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Effects of Biochar Application as a Carbon Substrate on Cotton Plant Growth and Some Soil Enzymes

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Abstract
Biochar is a soil amendment that can influence many biotic processes in the soil. When applied to soil with low organic matter content, it improves the physical, chemical, and biological properties of the soil, thereby enhancing soil quality. This study was conducted to examine the potential effects of different ratios of biochar applied to a calcareous soil on the growth of cotton plants and soil biology. The study comprised four treatments: a control group without biochar application and three different levels of biochar application (3%, 6%, 9%). As a result, biochar application increased the uptake of nitrogen, potassium, iron, and boron in cotton plants. It significantly increased the NDVI (Normalized Difference Vegetation Index) and SPAD values used to assess the plant's nitrogen status. By triggering an increase in the activity of nitrate reductase enzymes in the plant leaves, biochar application notably hindered nitrate accumulation, particularly in the case of the 6% biochar application. Additionally, biochar significantly increased the soil enzymes dehydrogenase and urease, contributing positively to the C and N cycles in the soil. The study results demonstrate that biochar application can enhance the uptake of plant nutrient elements from the soil and increase soil enzyme activity in cotton plants.

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1. Introduction

The global textile industry relies on cotton (*Gossypium hirsutum* L.) as a crucial raw material source. According to the report of the National Cotton Council, cotton, a key industrial crop, is of direct concern to 68 countries worldwide. It's reported that cotton is produced in 45 countries, while 23 countries solely consume cotton without producing it. Turkey ranks sixth in cotton production and fourth in cotton consumption worldwide. In 2022, it was reported that Turkey obtained an average yield of 500 kg ha^{-1} from 550,000 hectares of cultivated cotton fields, with a ginning efficiency of cotton at 36.4% (TUIK, 2022). Biocha is a carbon-rich, coal-like material obtained from organic material exposed to specific pyrolysis temperatures in an oxygen deprived environment (Lehmann, 2009). Due to its high carbon content, extensive surface area, and high cation exchange capacity, biochar has gained popularity as a subject of study in recent years (Wang and Wang, 2019). Biochar's inclusion of stable carbon in its composition has a positive impact on several soil properties (Rizwan et al., 2016). Compared to biochar derived from plant residues containing lignocellulosic materials, it can contain higher nutrient levels and have a higher surface area and pH values (Singh et al., 2010). Biochar serves as a significant soil conditioner that triggers the uptake of macro and micro plant nutrient elements by plants in the soil-plant system (Gul and Whalen, 2016). Many biochemical processes in soil are controlled by microorganisms. The application of biochar to soil, due to its high labile carbon content, provides a broader living space for soil microorganisms (Gul et al., 2015). Some studies have reported that applying biochar to soil increases nitrogen uptake by plants. For example, researchers have found that biochar obtained at a slow pyrolysis temperature (400 °C) increased nodulation by 45% in red clover roots and enhanced N_2 fixation in bean plants by 31% in clayey soils (Rondon et al., 2007; Mia et al., 2014). The application of biochar to acidic soils can increase soil pH from 6.3 to 7.2 significantly enhancing potassium uptake in clover plants (Quilliam et al., 2013). Soil enzymes, representing the biological properties of soils, are one of the most critical parameters for soil quality (Mierzwa-Hersztek et al., 2016). Adding external organic matter to the soil further stimulates the activities of soil microorganisms, leading to observed increases in soil enzyme activities. Biochar enhances soil microbial activities by improving the porous structure of the soil (Steinbeiss et al., 2009). The mineralization of organic matter in soil is provided by enzymes produced by soil organisms as a result of microbiological processes in soil (Kussainova et al., 2013). Enzymes produced by soil organisms are closely related to various factors, such as the microbial population in the soil, the cultivated crop, and the physical, chemical, and biological properties of the soil (Nielsen et al., 2014; Lehmann and Joseph, 2015). This study was conducted to investigate the effects of different levels of biochar applied to soil on cotton plant growth and some soil enzyme activities.

