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Foliar application of nano biochar solution elevates tomato productivity by counteracting the effect of salt stress insights into morphological physiological and biochemical indices

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Nano-biochar considers a versatile and valuable sorbent to enhance plant productivity by improving soil environment and emerged as a novel solution for environmental remediation and sustainable agriculture in modern era. In this study, roles of foliar applied nanobiochar colloidal solution (NBS) on salt stressed tomato plants were investigated. For this purpose, NBS was applied (0%, 1% 3% and 5%) on two groups of plants (control 0 mM and salt stress 60 mM). Tween-20 was used as a surfactant to prolong NBS effective stay on plant leaf surface. The results showed that 3% NBS application effectively improved the plant height, plant biomass, fruit count and fruit weight under non-stressed and stressed plants. In addition, 3% NBS application further increased the plant pigments such as chlorophyll by 72% and 53%, carotenoids by 64% and 40%, leaf relative water content by 4.1 fold and 1.07 fold under both conditions, respectively. NBS application stabilized the plasma membrane via reducing electrolyte leakage by 30% as well as reduced the lipid peroxidation rates by 46% and 29% under non-stressed and stressed plants, respectively. 3% NBS application also significantly enhanced the plants primary and secondary metabolites, as well as activities of antioxidant enzymes compared to control plants. Overall, NBS foliar application significantly improved all growth and yield indices, pigments, primary and secondary metabolites, leaf water content, antioxidant enzyme activities as well as reduced electrolyte leakage and lipid peroxidation rates in tomato to combat stress conditions. In future, studies on nano biochar interactions with soil microbiota, surface modifications, long-term environmental impacts, reduced methane gas emissions, and biocompatibility could provide insights into optimizing its use in sustainable agriculture.

Keywords Nanobiochar, Biochemical indices, Growth, Yield, Metabolites, Antioxidant enzymes

Abbreviations

MDA	Malonaldehyde
JA	Jasmonic acid
SOD	Superoxide dismutase
NBS	Nanobiochar colloidal solution
ROS	Reactive oxygen species
BC	Biochar

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NB Nanobiochar
NPs Nanoparticles
SA Salicylic acid
RWC Relative water content
EU Enzyme unit

Salinity is a significant environmental stressor and diminishes the plant's yield. It disturbs irrigated soils and reduce one-third of food production. Saline soil has an impact on ecosystems, land use, and agriculture productivity[1]. Salinity caused physiological mechanisms change which are responsible for growth and developmental regulations as it interferes with both nitrogen and carbon metabolism in plants[2]. Growth and physiological traits, including stem length, leaf area, relative water content, chlorophyll concentration, and yield output, decreased when exposed to salinity[3]. High salinity has been linked to the generation and formation of reactive oxygen species (ROS) in plant cells[4]. On the other hand, overproduction of reactive oxygen species damage membrane lipids causing membrane injury[5].

Additionally, a higher amount of Na^+ and Cl^- ions negatively impact protein synthesis, plant lipid metabolism, and enzyme functioning. Furthermore, the presence of such ions in excess amount can promote reactive oxygen species (ROS) production that impose very fatal oxidative stress on plants[6]. Salt exclusion through channel proteins and upregulation of ROS scavenging system are among the basic mechanisms for structural protection of cellular organelles [7].

In past, farmers have been utilized both organic and artificial fertilizers to improve production and maintain soil productivity[8]. The usage of chemical fertilizers harms the environment and crops by causing water pollution, greenhouse gas emissions, and N leaching[9]. A substance like charcoal called biochar is being used more frequently in agriculture with the goal of enhancing agricultural yield, reducing greenhouse gas emissions, and improving soil fertility[10]. Biochar has a high capacity for adsorbing salt, which could lower plant Na^+ absorption and mitigate the negative effects of soil salinity[11]. .

Additionally, it has been said that biochar can lower exchangeable acidity[12]. Biochar (BC) contains significant amounts of inorganic carbonates and minerals like calcium and magnesium that are good for plant growth. The properties of soil and carbon sequestration may be improved by biochar[13]. The beneficial effects of biochar may be ascribed to the fact that it increases the amount of carbon in the soil, which enhances soil quality, leads to an increase in relative water content, and increases plant height, leaf number, and leaf area per plant[14].

In recent era, nanobiochar (NB) is synthesized from ordinary biochar with different techniques. Generally, mechanical grinding is used for nanosize particle generation. Despite mechanical methods, direct NB manufacturing by flash heating resulted in graphitic nanosheets. Depending on the feedstock sources and pyrolysis temperature, the weight of the biochar nanoparticles (NPs) ranges from 1.6 to 2.6% of the total generated biochar particles[15]. In order to overcome the limitations of biochar, NB has been developed, increasing its physical, chemical, and structural properties. For instance, its greater surface area brought about by its nanosize and the abundance of functional groups with enhanced mechanical and thermal stability, as recently reported by Noreen and Abd-Elsalam, (2021)[16]. NB can be used to boost the growth of plants like wheat and rice by alleviating salt stress[17]. The best perspective has been provided by nanotechnology in agriculture for plant nutrition [18]. Previous literature has been highlighted the potential of nano-sized biochar to enhance the growth, biomass and mineral composition of raddish and carrot plants[19,20]. Further, foliar application of nano-biochar colloidal suspension ameliorated the negative effects of drought stress in tomato[21] and salinity stress in quinoa and wheat[22]. Recent reports also displayed that nano-biochar application enhanced the growth of wheat seedlings by activating the hydrolyzing and nitrogen metabolic enzymes under salt stress[23,24]. However, the effects of foliar applied nano-biochar colloidal solution in alleviation of salt stress through improved metabolites and antioxidant enzyme activities in vegetables remains unexplored yet.

The tomato is regarded as one of the more well-liked fruits or vegetables, with a wide distribution over the world and a significant economic significance[25]. Tomatoes are highly sensitive to environmental factors such as temperature, light, and changes in irrigation throughout the growth phase of the plant[26]. Tomato can be grown in areas with some soil salinization due to its moderate resistance towards soil salts[27]. However, the nutritional profiles of tomatoes which include vitamins, carotenoids, and phenolic compounds, are mostly effected by salt stress[28].

