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Biochar innovations for sustainable agriculture: Acidification and zinc enrichment strategies to improve calcareous soil fertility and wheat yield

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Abstract: Calcareous soils, typically characterized by low fertility, low organic matter and nitrogen content, and often deficient in phosphorus, zinc, and iron, as well as having low microbial activity, require the development of sustainable soil conditioners to improve fertility. To address these shortcomings and promote sustainable agriculture, biochar, especially with acidic character, may offer a promising solution. This study investigates the effects of modified biochar by H_2SO_4 and $ZnSO_4$ on soil properties and wheat yield under field conditions. For this purpose, biochar (B), acidified biochar (AB), Zn enriched biochar (BZn), and acidified-Zn enriched biochar (ABZn) were applied to the field at two different doses (0.5 and 1.0%) together with the control treatment (Ck) without biochar application. $AB_{1.0\%}$ was determined as the most effective treatment in decreasing soil pH (0.15 units), while B1.0% was determined as the most effective treatment in increasing organic carbon and cation exchange capacity, 13% and 32%, respectively. The effect of the treatments varied for specific nutrients. The highest antioxidant enzyme activities were found in acidified biochars where the lowest yields were obtained. Compared to the Ck, the highest catalase (CAT) (32%) was determined in $ABZn_{1.0\%}$, ascorbate peroxidase (APX) (56%) and glutathione peroxidase (GPX) (36%) were determined in $ABZn_{0.5\%}$, and superoxide dismutase (SOD) (28%) was determined in $AB_{0.5\%}$. The highest proline (PRO), with the least decrease in yield, was found in the $AB_{1.0\%}$ application, which is 205% more than Ck. B and BZn treatments all increased the grain yield, and the highest increase was 20% in $B_{1.0\%}$ when compared to the Ck.

Keywords: antioxidant enzyme activity; biochar; calcareous soil; nutrient enhancement; wheat; zinc

Calcareous soils in arid and semi-arid regions are typically low in soil organic matter and plant nutrients, especially microelements, which can seriously inhibit plant growth (Rengel 2015). These soils are often over-fertilised in order to get more yield per unit area. While fertilizers can increase productivity by 30–50% compared to non-fertilized conditions, long-term use of chemical fertilizers without the addition of organic matter can lead to a reduction in fertilizer effectiveness and environmental pollution (Chaudhary et al. 2017). It is important to increase the low organic matter at the same time with the elimination of the deficiency of the nutrients for sustainable agriculture in these soils. For this purpose, it is important to use organic-based fertilisers as an alternative to inorganic fertilisers. Organic-based fertilizers, such as nutrient-enriched manure, have gained popularity due to their cost-effectiveness compared to inorganic fertilizers

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(Leytem et al. 2011; Moore et al. 2014). The addition of organic matter to the soil can maintain soil organic matter content and improve soil physical and biochemical properties (Gülser 2006; Zhao et al. 2009). However, the rapid decomposition of applied organic matter can have a short-term and negative effect on greenhouse gas emissions (De Vries et al. 2012; Naeem et al. 2018), so more suitable alternatives need to be found. In this context, biochar has recently gained considerable attention.

Biochar, produced by heating the biomass in an oxygen-free, high-temperature environment (pyrolysis or gasification), is considered to be an additive that improves soil physical, chemical, and biological properties due to its structure, which is resistant to degradation, has a high specific surface area, and a negative surface charge (Madari et al. 2017; Zhang et al. 2017). Biochar application to soil has been reported to increase soil organic matter (Liu et al. 2017) and pH owing to its alkaline character, very porous structure, high carbon (C) content, and surface area (Chan et al. 2007; Gaskin et al. 2010; Laird et al. 2010) and affect the type and amount of microorganisms (Gul et al. 2015). However, studies have shown that the effect of biochar on productivity is highly variable (null or negative), possibly due to factors such as soil pH, biochar properties, and growing conditions (Jeffery et al. 2011; Biederman & Harpole 2013; Liu et al. 2013). The main issue with biochar application in arid regions is that it can cause soil alkalinity due to the high pH of biochar. Gunes et al. (2014) found that the addition of biochar to alkaline soils increases the nutritional status of nitrogen (N), phosphorus (P), and potassium (K) but has a negative effect on micronutrients. Zinc (Zn) deficiency is one of the most important factors that negatively affect the yield of wheat, especially in the calcareous soils of arid and semi-arid regions (Alloway 2008; Cakmak & Kutman 2018). In recent years, many studies have been carried out to eliminate the negative effects of lime in the soil and increase plant growth by applying acidified biochar to calcareous soils (Sahin et al. 2017; Demirkaya et al. 2021; Demirkaya & Gülser 2023). Although the pH of the biochar decreases with the acidification process, the solubility of its nutritional elements increases, and a more negative charge appears (Xu et al. 2021). With its large surface area and porous structure, biochar has a high ability to retain nutrients, especially heavy metals (Kılıc et al. 2013; Wang et al. 2017). Previous studies have revealed that the application of fertilisers (inorganic or organic) along with biochar increases crop yield (Yang et al. 2019). As an alternative to fertilizer, many researchers have focused on the production and application of nutrient-rich biochar to increase soil quality and productivity (Ippolito et al. 2015).

This study is important as it aims to develop a material that can serve two purposes: to act as a soil conditioner in calcareous soils with low organic matter and to positively influence plant growth by serving as a slow-release fertiliser enriched with zinc. Furthermore, the application of acidified biochar can be beneficial on calcareous soils with low biological activity and microelement content in arid regions. After acidification, biochar forms more negative surface charges that can retain cations, lowering soil pH and increasing the availability of plant nutrients (Sajjadi et al. 2019; Chen et al. 2021). However, it is important to consider the potential negative effects of biochar application on the soil and plant health (Brtnicky et al. 2021; Xiang et al. 2021). To address this, the antioxidant system of the plants can be monitored as an indicator of oxidative stress. Key antioxidant enzymes, such as catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione S-transferases (GST), and superoxide dismutase (SOD), play a critical role in protecting plants against oxidative damage. Additionally, proline (PRO) accumulation, a common response to stress, can be measured to assess the overall stress levels in plants. By assessing these parameters, we can gain insights into the plant's physiological responses and determine whether the biochar amendments are beneficial or induce stress responses.