2. Materials and Methods

2.1. Materials

In the study, biochar material was obtained by slow pyrolysis of burnt animal manure at 500°C. The burnt animal manure, initially considered as waste, was spread in an open area in the Buyukayrik village of Kızıltepe district in Mardin province and exposed to the sun for an extended period. It was subsequently used as a source of biochar. Some characteristics of the biochar material are presented in Table 1. The soil used in the experiment had a high lime content (32.91%), very low organic matter levels (0.83%), and an alkaline soil reaction (pH: 7.9).

Table 1. Characteristics of the biochar material used in the experiment

N	K	Ca	Mg	Fe	Zn	Cu	B	Mn
%				mg kg^{-1}				
-	6.1	5.9	0.9	439	47	21	286	42

2.2. Methods

The soil obtained from the field was sieved through a 2 mm mesh and thoroughly mixed in a large plastic container to ensure homogeneity. It was then combined with different proportions of biochar and transferred to 5 kg pots. The experiment included a control without biochar (CK) group and three different biochar application rates (3%, 6% and 9%). After sowing cotton seeds in the soil, all pots received a nitrogen fertilizer application (20 kg N). The pot experiment was conducted in May 2022 in the greenhouse of the Department of Field Crops at Harran University. The plant samples collected after harvest were first rinsed three times with distilled water, followed by rinsing with blotters. Subsequently, they were placed in paper bags and dried in an oven at 65°C for 48 hours. After drying, 5 ml of HNO_3 was added in the first stage, and 4 ml of concentrated $HClO_4$ acid was added in the second stage to the plant samples, which had been broken down with the help of a mixer. They were then placed on a hot plate at 200°C for 3 hours (Jones and Case, 1990). As a result of wet decomposition, the total elemental concentrations of K, Ca, Mg, Fe, Zn, Cu, and Mn in the plants were determined using an ICP-OES instrument. For total nitrogen analysis, a Kjeldahl tablet was added to the samples, which were then transferred to Kjeldahl distillation tubes. Next, 15 ml of H_2SO_4 was added to the tubes, and after digestion in the combustion unit for 3 hours, the samples were distilled using a distillation device (Bremner et al., 1982). Nitrate analysis in the leaves was determined using the salicylic acid method on dry matter via a spectrophotometer (Cataldo et al., 1975). 0.2 g of the plant sample was incubated with 5 ml of 0.1M phosphate buffer solution for 1 hour. After incubation, filtrates were obtained from the mixture. 0.4 ml was taken from the obtained filtrates. Sulfanilamide and N-(1-naphthyl)-ethylenediamine hydrochloride were added to the 0.4 ml filtrate. The resulting color was determined at a wavelength of 540 nm using a spectrophotometer (Jaworski, 1971). The chlorophyll content (SPAD values) of selected plant leaves was measured using a SPAD meter (Minolta 502). NDVI values were determined using a Greenseeker crop sensor handheld device. Dehydrogenase enzyme activity was assessed by incubating soil samples to which TTC (2,3,5-triphenyltetrazolium chloride) substrate solution had been added, at 25°C for 24 hours, and measuring the color formed using TPF (triphenylformazan) solution at 485 nm with a UV-VIS spectrophotometer (Optima SP-3000) (Tabatabi, 1982). Urease enzyme activity was determined after incubating urea as a substrate at 37°C for 1 hour and measuring the color formed by the amount of dissolved ammonium after adding a buffer solution at pH 6.7, at 578 nm using a UV-VIS spectrophotometer (Optima SP-3000) (Tabatabai and Bremner, 1972). Catalase enzyme activity was assessed by adding 10 ml of phosphate buffer (pH 7) and 5 ml of a 3% H_2O_2 substrate solution to 5 g of soil. The volume (in ml) of O_2 released as gasometric within 5 minutes at 20°C was determined (Beck, 1971).