In recent years, the use of nanotechnology in agriculture, a rapidly developing industry, has made significant progress[29], due to its capacity to offer viable and timely solutions essential for sustainable agriculture[30]. The best perspective on plant nutrition has been provided by nanotechnology applied as nanoparticles (NPs). The most popular way for giving plants the vital nutrients they need is soil application. In this instance, plant roots take up the provided nutrients. However, when sprayed as foliar sprays in the proper amounts, taller plants can absorb mineral nutrients more efficiently. While in most of the cases foliar fertilization have been seen as a visible, affordable technique to add nutrients to the plants for more effective fertilization[31]. Under challenging circumstances like biotic and abiotic stresses[32]and normal conditions as well the application of nanoparticles in the agriculture sector could be considered as one of the effective methods to increase agricultural output.

Materials and methods

Experiment detail

An experiment with the pots was designed at the wire-house of The University of Lahore field area in spring 2020–2021 to assess the effects of foliar applied nano-biochar (supplied by Shaanxi Dainong Huitai Biological Health Agricultural Technology Co., Ltd China). Characterization and elemental composition of this nano-biochar solution has been published earlier[33]. All laboratory analysis was carried out in General Botany Laboratory, Institute of Molecular Biology and Bio-technology, UOL Lahore. Tomato seedlings were supplied by The Vegetables Research Institute, AARI, Faisalabad, Pakistan. Briefly, five tomato seedlings with two to three leaves were transplanted into clay pots with loam-organic manure soil (3:1) weighing around 20 g per pot. Four replicates of each treatment were used for study the each parameter. The experiment was arranged in a completely randomized design (CRD).

Application of salt and NBS solution

After one week of transplantation, once the field capacity condition was achieved, tomato plants were irrigated by salt solution adjusted to the electrical conductivity of 6 dS/m by adding NaCl (equivalent to 60 mM). A screening experiment was conducted to identify an optimal salt stress level for tomato plants. 60 mM NaCl was chosen because it produced a notable stress response.

The control plants were not irrigated with salt solution. Following two weeks of salt stress treatment, both the control and salt groups received foliar applications of Nano-biochar solution (NBS) in four different concentrations: 0%, 1%, 3%, and 5%. Three applications of nano-biochar sprays were applied with an interval of five days. In order to guarantee effective spray penetration into leaves, 0.5% tween-20 was added to the nano-biochar solution.

Sample collection

Plant samples were collected after 20 days of initial foliar application of NBS for biochemical analysis and preserved at -20ºC in refrigerator. While fresh plant samples were uprooted at maturity (65 days after planting) to investigate the plant fresh and dry biomass and some physiological attributes. Yield indices were recorded at the end of the experiment.

Pigments analysis

Pigment analysis was performed as reported by Arnon, (1949)[34]to determine the contents of chlorophyll a, b, total chlorophyll, and carotenoids in the fresh leaves of tomato in both control as well as salt treated groups by determining the absorbance at 663, 645, and 480 nm wavelengths using the UV/VIS spectrophotometer (HALO SB-10). The chlorophyll a and b contents, total chlorophyll contents, and carotenoid contents were calculated using formulas as mentioned by Yoshida et al. (1980)[35]and results were expressed as (mg/g FW).

Estimation of membrane stability indices

The relative water content (RWC) was determined by using method of Barr and Weatherley, (1962)[36]. For this purpose, fully expanded topmost leaves were collected from each treatment groups. Then quickly weigh out the leaves to avoid moisture loss. After that, leaves were dipped in deionized water for 4 h. In next, leaves were taken out from water and placed on blotting paper to remove extra surface water and again weight out to determine turgid weight. In next step, leaves were placed in oven at 55 °C for 24 h and again weigh out to find the dry weight. The relative water content was recorded using following formula:

$$RWC\ (\%) = \frac{\text{Fresh weight- dry weight}}{\text{Turgid weight - dry weight (g)}}\ \times\ 100$$

Electrolyte leakage% was estimated using method as reported by Lutts, (1996)[37]. The proportion of electrolyte leakage (EL) in response to stress damage was calculated. In this method, fully expanded fresh leaves were collected and cut in to 5–10 leaf discs (about 1–1.5 cm diameter) without midrib or major veins and placed 6 leaf discs in each test tubes. 10 ml distilled water was added in each tube. Disc samples were incubated at room temperature for 24 h in shaker. In next day, electrical conductivity of test solutions was read which designated as EC_1. Sample solutions were than autoclaved at 120°C for 20 min and EC_2 was recorded afterwards.

EC % was calculated by using following formula:

$$\text{EC}\,\% = \frac{\text{L1}}{\text{L2}}\ \times\ 100$$

To determine the lipid peroxidation rates in terms of the content of malondialdehyde (MDA) production, the method proposed by Heath and Packer, (1968)[38]was adopted. For this purpose, 0.1 g leaf tissue from each test sample was homogenized in 0.5 ml of 0.1% TCA. Homogenate was centrifuged for 10 mints and supernatant was collected. After that, 0.5 ml of supernatant was mixed with 1.5 ml of 0.5% TBA solution which was diluted in 20% TCA. This mixture was incubated in water bath for 25 mints at 95 °C. The reaction was ended up by incubating the samples on ice bath. Development of Light pink color showed the presence of MDA contents. Absorbance was measured at 532 and 600 nm on spectrophotometer. MDA concentration was calculated by using the Lambert-Beer law with an extinction coefficient of $\varepsilon M = 155\ mM^{-1}cm^{-1}$.

Determination of primary metabolites

Leaf samples were extracted in phosphate buffer solution (0.2 M) for biochemical examination after 3 weeks of NBS treatments. Total free amino acids were estimated by Hamilton and Van Slyke (1943) [39]and OD measured at 570 nm. Total soluble sugars were determined by using the method of Riazi et al. (1985)[40] and measurements were taken at 620 nm using spectrophotometer.