The aim of this study is to investigate the effects of acidified and zinc-enriched biochar on the productivity of calcareous soils and the yield of wheat grown in arid and semi-arid regions. Additionally, the study aims to evaluate the possible negative effects of biochar on plant health by monitoring antioxidant systems in wheat, while also assessing its impact on soil chemistry.

MATERIAL AND METHOD

Experimental area and basic soil properties. The field experiment was conducted at Ondokuz Mayıs University, Faculty of Agriculture, Bafra Experiment Station in 2022. The coordinates of the experimental site are 41°33.36'N, 35°51.54'E and the altitude is 21 m. The soil is classified as Typic Ustifluvent, formed from Kızılırmak alluvial deposits (Sarıoğlu & Dengiz 2012). Monthly maximum and minimum

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temperatures and precipitation are given for the year the experiment was conducted (Figure 1).

The soil texture at the experimental site is sandy clay loam, with a 53% sand, 24% silt, and 23% clay. Other general properties of soil were as follows: pH of 7.9, electrical conductivity (EC) of 0.3 dS/m, organic matter (OM) content of 2.3%, $CaCO_3$ content of 14.9%, cation exchange capacity (CEC) of 26.3 cmol/kg and diethylenetriaminepentaacetic acid (DTPA) extractable Zn of 0.69 mg/kg at the beginning of the field experiment.

The soil texture was determined using the hydrometer method (Gee & Or 2002), pH and electrical conductivity were measured in soil-distilled water suspension at soil-water ratio of 1 : 1 (w : v). The same method was used to determine the pH and EC of the biochar, but biochar-water mixture ratio of 1 : 10 (w : v). The carbon content of organic matter was determined using potassium dichoramate ($K_2Cr_2O_7$) oxidable carbon based on the Walkley-Black method (Nelson & Sommers 1982) and the organic carbon content was multiplied by 1.724. Lime content was measured using a calcimeter (Soil type Calcimeter, ILDAM, Türkiye). In the soil and biochar, exchangeable cations were extracted using the 1 M ammonium acetate method and plant-available phosphorus was extracted using the sodium bicarbonate method (Olsen 1954). DTPA-extractable micronutrients in the soil and biochar was analysed using the DTPA method (Lindsay & Norvell 1978) by atomic absorption, but the solution ratio of biochar was 1 : 20 (w : v). CEC of soil and biochar was determined using the ammonium acetate (NH_4OAc) method at pH 7, where samples were saturated with NH_4^+, washed with ethanol to remove excess ions, and subsequently displaced with K^+ for quantification via atomic absorption spectroscopy (Rhoades 1982; Munera-Echeverri et al. 2018).

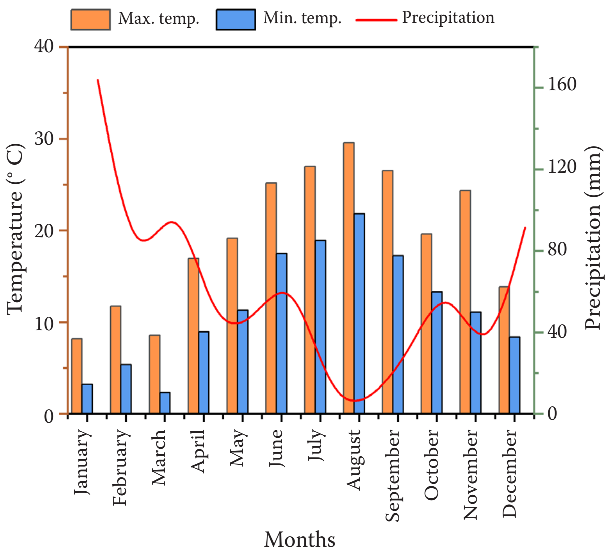

Figure 1. Monthly temperature and precipitation values in the experimental year

Antioxidant enzyme activity measurements. For the preparation of the homogenate, 1 g of fresh, fully developed youngest leaf samples were taken and ground in liquid nitrogen. The ground samples were then homogenized in 5 mL of 100 mM KH_2PO_4 per 0.5 mM EDTA buffer (pH 7.7) containing 1% (w/v) polyvinylpyrrolidone (PVP). The samples were vortexed for 10–20 seconds. The homogenate was then centrifuged at 15 000 × *g* for 20 min at 4 °C in a refrigerated centrifuge, and the supernatant was separated from the precipitant. The supernatant was aliquoted into 500 μL Eppendorf tubes for different analyses and stored at –20 °C until use.

CAT activity was determined according to the method of Aebi (1984). The enzyme activity was carried out in a final volume of 1 000 μL solution containing 835 μL of 50 mM KH_2PO_4 buffer solution, 155 μL of supernatant, and 150 μL of 120 mM H_2O_2. The decrease in absorbance at 240 nm, corresponding to the decomposition of H_2O_2, was monitored using a spectrophotometer. One unit of CAT activity was defined as the amount of enzyme that decomposes μmol of H_2O_2 per minute.

SOD activity was determined according to the method of Beauchamp and Fridovich (1971). The reaction mixture (1 000 μL) contained 400 μL of 50 mM KH_2PO_4/0.1 mM EDTA (pH 7.8), 100 μL of 120 mM L-methionine, 100 μL of 120 mM Na_2CO_3, 100 μL of 750 μM nitro blue tetrazolium (NBT), 200 μL 100 μM riboflavin, and 100 μL supernatant. The reaction was initiated by illuminating the reaction mixture with a fluorescent light. The reduction of NBT was followed by measuring the absorbance at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the NBT reduction rate.

The amount of PRO in leaf tissues was determined according to the method of Bates et al. (1973). A standard graph is needed for protein determination by this method. For this purpose, 20, 40, 60, 60, 80, 100, 200, 300, 300, 400, 500 and 600 μL of the stock solution containing 100 μg proline in 1 mL were taken into tubes and each tube was filled to 2 mL with distilled water. Then 2 mL of 96% glacial acetic acid and 2 mL

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of acid-ninhydrin solution were added to the tubes. The standard solutions were then incubated in a water bath set at 100 °C for 1 h. After one hour, 4 mL of cold toluene was added to each tube, kept on ice for 10 min and mixed for 20–30 s with a vortex. Then the upper phase in each tube was removed with an automatic pipette and the absorbance was measured at 520 nm. Toluene was used for the blind sample.

