (ROS) commonly triggered by water limitation. Furthermore, the induced antioxidant defense system contributes to maintaining membrane integrity and supports physiological stability, thereby promoting improved tolerance to drought-induced stress (Mastouri et al. 2012). Furthermore, the enhanced antioxidant activity is often accompanied by a coordinated modulation of gene expression related to stress responses, suggesting that *Trichoderma* exerts its beneficial effects via both direct and systemic mechanisms (Bashyal et al. 2021). This dual functionality is crucial for integrated pest management strategies that aim to reduce dependence on synthetic active ingredients and promote sustainable agriculture. In addition, the emission of bioactive VOCs by *Trichoderma* has been recognized as an important method to promote plant growth. These volatile compounds not

only suppress pathogenic species in the rhizosphere, but also activate growth-related signaling pathways in plants, thus increasing the resilience of plants to biotic and abiotic stress factors (Koza et al. 2022; Kumari et al. 2020; Lee et al. 2016). In addition, these microorganisms have been shown to regulate plant metabolic processes to trigger systemic resistance while promoting nutrient uptake and overall plant vigor under challenging environmental conditions (Yusnawan et al. 2021). For example, a strain of *Trichoderma harzianum* isolated from the rhizosphere of healthy plants of *Nicotiana tabacum* L. was reported to enhance plant growth and drought tolerance, highlighting its role in establishing effective root colonization that improves water uptake under drought conditions (Yin et al. 2025). Similarly, microbial seed priming using drought-resilient *Trichoderma harzianum* strains has been reported to postpone the activation of drought-related responses in wheat by enhancing key physiological parameters such as leaf gas exchange, photosynthetic efficiency, and chlorophyll activity (Rawat et al. 2016). These findings underscore the combined influence of enhanced root architecture, modulated hormonal activity, and strengthened antioxidant defense, thereby underscoring the potential role of *Trichoderma* as a sustainable microbial agent in enhancing plant resilience under water-deficient conditions. This versatile mode of action not only contributes to improved crop yields under drought conditions, but also provides an environmentally friendly strategy to improve plant resilience to increasing water scarcity.

Biochar, a carbon-dense substance produced through the thermal decomposition of biomass under limited oxygen conditions, has garnered attention in sustainable agriculture for its multifunctional benefits on soil quality and plant–microbe relationships (Gyawali 2024; Joseph et al. 2021). Through its role in modifying soil porosity, improving moisture-holding capacity, and elevating nutrient exchange potential, biochar fosters conditions that support root growth and microbial colonization (Ippolito et al. 2014). These improvements facilitate moisture retention and nutrient availability, which indirectly alleviates water stress and supports plant growth under difficult environmental conditions (Pandao et al. 2023). Moreover, organic amendments like biochar contribute to enhancing soil structure while simultaneously acting as a habitat for diverse microbial populations that are integral to nutrient transformation processes and maintaining plant vitality (Khan et al. 2023a, Pantelides et al. 2023). The highly porous nature of biochar creates protective niches for microorganisms, supporting their persistence and metabolic functions even under nutrient-deficient or drought-prone conditions (Ippolito et al. 2014; Laghari et al. 2016). This microbial enhancement facilitates the breakdown of organic residues and stabilizes soil particles, thereby strengthening nutrient retention and boosting

the functional capacity of agricultural systems. (Scott et al. 2014). Consequently, the synergistic effects of biochar on soil physical properties and microbial community dynamics emphasize its role as a sustainable soil amendment that supports the indirect resilience of plants to abiotic stressors such as drought.

Previous research has shown that combining *Trichoderma* strains with biochar can amplify the positive effects of each input, resulting in a cumulative influence on crop development and productivity. For instance, co-application of *Trichoderma aureoviride* and biochar was found to improve watermelon performance by enhancing soil conditions, underlining the role of biochar as a promising medium for *Trichoderma* delivery (Medeiros et al. 2020). In a similar vein, the joint use of *Trichoderma asperellum* and biochar in rice farming under the System of Rice Intensification (SRI) framework produced markedly greater yield outcomes compared to conventional fertilizer practices (Elita and Susila 2022). These findings indicate that the concurrent application of both agents may enhance key physiological responses and biochemical pathways in plants under stress, leading to more effective water utilization, improved nutrient assimilation, and enhanced adaptation to adverse conditions (Amanullah and Khan 2023). The beneficial interplay between *Trichoderma* and biochar is attributed to multiple complementary mechanisms. While biochar contributes to improving the structural quality of soil and offers a favorable environment for beneficial microorganisms, *Trichoderma* supports root growth and nutrient access by releasing various bioactive substances. Together, these effects form a dynamic feedback loop that strengthens soil microbial diversity and functionality, ultimately leading to more resilient plant systems that are better equipped to withstand abiotic stress factors such as drought and salinity. Therefore, this study aims to comparatively evaluate the effects of single *Trichoderma* spp., biochar, and their combined application (*Trichoderma* spp. + biochar) on the plant growth of peppers (*Capsicum annuum* L.) exposed to different levels of water stress.

The present research holds considerable relevance in advancing eco-conscious and resilient farming methodologies, especially in light of the increasing pressures that water scarcity and climate variability impose on crop performance. Considering the ecological and financial limitations linked to the use of traditional fertilizers and chemical-based stress alleviation methods, the integrated utilization of biochar with beneficial microorganisms such as *Trichoderma harzianum* offers a promising and environmentally responsible approach. This approach not only enhances soil microbial health but also strengthens plant resilience against abiotic stresses. Accordingly, the study holds strategic value not only at the mechanistic and physiological levels but also

for the agricultural sector, offering a promising pathway to improve production sustainability under drought conditions.

This research aims to elucidate the interactive effects of *Trichoderma* species and biochar on physiological responses and growth-related traits of plants subjected to restricted water availability. Considering the scarcity of comprehensive investigations on this subject, the current work endeavors to contribute novel perspectives regarding their combined application, thereby promoting innovative approaches for environmentally sound drought mitigation in agriculture.

Material methods

Experimental material and applications

The experiment was conducted in 2021 under controlled climate room conditions at the Faculty of Agriculture, Van Yüzüncü Yıl University. In the study, local green peppers registered with Sera Demre 8 were used as plant material. After the cotyledons developed, the peppers were transplanted into pots 14 cm high and 11 cm wide. The room was maintained at 25 °C with a photoperiod of 14/10 h (day/night) and 65±2% relative humidity. A total of 48 plastic pots were arranged according to a randomized factorial design. The treatments consisted of three irrigation regimes (100%, 75%, and 50% of field capacity) and four different treatments: control, biochar (B), *Trichoderma harzianum* (TH), and combined biochar+*Trichoderma harzianum* (BTH). Biochar was mixed with the soil at a dose of 6 g per pot. One green pepper (*Capsicum annuum* L.) seedling was planted in each pot. Each pot was filled with 462 g of sterilized garden soil. The biochar used in the experiment was obtained from 100% natural oak. A temperature of 450 degrees was applied.

Throughout the study, the following treatment codes were used:

C100: Control with 100% irrigation.

C75: Control with 75% irrigation.

C50: Control with 50% irrigation.

B100, B75, B50: Biochar-treated plants under 100%, 75%, and 50% irrigation.

TH100, TH75, TH50: *Trichoderma*-treated plants under 100%, 75%, and 50% irrigation.

BTH100, BTH75, BTH50: Combined biochar and *Trichoderma* treatments under 100%, 75%, and 50% irrigation.

Irrigation management and water stress imposition

The field capacity of the soil was determined gravimetrically. Irrigation volumes were calculated based on pot weight differences over time using the formula:

$$I = (W_i - 1 - -W_i) \times IR$$

Where,

I=irrigation volume (mL);

Wi-1=previous pot weight;

Wi=current pot weight and IR=irrigation ratio.

