IMPACT OF BIOCHAR AND COMPOST ON GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF MAIZE (*ZEA MAYS* L.)

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Abstract. This study was conducted to assess the influence of combined of biochar and compost application on the growth-related morphological attributes (root and shoot weights (fresh and dry), root and shoot lengths, height of plant, no of leaves, leaves areas, stem diameter), physiological (chlorophyll, proline, free amino acid, phenolic contents) of maize (*Zea mays* L.). The results showed that compost and biochar treatments doses considerably affected the morpho-physiological characteristics of maize. Comparing the control (B_0C_0, no biochar, no compost) the introduction of combined treatment (B_1C_1 1% biochar, 1% compost) improved root and shoot fresh weight, total weight of plant, root and shoot length, and total length of plant, number of leaves, leaves area, stem diameter and dry weight of crop. But the concentration of biochar and compost enhanced at the level of (B_2C_0 and B_0C_2, 2% biochar, no compost and no biochar, 2% compost respectively) also improved the growth characters. Furthermore, combined treatment of biochar and compost also showed impact on physiological attributes (chlorophyll contents, reactive oxygen species, total free amino acid and phenolic content) of crop. Our results showed that biochar-compost based soil practices can improve the growth of maize.
Keywords: *plant growth, yield attributes, biochar, compost*

Introduction

Maize (*Zea mays* L.) is known as third most vital cereal crops worldwide as human food, after rice and wheat crop. In developed countries, *maize* is mainly consumed as food and used as major component for livestock feed and also used in several industries (Erenstein et al., 2022). Maize is a globally significant crop, cultivated in diverse soil types and climates. A rapidly rising global population requires water-efficient agriculture techniques (Yang et al., 2022; Aslam et al., 2024). The crop residues without proper management will cause large-scale issues' as slash-and-burn farming, which involves burning crops without covering them, leads to air pollution, and harms human health (Hamzah et al., 2018). The bare areas of field soil left by burning processes are especially vulnerable to erosion and nutrient depletion (Lin et al., 2022). Declining soil organic matter (OM) levels reduced soil fertility, which negatively impacts sustainable crop production (Githongo et al., 2022). Thus, utilizing biomass from crop waste to create biochar can improve soil nutritional status and crop yield (Mustafa et al., 2022). However, overuse of fertilizer can be problematic since it causes serious environmental and ecological problems such soil contamination, which in turn results in low agricultural production (Bisht and Chauhan, 2020) loss of biodiversity (Jwaideh et al., 2022), and water pollution (Craswell, 2021), hindering the development of sustainable agriculture (Krasilnikov et al., 2022). Therefore, managing soil under limited irrigation is essential to achieve high water-fertilizer use efficiency and safeguarding the natural environment. Biochar exhibits multifunctional characteristics, including a porous nature with decreased particle size, high adsorption capacity, abundant surface area, less bulk density and potential for carbon sequestration (Kumar et al., 2023).

Many studies have demonstrated the biochar application can positively affect the physico-chemical characteristics of soil, growth and yield traits of crop, water uptake capacity and fertilizer use efficiency (Abukari et al., 2022; Yang et al., 2022; Cong et al., 2023). Khan et al. (2023) and Minhas et al. (2020) reported the biochar treatment was prominent for maize improvement.

Biochar on maize crop which significantly enhance the chlorophyll activity also found negative impact when the concentration exceeded 12 t ha^{-1} plant traits like leaf area, roots attributes, plant growth, morphological and yield-related parameters. Haider et al. (2022) found that biochar also capable of scavenging reactive oxygen species (ROS) it can activate the plant antioxidant status. According to Abideen et al. (2020) plants treated with biochar (0.75%) noticed less oxidative stress due to antioxidant system efficiently ensures detoxification of ROS. In addition to ROS's capacity to scavenge, osmolyte accumulation, such as proline, soluble sugars, and protein levels for osmotic regulation, can sustain cell turgidity, which is essential for crop development (Singh et al., 2022).

