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Research Article

Synergistic Biochar and Mycorrhizal Fungi Application Alleviates Cadmium Toxicity and Salinity Stress by Enhancing Soil Functioning and Tomato Productivity

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Abstract

Tomato production is increasingly constrained by cadmium (Cd) contamination and abiotic stresses, which impair plant growth, fruit quality, and food safety. Innovative and eco-friendly strategies are therefore needed to mitigate these constraints. Biochar and mycorrhizal fungi, applied individually or synergistically, have the potential to improve soil biochemical properties, reduce metal bioavailability and toxicity, and enhance tomato growth and fruit quality under stress conditions. This study evaluated the synergistic effects of biochar (0, 1%, and 2% w/w) and mycorrhizal fungi on soil biochemical functioning, Cd bioavailability, and tomato growth in saline soil. The combined application of mycorrhizal fungi and biochar at 2% (w/w) significantly increased soil respiration and phosphatase, nitrogenase, and dehydrogenase activities by 140%, 127%, 330%, and 130%, respectively, while reducing soil electrical conductivity and available Cd concentrations by 29.9% and 59%, respectively. This treatment also markedly decreased Cd accumulation in tomato shoots, roots, and fruits by 37%, 40%, and 93%, respectively, accompanied by reductions of 6% and 56% in the translocation factor and biological concentration factor, respectively. Furthermore, electrolyte leakage and the contents of proline, malondialdehyde, and hydrogen peroxide decreased by 77%, 50%, 94%, and 79.9%, respectively, whereas the activities of antioxidant enzymes, including polyphenol oxidase, peroxidase, catalase, and superoxide dismutase, increased by 129%, 80%, 81%, and 59%, respectively. These improvements in soil biochemical properties and plant physiological responses were associated with an 80.8% increase in tomato yield. Overall, the synergistic application of biochar and mycorrhizal fungi effectively alleviated Cd toxicity, improved soil biochemical functioning, enhanced antioxidant defense, and promoted tomato growth and productivity under saline stress.

Keywords: Biochar, Mycorrhizea, Cd toxicity, Salinity, Cd Stress

1. Introduction

Tomato (*Solanum lycopersicum* L.) production is increasingly threatened by soil contamination with heavy metals [1], particularly cadmium (Cd), and by saline stress [2], both of which severely impair plant growth, physiological functions, and fruit quality. These environmental challenges not only reduce crop yield but also compromise the nutritional and market value of the produce, posing serious concerns for sustainable agriculture and food security [3]. Traditional soil management practices often fail to alleviate heavy metal toxicity and salinity simultaneously, highlighting the need for innovative, eco-friendly approaches. The use of biochar, a carbon-rich soil amendment, and mycorrhizal fungi, a beneficial plant symbiont, has emerged as a promising strategy to improve soil health, mitigate metal toxicity, and enhance crop performance [4, 5]. This study was therefore designed to evaluate the synergistic potential of biochar and mycorrhizal fungi in improving tomato growth, fruit quality, and soil biochemical functioning under combined cadmium and saline stress. Environmental degradation has emerged as a major constraint to global food security, particularly through the deterioration of soil quality and the increasing prevalence of abiotic stressors in agricultural systems [6]. Among these stressors, heavy metal contamination and soil salinity pose serious threats to crop productivity, ecological stability, and food safety worldwide [7]. Cadmium (Cd) is considered one of the most toxic and environmentally mobile heavy metals, frequently accumulating in agricultural soils due to anthropogenic activities such as the application of phosphate fertilizers, sewage sludge amendments, and the use of industrial and municipal wastewater for irrigation [8]. Owing to its high mobility and bioavailability, Cd can be readily taken up by plants and transferred into the food chain, posing severe risks to human and animal health [9].

Cadmium toxicity severely disrupts plant physiological

and biochemical processes, resulting in inhibited growth, reduced photosynthetic efficiency, and impaired nutrient uptake [10]. Exposure to Cd induces oxidative stress by promoting the excessive production of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), which lead to membrane lipid peroxidation and electrolyte leakage [11]. Elevated ROS levels can damage proteins, nucleic acids, and cellular membranes, ultimately overwhelming the plant antioxidant defense system and accelerating cellular dysfunction [12].

Soil salinity is another widespread environmental constraint, affecting more than 800 million hectares of land globally and continuing to expand due to improper irrigation practices, poor drainage, and climate change–induced water scarcity [13]. Salinity stress negatively affects plant growth by disrupting osmotic balance, reducing water uptake, and limiting the availability of essential nutrients such as calcium and potassium [14]. Salinity exacerbates oxidative stress by suppressing antioxidant enzyme activities, thereby intensifying cellular damage and reducing crop productivity [15]. In saline soils, the mobility and bioavailability of heavy metals such as Cd are often enhanced, leading to greater metal uptake and toxicity in plants [16].

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops worldwide and plays a critical role in human nutrition due to its high content of vitamins, antioxidants, and bioactive compounds. However, tomato plants are highly s ensitive to both Cd toxicity and salinity stress, which can substantially reduce fruit yield and quality [17]. In many agricultural regions facing freshwater scarcity, tomatoes are frequently irrigated with marginal or wastewater sources, increasing the likelihood of combined exposure to salinity and heavy metal contamination [18]. Previous studies have demonstrated that the coexistence of salinity and Cd stress exerts synergistically negative effects on plant growth, antioxidant metabolism, and metal accumulation, causing greater damage than either stress alone [19, 20].

In this context, sustainable soil management strategies are urgently required to mitigate Cd toxicity and salinity stress simultaneously. Biochar, a carbon-rich material produced through the pyrolysis of biomass, has gained considerable attention for its ability to improve soil physicochemical properties, immobilize heavy metals, and enhance microbial activity [4, 4]. Biochar application can reduce Cd bioavailability through adsorption, surface complexation, and pH-mediated mechanisms, while also improving soil structure and nutrient retention [21]. In parallel, arbuscular mycorrhizal fungi (AMF) play a vital role in improving plant stress tolerance by enhancing nutrient uptake, regulating metal translocation, and stimulating antioxidant defense systems [22].

Recent evidence suggests that the combined application of biochar and mycorrhizal fungi can exert synergistic effects on soil biochemical functioning and plant performance under adverse environmental conditions [23, 24]. Biochar provides a favorable habitat for mycorrhizal colonization, while mycorrhizal fungi enhance plant access to nutrients and restrict Cd transport to aboveground tissues [25]. However, despite growing interest in this integrated approach, limited information is available regarding its effectiveness in regulating soil enzymatic activity, Cd bioavailability, and physiological responses of tomato plants under saline conditions. Emerging evidence indicates that the combined application of biochar and mycorrhizal fungi can exert synergistic effects on soil biochemical properties and plant performance [24]. Biochar-amended soils provide a conducive environment for mycorrhizal colonization by improving soil structure, reducing metal toxicity, and enhancing microbial habitat stability [25]. In turn, mycorrhizal fungi enhance plant nutrient uptake and physiological resilience, amplifying the beneficial effects of biochar under saline and Cd-stressed conditions [26].

Despite the growing recognition of this integrated approach, limited studies have systematically evaluated its impact on soil enzymatic activity, Cd bioavailability, and physiological responses of tomato plants under saline stress. Therefore, the present study aimed to evaluate the synergistic effects of biochar and mycorrhizal fungi on soil enzymatic activity, cadmium bioavailability, plant physiological responses, and tomato growth under saline stress. This research provides mechanistic insights into an integrated, eco-friendly strategy for improving crop productivity and soil health in Cd-contaminated saline soils.

2. Materials and Methods

2.1. Experimental design

A pot experiment was conducted at Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad 13100, Pakistan under controlled greenhouse conditions to investigate the effects of biochar and arbuscular mycorrhizal fungi (AMF) on tomato growth, soil properties and Cd availability under saline, Cd-contaminated irrigation. The experiment followed a completely randomized factorial design with two factors: biochar application and AMF inoculation. Biochar was incorporated at 0, 1 and 2% (w/w) of soil dry weight, while AMF treatments comprised non-inoculated (AMF0) and inoculated (AMF) plants. Each treatment combination was replicated four times.

2.2. Soil and plant material

Surface soil was air-dried, homogenized and passed through a 2-mm sieve before use. Each 10-L plastic pot (approximately 25 cm in diameter and 25 cm in height) was filled with 8 kg of soil. Initial soil physicochemical properties were determined before treatment application. The soil was slightly alkaline (pH 7.6) and contained 0.86 g kg^{-1} total nitrogen, 0.42 g kg^{-1} total phosphorus, 8.15 g kg^{-1} total potassium and 9.8 g kg^{-1} soil organic carbon.

Tomato (Solanum lycopersicum L.) seeds were surface-sterilized and germinated in nursery trays under greenhouse conditions. Uniform, healthy seedlings were transplanted individually into the prepared pots. All plants received the same basal fertilization and were maintained under identical greenhouse conditions throughout the experiment.