APX activity was determined by measuring the rate of ascorbate oxidation at 290 nm using a spectrophotometer. APX activity was measured by monitoring the decrease in absorbance at 290 nm due to the oxidation of ascorbate (ε: 2.8 mM^{-1} cm^{-1}) over a period of 3 min. The reaction mixture (1 000 µL) consisted of 725 µL of 40 mM KH_2PO_4 buffer (pH 6.0), 100 µL of 1 mM EDTA, 50 µL of 20 mM H_2O_2, 100 µL of 2.5 mM L(+) ascorbic acid (ASA), and 25 µL of supertanant (Cakmak & Marschner 1992; Uarrota et al. 2016).

GST activity was determined according to the method of Habig et al. (1974). The reaction mixture (1 000 µL) contained 500 µL of 0.1 M tris-HCl buffer (pH 7.0), 100 µL of 25 mM 1-chloro-2,4-dinitrobenzene (CDNB), 100 µL 20 mM reduced glutathione (GSH), 200 µL distilled water and 100 µL supernatant. The reaction was initiated by adding CDNB, and the formation of the GSH-CDNB conjugate was followed by measuring the increase in absorbance at 340 nm. One unit of GST activity was defined as the amount of enzyme that catalyses the conjugation of µmol of CDNB per minute.

GPX activity was determined according to the method of Şişecioğlu et al. (2010). Hydrogen peroxide (H_2O_2) and guaiacol were used as the substrate for POD. The reaction mixture (1 000 µL) contained 550 µL of 0.2 M KH_2PO_4 buffer (pH 7.0), 200 µL of 50 mM H_2O_2, 200 µL 100 mM guaiacol, and 50 µL supernatant. The reaction's absorbance value was measured in a spectrophotometer for 3 min at 470 nm.

The reaction mixture (1 000 µL) contained 50 mM Na-phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM NaN3, 1 mM reduced glutathione (GSH), 0.2 mM NADPH, 1 unit of glutathione reductase, and 0.1 mL enzyme extract in a final assay volume of 1 mL. The reaction was initiated by adding 0.1 mM H_2O_2. The decrease in absorbance at 340 nm, corresponding to the oxidation of NADPH, was monitored. One unit of GPX activity was defined as the amount of enzyme that catalyses the oxidation of µmol of NADPH per minute.

Preparation of biochar. In this study, hardwood-based gasified biochar (> 700 °C) was used which was obtained from the Kastamonu Entegre Factory. The biochar was ground and sieved through a 4.7 mm sieve. The average diameter of the biochar was determined as 2.09 mm.

To obtain acidified biochar with a pH of 4.0 ± 0.25, 100 mL of 2N H_2SO_4 was mixed with 100 g of raw biochar based on the results of the preliminary experiment. Zinc-enriched biochar was prepared by 3 mg of $ZnSO_4.7H_2O$ dissolving in 100 mL distilled water and adding it to 100 g of the original biochar. Acidified and zinc-enriched biochar was prepared by 3 mg of $ZnSO_4.7H_2O$ dissolving in 100 mL 2 N H_2SO_4 solution and added to 100 g of biochar. The zinc enrichment process consisted of adding 1.5 and 3.0 mg per kg of Zn to the soil with increasing doses of doses. All the biochars were kept under laboratory conditions for 48 h and then dried in an oven at 65 °C for 48 h. At the end of all the processes, we obtained four different types of biochar material: original (B), acidified (AB), zinc-enriched (BZn) and acidified zinc-enriched (ABZn).

Field experiment. In the field experiment, the biochars were transferred to the plots, and then the plots were ploughed to ensure that the biochar material was mixed into the soil (0–20 cm) using a rotary cultivator on 16 February 2022. For ease of application, the biochar was applied to the field when wet. The experiment (3 m length × 1 m plots) was conducted in a completely randomized block design with three replicates. The experiment consisted of a total of 36 plots [4 types of biochar (B, BZn, AB, ABZn) × 3 application doses (0, 0.5, 1.0%) × 3 replications = 36]. One week after biochar application, seeds were sown on 23 February 2022. The Altindane wheat (*Triticum aestivum*) variety was grown as a test plant in the experiment. During the heading stage of the plants, on 20 May 2022, 10 plant samples were taken from each plot to determine enzyme activities. At harvest maturity, the entire plots were harvested on 4 July 2022. The total biomass weight of the plants in each plot was measured and the biological yield was determined. After threshing, the grains were collected and weighed to calculate the grain yield. The harvest index was determined as the ratio of grain yield to biological yield.

Statistical analysis. One-way ANOVA was used to test by differences between treatments on examined parameters. When the ANOVA indicated a significant *F* value ($P \leq 0.05$) for treatment, multiple comparisons were made using the Tukey test ($P \leq 0.05$). Pearson´s

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Table 1. Some chemical properties of biochar materials

	pH	EC (dS/m)	OC (%)	CEC (cmol/kg)	Ca	Mg	K	P	Zn
					(g/kg)				
B	9.42	0.31	4.88	25.25	7.50	1.56	0.92	0.21	0.01
BZn	9.10	0.54	4.50	29.82	7.70	1.26	0.86	0.29	0.22
AB	3.98	4.38	4.65	37.09	17.40	3.00	1.45	0.58	0.03
ABZn	4.11	4.84	4.09	48.19	18.00	3.12	1.26	0.57	0.20

EC – electrical conductivity; OC – refers to organic carbon that can be oxidised with potassium dichromate; CEC – cation exchange capacity; B – biochar; BZn – Zn enriched biochar; AB – acidified biochar; ABZn – acidified-Zn enriched biochar

correlation analysis was performed on examined soil and plant properties.