Biochar influenced positively on amino acid and proline synthesis in different crops, thus supporting the osmotic regulation systems of plants in response to environmental challenges (Haider et al., 2022). However, studies with the effect of biochar application on physiological and biochemical traits of maize needs to further explore (Mahmoud et al., 2022). Furthermore, organic manure or compost, crumby rotted decomposed plant material used in agriculture help in improving soil attributes and increased plant growth (Gil et al., 2008). Composting is an efficient nutrient management approach that sustains nitrogen intake, reduces nitrogen loss, improves soil fertility and maize production (Zerssa et al., 2021). Furthermore, compost is a valuable source for returning the organic form of vital minerals back to the soil (Goldan et al., 2023). Cow manure compost application promotes the maize growth attributes thus promotes the shoot and yield traits (Naveed et al., 2023). Kandil et al. (2020) examined the compost potentially increased the grain and biomass production, grain protein contents in maize crop. Gomaa et al. (2016) found that compost manure application efficiently enhanced the grain yield of

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maize. The present research was investigated to evaluate the influence of biochar and compost applied alone or their combine application on growth, morphology, physiology and yield attributes of *maize* grown in pots in research area of University of Agriculture Faisalabad, Pakistan.

Methodology

Experimental design

The pot study was carried at University of Agriculture Faisalabad, Pakistan. A perforated plastics pots with diameter of 23*20 cm, with height of 20 cm was used in this experiment. Pots containing soil was comprised of rice residue based-biochar and organic based-compost i.e. B_0C_0 (no biochar, no compost), B_0C_1 (no biochar, 1% compost), B_0C_2 (no biochar, 2% compost), B_1C_0 (1% biochar, no compost), B_1C_1 (1% biochar, 1% compost), B_1C_2 (1% biochar, 2% compost), B_2C_0 (2% biochar, no compost), B_2C_1 (2% biochar, 1% compost), B_2C_2 (2% biochar, 2% compost). Rice residue was used to prepare biochar through pyrolysis process at 600 °C for 8 h and passed through 2 mm sieve before mixing into soil. Compost used for the study was prepared from animal waste material (organic). We sowed four maize seeds per pot at 4 cm and thinned them to two seedling five days after emergence. A period of nine weeks was maintained with controlled water condition, temperatures 35°C, and natural day length for nine weeks during July-August (summer season). In control, pots were irrigated twice a day and placed under suspended shade cover. Completely randomized design (CRD) was applied with 3 biological replications (*Table 1*).

Table 1. *Composition of soil, biochar and composted used in present study*

	Soil	**Biochar**	**Compost**
pH	7.92	9.2	7.20
N	0.8 g kg^{-1}	3.92 g kg^{-1}	2.33 g kg^{-1}
P	6.44 mg kg^{-1}	12.10 g kg^{-1}	7.12 g kg^{-1}
K	172 mg kg^{-1}	23.12 g kg^{-1}	18.20 g kg^{-1}

Morphological attributes

Various plant attributes (root and shoot weights (fresh and dry), root and shoot lengths, height of plant, no of leaves, leaves areas) were measured after 4 weeks of growth of maize.

Root and shoot weight

Uprooted the plants separated via blade and measure the weight by using analytical balance after dried the plant material in oven (65 ^{0}C) for 10 days and measured again for dry weights.

Root and shoot length and height

Scale measurement was carried out for root and shoot length and by adding the root and shoot length measure the height of plant.

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No of leaves and leaf area

Numbers of leaves from each plant were recorded. For calculating leaf area index, lengths and widths of leaves were measured using a scale and then multiplied it with the co-efficient factor (0.75) for leaf area.

Physiological attributes

Experimental plants were treated as physiological attributes i.e. chlorophyll, proline, phenolic, free amino acid.

Chlorophyll content

Chlorophyll content was measured by the technique of Arnon (1949). Fresh cleaned leaves 0.1 g was used to extract chlorophyll using 80% acetone (10 ml) after centrifugation (14000 rpm, 4 °C), the chlorophyll content of filtered solution was measured using the classical spectrophotometric method (645, 663, and 480 nm).

Proline

Leaf proline concentration was measured according to the acid ninhydrin method (Bates et al., 1973). Fresh leaves of plant used for proline concentration analysis. Leaf samples were placed at −80 ◦C for further analysis.

Hydrogen peroxide (H_2O_2)

H_2O_2 was investigated by the trichloro acetic acid (TCA) method recommended by Velikova et al. (2000). The leaf of maize crop was frozen in liquid nitrogen at -210°C and homogenized with trichloroacetic acid. Supernatant was mixed with phosphate buffer (0.5 mL) and potassium iodide solution (1 mL). The optical density was taken at 390 nm on a spectrophotometer.

