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Mitigating water deficit stress in lemon balm (*Melissa officinalis* L.) through integrated soil amendments: A pathway to sustainable agriculture

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Abstract

Lemon balm (*Melissa officinalis* L.) is a valuable medicinal plant, but its growth can be significantly impacted by drought stress. This study aimed to mitigate the adverse effects of water deficit stress on lemon balm biomass by integrating poultry manure compost, poultry manure biochar, NPK fertilizer, *Trichoderma harzianum*, *Thiobacillus thioparus*, and elemental sulfur as soil amendments. The experiment was conducted in a greenhouse using a completely randomized design with a factorial arrangement, consisting of three replicates. It included a water deficit stress factor at three levels (95–100%, 75–80%, and 55–60% of field capacity) and a soil amendment treatment factor with eleven different fertilizer levels. Treatments included control (no amendment), NPK fertilizer, poultry manure compost, poultry manure biochar, and combinations of these with *T. harzianum*, *T. thioparus,* and elemental sulfur under various water deficit levels. Water deficit stress significantly reduced photosynthetic pigments, gas exchange parameters, chlorophyll fluorescence, relative water content, and antioxidant enzyme activity, while increasing membrane permeability and lipid peroxidation in lemon balm plants. However, the integrated application of organic, biological, and chemical amendments mitigated these negative impacts. The combined treatment of poultry manure compost, poultry manure biochar, NPK fertilizer, *T. harzianum*, *T. thioparus*, and elemental sulfur was the most effective in improving the morpho-physiological properties (1.97–60%) and biomass (2.31–2.76 times) of lemon balm under water deficit stress. The results demonstrate the potential of this holistic approach to enhance the resilience of lemon balm cultivation in water-scarce environments. The integration of organic, biological, and chemical amendments can contribute to sustainable agricultural practices by improving plant morphological and physiological properties and plant performance under drought conditions.

Keywords Plant morpho-physiological properties, Poultry manure compost, Poultry manure biochar, *Trichoderma*, *Thiobacillus* and elemental sulfur

Introduction

Lemon balm (*Melissa officinalis* L.) is a perennial herb valued for its essential oils [1], but drought stress significantly impacts its growth. With climate change leading to decreased rainfall and increased drought, water scarcity poses a major challenge to agriculture [2, 3]. Drought stress can significantly alter the biochemical, physiological, and morphological traits of plants, as well as their

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quality characteristics, resulting in noticeable changes in structure, function, and growth patterns [4, 5]. Changes in the biochemical, physiological, and morphological characteristics of the plant caused by drought stress can affect lemon balm plant biomass [6]. Consequently, researchers are actively seeking solutions to enhance water productivity and soil fertility, particularly in arid and semi-arid regions, while minimizing costs and biological pollution.

Various programs have been proposed to address this challenge, with one prominent method being the implementation of organic amendments in soil management practices [7, 8]. In a study, it was found that drought stress negatively impacted the growth and physiological traits of mustard (*Brassica juncea*), as well as its macro- and micronutrient concentrations. However, the application of organic fertilizers significantly improved plant height, leaf area, relative water content, membrane stability index, and chlorophyll content, enabling the plants to better withstand drought conditions and enhancing their nutrient content [9]. In another study, it was found that drought stress increased essential oil content but decreased essential oil yield and other physiological traits of lemon balm; however, the application of vermicompost significantly improved these traits, especially relative water content, highlighting important interactions with irrigation treatments [10]. The incorporation of adequate amounts of organic amendments into soil also enhances its physical, chemical, and biological properties, thereby facilitating improved water infiltration, reduced soil evaporation, and increased water retention capacity [11–13].

One of the organic amendments commonly utilized in agriculture is poultry manure. Despite being a valuable source of nutrients (rich in N and significant quantities of P and K and trace elements) for plants, poultry manure presents certain drawbacks in agricultural practices (e.g., infectious diseases and pests such as *Escherichia coli, Salmonella,* etc. and nematodes, burning seedlings, toxic gas hazards, high salinity, slow fertilizer efficiency, pollution of the environment, soil hypoxia, nutritional imbalance**,** inconvenient transportation, etc.) [14–16]. To address these challenges, it is essential to implement proper composting, handling, enrichment, and application techniques. These measures are crucial for mitigating risks and ensuring the safe and effective utilization of poultry manure as an organic fertilizer. Various technologies exist for processing poultry manure into value-added products, with biological treatment methods like composting or anaerobic digestion being the most commonly used [14]. Conversion of poultry manure into compost has shown potential for soil and environmental applications, but it can also result in N loss through ammonia emission, ranging from 13 to 70% [17, 18]. The pyrolysis of poultry manure into biochar has also demonstrated potential for soil and environmental applications, although it has yet to be widely implemented and scaled up to operational installations [19, 20]. Biochar has been shown to mitigate ammonia emissions and N loss during poultry manure composting [21, 22]. Typically, poultry manure contains 1–14% carbon, resulting in a C:N ratio of 6–7 [23]. Hence, optimal utilization of poultry manure or poultry manure compost as a soil organic fertilizer may necessitate the addition of an external carbon source such as biochar to mitigate N and P loss through leaching. In addition, the utilization of biochar as a soil amendment has garnered increased attention in recent years, attributed to its significant contributions in mitigating climate change, enhancing soil fertility, improving water use efficiency, reducing greenhouse gas emissions, and boosting crop production [24]. Biochar application also improves soil conditions, fostering beneficial microorganisms like plant growth-promoting bacteria. These bacteria improve nutrient availability and produce plant growth-stimulating compounds, boosting plant performance, especially under drought stress [25–27].

Soil microorganisms, like *Trichoderma* spp., play key roles in soil functions, promoting plant growth by enhancing organic matter decomposition, nutrient availability, and soil enzyme activity. They thrive in the rhizosphere, stimulating nutrient mineralization and improving plant performance through increased nutrient absorption [28, 29].

Studies have explored methods to improve plant nutrition in calcareous soils. The use of acidic materials like sulfuric acid and elemental sulfur is a practical and effective approach, enhancing nutrient uptake by reducing soil pH around the roots. When elemental sulfur is applied, soil microorganisms (e.g., *Thiobacillus* bacteria) oxidize it to produce sulfuric acid, boosting nutrient absorption and plant growth [30].

The research addresses the pressing need for sustainable agricultural practices in the face of climate change-induced water scarcity. By combining poultry manure compost with poultry manure biochar, NPK fertilizer, *Trichoderma harzianum*, and *Thiobacillus thioparus* along with elemental sulfur, the study aimed to investigate the combined effects of these amendments on some morphological and physiological properties of the biomass of lemon balm under water deficit stress in calcareous soil. The goal was to assess the feasibility and sustainability of these combined treatments and to contribute to innovative strategies for sustainable agriculture in drought-prone environments. The innovation lies in the holistic approach of integrating multiple amendments to optimize soil fertility, water retention, and plant resilience. We hypothesized that poultry manure compost enriched with various biological and non-biological modifiers can improve the morphological and physiological properties of lemon balm biomass by mitigating water deficit stress.

Materials and methods

Soil collection and analysis

Soil samples were collected from the top 30 cm layer at the research farm of the Faculty of Agriculture of Lorestan University, Iran. After air-drying and passing through a two-millimeter sieve, various physical and chemical soil characteristics were determined (Table S1). Soil texture was analyzed using the hydrometer method [31], organic matter content was assessed via the wet oxidation method [32], and soil pH was measured in saturated paste extracts [33]. Electrical conductivity (EC) of saturated extracts was determined using an electrical conductivity meter [34], while soil cation exchange capacity was calculated according to a previous study [35]. Calcium carbonate equivalent was determined through acid neutralization [36], and available P was extracted with sodium bicarbonate [37]. Total N was measured via the Kjeldahl method [38], and cationic elements (Fe, Zn, Cu, and Mn) were extracted using the DTPA method [39]. Potassium content was determined by extraction with ammonium acetate, and readings were taken using a flame photometer [40].

Preparation of poultry manure compost and poultry manure biochar

Poultry manure compost and poultry manure biochar were prepared using poultry manure supplied from the complex of broiler breeding farms in Khorram Abad, Iran. The composting process was carried out aerobically according to a previous study [41]. The poultry manure was converted into biochar following a previously established method [42], with the conversion process conducted at a temperature of 300 °C. Subsequently, the compost and biochar derived from poultry manure were ground, and several chemical and physical characteristics were analyzed (Table S2). The pH and EC were measured in a one-to-ten ratio of samples to water [43], while organic carbon content was determined using the wet oxidation method [32], and total N was assessed via the Kjeldahl method [38]. Moreover, the functional groups of biochar were identified using Fourier-transform infrared spectroscopy (FTIR), and its morphology was examined using scanning electron microscopy (SEM) (Fig. 1Sa-d). For chemical analysis, 1 g of each sample was ashed individually in an electric furnace at 400 °C for four hours, dissolved in 2 N hydrochloric acid, and filtered with distilled water. The concentrations of Fe, Mn, Zn, and Cu were determined using an atomic absorption spectrophotometer, K concentration was measured with a flame photometer, and P concentration was determined using the ammonium molybdate vanadate method [44].