Irrigation volume corresponding to 100% field capacity was determined as 612 mL. Water stress was imposed by reducing this volume by 25% (for C75, B75, etc.) and 50% (for C50, B50, etc.). Tap water was used as an irrigation source.

Growth period and plant sampling

The growth period lasted 30 days. Harvesting was performed based on observable chlorosis and biomass differences in the shoots of stressed plants compared to controls. Plants were harvested, washed with tap and distilled water, oven-dried at 70 °C for 48 h to constant weight, and then ground for further analyses.

Microorganism inoculation

Selected *Trichoderma harzianum* isolates with demonstrated strong antagonistic potential were sourced from the culture collection maintained by the Phytopathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Van Yüzüncü Yıl University. A suspension containing 1×10^6 conidia/ml of *Trichoderma* spp. grown in PDA (Potato Dextrose Agar) medium was prepared from the colonies using a hemocytometer. The prepared *Trichoderma* spp. suspension was applied as 15 ml irrigation per plant during the seedling period.

Analysis of the soil

The chemical and physical characteristics of the growth substrate were evaluated and are presented in Table 1. Measurements included electrical conductivity following the method of (Rhoades 1996), pH and calcium carbonate

Table 1 Analytical data on the physical and chemical profile of the soil

Soil Properties	Unit	Analysis Result
pH		7.19 (Slightly alkaline)
EC	mS/cm	64.6 (Salt-free)
Calcareous	%	2.26 (Low calcif)
Organic Matter Content	%	0.87/0.044 N
Texture	%	Sand: 67.2 Clay: 17.6 Silt: 15.2

levels as described by and organic matter content according to (Nelson and Sommers 1996).

Analysis of the plant nutrient concentration

To determine the nutrient content of the plants, 1 g of dried leaf samples were digested in a three-step procedure with a mixture of 3 mL HNO_3 and 9 mL HCl in a microwave digestion device. The first step was performed at 145 °C with 75% microwave power for 5 min, the second step at 180 °C with 90% power for 10 min, and the third step at 100 °C with 40% power for 10 min. All digestion procedures were carried out in a microwave digestion chamber that can withstand a pressure of up to 40 bar (Speedwave MWS-2, Berghof Products+Instruments, Harresstr. 1, 72800 Eningen, Germany) (Mertens 2005). After preparation, the samples were analyzed for concentrations of plant nutrients using an inductively coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484–4794, USA).

Analysis of plant hormones

The extraction and purification of endogenous phytohormones—including indole-3-acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and cytokinins (CK)—were carried out using a combination of established methods with slight modifications. Initially, 1 g of fresh leaf tissue was homogenized in 80% methanol, precooled to –40 °C, and incubated for 10 min, following the procedure of Koshita et al. (1999). The homogenate was filtered, and the supernatant was evaporated at 35 °C until completely dry. The residue was dissolved in 0.1 M potassium dihydrogen phosphate (KH_2PO_4 , pH 8.0) buffer. For purification, a C18 Sep-Pak cartridge (waters) was used to eliminate interfering substances.

Subsequent hormone quantification was performed using high-performance liquid chromatography (HPLC) on an Agilent 1200 system equipped with a Zorbax Eclipse AAA C18 column. The mobile phase was composed of 13% acetonitrile adjusted to pH 4.98 (Kuraishi et al. 1991). IAA, GA, ABA, and SA were detected at 265 nm using a UV detector. JA was analyzed using a modified protocol from Gundlach et al. (1992). For cytokinin (zeatin), extraction and purification followed Li et al. (2016), and detection was carried out at 254 nm based on Sadeghipour (2017).

Antioxidant enzyme activity analysis

The activities of key antioxidant enzymes—superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)—were assessed using spectrophotometric methods with

protocol modifications based on Dhindsa et al. (1981) and Sairam and Srivastava (2001). For enzyme extraction, 0.5 g of fresh leaf tissue was homogenized in 3 mL of 50 mM potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at $15,000 \times g$ for 15 min at 4 °C, and the supernatant was collected and stored at –80 °C until analysis.

To improve enzyme stability and reduce phenolic interference, the extraction buffer was supplemented with 0.5% polyvinylpyrrolidone (PVP), 1 mM phenylmethylsulfonyl fluoride (PMSF) as a protease inhibitor, and 1 mM ethylenediaminetetraacetic acid (EDTA) as a chelating agent.

The enzyme activities were quantified based on changes in absorbance at specific wavelengths: SOD activity was measured at 560 nm, POD at 470 nm, and CAT at 240 nm. One unit (U) of SOD activity was defined as the enzyme amount required to inhibit 50% of the photochemical reduction of nitroblue tetrazolium (NBT). POD and CAT activities were expressed as the enzyme quantity that caused an increase in absorbance by 0.01 per minute under the assay conditions.

Analysis of the reactive oxygen species (ROS)

To quantify hydrogen peroxide (H_2O_2), 0.1 g of leaf tissue was finely ground and homogenized in 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) on ice. The subsequent analytical steps were carried out as outlined by Velikova et al. (2000). The H_2O_2 concentration was estimated using a standard curve prepared with known concentrations of H_2O_2 .

Lipid peroxidation levels were determined by measuring the malondialdehyde (MDA) content. For this purpose, 0.1 g of frozen leaf tissue was pulverized in liquid nitrogen and extracted with 3 mL of chilled ethanol, following the method of Yildirim et al. (2015). Thiobarbituric acid reactive substances (TBARS), indicative of lipid peroxidation and expressed as MDA equivalents, were quantified, and MDA levels were calculated using an extinction coefficient of $155 \text{ mmol}^{-1} \text{ L cm}^{-1}$, as described by Shams et al. (2019).

To assess sucrose concentration, 0.1 g of dried tissue was incubated in 10 mL of 0.1 M phosphate buffer (pH 5.4) at 22 °C for 24 h. An aliquot of 0.2 mL from the supernatant was mixed with 1.0 mL of invertase solution (10 U/mL) and distilled water, then incubated in a water bath at 50 °C for 1 h according to the method of Shams et al. (2019). Quantification of sucrose was performed based on the protocol by Chopra et al. (2000).

Proline (Pro) levels were determined spectrophotometrically at 520 nm following the approach of Bates et al. (1973). Briefly, 50 mg of frozen leaf tissue was ground under liquid nitrogen using a mortar and pestle, then homogenized on ice in 4.5 mL of 3% sulfosalicylic acid. The homogenate was

centrifuged at $12,000 \times g$ for 15 min at 4 °C, following the procedure reported by Shams et al. (2019).

Statistical analysis

Data were statistically evaluated using the SPSS program (v22.0). A two-way variance analysis was applied to determine the influence of different treatments. Post-hoc comparisons between group means were made using Duncan's multiple range procedure with a significance level set at $p < 0.05$. Furthermore, principal component evaluation was performed to explore complex relationships among physiological, biochemical, and growth-related traits.

Results

Analysis of the findings revealed statistically meaningful differences ($p < 0.05$) in the concentration of macrolelements (N, P, K, Ca, Mg, S) and microelements (Mn, Fe, Zn, B, Cu) in the shoot biomass of pepper plants subjected to varying levels of water stress. The nitrogen (N) concentration in the shoot biomass of pepper plants was lowest in treatments C75 (2.05%) and C50 (2.09%). This decrease was statistically significant compared to the other treatment groups. However, the highest N concentrations were observed in the BTH treatments (BTH100, BTH75, and BTH50) with values of 4.46%, 4.60%, and 4.54%, respectively. These increases were highly significant compared to the other applications. Phosphorus concentration of the plants also showed significant differences depending on the treatment. Among the treatments, BTH75 (0.70%) and BTH50 (0.69%) exhibited the highest phosphorus concentrations, whereas TH75 showed the lowest level at 0.16%. In terms of potassium, the most elevated concentration was determined in BTH100 (2.28%), while TH50 resulted in the minimum value (0.62%). For Ca, the highest concentration was observed in the BTH50 group (2.18%), while the lowest Ca concentration was observed in the TH50 treatment (0.74%). The levels of Mg and S remained relatively stable in the control and organic cabbage groups. However, a significant increase in the concentrations of Mg (0.55%) and S (0.36%) was observed in the TH50 treatment. The lowest Mg and S levels (0.17% and 0.13%, respectively) were observed in the C75 treatment.