Total phenolic content

The leaf of maize crop was extracted with aqueous acetone solution (80%). The supernatant (100 μL) was mixed with 0.5 mL (Folin-Ciocalteu reagent) and added 2 mL of sodium carbonate solution (20%) (Julkunen-Tiitto, 1985). Optical density measurement was investigated by spectrophotometer (750 nm).

Total free amino acid (TFAA)

An assay for ninhydrin, as detailed by Hamilton and Van Slyke (1943), was employed to determine the TFAA. For this, 1 ml of homogenized mixture of leaf samples in cold phosphate buffer (PH 7.0) was centrifuged at 10,000 rpm, and then 1 ml of 10% pyridine and 1 ml of 2% ninhydrin solutions were mixed in test tubes. After that, diluted the solution with dH2O to a level of 50 ml and incubated it in a water bath for half hour. Following this, spectrophotometers were used to measure absorbance at 570 nm. By creating a leucine standard curve, TFAA was evaluated.

Statistical analysis

Two-way ANOVA was implemented using the least significant difference (LSD) test (0.05) by Statistix-8.1 to compare the treatment means of recorded observations (Steel and Torrie, 1980).

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Results

The effect of biochar and compost applied alone or in combination on the growth and physiological performance of maize crop as dry, fresh weight of both root and shoot, height characters of root and shoot, number of leaves, leaf area, photosynthetic characters, reactive oxygen species, phenolic contents, proline and total free amino acid of maize cultivar. The different levels of biochar and compost compositions i.e. B_0C_0 (no biochar, no compost), B_0C_1 (no biochar, 1% compost), B_0C_2 (no biochar, 2% compost), B1C0 (1% biochar, no compost), B_1C_1 (1% biochar, 1% compost), B_1C_2 (1% biochar, 2% compost), B_2C_0 (2% biochar, no compost), B_2C_1 (2% biochar, 1% compost), B_2C_2 (2% biochar, 2% compost) were applied in soil to nourish the maize crop. Biochar and compost application (B_1C_1, 1% biochar, 1% compost) significantly increased the fresh biomass (root, shoot and plant), length (root, shoot, and plant), leaf number; area, and stem diameter as compared to B_0C_0 (no biochar, no compost) treatment. When the concentrations of biochar and compost enhanced in treatments such as (B_0C_2, no biochar, 2% compost), and (B_2C_0, 2% biochar, no compost) also improves the growth characters as compared to B_0C_0 (no biochar, no compost) and other treatments of biochar and compost. However, in (B_0C_1, no biochar, 1% compost) treatment caused reduction in growth attributes overall compared to control treatment. The treatments (B_1C_0, 1% biochar, no compost) and (B_2C_0, 2% biochar, no compost), have been improved the growth traits of maize as compared to control ones. While the treatment (B_2C_1, 2% biochar, 1% compost), showed less response but (B_2C_2, 2% biochar, 2% compost) treatment not more response as compared to control as presented (*Figure 1* and *Figure 2*).