3. Results and Discussion

The addition of biochar material to the soil has led to an increase in various soil enzyme activities, as well as the concentration of macro and micronutrient elements in cotton plant leaf tissues. This increase can be attributed to the high carbon content of the biochar material and its potential to improve the low levels of soil organic matter in the soil. Biochar serves as a significant nutrient source for soil microorganisms, playing a crucial role in both increasing their populations and providing them with new habitats. The impact of the applications on the uptake of macro plant nutrient elements by the cotton plant is presented in Table 2. Statistical comparisons of the applications indicate that the BC application has the potential to increase nitrogen uptake by the plant (Figure 1).

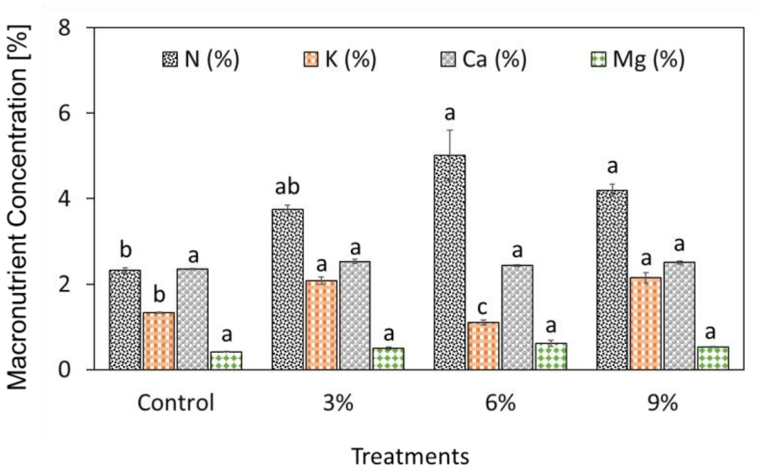

Figure 1. Concentrations of macro plant nutrient elements in cotton plant leaf tissues

Letters indicate significant differences at the $p < 0.05$ level between treatments

The BC applications resulted in higher nitrogen content in the plant compared to the control group. Notably, the 6% BC application provided the highest nitrogen uptake among the BC applications (3% and 9%) (Table 2). Regarding potassium content in the plant, both the 3% and 6% BC applications increased potassium uptake, while the 3% BC application, interestingly, showed a lower level than both the other two BC applications and the control group. There were no statistically significant differences in calcium and magnesium content in the plant ($P < 0.05$).

Table 2. Concentrations of macro and micronutrient elements in cotton plant leaf tissues

	N	K	Ca	Mg	Fe	Zn	Cu	B	Mn
Treatments	%				mg kg^{-1}				
Control	2.31	1.33	2.35	0.42	133.90	17.93	5.75	94.15	41.40
3%	3.73	2.08	2.50	0.49	176.45	2.55	3.75	99.30	59.75
6%	5.00	1.07	2.44	0.61	114.32	4.75	4.20	151.00	56.75
9%	4.19	2.15	2.50	0.53	104.00	2.00	2.70	112.05	54.95

When examining the concentrations of micronutrient elements in the plant, it was found that the iron content was only higher in the 3% BC application compared to the control group, while the other two applications had lower concentrations compared to the control (Table 2). Zinc concentrations in the plant were measured at lower levels in the BC applications compared to the control group. Plant boron contents were found to be higher in BC applications compared to the control group, indicating that BC increased boron uptake by the plant. While there was no statistical difference in manganese concentration in the plant, BC applications showed relatively higher levels compared to the control group (Figure 2).

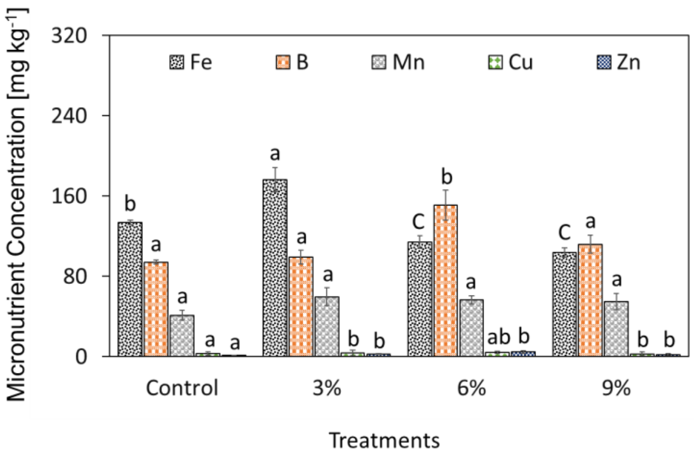

Figure 2. Concentrations of micronutrient elements in cotton plant leaf tissues

Letters indicate significant differences at the $p < 0.05$ level between treatments.

