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Synergistic effects of soil and foliar nano-biochar on growth, nitrogen metabolism and mineral uptake in wheat varieties

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ABSTRACT

Soil amendment and foliar application of nanobiochar (NBC: a unique nanomaterial) may enhance soil fertility which ensures that nutrients are available to plants, and increase crop production because it is a suitable source of macro- and micronutrients. From this study, NBC induced modification in two wheat varieties (Akbar, Zincol) yield, growth, physio-biochemical and ionic profiles have been studied. The soil application of nanobiochar was applied in four concentrations [control (0 %), S_1 (1 %), S_3 (3 %), and S_5 (5 %)] at the sowing stage while after 30 days of germination, the foliar application of same NBC concentrations with surfactant (0.1 % Tween-20) was applied in the pots. Each treatment has three replications and experiment was arranged in a completely randomized design (CRD). Results indicated that growth attributes, physio-biochemical parameters and ion contents were significantly increased in both varieties than their respective controls with Akbar variety exhibiting better improvement in Akbar. However, the application of NBC increased the plant height, shoot–root dry biomass and shoot length at the S_3F_3 level (NBC in soil 5 % + NBC as foliar 3 %) while root length, plant and shoot fresh weight at the S_5F_5 level (NBC in soil 5 % + NBC as foliar 5 %) root fresh weight and plant dry weight S_3F_1 level (NBC in soil 3 % + NBC as foliar 1 %), leaf area at S_3F_3 level (NBC in soil 3 % + NBC as foliar 3 %), number of tillers at S_1F_5 level (NBC in soil 1 % + NBC as foliar 5 %). On the other hand, both methods of NBC applications significantly enhanced the photosynthetic pigments in both varieties but the Zincol variety showed the highest rate of pigments. Moreover, both applications of NBC significantly affect the primary metabolites (total soluble sugar, protein, and free amino acids), secondary metabolites (phenolic and flavonoid content), antioxidants (Catalase and peroxidase) and nitrogen metabolic enzyme (Nitrite and Nitrate) of both varieties with comparatively higher rate in the Akbar variety. In conclusion, these results showed that the combined applications of NBC as soil and foliar form could be used as a suitable source of fertilizer because of its beneficial effects and high nutritional content.

1. Introduction

Biochar is a solid carbonized product produced by pyrolysis from biomass feedstock such as agricultural waste, animal waste, and other lignocellulosic materials (Salama et al., 2021). It improves plant growth, soil health, and fertility while reducing climate change, while climate changes are region and site-specific which in turn predict the shifting of annual precipitation (varying with season) and increase in temperature leading to disturbance in climatic conditions (Wolf et al., 2023). Biochar is high in macronutrients and promotes soil nutrient availability, resulting in better plant development and crop output. Biochar pyrolysis temperature, duration, and feedstock all influence nutrient content. Its

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particle sizes range from micrometers to centimeters, depending on the pyrolysis process (Mansoor et al., 2021). Decreasing particle size in the micron range (10–600 m) enhanced the number of accessible adsorption sites, resulting in improved adsorption capacity. Further reducing the size of biochar particles to the nanoscale, down to 100 nm or less, improved their characteristics, resulting in a larger surface-to-volume ratio, which increased surface energy, adsorption potential, and biological effectiveness (Naghdi et al., 2018).

Many variations exist in the particle size of biochar such as macro-biochar or residual biochar having 1 m or greater, whereas colloidal biochar has 100 nm to 1000 nm and nanobiochar has less than 100 nm (Mansoor et al., 2021). Nanobiochar is a new type of nanostructured material. It is produced from bulk BCs using various top-down techniques and physical degradation. Ball milling is the most widely utilized method for mechanically breaking down bulk BC into tiny particles with diameters less than 100 nm. It has a greater surface area, microporosity, hydrophobicity and more functional groups with higher mechanical and thermal stability as compared to bulk biochar making it a suitable solution for the removal of pollutants, soil amendments, effective enzyme transporter and greater affinity of absorbent (Noreen and Abd-Elsalam 2021).

Industrialization, deforestation, overgrazing, and other human activities have dramatically reduced soil nutrient levels and it affects food security (Ramadan and Abd-Elsalam, 2020). Using chemical fertilizers to enhance the soil fertility badly effects the soil health as they leach down causing soil pollution and affecting soil microbiota, nanobiochar is an alternative approach to minimize the loss of nutrients and utilizing chemical fertilizer while the combination of artificial fertilizers with NBC boosts the production of crop by 10 % to 20 % and reduce the utilization of chemical fertilizers by 30 % to 50 % (Zhu et al., 2021).

In the literature, there is limited knowledge available of the impact of NBC on the growth, physiological, biochemical, and yield attributes in different crops. Hence, to understand how different applications of NBC such as soil amendments and foliar applications affect the morphological, biochemical, and physiological attributes like root length, root tissue mass density, and root diameter, as well as aboveground plant traits like leaf area, net assimilation rate, and dry matter accumulation in wheat plants. Wheat (*Triticum aestivum* L.) is a staple and cereal crop that belongs to the Poaceae family. The grain of this crop has great importance due to the presence of protein, minerals (P, Mg, Fe, Cu, and Zn), vitamins (thiamine, riboflavin, niacin, and vitamin E) and amino acids in addition to carbohydrates with unique chemical and physical properties (Khalid et al., 2023). This study has the objective to estimate the effect of nanobiochar application as soil amendments and foliar spray to what extent they alter the Physio-biochemical and nutrient uptake activities in wheat crops.

2. Materials and methods

2.1. Experimental setup

During the Rabi season 2020–2021, a pot experiment was done at The University of Lahore's research area to investigate the effects of varied applications of nanobiochar as foliar applied and soil supplements on growth, yield, and nutrient uptake in two wheat types. The General Botany Laboratory, Institute of Molecular Biology and Biotechnology, UOL Lahore, conducted the laboratory analysis. Shaanxi Dainong Huitai Biological Health Agricultural Technology Co., Ltd China provided the nano-biochar. Wheat seeds (Akbar and Zincol) were received from Pakistan's Nuclear Institute of Agriculture and Biology (NIAB). Each plastic pot was filled with loamy soil and ten seeds were sown in it. At two weeks old seedlings stage, the plants were thinned to keep five plants in one pot and preserve the recommended space between plants. This experiment used a completely randomized design (CRD).

2.2. Nanobiochar as soil additives and foliar applications

Nanobiochar (provided by Shaanxi Dainong Huitai Biological Health Agricultural Technology Co., Ltd China) after 20 days of germination. The elemental content and characterization of this nano-biochar solution have already been published (Khaliq et al., 2023). was applied to the soil at concentrations of 0, 1, 3, and 5 % (w/w soil: nanobiochar), designated as S0, S1, S3, and S5, with a foliar spray of nanobiochar at concentrations of 0, 1, 3, and 5 % (designated as F0, F1, F3, and F5; v/v water: nanobiochar). Nanobiochar sprays were applied three times at five-day intervals. As a surfactant, 0.1 % Tween-20 which is a gel like substance was mixed in the colloidal solution of Nanobiochar and was used to ensure that the spray gets into the leaves. After 40 days of spraying nanobiochar, data for several physio-biochemical characteristics were collected. Plant samples were also taken and stored at −20 °C for subsequent analysis after 40 days of the first foliar application of nanobiochar solution. The biomass and yield traits were documented after harvesting.