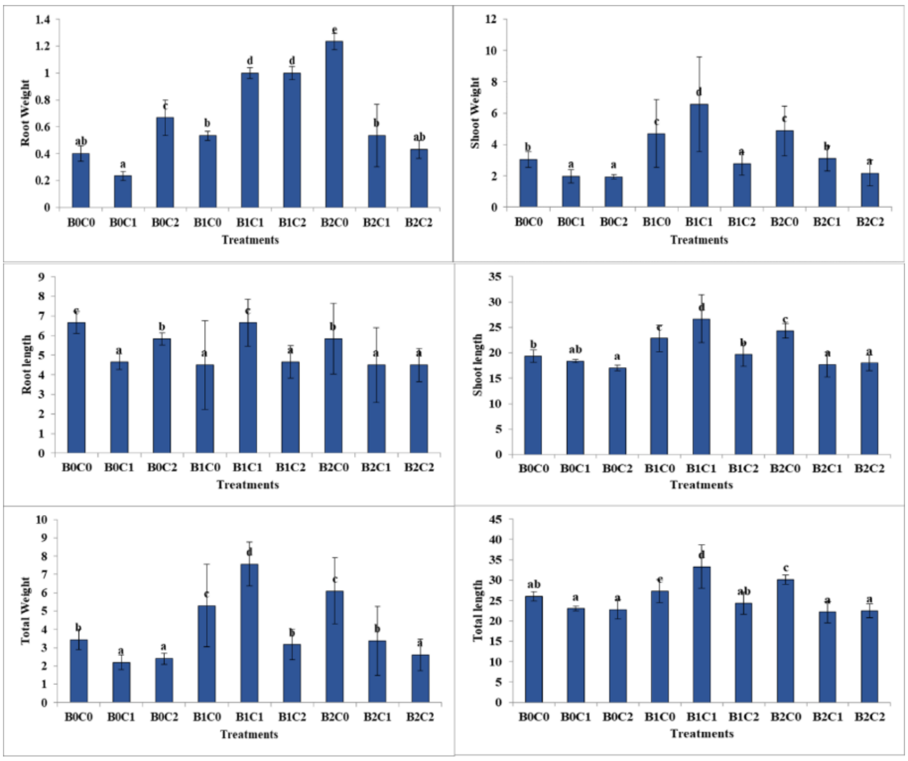

Figure 1. *Biochar and compost treatment on growth attributes as root weight (g), shoot weight (g), root length (cm), shoot length (cm), total weight (g), total height (cm) of maize*

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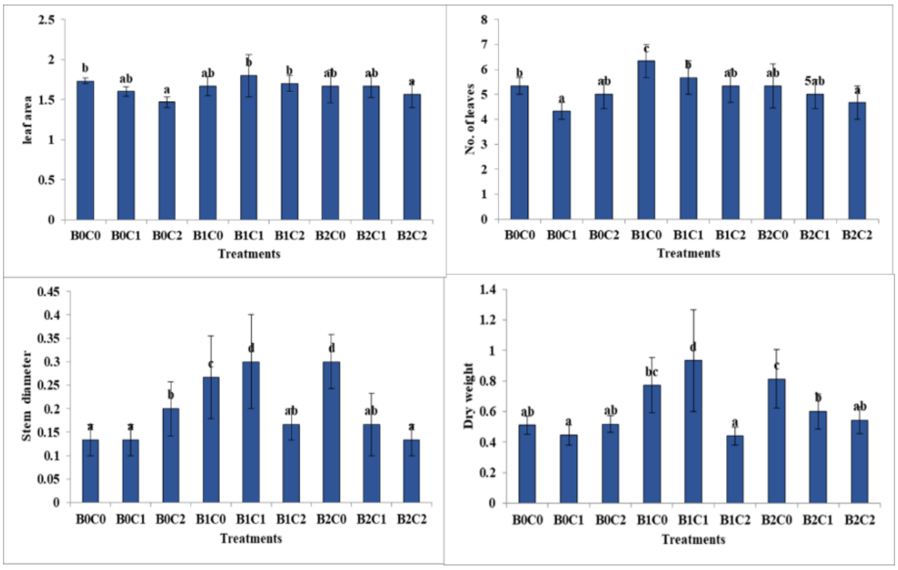

Figure 2. *Biochar and compost treatment on growth attributes as leaf area, number of leaves, stem diameter (cm), dry weight (g) of maize*

Photosynthetic attributes as chlorophyll a content showed increasing trend using biochar and compost treatments as compared to control (B_0C_0, no biochar, no compost). However, the highest level obtained in (B_2C_1, 2% biochar, 1% compost), (B_2C_2, 2% biochar, 2% compost) both treatments as compared to all other treatments applied on maize. Furthermore, chlorophyll b content and carotenoids of maize improved via biochar and compost treatments however, the highest level achieved in B_2C_0, 2% biochar, no compost) and lowest level in (B_0C_0, no biochar, no compost). In case of total chlorophyll enhanced via biochar and compost treatments, the highest level obtained in (B_2C_1, 2% biochar, 1% compost), (B_2C_2, 2% biochar, 2% compost) and the lowest level in (B_0C_0, no biochar, no compost) treatment (*Figure 3*).