2.3. Irrigation water and Cd exposure

Irrigation water was characterized before the experiment and had an electrical conductivity (EC) of 0.60 ± 0.01 dS m^{-1}, pH of 8.31 ± 0.02 and sodium adsorption ratio (SAR) of 1.50 ± 0.03. The concentrations of Cl^-, Na^+, NH^{4+}, $SO4^{2-}$, Pb, Cd and Ni were 3.51 ± 0.06, 2.01 ± 0.05, 1.69 ± 0.02, 0.14 ± 0.02, 0.589 ± 0.05, 0.098 ± 0.03 and 0.088 ± 0.06 mg L^{-1}, respectively. The Cd concentration was approximately twice the FAO guideline value of 0.05 mg L^{-1} for irrigation water [27].

Following transplanting, all pots were irrigated exclusively with the characterized water throughout the experiment. Irrigation was applied at regular intervals according to plant water requirements, and soil moisture was maintained close to field capacity. An equal volume of irrigation water was supplied to all pots during each irrigation event to ensure comparable exposure to salinity and trace metals.

2.4. Biochar production and characterization

Biochar was produced from a 1:1 (w/w) mixture of wheat straw and peanut shells by pyrolysis at 500 °C for 4 h under oxygen-limited conditions. The resulting biochar had an EC of 0.69 ± 0.02 dS m^{-1}, pH of 7.79 ± 0.03, bulk density of 0.19 ± 0.04 g cm^{-3} and CaCO3 content of 1.6 ± 0.02%. Its specific surface area was 41.0 ± 3.09 m^2 g^{-1} and water-holding capacity was 29.24 ± 1.91%. Moisture content was 9.8 ± 1.11%, while total N, P and K concentrations were 24.19 ± 3.02, 7.59 ± 0.79 and 14.19 ± 1.39 mg kg^{-1}, respectively. The Cd concentration of the biochar was 0.07 mg kg^{-1}.

Biochar was thoroughly homogenized with the soil before transplanting at rates of 0, 1 and 2% (w/w). The 0% treatment served as the biochar control.

2.5. AMF inoculation

A commercial AMF inoculum containing predominantly Rhizophagus intraradices and Funneliformis mosseae was used. The inoculum contained approximately 1,200 infective propagules per 100 g and was applied at 10 g per plant at transplanting. The inoculum was placed directly into the planting hole to facilitate early root colonization. Plants assigned to the non-inoculated treatment received an equivalent quantity of sterilized inoculum carrier.

2.6. Plant management

Phosphorus was incorporated into the soil before transplanting as calcium superphosphate. Nitrogen and potassium were supplied as ammonium nitrate and potassium sulfate, respectively, using split applications during the early vegetative and flowering stages. All treatments received identical fertilizer inputs. Standard greenhouse management practices were followed for weed and pest control, and no additional microbial inoculants were applied during the experiment.

2.7. Sampling and analytical measurements

At harvest, plant growth parameters, physiological traits, and yield attributes were recorded. Soil samples were collected for determination of soil enzymatic activities, electrical conductivity, and available Cd concentration. Plant tissues were separated into roots, shoots, and fruits for analysis of Cd accumulation and antioxidant enzyme activities following standard protocols.

2.6.1. Soil pH, electrical conductivity, and available Cadmium

Soil samples for physicochemical characterization were collected at crop harvest (90 days after transplanting). From each experimental unit, three soil cores (5 cm diameter, 0–20 cm depth) were randomly collected and composited to obtain a representative sample. Visible plant residues, stones, and debris were removed, after which the samples were air-dried, homogenized, and passed through a 2-mm mesh sieve. The processed samples were stored in polyethylene bags at room temperature until chemical analysis.

Soil pH was measured in a soil-to-deionized water suspension (1:2.5, w/w) using a calibrated pH meter, while soil electrical conductivity (ECe) was determined from a saturated soil paste extract at 25 °C using an EC meter. These procedures are commonly used for assessing soil physicochemical properties and salinity status [28]. Available Cd was extracted using ethylenediaminetetraacetic acid (EDTA) and quantified by atomic absorption spectrophotometry (AAS; BK-AA320N; detection limit, 10 ppb), following established procedures for evaluating bioavailable/extractable Cd in soil [29, 30].

2.8. Soil biological properties

2.7.1. Soil respiration

Soil samples intended for biological analysis were collected at the flowering stage (45 days after transplanting), immediately placed in sterile polyethylene bags, transported to the laboratory in ice boxes, and stored at −20 °C until analysis. Prior to analysis, frozen samples were thawed at 4 °C for 24 h. Soil respiration was determined using a glucose-induced respiration approach based on established substrate-induced respiration methods [31]. Briefly, soil samples were amended with glucose (80 mg g^{-1} soil), and soil moisture was adjusted to 60% of water-holding capacity. Samples were incubated at 35 °C for 10 days, and the evolved CO_2 was trapped in NaOH and quantified by titration. Respiration was expressed as mg CO_2 per 100 g dry soil over 24 h.

2.7.2. Soil enzyme activities

Soil dehydrogenase activity (μg TPF g^{-1} dry soil 24 h^{-1}) was determined based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC, 3% w/v) to triphenyl formazan (TPF), following the method of Casida et al. [32]. Phosphatase activity (mg phenol g^{-1} dry soil 24 h^{-1}) was assessed using a colorimetric method based on the hydrolysis of an appropriate phosphatase substrate, following Tabatabai and Bremner [33]. These enzymatic indicators were selected because of their sensitivity to changes in soil microbial

activity and biochemical functioning under environmental stress.

2.9. Plant sampling and processing

2.8.1. Collection of tomato plant samples

At harvest, five tomato plants were randomly selected from each replicate, resulting in 20 plants per treatment. Plants were carefully uprooted and separated into roots, shoots, and fruits. The tissues were thoroughly washed with tap water followed by deionized water to remove adhering soil particles. Root samples were subsequently rinsed with 1 M HCl to remove surface-adsorbed Cd and then repeatedly washed with deionized water. All plant tissues were oven-dried at 65 °C to constant weight, ground into a fine powder using a stainless-steel grinder, and stored in airtight polyethylene containers until chemical analysis..

2.10. Determination of Cd concentration in plant tissues

Cadmium concentration in tomato plant tissues was determined following wet acid digestion. Briefly, 500 mg of dried and ground plant material was transferred to a 300-mL Kjeldahl digestion tube, and 10 mL of concentrated nitric acid (HNO_3) was added. Samples were digested at 121 °C for 1 h using a digestion block. After cooling to below 100 °C, 3 mL of perchloric acid ($HClO_4$) was added, and digestion was continued at 140 °C for an additional 1 h until a clear solution was obtained. The digested samples were diluted to a final volume of 30 mL with deionized water and filtered through Whatman No. 42 filter paper. Cadmium concentration was determined by atomic absorption spectrophotometry (AAS; BK-AA320N), with a detection limit of 10 ppb, following established procedures for acid digestion and elemental analysis of plant tissues [34, 35].

2.11. Relevance to biochar and mycorrhizal treatments

The selected soil chemical, biological, and plant parameters were chosen to comprehensively evaluate the role of biochar and arbuscular mycorrhizal fungi in regulating soil enzymatic activity, reducing Cd bioavailability, and enhancing tomato growth under combined salinity and Cd stress conditions. These indicators collectively reflect soil health, microbial functionality, metal immobilization efficiency, and plant metal uptake dynamics, which are central to the objectives of this study.

2.12. Analysis of BAC, TF, and BCF indices

The biological accumulation co-efficient (BAC), translocation factor (TF) and biological concentration factor (BCF) were calculated using the following formulas (1, 2 and 3):

$$BAC = \frac{Cd\ level\ in\ plant\ shoot}{Cd\ level\ in\ soil} \quad (1)$$

$$TF = \frac{Cd\ level\ in\ plant\ shoot}{Cd\ level\ in\ root} \quad (2)$$

$$BCF = \frac{Cd\ level\ in\ plant\ root}{Cd\ level\ in\ soil} \quad (3)$$

2.13. Stomatal conductance, H_2O_2, MDA, Proline level, EL, and RWC determination

Physiological and biochemical analyses were conducted using fully expanded, healthy leaves collected from the upper canopy of tomato plants at 60 days after transplanting. Sampling was performed between 11:30 and 13:30 h to minimize diurnal variation and ensure uniform light exposure across treatments. Stomatal conductance (gs) was measured on the youngest fully expanded leaf using a portable photosynthesis system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) under ambient CO_2 concentration and natural light conditions. Relative water content (RWC, %) was determined using leaf discs (1 cm^2). Fresh weight (FW) was recorded immediately after excision, followed by immersion of discs in deionized water for 5 h at room temperature to obtain turgid weight (TW). Dry weight (DW) was measured after oven-drying samples at 65 °C for 36 h. RWC was calculated using the following equation:

$$RWC\ (\%) = \frac{FW-DW}{2TW-DW} \times 100 \quad (4)$$

Hydrogen peroxide (H_2O_2) content was determined colorimetrically following the method of Velikova et al. [37]. Briefly, fresh leaf tissue was homogenized in 0.1% (w/v) trichloroacetic acid (TCA) under chilled conditions and centrifuged at 6000 × g for 15 min. The absorbance of the resulting supernatant was measured at 426 nm using a UV–visible spectrophotometer, and H_2O_2 concentration was calculated against an appropriate standard curve and expressed as µmol g^{-1} fresh weight. Lipid peroxidation was assessed by determining malondialdehyde (MDA) content using the thiobarbituric acid-reactive substances (TBARS) assay. The assay was performed according to the method of Hodges et al. [38], with absorbance measured at 532 and 600 nm to correct for nonspecific turbidity. MDA concentration was expressed as nmol g^{-1} fresh weight. Heath and Packer (1968) may be cited as the original basis of the TBA assay, but Hodges et al. [38] is preferable for plant leaf tissues because it accounts for interfering compounds.