2.3. Growth and yield estimation

At maturity, plants were removed, the tillers were counted, and grain weight was measured. The plant height, root and shoot length, and fresh weight of plants were measured using a measuring scale (cm), and the dry biomass was assessed by drying the plants in an oven at 50 °C for 72 h.

2.4. Photosynthesis pigment identification

The chlorophyll *a*, b, total, and carotenoid levels were determined using the extraction method, and the OD was measured at 663, 645, and 480 nm using the Arnon (1949) and the findings were given as (mg/g FW).

2.5. Primary metabolite determination

After 40 days, leaves (0.5 g in 10 ml buffer) were used to determine certain primary metabolites such as total soluble protein, total soluble sugar, and total free amino acids. Before analyzing primary metabolites, leaves were extracted in 10 ml of phosphate buffer solution (0.2 M, 7 pH) and centrifuged at a rate of 7000 rpm for 10 min. Total soluble protein levels were determined using the Lowry et al. (1951) technique and a spectrometer at OD 620 nm. Total free amino acids were quantified using the Hamilton and Slyke (1943) method and measured using a spectrophotometer set to OD 570 nm. The total soluble sugar was determined using the (Riazi et al., 1985) method, and the absorbance was measured at 625 nm with a spectrophotometer.

2.6. Secondary metabolite determination

Different procedures were used to quantify secondary metabolites such as total phenolic and flavonoid content. The Folin-Ciocalteau reagent established by Singleton and Rossi (1965) was used to calculate the total phenolic content of leaves. A HALO-SB 10 UV–VIS single-beam spectrophotometer was used to determine the absorbance of the produced blue color at 725 nm.

2.7. Nitrogen metabolic enzyme activity determination

Fresh leaves of the plants were used to determine the activity nitrogen metabolic enzymes. The method proposed by Sym (1984) was used to evaluate the nitrate reductase activity while the nitrite reductase activity was measured by following the method of Ramarao et al. (1983).

2.8. Antioxidant enzyme activity determination

In 5 cc of 50 mM phosphate buffer (pH=7.8), 0.25 g of fresh leaf material was regimented. The extract was then filtered and centrifuged for 20 min at 15,000 g. The supernatant was stored at −20 °C in Eppendorf tubes and used to assess the activity of different antioxidants, including peroxidase (POD) and catalase (CAT). As a result, Chance and Maehly (1955) calculated CAT activity by measuring the rate of hydrogen peroxide conversion to water and oxygen molecules. POD activity was determined by detecting hydrogen peroxide peroxidation in the presence of guaiacol as an electron donor (Chance and Maehly, 1955).

2.9. Mineral content determination

The mineral content of dry wheat grain was evaluated using acid digestion method, which used sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2). Potassium (K) was determined using the salinity laboratory staff method, and the reading was taken with a flame photometer. While total available nitrogen (N) was assessed using the micro kjeldahl's method, Phosphorous (P) content was determined using Barton's reagents, and the final measurement of N and P was taken using a spectrophotometer.

2.10. Statistical investigation

A computer based software, Statistix 8.1 software was used to implement an Analysis of Variance (ANOVA) on all obtained data. The Least Significant Difference (LSD) test was employed to assess significant differences between treatments, and mean values were compared at 5 % probability.

3. Results

3.1. Vegetative growth

Different applications of nanobiochar such as soil amendments and foliar application significantly affect the growth attributes, biomass and yield of wheat plants (Table 1a and b). The results showed that plant height increased by 29.0 and 29.6 % in combination of S_5F_3 and S_5F_1, shoot length increased by 26 and 28 % in combination of S_5F_3 and S_3F_3 and root length increased by 40 and 39 % in combination of S_5F_5 and S_5F_1 compared to control under Akbar and Zincol varieties, respectively. NBC application also significantly influenced plant biomass as S_5F_3 in Akbar and S_5F_3 in Zincol improved the shoot fresh weight by 57 and 45 % while S_5F_3 and S_5F_3 treatments improved the shoot dry weight by 80 and 58 % compared to non-treated plants. Similar increasing trend was observed in root fresh and dry weights as these parameters increased in Akbar by 76 and 85 % in S_5F_5 and S_3F_1 treatments while 77 and 47 % increase was observed in Zincol under S_1F_5 and S_5F_1 treatments, respectively.

NBC application showed positive effects on leaf area and tillers production of wheat plant. It was counted that no of tillers increased by 80 and 65 % under S_3F_3 and S_1F_5 treatments in Akbar and Zincol, respectively. In same trend, leaf area improved by 35 and 40 % under S_5F_3 and S_5F_5 treatments compared to control plants S_0F_0 in Akbar and Zincol, respectively. Overall, Akbar variety displayed better growth attributes such as plant height, fresh and dry biomass production and tillers count as compared to Zincol, while leaf area in Zincol variety plants was higher.

3.2. Photosynthetic pigments

Different applications of NBC such as soil amendments and foliar application significantly affect the photosynthetic pigments of wheat leaves (Fig. 1). In Akbar variety, the higher values of chlorophyll *a* (83 %), chlorophyll *b* (82 %), and total chlorophyll (81 %) were recorded specifically in combination with S_1F_3, S_3F_5 and S_3F_5 respectively, as compared to control plants (S_0F_0). Moreover, the highest carotenoid content (63 %) was observed specifically in combination with S_5F_3 as compared to control plants (S_0F_0). On the other hand, in variety (Zincol) the chlorophyll *a* content increased by (67 %), chlorophyll *b* (64 %) and total chlorophyll (67 %) specifically in the combination of S_1F_5, S_3F_3, and S_1F_3 respectively, as compared to control plants (S_0F_0). However, the highest carotenoid content (61 %) was observed specifically in combination with S_1F_3 as compared to control plants (S_0F_0). The wheat varieties (Akbar and Zincol) responded differently under present conditions. The Variety (Zincol) maintained higher leaf pigments of wheat than that of Variety (Akbar).

3.3. Primary metabolites

Different applications of NBC in form of soil amendments and foliar application significantly affect the production of primary metabolites such as total soluble protein, total soluble sugar and total free amino acids of wheat leaves (Fig. 2). In (Zincol) variety, the highest total soluble protein content (45 %) was observed specifically in combination with S_5F_1 application as compared to control plants S_0F_0. However, the (Akbar) variety displayed the highest protein content (43 %) in combination with S_3F_5 as compared to control plants (S_0F_0). Moreover, in

Table 1a
The effect of soil and foliarly applied nano-biochar on growth attributes of wheat plants (Zincol and Akbar).