Determination of secondary metabolites

The method described by Pekal and Pyrzynska, (2014)[41] was used to calculate the total flavonoid contents at 465 nm OD. Further the total phenolic content of leaves was assessed using the Folin-Denis reagent as reported by Julkunen-Titto (1985)[42]and OD was measured at 765 nm.

Antioxidant enzyme activities

Catalase and peroxidase enzyme activities were tested using the Chance and Maehly (1955)[43]method. The enzyme activities of catalase (CAT) and peroxidase (POD) were measured as enzyme units (EU) per gram or ml of fresh tissue weight (EU/g FW) and per milligram of protein (EU/mg protein). Superoxide dismutase activity was measured using the Dhindsa et al. (1981)[44] approach. The superoxide dismutase activity was determined as enzyme unit (EU) on per g fresh basis and per mg protein.

Statistical analysis

The data from 4 repeats were subjected to two-way analysis of variance (ANOVA) and analyzed by computer based software STATISTIX 8.1. Significant differences among treatments were analyzed by the Least Significant Difference (LSD) test. The least significant difference was used to compare means at $p \leq 0.05$.

Results

NBS foliar application promoted growth indices under salt stress

To investigate the effects of NBS colloidal solution on tomato growth, yield and biomass, various parameters were studied as shown in Table 1. It was noted that salinity reduced the root and shoot lengths by 21.6% and 16.6%, respectively, as compared to control plants. Biomass of a plant comprises of root and shoot dry as well as fresh weights. In current study, plant fresh biomass decreased by 53.9% and plant dry biomass by 64.5% in only saline stressed plants as compared to control plants. However, the foliar application of NBS solution accelerated the growth characteristics under both stressed and non-stressed. Foliar application of 3% NBS solution increased the shoot length by 44 and 53%, and root length by 35 and 36% under non-stressed and stressed plants, respectively compared to their respective controls. While 1% NBS application increased the fresh biomass by 26 and 67% and plant dry biomass by 67 and 80.3% under non-stressed and stressed plants, respectively when compared to their respective control samples.

The foliar application of NBS solution also accelerated yield characteristics of tomato under both stressed and non-stressed conditions (Table 2).

It is noted that only salt treated plants exhibited reduction in yield attributes compared to control plants like the number of leaves reduced by 20.9%, flowers by 6.25%, fruits by 25% and fruit weight per plant by 41% as compared to plants which are not supplemented with salt. However, 1% NBS application increased the number of leaves by 19.2% and 35.3%, number of flowers by 56% and 47.7% under non-stressed and stressed plants, respectively compared to their respective control. While 3% NBS application exerted stimulatory effect on number of fruits as fruit no increased by 50% and 25%, and fruit weight per plant was increased by 57% and 1.04 fold, under non-stressed and stressed plants, respectively compared to respective control plants. Figure 1a, b showed the effects of different concentrations of NBS on plant morphology, fruit size and number under non-stressed and stressed conditions. These results suggest that NBS at 1% and 3% concentration exerted significant positive effects on plant growth, biomass and fruit count.

Condition	Foliar (%)	Shoot length (%)	Root length (%)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	0	16.6^{c}	9.6^{bc}	20.9^{c}	5.0^{cd}	3.7^{b}	1.4^{bcd}
	1	21.0^{b}	11.2^{b}	25.4^{b}	8.7^{a}	5.8^{a}	1.8^{abc}
	3	24.6^{a}	13.0^{a}	25.0a	8.9^{a}	5.6^{a}	1.8^{ab}
	5	17.3^{c}	10.8^{bc}	19.9^{c}	7.3b	3.9^{b}	1.3^{cd}
Salinity (60 mM)	0	13.0^{d}	8.0^{d}	12.42^{d}	4.0^{d}	2.4^{d}	1.1^{d}
	1	18.0^{c}	10.0^{bc}	20.47^{c}	7.7^{ab}	4.4^{b}	1.5^{abc}
	3	20.0^{b}	10.9^{bc}	19.0^{b}	7.2b	4.1^{bc}	1.5^{abcd}
	5	10.0^{e}	9.4^{cd}	16.3^{e}	5.6^{c}	3.0^{c}	1.2^{cd}
ANOVA (F Value)							
Salinity (S)		***	***	***	***	***	***
Foliar (F)		***	***	***	***	***	***
Salinity*foliar		**	ns	**	ns	ns	ns

Table 1. The effect of foliar application of NBS on growth and biomass of tomato under salt stress. Each value is a mean of three replicates; different alphabetic letters indicate significant differences ($P \leq 0.05$) among treatments, * and *** indicate significant at $p \leq 0.05$ and $p \leq 0.001$ respectively; ns indicate non-significant difference.

Condition	Foliar (%)	Number of leaves (n)	Number of flowers (n)	Number of fruits(n)	Fruit weight per plant (g)
Control	0	14.3^{bc}	9.6^{cde}	4.0^{bc}	11.2^{c}
	1	17.0^{a}	15.0^{a}	5.0^{ab}	15.1^{b}
	3	13.0^{cd}	11.3^{bc}	6.0^{a}	17.6^{a}
	5	11.6^{de}	8.0^{e}	4.0^{bc}	13.6^{b}
Salinity	0	11.3^{de}	9.0^{be}	3.0^{c}	6.6^{d}
	1	15.3^{ab}	13.3^{ab}	4.0^{bc}	10.1^{c}
	3	12.6^{cde}	10.6^{cd}	5.0^{ab}	13.5^{b}
	5	10.6^{e}	7.6^{e}	3.0^{c}	9.4^{c}
ANOVA (F_Value)					
Salinity (S)		***	***	***	***
Foliar (F)		***	**	***	***
Salinity*foliar		*	ns	ns	ns

Table 2. The effect of foliar application of NBS on yield attributes of tomato under salt stress. Each value is a mean of three replicates; different alphabetic letters indicate significant differences ($P \le 0.05$) among treatments, * and *** indicate significant at $p \le 0.05$ and $p \le 0.001$ respectively; ns indicate non-significant difference.