Electrolyte leakage (EL) was determined as an indicator of cellular membrane integrity. Uniform leaf discs were thoroughly rinsed with deionized water and immersed in a known volume of deionized water. The initial electrical conductivity (C_1) of the bathing solution was measured after incubation at 60 °C for 30 min. The same samples were subsequently heated at 90 °C for 15 min to release the remaining electrolytes, after which the final electrical conductivity (C_2) was recorded. Electrolyte leakage was calculated following equation (5).

$$EL\ (\%) = \frac{EC1}{EC2} \times 100 \quad (5)$$

where C_1 represents the electrical conductivity measured after the first incubation and C_2 represents the total electrical conductivity measured after the second heat treatment.

Free proline content was determined using the acid-ninhydrin method of Bates et al. [39]. Briefly, 0.8 g of fresh

leaf tissue was homogenized in the appropriate extraction medium and centrifuged at 10,000 × g for 7 min. The resulting extract was reacted with acid ninhydrin and glacial acetic acid and incubated to develop the proline–ninhydrin chromophore. The chromophore was subsequently extracted with toluene, and absorbance was measured at 520 nm using a UV–visible spectrophotometer. Proline concentration was calculated from an L-proline standard curve and expressed as μmol g^{-1} fresh weight. The use of acid ninhydrin, toluene extraction and measurement at 520 nm follows the established method of Bates et al. [39].

Lipid peroxidation was assessed by determining malondialdehyde (MDA) concentration using the thiobarbituric acid-reactive substances (TBARS) assay. Approximately 450 mg of fresh leaf tissue was homogenized under liquid-nitrogen conditions and extracted with trichloroacetic acid (TCA). The extract was reacted with thiobarbituric acid (TBA) and heated at 90 °C. After cooling and centrifugation at 12,000 × g for 15 min, absorbance was measured at 532 and 600 nm using a UV–visible spectrophotometer. MDA concentration was calculated after correction for nonspecific turbidity and expressed as nmol g^{-1} fresh weight, following the plant-tissue TBARS procedure of Hodges et al. [38].

Hydrogen peroxide (H_2O_2) content was determined spectrophotometrically following the method of Shan and Ou [40]. Approximately 1.5 g of fresh leaf tissue was homogenized under liquid-nitrogen conditions in 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 6500 × g for 10 min, and the resulting supernatant was used for colorimetric determination of H_2O_2. Absorbance was measured at 426 nm using a UV–visible spectrophotometer, and H_2O concentration was calculated against an appropriate standard curve and expressed as μmol g^{-1} fresh weight.

These physiological and biochemical parameters were used to evaluate plant water status, membrane stability, osmotic adjustment, and oxidative stress responses under combined salinity and Cd stress and to assess the potential protective effects of biochar and AMF application.

2.14. Photosynthetic pigments and photosynthetic rate

Photosynthetic pigments were determined at 60 days after transplanting using fully expanded leaves from the upper canopy. Fresh leaf tissue was extracted in an appropriate solvent, and absorbance was recorded at 663, 645 and 470 nm using a UV–visible spectrophotometer. Chlorophyll a, chlorophyll b and total carotenoid concentrations were calculated using established spectrophotometric equations and expressed on a fresh-weight basis.

Net photosynthetic rate ((Pn)) was measured at 60 days after transplanting using fully expanded upper-canopy leaves and a portable photosynthesis system (LI-6400; LI-COR Biosciences). Measurements were conducted between 11:30 and 13:30 h. Photosynthetically active radiation was maintained at 1000 μmol m^{-2} s^{-1}, leaf-chamber temperature was maintained at 32 °C, and ambient CO_2 concentration ranged from 350 to 400 μmol mol^{-1}. Measurements were recorded after the gas-exchange parameters had stabilized.

2.15. Antioxidant enzyme activity

Antioxidant enzyme activities were determined in fresh leaf tissue collected at 60 days after transplanting. Approximately 0.5 g of fresh leaf tissue was homogenized under chilled conditions in 50 mM phosphate buffer (pH 7.7) using a pre-cooled mortar and pestle under liquid nitrogen. The homogenates were centrifuged at 11,000 × g for 10 min at 5 °C, and the resulting supernatants were used for enzyme assays.

Catalase (CAT) activity was determined by monitoring the decrease in absorbance at 240 nm resulting from the decomposition of H_2O_2. Peroxidase (POD) activity was determined spectrophotometrically by monitoring the oxidation of an appropriate electron donor in the presence of H_2O_2. Superoxide dismutase (SOD) activity was determined using a standard inhibition-based spectrophotometric assay. Polyphenol oxidase (PPO) activity was determined by monitoring the oxidation of an appropriate phenolic substrate. Enzyme activities were expressed on a fresh-weight basis as units g^{-1} fresh weight.

2.16. Fruit yield and yield components

At physiological maturity, five representative tomato plants were randomly selected from each replicate. Fruits were harvested, cleaned to remove adhering soil and debris, and weighed using an analytical balance to determine fresh fruit yield per plant. Where dry biomass was required, fruit samples were subsequently oven-dried at 65 °C to constant weight. Yield components were recorded for each experimental unit and used to assess the effects of biochar and AMF on tomato productivity under saline and Cd-contaminated irrigation.

2.17. Statistical analysis

The experiment was analyzed using a two-factor factorial design, with biochar application rate and arbuscular mycorrhizal fungi (AMF) inoculation as fixed factors. Data were subjected to analysis of variance (ANOVA) to determine the main effects of biochar, AMF inoculation, and their interaction. The assumptions of normality and homogeneity of variance were assessed before analysis. When significant treatment effects were detected, means were separated using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. Results are presented as means ± standard deviation (SD) of four biological replicates. All statistical analyses were performed using Statistix 8.2 (Analytical Software, Tallahassee, FL, USA).

3. Results

3.1. Variations in soil chemical properties and biochemical reactions

The application of biochar (BC) and mycorrhizal fungi markedly influenced soil chemical properties and enzymatic activities under saline and Cd stress (Table 1). Control plots (CL), irrigated with Cd-contaminated water without any amendments, showed minimal changes in soil pH relative to

initial levels. Among the treatments, the combined application of mycorrhizal fungi and BC at 2% (w/w) produced the greatest reduction in soil pH, indicating enhanced amelioration of soil alkalinity. Similarly, soil electrical conductivity (ECe) was elevated in control plots compared to pre-trial measurements, whereas all BC and mycorrhizal treatments effectively reduced ECe, with the mycorrhizal fungi + BC (2% w/w) combination yielding the lowest ECe (3.29 dS m^{-1}). Irrigation with Cd-contaminated water increased the available soil Cd concentration from 0.98 mg kg^{-1} to 1.10 mg kg^{-1} in control plots. The application of BC and mycorrhizal fungi significantly reduced soil Cd availability, with the most pronounced decrease observed under the mycorrhizal fungi + BC (2% w/w) treatment. Soil respiration rates were enhanced by both BC and mycorrhizal inoculation. Plots treated with BC at 1% and 2% (w/w) showed soil respiration rates of 36.1 and 44.2 mg CO_2 100 g^{-1} soil 24 h^{-1}, respectively, compared to 27.3 in control plots. Among fungal treatments, mycorrhizal fungi alone exhibited higher soil respiration, with the maximum rate recorded under mycorrhizal fungi + BC (2% w/w) (50.4 mg CO_2 100 g^{-1} soil 24 h^{-1}). Similarly, soil dehydrogenase activity increased with BC and mycorrhizal inoculation. While BC (2% w/w) alone enhanced dehydrogenase activity to 38.2 µg TPF g^{-1} soil 24 h^{-1}, the combination of mycorrhizal fungi + BC (2% w/w) resulted in the highest activity (42.4 µg TPF g^{-1} soil 24 h^{-1}), surpassing both control and individual treatments. Soil nitrogenase activity exhibited a dose-dependent response to BC application. BC (2% w/w) enhanced nitrogenase activity to 181 µg N g^{-1} soil 24 h^{-1}, whereas control plots showed an activity of 78 µg N g^{-1} soil 24 h^{-1}. Mycorrhizal fungi treatments also significantly improved nitrogenase activity, with the combined mycorrhizal fungi + BC (2% w/w) treatment producing the maximum activity, demonstrating the synergistic effect of biochar and beneficial fungi in stimulating N-cycling processes under Cd stress. Phosphatase activity followed a similar trend. The highest activity was observed in plots receiving mycorrhizal fungi + BC (2% w/w) (0.89 mg phenol g^{-1} soil 24 h^{-1}), whereas control plots exhibited the lowest activity (0.60 mg phenol g^{-1} soil 24 h^{-1}). While mycorrhizal fungi alone increased phosphatase activity to 0.79 mg phenol g^{-1} soil 24 h^{-1}, the combination with BC at 2% (w/w) maximized enzyme activity, highlighting the positive interaction between biochar and mycorrhizal inoculation in enhancing soil biochemical functioning under saline and Cd stress.