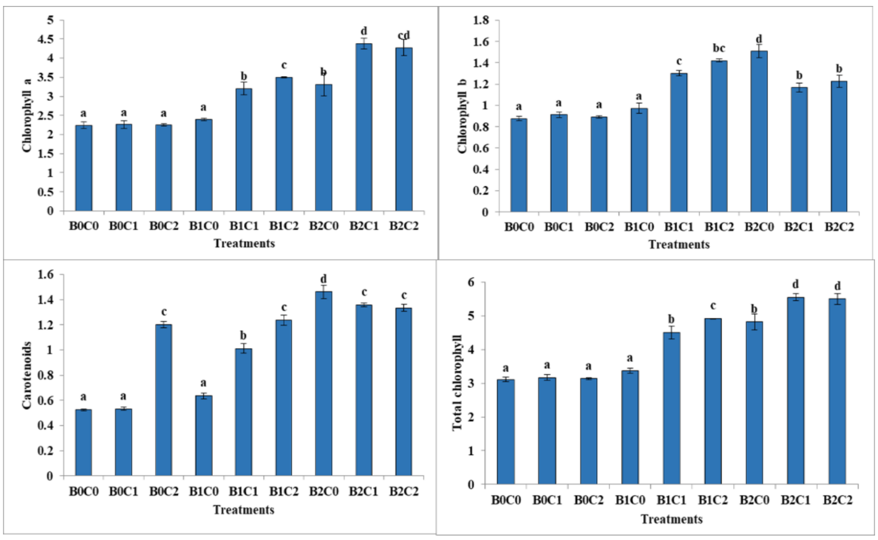

Figure 3. *Biochar and compost treatment on physiological attributes as chlorophyll a (mg g^{-1}), chlorophyll b (mg g^{-1}), carotenoids, total chlorophyll (mg g^{-1}) of maize*

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The level of reactive oxygen species showed reducing trend while the level of phenolic contents, total free amino acid and proline showed enhancing trend via the use of biochar and compost treatments on maize. The highest reducing level of hydrogen peroxide was recorded in (B_1C_0, 1% biochar, no compost) treatment while less reduction was noted in (B_1C_1, 1% biochar, 1% compost). The highest level of total phenolic content, proline and total free amino acid were noted in B_2C_1 (2% biochar, 1% compost), B_2C_2 (2% biochar, 2% compost) treatments than control (*Figure 4*).

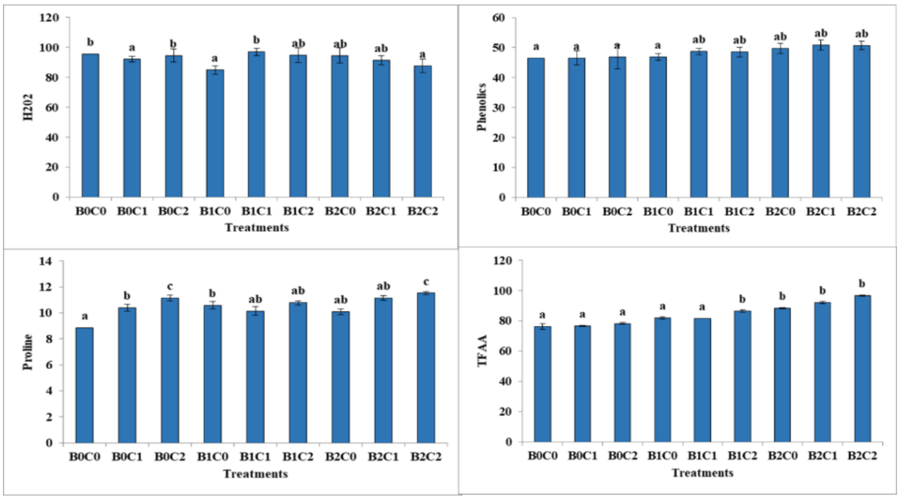

***Figure 4.** Biochar and compost treatment on physiological attributes as H_2O_2 (g) , phenolic (g), proline (cm), total free amino acid (g) of maize*

Discussion

In this study we found that biochar and compost treatments improve the growth characters root shoot weight, root shoot length, dry weight, stem diameter, total weight, total length, leaf area, number of leaves of maize (*Table 2*). The significant improvement in plant growth attributes could because of biochar treatment which significantly improve the soil characters as composition, fertility, carbon contents, water holding capacity and efficiency (Alkharabsheh et al., 2021). Furthermore, organic manure or compost in the presence of biochar decompose faster in the soil, leading to higher quantity of nutrients (Agarwal et al., 2022) ultimately improving plant growth characters. Similarly, the treatment of biochar and compost in maize crop positively enhanced the growth attributes as plant height, LAI, CGR, leaf number, stem diameter, and dry biomass of crop (Khan et al., 2022). Furthermore, biochar-compost treatments could strongly impact the plant height and dry weight characters during the plant growth (Izilan et al., 2022). Different doses of biochar treatment significantly improve the stem diameter, number of leaves, plant dry weight and height characters (Rahayu et al., 2022). Conversely, maize growth traits were increased with biochar application. Our results correspond with Cong et al. (2023) who stated a significant improvement in growth and physiological attributes of maize with use of biochar. Similarly, Irfan et al. (2021) investigated the shoot and root dry weights significantly improved by applying biochar and compost treatment on maize. Simiele et al. (2022) found that compost treatment alone plant height character and root length of plant. Khan et al. (2022) stated higher LAI could be due to the cell expansion under ample nutrient supply with addition of biochar (Hasnain et al., 2023).