NBS Soil (%)	NBS Foliar (%)	Plant height (cm)		Shoot length (cm)		Root length (cm)		Leaf area (cm^2)		No of tillers (n)	
		Variety 1 (Akbar)	Variety 2 (Zincol)	Variety 1 (Akbar)	Variety 2 (Zincol)	Variety 1 (Akbar)	Variety 2 (Zincol)	Variety 1 (Akbar)	Variety 2 (Zincol)	Variety 1 (Akbar)	Variety 2 (Zincol)
S_0	F_0	79.6^{DE}	77.8^{E}	70.3^{CD}	68.3^{D}	9.33^{B}	9.5^{B}	39^{F}	38.3^{F}	0.73^{G}	1.16^{FG}
S_1	F_1	102.0^{ABC}	94.6^{CD}	87.0^{AB}	82.6^{ABC}	15.0^{A}	12.0^{AB}	67.0^{A}	61.5^{ABC}	2.66^{BCD}	2.66^{BCD}
	F_3	101.3^{ABC}	107.6^{ABC}	87.3^{AB}	93.3^{AB}	14.0^{A}	14.3^{A}	$48.8^{B\text{-}F}$	$57.3^{A\text{-}F}$	1.66^{EF}	$2.0^{C\text{-}F}$
	F_5	103.3^{ABC}	82.1^{DE}	90.3^{AB}	70.0^{CD}	13.0^{AB}	12.0^{AB}	$47.8^{C\text{-}F}$	$54.6^{A\text{-}F}$	3.66^{A}	3.33^{AB}
S_3	F_1	107.0^{ABC}	99.3^{ABC}	92.3^{AB}	86.0^{AB}	14.6^{A}	13.3^{AB}	38.2^{F}	43.9^{DEF}	2.83^{ABC}	1.83^{DEF}
	F_3	111.3^{A}	107.3^{ABC}	95.3^{A}	95.3^{A}	16.0^{A}	12.0^{AB}	$60.2^{A\text{-}D}$	$53.9^{A\text{-}F}$	2.33^{CDE}	2.83^{ABC}
	F_5	103.3^{ABC}	106.0^{ABC}	89.1^{AB}	93.0^{AB}	14.3^{A}	13.0^{AB}	39.5^{F}	65.4^{A}	1.66^{EF}	2.66^{BCD}
S_5	F_1	95.0^{BCD}	110.6^{AB}	81.0^{BCD}	95.0^{AB}	14.0^{A}	15.0^{A}	43.2^{EF}	$54.4^{A\text{-}F}$	1.33^{FG}	2.33^{CDE}
	F_3	112.3^{A}	105.0^{ABC}	96.3^{A}	92.6^{AB}	16.0^{A}	12.0^{AB}	44.3^{DEF}	64.9^{AB}	2.66^{BCD}	2.66^{BCD}
	F_5	111.6^{A}	98.6^{ABC}	96.0^{A}	86.6^{AB}	15.0^{A}	12.0^{AB}	$36.1^{A\text{-}E}$	64.06^{ABC}	2.33^{CDE}	1.33^{FG}
ANOVA	Variety	ns		ns		***		***		ns	
	Treatment	***		***		***		***		***	
	Variety* Treatment	ns		ns		ns		ns		*	

Each value is a mean of 4 replicates ± standard errors; different alphabets indicate significant difference between treatments; *, **, and *** indicated significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ respectively; ns indicated non-significant difference.

Table 1b
The effect of soil and foliarly applied nano-biochar on growth attributes of wheat plants (Zincol and Akbar).

NBS soil (%)	NBS foliar (%)	Plant fresh weight (g)		Plant dry weight (g)		Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Root dry weight (g)	
		Variety 1 (akbar)	Variety 2 (zincol)	Variety 1 (akbar)	Variety 2 (zincol)	Variety 1 (akbar)	Variety 2 (zincol)	Variety 1 (akbar)	Variety 2 (zincol)	Variety 1 (akbar)	Variety 2 (zincol)	Variety 1 (akbar)	Variety 2 (zincol)
S_0	F_0	6.83^{I}	7.73^{HI}	0.95^{H}	1.37^{GH}	6.5^{I}	7.38^{HI}	0.82^{G}	1.17^{FG}	0.33^{G}	0.35^{G}	0.12^{G}	0.19^{FG}
S_1	F_1	14.5^{BC}	$8.78^{F\text{-}I}$	2.87^{BCD}	2.96^{BCD}	13.7^{BC}	8.15^{GHI}	2.41^{BCD}	2.74^{BCD}	0.79^{EF}	0.63^{FG}	0.45^{CDE}	0.22^{EFG}
	F_3	$8.87^{F\text{-}I}$	10.7^{EFG}	$2.55^{C\text{-}F}$	$2.16^{D\text{-}G}$	$7.64\pm^{HI}$	$10.14^{E\text{-}H}$	1.95^{C}	1.92^{DEF}	$1.23^{A\text{-}D}$	0.59^{FG}	0.6^{BC}	0.24^{EFG}
	F_5	$8.82^{F\text{-}I}$	14.0^{BCD}	$2.45^{C\text{-}F}$	3.00^{BCD}	7.96^{GHI}	12.5^{CDE}	$2.11^{B\text{-}E}$	2.47^{BCD}	0.85^{EF}	1.52^{A}	$0.33^{D\text{-}G}$	0.53^{BCD}
S_3	F_1	12.4^{CDE}	8.30^{GHI}	3.71^{B}	$1.79^{E\text{-}H}$	11.02^{CDEF}	7.63^{HI}	2.98^{B}	1.42^{EFG}	1.40^{AB}	0.66^{FG}	0.73^{AB}	$0.37^{C\text{-}F}$
	F_3	$10.11^{E\text{-}H}$	14.3^{BC}	3.25^{BC}	2.98^{BCD}	9.54^{FGH}	$12.8^{B\text{-}E}$	2.85^{BC}	2.60^{BCD}	0.57^{FG}	1.45^{A}	$0.40^{C\text{-}F}$	$0.37^{C\text{-}G}$
	F_5	$9.82^{E\text{-}H}$	14.2^{BC}	$2.29^{C\text{-}G}$	$2.56^{C\text{-}F}$	$8.99^{F\text{-}I}$	13.2^{BCD}	1.93^{DEF}	$2.28^{B\text{-}E}$	0.83^{EF}	$0.93^{C\text{-}F}$	$0.36^{C\text{-}G}$	0.27^{EFG}
S_5	F_1	$11.1^{D\text{-}G}$	$11.6^{C\text{-}F}$	$2.28^{C\text{-}G}$	$2.51^{C\text{-}F}$	10.4^{EFG}	$10.6^{D\text{-}G}$	$2.04^{C\text{-}F}$	$2.12^{B\text{-}E}$	0.67^{EFG}	$1.04^{B\text{-}E}$	0.24^{EFG}	$0.38^{C\text{-}F}$
	F_3	18.5^{A}	14.47^{6BC}	5.04^{A}	3.20^{BC}	17.0^{A}	13.5^{BC}	4.17^{A}	2.85^{BC}	1.54^{A}	0.9^{DEF}	0.86^{A}	$0.35^{D\text{-}G}$
	F_5	16.73^{AB}	$8.87^{F\text{-}I}$	2.64^{CDE}	1.60^{FGH}	15.44^{AB}	8.1^{GHI}	$2.21^{B\text{-}E}$	1.39^{EFG}	1.29^{ABC}	0.68^{EFG}	$0.43^{C\text{-}F}$	0.21^{EFG}
ANOVA	Variety	ns		*		ns		ns		ns		***	
	Treatment	***		***		***		***		***		***	
	Variety* Treatment	***		***		***		**		*		***	