Preparation of fungal and bacterial inoculum

Trichoderma harzianum (1×10^7 spores mL^{-1}) was obtained from the Gene Bank of the Department of Plant Protection, Faculty of Agriculture, Lorestan University, Iran. To initiate the inoculum, 500 g of wheat grains were combined with 500 mL of distilled water in one-liter jars. Subsequently, the jars underwent autoclaving for two consecutive days at 121 °C and a pressure of 1 atmosphere to ensure sterilization. Following cooling under sterile conditions, 10^7 spores of *T. harzianum* per milliliter or 4 plugs of 2 cm from the margin of actively growing *T. harzianum* culture were added to each jar containing sterilized wheat seeds. The inoculated jars were then stored at 25 °C and incubated until complete coverage of all culture media by *T. harzianum* was achieved. Daily gentle shaking of the flasks ensured uniform fungal growth. The prepared inoculum was used as a powder [45]. The inoculum of *T. thioparus* bacterum, in powder form with a population of 10^8 CFU g^{-1}, was obtained from the Biology Department of the Agricultural and Natural Resources Research Center of Golestan province, Iran.

Experimental set up and treatments

This study was conducted in 2021 at the research greenhouse of the Faculty of Agriculture, Lorestan University, Iran. The experiment followed a factorial design based on a completely randomized setup with three replications. In this assessment, poultry manure compost was enriched with poultry manure biochar, *T. harzianum*, *T. thioparus* + elemental sulfur, and NPK chemical fertilizers, either separately or in combination. Each fertilizer package (consisting of poultry manure compost with other modifiers, combined or separate) was added to pots containing 4 kg of soil at a rate of half a percent, equivalent to 20 tons per hectare. The experimental treatments comprised three levels of water deficit stress (95–100 (D0), 75–80 (D1), and 55–60 (D2) percent of field capacity) and eleven soil amendment treatments as fertilizer, including: control (F0); poultry manure compost (0.5%,*w/w*) (F1); F1 + poultry manure biochar (10% of poultry manure compost) (F2); F1 + *T. harzianum* (0.5% of poultry manure compost) (F3); F1 + *T. thioparus* (0.5% of poultry manure compost) + sulfur (5.5% of poultry manure compost) (F4); F1 + NPK chemical fertilizer (F5); F1 + poultry manure biochar + *T. harzianum* (F6); F1 + poultry manure biochar + *T. thioparus* + sulfur (F7); F1 + poultry manure biochar + NPK chemical fertilizer

(F8); F1 + poultry manure biochar + *T. harzianum* + *T. thioparus* + sulfur (F9); and F1 + poultry manure biochar + *T. harzianum* + *T. thioparus* + Sulfur + NPK chemical fertilizer (F10). NPK chemical fertilizers were sourced from urea, triple superphosphate, and potassium sulfate, respectively, with each fertilizer applied at a rate of 50 kg per hectare.

To carry out the experiment, the soil was mixed with 0.5% by weight of various fertilizer treatments. After thorough mixing, the soil was then transferred to 4 kg pots, which are plastic pots weighing 210 ± 10 g when empty, with a diameter of 15.5 cm and a height of 18 cm. Except for NPK chemical fertilizer, the other modifiers were thoroughly mixed with poultry manure compost and added to pots containing soil. The mixture was then well-incorporated and incubated at 25 °C for 20 days. During the incubation period, experimental treatments were weighed every two days, and any weight loss due to water evaporation was compensated by adding water. Following the incubation period, one healthy lemon balm sprout obtained from Specialized Service Center for Production and Propagation of Medicinal Plants, Lorestan University, Iran, were planted in each pot containing the respective treatments. This wild plant (with herbarium code of Lu1000) was identified botanically by Dr. Faraj Allah Tarnian at the Pasture and Watershed Management, Lorestan University, Khorramabad, Iran.

Initially, to prevent potential nutrient deficiencies based on the soil test results, zinc, copper, and iron were incorporated into the soil at rates of 150, 1, 2, and 5 mg kg^{-1} soil, respectively, sourced from zinc sulfate, copper sulfate, and iron sequestrene as a solution, respectively. Water deficit stress was not imposed until the six-leaf stage. Following this stage, irrigation was conducted daily throughout the growing season to maintain soil moisture levels consistent with the specified values. Daily measurements of pot weights were recorded using a digital scale to calculate moisture levels. This involved comparing the initial weight of each pot (on the day of watering) with its daily weight. Irrigation adjustments were made as necessary to address any soil water deficiencies according to the assigned treatments. It is important to note that by positioning a series of pots without plants alongside those containing plants, we were able to measure the water required to maintain moisture levels based on the weight of the empty pots. This method effectively accounts for water loss due to evaporation and other factors (transpiration and absorption), eliminating the interference of the increasing weight of the plants as they grow. The average daily temperature ranged from 25 °C to 32 °C, while the night temperature ranged from 20 °C to 16 °C. Relative humidity levels were maintained between 60 and 70%. Light intensity in the greenhouse was measured at 400–500 micromoles per square meter per second. The length of the plant growth period in greenhouse was seven months.

Measurements

At the end of the experiment, the height of lemon balm plant was measured using a ruler. The shoot of the plant was harvested from the soil surface using garden shears, immediately transferred to the laboratory. The plant material was first washed with tap water and then rinsed with distilled water. To determine the dry weight, the plant material (shoot and root) was placed in a paper envelope and dried in an oven at 65 °C for 72 h. After the drying period, the dry weight was also measured using a digital scale accurate to 0.001 g.

Chlorophyll (*a* and *b*) and carotenoid were measured according to a previous method [46]. The chlorophyll fluorescence of the plants was quantified using a fluorimeter device (Hanon tech, Pocket PEA, England), and the maximum PSII quantum efficiency (Fm/Fv) was subsequently computed [47]. Gas exchange parameters were assessed in the upper leaves using a portable gas exchange measuring device (CI-340 model, USA). Measurements included photosynthesis (Pn) in micromoles of carbon dioxide per square meter per second, stomatal conductance (gs) in millimoles of water per square meter per second, CO_2 concentration within the stomatal chamber (Ci) in micromoles per mole, and transpiration (E) in millimoles of water per square meter per second. Data collection occurred between 9 to 11 a.m. on the most developed upper leaf in each replicate. Each leaf was placed in the gas exchange chamber for 60 s, with room temperatures maintained between 20 °C to 30 °C.

To assess the relative water content of the leaves, a sample was chosen from the growing young leaves. Initially, the fresh weight of the sample was measured. Subsequently, the sample was immersed in distilled water for 24 h, and the rehydrated weight was recorded. For the determination of the dry weight, the sample was oven-dried for 48 h at 80 °C. Finally, the relative water content of the leaf was calculated using these measurements [48]. In order to assess the electrolyte leakage of leaf cells, discs of uniform size were prepared and washed with distilled water. These samples were then placed in glass tubes filled with 10 mL of distilled water. After 24 h, the electrical conductivity of the solution was measured using an electrical conductivity meter. Subsequently, the tube containing the sample was subjected to autoclaving at 120 °C for 20 min. Following a cooldown period, the electrical conductivity was measured once more. Finally, the percentage of electrolyte leakage was calculated based on these measurements [49]. To measure malondialdehyde concentration, 0.5 g fresh leaf tissue was ground with

5 mL 20% trichloroacetic acid and 0.5% thiobarbiotic. The extract was centrifuged, then heated at 80 °C for 25 min, cooled, and centrifuged again. The malondialdehyde-thiobarbiotic rust-red substance was measured at 532 nm using a spectrophotometer. Other pigments' absorption was read at 600 nm. Malondialdehyde concentration was calculated in micromoles per gram of leaf fresh weight [50]. For catalase enzyme extraction, 1.5 mL of potassium phosphate buffer with PVP and EDTA was mixed with 0.3 g of powdered leaf tissue. The resulting suspension underwent centrifugation for 20 min at 14,000 rpm and 4 °C. The enzyme assay was conducted at a wavelength of 240 nm, monitoring optical absorption changes at 10-s intervals. Enzyme activity was determined by the amount of hydrogen peroxide inactivated per minute per gram of leaf tissue [51]. For peroxidase enzyme extraction, 1.5 mL of pH 7 potassium phosphate buffer was added to 0.3 g of powdered leaf tissue. After centrifugation for 20 min at 14,000 rpm and 4 °C, the peroxidase enzyme activity was monitored by measuring light absorption changes every 10 s for two minutes at 475 nm. The enzyme activity was quantified based on the inactivation of oxygen in one minute per gram of fresh tissue weight [52].

Data statistical analysis

Data statistical analysis (two way-ANOVA) was conducted to evaluate the impact of various treatments on various parameterizes measured in this assay. Means were compared using Tukey's test at a significance level of 0.05, facilitated by SAS 9.2 software. Correlation analysis was performed using Pearson's coefficients, and the correlation diagram between traits was created using the R-4.4.0 program. Additionally, principal component analysis and cluster analysis were conducted using the factoextra and heatmaps packages in the same software. Graphical representation of the results was generated using GraphPad Prism 8 software.