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Table 2. *Means of growth-related morphological attributes of maize*

Treatments	Root weight (g)	Shoot weight (g)	Plant weight (g)	Root Length (cm)	Shoot Length (cm)	Plant Length (cm)	Stem Diameter	Leaf Area	No. of leaves	Dry weight (g)
B_0C_0	0.3	2.3	2.6	4	20	24	0.1	1.8	5	0.47
B_0C_0	0.5	4	4.5	7	21	28	0.1	1.7	6	0.43
B_0C_0	0.4	2.8	3.2	9	17	26	0.2	1.7	5	0.63
B_0C_1	0.2	2.8	3	4	19	23	0.2	1.7	5	0.37
B_0C_1	0.3	1.6	1.9	6	18	24	0.1	1.6	4	0.58
B_0C_1	0.2	1.5	1.7	4	18	22	0.1	1.5	4	0.39
B_0C_2	0.8	1.8	2	3	16	19	0.1	1.4	5	0.61
B_0C_2	0.4	2.2	3	9	18	27	0.3	1.6	6	0.42
B_0C_2	0.8	1.8	2.2	5.5	17	22.5	0.2	1.4	4	0.52
B_1C_0	0.5	9	9.8	5	28	33	0.4	1.9	7	1.13
B_1C_0	0.5	3	3.5	3.5	20.5	24	0.3	1.6	7	0.62
B_1C_0	0.6	2.1	2.6	5	20	25	0.1	1.5	5	0.56
B_1C_1	2	3.2	3.8	6	21.5	27.5	0.2	2.2	5	0.63
B_1C_1	0.4	12.6	14.6	8	36	44	0.5	1.9	7	0.57
B_1C_1	0.6	3.9	4.3	6	22.5	28.5	0.2	1.3	5	1.6
B_1C_2	2	1.3	1.5	4	15	19	0.1	1.5	4	0.56
B_1C_2	0.5	3.5	4	5	22	27	0.2	1.8	6	0.38
B_1C_2	0.5	3.5	4	5	22	27	0.2	1.8	6	0.38
B_2C_0	2.5	8	10.5	5.5	27	32.5	0.4	2	7	0.75
B_2C_0	0.4	2.8	3.2	5	24	29	0.2	1.3	5	0.52
B_2C_0	0.8	3.8	4.6	7	22	29	0.3	1.7	4	1.17
B_2C_1	0.3	3.8	3.3	3.5	22	25.5	0.1	1.7	5	0.57
B_2C_1	1	4	5	6.5	17.5	24	0.3	1.9	6	0.81
B_2C_1	0.3	1.5	1.8	3.5	13.5	17	0.1	1.4	4	0.42
B_2C_2	0.3	1.4	1.7	5	16	21	0.1	1.4	4	0.45
B_2C_2	0.5	3.8	4.3	5	21	26	0.2	1.9	6	0.72
B_2C_2	0.5	1.3	1.8	3.5	17	20.5	0.1	1.4	4	0.46

The latest findings (Rasool et al., 2021; Tanveer et al., 2022; Kumar et al., 2022) demonstrated that compost and biochar treatment considerably influenced growth-related attributes. Our findings showed that chlorophyll contents were improved as a result of combine (biochar and compost) application (*Table. 3, Figure 3* and *Figure 4*). Results from this study show that biochar and compost applied together increased soil and crop attributes for better crop yield. Liu et al. (2021) found that chlorophyll contents roselle plant was significantly responded to the added biochar and compost in soil. He et al. (2020) investigated that biochar supplement significantly improved the photosynthesis and chlorophyll contents in C_3 and C_4 plants. Furthermore, Hafeez et al. (2017) reported high chl contents and TFAA in soybean with biochar application. In our findings the reactive oxygen species, decreased while proline contents, total free amino acids contents and phenolic contents were increased in maize due to applied combined compost and biochar treatments. Roy et al. (2022) investigated that applied biochar and cattle manure compost enhanced osmotic adjustment by accumulating osmolytes and antioxidants decreasing the oxidative stress as verified by low level of hydrogen peroxide in plant leaves. Biochar and compost can protect plants from oxidative stress by increasing concentrations of phenolic acids and facilitating the synthesis of phenolic compounds, which in turn helps plants detoxify ROS (Zulfiqar et al., 2021).