RESULTS

Chemical composition of biochar. The properties of the raw and modified biochars are shown in the Table 1. As a result of the acidification process, pH and organic carbon content of the biochar decreased, while EC, CEC, Ca, Mg, K, P and Zn increased. The acidification process increased the EC of biochar by 14 to 15 times. Zn enrichment slightly reduced the pH, Mg, and K content according to the original biochar, but the zinc content of the biochar increased by approximately 20-fold following the zinc enrichment process.

Soil chemical properties. The effect of the treatments on soil pH ($P < 0.01$), EC ($P < 0.01$), OM ($P < 0.05$) and CEC ($P < 0.01$) were statistically significant (Figure 2). pH values were slightly alkaline, showing little variability across treatments, rang-

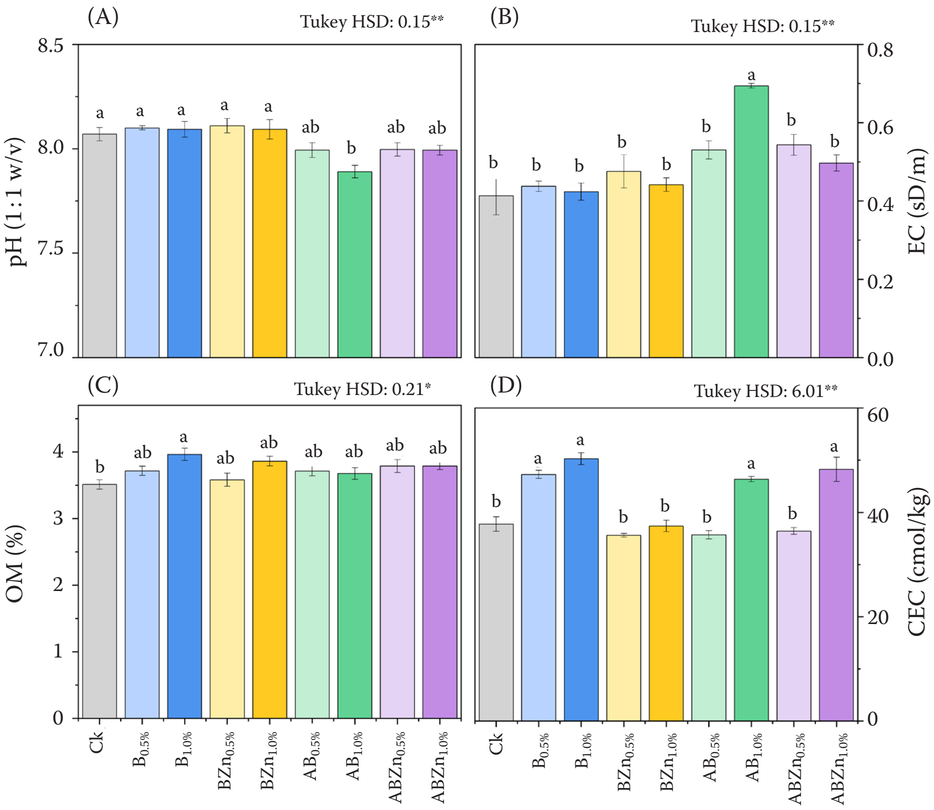

Figure 2. The effect of treatments on soil pH (A), electrical conductivity (EC) (B), organic matter (OM) (C), and cation exchange capacity (CEC) (D)

Ck – control; B – biochar; AB – acidified biochar; BZn – Zn enriched biochar; ABZn – acidified-Zn enriched biochar; *, **$P < 0.05$, 0.01, ns – non-significant ing from 7.89 to 8.11. $AB_{1.0\%}$ showed the lowest pH at 7.89 according to Ck (Figure 2A). All treatments demonstrated an increase in EC compared to Ck and $AB_{1.0\%}$ showed the highest increment as 68% (Figure 2B). Biochar applications had a positive effect on soil organic carbon content. The highest increase in organic carbon compared to Ck was determined as 13% in $B_{1.0\%}$ (Figure 2C). The treatments had varied effects on soil CEC. The $B_{1.0\%}$ showed the maximum increase in CEC, increasing by 33% compared to Ck (Figure 2D).

Nutrient availability. The effect of the treatments on exchangeable Ca ($P < 0.01$), Mg ($P < 0.01$), K ($P < 0.01$), plant available P ($P < 0.01$) and DTPA extractable Zn ($P < 0.01$) were statistically significant (Figure 3). While AB decreased the amount of exchangeable Ca compared to Ck, the effects of B and ABZn varied depending on the doses and BZn increased it. The highest increase of exchangeable Ca compared to the control was 19% in $BZn_{1.0\%}$ (Figure 3A). All treatments positively affected the exchangeable Mg and K content. $BZn_{0.5\%}$ and $B_{1.0\%}$ significantly increased Mg and K content by 123% and 31% in comparison with Ck, respectively (Figure 3B, D). With the exception of the $AB_{1.0\%}$, the treatments either did not affect or reduced the plant