Results

Effect on photosynthetic activity

As water deficit stress increased, photosynthesis rates decreased. However, the application of enriched fertilizers, particularly the F10 treatment, positively impacted photosynthetic activity across all moisture levels. Compared to no fertilizer, photosynthesis rates at moisture levels of 95–100% (D0), 75–80% (D1), and 60–55% (D2) increased by 2.28, 2.24, and 2.34 times, respectively. The highest photosynthesis rate—28.13 μmol m^{-2} s^{-1}—was recorded under no stress with F10 fertilizer, while the lowest (10.86 μmol m^{-2} s^{-1}) occurred at the 55–60% stress level without fertilizer (Fig. 1A). Chlorophyll *a* content decreased with increasing stress across all fertilizer treatments, although F10 application significantly enhanced chlorophyll a levels compared to no fertilizer. Increases were observed with F10 application at 52.41%, 55.41%, and 60.00% for water levels D0, D1, and D2, respectively (Fig. 1B). The highest chlorophyll *a* content (10.14 mg g^{-1} leaf fresh weight) was noted at 95–100% field capacity with F10, whereas the lowest (5.696 mg g^{-1} leaf fresh weight) was observed at D2 without fertilizer. Chlorophyll *b* content also followed a similar trend, declining with greater stress, but the negative effects were mitigated by fertilizer treatments, particularly F10, which increased chlorophyll *b* levels by 24.44%, 22.20%, and 31.69% for D0, D1, and D2 (Fig. 1C). The highest chlorophyll *b* content (5.961 mg g^{-1} leaf fresh weight) was observed under no stress with F10, compared to the lowest (4.250 mg g^{-1} leaf fresh weight) at the D2 stress level without fertilizer. Carotenoid levels similarly decreased under stress. However, F10 fertilizer produced the most favorable results, with increases of 77.54%, 75.59%, and 94.97% at moisture levels D0, D1, and D2 (Fig. 1D). The peak carotenoid content (3.107 mg g^{-1} fresh weight) was achieved without stress using F10. The lowest was found at the D2 level without fertilizer (1.414 mg g^{-1} fresh weight).

Water deficit stress resulted in increased sub-stomatal CO_2 concentrations, while enriched fertilizers lowered these levels. With the F10 treatment, sub-stomatal CO_2 decreased by 50.77%, 49.82%, and 43.03% at moisture levels D0, D1, and D2 (Fig. 2A). The highest concentration (361 μmol mol^{-1}) occurred without fertilizer at D2, while the lowest (169 μmol mol^{-1}) was seen with F10 at D0. Quantum efficiency of photosystem II (Fm/Fv) declined under stress but improved with F10 application—18.53%, 17.09%, and 16.15% increases at D0, D1, and D2 compared to no fertilizer (Fig. 2B). The highest efficiency was recorded at D0 with F10. The influence of water deficit stress on stomatal conductance was significant; however, fertilizer application enhanced this trait. Both D1 and D2 significantly reduced stomatal conductance, while all fertilizer levels showed improvement over the control treatment, most notably with F10 (Fig. 2C and D).

Similarly, while water deficit stress substantially decreased transpiration rates, the application of F10 fertilizer significantly boosted these rates. Each fertilizer level surpassed the control treatment, but no significant differences were found between lower fertilizer levels and the control (Fig. 3A and B). The interaction of water deficit stress and fertilizer treatments showed no significant effect on leaf relative water content ($p < 0.05$). Water deficit stress led to notable reductions in leaf relative water content, with D1 and D2 resulting in decreases of 3.43% and 7.48% compared to the control treatment (no stress). Application of enriched fertilizers increased relative leaf water content significantly, with F1 through F10

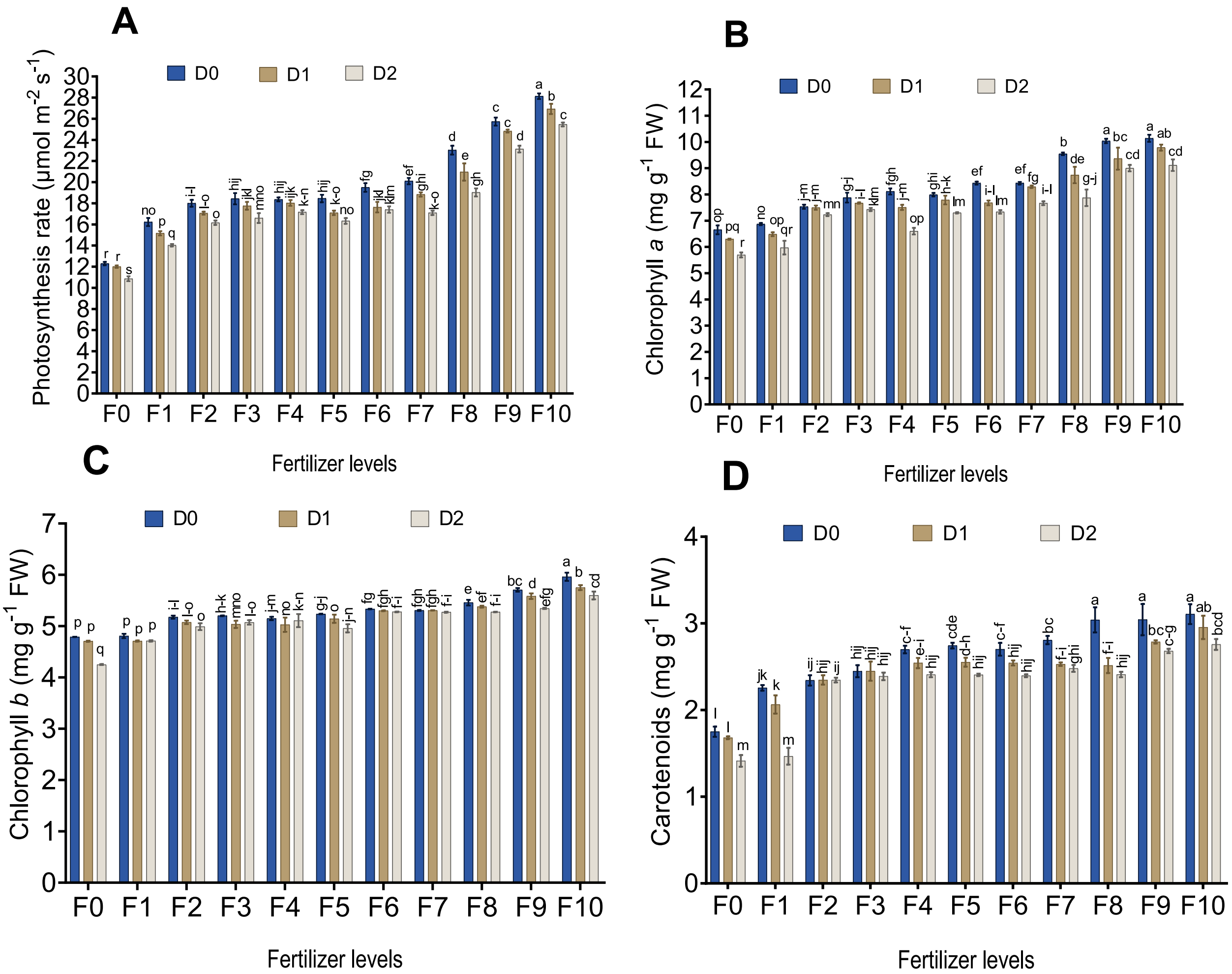

Fig. 1 Effect of fertilizer treatments on photosynthesis rate (**A**), chlorophyll *a* (**B**), chlorophyll *b* (**C**), and carotenoids (**D**) of lemon balm under water deficit D0, water holding capacity at 95–100 percent of field capacity; D1, water holding capacity at 75–80 percent of field capacity; D2, water holding capacity at 55–60 percent of field capacity; control (F0); poultry manure compost (F1); F1 + poultry manure biochar (10% of poultry manure compost) (F2); F1 + *T. harzianum* (F3); F1 + *T. thioparus* + sulfur (F4); F1 + NPK chemical fertilizer (F5); F1 + poultry manure biochar + *T. harzianum* (F6); F1 + poultry manure biochar + *T. thioparus* + sulfur (F7); F1 + poultry manure biochar + NPK chemical fertilizer (F8); F1 + poultry manure biochar + *T. harzianum* + *T. thioparus* + sulfur (F9); and F1 + poultry manure biochar + *T. harzianum* + *T. thioparus* + Sulfur + NPK chemical fertilizer (F10). The means ± SD ($n = 3$) that share the same letters are not significantly different as determined by Tukey's multiple range test at a significance level of $p < 0.05$

demonstrating increases ranging from 6.87% to 18.69% compared to controls (F0), with the highest relative water content recorded in the F10 treatment, although not significantly different from F9 (Fig. 3C and D).

Effects on oxidant and antioxidant activity

The interaction between water deficit stress and fertilizer treatment demonstrated that malondialdehyde levels increased with water deficit stress, while fertilizer applications, particularly the F10 treatment, effectively reduced malondialdehyde levels in plants (Fig. 4A). At moisture levels of 95–100% (D0), 75–80% (D1), and 55–60% (D2) of field capacity, malondialdehyde levels decreased by 49.95%, 47.37%, and 46.56%, respectively, with F10 compared to no fertilizer application at the same moisture levels. The highest malondialdehyde concentration (4.793 µmol g^{-1} fresh weight) was observed at the D2 stress level without fertilizer, while the lowest (2.187 µmol g^{-1} fresh weight) occurred under no stress with F10 treatment (Fig. 4A).