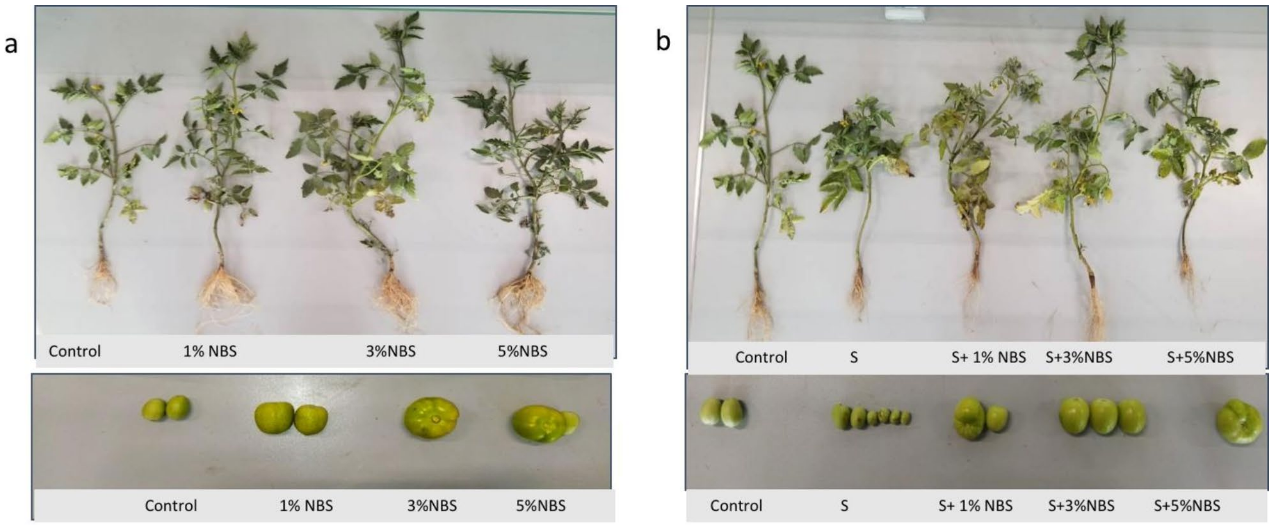

Fig. 1. Effect of different concentrations of NBS on (**a**) plant morphology, fruit size and number without stress (**b**) with stress NBS; nanobiochar solution, S; salinity (60 mM).

Foliar application of NBS improved photosynthetic pigments

In order to further investigate the association of NBS with photosynthesis, photosynthetic pigments were analyzed. Data in Fig. 2 showed the response of foliar application of NBS solution on plant photosynthetic pigments under non-stressed and stressed conditions. As salinity had a considerable negative effect on tomato leaf pigments. In salt stress, chlorophyll a was reduced by 25%, chlorophyll b by 46%, total chlorophyll by 40%, and carotenoids content by 26.73% compared to control plants (Fig. 2a, b, c, d). But exogenous application of 3% NBS enhanced the plant pigments such as chlorophyll a by 1.76 fold and 1.82 fold, chlorophyll b by 79 and 95%, total chlorophyll by 53 and72%, and carotenoids contents by 40and 64% under non-stressed and stressed plants, respectively compared to their respective control plants. These results clearly suggest that 3% NBS application significantly improved the photosynthetic machinery which ultimately improved the growth and yield attributes of tomato plants.

Foliar NBS improved the plasma membrane stability in salt stressed plants

As salinity stress is well known for membrane system destructions, ionic toxicity as well as increase in membrane permeability which leads towards high relative conductivity in plants. To evaluate the foliar applied NBS efficiency on plant plasma membrane stability under stressed conditions, various parameters such as leaf RWC%, EL% as well as MDA contents were investigated (Fig. 3a, b, c). The leaf water status of the tomato plant was significantly influenced by salt stress. Salt stress reduced the leaf RWC (%) by 54% as compared to control plants. But 1% NBS application raised the water content by 69% and 1.78 fold while 3% NBS increased the RWC(%) by 1.46 fold and

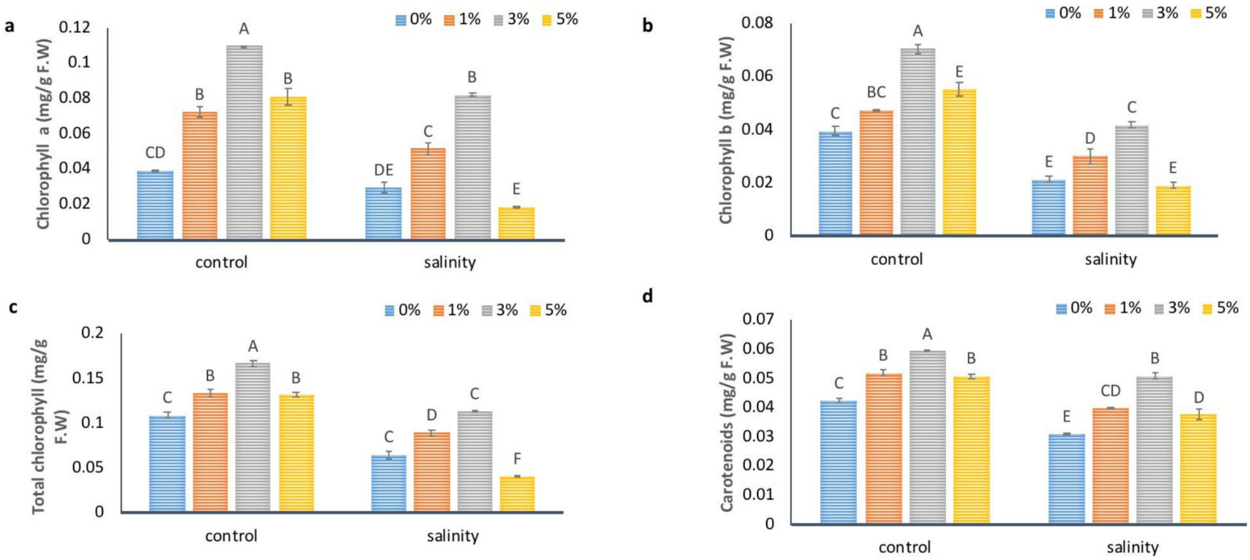

Fig. 2. The effect of different concentrations of foliar application of NBS on leaf pigments (**a**) chlorophyll a, (**b**) chlorophyll b, (**c**) total chlorophyll and (**d**) carotenoid contents in leaves of tomato under salinity. Mean values are the average of three replicates. The upper case alphabetic letters indicate significant differences ($P \le 0.05$) among treatments using Tukey's test.

