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Influence of Carbon Nanoparticle Concentrations and Application Stages on Growth and Quality of Broccoli (*Brassica Oleracea*)

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ABSTRACT

In pursuit of enhancing agricultural productivity, the application of nanotechnology, particularly carbon Nanoparticles (CNP), has emerged as a promising approach to improve crop growth and quality. This study, conducted during 2022-23 at the Horticulture Research Farm and Biotechnology Laboratory of Abdul Wali Khan University Mardan, Pakistan, investigated the effects of CNP Concentrations and application stage on broccoli (*Brassica oleracea* var. Marathon). The experiment utilized a Randomized Complete Block Design, with foliar applications of CNPs at 0, 10, 20, 30, and 40 mg L^{-1} applied at three growth stages (4-leaf, 8-leaf, and head stage). Data were collected on growth parameters (leaf number, plant height, stem diameter, days to head initiation), yield, and quality traits (chlorophyll A and B, carotenoids, total soluble solids (TSS), ascorbic acid, and head diameter). Results revealed that CNPs significantly enhanced broccoli performance. The highest leaf number (21.89) was recorded with 40 mg L^{-1} at the 4-leaf stage, while maximum plant height (30.59 cm) and stem diameter (1.92 mm) were observed with 40 mg L^{-1} at the 8-leaf stage. The fewest days to head initiation (80.44) occurred with 30 mg L^{-1} at the 4-leaf stage. Chlorophyll A (4.84 mg/g) and B (4.43 mg/g) peaked with 30 mg L^{-1}, while carotenoids (13.51 mg/g) and ascorbic acid (0.51 mg/g) were highest at 10 and 20 mg L^{-1}, respectively. Yield (90.25 g/plant), head diameter (22.99 cm), and TSS (7.60 °Brix) were maximized with 30–40 mg L^{-1} at the 8-leaf stage. Thus, applying 30 mg L^{-1} CNPs at the 8-leaf stage is recommended to optimize broccoli growth and quality.

INTRODUCTION

Broccoli (*Brassica oleracea*), a biennial vegetable of the Brassicaceae family, is globally valued for its rich nutritional profile, distinctive flavor, and health-promoting properties (Wang *et al*., 2025). In 2024, global production of broccoli and cauliflower reached 26.5 million tonnes, with China and India contributing 65% of the total, followed by secondary producers like the United States, Spain, and Mexico, each yielding approximately one million tonnes annually (FAOSTAT, 2024). Broccoli is a powerhouse of bioactive compounds, including glucosinolates, flavonoids, hydroxycinnamic acids, ascorbic acid, minerals (potassium, magnesium, selenium), and dietary fibers, which confer anti-inflammatory, anticancer, and antioxidant benefits (Gudiño *et al*., 2024; Jaiswal, 2020). Epidemiological studies link broccoli consumption to reduced risks of lung and colorectal cancers, attributed to its high glucosinolate content and phytochemicals like vitamin C, which also support cardiovascular health (Zhao *et al*., 2007; Gliszczyńska *et al*., 2006; Higdon *et al*., 2007; Podsedek, 2007; Wargovich, 2000; Keck *et al*., 2004). Despite its global popularity, broccoli cultivation in Pakistan remains limited, primarily as a salad crop, with minimal scientific research to optimize its production (Ahmed *et al*., 2004). Mineral fertilizers (N, P, K) and organic manures enhance broccoli growth, yield, and quality by improving soil properties and nutrient availability (Nkoa *et al*., 2002; Chaterjee *et al*., 2005). However, emerging technologies like nanotechnology offer innovative solutions to further improve crop performance. Carbon Nanoparticles (CNPs), including single-walled (SWCNP) and multi-walled (MWCNP) variants, have gained attention in phyto-nanotechnology for their unique structural and functional properties (Anzar *et al*., 2020). CNPs, composed of hexagonally arranged sp2-hybridized carbon atoms with diameters of a few nanometers and lengths in the micrometer range, can regulate plant growth, enhance seed germination, and improve photosynthetic efficiency by increasing pigment content and enzymatic activity (Khodakovskaya *et al*., 2009, 2012; Rahmani *et al*., 2020). Additionally, CNPs exhibit antimicrobial properties, offering potential for pathogen control through membrane disruption and oxidative stress (Maksimova *et al*., 2019). CNPs also serve as delivery systems for agrochemicals, enhancing disease resistance and nutrient uptake (Jordan *et al*., 2020). Given the limited research on broccoli in Pakistan and the potential of CNPs to revolutionize crop production, this study aimed to evaluate the effects of CNP Concentrations and application timing on the growth, yield, and quality of broccoli (*Brassica oleracea* var. Marathon). This study investigate to assess the impact of foliar-applied CNPs on morphological (leaf number, plant height, stem diameter, head diameter), physiological (chlorophyll, carotenoids, ascorbic acid), and yield-related traits, with the goal of identifying optimal CNP Concentrations and application stages for enhancing broccoli production in Pakistan.

METHODOLOGY

Experimental site

An experiment titled "Influence of Carbon Nanoparticles Concentrations and Application Stage on Growth and Quality of Broccoli (*Brassica oleracea*)" was conducted in 2022 at the Horticulture Research Farm and Biotechnology Laboratory, Abdul Wali Khan University, Mardan, Pakistan, located at 310 meters above sea level (34.1481° N latitude, 72.0972° E longitude). The study employed a two-factor Randomized Complete Block Design (RCBD) with three replications. Factor A consisted of five carbon nanotube (CNP) Concentrations (0, 10, 20, 30, and 40 mg L^{-1}), sourced from Sigma Company via the Postgraduate Laboratory, Department of Biotechnology, AWKUM. Factor B included three application stages (4-leaf, 8-leaf, and head stage). The broccoli variety Marathon was used, with treatments applied to plants grown in 225 pots (1.4 feet diameter, 1 foot height), spaced 18 inches apart within and between three blocks. Data were collected on growth, yield, and quality parameters and analyzed statistically to evaluate the effects of CNP Concentrations and application stage.