Of the micronutrients, the highest concentration of Mn was found in the TH50 treatment (72.56 mg/kg). In contrast, the concentrations of Fe and Zn were significantly reduced in the TH and BTH treatments compared to the control groups. The lowest Fe concentrations were observed in the BTH100, BTH75, and BTH50 groups, with values of 11.75, 11.15, and 12.52 mg/kg, respectively. The B

content remained relatively stable in all treatments; however, the highest B concentration (21.21 mg/kg) was measured in treatment BTH50. The Cu content also increased significantly in the TH50 group (6.37 mg/kg). Overall, under severe water stress (50% irrigation), nutrient uptake decreased in the control group (C) and the single application groups (biochar (B) and *Trichoderma* spp. (TH), while the BTH treatments had the highest concentrations of macro- and micronutrients (Table 2).

In the aerial parts of pepper plants, significant variations ($p < 0.05$) were detected in the activities of antioxidant enzymes—catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD)—as well as in reactive oxygen species levels (H_2O_2 and MDA), proline accumulation, and sucrose concentration across the treatments applied under water deficit conditions. CAT activity peaked in the BTH50 treatment (835.59 U/g FW), whereas the TH50 group exhibited the lowest level (216.35 U/g FW). For POD activity, the C50 application recorded the highest enzyme level (16,149.12 U/g FW), while the TH50 treatment displayed the minimal activity (1,423.44 U/g FW). The highest SOD activities were measured in treatments B75 (1,984.83 U/g FW) and B100 (1,880.55 U/g FW). Conversely, the lowest SOD activities were found in the groups BTH75 (558.46 U/g FW) and BTH100 (601.50 U/g FW). As for the H_2O_2 content, the highest value was found in the B100 treatment (26.79 $\mu\text{mol/g}$ FW), while the lowest value was observed in the BTH50 group (14.39 $\mu\text{mol/g}$ FW). Regarding the accumulation of malondialdehyde (MDA), MDA levels remained relatively similar in the control and single treatment groups (between 11 and 17 mg/g FW). However, the highest MDA concentrations were found in the BTH100 (45.74 mg/g FW), BTH75 (39.34 mg/g FW), and BTH50 (49.40 mg/g FW) treatments. For proline content, the highest accumulation was observed in the BTH100 group (1.19 $\mu\text{mol/g}$ FW), while the lowest proline content concentration was found in the C100 treatment (0.11 $\mu\text{mol/g}$ FW). As for sucrose content, the highest value was observed in the BTH75 group (5.83 mg/g FW), followed by high values in the BTH50 (5.09 mg/g FW) and BTH100 (4.01 mg/g FW) treatments. In contrast, the sucrose content remained low in all other groups. Overall, BTH application significantly increased the accumulation of stress-induced metabolites such as proline and sucrose (Table SI.1 and Figs. 1, 2 and 3).

Significant effects ($p < 0.05$) on the hormone profiles of pepper plants were observed depending on the treatments applied under water stress conditions. IAA levels increased significantly, especially in BTH treatments. The highest IAA concentration was measured in the BTH100 group (0.25 $\mu\text{g/g}$ FW), followed by BTH75 (0.16 $\mu\text{g/g}$ FW) and BTH50 (0.17 $\mu\text{g/g}$ FW). The lowest IAA content was measured in the C50 group (0.01 $\mu\text{g/g}$ FW). ABA concentrations

Table 2 Effect of irrigation levels and microbial treatments on the concentrations of essential macro- and micronutrients in the aerial parts of pepper (*Capsicum annuum* L.) plants

	N	P	K	Ca	Mg	S	Mn	Fe	Zn	B	Cu
C100	2.92±0.73 ^c	0.30±0.08 ^b	1.83±0.51 ^{ab}	1.36±0.43 ^b	0.29±0.08 ^{bc}	0.19±0.04 ^{cd}	31.53±8.68 ^b	73.02±14.98 ^a	19.91±5.41 ^a	8.59±1.30 ^{cd}	1.94±0.62 ^{bc}
C75	2.05±0.19 ^{cd}	0.20±0.02 ^{bcd}	1.27±0.06 ^c	0.95±0.12 ^{cd}	0.17±0.02 ^c	0.13±0.02 ^d	22.45±0.67 ^b	49.82±3.73 ^b	16.34±4.16 ^{ab}	4.92±0.45 ^d	0.82±0.30 ^c
C50	2.09±0.27 ^{cd}	0.21±0.03 ^{bcd}	1.27±0.15 ^c	0.93±0.05 ^{cd}	0.21±0.06 ^{bc}	0.14±0.02 ^d	22.41±4.49 ^b	52.45±6.78 ^b	14.99±3.75 ^{abc}	7.63±2.57 ^d	1.51±0.42 ^{bc}
B100	2.16±0.60 ^{cd}	0.23±0.05 ^{bcd}	1.35±0.26 ^c	0.94±0.23 ^{cd}	0.24±0.07 ^{bc}	0.14±0.04 ^d	24.25±7.68 ^b	53.71±15.58 ^b	17.78±7.74 ^{ab}	7.19±2.90 ^d	1.48±0.96 ^{bc}
B75	2.95±0.42 ^{bc}	0.29±0.05 ^{bc}	1.84±0.20 ^{ab}	1.29±0.21 ^{bc}	0.28±0.04 ^{bc}	0.19±0.04 ^{cd}	31.43±4.75 ^b	72.51±12.66 ^a	21.65±5.31 ^a	8.11±1.49 ^{cd}	1.18±0.48 ^c
B50	2.32±0.64 ^{bcd}	0.23±0.05 ^{bcd}	1.49±0.38 ^{bc}	1.01±0.29 ^{bcd}	0.23±0.08 ^{bc}	0.17±0.03 ^d	25.95±7.6 ^b	59.02±12.63 ^{ab}	19.07±6.59 ^{ab}	8.33±3.20 ^{cd}	1.41±0.41 ^{bc}
TH100	1.66±0.31 ^d	0.18±0.03 ^{cd}	1.03±0.15 ^{cd}	0.79±0.14 ^d	0.15±0.03 ^c	0.11±0.02 ^d	17.20±3.10 ^b	43.20±7.36 ^{bc}	11.68±1.89 ^{bcd}	3.99±0.64 ^d	0.54±0.09 ^c
TH75	1.93±1.25 ^{cd}	0.16±0.02 ^d	0.75±0.09 ^d	0.74±0.21 ^d	0.29±0.30 ^{bc}	0.20±0.17 ^{bcd}	31.03±32.59 ^b	31.62±1.91 ^{cd}	8.01±2.69 ^{cde}	4.88±2.25 ^d	1.74±1.89 ^{bc}
TH50	3.19±0.13 ^b	0.19±0.02 ^{cd}	0.62±0.01 ^d	0.89±0.07 ^d	0.55±0.06 ^a	0.36±0.02 ^a	72.56±3.57 ^a	25.56±3.00 ^{de}	5.20±0.44 ^{de}	4.87±2.01 ^d	6.37±3.41 ^a
BTH100	4.46±0.42 ^a	0.67±0.08 ^a	2.28±0.21 ^a	2.06±0.15 ^a	0.49±0.10 ^a	0.30±0.02 ^{ab}	56.99±0.34 ^a	11.75±0.70 ^c	2.61±0.59 ^e	16.69±1.27 ^{ab}	2.92±1.05 ^{bc}
BTH75	4.60±0.12 ^a	0.70±0.12 ^a	2.06±0.35 ^a	2.05±0.18 ^a	0.40±0.03 ^{ab}	0.29±0.06 ^{bc}	53.05±12.77 ^a	11.15±1.32 ^c	2.89±0.43 ^e	13.20±3.66 ^{bc}	2.11±1.21 ^{bc}
BTH50	4.54±0.28 ^a	0.69±0.06 ^a	2.18±0.35 ^a	2.16±0.08 ^a	0.53±0.15 ^a	0.33±0.06 ^a	55.44±6.81 ^a	12.52±1.48 ^c	3.27±0.16 ^e	21.21±7.13 ^{ab}	3.91±0.95 ^b