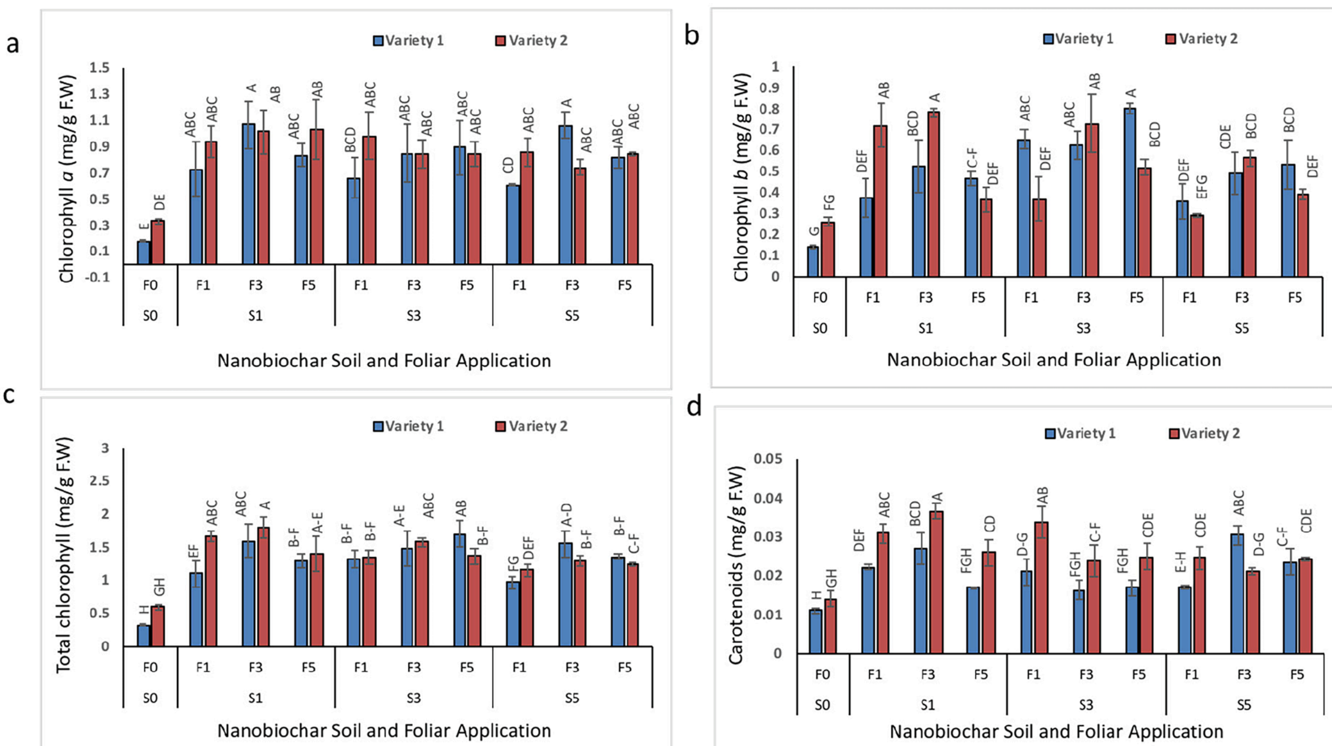

Fig. 1. Pigment analysis of wheat leaves. Determination of (a) chlorophyll *a* (b) chlorophyll *b* (c) total chlorophyll (d) carotenoids under soil and foliarly applied nano-biochar in wheat leaves. F0, F1, F3, and F5 represents 0%, 1%, 3% and 5% of foliarly applied nano-biochar solution and S0, S1, S3, and S5 represents 0%, 1%, 3% and 5% of powdered nano-biochar in soil. Variety 1 denotes Akbar and variety 2 denotes Zincol. Each value is a mean of 4 replicates; different alphabets indicate significant differences between treatments.

variety (Akbar) higher values of total soluble sugar content (57 %) were recorded, under the combined effect of S_3F_5 as compared to control plants (S_0F_0). But in variety (Zincol) the application of S_5F_3 increased the sugar content in wheat leaves specifically in combination with F_3 (37 %) as compared to control plants (S_0F_0). Furthermore, total free amino acids content was increased by (68 %) specifically in combination with S_5F_1 as compared to control plants (S_0F_0) in Akbar. However, in the case of variety (Zincol) the application of S_3 increased the amino acid content by (53 %) under the combined effect of F_3 as compared to control plants (S_0F_0). Both varieties (Akbar and Zincol) responded differently under present conditions. But overall, Akbar maintained higher primary metabolites production (Total soluble sugar, total soluble protein) than that of Variety (Zincol) but total free amino acids were higher in Zincol than Akbar variety.

3.4. Secondary metabolites

To further investigate the effects of different applications of NBC in form of soil amendments and foliar application on secondary metabolites production, total phenolic content and flavonoid contents were calculated from wheat leaves (Fig. 3). In variety (Akbar) higher values of total phenolic content (64 %) were recorded specifically in combination of S_1F_1 as compared to control plants (S_0F_0). But in variety (Zincol) higher the total phenolic content (59 %) under the combined effect of