3.2. Cd concentrations in plant tissues and their accumulation characteristics

The application of mycorrhizal fungi (MF) and biochar (BC) significantly reduced Cd concentration in tomato plant tissues (Table 2).

Table 1. Variations in soil chemical properties and biochemical activities with biochar applications and mycorrhizea treatments.

Factors	Treatment	pH	ECe (dSm^{-1})	Cd(mg.kg^{-1})	Soil respiration (mg.CO_2 100g^{-1} 24 h^{-1})	Dehydrogenase (µg TPF g^{-1} dry soil 24 h^{-1})	Nitrogenase (µM.C_2H_4 kg^{-1} soil h^{-1})	Phosphates (mg phenol g^{-1} soil 24 h^{-1})
BC	0	8.09±0.02a	4.19±0.02a	0.69±0.01a	27.3±3.07c	21.9±1.80c	78±20.19c	0.60±0.09c
	1% w/w	8.06±0.02b	3.99±0.03b	0.49±0.02b	36.1±4.50b	30.2±2.79b	130±28.09b	0.69±0.11b
	2% w/w	8.01±0.01b	3.90±0.03c	0.44±0.03c	44.2±3.94a	38.2±2.39a	181±19.12a	0.90±0.10a
AMF	Control	8.12±0.02a	4.70±0.03a	0.70±0.01a	27.9±8.09b	25.1±7.10b	93.8±39.70b	0.59±0.10b
	AMF-1	8.10±0.02b	4.19±0.03b	0.49±0.02b	34.9±5.80b	30.2±7.01ab	130±38.69ab	0.69±0.09ab
	AMF-2	8.09±0.02c	3.79±0.03c	0.48±0.01b	37.3±8.10b	30.9±7.31ab	135±36.74ab	0.74±0.08b
	AMF-3	8.08±0.01c	3.50±0.04d	0.44±0.02c	39.9±8.81a	32.9±7.30a	149±43.39a	0.79±0.09a
Combined Interactions								
BC-0%	Control	8.20±0.02a	4.90±0.04a	1.10±0.02a	21.1±0.89g	18.1±0.50k	47±1.39i	0.39±0.01h
	AMF-1	8.15±0.02bc	4.39±0.02c	0.63±0.01b	26.9±0.29f	22.3±0.49j	80±1.79h	0.59±0.02g
	AMF-2	8.09±0.02cd	3.89±0.02f	0.59±0.02b	28.4±0.50i	22.8±0.50i	85±1.50h	0.61±0.02f
	AMF-3	8.08±0.02de	3.70±0.03h	0.49±0.001c	24.9±0.40h	24.9±0.40h	98±0.80g	0.66±0.02g
BC-1%	Control	8.12±0.02ab	4.59±0.02b	0.55±0.03f	26.9±0.60f	23.9±0.20i	89±1.19g	0.64±0.02f
w/w	AMF-1	8.11±0.02cd	4.20±0.05g	0.49±0.01d	35.8±0.59d	29.2±0.49g	125±1.80f	0.69±0.02e
	AMF-2	8.10±0.02de	3.79±0.06f	0.47±0.02g	36.9±0.10d	31.2±0.70f	129±2.14e	0.71±0.02e
	AMF-3	8.07±0.05d	3.39±0.03i	0.46±0.02h	39.4±0.70c	33.9±0.29d	166±2.80c	0.79±0.01c
BC-2%	Control	8.12±0.02ab	4.39±0.02c	0.47±0.02f	37.1±1.10d	31.9±0.20e	147±2.90d	0.75±0.02d
w/w	AMF-1	8.10±0.02cd	4.10±0.02e	0.44±0.02g	43.2±0.80bc	38.1±0.19c	179±3.79b	0.82±0.02b
	AMF-2	8.09±0.02d	3.80±0.06h	0.41±0.02h	43.9±0.70b	38.9±0.20b	190±1.29b	0.84±0.02b
	AMF-3	8.04±0.02de	3.29±0.06i	0.39±0.02i	50.4±0.30a	42.4±0.30a	198±2.20a	0.89±0.02a

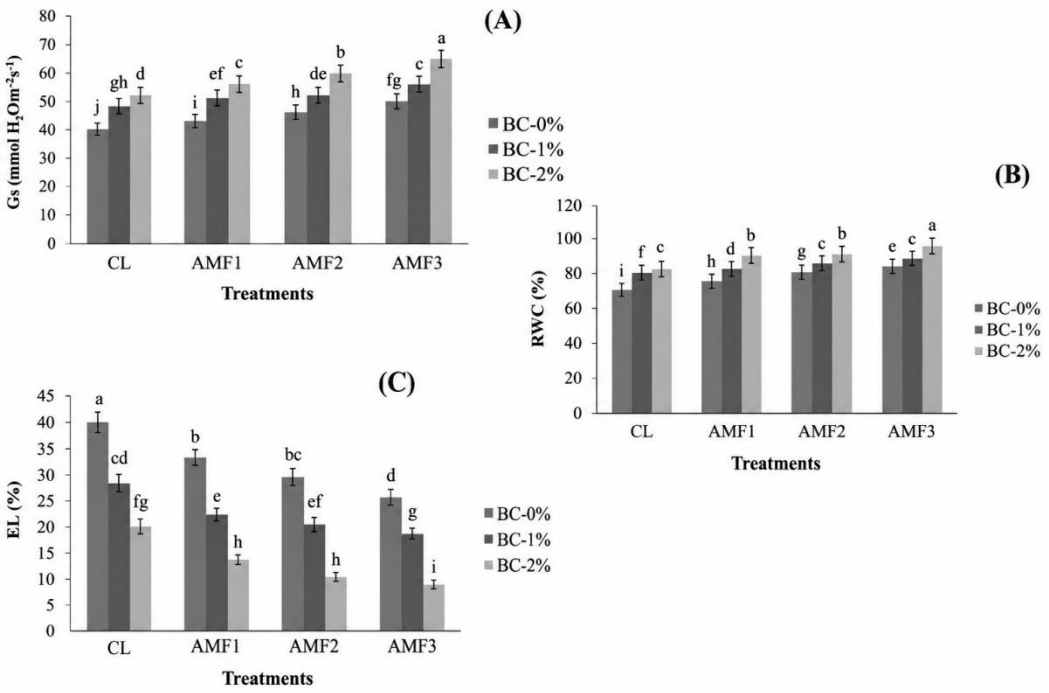

Figure 1. Physiochemical changes, stomatal conductance (A), RWC (B), EL (C), proline concentration (D) malondialdehyde concentration (E), H2O2 concentration (F) in tissues under Cd stress, with biochar applications and AMF treatments.

Table 2. Cd distribution and accumulation metrics with biochar applications and mycorrhizea treatments.

Factors	Treatments	Root Cd level (μgg^{-1})	Shoot Cd level (μgg^{-1})	Seed Cd level (μgg^{-1})	BCF value	TF value	BAC value
BC	0	10.39±1.20a	8.60±0.69a	2.49±0.39a	2.39±0.29a	0.90±0.03a	2.19±0.20a
	1% w/w	7.50±0.69b	6.79±0.69b	1.60±0.39b	1.89±0.20b	0.88±0.03a	1.80±0.20b
	2% w/w	5.80±0.70c	5.40±0.49c	0.59±0.40c	1.49±0.20c	0.89±0.01a	1.39±0.10c
AMF	Control	8.80±1.89a	7.89±1.49a	2.19±0.79a	2.30±0.49a	0.89±0.02b	2.10±0.39a
	AMF-1	7.39±1.40b	6.90±1.40a	1.49±0.79b	1.94±0.40b	0.94±0.02b	1.80±0.40a
	AMF-2	7.19±1.40b	6.69±1.40a	1.39±0.69b	1.90±0.29b	0.94±0.03b	1.69±0.40a
	AMF-3	6.49±1.40b	6.20±1.29a	1.20±0.79b	1.69±0.40b	0.97±0.01a	1.59±0.29a
Combined Interactions							
BC-0	Control	11.09±0.20a	9.70±0.30a	3.20±0.06a	2.90±0.06a	0.97±0.02a	2.49±0.10a
	AMF-1	8.89±0.04b	8.50±0.03b	2.50±0.05b	2.29±0.02b	0.97±0.02a	2.19±0.01b
	AMF-2	8.70±0.07c	8.29±0.06b	2.30±0.02b	2.24±0.03c	0.96±0.02ab	2.17±0.01b
	AMF-3	8.09±0.09d	7.80±0.10c	2.10±0.02c	2.10±0.02d	0.88±0.01f	1.99±0.03b
BC-1% w/w	Control	8.39±0.02c	7.90±0.09c	2.20±0.03c	2.20±0.02c	0.95±0.02b	2.10±0.02c
	AMF-1	7.60±0.10e	6.80±0.09d	1.49±0.03d	1.99±0.04e	0.89±0.01de	1.80±0.04d
	AMF-2	7.39±0.06e	6.60±0.10d	1.39±0.06d	1.89±0.02e	0.87±0.01c	1.69±0.02d
	AMF-3	6.50±0.02f	6.01±0.04e	1.09±0.06e	1.70±0.01f	0.87±0.01e	1.54±0.01d
BC-2% w/w	Control	6.69±0.10f	6.09±0.10e	1.19±0.06e	1.80±0.02f	0.97±0.02a	1.59±0.03e
	AMF-1	5.80±0.10g	5.40±0.10f	0.59±0.06f	1.49±0.03g	0.94±0.01c	1.39±0.03f
	AMF-2	5.60±0.02g	5.19±0.01	0.50±0.02f	1.46±0.01g	0.94±0.01c	1.34±0.02f
	AMF-3	4.89±0.09h	4.69±0.10g	0.26±0.03g	1.29±0.03h	0.89±0.01cd	1.19±0.03g

Note: BC-Biochar; AMF-Arbuscular Mycorrhizal Fungi; Cd-Cadmium; BCF-Bioconcentration Factor; TF-Translocation Factor; BAC-Bioaccumulation Coefficient; ± standard error (SE); Means within the same column followed by different lowercase letters indicate statistically significant differences among treatments ($P < 0.05$).