Catalase activity increased in response to both water deficit stress and fertilizer treatments. Among the fertilizers, F10 led to the most significant enhancement in catalase activity, which increased by 2.10, 2.49, and 2.31 times at moisture levels D0, D1, and D2, respectively, compared to no fertilizer application. The highest

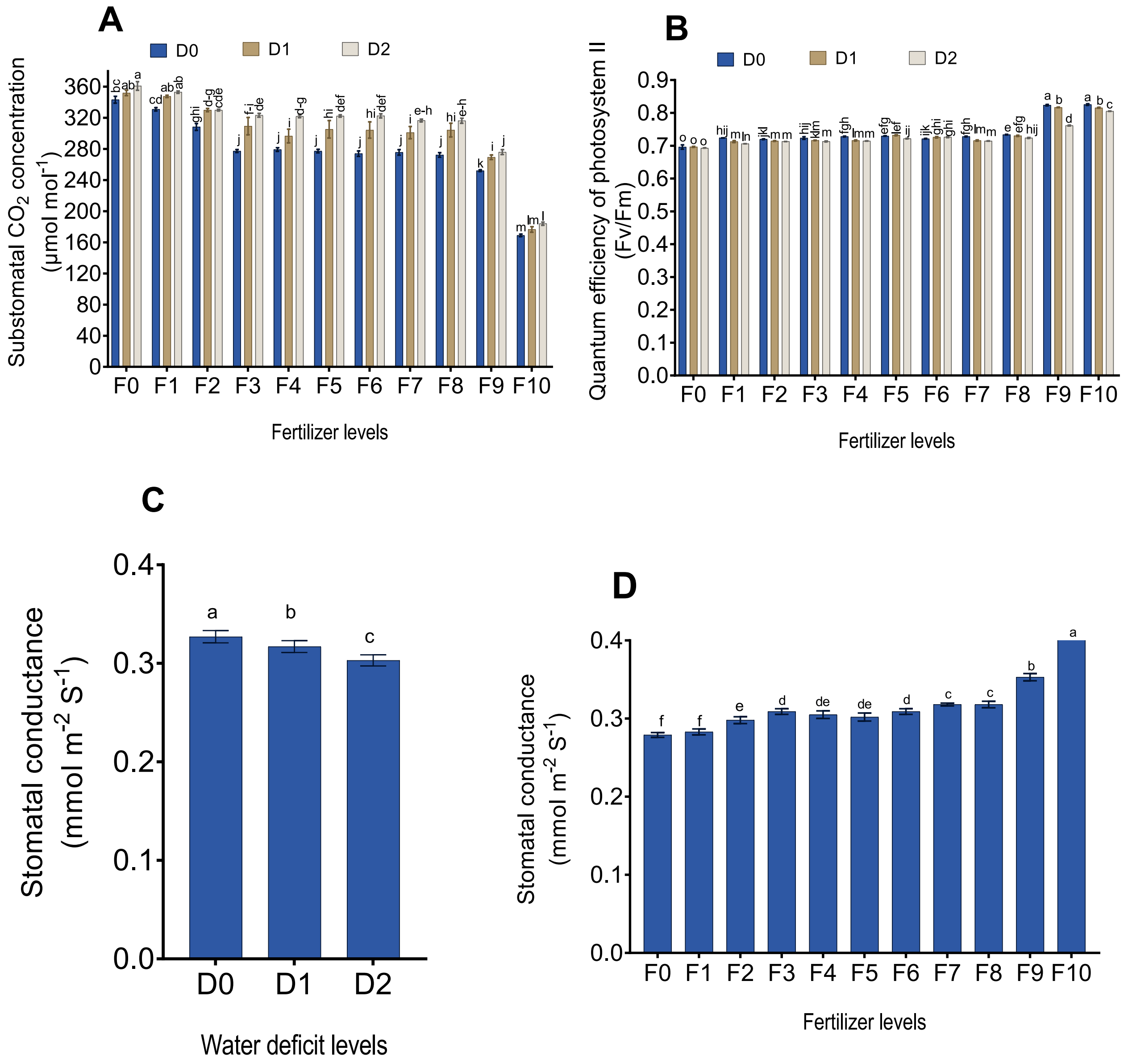

Fig. 2 Effect of fertilizer treatments on substomatal CO_2 concentration (**A**) and quantum efficiency of photosystem II (**B**) of lemon balm under water deficit stress and the effect of water deficit stress and fertilizer treatments on stomatal conductance (**C** and **D**) of lemon balm. Refer to the caption of Fig. 1 for a description of the treatments. The means ± SD ($n = 3$) that share the same letters are not significantly different as determined by Tukey's multiple range test at a significance level of $p < 0.05$

catalase enzyme activity was noted at the D2 stress level with F10 (2.442 µmol min^{-1} fresh weight), while the lowest activity (0.840 µmol min^{-1} fresh weight) was recorded under no stress with no fertilizer (Fig. 4B). Peroxidase enzyme activity also increased with water deficit stress and fertilizer applications. The F10 fertilizer exhibited the most substantial impact, resulting in increased peroxidase activity by 1.79, 1.97, and 2.07 times at D0, D1, and D2, respectively, compared to controls. The maximum peroxidase activity was observed at D2 with F10 treatment (2.754 µmol min^{-1} fresh weight), while the minimum (1.177 µmol min^{-1} fresh weight) was seen in the control group without fertilizer (Fig. 4C).

The interaction between water deficit stress and fertilizer treatments did not significantly affect ion leakage ($p < 0.05$). Electrolyte leakage increased with drought stress, particularly at stress levels D1 and D2, which exhibited increases of 3% and 4.06%, respectively, compared to the no stress control. Conversely, enriched fertilizer treatments reduced electrolyte leakage significantly,

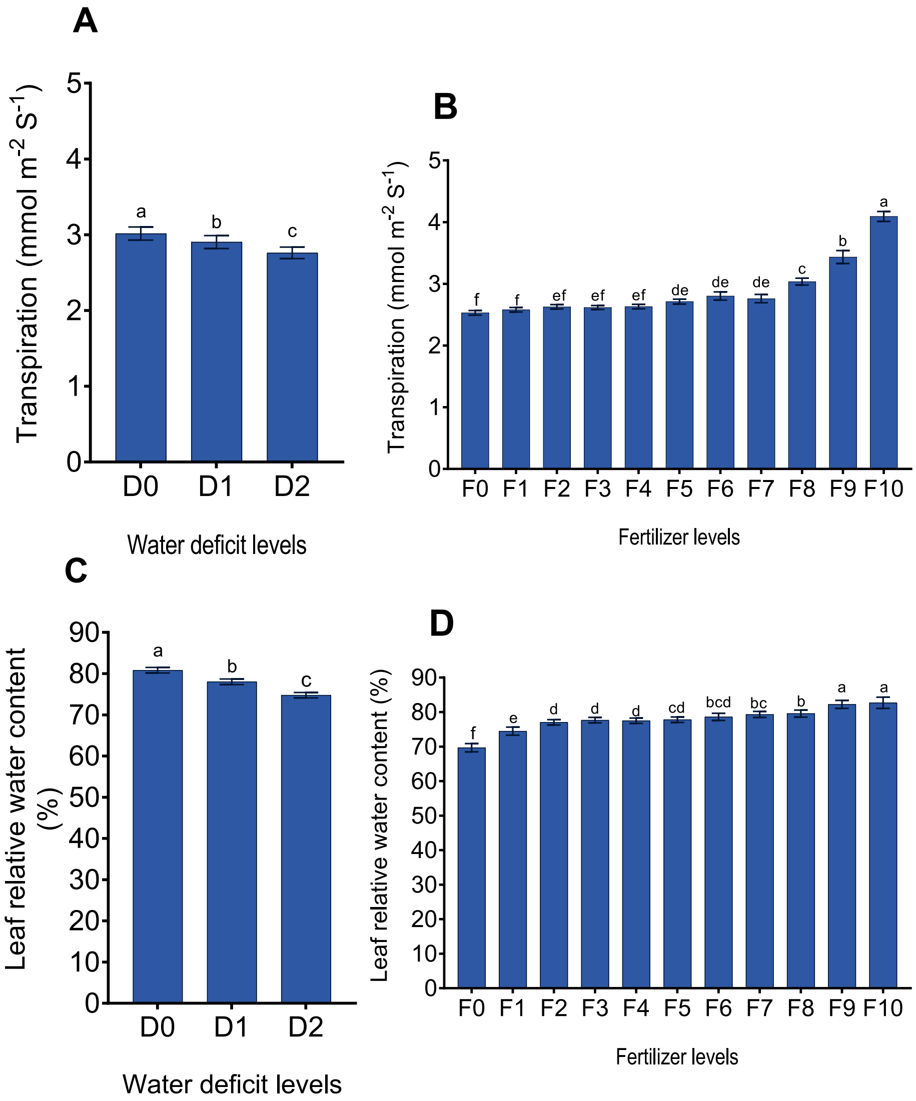

Fig. 3 Effect of water deficit stress and fertilizer treatments on transpiration rate (**A** and **B**) and leaf relative water content (**C** and **D**) of lemon balm. Refer to the caption of Fig. 1 for a description of the treatments. The means ± SD ($n=3$) that share the same letters are not significantly different as determined by Tukey's multiple range test at a significance level of $p < 0.05$

with reductions of 4.20% to 24.69% across different fertilizer levels compared to controls. However, no significant differences were found between the F1 treatment and the control (Fig. 4D and Fig. 5A).