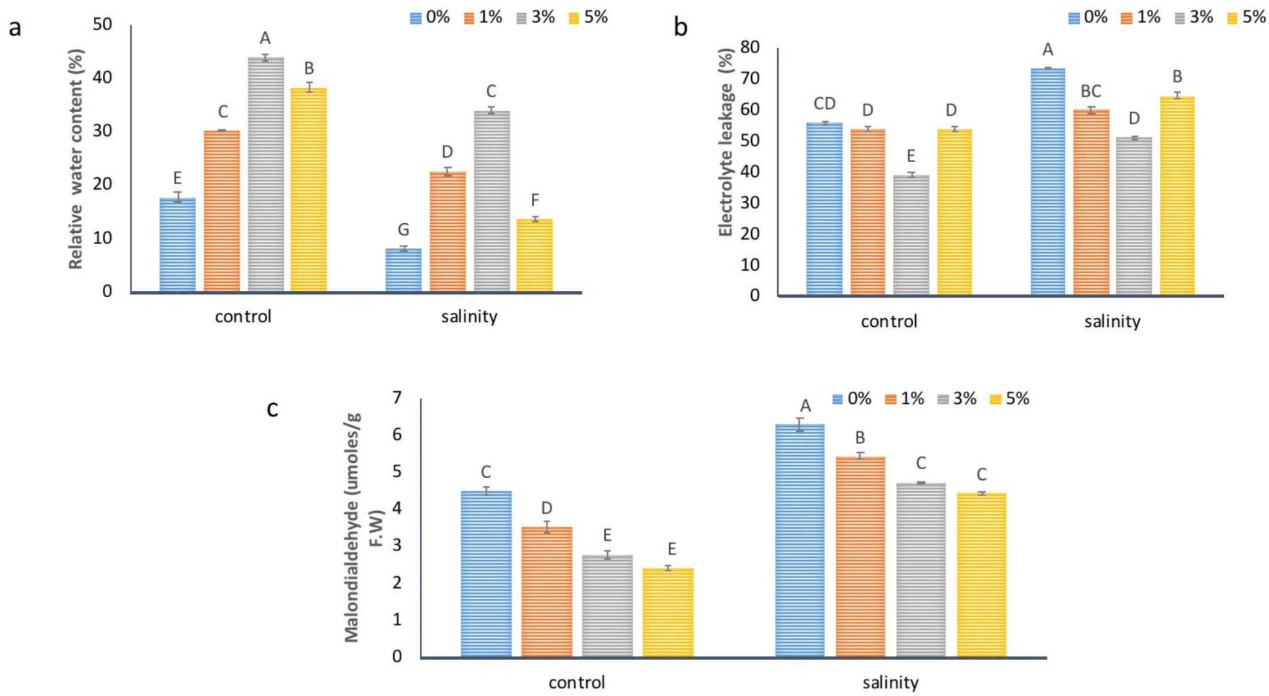

Fig. 3. The effect of different concentrations of foliar application of NBS on membrane stability parameters (**a**) relative water content (**b**) Electrolyte leakage % (**c**) Malondialdehyde contents in leaves of tomato under salinity. Mean values are the average of three replicates. The upper case alphabetic letters indicate significant differences ($P \le 0.05$) among treatments using Tukey's test.

2.55 fold under non-stressed and stressed plants, respectively when compared to their respective control groups. Further, salt stress increased the EL(%) by 32% compared to control plants. Moreover, foliar applied 3% NBS significantly reduced the EL (%) by 28% under non-stressed and stressed plants, respectively compared to their respective control samples.

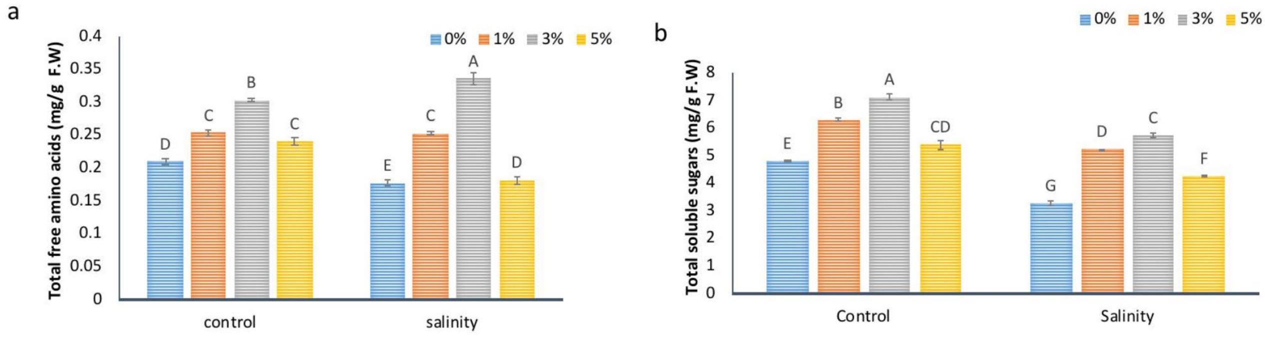

Fig. 4. The effect of different concentrations of foliar application of NBS on primary metabolites in leaves of tomato under salinity. Mean values are the average of three replicates. The upper case alphabetic letters indicate significant differences ($P \le 0.05$) among treatments using Tukey's test.