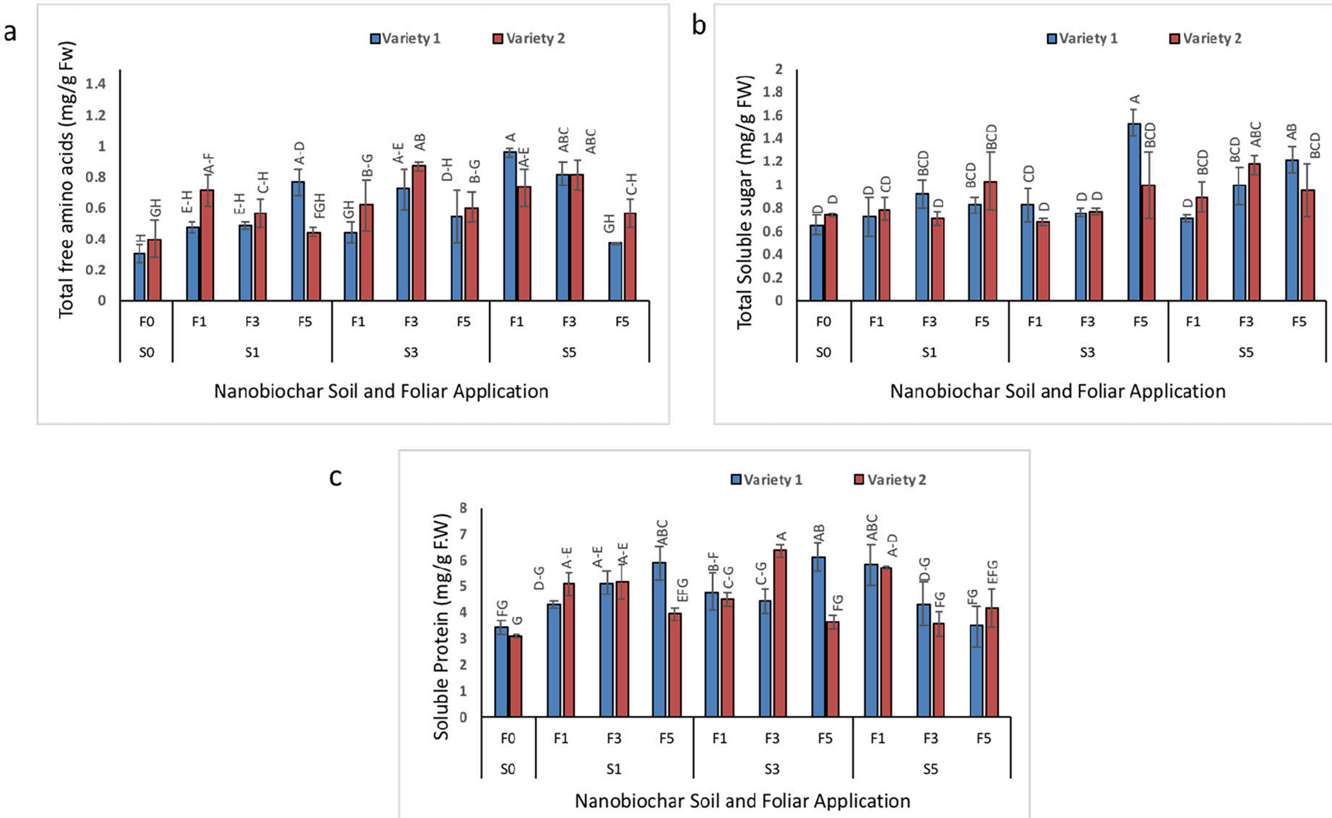

Fig. 2. The impact of different concentrations of soil and foliarly applied nano-biochar on primary metabolites (a)total free amino acids (b)total soluble sugars and (c)soluble proteins in wheat leaves. F0, F1, F3, and F5 represents 0%, 1%, 3% and 5% of foliar applied nano-biochar solution and S0, S1, S3, and S5 represents 0%, 1%, 3% and 5% of powdered nano-biochar in soil. Variety 1 denotes for Akbar and variety 2 denotes for Zincol. Each value is a mean of 4 replicates; different alphabets indicate significant differences between treatments.

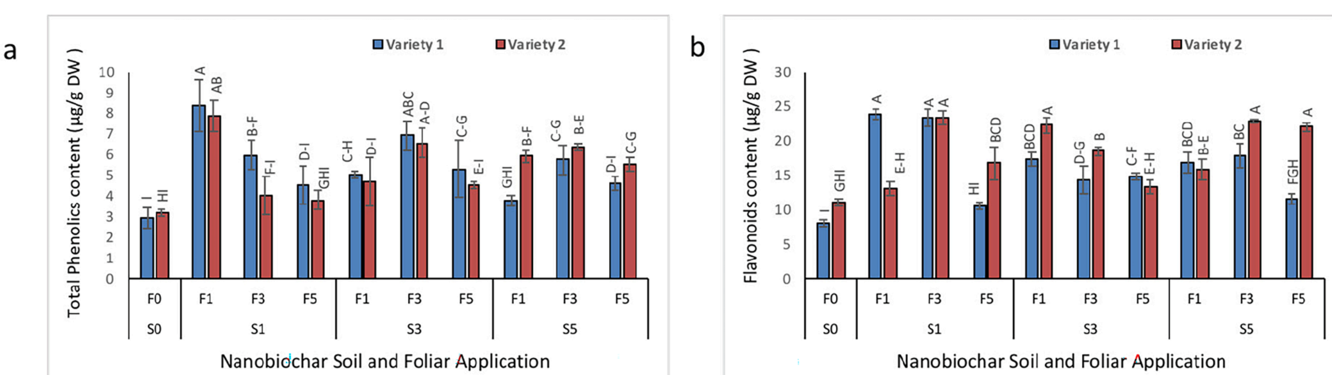

Fig. 3. The impact of different concentrations of soil and foliarly applied nano-biochar on secondary metabolites (a)total phenolics (b)flavnoids contents in wheat leaves. F0, F1, F3, and F5 represents 0%, 1%, 3% and 5% of foliar applied nano-biochar solution and S0, S1, S3, and S5 represents 0%, 1%, 3% and 5% of powdered nano-biochar in soil. Variety 1 denotes for Akbar and variety 2 denotes for Zincol. Each value is a mean of 4 replicates; different alphabets indicate significant differences between treatments.

S_1F_1 as compared to control plants (S_0F_0). On the other hand, in the case of flavonoid content, the application of S_1 increased (65 %) specifically in combination with F_3 as compared to control plants (S_0F_0) in variety (Akbar). Moreover, the higher values of flavonoids (50 %) were recorded under the combined effect of S_3F_1 as compared to control plants (S_0F_0) in variety (Zincol). Overall, Akbar variety displayed higher phenolics contents while Zincol was proved good to exhibit flavonoids contents in leaves.

3.5. *Nitrogen metabolic enzyme activity*

The application of NBC as a soil amendment in the combination of nanobiochar as foliar application significantly influenced the activation of nitrogen metabolic enzymes activities such as nitrite reductase and nitrate reductase in wheat leaves (Fig. 4). In variety (Akbar) the higher values of nitrite reductase (74 %) were recorded specifically in combination with S_5F_1 as compared to control plants (S_0F_0). However, in the case of variety (Zincol) the application of S_5 increased the nitrite reductase specifically in combination with F_5 (68 %) as compared to the