In roots, Cd concentration decreased from 10.39 µg g^{-1} in control plants to 6.79 µg g^{-1} in BC (2% w/w)-treated plants. Tomato plants inoculated with mycorrhizal fungi alone showed a root Cd concentration of 7.49 µg g^{-1}, whereas the combined application of mycorrhizal fungi + BC (2% w/w) achieved the lowest root Cd level of 5.43 µg g^{-1}. Shoot Cd concentration followed a similar trend. Control plants exhibited the highest shoot Cd concentration (10.81 µg g^{-1}), while the combined treatment of mycorrhizal fungi + BC (2% w/w) markedly reduced shoot Cd to 5.71 µg g^{-1}. In fruits, Cd levels were lower than in roots and shoots. The maximum Cd concentration in fruits was observed in control plants (4.20 µg g^{-1}), whereas the synergistic treatment of mycorrhizal fungi + BC (2% w/w) reduced fruit Cd to 0.26 µg g^{1}, demonstrating effective mitigation of Cd accumulation in edible tissues. Bioconcentration factor (BCF) values exceeded 1 across all treatments; however, control plants exhibited the highest BCF (2.93). Increasing BC application reduced BCF, reaching a minimum of 1.49 with BC (2% w/w). Mycorrhizal inoculation alone also significantly decreased BCF, with the lowest BCF (1.41) recorded in plants receiving mycorrhizal fungi + BC (2% w/w). Translocation factor (TF) values remained below 1 in all treatments, indicating limited upward movement of Cd from roots to shoots and fruits. Control plants showed a TF of 0.89, whereas mycorrhizal-treated plants exhibited slightly higher TF (0.97). The lowest TF (0.90) was observed in the combined mycorrhizal fungi + BC (1% w/w) treatment, indicating that biochar strongly restricted Cd translocation to aerial parts. Bioaccumulation coefficient (BAC) exceeded 1 in all cases, with control plants showing the highest BAC (2.19). Application of BC alone reduced BAC, reaching 1.39 in BC (2% w/w)-treated plants. Mycorrhizal fungi alone also reduced BAC compared to control, with the lowest BAC (1.18) recorded in plants treated with mycorrhizal fungi + BC (2% w/w). These results clearly indicate that the combined use of biochar and mycorrhizal fungi is the most effective strategy to reduce Cd uptake and accumulation in tomato plants under saline and Cd stress.

3.3. Stomatal conductance, relative water content, and oxidative stress markers in tomato leaves

The stomatal conductance (mmol H_2O m^{-2} s^{-1}) of tomato plants varied significantly depending on the application of mycorrhizal fungi (MF) and biochar (BC) (Figure 1). Control plants exhibited a stomatal conductance of 40.02, which increased to 48.89 following inoculation with mycorrhizal fungi alone, and further improved to 49.87 with BC (2% w/w) application. The combined treatment of mycorrhizal fungi + BC (2% w/w) resulted in the maximum stomatal conductance of 62.09, indicating enhanced gas exchange under Cd and saline stress. Relative water content (RWC, %) of control plants was 70.14%, increasing to 79.8% with mycorrhizal fungi inoculation, and further to 88.3% under BC (2% w/w) treatment. The highest RWC (92.8%) was observed in plants receiving the combined mycorrhizal fungi + BC (2% w/w) treatment, reflecting improved water status and turgor maintenance in stressed tomato plants. Proline concentration (mg 100 g^{1} FW), an osmolyte indicative of stress, was highest in control plants (9.19 mg 100 g^{1} FW). Application of BC alone at 1% and 2% w/w reduced proline levels to 7.21 and 6.39, respectively.

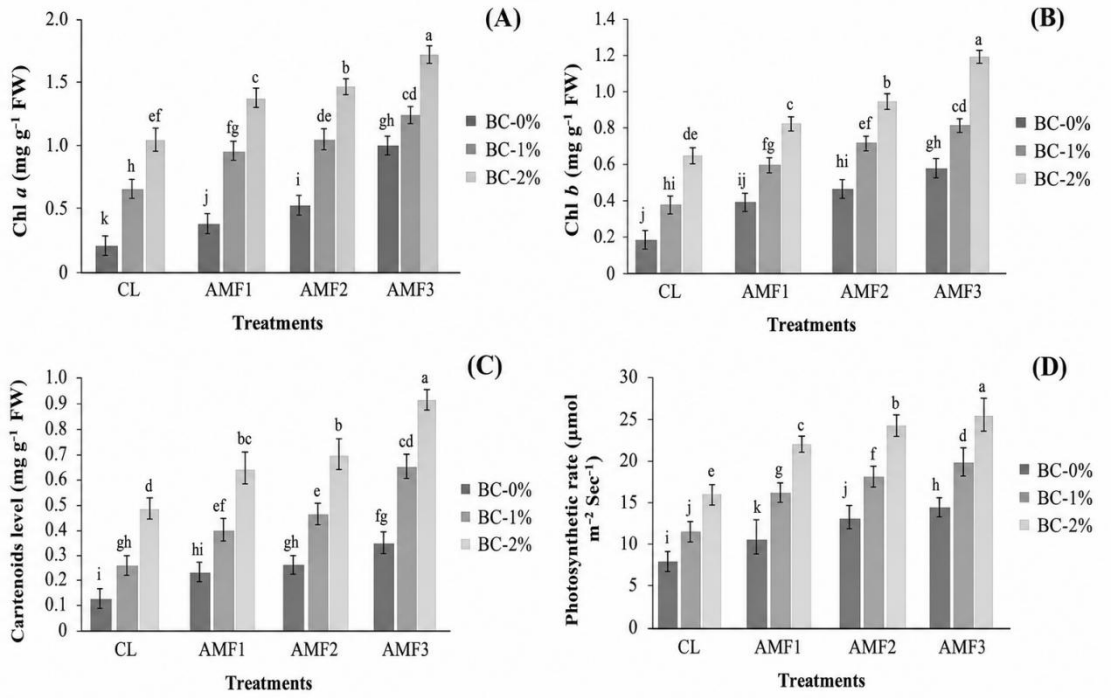

Figure 2. Performance of photosynthetic apparatus, chlorophyll a (A), chlorophyll b (B), carotenoids (C) , and rate of photosynthesis (D) under Cd stress with biochar applications and Arbuscular Mycorrhizal Fungi (AMF) treatments. Different lowercase letters on the bars indicate statistically significant differences among treatments ($P < 0.05$).

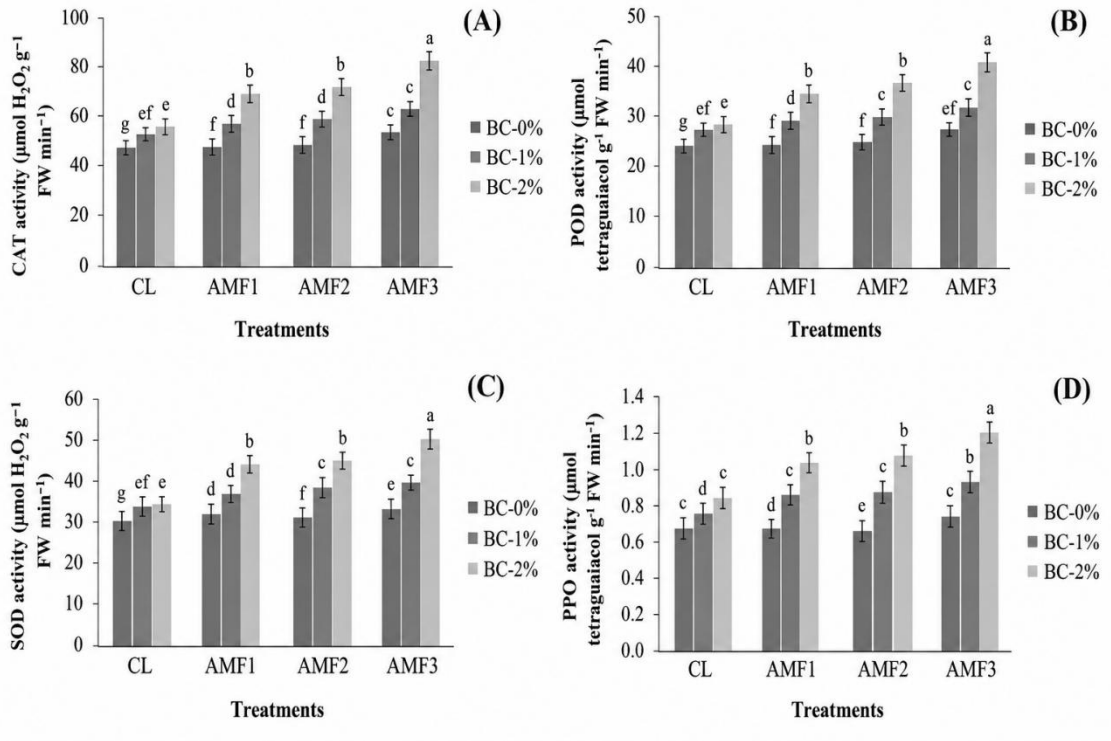

Figure 3. Performance of enzymatic-antioxidant ability, CAT (A), POD (B), SOD (C), PPO (D) under Cd stress with biochar applications and AMF treatments. Different lowercase letters on the bars indicate statistically significant differences among treatments ($P < 0.05$).