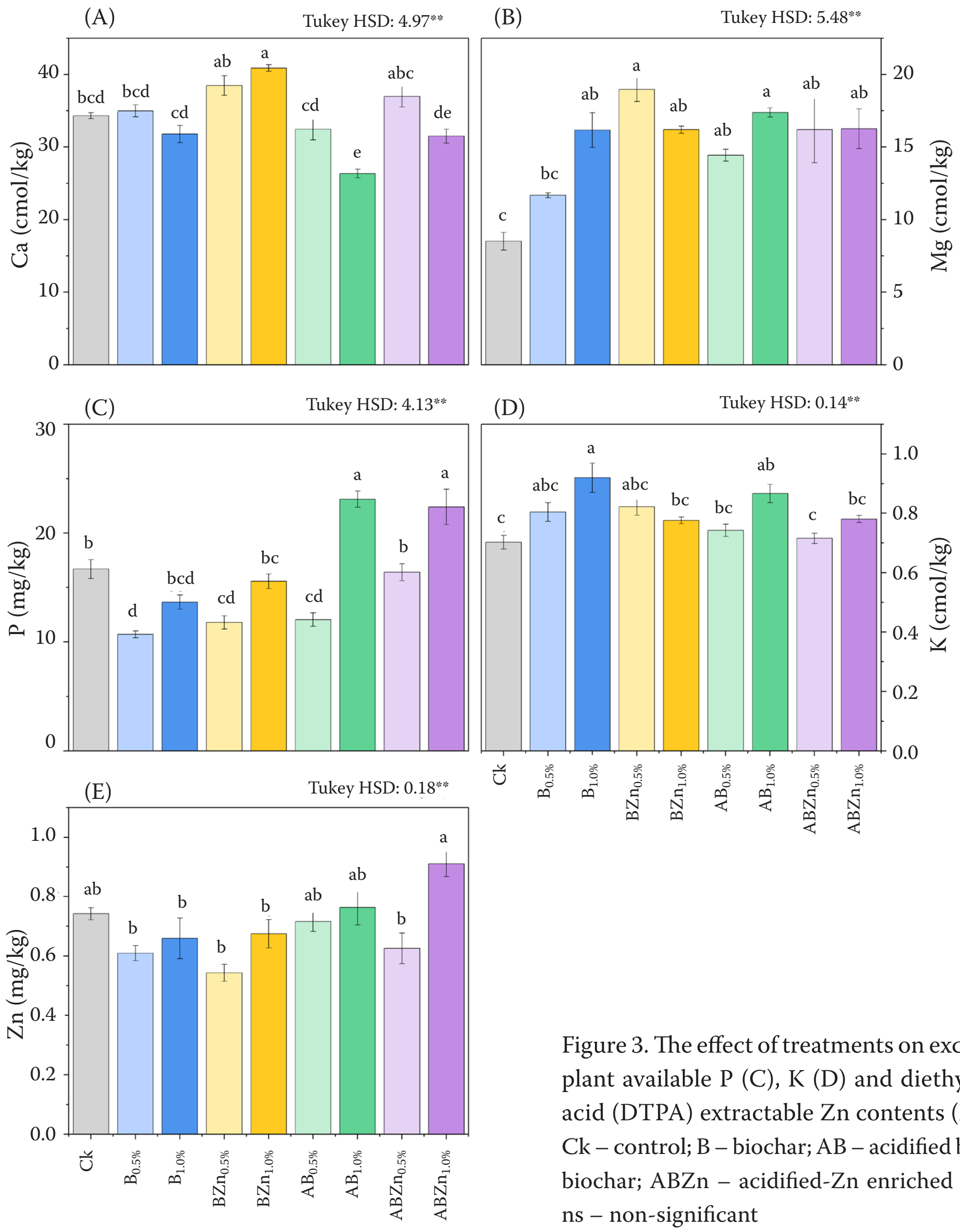

Figure 3. The effect of treatments on exchangeable Ca (A), Mg (B), plant available P (C), K (D) and diethylenetriaminepentaacetic acid (DTPA) extractable Zn contents (E)

Ck – control; B – biochar; AB – acidified biochar; BZn – Zn enriched biochar; ABZn – acidified-Zn enriched biochar;*, **$P < 0.05$, 0.01, ns – non-significant

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available P in the soil. Similar results were observed in DTPA Zn contents as in plant available P. Only the $ABZn_{1.0\%}$ significantly increased the DTPA Zn content. $AB_{1.0\%}$ increased the amount of P by 38% compared to the Ck, while $ABZn_{1.0\%}$ increased the amount of Zn by 23% compared to the Ck (Figure 3C, E).

Antioxidant enzyme activity. The application of biochar had a statistically significant effect on all antioxidant enzyme activities except for GST ($P < 0.01$) (Figure 4). Only the original biochar application decreased CAT enzyme activity compared to the control. The highest CAT activity was observed in $ABZn_{1.0\%}$, which was 32% higher than the Ck (Figure 4A). The effect of the treatments for APX, GPX, and SOD showed variability depending on the doses. For APX and GPX, the highest activity was determined in the $ABZn_{0.5\%}$ treatment, 56% and 36% higher than the Ck respectively (Figure 4B, C). The highest GST enzyme activity was found in $BZn_{0.5\%}$, but there was no statistical difference between the