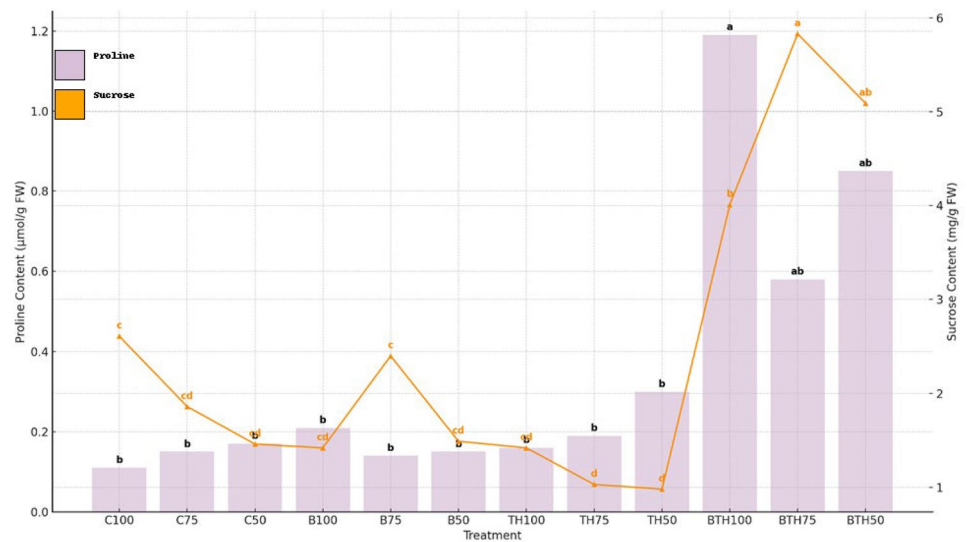
Different superscript letters following the means denote significant differences determined by Duncan's test ($p < 0.05$), while the absence of such letters reflects no statistically significant variation ($p > 0.05$)

were high in the C50 (4449.00 ng/g FW) and C100 (3112.65 ng/g FW) groups, where water stress was more pronounced. In contrast, ABA levels decreased significantly in the BTH treatments, reaching the lowest concentrations in BTH50 (9.49 ng/g FW), BTH75 (13.16 ng/g FW), and BTH100 (19.14 ng/g FW). GA levels were highest in treatments BTH75 (1.64 µg/g FW) and BTH50 (1.87 µg/g FW), and this increase was statistically significant compared to the other groups. In contrast, GA concentrations in the other groups remained relatively low (between 0.06 and 0.15 µg/g FW), and no statistically significant differences in GA concentrations were observed between the groups. As for SA, the highest concentration was measured only in the TH50 group (0.12 µg/g FW), which was statistically significant. In all other treatments, the SA content was between 0.02 and 0.06 µg/g body weight. The cytokinin content reached its highest value in the TH50 group (0.14), while it fluctuated between 0.03 and 0.07 in the other groups. Zeatin concentrations were similarly high in the TH50 (0.16), BTH100 (0.16), BTH75 (0.17), and BTH50 (0.19) treatments, while in groups such as C50 and B75, zeatin concentrations dropped to as low as 0.01%. The concentrations of jasmonic acid were highest in treatments B75 (0.95 µg/g FW) and C100 (0.87 µg/g FW). In the BTH groups, however, the jasmonic acid content decreased significantly and remained at around 0.02% (Table SI.2 and Figs. 4 and 5).

Discussion

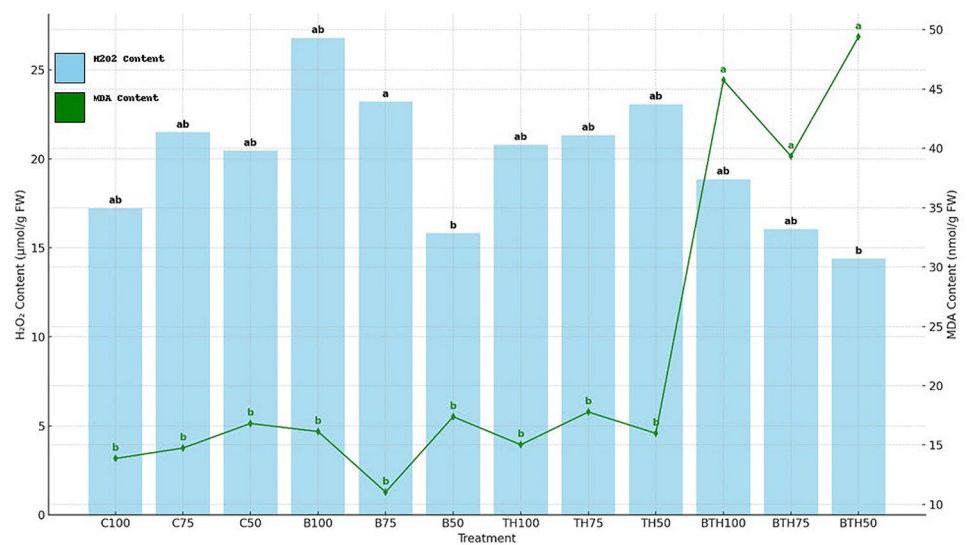
Based on the findings of this research, the concentrations of both macro- and micronutrients in pepper plants exhibited statistically significant variations depending on the applied treatments under water-deficit conditions. Overall, increased water stress reduced nutrient uptake in pepper plants. Under limited water availability, the movement of nutrients toward the root zone is reduced, root absorption efficiency declines, and internal distribution of these elements throughout the plant body becomes constrained. This is primarily due to reduced transpiration flux and reduced active transport (Bista et al. 2020; Hoang et al. 2014; Htoon et al. 2014; İstanbullu et al. 2020; Özkutlu et al. 2019). Our results showed that nutrient concentrations decreased significantly in the control groups at 100%, 75% and 50% irrigation, while biochar, *Trichoderma harzianum* and their combined application attenuated these reductions. In the C75 group, N decreased by about 29.8%, P by 33.3%, K by 30.6% and Ca by 30.1% compared to C100. Similar decreases continued in the C50 group. However, significant increases were achieved in biochar treatment. In the B75 group, the N concentration increased by 43.9%, P by 45%, K by 44.9% and Ca by 35.8% compared to C75. This improvement is

Fig. 1 Effect of different treatments on proline and sucrose concentrations in pepper (*Capsicum annuum* L.) plants under varying irrigation regimes



Statistical differences are shown with different letters (Duncan's test, $p < 0.05$).