The NDVI value, considered an important indicator of plant development, significantly increased with the BC application, as evidenced in Table 3 and Figure 3. This increase was also statistically significant.

Table 3. The effect of applications on certain biochemical characteristics of cotton plants

Treatments	NDVI	SPAD	L-NRA	L-NO_3
Control	0.57	17.35	1.28	166.63
3%	0.64	26.43	2.56	153.60
6%	0.72	24.81	1.70	263.40
9%	0.67	26.30	2.62	141.23

L-NRA: Leaf nitrate reductase, L-NO_3: Leaf nitrate content

The highest NDVI value was measured in the BC 6% application, followed by the 9% and 3% BC applications, in that order. While there was no statistical difference in SPAD values among BC applications, SPAD values were higher compared to the control group (Table 3; Figure 3). Nitrate concentrations in cotton plant leaves collected before harvest were determined, with the highest nitrate concentration measured in the 9% BC application, followed by the 3% and 6% applications (Table 3; Figure 3). The nitrate reductase enzyme activity in plant leaves was recorded as the highest in the 9% BC application and the lowest in the control group (Table 3; Figure 3).

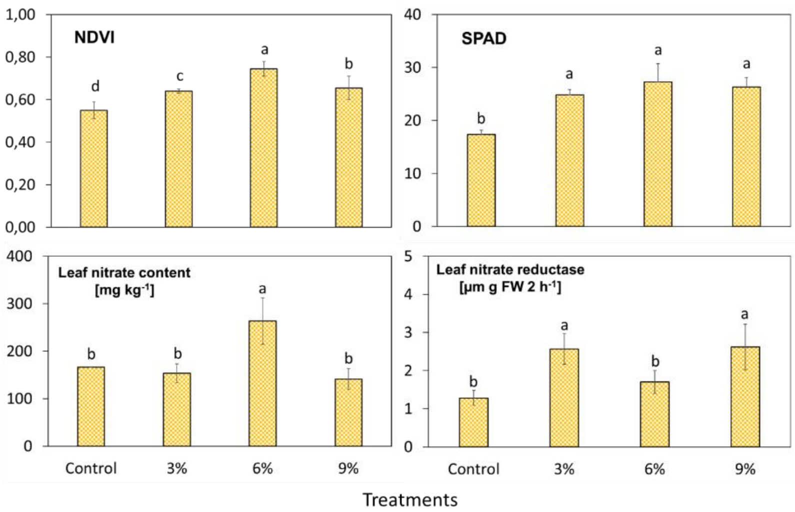

Figure 3. The effect of the applications on certain biochemical characteristics of cotton plants

Letters indicate significant differences at the $p < 0.05$ level between treatments

Soil enzymes, being highly sensitive parameters of soil biology that respond promptly to external interventions in the soil, were significantly influenced by the biochar application (Figure 4). Catalase enzyme activity was observed to be the lowest in the 3% BC application, followed by the 9% and 3% BC applications, respectively (Table 4).

Table 4. The effect of biochar applications on certain soil enzymes

Treatments	Catalase	Urease	Dehydrogenase
Control	96.00	69.27	15.60
3%	89.00	70.00	25.62
6%	92.50	78.41	31.70
9%	90.00	81.28	22.96

Soil urease enzyme activity significantly increased with the BC applications when compared to the control. The highest urease enzyme activity was recorded in the 9% BC application, while the lowest activity was measured in the 3% BC application (Table 4; Figure 4). Soil dehydrogenase enzyme activity, which is closely related to the activity of soil microorganisms, also significantly increased with biochar application (Table 4; Figure 4). All BC applications resulted in an increase compared to the control group, with the highest increases seen in the 6% BC application, followed by the 3% and 9% BC applications, respectively.