Effect on plant biomass

The interaction between water deficit stress and fertilizer treatments revealed that plant height decreased under stress, while fertilizer applications, particularly F10, significantly enhanced this trait (Fig. 5B). At moisture levels of 95–100% (D0), 75–80% (D1), and 60–55% (D2), plant height increased by 2.76, 2.72, and 2.12 times, respectively, with F10 compared to no fertilizer. The maximum height (38 cm) was observed at D0 with F10, while the minimum (17 cm) occurred at D2 without fertilizer. Water deficit stress also reduced shoot dry weight (Fig. 5C). However, all fertilizer treatments improved shoot dry weight, with F10 showing the most significant effects, increasing dry weight by 2.76, 2.72, and 2.71 times at D0, D1, and D2, respectively. The highest dry weight (76.6 g pot^{-1}) was recorded at D0 with F10, while the lowest (24.73 g pot^{-1}) was at D2 without fertilizer. Root dry weight decreased under water deficit stress, but fertilizer treatments mitigated these negative impacts. F10 significantly increased root dry weight by 2.31, 2.36, and 2.51 times at D0,

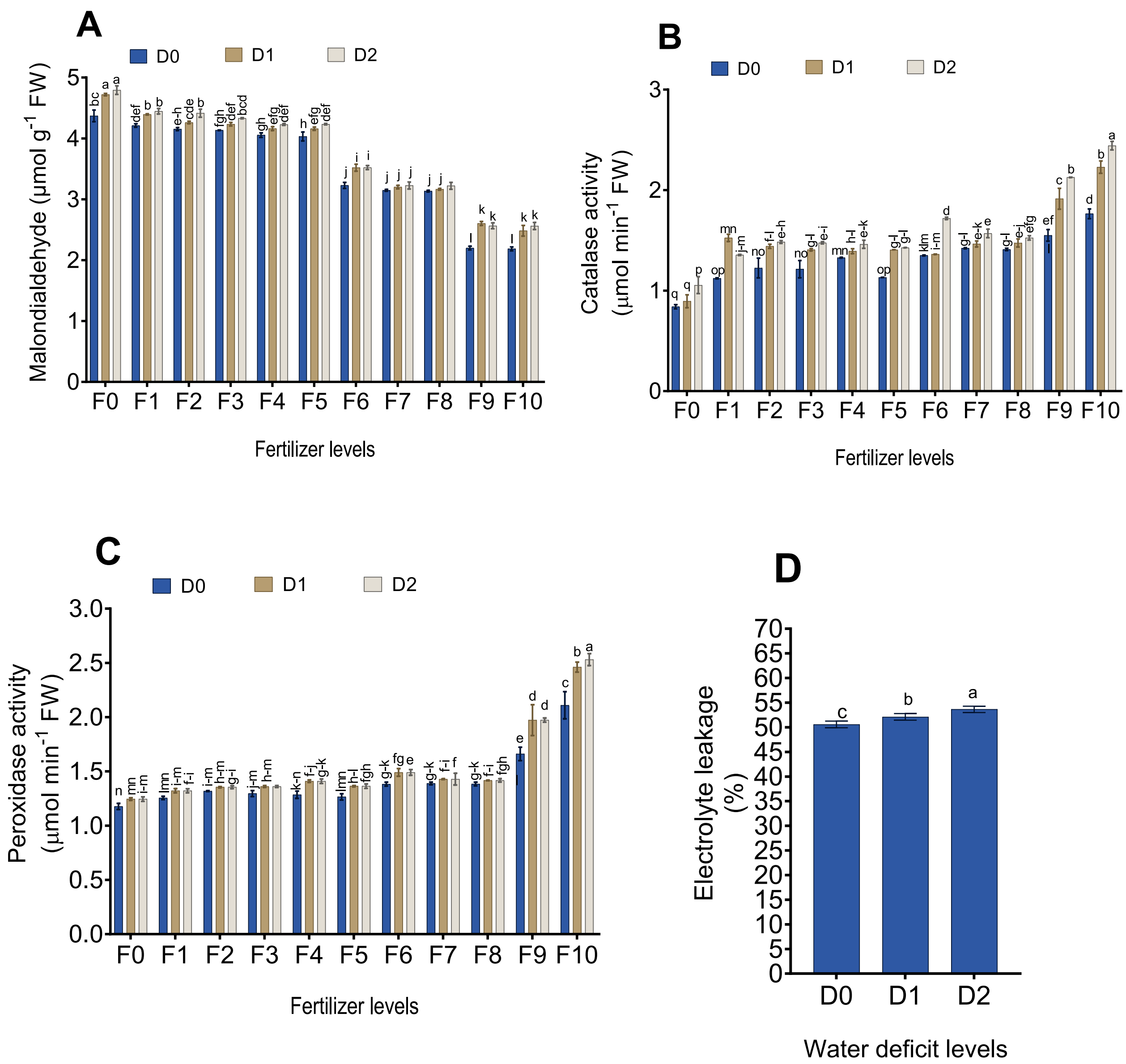

Fig. 4 Effect of fertilizer treatments on malondialdehyde (**A**), catalase activity (**B**), and peroxidase activity (**C**) of lemon balm under water deficit stress and the effect of water deficit stress on electrolyte leakage (**D**) of lemon balm. Refer to the caption of Fig. 1 for a description of the treatments. The means ± SD ($n = 3$) that share the same letters are not significantly different as determined by Tukey's multiple range test at a significance level of $p < 0.05$

D1, and D2, respectively (Fig. 5D). The highest root dry weight (50.5 g pot^{-1}) was at D0 with F10, with no significant difference from F9. The lowest root weight (17.43 g pot^{-1}) was observed at D2 without fertilizer, showing no significant difference from D1. In conclusion, the application of enriched fertilizers, especially F10, significantly enhanced plant biomass under water deficit stress levels (Fig. 6).

Correlation and principal component analysis

The correlation analysis revealed that sub-stomatal CO_2 concentration, malondialdehyde, and electrolyte leakage exhibited positive correlations among themselves while negatively correlating with other traits. The strongest positive correlations were found between chlorophyll a content and photosynthesis rate (r = 0.96), plant height and shoot dry weight (r = 0.96), and transpiration rate

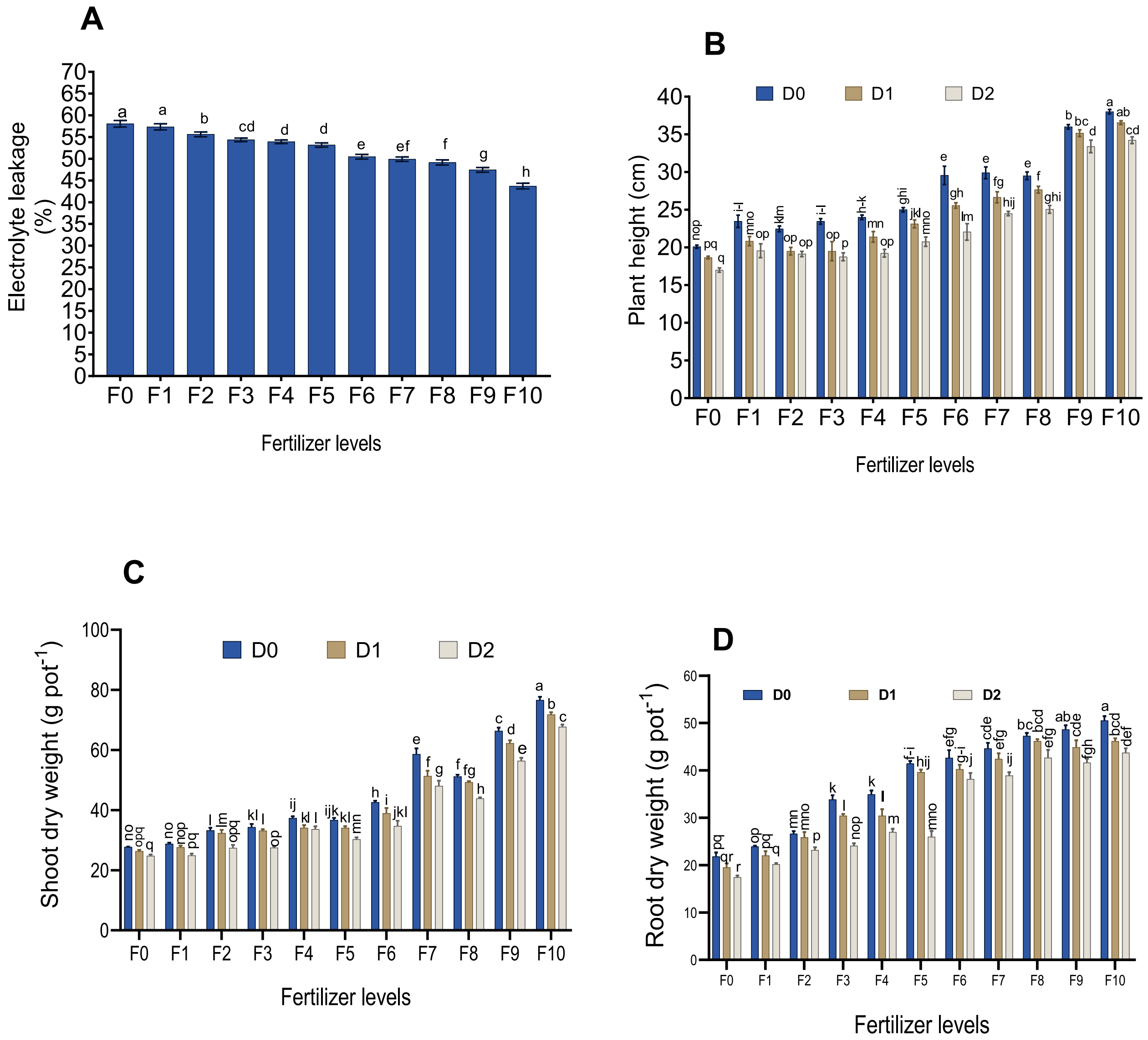

Fig. 5 Effect of fertilizer treatments on electrolyte leakage (**A**) of lemon balm and effect of fertilizer treatments on height (**B**), shoot dry weight (**C**), and root dry weight (**D**) of lemon balm under water deficit stress. Refer to the caption of Fig. 1 for a description of the treatments. The means ± SD (*n* = *3*) that share the same letters are not significantly different as determined by Tukey's multiple range test at a significance level of $p < 0.05$