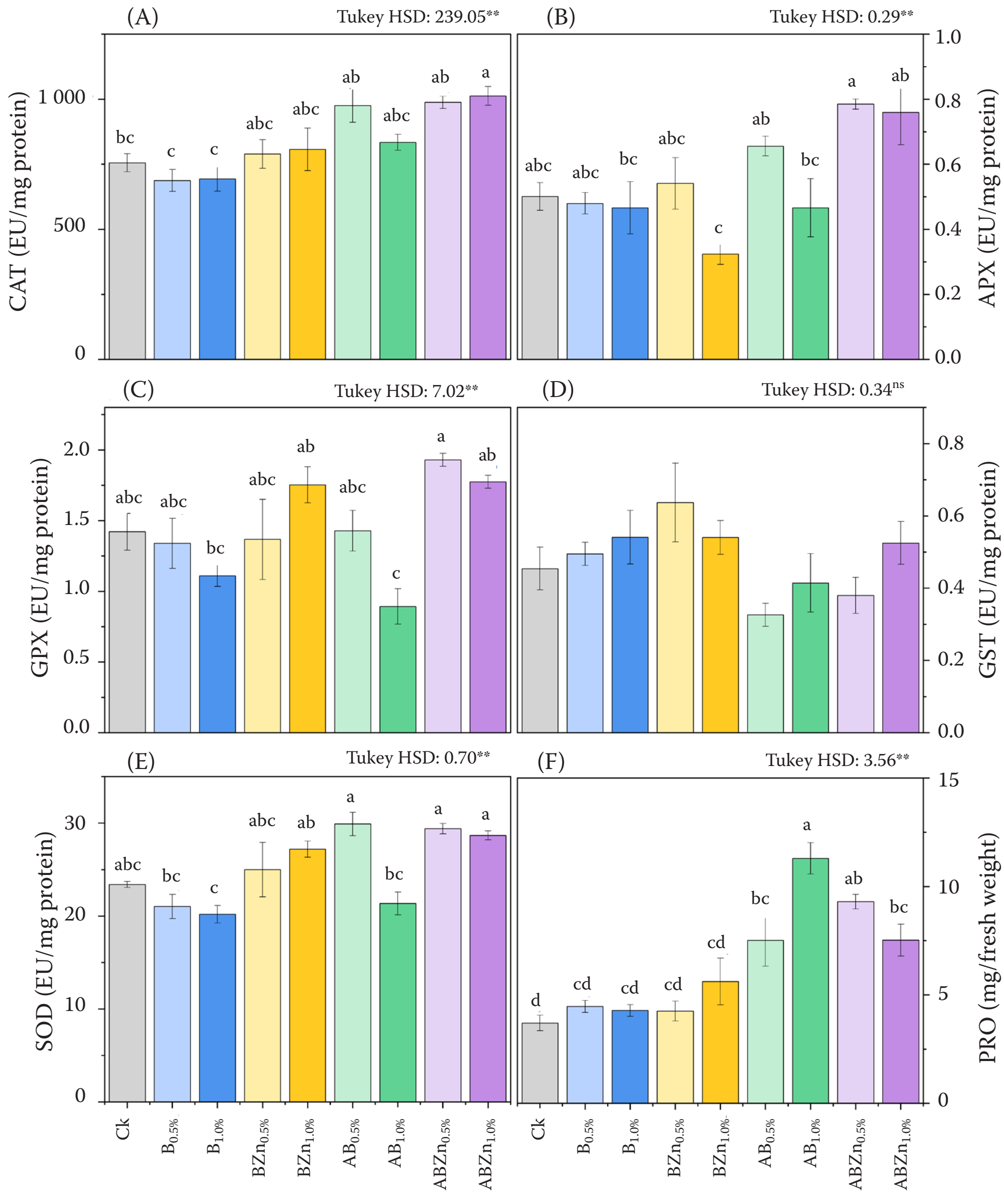

Figure 4. The effect of treatments on antioxidant enzyme activity catalase (CAT) (A), ascorbate peroxidase (APX) (B), glutathione peroxidase (GPX) (C), glutathione S-transferase (GST) (D), superoxide dismutase (SOD) (E) and proline (PRO) (F) Ck – control; B – biochar; AB – acidified biochar; BZn – Zn enriched biochar; ABZn – acidified-Zn enriched biochar; *, ** $P < 0.05$, 0.01, ns – non-significant

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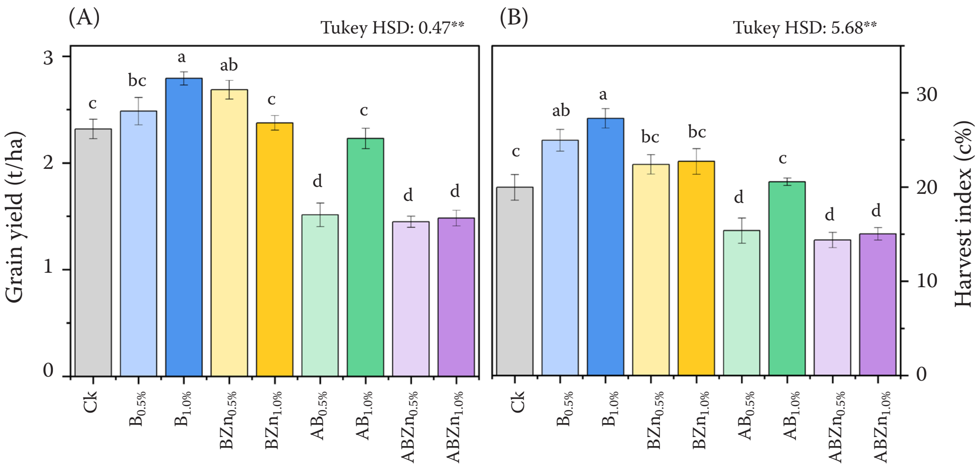

Figure 5. The effect of treatments on grain yield (A) and harvest index (B)

Ck – control; B – biochar; AB – acidified biochar; BZn – Zn enriched biochar; ABZn – acidified-Zn enriched biochar; *, ***P* < 0.05, 0.01, ns – non-significant

treatments (Figure 4D). The highest SOD enzyme activity was found in $AB_{0.5\%}$ and 28% higher than the Ck (Figure 4E). While all treatments increased the proline content compared to the control, this increase was very significant in the $AB_{1.0\%}$ and was realized by 205% compared to the Ck (Figure 4F).

Grain yield and harvest index. Grain yield was positively affected by the original and zinc enriched biochar treatments, while acidified biochar treatments had a decreasing effect compared to the control. Similar effects were observed in harvest index in general, but $AB_{1.0\%}$ showed an increase compared to Ck. The highest increase in grain yield and harvest index compared to the control was 20% and 36.5% in $B_{1.0\%}$, respectively (Figure 5A, B).

DISCUSSION

Effect of acidification and Zn enrichment on biochar. The properties of biochar are greatly altered by the acidification process. In particular, the EC and nutrient content increased, and the organic carbon value decreased. The excessive increase in EC is due to the increased solubility of nutrients in the biochar after acid addition and the slight increase after zinc enrichment is due to sulphate (de la Fuente et al. 2007). The increase in CEC can be explained by the fact that oxidation improves the surface adsorption properties of biochar, resulting in an improvement in cation exchange capacity and an increase in oxygenated functional groups (Han et al. 2016; Li et al. 2017). As expected, the mineral components in the biochar structure dissolved after acidification, as demonstrated by Xu et al. (2021).

Soil chemical properties. It was observed that non-acidified biochar increased soil pH, whereas acidified biochar decreased it. The application of biochar is known to increase soil pH due to its alkaline nature and high alkaline metal content. When biochar is applied to the soil, the alkaline metals in the structure of the biochar are gradually released into the soil solution and, as a result, the pH of the soil is increased (Xu et al. 2011; Müller-Stöver et al. 2012; Yang et al. 2019). The decrease in soil pH can be attributed to the introduction of H_2SO_4 into the soil solution through the acid-modified biochars (Sultan et al. 2020; Demirkaya et al. 2021).

The addition of acid-modified biochar decreased soil pH and increased the amount of plant-available phosphorus in the soil (Figure 2A and Figure 3C). Acidification can enhance the solubility of biochar nutrients, thereby raising soil EC; alternatively, the introduction of H_2SO_4 into the soil solution with acidified biochars can also contribute to an increased soil EC (de la Fuente et al. 2007).