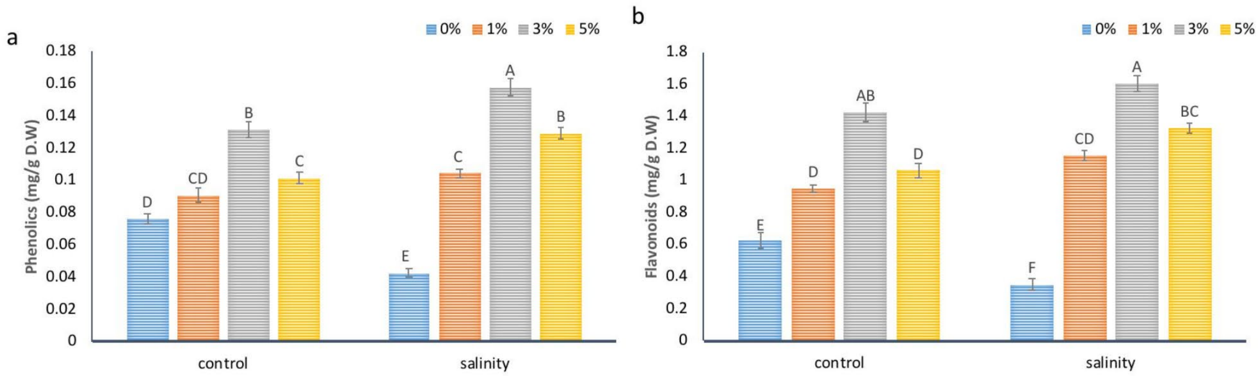

Fig. 5. The effect of different concentrations of foliar application of NBS on secondary metabolites (**a**) phenolics (**b**) flavonoids in leaves of tomato under salinity. Mean values are the average of three replicates. The upper case alphabetic letters indicate significant differences ($P \le 0.05$) among treatments using Tukey's test.

Since MDA is a byproduct of membrane lipid peroxidation and is regarded as a reliable indicator of oxidative stress, higher MDA contents indicate greater levels of oxidative stress. In this study, salinity stress increased the MDA contents by 39% as compared to the control. But foliar application of 5% NBS greatly reduced the MDA contents by 46% and 29% under non-stressed and stressed conditions. MDA contents decreased gradually with increasing concentration of NBS under both conditions. These results suggests, that NBS application stabilized the plasma membrane via lowering lipid peroxidation rate, reduced leakage of electrolytes as well as improvement in leaf water status.

NBS application altered the levels of plant metabolites

To further evaluate the influence of NBS on plant metabolism under stressed and non-stressed conditions various metabolites contents were calculated such as total free amino acids, total soluble sugars, total phenolics contents and flavonoids contents. Data in Fig. 4a, b showed the effect of NBS foliar application on plant primary metabolites such as free amino acids and soluble sugars. It is noted that the total amount of soluble sugars and amin acids slightly decreased in salt stressed plants, but significant improvement was noticed when treated with NBS. In salt stress, the contents of total soluble sugars and total free amino acids were reduced by 31% and 45%, respectively, as compared to control plants The foliar application of NBS, on the other hand, showed a rising tendency for both soluble sugars and total free amino acids. At 3% NBS, total soluble sugars increased by 48% and 75% under non-stressed and stressed plants, respectively. Further, total free amino acids contents were checked. Salt stress decreased the total free amino acids by 15% however, 3% NBS application raised levels of free amino acids by 42% and 44% under non-stressed and stressed plants, respectively as compared to their respective control groups.

NBS application also altered the contents of plant secondary metabolites as shown in Fig. 5a, b. Salt stress decreased the total phenolics and flavonoids contents by 44% and 43% as compared to control plants, respectively. But 3% NBS application improved the phenolics contents by 71% and 2.76 fold as well as flavonoids contents by 1.29 fold and 3.5 fold under non-stressed and stressed plants, respectively compared to their respective control samples. NBS application rapidly increased the levels of secondary metabolites especially under salt stress

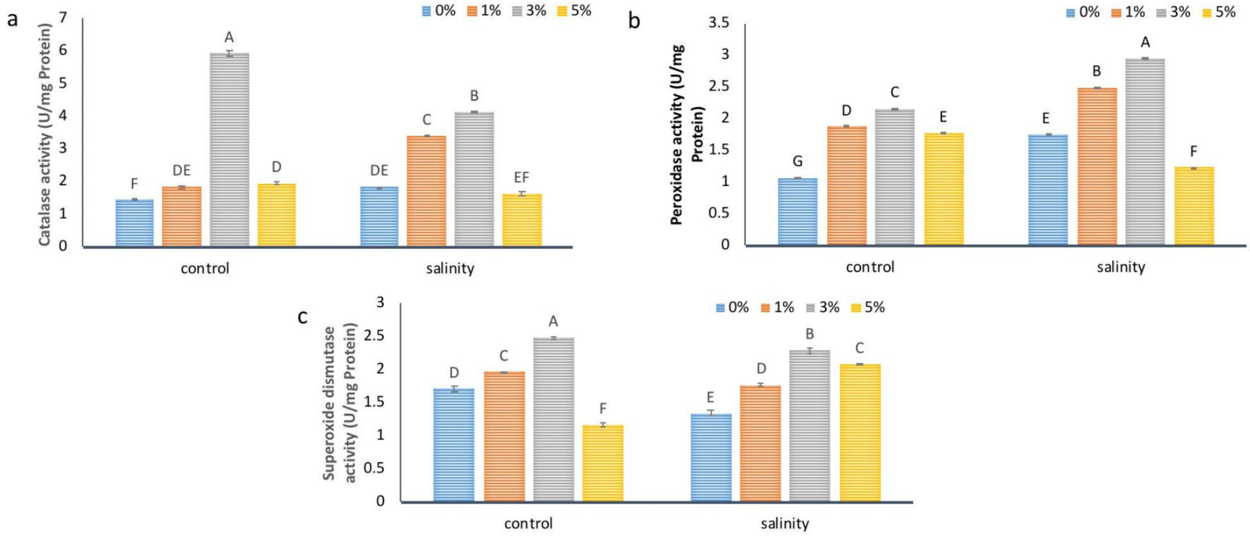

Fig. 6. The effect of different concentrations of foliar application of NBS on antioxidant enzyme activities (**a**) catalase (**b**) peroxidase (**c**) superoxide dismutase in leaves of tomato under salinity. Mean values are the average of three replicates. The upper case alphabetic letters indicate significant differences ($P \leq 0.05$) among treatments using Tukey's test.

conditions. These results strongly suggested that NBS application worked very well at all basic cellular levels of plant growth, as healthy plant metabolism and rapid production of primary and secondary metabolites lead towards stress tolerance in plants.