Fig. 2 Effect of different treatments on MDA and H_2O_2 concentrations in pepper (*Capsicum annuum* L.) plants under varying irrigation regimes

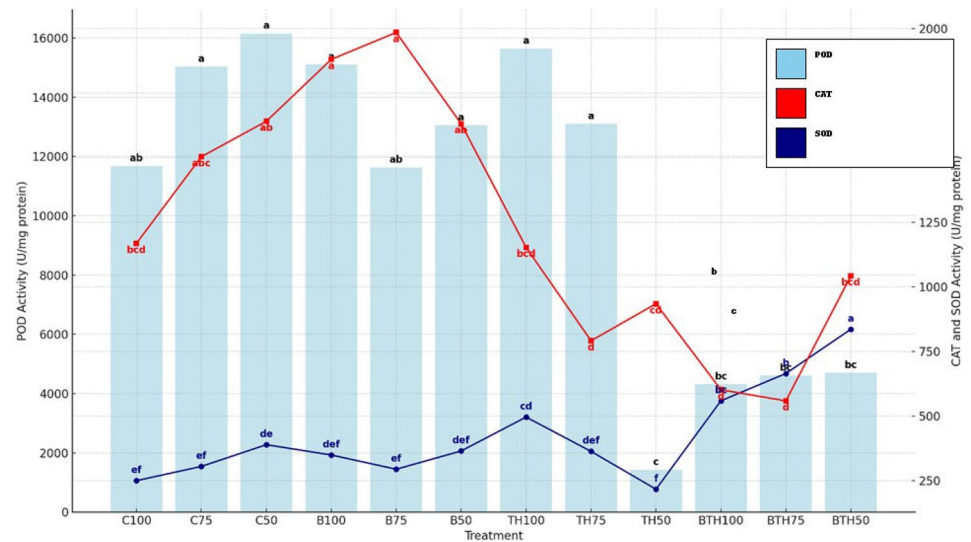


Statistical differences are shown with different letters (Duncan's test, $p < 0.05$).

attributed to the biochar's ability to bind water and nutrients better (Jeffery et al. 2011). Due to its highly porous structure, biochar contributes to enhancing soil's chemical and physical characteristics. Under water-limited conditions, it enhances moisture retention, ensures more consistent water availability for root systems, and mitigates premature wilting caused by drought stress (Jeffery et al. 2011; Lehmann and Joseph 2024; Rathinapriya et al. 2025). In fact, pepper seedlings grown in biochar-enriched soils have been reported to have darker green leaves, healthier stems and better overall plant condition during drought periods compared to control soils (Yan and Shi 2025). Thus, the biochar-enriched pots provided more water and nutrients in the root zone even under water stress, helping to maintain the nutritional status of the plants. Furthermore, biochar application

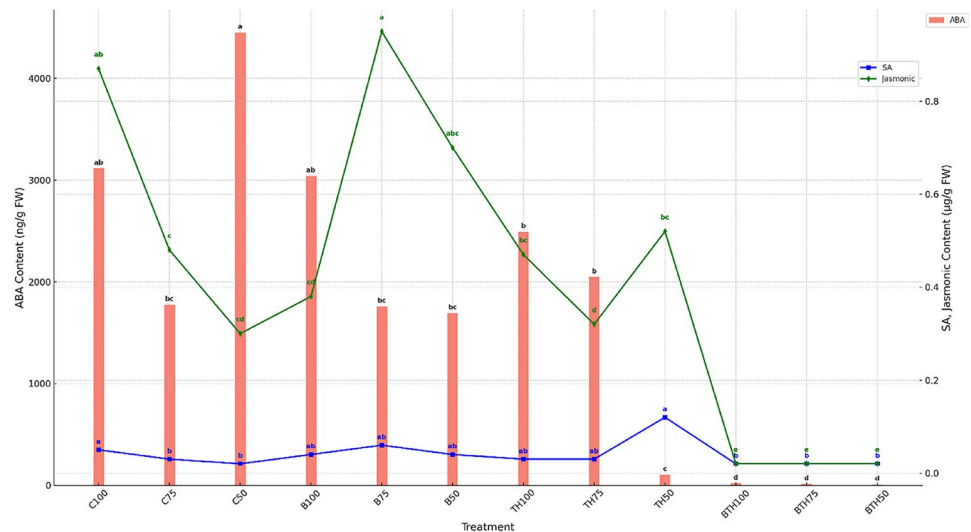
has been reported to alleviate drought-associated reductions in plant growth, thereby enhancing both yield and product quality (Hareem et al. 2024). It also contributes to improved soil nutrient retention and facilitates the effective assimilation of key elements such as nitrogen and phosphorus, while minimizing the leaching of nitrate and phosphate, while reducing nitrate and phosphate leaching. Studies on peppers and tomatoes have shown that the application of biochar significantly improves the efficiency of N and P uptake and reduces nitrate-phosphate leaching into the drainage water (Yang et al. 2025). Biochar acts as a buffer, especially under water-scarce conditions, by retaining important nutrients for plants. In their study on drought-stressed chili peppers (*Capsicum annuum* L.), (Hareem et al. 2024) revealed that mango peel-derived biochar led to notable enhancements

Fig. 3 Effect of different treatments on CAT, POD, and SOD enzyme activities in pepper (*Capsicum annuum* L.) plants under varying irrigation levels.



Statistical differences are shown with different letters (Duncan's test, $p < 0.05$).

Fig. 4 Effect of different treatments and irrigation levels on salicylic acid and jasmonic acid concentrations in pepper (*Capsicum annuum* L.) plants

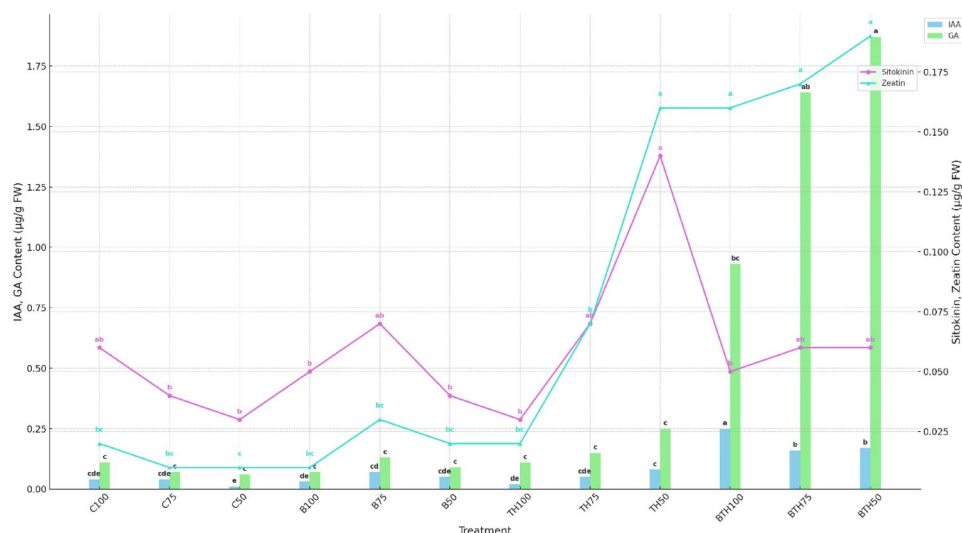


Statistical differences are shown with different letters (Duncan's test, $p < 0.05$).

in leaf nitrogen, phosphorus, and potassium concentrations when compared to untreated plants. In line with this, (Gaber et al. 2024) indicated that biochar incorporation into pepper cultivation supported shoot and root biomass development under water-limited conditions and helped reduce productivity decline. In the TH50 group, the Mg concentration increased by 161.9% and the Mn concentration by 223% compared to the C50 group. *Trichoderma* species are known to stimulate root system expansion by colonizing the rhizosphere, which enhances lateral root initiation and contributes to higher root mass, thereby facilitating more efficient access to soil nutrients (Halifu et al. 2019). *Trichoderma harzianum* establishes a mutualistic association with plant roots, supporting growth through multiple mechanisms. It releases organic acids that mobilize otherwise inaccessible