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Table 3. *Means of physiological attributes of maize*

Treatments	Chl a (mg g^{-1} f. wt.)	Chl b (mg g^{-1} f. wt.)	Carotenoids (mg g^{-1} f. wt.)	Total Chl (mg g^{-1} f. wt.)	H_2O_2 (µmol g^{-1} f. wt.)	Phenolic (mg g_{-1} f. wt.)	Proline	TFAA
B_0C_0	2.414	0.835	0.535	3.249	97.38	46.42	9.351	72.66
B_0C_0	2.174	0.893	0.527	3.067	97.86	50.60	8.727	79.28
B_0C_0	2.132	0.897	0.509	3.029	91.62	42.28	8.519	76.87
B_0C_1	2.470	0.882	0.558	3.353	98.42	48.15	10.45	76.07
B_0C_1	2.176	0.895	0.521	3.072	94.42	38.94	10.73	76.67
B_0C_1	2.133	0.957	0.518	3.090	83.78	52.63	9.974	76.87
B_0C_2	2.306	0.876	1.173	3.183	91.70	44.60	10.73	77.67
B_0C_2	2.215	0.911	1.180	3.126	91.78	48.32	11.70	79.28
B_0C_2	2.212	0.884	1.254	3.097	100.1	47.63	11.01	77.67
B_1C_0	2.461	0.996	0.671	3.458	80.34	45.04	9.905	83.50
B_1C_0	2.334	0.880	0.594	3.215	85.70	48.43	10.80	81.69
B_1C_0	2.391	1.042	0.640	3.434	88.82	47.25	11.01	80.69
B_1C_1	3.235	1.311	0.987	4.547	88.10	46.39	9.835	81.49
B_1C_1	2.899	1.251	1.086	4.151	98.74	47.94	10.32	81.49
B_1C_1	3.466	1.340	0.957	4.806	104.4	51.77	10.25	81.49
B_1C_2	3.524	1.390	1.200	4.914	103.9	45.56	11.22	84.90
B_1C_2	3.468	1.433	1.312	4.901	86.26	48.70	10.59	87.51
B_1C_2	3.491	1.438	1.194	4.930	94.26	51.39	10.52	86.71
B_2C_0	3.494	1.484	1.544	4.979	89.94	53.12	9.766	88.71
B_2C_0	2.714	1.625	1.359	4.340	93.30	47.94	10.32	88.91
B_2C_0	3.717	1.414	1.480	5.131	100.5	47.91	10.18	87.31
B_2C_1	4.419	1.156	1.387	5.575	100.1	49.70	11.35	90.72
B_2C_1	4.604	1.103	1.349	5.707	85.38	49.01	11.01	91.72
B_2C_1	4.116	1.242	1.331	5.359	88.74	53.77	11.01	93.73
B_2C_2	4.579	1.133	1.366	5.712	88.74	46.15	11.22	95.54
B_2C_2	4.373	1.219	1.276	5.592	85.38	52.63	11.70	97.14
B_2C_2	3.858	1.332	1.358	5.191	88.74	53.39	11.63	97.14

Tanveer et al. (2022) also found that biochar and compost treatment maintained the level of proline contents in *Spinacia oleracea* leaves. Pavlíkova et al. (2022) found that proline content and total free amino acid contents were enhanced after the applied biochar application on spinach and mustard plants. Khan et al. (2023) noted during findings the application of biochar increased the level of total free amino acid contents in plant. Zulfiqar et al. (2021) examined the biochar and compost positive influence related to antioxidant enzyme, total phenol and phenolic contents medicinal plants.

Conclusion

The present experiment showed that treatments of biochar and compost different doses positively influenced various growth-related morphological attributes, physiological traits (chlorophyll contents, hydrogen peroxide, proline, phenolic contents and total free amino acid) in maize plant. It was observed that combined biochar and compost amendments stabilize the cell membrane, improve the water- and nutrient-uptake, photosynthesis and crop performance (growth of plant). We concluded that the combined biochar-compost based soil management strategies can improve the maize growth.

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