Similarly, mycorrhizal fungi treatment alone lowered proline to 6.78 mg 100 g^1 FW. The most pronounced reduction occurred with mycorrhizal fungi + BC (2% w/w), yielding a minimum proline concentration of 4.39, indicating mitigation of osmotic stress in tomato leaves. Malondialdehyde (MDA, nmol g^{-1} FW), a marker of lipid peroxidation, was highest in control plants (25.3 nmol g^{-1} FW) and decreased to 15.2 with mycorrhizal fungi alone, and to 9.4 with BC (2% w/w) treatment. The lowest MDA level (1.4 nmol g^{-1} FW) was observed in plants receiving combined mycorrhizal fungi + BC (2% w/w), reflecting reduced membrane damage under Cd stress. Hydrogen peroxide (H_2O_2, µmol g^{-1} FW) levels were also significantly reduced by treatments. Control plants had 3.98 µmol g^{-1} FW H_2O_2, which decreased to 1.60 with mycorrhizal fungi inoculation and to 2.10 under BC (2% w/w) treatment. The combined application of mycorrhizal fungi + BC (2% w/w) resulted in the lowest H_2O_2 concentration (0.79 µmol g^{-1} FW), indicating a strong alleviation of oxidative stress in tomato leaves. The results demonstrate that biochar and mycorrhizal fungi act synergistically to improve stomatal function, enhance leaf water status, and reduce oxidative stress markers under saline and Cd-contaminated conditions in tomato plants.

3.4. Photosynthetic rate and pigment content in tomato leaves

Tomato plants irrigated with Cd-contaminated water exhibited a significant reduction in photosynthetic rate (µmol m^{-2} s^{-1}), indicating impaired photosynthetic efficiency under metal stress (Figure 2). Application of mycorrhizal fungi (MF) and biochar (BC) markedly alleviated Cd-induced stress, improving the photosynthetic apparatus. Control plants showed a photosynthetic rate of 8.08 µmol m^{-2} s^{-1}, which increased to 14.40 µmol m^{-2} s^{-1} with BC (1% w/w) and further to 17.08 µmol m^{-2} s^{-1} with BC (2% w/w). Mycorrhizal fungi alone also enhanced photosynthetic rate, ranging from 11.09 µmol m^{-2} s^{-1} (MF-1) to 14.11 µmol m^{-2} s^{-1} (MF-3). The highest photosynthetic rate (22.39 µmol m^{-2} s^{-1}) was observed with the combined application of mycorrhizal fungi + BC (2% w/w), demonstrating a synergistic effect in alleviating Cd stress in tomato leaves. Similarly, photosynthetic pigment content improved under mycorrhizal fungi and biochar treatments. Control plants showed the lowest concentrations of total carotenoids (0.08 mg g^{-1} FW), chlorophyll a (0.15 mg g^{-1} FW), and chlorophyll b (0.08 mg g^{-1} FW). Biochar amendment at 2% w/w increased carotenoids, chlorophyll a, and chlorophyll b concentrations to 0.47, 0.99, and 0.50 mg g^{-1} FW, respectively. The combined application of mycorrhizal fungi + BC (2% w/w) produced the highest pigment levels, with total carotenoids at 0.76 mg g^{-1} FW, chlorophyll a at 1.70 mg g^{-1} FW, and chlorophyll b at 0.96 mg g^{-1} FW, indicating enhanced photosynthetic capacity and improved light-harvesting efficiency under Cd and saline stress. These results indicate that biochar and mycorrhizal fungi act synergistically to restore photosynthetic performance and pigment accumulation in Cd-stressed tomato plants.

Table 3. Attributes of tomato with biochar applications and mycorrhizea treatments.

Factors	Treatments	Average fruit weight (g)	Fruit plant^{-1}picking^{-1}
BC	0	35.70±0.11b	4.70±0.02d
	1% w/w	36.49±0.15b	4.72±0.02c
	2% w/w	40.86±0.21a	6.87±0.03a
AMF	Control	34.71±0.11b	4.77±0.03i
	AMF-1	41.12±1.14ab	4.89±0.04b
	AMF-2	44.43±1.37ab	4.97±0.03b
	AMF-3	47.87±0.31a	5.44±0.04a
Combined Interactions			
BC-0	Control	33.02±0.14b	4.80±0.03i
	AMF-1	40.18±1.15ab	4.97±0.04b
	AMF-2	45.40±1.40ab	5.01±0.03b
	AMF-3	46.99±0.29a	5.08±0.04a
BC-1% w/w	Control	36.90±0.10b	5.11±0.03c
	AMF-1	42.20±1.20ab	5.24±0.03d
	AMF-2	44.38±1.77ab	5.46±0.03b
	AMF-3	49.01±0.39a	6.88±0.04a
BC-2% w/w	Control	38.47±1.11b	8.02±0.04c
	AMF-1	42.20±1.39ab	8.05±0.02d
	AMF-2	44.38±1.75ab	8.11±0.04c
	AMF-3	57.47±1.97a	10.23±0.06a

3.5. Antioxidant enzymatic activities in tomato leaves

The application of mycorrhizal fungi (MF) and biochar (BC) significantly enhanced the antioxidant enzymatic activities in tomato leaves, including polyphenol oxidase (PPO, µM tetraguaiacol g^{-1} FW min^{-1}), superoxide dismutase (SOD, µmol H_2O_2 g^{-1} FW min^{-1}), peroxidase (POD, µM tetraguaiacol g^{-1} FW min^{-1}), and catalase (CAT, µmol H_2O_2 g^{-1} FW min^{-1}) (Figure 3). Control plants exhibited the lowest CAT activity (39.6 µmol H_2O_2 g^{-1} FW min^{-1}). Mycorrhizal fungi alone increased CAT activity, with MF-3-treated plants showing 47.6 µmol H_2O_2 g^{-1} FW min^{-1}. The combined treatment of MF-3 and BC (2% w/w) resulted in the highest CAT activity (74.3 µmol H_2O_2 g^{-1} FW min^{-1}), highlighting the synergistic effect of biochar and mycorrhizal fungi. Similarly, POD activity increased under MF and BC treatments. Control plants exhibited 21.3 µM tetraguaiacol g^{-1} FW min^{-1}, whereas MF-3 alone improved POD to 24.3 µM, and BC (2% w/w) alone raised it to 28.4 µM. The maximum POD activity (38.4 µM) was observed in plants receiving MF-3 + BC (2% w/w). SOD activity also improved substantially. Control plants showed 28.4 µmol H_2O_2 g^{-1} FW min^{-1}, while MF-3 alone increased SOD to 36.7 µmol H_2O_2 g^{-1} FW min^{-1}. The combination of MF-3 and BC (2% w/w) nearly doubled SOD activity to 44.3 µmol H_2O_2 g^{-1} FW min^{-1}, indicating enhanced ROS scavenging under Cd and saline stress. The PPO activity followed similar trends. Control plants showed a PPO activity of 0.50 µM tetraguaiacol g^{-1} FW min^{-1}. Biochar or mycorrhizal fungi alone significantly enhanced PPO activity, but the combined application of MF-3 and BC (2% w/w) exhibited the highest PPO activity at 1.11 µM, representing a ~2-fold increase compared to control. These results demonstrate that biochar and mycorrhizal fungi synergistically enhance the antioxidant defense system in tomato plants under Cd and saline stress, effectively reducing oxidative damage and supporting plant growth.

3.6. Fruit Growth and Quality Attributes

The application of mycorrhizal fungi (MF) and biochar (BC) significantly improved tomato fruit growth and quality under Cd-contaminated irrigation (Table 3). The average fruit diameter increased from 4.8 cm in control plants to 5.9 cm in plants treated with BC (2% w/w). MF-3-inoculated plants also showed enhanced fruit diameter (6.2 cm), while the combined MF-3 + BC (2% w/w) treatment produced the largest fruits, with an average diameter of 6.8 cm. The average fruit length followed a similar trend, increasing from 5.1 cm in control to 6.3 cm with BC (2% w/w), 6.6 cm with MF-3, and 7.1 cm in MF-3 + BC-treated plants. Fruit firmness, an important quality trait, improved with treatments. Control fruits showed firmness of 2.8 N, whereas BC (2% w/w) and MF-3-treated plants exhibited 3.5 N and 3.8 N, respectively. The maximum firmness (4.2 N) was observed in the combined MF-3 + BC treatment. Additionally, fruit soluble solids content (°Brix) increased from 4.2 in control fruits to 5.1 with BC (2% w/w), 5.4 with MF-3, and 6.0 in the combined MF-3 + BC treatment. The titratable acidity decreased slightly in treated fruits, indicating improved fruit quality under stress mitigation. The combined application of mycorrhizal fungi and biochar enhanced tomato fruit size, firmness, and quality traits, suggesting a synergistic role in mitigating Cd toxicity and improving plant physiological performance.