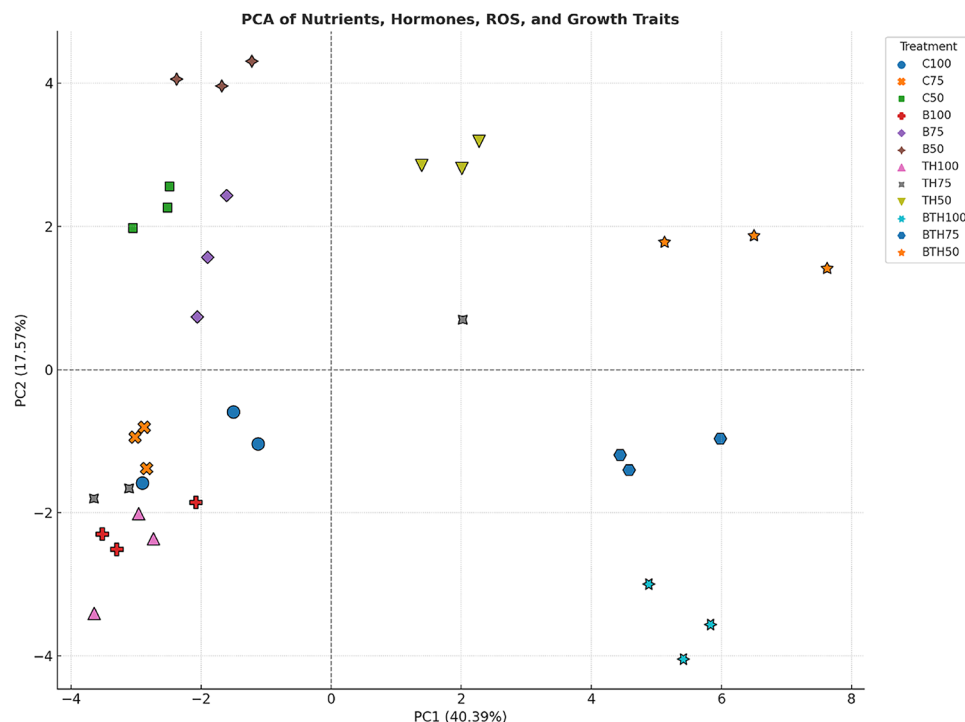
mineral elements in the soil and transforms them into more plant-accessible forms, while also chelating micronutrients to increase their biological availability (Benítez et al. 2004; Harman et al. 2004; Mukherjee et al. 2013). Our results showed that the combined BTH applications produced the highest performance. In the BTH75 group, the N concentration increased ~2.2-fold (from 2.05% in C75 to 4.60%), the P concentration increased 3.5-fold (from 0.20% to 0.70%) and the K concentration increased ~1.6-fold (from 1.27% to 2.06%) compared to C75. In the BTH50 group, the Ca concentration increased ~2.3-fold (from 0.93% to 2.18%) and the Mg and S concentrations increased ~2.5-fold (from 0.21% to 0.53%) and ~2.4-fold (from 0.14 to 0.33%) respectively. Previous findings indicate that organic acids excreted by *Trichoderma* contribute to mobilizing immobilized

Fig. 5 Effect of irrigation levels and treatment applications on the concentrations of growth-related hormones (IAA, GA, cytokinin, and zeatin) in pepper (*Capsicum annuum* L.) plants.



Statistical differences are shown with different letters (Duncan's test, $p < 0.05$).

Fig. 6 Component-based multivariate assessment of nutrient acquisition, hormone regulation, antioxidant response, and redox-related indicators in pepper subjected to varying irrigation and treatment regimes



phosphorus fractions in the rhizosphere, thereby enhancing nutrient availability for plant absorption and expediting nutrient turnover processes. Moreover, *Trichoderma* enhances the contact area between roots and surrounding soil via intensive root colonization and promotes the activity of soil enzymes such as sucrase, urease, and phosphatase, which are critical for effective nutrient mineralization (Harman 2011; Khan et al. 2017; Maeda et al. 2015). Such mechanisms enhance the accessibility of nitrogen in its ammonium and nitrate forms and promote the breakdown of organic substrates, thereby facilitating nutrient liberation.

For example, studies by Wagner et al. (2016) and Yadav et al. (2009) have shown that the application of *Trichoderma* increases plant-available nutrient concentration and microbial biomass in soils, suggesting that this fungus enriches both plant nutrition and soil microbial communities (Wagner et al. 2016; Yadav et al. 2009).

Study results suggest that biochar acts as a “reservoir” by enhancing water and nutrient retention around plant roots under drought conditions (Yan and Shi 2025; Yang et al. 2025), while *Trichoderma harzianum* facilitates the uptake of these nutrients through enzymatic and biochemical

support. Consequently, pepper plants could maintain relatively better nutrient uptake even under water scarcity. While water stress suppresses nutrient uptake, biochar and *Trichoderma* acted as balancing factors and supported plant nutrition. Especially under moderate water stress (75% irrigation), the limited decrease in nutrient concentration indicates that biochar and *Trichoderma* played a role in maintaining plant nutrition. Even under severe water stress, plant survival and continued nutrient uptake demonstrate the protective effect of these treatments. In summary, although water stress significantly limited the uptake of N, P, K, Ca, Mg, S, and micronutrients such as Fe, Zn, Mn, B, and Cu in pepper plants, the application of biochar and *Trichoderma harzianum* significantly mitigated these negative effects. Therefore, encouraging the implementation of such approaches in agricultural practices under limited water availability appears to be a viable strategy for sustaining plant development, nutritional status, and overall performance.

Despite the increase in CAT, POD and SOD enzyme activities in pepper plants exposed to water limitation under C75 and C50 treatments, these improvements did not result in adequate antioxidant protection, as the formation of H₂O₂ and MDA persisted. In the C50 group, CAT activity increased ~1.6-fold compared to C100 (from 537.0 to 835.6 U/g FW), while MDA concentration increased ~1.2-fold (from 4.6 to 17.7 mg/g FW). This indicates that although CAT activity was increased under water stress in the control groups, it was not able to sufficiently reduce H₂O₂ and MDA accumulation. In contrast, CAT activity increased ~2.1-fold (from 388.6 to 835.6 U/g FW) when treated with BTH50, while H₂O₂ and MDA concentrations decreased ~1.4-fold (from 20.5 to 14.4 µmol/g FW) and ~2.9-fold (from 16.8 to 49.4 mg/g FW), respectively. These results suggest that the combined effect of enhanced antioxidant gene expression by *Trichoderma* (Bashyal et al. 2021; Mastouri et al. 2012) and improved water binding by biochar (Xu and Mou 2015) contributes to effective detoxification of ROS.

POD activities increased in the control groups under water stress; however, ROS accumulation was suppressed by ~2.5-fold (from 36.0 to 14.4 µmol/g FW) in the BTH groups. In plants inoculated with *Trichoderma harzianum*, the activities of antioxidant enzymes further increased, and the accumulation of peroxide decreased. Bashyal et al. (2021) show that inoculation of rice with *Trichoderma harzianum* under drought stress leads to exclusive expression of peroxidase enzymes and enrichment of metabolic pathways associated with glutathione metabolism (Bashyal et al. 2021). This beneficial fungal application is reported to strengthen the plant's internal defense against oxidative stress, accelerating H₂O₂ detoxification and thereby reducing MDA levels (Lian et al. 2023; Seleiman et al.