Pots Preparation

In this experiment, pots with dimensions of 40 cm in height and 30 cm in width were filled with soil. Each pot had a single hole in the center of the base that was filled with pieces of other clay pots. Before use, the pots were carefully rinsed in tap water and dried in the sun. In the experiment, fertilizers were mixed into the soil. The last check included the elimination of any plant propagules, inert objects, visible insects, and pests.

The factors and treatments detail were as under:

The experiments were consisting of 2 factors:

Factor A: Different levels of CNP

(C_0: 0mg/L, C1: 10mg/L, C2: 20mg/L, C3: 30mg/L, C4: 40mg/L)

Factor B: Different Growth Stages (GS)

GS 1= 4 leaves, GS 2= 8 leaves, GS 3= Head Stage

Determination of Morphological and biochemical parameters of Broccoli (*Brassica oleracea*)

Plant height (cm) was measured using a measuring tape, averaging the heights of five randomly selected plants per treatment. Stem diameter (mm) was determined with a Vernier caliper, calculating the mean from five randomly chosen plants per treatment. Days to head initiation were recorded by counting the days from planting to head formation through regular field observations. Head diameter (cm), a critical factor for assessing marketable yield, was measured with a measuring tape, averaging the diameters of five randomly selected broccoli heads per treatment. Broccoli yield per plant (g) was quantified by weighing each head on a digital balance and dividing the total weight by the number of surviving plants in each treatment.

Biochemical analysis

The broccoli experiment assessed chlorophyll A, B, carotenoids, total soluble solids (TSS), and ascorbic acid (vitamin C) to evaluate the impact of carbon nanotube (CNP) treatments on nutritional and physiological quality. Chlorophyll A, B, and carotenoids were quantified using Sumanta *et al*. (2014), where 0.5 g of fresh leaves were ground, mixed with 10 mL of 80% acetone, centrifuged at 3000 rpm for 25 minutes, and supernatant optical density was measured at 480, 645, and 663 nm with a Shimadzu spectrophotometer to assess photosynthetic efficiency and antioxidant potential. TSS, indicating sweetness, was measured by placing a drop of broccoli head juice on a digital refractometer to record °Brix at room temperature. Ascorbic acid content was determined per Jagota and Dani (1982) by grinding 0.1 g of broccoli heads in 15 mL of 10% TCA, centrifuging, diluting 0.2 mL of supernatant to 2 mL, mixing with 0.2 M folin reagent, and measuring absorbance at 760 nm after 10 minutes, reflecting nutritional quality.

Scanning of electron microscope

For investigating the presence of carbon nano-particles in various broccoli plant parts i.e., leaves and head electron microscope model: Stereo Scan S360, Oxford, UK, available at national Centre of Excellence, University of Peshawar was used as per the procedure of Jones (2012).

Statistical analysis

The data collected was subjected to analysis of variance (ANOVA) for analysis. The computer software, Statistix 8.1 was used for the analysis purpose. The least significant difference (LSD) test was applied for separation of means in case of significant differences (Steel and Torrie, 1980).

RESULTS

Plant Height (cm):

Plant height, a key growth parameter, was evaluated across five carbon Nanoparticles (CNP) Concentrations (C0: 0 mg/L, C1: 10 mg/L, C2: 20 mg/L, C3: 30 mg/L, C4: 40 mg/L) and three growth stages (GS1: 4-leaf stage, GS2: 8-leaf stage, GS3: head stage). The results indicated that CNP Concentrations significantly influenced plant height, with the maximum height of 30.59 cm recorded at 40 mg/L (C4), while the minimum height of 22.06 cm was observed in the control (C0) shown in (**Fig 1).** Among the growth stages, the highest average plant height of 28.19 cm was achieved at GS2 (8-leaf stage), followed by 27.75 cm at GS3 (head stage), with the lowest height of 25.25 cm recorded at GS1 (4-leaf stage). These findings highlight the positive effect of higher CNP Concentrations and the 8-leaf stage on enhancing broccoli plant height.

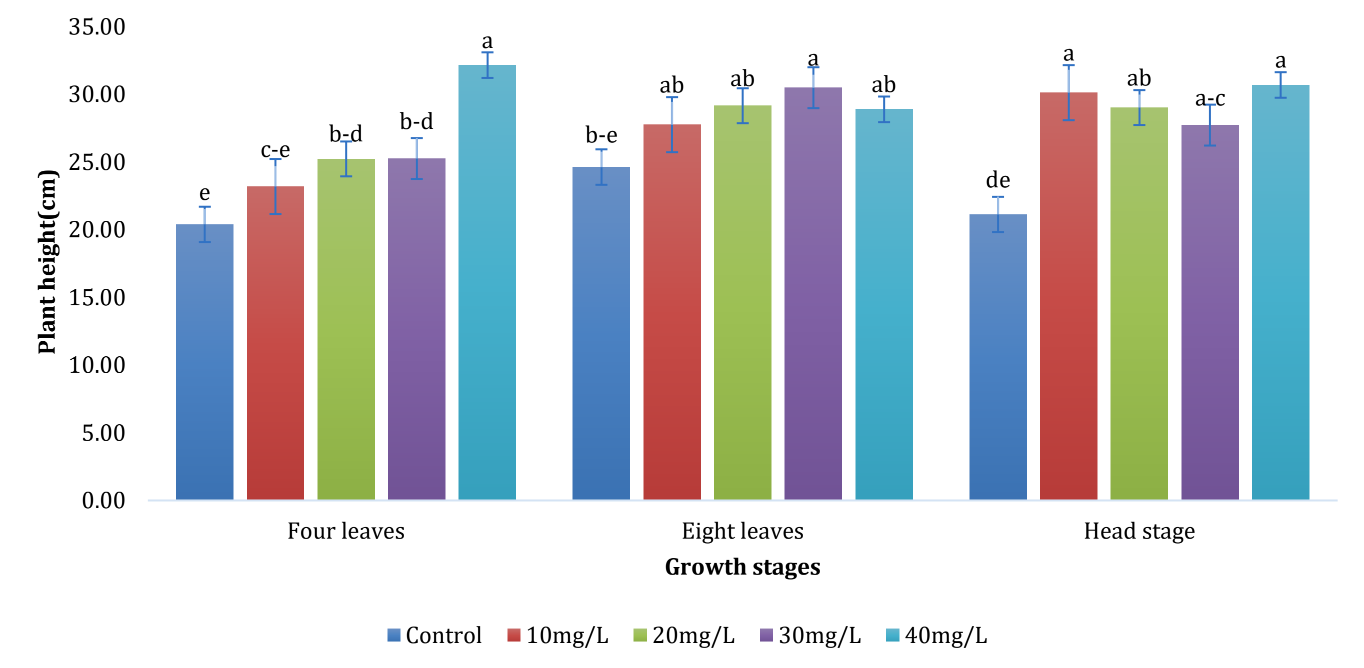

Figure 1: Plant height (cm) as affected by carbon Nanoparticles applied at different growth stages