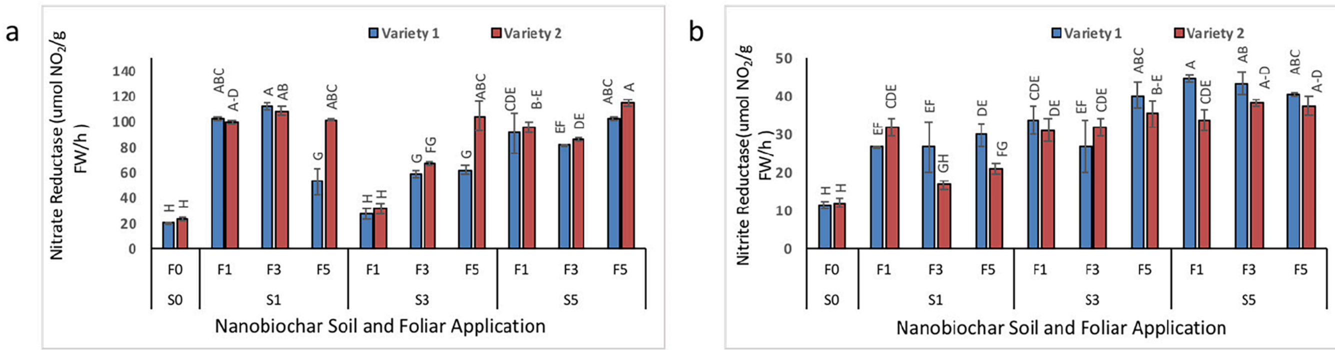

Fig. 4. The impact of soil and foliar applied nano-biochar on Nitrogen metabolic enzyme activities (a) Nitrate reductase (b) Nitrite reductase in wheat leaves. F0, F1, F3, and F5 represents 0%, 1%, 3% and 5% of foliarly applied nano-biochar solution and S0, S1, S3, and S5 represents 0%, 1%, 3% and 5% of powdered nano-biochar in soil. Variety 1 denotes for Akbar and variety 2 denotes for Zincol. Each value is a mean of 4 replicates; different alphabets indicate significant differences between treatments.

control plants (S_0F_0). On the other hand, in variety (Akbar) higher values of nitrate reductase (81 %) were noted under the combined effect of S_1F_3 as compared to control plants (S_0F_0). However, in the case of variety (Zincol) the highest nitrate reductase (79 %) was found specifically in combination with S_5F_5 as compared to control plants (S_0F_0). The wheat varieties (Akbar and Zincol) responded differently under present conditions. The variety (Akbar) maintained the highest nitrite reductase than that of the variety (Zincol). However, the variety (Zincol) maintained the highest nitrate reductase than that of the variety (Akbar).

3.6. Antioxidants enzyme activities

Further, the possible effects of NBC application in form ofsoil amendment and foliar application were investigated on antioxidant enzyme activities (Peroxidase and Catalase) in wheat leaves (Fig. 5). In variety (Akbar) higher reduction of peroxidase (47 %) was observed in the combination of S_5F_1 than in control plants (S_0F_0). However, in the case of variety (Zincol) the highest reduction of peroxidase (50 %) was noted specifically in the combination of S_5F_1 as compared to control plants (S_0F_0). On the other hand, in variety (Akbar) higher reduction of catalase (23 %) was observed in the combination of S_1F_1 than in control plants (S_0F_0). However, in the case of variety (Zincol) the highest reduction of catalase (9 %) was noted specifically in the combination of S_5F_3 as compared to control plants (S_0F_0). These results clearly depict the involvement of NBC to strengthen defense system of plant under external harsh environmental conditions.

3.7. Mineral contents

Plant health is always depending on its efficient nutrient uptake ability from soil. To explore the role of NBC treatments on minerals contents, leaf ionic analysis was performed. The nanobiochar (NBC) as a soil amendment in the combination of nanobiochar as foliar application significantly affects the mineral contents such as potassium (K), Nitrogen (N) and phosphorous (P) contents of wheat leaves (Fig. 6). In variety (Akbar) higher values of K content (61 %) were recorded specifically in combination of S_1F_5 as compared to control plants (S_0F_0). But in Zincol variety the K content was higher (50 %) under the combined effect of S_1F_3 as compared to control plants (S_0F_0). Furthermore, in variety (Akbar) the application of S_1 increased the N content specifically in combination with F_5 (66 %) as compared to control plants (S_0F_0). However, in the case of variety (Zincol) higher values of N content (41 %) were recorded under the combined treatment of S_3F_5 as compared to control plants (S_0F_0). Moreover, in variety (Akbar) the higher values of P content (53 %) were recorded specifically in combination with S_1F_5 as compared to control plants (S_0F_0). However, in the case of variety (Zincol) the application of S_3 increased the P content specifically in combination with F_5 (51 %) as compared to control plants (S_0F_0). The wheat varieties (Akbar and Zincol) responded differently under present conditions. So, The Zincol variety maintained the highest mineral contents as compared to the Akbar variety.

4. Discussion

Different applications of nanobiochar have a favorable impact on soil

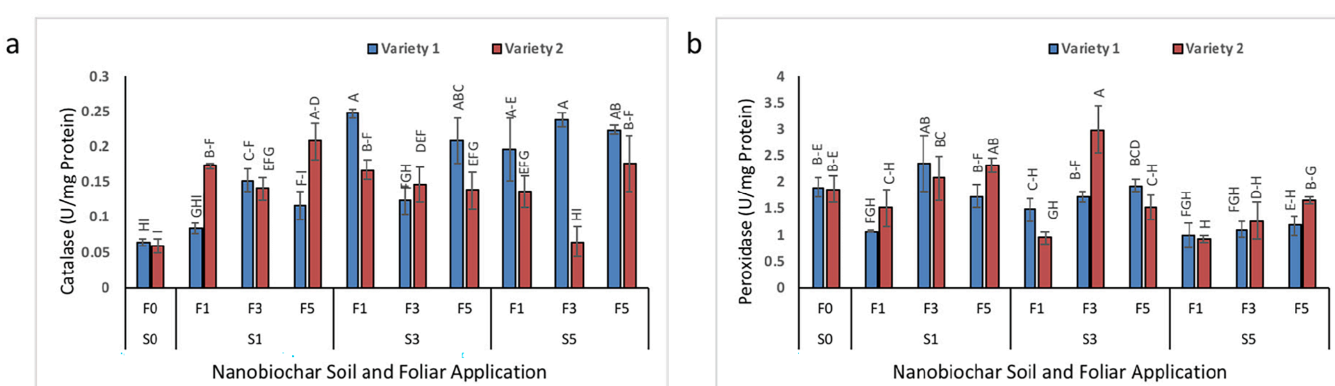

Fig. 5. The impact of soil and foliar applied nano-biochar on antioxidant enzyme activities (a)Catalase (b)Peroxidase in wheat leaves. F0, F1, F3, and F5 represents 0%, 1%, 3% and 5% of foliarly applied nano-biochar solution and S0, S1, S3, and S5 represents 0%, 1%, 3% and 5% of powdered nano-biochar in soil. Variety 1 denotes for Akbar and variety 2 denotes for Zincol. Each value is a mean of 4 replicates; different alphabets indicate significant differences between treatments.