with stomatal conductance (r = 0.96). Conversely, the most significant negative correlation occurred between stomatal conductance and sub-stomatal CO2 concentration (r = -0.97), indicating that reduced stomatal conductance corresponds with an increase in CO_2 concentration (Fig. 7).

Principal Component Analysis (PCA) showed that the first component accounted for over 80% of the variation in the data, with the first and second components together explaining 91.7% of the total variance. This allowed for more confident interpretation of the results. The PCA indicated two distinct groups of traits differentiated by the first component, with sub-stomatal CO_2 concentration, malondialdehyde, and electrolyte leakage forming one group, while other traits formed a second group. The closely aligned vectors for catalase and peroxidase indicated minimal variation in response to treatments (Fig. 8).

In the biplot analysis, treatments such as F7D2, F8D2, F6D1, and F6D2 were positioned near the origin, indicating minimal effects on traits. In contrast, treatments like F10D0, F9D0, F10D1, F10D2, F0D2, F9D2, and F10D2 exerted more significant impacts and were located further from the origin (Fig. 9). The

Fig. 6 An image depicting lemon balm plant growth under varying levels of water deficit stress, both with and without the application of a soil amendment (F10) and the effects of water deficit stress and the soil amendment on the morphophysiological parameters measured in this study. Upward arrows represent an increasing effect, while downward arrows indicate a decreasing effect

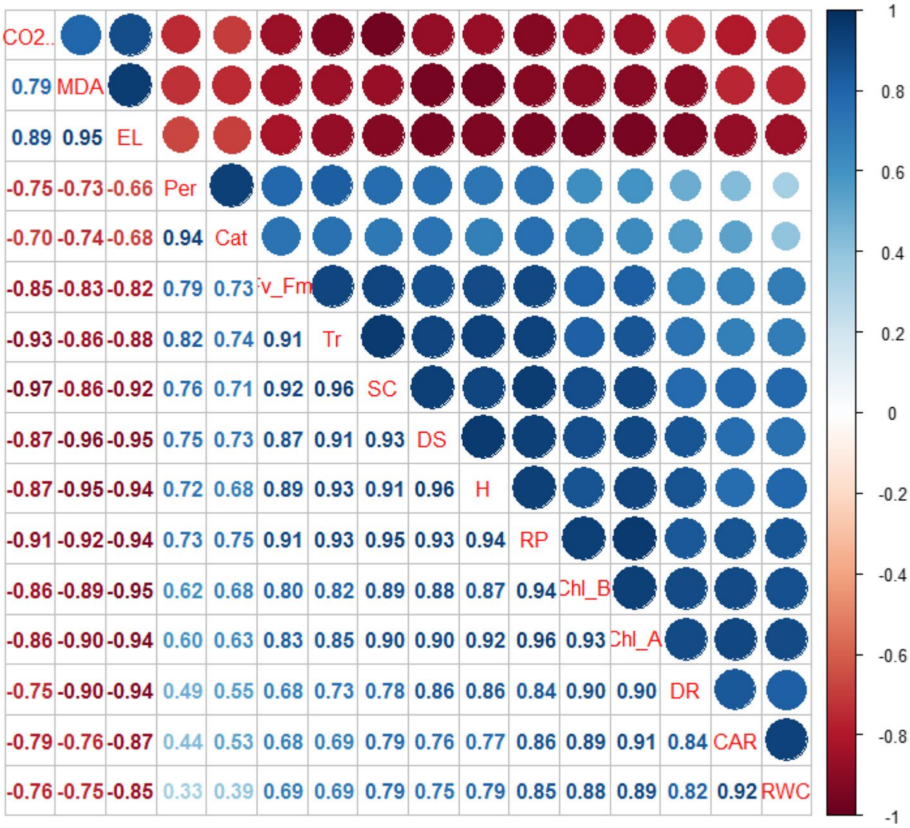

Fig. 7 Pearson's correlation analysis of the impacts of fertilizer fertilizers on morphophysiological parameters of lemon balm in this study under different levels of water deficit stress. CO_2: substomatal CO_2 concentration, MDA: malondialdehyde, EL: electrolyte leakage, Per: peroxidase activity, Cat: catalase activity, Fv-Fm: quantum efficiency of photosystem II, Tr: transpiration rate, SC: stomatal conductance, DS: shoot dry weight, H, plant height, RP: photosynthesis rate, Chl_B: chlorophyll *b*, Chl_A: chlorophyll *a*, DR: root dry weight, CAR: carotenoids, and RWC: leaf relative water content

first component effectively separated factor F levels, with negative values corresponding to treatments F0 through F4 and positive values for treatments F6 through F10. The second component distinguished factor D levels, with D0, D1, and D2 showing negative, zero, and positive values, respectively.

Cluster analysis further categorized the studied traits into two groups. The first group consisted of sub-stomatal CO_2 concentration, malondialdehyde, and electrolyte leakage, while the second group included the remaining traits. Treatments grouped into four clusters, with specific treatments exhibiting varying traits: F10D1 and similar treatments had lower trait values, while treatments F0D2, F1D2, and F0D1 showed higher averages for CO_2 concentration and related traits but lower values for the others (Fig. 10). The findings highlight the complex interrelations among physiological traits and their responses to different treatments. Significant correlations and clear separations among traits suggest targeted avenues for further research on optimizing plant responses to environmental conditions.

Discussion

This study explored the integration of various soil amendments to mitigate water deficit stress in lemon balm, a valuable medicinal plant, through a comprehensive greenhouse experiment. Water deficit stress is a primary challenge for agriculture, particularly in arid and semi-arid regions, where it can dramatically affect lemon balm growth and physiological functions [53, 54]. Our findings underscore the potential of combining organic, biological, and chemical amendments to enhance the resilience of lemon balm under water deficit conditions.

A reduction in water availability significantly disrupts plant functioning by impairing physiological activities [55]. Photosynthetic pigments like chlorophyll and carotenoids are vital indicators of plant health and growth, with their levels directly affecting photosynthesis [56]. Drought reduces chlorophyll and carotenoid content by inhibiting biosynthetic enzymes and increasing oxidative stress due to free radicals [57, 58]. Our findings confirmed that fertilizer treatments significantly improved pigment levels under water deficit stress, with organic

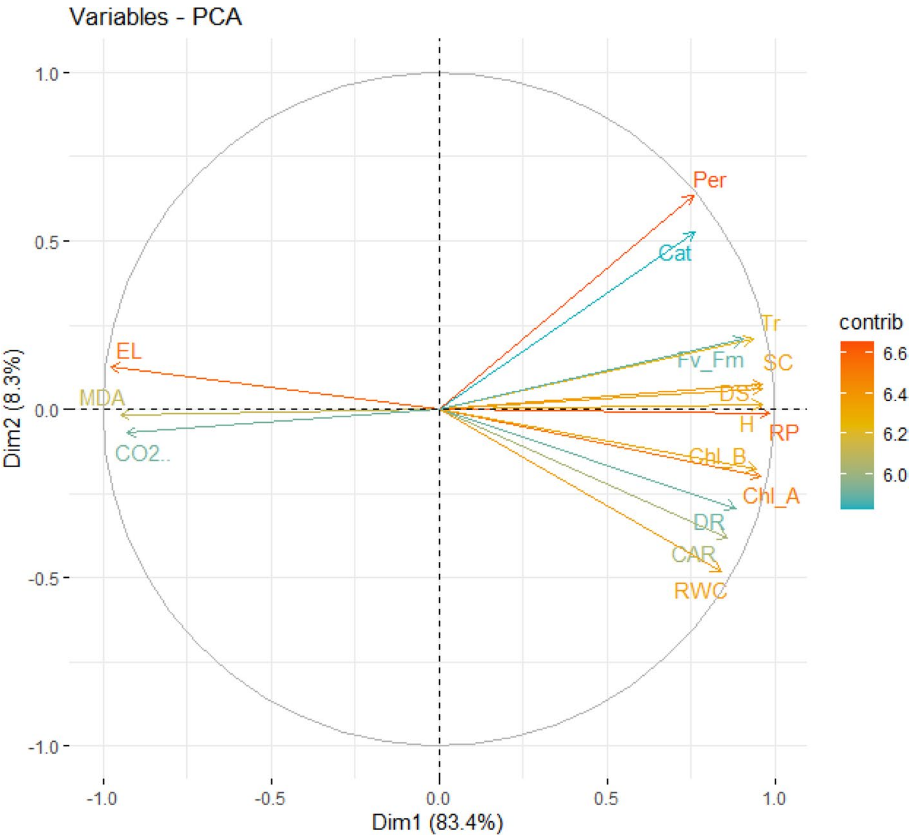

Fig. 8 Principal component analysis (PCA) based on treatments and studied parameters. Refer to the caption of Fig. 7 for a description of the parameters