Stem diameter (mm)

Stem diameter (mm), an important indicator of structural strength, was assessed under five carbon Nanoparticles (CNP) Concentrations (C0: 0 mg/L, C1: 10 mg/L, C2: 20 mg/L, C3: 30 mg/L, C4: 40 mg/L) and three growth stages (GS1: 4-leaf stage, GS2: 8-leaf stage, GS3: head stage). The results demonstrated that CNP Concentrations significantly enhanced stem diameter, with the maximum diameter of 1.92 mm recorded at 40 mg/L (C4), followed by 1.82 mm at 30 mg/L (C3) and 1.72 mm at 20 mg/L (C2), while the minimum diameter of 1.34 mm was observed in the control (C0). However, the effects of growth stages and their interaction with CNP Concentrations were non-significant (**Fig 2**). These findings underscore the substantial role of higher CNP Concentration in improving broccoli stem diameter, independent of the application stage.

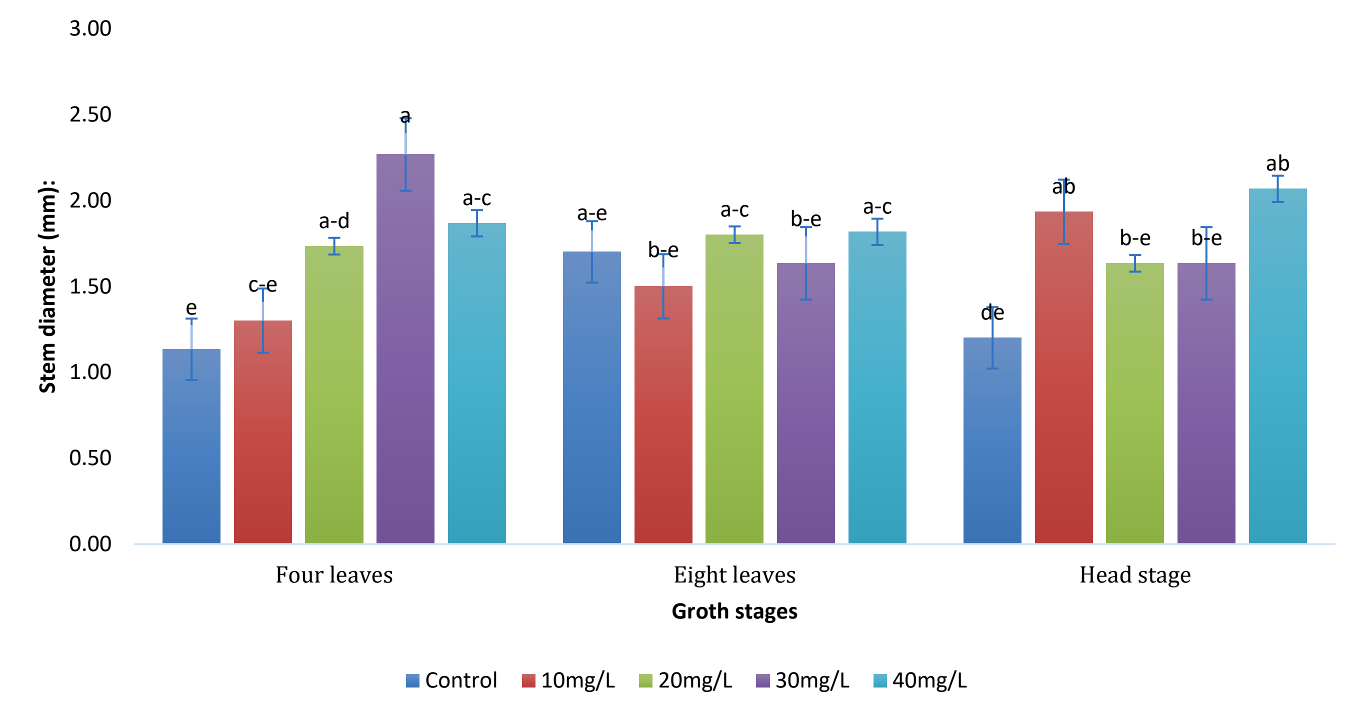

Figure 2: Stem diameter (mm)as affected by carbon Nanoparticles applied at different growth stages

Days to head initiation

The initiation time for head development, a critical factor in broccoli maturity, was examined across five Concentrations of carbon Nanoparticles (CNP) (C0: 0 mg/L, C1: 10 mg/L, C2: 20 mg/L, C3: 30 mg/L, C4: 40 mg/L) and three growth stages (GS1: 4-leaf stage, GS2: 8-leaf stage, GS3: head stage). The findings indicated a notable impact of CNP Concentrations on the reduction of days to head initiation, with the minimum duration recorded at 80.56 days for 40 mg/L (C4), followed by 80.44 days for 30 mg/L (C3) and 81.78 days for 20 mg/L (C2), in contrast to the maximum duration of 86.22 days observed in the control group (C0). The interaction between growth stages and CNP levels was not significant. Higher CNP Concentrations significantly accelerate broccoli head development, regardless of the timing of application (**Fig 3**).