4. Discussion

4.1. Effects of Cd-contaminated irrigation water on soil properties

Cadmium contamination through irrigation water can adversely affect soil physicochemical and biological properties and subsequently impair plant growth and nutrient acquisition. In the present study, irrigation with Cd-contaminated water decreased soil pH from 8.31 to 8.20 and increased electrical conductivity (EC) by 5.9%, while available soil Cd increased by 11%. These changes indicate that repeated application of Cd-contaminated irrigation water can modify the soil chemical environment and increase the pool of potentially available Cd. The behavior of Cd in soil is strongly regulated by pH, organic matter, clay content, ionic composition, and the chemical form of Cd present in the soil solution [41, 42].

The relatively small decline in soil pH observed in this study may reflect changes in ion exchange and rhizosphere processes associated with Cd-contaminated irrigation rather than direct acidification by Cd alone. Soil pH is particularly important because it controls Cd sorption, precipitation, complexation, and dissolution reactions. Generally, increasing soil pH promotes Cd immobilization, whereas acidification can increase Cd mobility and bioavailability [42, 43]. Therefore, even a relatively small change in pH may influence Cd availability in saline-alkaline soils.

The increase in EC following Cd-contaminated irrigation is also relevant because saline conditions can interact with Cd toxicity. Salinity modifies ionic balance, osmotic potential, membrane stability, and nutrient uptake, thereby increasing the physiological burden imposed by Cd stress. Yang et al. [44] demonstrated that salinity can increase Cd bioaccumulation under combined Cd and salt exposure, highlighting the importance of considering Cd and salinity as interacting rather than independent stresses. Similar interactions between salinity and heavy-metal toxicity have also been reported in plants exposed simultaneously to NaCl and Cd [45, 46].

The present study further showed that Cd-contaminated irrigation reduced soil respiration and enzyme activities. The control treatment exhibited the lowest soil respiration (21.1 mg CO_2 100 g^{-1} soil 24 h^{-1}), suggesting that Cd exposure negatively affected microbial functioning. Heavy metals can suppress microbial biomass and activity by interfering with cellular metabolism and enzyme systems, thereby affecting carbon and nutrient cycling [7]. Dehydrogenase activity is particularly useful as an indicator of overall microbial metabolic activity, whereas phosphatase and nitrogen-related enzymes are associated with P and N transformations. The reductions observed under Cd stress therefore indicate a deterioration of biological soil functioning.

4.2. Improvement of soil properties by biochar and AMF

Biochar and AMF substantially improved several soil properties under Cd stress. In the present experiment, biochar application produced a slight reduction in soil pH, while the combined biochar + AMF treatment resulted in a greater decrease. This response differs from the commonly reported alkalizing effect of many biochars and may be related to the relatively low pH of the biochar used in the present study (pH 7.79) compared with the initial soil pH (8.31). Therefore, the direction and magnitude of pH change following biochar application should be interpreted in relation to both biochar characteristics and initial soil conditions rather than assuming that all biochars increase soil pH.

Biochar can modify soil pH, CEC, organic carbon content, water-holding capacity, and the chemical environment surrounding soil particles. Its effects depend strongly on feedstock, pyrolysis temperature, application rate, and the initial properties of the soil [42, 47]. Importantly, biochar does not immobilize Cd through a single mechanism. Cd retention can involve surface adsorption, ion exchange, complexation with oxygen-containing functional groups, precipitation, and interactions with mineral phases. These mechanisms collectively decrease the fraction of Cd that remains readily available for plant uptake [42, 48].

A major finding of the present study was the substantial reduction in available soil Cd following combined biochar and AMF application. Available Cd declined to approximately 0.40 mg kg^{-1}compared with approximately 1.0 mg kg^{-1} in the control. This result is consistent with evidence that biochar can decrease Cd bioavailability through immobilization and redistribution of Cd into less mobile soil fractions. A meta-analysis reported that biochar application generally reduced available Cd and increased the proportion of less bioavailable Cd fractions, although the magnitude of the response depended strongly on soil pH and biochar properties [43]. Thus, the pronounced reduction observed in the present saline-alkaline soil may reflect the combined effects of biochar-mediated Cd sorption and the chemical environment created by the amendment.

The improvement was further strengthened when AMF was applied together with biochar. AMF can modify the rhizosphere through extensive extraradical hyphal networks, altered nutrient acquisition, organic compound release, and interactions with soil microorganisms. These processes may contribute to reduced Cd mobility and improved plant nutritional status. A meta-analysis of 194 studies found that AMF inoculation reduced Cd accumulation in both roots and shoots and could also decrease soil Cd bioavailability, although the magnitude of the response depended on plant species, soil properties, AMF identity, and experimental conditions [49]. More recent evidence also confirms that AMF can reduce Cd toxicity by modifying Cd uptake and transport, improving nutrient status, and regulating plant antioxidant responses [50].

The combined treatment also markedly enhanced soil respiration and enzyme activities. Biochar increased soil phosphatase, nitrogenase, and dehydrogenase activities by 86%, 220%, and 80%, respectively, whereas the combined AMF + biochar treatment increased these activities by 127%, 330%, and 130%, respectively. These improvements suggest that the combined amendment restored microbial functioning under Cd stress. Biochar provides carbon-rich microsites and porous habitats that can support microbial colonization, while AMF establishes an important plant–microbe interaction in

the rhizosphere. The stronger response under co-application therefore suggests a complementary interaction between the two amendments rather than an effect attributable to either amendment alone.

4.3. Effects of AMF and biochar on Cd uptake and translocation

Cadmium accumulation differed markedly among tomato organs. In the present study, Cd concentrations were highest in roots, followed by leaves, whereas fruits contained the lowest concentrations. This distribution indicates that a substantial proportion of absorbed Cd was retained belowground, limiting its movement toward edible reproductive tissues. Such root retention is important from a food-safety perspective because tomato fruit is the harvested component of the crop.

Biochar application significantly decreased Cd accumulation in tomato tissues. The 2% biochar treatment reduced Cd concentrations in roots, shoots, and fruits by 37%, 40%, and 59.9%, respectively. These results agree with previous work showing that biochar can reduce Cd accumulation in tomato by decreasing Cd availability in the soil. Abid et al. [51], for example, reported that 1% cotton-stalk biochar reduced shoot Cd concentration in tomato grown under Cd-contaminated irrigation while simultaneously improving biomass and photosynthetic pigments.

The stronger response observed following combined AMF and biochar application is particularly important. In the present study, AMF + biochar reduced Cd concentrations in tomato roots and fruits by 57% and 93%, respectively. This pronounced reduction in fruit Cd indicates that the combined treatment was particularly effective in restricting Cd movement from the soil–root interface toward the edible organs. The result is consistent with meta-analytical evidence showing that AMF generally decreases Cd accumulation in plant tissues, although the magnitude of the effect varies according to soil characteristics, host plant, AMF species, and experimental conditions [49].

Several complementary processes may explain the lower Cd accumulation under co-application. Biochar can immobilize Cd in soil through adsorption, ion exchange, surface complexation, and precipitation, thereby reducing the Cd concentration in the soil solution. AMF can further influence Cd uptake by modifying the rhizosphere, improving nutrient acquisition, altering Cd transport and compartmentalization, and retaining a proportion of the metal within fungal structures or the root system [48]. Thus, the combined treatment may establish both a soil-based barrier to Cd availability and a biological barrier to Cd transfer into shoots and fruits.

The reductions in BAC, TF, and BCF observed in the combined treatment provide additional evidence of reduced Cd mobility within the soil–plant system. The AMF + biochar treatment reduced BAC, TF, and BCF by 51%, 6%, and 56%, respectively. Because these indices integrate Cd concentration in soil and plant tissues, their decline indicates that the combined amendment reduced both Cd uptake and internal transfer. Nevertheless, the relatively small reduction in TF compared with BAC and BCF suggests that the major effect of the treatment occurred at the soil–root interface and during overall Cd uptake rather than exclusively at the root-to-shoot transport stage.

4.4. Effects of AMF and biochar on plant water relations and oxidative stress

Cadmium stress substantially altered the physiological status of tomato plants. Control plants exposed to Cd-contaminated irrigation exhibited lower relative water content (RWC) and stomatal conductance, together with increased electrolyte leakage (EL), proline, malondialdehyde (MDA), and H_2O_2. These responses are characteristic of stress-induced disturbances in cellular water relations, membrane stability, and redox homeostasis. Cd can interfere with nutrient balance, photosynthetic metabolism, membrane function, and antioxidant systems, ultimately increasing reactive oxygen species (ROS) production [1, 12].