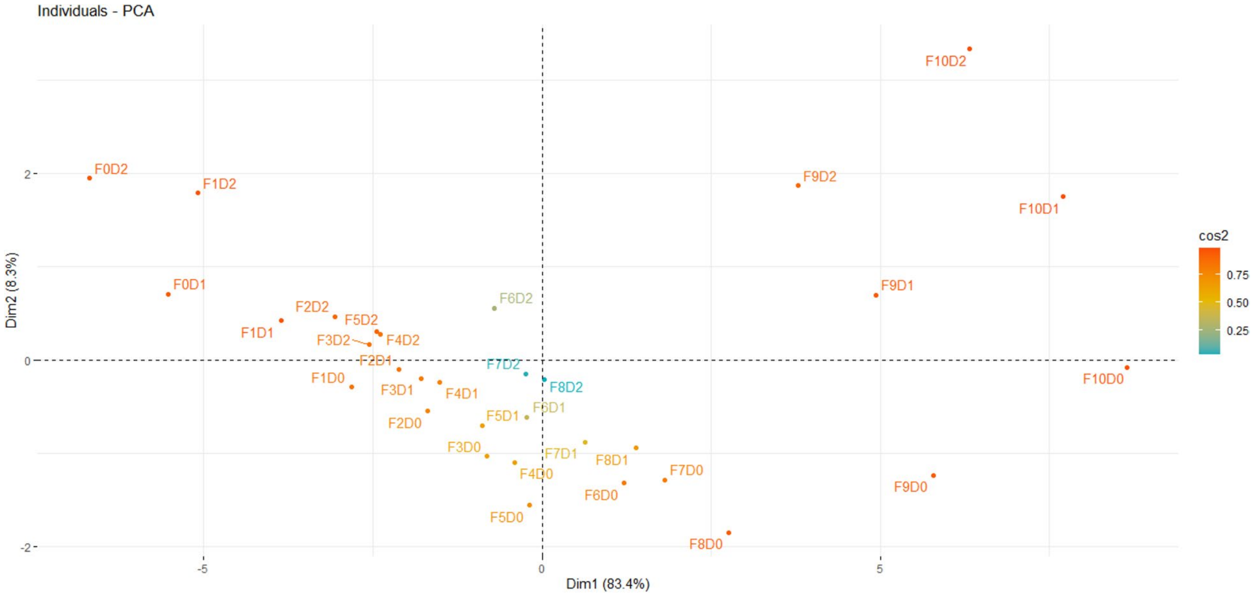

Fig. 9 Distribution of the studied treatments using principal component analysis (PCA). Refer to the caption of Fig. 1 for a description of the treatments

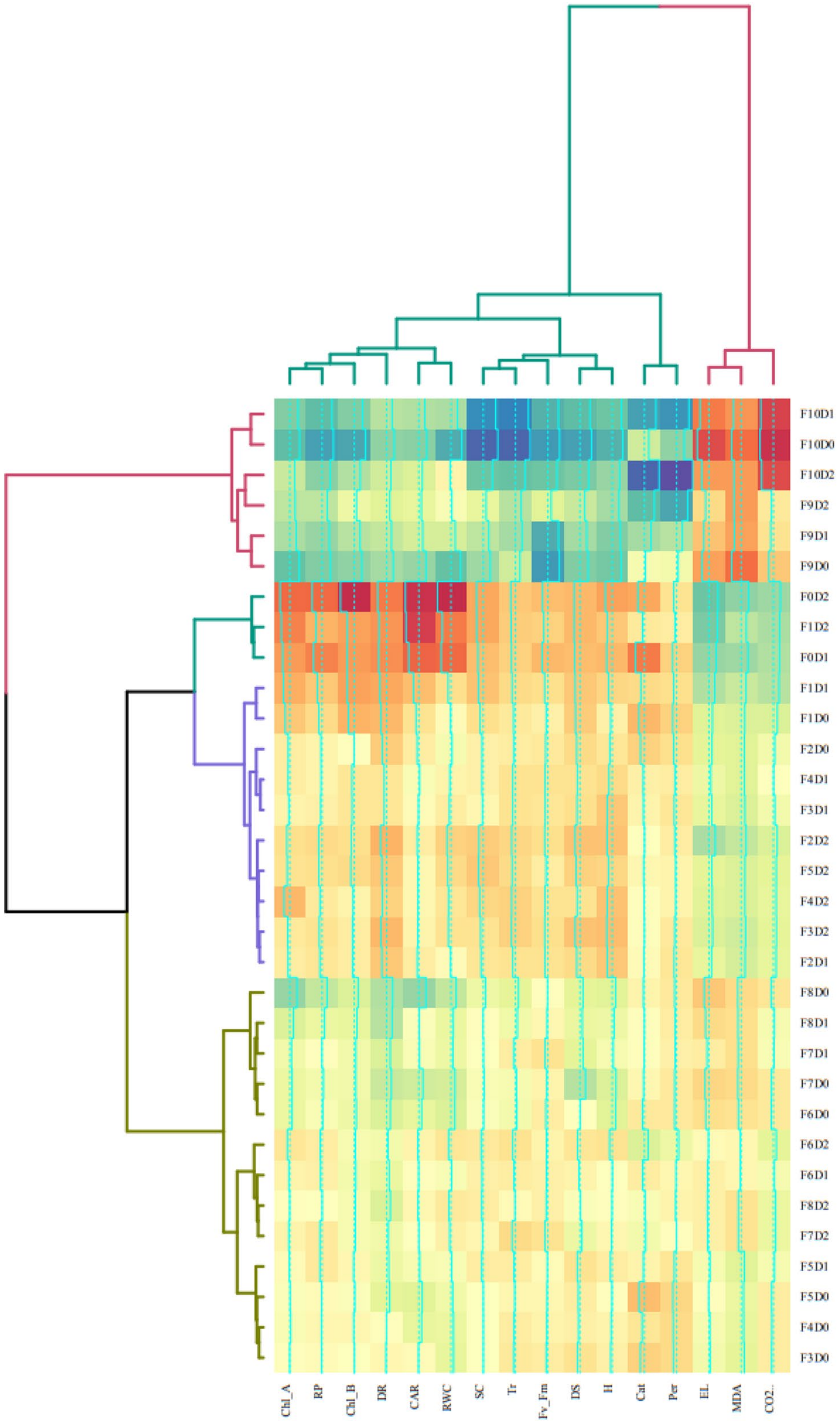

Fig. 10 Heatmap analysis for grouping the studied treatments and measured parameters in this study. Refer to the caption of Fig. 1 and 7 for a description of the treatments and measured parameters in this study, respectively

fertilizers enhancing the availability of essential nutrients for chlorophyll synthesis [59, 60]. According to previous studies, organic fertilizers such as poultry manure compost and poultry manure biochar enhance the availability of essential nutrients for chlorophyll synthesis through several synergistic mechanisms. These organic amendments improve the release and retention of key nutrients like nitrogen, phosphorus, and potassium in the soil. Biochar, in particular, possesses a high cation exchange capacity, which aids in retaining nutrients and preventing their leaching, thereby making them more accessible to plants [61]. Additionally, the incorporation of biochar and compost stimulates microbial activity, which is crucial for the mineralization of organic matter. This microbial process releases nutrients in forms that plants can readily absorb, enhancing nitrification and nitrogen availability [62]. Furthermore, biochar improves soil structure by increasing porosity and water retention, facilitating better root growth and nutrient uptake, which are essential for chlorophyll synthesis. Microorganisms like *Trichoderma* enhance nutrient absorption and chlorophyll levels [63, 64] by solubilizing phosphorus and other nutrients, thereby increasing their availability to plants [65]. This effect is particularly beneficial when using organic amendments such as poultry manure compost and biochar, which can be enriched with nutrients through microbial activity. Additionally, *Trichoderma* spp. boost soil enzyme activities, including phosphatase, facilitating the mineralization of organic phosphorus and improving nutrient uptake by plants [65]. Their presence also alters the soil microbial community, promoting beneficial microbes that enhance nutrient cycling and availability, especially in soils amended with organic materials like poultry manure [62]. The sulfur supplied in fertilizers aids in amino acid production vital for chlorophyll synthesis and overall plant health [66].