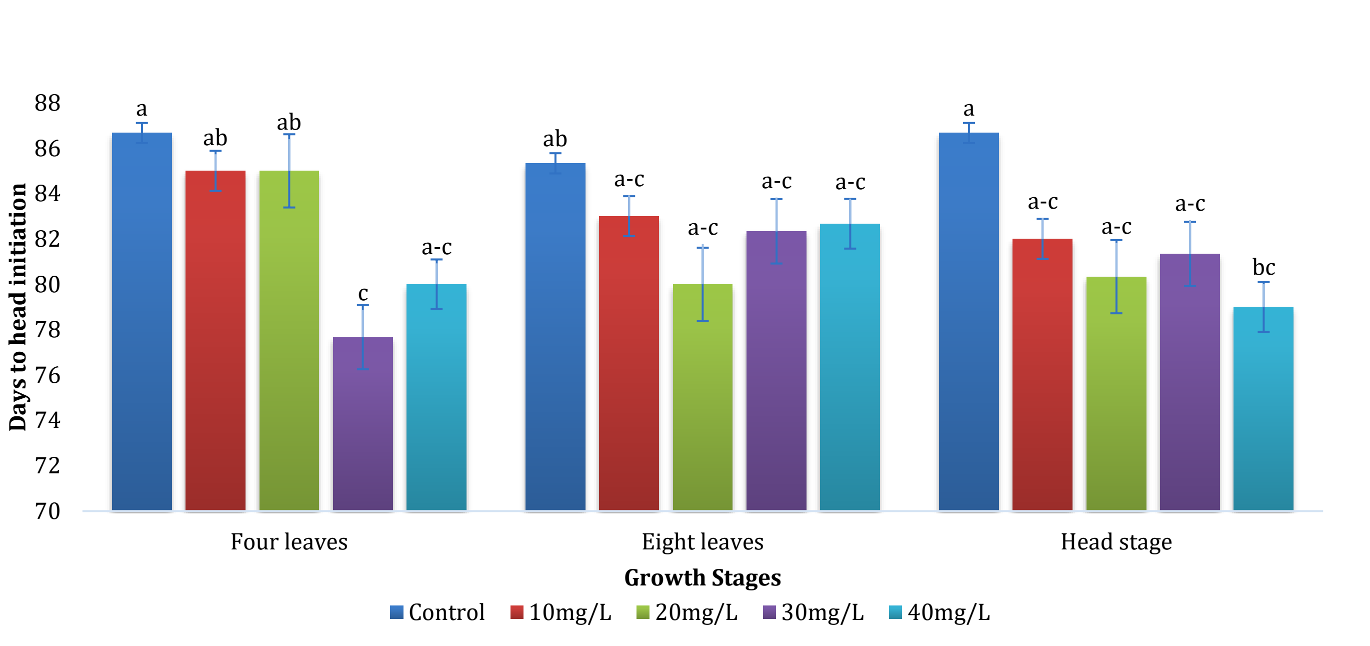

Figure 3: Days to head initiations as affected by carbon Nanoparticles applied at different growth stages

Head diameter (cm)

The study demonstrated that the application of carbon Nanoparticles (CNP) significantly increased the head diameter of broccoli, measured in centimeters (cm), while the effects of growth stages and their interaction with CNP levels were not significant (**Fig 4**). Data from the means table showed that the largest head diameter of 22.99 cm was recorded at 40 mg/L, followed by 21.6 cm at 30 mg/L, with the smallest diameter of 11.77 cm observed in the control. These findings highlight the pronounced effect of higher CNP Concentrations, including the 30 mg/L treatment, on enhancing broccoli head size, irrespective of the application stage.

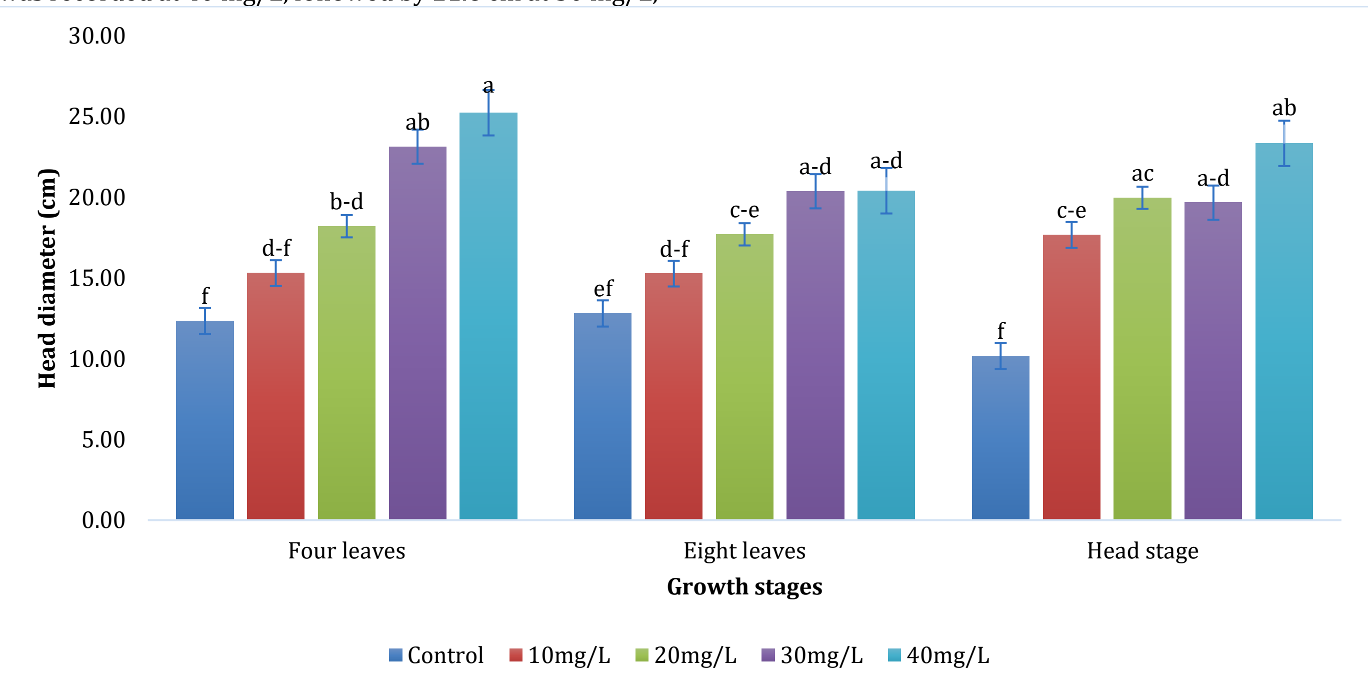

Figure 4: Head diameter (cm)as affected by carbon Nanoparticles applied at different growth stages