NBS application improved the antioxidant defense system in tomato

Different antioxidant enzyme activities were checked to further evaluate the roles of NBS application in plant defense, especially ROS scavenging capability (Fig. 6a, b,c). Data showed that salt stress increased the CAT and POD enzyme activities by 27% and 63% while SOD activities decreased by 21% as compared to control plants, respectively. Meanwhile, 3% NBS application increased the CAT activity by 3.11 fold and 1.26 fold, POD activity by 1.01 fold and 68%, and SOD activity was increased by 45% and 70% under non-stressed and stressed plants, respectively compared to their respective controls. 1% NBS and 5% NBS application also improved the antioxidant enzyme activities as compared to non-NBS treated plants under both conditions which clearly showed the positive role of NBS in activation of antioxidant defense system in plant defense against stress conditions.

Discussion

Application of NBS may benefit crop production by altering the ability of soil to hold water, fostering biotic interactions as the foliar application of NBS is another sources of macro and micronutrients supplementation to the plants. Due to its capacity to offer prospective and quick solutions essential for sustainable agriculture, the application of nanotechnology in agriculture is a subject that is rapidly evolving, has gained significant ground recently[29,30]. In general, nanoparticles are prospective materials for the site-specific delivery of nucleotides, proteins, and chemicals to accomplish important objectives including enhancing crop growth and yield as well as resistance to stressful signals[45]. Reported data suggests that adding nanoparticles to plants can considerably reduce the negative impacts of certain severe circumstances, such as salt stress, and hence control plant adaptations[32,46].

As nano-carbon is the major constituent of this NBS solution, and it adsorb nitrogen from ammonia as well as release hydrogen ions which enhance water and mineral absorption of plants. Due to this phenomenon, nutrients uptake such as N, P and K can be increased which has been proven by field experiment in which interactive application of N and nano-carbon boosted the quality and output of rice[45,47]. In terms of reducing salt stress, NBS have thus far provided encouraging results[23,24]. In this study, we summaries recent ground-breaking progresses in the development of salt stress tolerance in tomato plants using NBS.

Abiotic stresses including salinity stress showed negative effects reduced the growth and yield parameters of plant. Findings of the current study demonstrated that the growth metrics including the height of the plant, the length of the shoots and roots, the fresh and dry weights of the shoots and roots, the quantity of leaves, flowers, and fruits, and the weight of the fruits per plant were decreased under the salt stressed condition. While an increase in these parameters have been observed by the foliar application of NBS at the levels of 1%, 3%, and 5% under both stressed and non-stressed conditions. Although, 3% NBS showed maximum increase in plant height, root length, and shoot length. A decrease in the plant FW, root length, biomass, number of leaves, yield, stem length and root length of tomato plant has been reported in response to salinity[45,46]. And the amendments of soil with biochar increased a number of characteristics in tomato plant, including plant height, number of leaves, fresh and dried weights of both above- and below-ground plant sections, and leaf relative water content (LRWC)[48,49].

Our results are in accordance to the findings of above described researchers. In another study, fertigation and foliar application of three nano-fertilizers revealed higher number of leaves and flowers by the application of 1% NBS while 3% NBS exhibited an increase in fruit weight per plant and fruit number per plant in tomato plants grown in saline environment[50]. Furthermore, carbon nanotubes also altered the lipid content, flexibility, and permeability of the root plasma membranes which resulted in greater aquaporin transduction under salt-stressed conditions[48].

The current study revealed that reduction in photosynthetic pigments in tomato under salt stress were significantly improved by the foliar application of NBS, although an increase in pigments was also observed in non-stressed plants under the influence of NBS application. These results are in accordance to the findings of[51], they worked on olive and reported an increase in total chlorophyll, chlorophyll *a* and *b*, and total soluble sugars by the application of BNP. An increase in carotene in fruits of tomato plants was observed under the effect of CuNPs[52]. These findings strongly suggest that carbon based nano-biochar has ability to dense the stomata and longer roots which in turns enhance water absorption and mineral uptake in plants. It has been proven by a latest report in which multiwalled carbon nano-tubes increased the chlorophyll contents and photosynthetic activity in rice[53,54].

Previously, Nano materials application have both positive and negative effects on different plant species depends on nature, concentration and type of plants. Therefore, the effects of NBS on membrane stability indices were investigated. Results of this study showed that a decrease in electrolyte leakage was observed by the foliar treatment at 5% NBS, while, relative water content increased at 3% NBS in both the stressed and non-stressed tomato plants. These results are in accordance to the reports of Sassine[50]et al., (2020), who worked on tomato plants and experienced more cellular leakage under the effect of nanofertilizer in saline condition.

Similar outcomes were exhibited under the impact of carbon nanotubes (MWCNTs) on broccoli grown in saline regimes where the rate of photosynthesis and water uptake increased[51]. It might be due to fact that, NBS has ability to produce more pores in cell wall and plasma membrane which can enhance water transport as well as transduction of aquaporins to alleviate harmful effects of salt stress.