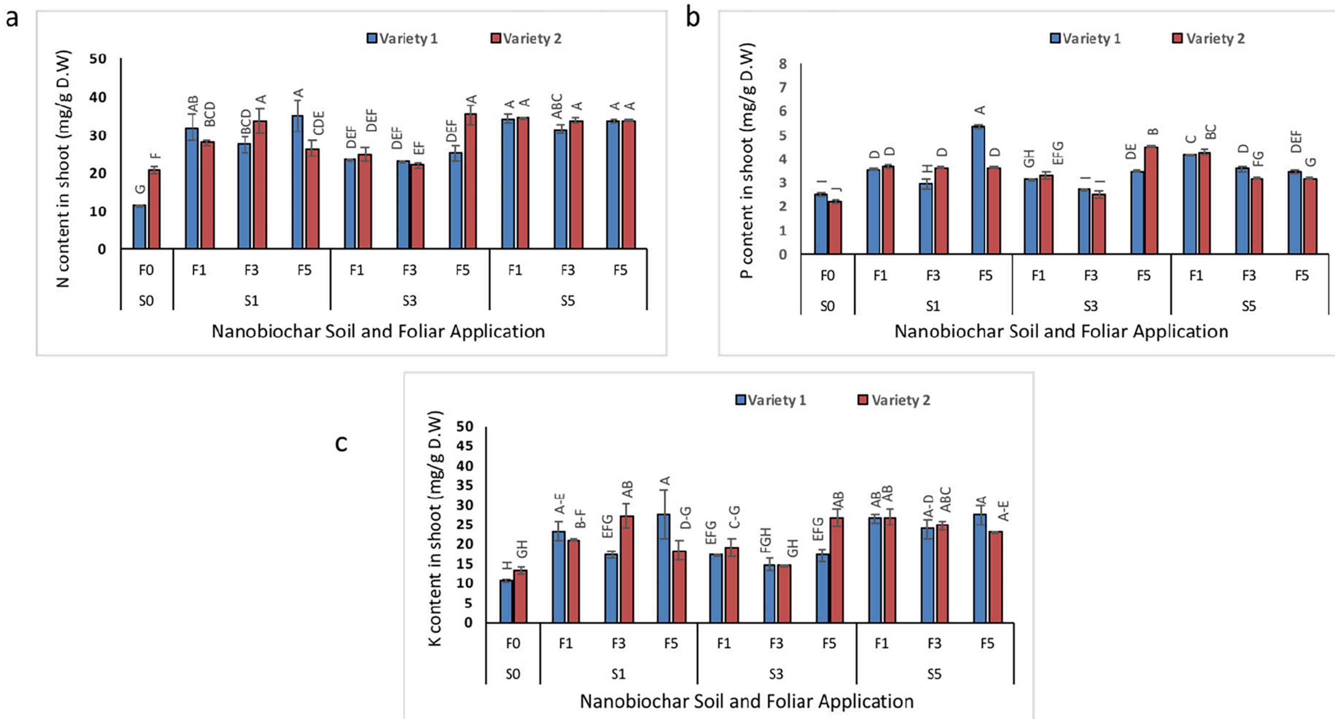

Fig. 6. The impact of soil and foliarly applied nano-biochar on mineral contents (a)Nitrogen in shoot (b)Phosphorous in shoot and (c)Potassium in wheat shoots. F0, F1, F3, and F5 represents 0%, 1%, 3% and 5% of foliar applied nano-biochar solution and S0, S1, S3, and S5 represents 0%, 1%, 3% and 5% of powdered nano-biochar in soil. Variety 1 denotes for Akbar and variety 2 denotes for Zincol. Each value is a mean of 4 replicates; different alphabets indicate significant differences between treatments.

properties since it not only increases the water holding capacity, permeability, and soil fertility, but it also has large amounts of nutrients due to its high charge density, resulting in higher crop yields. The foliar application of NBS is an alternative source of micro and macronutrient supplementation for plants (Zulfiqar and Ashraf, 2021). Nanoparticles are commonly used for the site-specific distribution of chemicals, nucleotides, and proteins in order to achieve essential goals such as building tolerance to abiotic and biotic stress and improving crop growth and output (Rastogi et al., 2019). The purpose of this study was to see how different applications of different concentration of NBC as soil amendments and foliar application affected the vegetative, physiological, biochemical, and nutritional uptake in wheat plants. The findings of this study demonstrated that the foliar application of nanobiochar, as well as soil amendments containing nanobiochar at levels of 1 %, 3 %, and 5 %, increased plant growth parameters such as plant height, root and shoot length, fresh and dry biomass of the plant, tillers count, and leaf area. However, the most significant influence of the combined effect of nanobiochar applications was increased plant height, shoot dry weight and root dry weight and shoot length at S_3F_3 level (NBC in soil 5 % + NBC as foliar 3 %), root length, shoot fresh weight and plant dry weight at S_5F_5 level (NBC in soil 5 % + NBC as foliar 5 %), root fresh weight and plant dry weight S_3F_1 level (NBC in soil 3 % + NBC as foliar 1 %), leaf area at S_3F_3 level (NBC in soil 3 % + NBC as foliar 3 %), number of tillers at S_1F_5 level (NBC in soil 1 % + NBC as foliar 5 %) as compared to control (NBC in soil 0 % + NBC as foliar 0 %) plants. It means that the combination of nanobiochasr as soil amendments and as a foliar application contained the essential nutrients required to promote growth. There are many reports which also indicated that the application of nutrients in proper combination at the proper time improves plant growth and plant productivity. Similarly, an earlier study conducted to test the effect of NBC application showed that the higher increase in yield parameters of wheat plants for weight of dry shoot, dry root, fresh shoot, fresh root, total fresh plant, fresh shoot, fresh root, total fresh plant, fresh shoot, fresh root noted in soils treated with NBC as compared to that of control (Kanwal et al. 2020). This is because biochar has the potential to improve soil physicochemical properties; consequently, an increase in growth and yield attributes is obvious. Biochar increases the ability of soil to exchange cation along with this it reduces the effect of heavy metals in the soil by increasing pH, CEC and organic carbon (Almaroai and Eissa, 2020). It is also suggested that biochar treatment to soil protected the soil moisture and improved barley growth. Earlier findings also indicated that plant growth characteristics are improved due to the resealed macronutrient and micronutrient by applied NBC and improved moisture-holding ability (Qian et al., 2019). Another report also suggested higher yields in biochar-treated soils due to a larger water-holding capacity and more plant-available water in the sandy soil because biochar increased the porosity od the soil which in turn increase the water retention and aeration, thus help in holding more water and nutrients and also make them available for the plants (Yadav et al., 2019). Kapoor et al. (2024) confirmed that biochar application along silicon increased the water retention capacity of the soil thus enhancing the growth of pepper. As the NBC also acts as a soil conditioner, so, the improvement in wheat plant growth was the result of this ability (Turner et al., 2020). Similar, findings were also reported for nanocarbons showing a positive effect on plant growth (Ramadan and Abd-Elsalam, 2020). The growth and productivity of plants also depend on several metabolic and physiological processes occurring in the cell, including, ion uptake, photosynthesis, growth promoters, nutrition metabolism, and respiration any disturbance in these processes negatively affected plant growth and productivity. But due to high surface area, porosity, and carbon sequestration, nanobiochar application has the ability to improve the soil properties that ultimately enhance the growth and yield of crops. As the biochar sequester the carbon in the soil for longer period of time which alleviate the climatic stress and increase the soil fertility. This added organic carbon from the baiochar improve the soil health and fertility (Diatta et al., 2020).