Broccoli yield per plant^{-1} (g)

The study demonstrated that the application of carbon Nanoparticles (CNP) significantly increased the broccoli yield per plant (measured in grams, g), while the effects of growth stages and their interaction with CNP levels were not significant. Data from the means table showed that the highest broccoli yield of 90.25 g was recorded at 30 mg/L, followed by 83.98 g at 40 mg/L, with the lowest yield of 44.15 g observed in the control (**Fig 5**). These findings highlight the pronounced effect of higher CNP Concentrations, including the 30 mg/L treatment, on enhancing broccoli yield, irrespective of the growth stage.

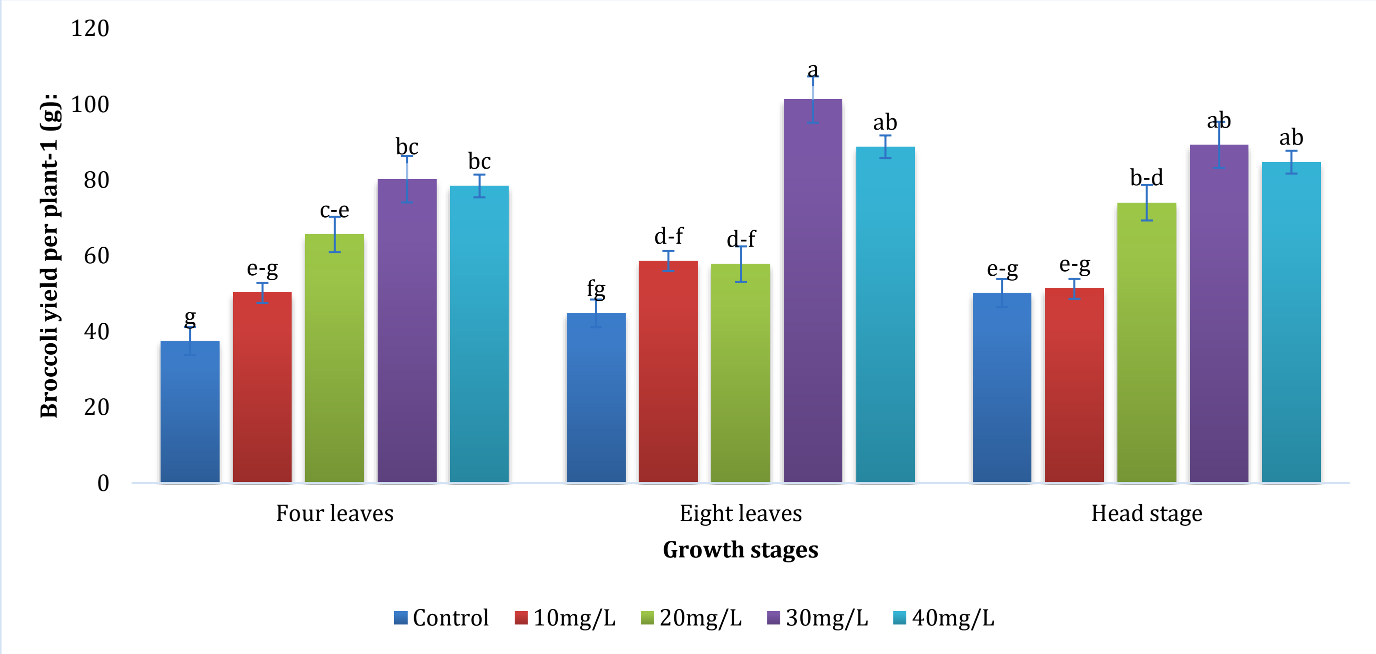

Figure 5: Broccoli yield per plant-1 (g)as affected by carbon Nanoparticles applied at different growth stages

Chlorophyll A content (mg/g)

The study revealed that the application of carbon Nanoparticles (CNP) at varying Concentrations, different growth stages, and their interactions significantly enhanced the chlorophyll "a" content in broccoli (measured in mg/g). Data from the means table indicated that among the five CNP levels, the highest chlorophyll "a" content (4.84 mg/g) was observed at 30 mg/L, while the lowest (3.61 mg/g) was recorded in the control. Regarding growth stages, the maximum chlorophyll "a" content (4.40 mg/g) was noted at growth stage 1 (four leaves stage), with the minimum (3.76 mg/g) occurring in treatments where CNP were applied at the eight leaves stage. The interactions showed the highest chlorophyll "a" content (5.83 mg/g) in plants treated with 10 mg/L CNP during growth stage 3 (head stage), while the lowest (1.07 mg/g) was observed with the same CNP Concentrations applied at growth stage 1 (four leaves stage) (**Fig 6**).