Application of NBS exhibited an increase in amino acids and total soluble sugars in stressed and non-stressed tomato plants, although maximum value was observed under the effect 3%NBS. The increased protein content may result from the up-regulation of N metabolism-related enzymes and better photosystems I and II photosynthetic efficiency[55]. Similarly, Shafiq et al., (2019)[56] found that pretreatment of wheat seedlings with polyhydroxy fullerene NPs decreased salt stress by elevating antioxidant activities, amino acids, chlorophyll content, soluble sugars, and K^+ and P contents. As stress increased the ROS production in plants, therefore to cope with its deleterious effects, phenolic compounds synthesis, activation of PAL enzymes (phenylalanine ammonium lyase) and flavonoids are major processes which are promoted by NB application[57]. According to the current findings, the phenolic and flavonoids were increased in stressed tomato plants than the non-stressed plants. Although, the maximum flavonoid content in leaves were observed at 1% NBS, while, the total phenolic were maximum in plants when treated with 3% NBS, foliarly. Similar to this, potassium treatment has been shown to improve the quality of figs by significantly increasing the antioxidant activity and phenolic components in fig fruit[58]. Khan (2016)[59] in addition to total phenols demonstrated that these NPs boost SOD and POX activity in tomato grown under salt stress[59,60] In compliment to these results, Guerrero et al., (2017)[61] reported that CuNPs adsorbed on CsPVA raised the overall phenol and flavonoid content in jalapeo peppers.

Antioxidant enzyme activity enhanced under stress condition[62], similar findings were observed in the current study that a considerably higher POD and CAT activity, while a slight decrease in SOD activity was recorded in stressed tomato plants as compared to the non-stressed plants. The activity of above said antioxidants increased in 3% of a NBS treated tomato plants under saline environment. These results are in agreement to the findings of Hernandez et al., (2018)[52] as they have observed an increasing expression of SOD and jasmonic acid (JA) genes led to reduced ionic and oxidative stress when CuNPs were tested on tomato plants under salt stress.

In *Helianthus annuus*, the foliar supply of FeNPs boosted the activities of polyphenol oxidase, CAT, and POD when grown under saline environment[61]. Moreover, application Fe_3O_4 and ZnONPs on the leaves of Moringa reduced the deleterious effects of salt stress by increasing the activity of certain enzymes[63]. It can be corelated at transcriptomics level as DRE/CRT is a well knows drought responsive Cis-element in DREB transcription factor family which showed upregulation under abiotic stresses and application of nano-particles[64]. Intriguingly, it was noted that NBS at low concentrations such as 1% and 3% significantly enhanced the plant secondary metabolites and antioxidant enzyme activities. It might be due to fact that C nanoparticles in low concentrations protected the tomato plants from salt stress, maintained the levels of ROS and other neutralizing factors and stimulates the antioxidant defense system[65]. Foliar application of NBS showed significant effect on malonaldehyde(MDA) content under salinity as 5% NBS reduced the level of MDA in leaves of stressed and non-stressed tomato plants. Similar results were found by the application of TiO_2 nanoparticles (rutile phase), they improved oxidative stress tolerance by modifying a number of processes, including the formation of superoxide radicals, hydrogen peroxide, MDA content, and also induced antioxidant enzymes activities i.e. superoxide dismutase, catalase, ascorbate peroxidase, and guaiacol peroxidase on the photochemical reaction of chloroplasts of *Spinacia oleracea* plants[64]. Lipid peroxidation, membrane degradation are harmful effects of ROS[66]. The production of ROS should be controlled, not only to prevent the injurious effects of ROS but also to ensure proper execution of their signaling functions[67].

A promising method for improving soil health and remediating contaminated soil is nanobiochar. It is necessary to carefully evaluate its possible effects on the environment, especially the mobility and deposition of nanoparticles in soil. Even though it can increase microbial activity and immobilize heavy metals. But, its transportation and effects on soil microbial communities needed careful management techniques and continued research. According to Chen et al. (2017)[68], biochar nanoparticles can migrate pollutants throughout the soil profile, which may harm groundwater. Because of its special qualities and uses, nano black carbon can be applied as a soil amendment, suspending agent, and nano fertilizer in a variety of environmental platforms[69]. Compared to regular biochar, nanobiochar has a stronger ability to migrate, improving soil texture, moisture, and flow rate but getting weaker with depth[70]. According to Mahmoud et al. (2024)[71], nanobiochar provides an environmentally friendly platform for crop improvement while also greatly enhancing soil health and maize production[72]. Compared to pure biochar, nanobiochar-based composites are far more successful at improving soil for heavy metal remediation[73]. However, there are a number of advantages to using nanobiochar for agricultural productivity and soil remediation. The long-term effects must be thoroughly investigated in order to create safe and efficient soil application techniques.

Conclusion

Our study findings indicated that foliar application of NBS at low concentrations such as 1% and 3% positively contributed towards the development of various growth and yield attributes in tomato by stabilizing the plasma membrane, improved water uptake and photosynthetic pigments, by production of total free amino acids, sugars, total phenolics and flavonoids both under stressed and non-stressed environments. Higher production of primary and secondary metabolites further supported the ROS scavenging systems by activating antioxidant enzymes. NBS foliar applied plants also exhibited less lipid peroxidation rates compared to non-NBS treated plants under stress conditions. Thus we believe that Despite the potential benefits of nanomaterials, their effects on plants require thorough evaluation as nano particles in higher concentrations may exert negative effects on all levels of ecosystem. Currently, several researchers are focusing on the interaction of carbon based nano materials with plant systems; however, this field is still in the nascent stage, and further research is required to understand the molecular mechanisms that influence plant growth and toxicity. Despite the potential benefits of nanomaterials, their effects on plants require thorough evaluation.

Data availability

All data is presented in this manuscript. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions
[JS], [ZN], [NA], idea formulation and design the experiment. [MYA], [CC] added valuable suggestions during experiment and manuscript improvement. [AAS], [ZN], contributed various inputs during the manuscript preparation and supervision. [MI], [NA], performed data analysis and design the manuscript. Validation, funding contributed by [SS]. [MI] involved in drafting article, revision, arranged the figures, tables and manuscript description. [MKG] validation, reviewing, funding, drafting. All the authors read and approved the finalized manuscript for publication.

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Declarations

Competing interests
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