2021). Similarly, the addition of biochar has been shown to increase antioxidant capacity and protect plant cells by reducing ROS formation. For example, biochar application under drought conditions was reported to significantly increase CAT, POD and SOD activities and reduce MDA accumulation compared to stressed controls (Hafez et al. 2020; Hashem et al. 2019). It should also be noted that MDA levels were relatively higher in some BTH treatments compared to other groups. While this seems contradictory to the reduction in H₂O₂, it can be attributed to the fact that BTH promoted enhanced metabolic activity and membrane lipid turnover, which may have resulted in elevated MDA despite an overall improvement in oxidative balance. Previous studies have also highlighted that MDA accumulation can vary depending on stress duration and tissue specificity and therefore may not always linearly reflect the antioxidant responses (Hashem et al. 2019; Seleiman et al. 2021). Although the BTH treatments improved overall antioxidant protection, it should be noted that SOD activity remained lower in BTH75 and BTH100 than in the biochar-only treatments (B75 and B100). This suggests that BTH exerts strong synergistic effects under severe stress (50% irrigation), while the application of biochar alone may be more effective in stimulating SOD activity under non-severe conditions. The most pronounced effects were observed in the BTH applications. In the BTH50 group, CAT activity increased by 114.9%, proline accumulation by 400% and sucrose content by 248% compared to C50. The findings indicate that fungal inoculation elicits a pronounced antioxidant defense, effectively contributing to ROS detoxification. In the B75 treatment group, SOD activity was elevated by ~1.7-fold (from 1,168 to 1,985 U/g FW), highlighting biochar's contribution to improving free radical neutralization capacity (Hashem et al. 2019).

The application of biochar significantly increased the activities of antioxidant enzymes and mitigated oxidative damage compared to the control groups. In the B100 group, SOD activity increased 1.6-fold compared to C100 (from 1,168 to 1,881 U/g FW), while in the B75 group, MDA levels decreased 1.3-fold (from 25.6 to 20.4 mg/g FW). In particular, treatment with *Trichoderma harzianum* led to a remarkable increase in CAT activity, with the TH100 group showing a ~2-fold increase (from 417 to 828 U/g FW) compared to C100. In the TH50 group, proline accumulation increased 1.8-fold (from 0.17 to 0.31 µmol/g FW) compared to C50, supporting osmotic balance. The most pronounced effects were observed in the BTH applications. In the BTH50 group, CAT activity increased ~2.1-fold (from 388.6 to 835.6 U/g FW), proline accumulation increased ~5-fold (from 0.24 to 1.19 µmol/g FW) and sucrose content increased ~3.5-fold (from 0.24 to 1.19 µmol/g FW) compared to C50. Water deficit conditions stimulate the synthesis

of Osmo protective compounds like proline and carbohydrates such as sucrose, which play a role in minimizing cellular dehydration and preserving osmotic balance (Khoyerdi et al. 2016). Erol (2023) highlighted that drought severity is closely associated with a significant elevation in proline levels within the leaf tissues of pepper plants (Erol 2023). Similarly, studies on various plant species, such as peanut genotypes, have found a significant increase in proline and total soluble sugars under water stress compared to control conditions (Khoyerdi et al. 2016). Proline acts as an Osmo protectant and protects cells from oxidative damage, while soluble sugars such as sucrose lower the osmotic potential of cells and support water retention (Yildirim et al. 2021). This mechanism helps plants to partially maintain cell turgor and continue metabolic activities under drought stress. However, excessive ROS production can impair the function of these osmolytes. Therefore, the coordinated action of osmolyte accumulation and antioxidant protection is crucial for effective stress tolerance.

In plants treated with *Trichoderma harzianum*, the proline content under water stress was higher than in control plants. This increase indicates that the non-mycorrhizal beneficial fungus improved osmotic regulation and contributed to osmotic adjustment under drought conditions. It has been reported that *Trichoderma harzianum* can increase proline accumulation in drought-stressed plants, which facilitates intracellular water storage (Pandey et al. 2016). In addition, H₂O₂ levels in the BTH50 group decreased 1.4-fold (from 20.5 to 14.4 µmol/g FW) and MDA levels decreased 2.9-fold (from 16.8 to 49.4 mg/g FW) compared to C50. The combination of biochar and *Trichoderma* has been reported to synergistically support antioxidant systems and osmotic adjustment (Lian et al. 2023; Mastouri et al. 2012; Rawat et al. 2016; Seleiman et al. 2021). Overall, drought stress triggered ROS accumulation and lipid peroxidation in pepper plants, while biochar and *Trichoderma* application significantly attenuated oxidative damage by activating antioxidant enzyme systems, increasing osmolyte accumulation, and supporting osmotic adjustment. Notably, BTH treatments enhanced the activation of antioxidant enzymes while limiting H₂O₂ and MDA buildup, thereby contributing to improved drought resilience in pepper. Relative to the C100, C75, and C50 groups, BTH applications led to elevated CAT and SOD activities, reduced oxidative marker accumulation, and stimulated the synthesis of key Osmo protectants like proline and sucrose. These findings indicate that the co-application of biochar and *Trichoderma* exhibits a complementary interaction in alleviating oxidative stress and conserving water content during limited moisture availability. Accordingly, employing both agents in tandem under water-limiting scenarios may represent an effective and sustainable approach for resilient crop management.

Water stress had a pronounced effect on the hormone profiles of pepper plants. It is known that drought stress disturbs the hormonal balance and thus impairs vital physiological processes. In general, drought increases levels of ABA, while concentrations of growth-promoting hormones such as IAA, GA and CK decrease (Claeys and Inzé 2013; Cutler et al. 2010; Peleg and Blumwald 2011; Verslues and Juenger 2011). As a result, adaptive responses such as stomatal closure, growth inhibition and accelerated leaf senescence are frequently observed. In our study, ABA concentrations rose by 43% in the C50 treatment relative to C100, highlighting its involvement in early signaling pathways triggered by reduced water availability (Verslues and Juenger 2011; Vishwakarma et al. 2017). However, in the BTH50 treatment, ABA content decreased by 99.8% compared to C50, reaching the lowest levels observed. This result indicates that the application of *Trichoderma* and biochar effectively limited ABA accumulation and mitigated growth limitations (Akhtar et al. 2015; Boorboori and Zhang 2023; Poveda 2020; Yu et al. 2023). Biopriming seeds with drought-tolerant isolates of *Trichoderma harzianum* in *Triticum aestivum* have been shown to downregulate genes responsible for ABA biosynthesis, thereby alleviating drought stress. The resulting lower ABA levels reduced the growth inhibitory effects typically associated with high ABA accumulation under water deficit and promoted better plant development (Shukla et al. 2014). Similarly, inoculation with *Trichoderma harzianum* in wheat has been reported to not only promote overall plant growth and root development, but also delay the onset of drought responses (Rawat et al. 2016).

Regarding the dynamics of IAA, drought stress decreased the IAA content in the C50 group by 75 compared to C100. However, the IAA concentration in the BTH50 group increased 17-fold (from 0.01 to 0.17 µg/g) compared to C50, reaching the highest auxin levels. This result shows that *Trichoderma* promotes IAA production in plant roots (Halifu et al. 2019; Salas-Marina et al. 2011) and that biochar indirectly supports auxin homeostasis (Chang et al. 2021; Yang et al. 2021). It has been reported that *Trichoderma* can stimulate endogenous IAA production when it colonizes plant roots and increases auxin levels even under drought conditions (Çakmakçı et al. 2022; Halifu et al. 2019; Nikpour-Rashidabad et al. 2019; Salas-Marina et al. 2011). For example, tomato seedlings treated with *Trichoderma harzianum* under drought stress showed increased IAA content compared to untreated controls (Rubio et al. 2012). Biochar also indirectly contributes to the maintenance of higher IAA content by improving soil moisture and nutrient status. Studies under saline conditions have shown that biochar application helps to maintain higher levels of growth hormones, including IAA, compared to stressed

controls (Chang et al. 2021; Hannachi et al. 2023; Nikpour-Rashidabad et al. 2019; Yang et al. 2020).