Drought stress lowers stomatal conductance, restricting CO_2 supply and subsequently limiting photosynthesis and plant growth. Abscisic acid levels increase in response to drought, leading to stomatal closure [67, 68]. Interestingly, enriched compost improved stomatal conductance, likely due to enhanced root growth and water access resulting from organic fertilizers [69]. Transpiration rates are crucial for understanding water uptake. Under drought, stomata close to conserve water, leading to reduced transpiration and photosynthesis. Our study highlighted that prolonged drought stress can severely diminish photosynthetic efficiency and biomass through stomatal closure [70]. According to previous studies, the soils treated with compost and biochar also enhanced leaf photosynthetic efficiency, promoting increased Rubisco activity and carbohydrate production [71, 72]. Based on the findings of previous studies, compost and biochar enhance photosynthetic efficiency through several key mechanisms. Biochar improves soil structure by increasing porosity and water retention, facilitating better root growth and nutrient uptake, which are essential for photosynthesis. It also adsorbs nutrients like nitrogen and phosphorus, crucial for chlorophyll synthesis. Compost enriches the soil with organic matter, boosting microbial activity and nutrient cycling. Together, these amendments improve microbial communities, reduce nutrient losses, and enhance nutrient availability, collectively contributing to greater photosynthetic efficiency in plants [73–75].

Substomatal CO_2 concentrations reflected reduced carbon fixation due to drought stress, indicating damage to the photosynthetic apparatus [76]. However, the application of various fertilizers improved stomatal conductivity, leading to a decrease in substomatal CO_2 levels, suggesting a recovery in photosynthetic function during optimal conditions. The Fv/Fm index is a critical measure of photosynthetic capacity under stress, and drought negatively impacts this metric due to damage to Photosystem II (PSII) [77, 78]. Supplementing soil fertility enhances chlorophyll synthesis and stabilizes thylakoid membranes, which are crucial for efficient photosynthesis. Adequate nutrients, particularly nitrogen, are essential for producing chlorophyll, allowing plants to capture light energy more effectively. Additionally, a nutrient-rich environment helps maintain the integrity of thylakoid membranes, ensuring optimal functioning of the photosynthetic machinery. This stability leads to reduced chlorophyll fluorescence, a stress indicator that increases when plants face nutrient deficiencies. By maintaining soil fertility, plants experience less stress, which allows them to sustain higher photosynthetic efficiency and overall productivity [79, 80]. Organic fertilizers, like enriched poultry compost, enhance micronutrient absorption, which is vital for photosynthesis, even under drought conditions. These fertilizers provide essential micronutrients such as zinc, iron, and manganese, which play critical roles in chlorophyll synthesis and enzyme functions related to photosynthesis. The organic matter in poultry compost improves soil structure and water retention, helping plants access moisture more effectively during dry periods. Additionally, the microbial activity stimulated by compost aids in the solubilization and availability of these micronutrients, ensuring that plants can uptake the necessary elements for optimal photosynthetic performance. This combination of improved nutrient availability and enhanced soil health allows plants to maintain photosynthesis even when water is limited [81, 82].

Relative water content (RWC) serves as a crucial indicator of drought tolerance. Plants under drought typically experience reduced RWC, leading to diminished photosynthesis and severe physiological effects [83, 84]. Our study revealed that both simple and enriched compost positively influenced RWC, showing the highest improvements with organic and biological amendments. Compost and biochar improved soil structure and moisture retention, ultimately supporting higher leaf RWC [85, 86]. Enhanced RWC promotes better nutrient absorption and overall plant health, critical for drought resistance [87, 88]. Membrane integrity, indicated by ion leakage, is essential in assessing plant stress response. Drought induces elevated reactive oxygen species levels, leading to increased ion leakage and cell membrane damage [89]. Our findings showed that enriched compost reduced ion leakage, indicating improved membrane stability. *Trichoderma* also contributed to maintaining membrane integrity through enhanced antioxidant activity, minimizing ion leakage [90, 91]. Paryan et al. [92] also reported that the application of farmyard manure under drought stress enhances plant growth while reducing electrolyte leakage and protein content in maize tissues and grains.

Water deficit also enhances reactive oxygen species stress in plants by elevating the production of catalase, superoxide dismutase, ascorbate peroxidase, peroxidase, and phenolic compounds [55, 93]. Catalase and peroxidase enzymes play pivotal roles in detoxifying hydrogen peroxide, particularly under drought-induced stress [94, 95]. The present study also revealed a significant increase in the production of antioxidants, specifically catalase and peroxidase, under conditions of water deficit stress. A similar response was noted by Cia et al. [96] and Hussain and Shah [97], where drought-stressed plants exhibited higher levels of catalase and ascorbate peroxidase compared to drought non-stressed plants. These reactive oxygen species generate hydrogen peroxide, which disrupts plant physiological activities through the subsequent production of hydroxyl radicals. These uncharged hydroxyl radicals react with cell membranes, leading to the formation of malondialdehyde compounds [98]. Malondialdehyde content served as a marker for oxidative stress, and increased malondialdehyde levels indicated significant cell damage during drought [99]. The application of compost reduced malondialdehyde levels, suggesting improved antioxidant defenses against oxidative stress [100]. This aligns with findings that organic fertilizers enhance photosynthetic capacity while mitigating oxidative damage [101]. Our study showed that the application of enriched compost significantly elevated these antioxidant enzyme activities, enhancing the plant's ability to cope with oxidative stress [27, 102]. A study on rice plants also found that microbial inoculation with *Trichoderma* and *Pseudomonas* reduced drought stress effects. This treatment increased the activity of antioxidant enzymes such as peroxidase, ascorbate peroxidase, and glutathione reductase, demonstrating that microbial inoculants can enhance the biochemical and molecular resilience of rice plants under drought conditions [103]. The synergistic benefits of organic fertilizers and beneficial microorganisms like *Trichoderma* strengthen the plant's defense mechanisms against drought-induced oxidative damage, enhancing resilience [104]. The increase in antioxidant enzyme activity in response to drought stress and further enhancement by various modifiers suggests that these treatments could be effective strategies for improving plant resilience under challenging conditions. This aligns with the concept of stress acclimatization, where plants adapt to their environment through physiological and biochemical changes.

Water deficit stress also significantly affected plant growth metrics, including plant biomass (shoot and root dry weights) and height, due to limited photosynthesis and stomatal closure [105]. In previous studies, biochar, farmyard manure, and poultry manure enriched with mineral nutrients also promoted root hair development and expanded the root exploration area, facilitating enhanced water uptake [55, 92, 97]. Notably, biochar application yielded significantly better results compared to farmyard manure, and poultry manure, likely due to its superior nutrient retention capacity. Additionally, biochar enhances soil hydro-physical properties, contributing to improved overall soil health [106, 107].

Overall, the combination of poultry manure compost enriched with poultry manure biochar, *T. harzianum*, *T. thioparus*, sulfur, and NPK fertilizer effectively mitigated water deficit stress on lemon balm through several mechanisms. According to the findings of previous studies, poultry manure compost enriches the soil with nutrients and improves soil structure, enhancing water retention and microbial activity, which supports plant growth during drought [95]. Poultry manure biochar enhances soil aeration and moisture retention, reducing nutrient leaching and improving nutrient availability [108]. *T. harzianum* promotes root development and nutrient uptake, particularly phosphorus, while producing growth-promoting substances that help plants tolerate stress [109]. *Thiobacillus thioparus* converts elemental sulfur into sulfate, making sulfur more available, which is essential for synthesizing amino acids and proteins vital for stress response [110]. Sulfur enhances chlorophyll production, supporting photosynthesis [111], while NPK fertilizer provides a balanced supply of nitrogen, phosphorus, and potassium, promoting overall plant health [112, 113]. Together, these components create a synergistic effect, boosting lemon balm's morpho-physiological properties and biomass, allowing the plants to better withstand water deficit stress. This research highlights the efficacy of integrated soil amendments in enhancing plant performance and offers insights for sustainable agriculture in water-scarce environments.

Conclusions

This study demonstrated the effectiveness of integrating multiple soil amendments to mitigate the adverse effects of water deficit stress on lemon balm. The combined application of poultry manure compost, poultry manure biochar, NPK fertilizer, *Trichoderma harzianum*, *Thiobacillus thioparus*, and elemental sulfur significantly enhanced the plant's morpho-physiological properties and overall biomass, particularly under moderate water deficit conditions (75–80% of field capacity). The results revealed that under these moderate drought conditions, the synergistic effects of these amendments dramatically improved chlorophyll content, photosynthesis rates, stomatal conductance, and antioxidant enzyme activity, thereby reinforcing the plant's resilience to stress. The enhancement of physiological traits was less pronounced under severe drought conditions (55–60% of field capacity), indicating that the integrated amendments are more effective when plants experience moderate water deficits. Overall, these findings emphasize the potential of a holistic approach to soil management, combining organic, biological, and chemical amendments to promote sustainable agriculture and enhance the cultivation of medicinal plants like lemon balm in water-scarce environments. Future research should further explore these integrated strategies and assess their long-term impacts on soil health and plant performance under varying environmental conditions.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
Conceptualization, Z.B., M.F., and H.E.; methodology, Z.B., software, Z.B., and H.E., resources, M.F., and H.E.; data curation, Z.B; writing—original draft preparation, Z.B.; and H.B., writing—review and editing, Z.B.; M.F.; H.E.; and L.S.K., supervision, M.F., L.S.K, and H.E., All authors have read and agreed to the published version of the manuscript.

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Declarations

Ethics approval and consent to participate
All methods were performed in accordance with the relevant guidelines and regulations. We have obtained permissions to collect plant material and seedlings.

Consent for publication
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Competing interests
The authors declare no competing interests.

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