The increase in MDA and EL indicates enhanced membrane damage under Cd stress. MDA is commonly associated with lipid peroxidation, whereas electrolyte leakage reflects loss of membrane integrity. The simultaneous accumulation of H_2O_2 further indicates an imbalance between ROS generation and detoxification. Such oxidative disturbances are particularly important under combined Cd and salinity stress because Na^+ accumulation and osmotic stress can further disrupt cellular homeostasis [45, 46].

Application of biochar and AMF markedly alleviated these responses. The combined AMF + biochar treatment increased RWC and stomatal conductance by 35% and 58%, respectively, while decreasing EL, proline, MDA, and H_2O_2 by 77%, 50%, 94%, and 79.9%, respectively. These results indicate improved water status and substantially lower cellular oxidative damage. The positive response may be associated with reduced Cd availability, improved soil water retention, enhanced nutrient acquisition, and improved rhizosphere functioning.

The role of AMF in improving plant performance under Cd stress is supported by recent meta-analyses. AMF inoculation has been shown to increase plant biomass and photosynthetic pigments while reducing Cd accumulation and membrane lipid peroxidation [49]. AMF may also improve plant water relations and nutrient acquisition through its extraradical hyphal network [52]. In parallel, biochar can improve soil physical properties and create microsites that retain water and nutrients [53]. Their combined application may therefore alleviate both the chemical stress caused by Cd and the osmotic stress associated with saline irrigation.

The observed reduction in MDA and H_2O_2 also indicates improved redox regulation. However, increased proline should not automatically be interpreted as evidence of improved stress tolerance because proline can accumulate as both a stress response and a protective osmolyte. In the present study, the reduction in proline under AMF + biochar treatment, together with lower MDA andH_2O_2 and improved RWC, suggests that plants experienced lower physiological stress rather than simply increasing osmotic adjustment.

4.5. Effects of AMF and biochar on photosynthetic performance

Cadmium stress significantly impaired tomato photosynthetic performance, as indicated by reductions in photosynthetic rate and photosynthetic pigments. Cd can interfere with chlorophyll biosynthesis, pigment stability, stomatal regulation, electron transport, and nutrient balance, ultimately reducing carbon assimilation. Salinity can further aggravate these effects through osmotic stress and ionic imbalance [46, 46].

The combined application of AMF and biochar substantially improved photosynthetic performance. In the present study, the AMF + biochar treatment increased photosynthetic rate, carotenoids, chlorophyll a, and chlorophyll b by approximately 4-, 11-, 13-, and 14-fold, respectively, compared with the Cd-stressed control. These pronounced improvements indicate that the combined treatment effectively protected the photosynthetic apparatus from Cd-induced damage.

The observed response is consistent with previous evidence that biochar can improve tomato photosynthesis under Cd-contaminated irrigation. Abid et al. [51] reported increased root and shoot biomass and improved photosynthetic pigments following biochar application to tomato exposed to Cd-contaminated irrigation water. Similarly, AMF has been associated with improved chlorophyll formation and plant nutritional status under Cd stress. A large meta-analysis of AMF effects on Cd-stressed crops found that AMF increased photosynthetic pigments and nutrient uptake while reducing Cd concentration in plants [49].

The superior performance of the combined treatment probably resulted from complementary effects. Biochar reduced Cd availability in the soil, while AMF improved nutrient acquisition and physiological resilience. Reduced Cd exposure would decrease damage to chloroplasts and photosynthetic membranes, whereas improved acquisition of nutrients such as N, P, Fe, and Mg would support pigment synthesis and photosynthetic metabolism. Therefore, the strong increase in photosynthetic traits under AMF + biochar suggests that the treatment improved both the soil chemical environment and plant physiological functioning.

4.6. Effects of AMF and biochar on antioxidant defense

Cadmium exposure is closely associated with oxidative stress because Cd can indirectly stimulate ROS accumulation by disrupting photosynthesis, electron transport, cellular metabolism, and antioxidant defense. Plants respond by activating enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO), which collectively contribute to ROS detoxification [1, 12].

In the present study, Cd-contaminated irrigation reduced PPO, POD, CAT, and SOD activities in tomato leaves, indicating that the antioxidant defense system was insufficient to prevent oxidative damage under the imposed stress. In contrast, application of AMF and biochar significantly enhanced antioxidant activity. The combined treatment increased PPO, POD, CAT, and SOD activities by 129%, 80%, 81%, and 59%, respectively.

The enhanced antioxidant response under AMF + biochar treatment may be related to the lower Cd availability observed in soil and plant tissues. By reducing Cd uptake, biochar can decrease the primary source of Cd-induced cellular disturbance, while AMF can improve nutritional status and stimulate plant defense pathways. AMF-mediated improvement of antioxidant capacity under heavy-metal stress has been widely reported, although the magnitude and direction of individual enzyme responses may vary according to plant species, AMF identity, Cd concentration, and environmental conditions [49].

The present findings should therefore be interpreted as an integrated response rather than assuming that higher antioxidant enzyme activity is always beneficial. Under severe stress, antioxidant enzymes may initially increase as a defense response, whereas prolonged or excessive stress can suppress enzyme activity. The simultaneous reduction in H_2O_2 and MDA and enhancement of SOD, CAT, POD, and PPO in the AMF + biochar treatment provides stronger evidence that the combined amendment improved redox homeostasis and reduced oxidative injury.

4.7. Effects of AMF and biochar on tomato fruit development

Cd stress adversely affected tomato fruit development, as reflected by the lower fruit parameters observed in the control treatment. The combined AMF + biochar treatment increased fruit weight and fruit quality parameters by 120.3% and 80.8%, respectively. These improvements demonstrate that mitigation of soil Cd stress was translated into improved reproductive performance.

The positive effect of biochar on tomato productivity under metal-contaminated irrigation has previously been reported. Biochar can improve soil physical and chemical properties, reduce Cd bioavailability, and enhance plant nutrient and water acquisition, thereby supporting vegetative growth and reproductive development [47, 54]. In the present study, these benefits were further strengthened by AMF inoculation.

AMF can improve plant P acquisition and other mineral nutrients through extraradical hyphae that extend beyond the nutrient-depleted zone surrounding roots [55]. This enhanced nutrient acquisition is particularly important under Cd stress, where nutrient imbalance can contribute to reduced growth and yield. Meta-analytical evidence indicates that AMF can substantially increase nutrient uptake and biomass while reducing Cd accumulation in plants exposed to Cd stress [49]. The reduction in fruit Cd concentration is especially important because tomato is consumed directly and therefore represents a potential pathway for Cd transfer into the human food chain. The 93% reduction in fruit Cd under combined AMF + biochar treatment suggests that this approach has considerable potential for reducing the food-safety risk associated with Cd-contaminated irrigation. However, the effectiveness of such treatments should be validated under field conditions because biochar performance is influenced by soil texture, pH, organic

matter, application rate, biochar properties, irrigation regime, and environmental aging [42, 48].

Overall, the present findings demonstrate that the co-application of biochar and AMF was more effective than individual treatments in improving soil biological functioning, reducing available Cd, restricting Cd accumulation and translocation, improving plant water relations and photosynthesis, strengthening antioxidant defense, and enhancing tomato fruit performance. The results support the concept that biochar and AMF act through complementary mechanisms: biochar primarily modifies the soil chemical and physical environment and immobilizes Cd, whereas AMF contributes to improved nutrient acquisition, rhizosphere functioning, and plant stress tolerance. The combined strategy therefore represents a promising approach for managing tomato production under saline-alkaline conditions affected by Cd-contaminated irrigation.

5. Conclusions

The synergistic application of mycorrhizal fungi and biochar showed substantial potential for alleviating the adverse effects of irrigating tomato plants with Cd-contaminated water in saline soils. This integrated strategy enhanced soil physicochemical properties, including water retention, nutrient availability, and pH buffering, which collectively reduced Cd bioavailability in the soil. As a result, Cd uptake by tomato plants was lowered, mitigating Cd-induced toxicity, oxidative stress, and cellular damage. The association of mycorrhizal fungi with tomato roots improved nutrient acquisition, stimulated root growth, and enhanced plant resilience to both Cd and drought stress. This symbiosis also promoted the synthesis of stress-related compounds such as proline and reinforced antioxidant defense mechanisms, including SOD, CAT, POD, and PPO activities. Combined treatment with biochar further supported photosynthetic efficiency by increasing chlorophyll content, carotenoids, and photosynthetic rate, contributing to higher fruit biomass and improved quality attributes. Despite these benefits, future research should aim to determine the optimal rates and combinations of mycorrhizal fungi and biochar for different soil types and tomato cultivars. Long-term studies are essential to assess the sustained effects on soil health, plant performance, and heavy metal immobilization. Additionally, evaluating the economic feasibility and field-scale application of these strategies is crucial for their practical implementation in sustainable tomato production。

Author contributions

Aneeta Aslam: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. Hayat Zada: Conceptualization, Supervision, Validation, Visualization, Writing – original Writing – review & editing,. All authors read and approved the final manuscript.

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Not applicable.

Conflicts of Interest

The authors report no conflicts of interest.

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Declaration of Generative AI and AI-assistedtechnologies in the writing process

During the preparation of this work the author(s) used AI-assisted tools (specifically GPT-5) in order to polish the English language and enhance clarity. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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