Under water deficit conditions, GA content declined by 45% in the C50 group compared to C100. Nevertheless, the BTH50 group exhibited a 301% elevation in GA concentration relative to C50. This indicates that *Trichoderma* stimulates GA production and helps to prevent the complete growth arrest normally induced by drought stress (Illescas et al. 2021; Jaroszuk-Ścisiel et al. 2019). Previous studies indicated that *Trichoderma* spp. may induce GA production or enhance its biosynthesis in host plants (Illescas et al. 2021; Jaroszuk-Ścisiel et al. 2019; Muhae-Ud-Din et al. 2018). Indeed, plants inoculated with *Trichoderma harzianum* have been reported to maintain higher GA levels that support vertical growth even under stress conditions. Similarly, biochar applications were found to increase GA levels by improving soil properties. In stressed pea seedlings, GA content was almost twice as high in biochar-enriched soils compared to unenriched controls (Dancewicz et al. 2017; Güllap et al. 2024; Khatun et al. 2021). Our results suggest that the application of B and especially BTH helps to maintain the partially elevated GA levels in pepper plants under drought stress and thus attenuate growth inhibition.

In the control groups, significant decreases in the concentrations of cytokinin (CK) and zeatin were observed with decreasing irrigation. However, zeatin concentration increased by 533% in the BTH50 group compared to C50. This improvement is probably due to the fact that *Trichoderma* promotes cytokinin biosynthesis (Illescas et al. 2021; Martínez-Medina et al. 2014) and biochar improves water and ion balance (Aydin et al. 2025; Архипова et al. 2007). *Trichoderma* has been reported to promote cytokinin synthesis or provide cytokinin-like compounds when it enters a mutualistic relationship with plants. *Trichoderma* colonization has been shown to increase levels of trans-zeatin and other cytokinins, thereby promoting root and shoot development (Gao et al. 2022; Illescas et al. 2021; Martínez-Medina et al. 2014). For salicylic acid (SA), drought stress led to a 60% decrease in SA concentrations in the C50 group compared to C100. In contrast, the SA content almost doubled in the TH75 and BTH75 treatments compared to C50. This suggests that *Trichoderma* activates SA signaling pathways to boost antioxidant defenses (Khan et al. 2023b; Leonetti et al. 2017; Miura and Tada 2014; Sidaiah et al. 2017). As for jasmonic acid (JA), the JA content increased in the C75 plants, while it decreased in the C50 group under severe stress. However, JA content increased by 216% in the BTH75 treatment compared to C50, suggesting that *Trichoderma* increases JA-related gene expression and stress tolerance (Cho et al. 2013; Yue et al. 2022; Zhao et al. 2016) and that biochar can indirectly support JA accumulation. Our results show that drought stress severely

disrupted the hormone balance of pepper plants. However, treatments with B, TH and especially BTH optimized the hormone profiles. The application of BTH maintained growth by keeping ABA levels low (99.8% reduction) and IAA (1600% increase), GA (301% increase) and CK (533% increase) levels high, while promoting defense mechanisms through increased SA and JA levels.

Overall findings highlight that jointly applying biochar and *Trichoderma harzianum* offers a promising approach for enhancing pepper development and resilience in water-restricted environments. Ultimately, the implemented treatments modulated the hormonal profile of pepper plants exposed to limited moisture, thereby improving their tolerance to drought. As expected, drought stress under control conditions resulted in a strong growth inhibition characterized by high ABA and low IAA/GA/CK levels. In contrast, biochar improved the soil water balance to physically support the plants, while *Trichoderma harzianum* biochemically reprogrammed plant signaling pathways and prepared the plants for stress conditions. The combined BTH treatment combined the benefits of both approaches by maintaining the most balanced hormone profile: Minimizing ABA accumulation, maintaining elevated levels of growth hormones (IAA, GA, CK) and increasing JA and SA levels to protect plant tissue from damage. Thus, the integrated application of biochar and *Trichoderma* can be considered as a promising and sustainable strategy to improve the resistance of plants under drought stress.

The biochemical, physiological and morphological responses of plants under water stress involve complex and interrelated processes, making drought a complex stress factor that affects multiple physiological pathways simultaneously. Water stress affects nutrient uptake, the antioxidant defense system, hormone balance and growth performance. In this context, the evaluation of several parameters determined in this study (e.g. IAA, ABA, GA, SOD, CAT, MDA, proline, Fe, Zn, N, P, K, etc.) requires not only the comparison of individual mean values, but also an understanding of the relationships and interaction patterns between these parameters. Therefore, a principal component analysis (PCA) was performed to obtain a clearer interpretation of the complex biological responses resulting from the combined effects of water stress and different treatments (Fig 6).

In the PCA analysis, the BTH treatments separated along the positive PC1 axis. This separation was primarily associated with higher levels of antioxidant enzymes (CAT, POD, SOD), osmolytes (proline, sucrose) and growth-promoting hormones (IAA, GA), suggesting that the BTH treatments were characterized by a different biochemical profile compared to the other groups. It is hypothesized that this separation is due to the synergistic effects of biochar, which improves soil structure, and *Trichoderma*, which improves

hormone production and antioxidant protection. In the BTH groups, growth parameters (plant height, leaf number, shoot and root weight) and levels of IAA, GA, cytokinin and zeatin increased, while MDA and H₂O₂ levels decreased. These results indicate that the combined application stimulated growth, supported redox homeostasis and suppressed cellular damage in an integrated manner. In addition, the activities of SOD, POD, catalase and proline accumulation were increased in the BTH treatments, which contributed to both the suppression of oxidative stress and the maintenance of osmotic protection.

In terms of nutrient uptake, elements such as Zn, Fe, K, Ca, and Mg were associated with growth and hormonal responses and grouped with positive PC1 loadings in the PCA plot. Strong positive correlations, such as between Zn–IAA and Fe–zeatin, showed synchronized regulation between nutrient intake and hormone balance. Conversely, control groups under water stress (C50 and C75) were clustered in the negative PC1/PC2 regions of PCA, which were characterized by lower growth, lower hormone levels, and higher ROS accumulation. While single applications of TH and B led to moderate improvements, the BTH combination resulted in stronger correlations between all parameters, leading to a more holistic stress tolerance response. In summary, the joint evaluation of PCA and correlation analyses showed that the application of BTH regulated growth, hormone balance, antioxidant protection, and nutrient uptake simultaneously, creating an effective and integrated protective mechanism against water stress.

Conclusion

This study shows that the combined application of biochar and *Trichoderma harzianum* (BTH) effectively improves the drought tolerance of *Capsicum annuum* L. plants. BTH treatments improved nutrient uptake, activated antioxidant enzymes (CAT, POD, SOD) and reduced oxidative stress indicators such as H₂O₂ and MDA. In addition, BTH increased the accumulation of proline and sucrose and modulated hormonal responses by lowering ABA levels and increasing levels of IAA, GA, cytokinin, salicylic acid and jasmonic acid. Principal component analysis confirmed that the BTH treatments differed significantly from the other groups due to differences in several physiological and biochemical characteristics. While the results provide promising insights, the study was conducted under controlled climate room conditions and limited to a short-term growth period. Therefore, the findings may not fully reflect responses under variable field conditions or long-term applications. Further field studies in different soil types and climates are needed to validate these results. Although BTH

seems promising, the present results should be interpreted with caution as this study was of short duration, conducted in pots and only one soil type was used.

In conclusion, the integrated use of biochar and *Trichoderma harzianum* offers a sustainable strategy to enhance plant resilience under drought stress. This approach may contribute to improved crop performance and resource use efficiency in water-limited agricultural systems.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10725-025-01423-2>.

Author contributions All authors contributed to the development of the research idea and the planning of the study. Material preparation and data collection were performed by (S.K.B.), while (O.E.A.), (M.T.), (F.Ö.), and (A.K.) were responsible for data analysis. The initial draft of the manuscript, including the interpretation of all measured parameters, was written by (A.K.). All authors critically revised the manuscript and gave their consent to its final form.

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Data availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

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