The finding of the present experiment indicated that chlorophyll *a*, *b*, total and carotenoids increased by the soil and foliar application of nanobiochar. Maximum values for chl *a*, chl *b*, total chl and carotenoids were found at S_1F_3 (NBC in soil 1 % + NBC as foliar 3 %), S_3F_5 (NBC in soil 3 % + NBC as foliar 5 %) and S_5F_3 (NBC in soil 5 % + NBC as foliar 3 %) respectively while the minimum was found at control (NBC in soil 0 % + NBC as foliar 0 %). Similar results revealed that photosynthetic pigments were increased in wheat and *Zea mays* by applying nanobiochar as soil amendments (Romdhane et al., 2019). Similarly, biochar has beneficial impacts on physiological activities, such as increasing photosynthetic rate and capacity (Yildirim et al., 2021). The current field trials revealed that adding biochar to the soil improved the amount of chlorophyll and carotenoids in tomato leaves compared to the control soil. This is because the rate of biochar greatly increased N and K uptake compared to the control (Almaroai and Eissa, 2020). This is because carotenoids, like chlorophylls, are recognized to be an accessory pigment in photosynthesis. It is also claimed that in higher plants, chlorophylls and carotenoids are bound to particular proteins to form reaction center pigment-protein complexes or photosystem PSI and PSII light-harvesting pigment–protein complexes (Xu et al., 2018). The photosynthetic machinery is made up of a number of gaseous exchange systems, photosynthetic pigments, photosystems, electron transport systems, carbon reduction routes, and enzyme systems, each of which has the potential to reduce the crop's photosynthetic activity, growth, biomass production, and nutrient co-ordination if one or more of these processes is impaired (Asante et al., 2019).

The soil and foliar application of nanobiochar improved catalase, peroxidase, phenolic, flavonoids, nitrite and nitrate reductase activity. Overall, the highest phenolic and flavonoid content in leaves was found at S_1F_1 (NBC in soil 1 % + NBC as foliar 1 %) and S_1F_3 (NBC 1 % + NBC as foliar 3 %) respectively, catalase and peroxidase were found at S1F1 (NBC in soil 1 % + NBC as foliar 1 %) and S_5F_1 (NBC in soil 5 % + NBC as foliar 1 %) respectively, nitrite and nitrate reductase were found at S_5F_1 (NBC in soil 5 % + NBC as foliar 1 %) and S_1F_3 (NBC 1 % + NBC as foliar 3 %) respectively while the lowest content in leaves was found at control (NBC in soil 0 % + NBC as foliar 0 %). Similarly, after the addition of biochar, secondary metabolites revealed dramatic changes in plants. Secondary metabolism produces phenolic chemicals, which play an important role in plant growth and development (Barracosa et al., 2020). Genotypes, production systems, water availability, and salinity are all factors that influence the quantities of phenolic compounds in tomatoes. Increased phenolic in organic farming could provide protection against pests and diseases (Dima et al., 2020). This is due to the fact that high levels of polyphenols and flavonoids detected in tomatoes grown on substrates treated with various biochars may be attributed to changes in the physical and chemical properties of the feedstock (Massa et al., 2019). According to Khadem and Raiesi (2019), variables other than nutrition play a significant impact in the production of phenolic compounds in response to biochar application.

In wheat plants, the presence of nanobiochar in soil and as a foliar increased total free amino acid, soluble sugar, and total soluble protein. Overall, the highest level of total soluble sugar was found at S_3F_5 (NBC in soil 3 % + NBC as foliar 5 %), while total free amino acid and total protein were observed in S_5F_1 (NBC in soil 5 % + NBC as foliar 1 %) which was greater than control, while the lowest levels were at control (NBC in soil 0 % + NBC as foliar 0 %). Similarly, when digestate was combined with biochar, it resulted in a large increase in amino acids in lupine seeds also discovered that the total amino acid content of spinach (*Spinacia oleracea*) and fenugreek (*Trigonella corniculata*) plants rose in the presence of biochar (Wiedner et al., 2019). The addition of rape straw biochar (RB), paddy straw biochar (PB), wheat straw biochar (WB), and corn straw biochar (MB) improved the soluble sugar concentration in stems of peach seedlings compared to the control (Sun et al., 2019). This is because amino acids are necessary for plant metabolism regarding osmotic adjustment. As the earliest process of photosynthesis and nitrogen assimilation, amino acids constitute a crucial link between carbon and nitrogen metabolism. Amino acid biosynthesis is linked to activities that are influenced by environmental conditions, plants respond to environmental stress by increasing their amino acid content (Arshad et al., 2019).

In the present study the application of both soil and foliar NBC improved the uptake of mineral contents such as NPK as compared to control in wheat plants. Overall, the maximum N, P and K contents were found at S_1F_5 (NBC in soil 1 % + NBC as foliar 5 %) as compared to control (NBC in soil 0 % + NBC as foliar 0 %). The application of biochar and nanobiochar change the productivity of crop (Dai et al., 2020). Literature indicated that the application of NBC increased soil ability to hold water by modifying the physical and chemical property of the soil enhancing in the soil's cation exchange capacity and allows nutrients, such as K, to be retained in the soil (Mansoor et al., 2021). Similar results have also been reported by Biswas et al., (2020) that the application of NPK in combination with vermicompost and biochar resulted in the highest values for all growth and yield parameters. With greater porosity, reduced bulk density, and improved nutrient uptake, moderate biochar presence in soil stimulates root elongation. This is owing to the fact that biochar improves soil fertility, water retention, and microbial activity, all of which lead to enhanced plant output (Knox et al., 2018). The use of nanobiochar in soil and foliar applications offers important nutrients to plants such as nitrogen, potassium, and phosphate, which aid in growth.

5. Conclusion

Nanobiochar is beneficial to all plant parameters due to its higher nutritional content. The addition of nanobiochar in soil (Control, S_1, S_3, S_5) and as a foliar (control, F_1, F_3, F_5) at the concentration of 0, 1, 3 and 5 % increased growth, physiological and biochemical parameters such as shoot length, root length, total soluble protein, total sugar, and free amino acid, nitrate, nitrite as well as photosynthetic pigments. In future research, NBC may be contrasted with conventional nutrient solutions and inorganic chemical fertilizers and providing an essential source of nutrients.

CRediT authorship contribution statement

Hafiz Muhammad Mehmood: Methodology, Investigation, Conceptualization. **M. Yasin Ashraf:** Methodology, Investigation, Conceptualization. **Hafiza Iqra Almas:** Investigation, Conceptualization. **Zaib-un-Nisa:** . **Naila Ali:** Writing – review & editing, Methodology, Conceptualization. **Beenish Khaliq:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Mushtaq Ahmad Ansari:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Rattandeep Singh:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Summia Gul:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103392.

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