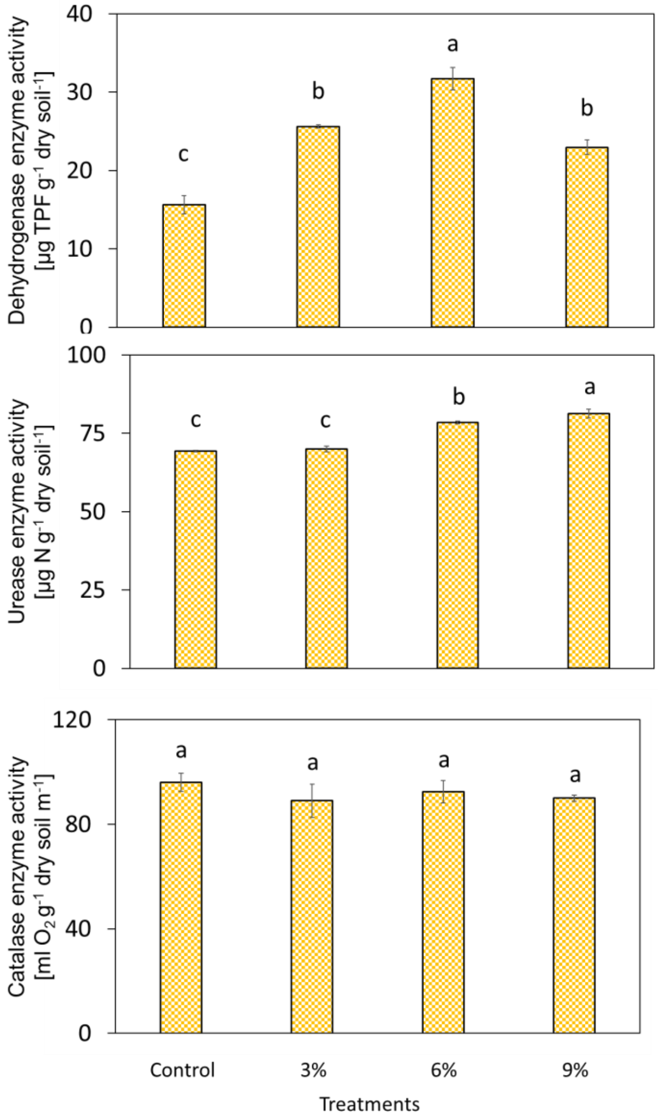

Figure 4. Biochar uygulamasının bazı toprak enzim aktivitelerine etkisi

Letters indicate significant differences at the $p < 0.05$ level between treatments