Only $B_{1.0\%}$ treatment was effective in increasing the amount of organic matter in the soil (Figure 2C). It has been shown in many studies that adding biochar to soils increases soil organic matter content (Cooper et al. 2020; Shi et al. 2021; Khan et al. 2023). This is consistent with previous research showing that biochar can contribute to a recalcitrant soil carbon pool due to its stability and resistance to decomposition in the soil environment (Laird et al. 2010). The

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contributions of biochars to soil organic matter vary due to differences in their degree of decomposition, which is influenced by the feedstocks and production temperatures.

Some treatments had a negative effect on CEC, but these were not statistically significant (Figure 2D). Acidification is known to increase the CEC of biochar, but the application dose seems to be important. It is generally known that biochar increases the cation exchange capacity of soils thanks to its large surface area and functional groups on the surface (Liang et al. 2006; Atkinson et al. 2010; Lorenz & Lal 2014), but some studies reported decreases in some cases (Prommer et al. 2014; Hailegnaw et al. 2019). Biochar CEC depends on feedstock or pyrolysis temperature, and biochars produced from woody materials and high temperatures have low CEC (Lee et al. 2010; Luo et al. 2011). Domingues et al. (2020) mentioned that the reason why biochar application reduces soil CEC may be due to the dilution effect when biochar with lower CEC than the soil is applied.

Nutrients availability. Only acidified biochar reduced the amount of exchangeable Ca, and the effect of the others varied with dose (Figure 3A). This could be due to the chelation and mobilization of calcium ions by the acidic components of biochar, making them less available in the soil matrix. The reduction in Ca may be beneficial in soils where calcium levels are too high, potentially freeing up binding sites for other essential nutrients.

The amount of exchangeable Mg significantly increased in all treatments except $B_{0.5\%}$ compared to the control (Figure 3B). Exchangeable potassium increased significantly only in the B1.0% and $AB_{1.0\%}$ treatments in comparison with the control (Figure 3D). In our soil, high Ca content is an important problem in terms of causing Mg and K deficiency. Increasing the amount of Mg and K plays an important role in eliminating the imbalance between these cations (Brady et al. 2008). The effects of biochar addition on soil cations can be realized directly or indirectly. Mg and K in the structure of the biochar may become useful in the soil or the biochar may increase the usefulness of these nutrients by regulating components such as OM, CEC, pH in the soil (Jeffery et al. 2016; Demirkaya & Gülser 2023). Acidified biochars applied at high doses positively affected the P and DTPA Zn availability in the soil, while the other treatments negatively affected it (Figure 3C, E). Acidified biochar increased the availability of these elements by regulating soil pH. The relationship between soil pH and phosphorus and micronutrients has been demonstrated by many studies (Lindsay & Cox 1985; Sharma et al. 2004). Gunes et al. (2015) reported that biochar produced at low temperatures increased Mn, Zn, and Cu availability in calcareous soil. (Vahedi et al. 2022) have shown that biochar, particularly when combined with microbial inoculants, can improve the bioavailability of Zn in calcareous soils. Asap et al. (2018) reported that phosphorus fertiliser applied to acid soils with chicken litter biochars increased phosphorus availability and decreased the amount of Al^{3+} and Fe^{2+}.

Antioxidant enzyme activity and proline content. In the present study, the activities of several key antioxidant enzymes, such as CAT, APX, GPX, GST, and SOD, were assessed under various treatments. Additionally, proline content was measured to evaluate its role in osmotic adjustment and stress tolerance. Each enzyme and PRO measurement provides insights into the oxidative stress response and adaptive mechanisms in the studied plant.

The decreased grain yield in treatments $AB_{0.5\%}$, $ABZn_{0.5\%}$, and $ABZn_{1.0\%}$ could be related to the higher activities of certain antioxidant enzymes, indicating a stress response. The highest CAT, APX, and SOD enzyme activities were determined in these treatments where the lowest yields were obtained (Figure 4). Generally, antioxidant enzymes are part of the plant's defense mechanism against stress, and increased activity can sometimes correlate with stress conditions that negatively impact growth and yield (Zhang et al. 2020; El-Sharkawy et al. 2022).

Catalase activity, as shown in Figure 4A, exhibited significant variations among the treatments. The highest CAT activity was observed in the $ABZn_{1.0\%}$ treatment, indicating an enhanced detoxification of H_2O_2 under this condition. This increase in CAT activity is crucial for protecting cells from oxidative damage by converting H_2O_2 into water and oxygen, thereby mitigating the harmful effects of reactive oxygen species (Garg & Manchanda 2009).

APX activity, depicted in Figure 4B, was significantly higher in the $ABZn_{0.5\%}$ treatment compared to other treatments. APX plays a pivotal role in scavenging H_2O_2 and protecting plant cells from oxidative stress (Foyer & Noctor 2005). The elevated APX activity suggests an adaptive response to increased oxidative stress, helping to maintain cellular redox homeostasis.

Figure 4D shows the GPX activity, which was markedly increased in the $AB_{1.0\%}$ treatment. GPX is involved in the reduction of lipid hydroperoxides and free hydrogen peroxide, thus protecting the cell membrane from peroxidative damage (Waszczak et al. 2018). The higher GPX activity indicates an effective defense mechanism against lipid peroxidation under stress conditions.

The GST activity (Figure 4D) did not show significant differences across most treatments, except for a notable increase in the $BZn_{0.5\%}$ treatment. GSTs are important for detoxification processes, catalysing the conjugation of glutathione to various substrates (Rezaei et al. 2013). This suggests that under certain treatments, the detoxification of toxic compounds is enhanced, providing additional protection to the plants. SOD activity (Figure 4) was significantly higher in the $AB_{0.5\%}$, $ABZn_{0.5\%}$, and $ABZn_{1.0\%}$ treatments. SOD is a key enzyme that catalyses the dismutation of superoxide radicals ($O^{2\cdot-}$) into hydrogen peroxide and oxygen, thus providing the first line of defence against ROS (Berwal & Ram 2018). The elevated SOD activity highlights its crucial role in managing oxidative stress in the plants. Proline content, as illustrated in Figure 4F, showed a significant increase in the $AB_{1.0\%}$ treatment. Proline acts as an osmoprotectant, stabilizing proteins and membranes, and scavenging free radicals (Ashraf & Foolad 2007). The higher proline levels indicate its important role in osmotic adjustment and protection against oxidative stress under adverse conditions.