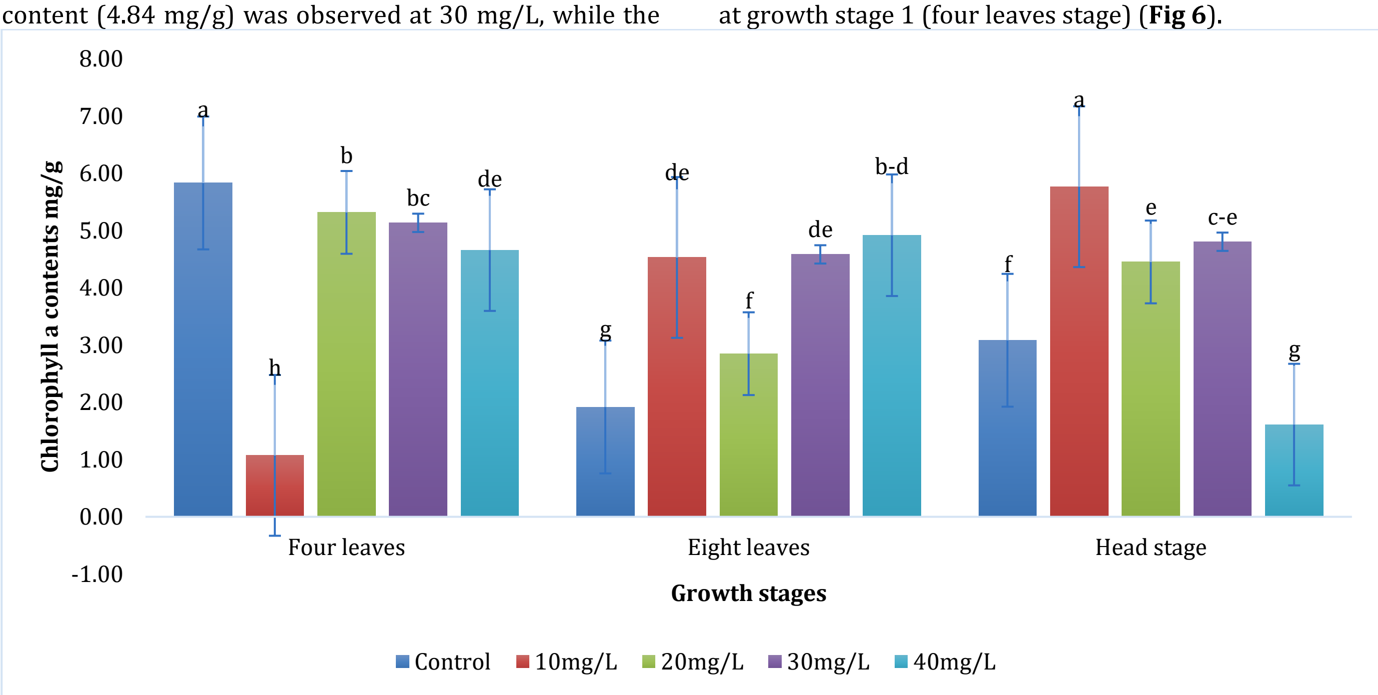

Figure 6: Chlorophyll A contents as affected by carbon Nanoparticles applied at different growth stages

Chlorophyll B content (mg/g

The study demonstrated that the application of carbon Nanoparticles (CNP) via foliar spray, across different growth stages, and their interactions significantly increased the chlorophyll "b" content in broccoli (measured in mg/g). The results showed the highest chlorophyll "b" content (4.43 mg/g) in plants treated with 30 mg/L, and the lowest (1.98 mg/g) in the control. Among the growth stages, the maximum chlorophyll "b" content (3.64 mg/g) was recorded at growth stage 2 (eight leaves stage), while the minimum (3.45 mg/g) was observed in plants treated at the eight leaves stage (**Fig 7**). The interaction between CNP and growth stages revealed the highest chlorophyll "b" content (5.61 mg/g) when CNP were applied at 20 mg/L during the four leaves stage, with the lowest (0.30 mg/g) occurring in treatments with 10 mg/L applied at growth stage 1.

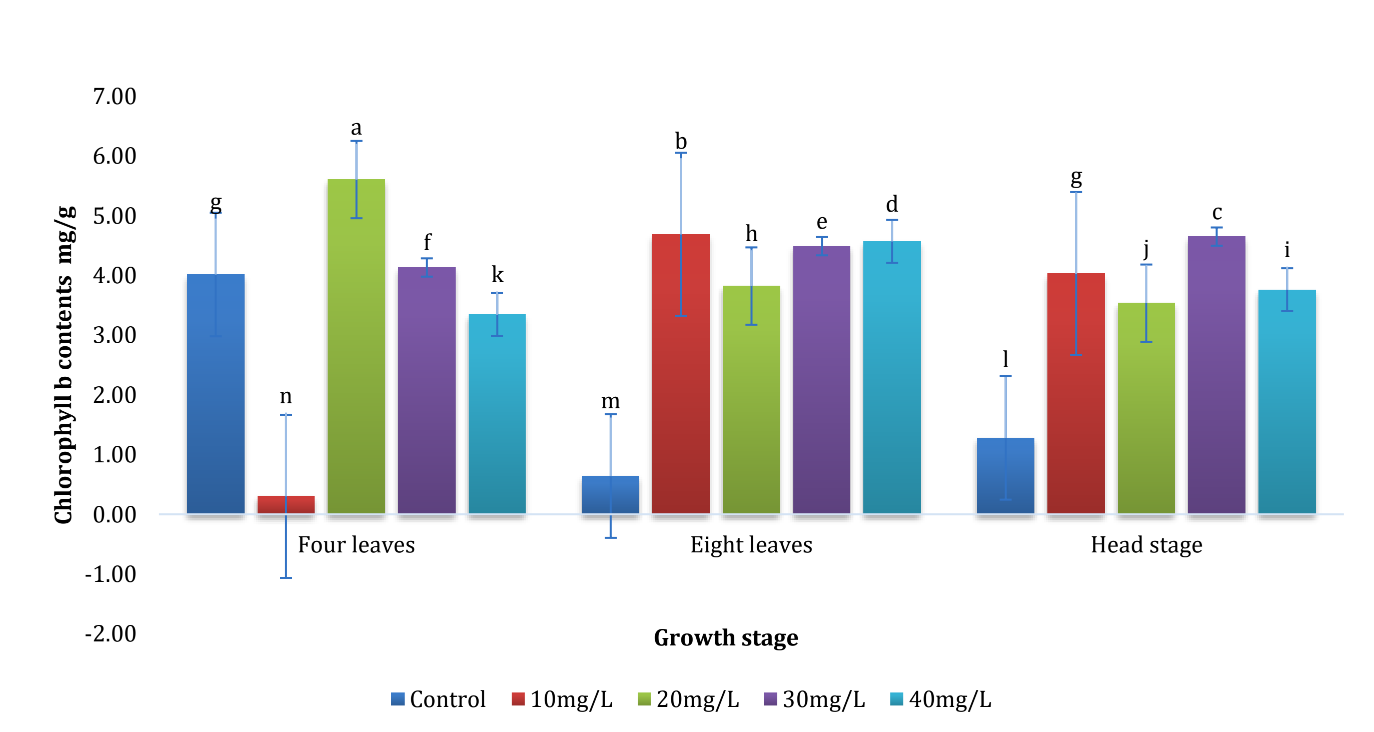

Figure 7: Chlorophyll B contents as affected by carbon Nanoparticles applied at different growth stages