Soil conditioners like biochar, when applied to soils, have the potential to improve the physical and chemical properties of the soil. In addition to enhancing soil quality and crop yields, biochar also serves as a long-lasting and partially recalcitrant carbon source in the soil (Asai et al., 2009; Hossain et al., 2010). Biochar can have a positive influence on the availability of nitrogen in the soil. According to our study results, the significant increase in nitrogen uptake in plants with the 6% biochar application may be attributed to its impact on certain biotic processes in the soil. Biochar can affect various biotic processes in the soil, including adsorption, mineralization, nitrification, and denitrification (Nguyen et al., 2017; Levesque et al., 2018). The combined use of biochar, particularly in combination with nitrogen fertilizers, has been reported to increase yields in various studies. For example, in a field trial conducted in Indonesia where biochar obtained from tree bark was used alongside nitrogen fertilizers for cultivating corn and peanuts, significant yield increases were observed (Yamato et al., 2006). Successful nitrogen management in rice production was achieved through the use of biochar (Asai et al., 2009). Another study conducted in potted experiments for growing radishes reported higher biological nitrogen fixation in soils with biochar application (Chan et al., 2007). These findings underscore the potential of biochar in improving nitrogen management and enhancing crop yields. Biochar material significantly increased the potassium content of cotton plants. Biochar has the potential to serve as a good source of potassium, potentially replacing a substantial portion of potassium fertilizers applied to soils (Angst and Sohi, 2013). The extent to which biochar can replace potassium fertilizers in the soil depends on factors such as the release rate of biochar, soil type, clay content, and the existing potassium balance in the soil (Li et al., 2009). Additionally, biochar facilitates the dissolution of potassium-containing minerals in the soil by providing organic anions and promoting potassium-dissolving bacteria, such as Bacillus mucilaginosus and Bacillus edaphicus (Sheng et al., 2008). In a study conducted on cotton plants with biochar application to the soil, significant increases were reported in the growth, boll count, and yield of the plants (Wu et al., 2019). Biochar application also had a positive impact on biochemical characteristics such as SPAD and NDVI. NDVI and SPAD values measured in plants are two important parameters that can assess the plant's nitrogen status. In tobacco plants with biochar-based fertilization, it was similarly reported that both SPAD and NDVI values significantly increased (Ren et al., 2023). The combination of biochar with fertilizers has been shown to significantly increase both SPAD and NDVI values in plants, thereby enhancing photosynthetic efficiency (Hu et al., 2010). Plants have the ability to reduce nitrate in their roots, shoots, and leaves, with leaves being more efficient in reducing nitrate compared to other plant parts (Marschner, 1995). Plants store the nitrate they uptake in their vacuoles, and the nitrate concentration in plants is closely related to nitrate reductase enzyme activity. According to the study's results, an inverse relationship was observed between nitrate content in plant leaves and the measured nitrate reductase enzyme activity. The interpretation of the relationship between plant nitrate content and nitrate reductase enzyme activity may vary among different researchers. In a study conducted on spinach, radish, and bok choy, it was reported that there is a threshold level for nitrate reductase to reduce nitrate, and under conditions of high nitrate concentration, there could be low nitrate reductase activity (Chen et al., 2004). However, in maize (Sivasankar et al., 1997) and wheat (Brunetti and Hageman, 1976), a positive relationship between plant nitrate content and nitrate reductase enzyme activity was observed. The nitrate reductase enzyme activity in plants is often observed to work inversely with the nitrate concentration in plants, and this relationship can vary depending on plant species and nitrate levels. Some mechanisms in this regard remain not fully understood (Hu et al., 1992). Biochar application significantly increases the activities of dehydrogenase and urease enzymes in the soil. Soil dehydrogenase activity, the most sensitive and vital soil biology indicator, is influenced by various soil properties (Paz-Ferreiro et al., 2012; Błońska et al., 2017). Reports indicate that the application of biochar at rates of 30 and 45 t ha-1 significantly increases soil dehydrogenase enzyme activity (Oleszczuk et al., 2014). Sopeña and Bending (2013) attribute this to the potential protective effect of biochar on soil dehydrogenase enzymes. Soil dehydrogenase enzyme activity can vary depending on the type of biochar, soil type, and environmental conditions, and it enhances essential parameters like organic matter content, microbial activity, and soil fertility (Brzezinska and Wlodarczyk, 2005). Soil urease enzyme activity is closely related to soil organic carbon and nitrogen content. In a field trial conducted by Futa et al. (2020), on winter rye (*Secale cereale* L.), they reported that the application of biochar at different rates significantly increased soil urease enzyme activity. In a meta-analysis by Pokharel et al. (2020), which compiled results from several studies, biochar was found to generally lead to a 23.1% increase in urease enzyme activity and a 19.8% increase in dehydrogenase enzyme activity in biochar-applied soils.

4. Conclusions

The study conducted in a soil with high calcium content and very low organic matter has shown that biochar application has positive contributions to the growth of cotton plants. Moreover, it has increased soil enzyme activities. Biochar, especially enhanced the uptake of essential plant nutrients such as nitrogen, potassium, iron, and boron, while also improving the plant's biochemical properties. It notably increased parameters such as the NDVI, which is a criterion for photosynthetic efficiency, and the SPAD values closely related to the plant's nitrogen status. Biochar application has also significantly increased urease and dehydrogenase enzyme activities in the soil.

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