Plant performance. We hypothesized that acidified biochar would regulate soil pH, increase the availability of plant nutrients and microbial activity, and positively affect plant yields. In general, it was successful in influencing soil properties, especially in lowering pH and increasing CEC, plant-available P, K and Zn when applied at high doses, but negatively affected plant yield (Figure 5A). Original and zinc-enriched biochar treatments increased yields even though they negatively affected soil P and Zn contents. It was observed that H_2SO_4 added to the soil with acidified biochar negatively affected plant growth, which correlated with increases in antioxidant enzyme levels. Correlation matrix provides insightful relationships between soil properties and plant yield, critical for effective soil management and enhancement strategies. The correlation matrix between examined soil properties and plant yield is given in Figure 6. Due to the harvest index being

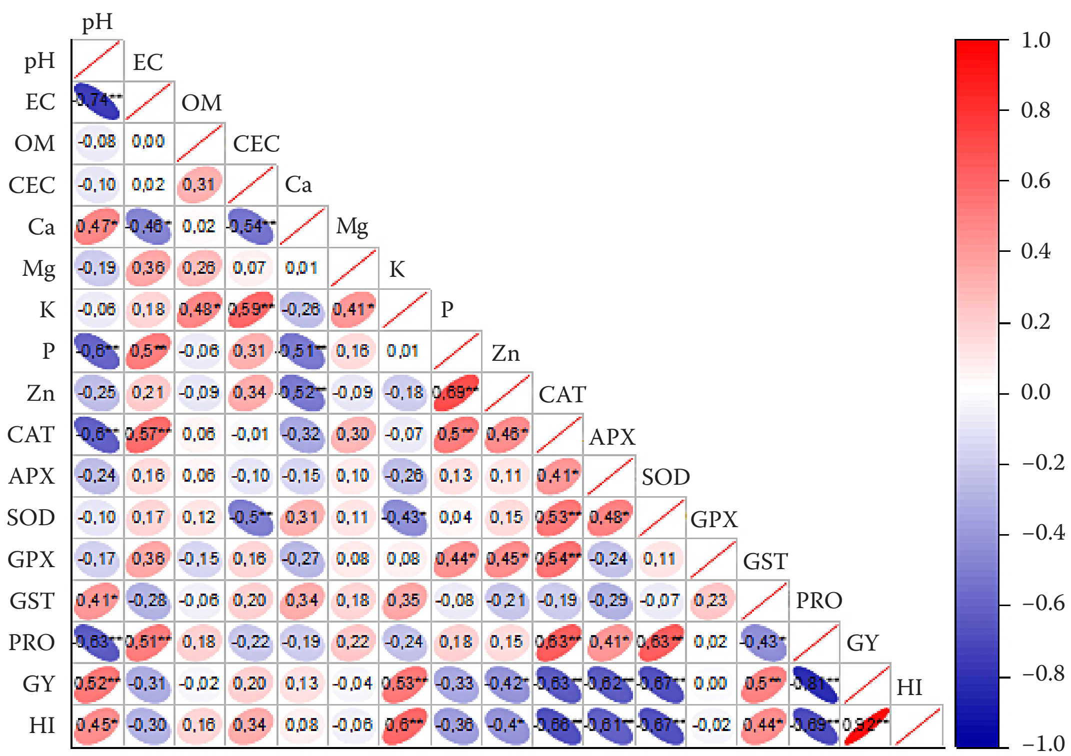

Figure 6. Correlation matrix between soil properties and plant yield and antioxidant enzyme activity

EC – electrical conductivity; OM – organic matter; CEC – cation exchange capacity; CAT – catalase; APX – ascorbate peroxidase; SOD – uperoxide dismutase; GPX – glutathione peroxidase; GST – glutathione S-transferase; PRO – proline; GY – grain yield; HI – harvest index; *,***P* ≤ 0.05, 0.01

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a function obtained using grain yield, there is naturally a very high positive correlation between yield and harvest index (r = 0.92) (Figure 6). Apart from the harvest index, the highest positive relationship with grain yield was found in exchangeable K content (r = 0.53) (Figure 6). The imbalance in soil K in favour of Ca and Mg has caused potassium deficiency in the plant. This is confirmed by the highest yield being obtained in the $B_{1.0\%}$, where the highest K levels were determined. For potassium, it should not be ignored that the imbalances of these ratios between exchangeable cations can cause serious losses in yield (White & Karley 2010).

Grain yield showed very high negative correlations with antioxidant enzymes such as CAT, APX, and SOD, as well as with PRO (Figure 6). The highest amount of proline in the $AB_{1.0\%}$ treatment may be a possible explanation for the lower decrease in yield compared to the other acidified biochar treatments (Szabados & Savouré 2010; Verslues & Sharma 2010).

CONCLUSION

In this study, the effects of biochars, which were acidified and enriched with zinc, on the chemical properties and nutrient contents of calcareous soil, as well as the yield of winter wheat were investigated. Acidified biochars were effective in regulating soil pH, while all biochar applications increased the amount of organic matter. The original biochar application increased CEC, whereas the effects of other applications varied depending on the doses. All treatments increased the exchangeable Mg and K content, but the effects on Ca, P, and Zn contents varied depending on the doses. When antioxidant enzyme activities were examined, the highest enzyme activities, especially CAT, APX, and SOD, were generally determined in acidified biochar applications where the lowest yield was obtained. While zinc-enriched biochar improved yields, the overall findings highlight the necessity for careful management of biochar amendments. Future research should focus on optimizing biochar application rates and combinations with other amendments to mitigate negative impacts on plant growth. Long-term field studies are essential to fully understand the ecological implications and practical benefits of biochar in sustainable agriculture. As a result, although acidified biochar applications positively affected soil properties, they did not show the same effect on plant yield.

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