Carotenoids content mg/g:

The study demonstrated that the application of carbon Nanoparticles (CNP) at varying Concentrations, different growth stages, and their interactions significantly enhanced the carotenoid content in broccoli (measured in mg/g). The results indicated the highest carotenoid content (13.51 mg/g) at 10 mg/L, and the lowest (11.84 mg/g) at 20 mg/L. Among the growth stages, the maximum carotenoid content (13.4 mg/g) was recorded at growth stage 3 (head stage), while the minimum (12.53 mg/g) was observed in plants treated at the eight leaves stage (**Fig 8**). The interactions revealed the highest carotenoid content (14.41 mg/g) in plants treated with 40 mg/L CNP at the head stage, with the lowest (10.32 mg/g) occurring when 20 mg/L CNP were applied at the eight leaves stage.

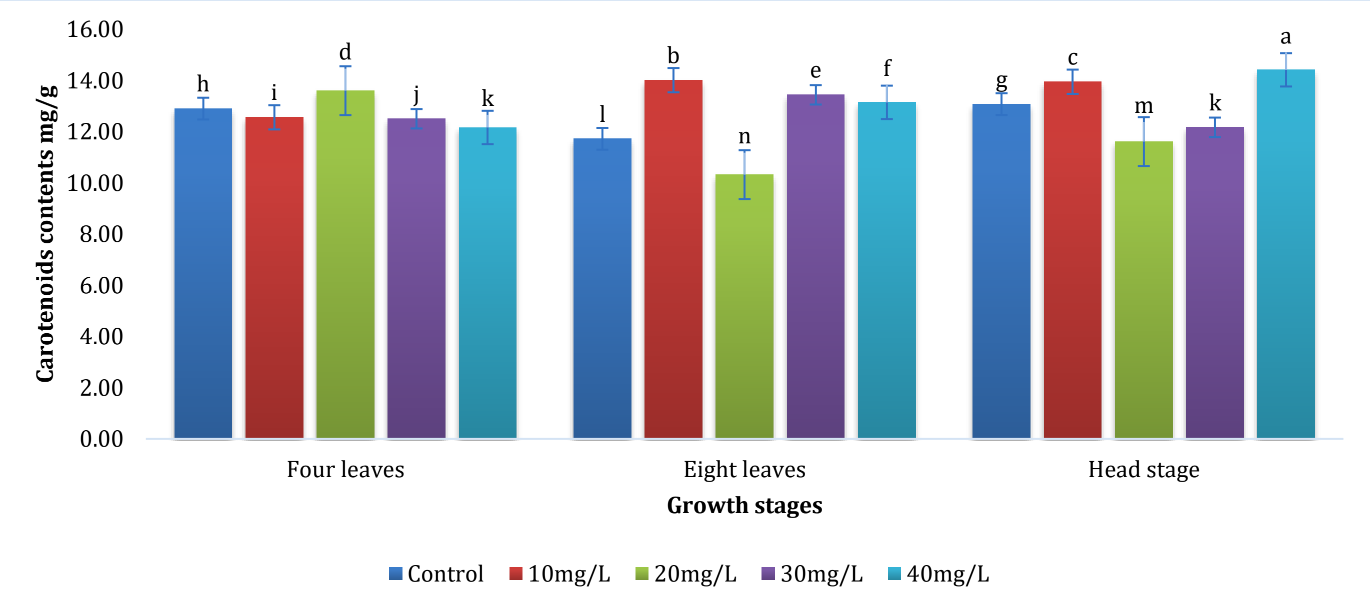

Figure 8: Carotenoids contents as affected by carbon Nanoparticles applied at different growth stages

Broccoli head total soluble solids (^{0}Brix):

The study demonstrated that the application of carbon Nanoparticles (CNP) at varying Concentrations, different growth stages, and their interactions significantly increased the total soluble solids (TSS) of broccoli (measured in °Brix) (**Fig 9**). The data showed that among the five CNP levels, the highest TSS (7.600 °Brix) was recorded at 20 mg/L, followed by 7.290 °Brix, 7.280 °Brix, and 7.220 °Brix at 10 mg/L, 30 mg/L, and 40 mg/L respectively, which were not significantly different from each other, while the lowest TSS (6.060 °Brix) was observed in the control. Among the growth stages, the maximum TSS (7.600 °Brix) was noted at growth stage 2 (eight leaves stage), with the minimum (6.500 °Brix) occurring in plants treated at the four leaves stage. The interactions revealed the highest TSS (8.500 °Brix) in plants treated with 40 mg/L CNP at growth stage 2 (eight leaves stage), while the lowest TSS (5.670 °Brix) was recorded in the control at growth stage 1 (four leaves stage).

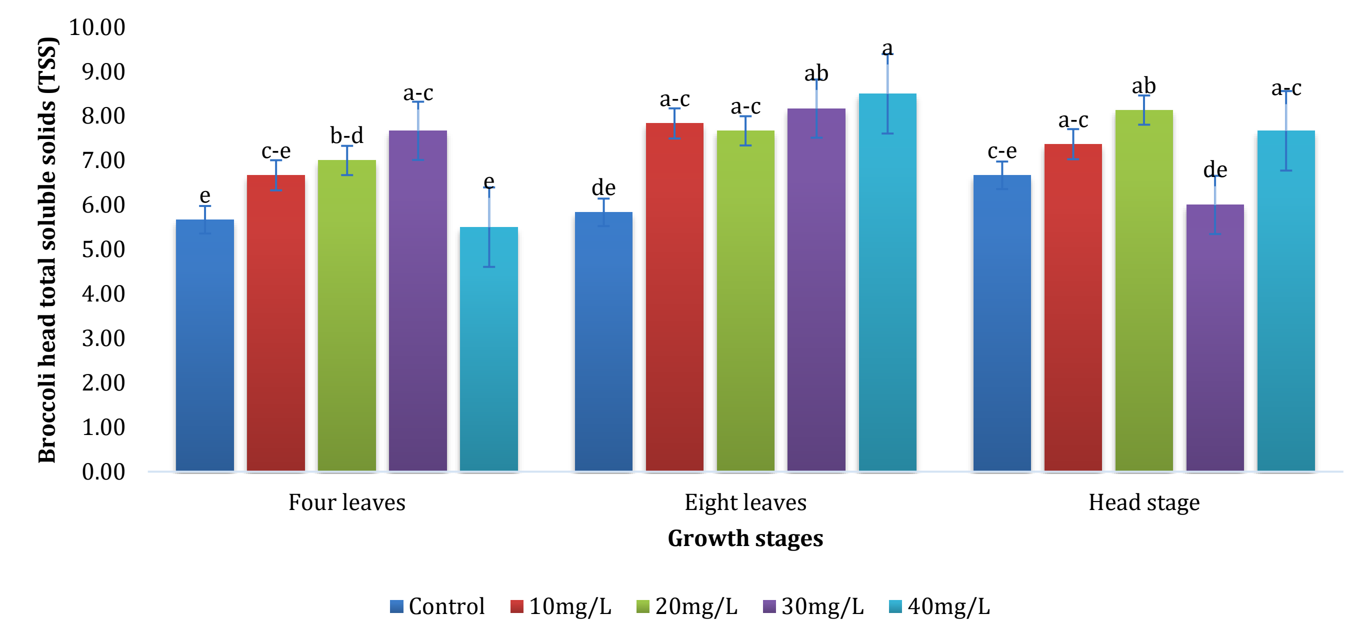

Figure 9: Broccoli head total soluble solids (^{0}Brix) as affected by carbon Nanoparticles applied at different growth stages

Ascorbic acid (Vitamin C):

The study indicated that growth stages and their interactions significantly influenced the vitamin C content in broccoli (measured in mg/g), while carbon nanotube (CNP) Concentrations showed no significant effect (**Fig 10**). The data revealed the highest vitamin C content (0.51 mg/g) at growth stage 1 (four leaves stage), followed by 0.39 mg/g at growth stage 2 (eight leaves stage), and the lowest (0.18 mg/g) at the head stage. In terms of interactions, the highest vitamin C content (1.22 mg/g) was observed with 20 mg/L CNP applied at growth stage 1 (four leaves stage), while the lowest (0.05 mg/g) was recorded in the control at growth stage 3 (head stage).

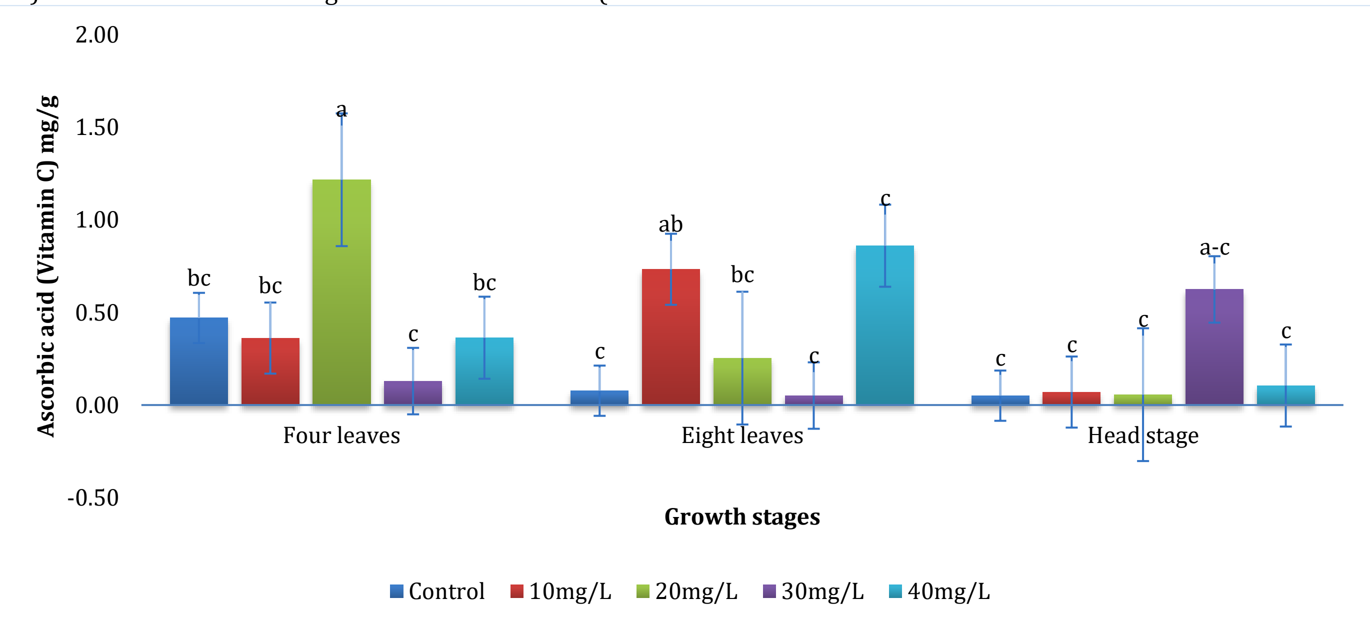

Figure 10: Ascorbic acid (vitamin C) as affected by carbon Nanoparticles applied at different growth stages

Scanning electron Microscope (SEM):

For detection of CNP in the vegetative parts i.e. head and leaves SEM was performed at national Centre of Excellence, University of Peshawar. CNP can be seen attached to the leaves and head of treated plants, which appear in white color in picture however, the control plants depicted no presence of such materials. Plants which were treated with 40mgL^{-1} showed the presence of more particles (**Fig 11**). The Concentrations of CNP in pictures is higher in higher doses. It was also noted that no CNP were detected in plants which were treated at early stage i.e. 4 leaves stage. The pictures showed more CNP attached to the plant parts when applied at head stage.

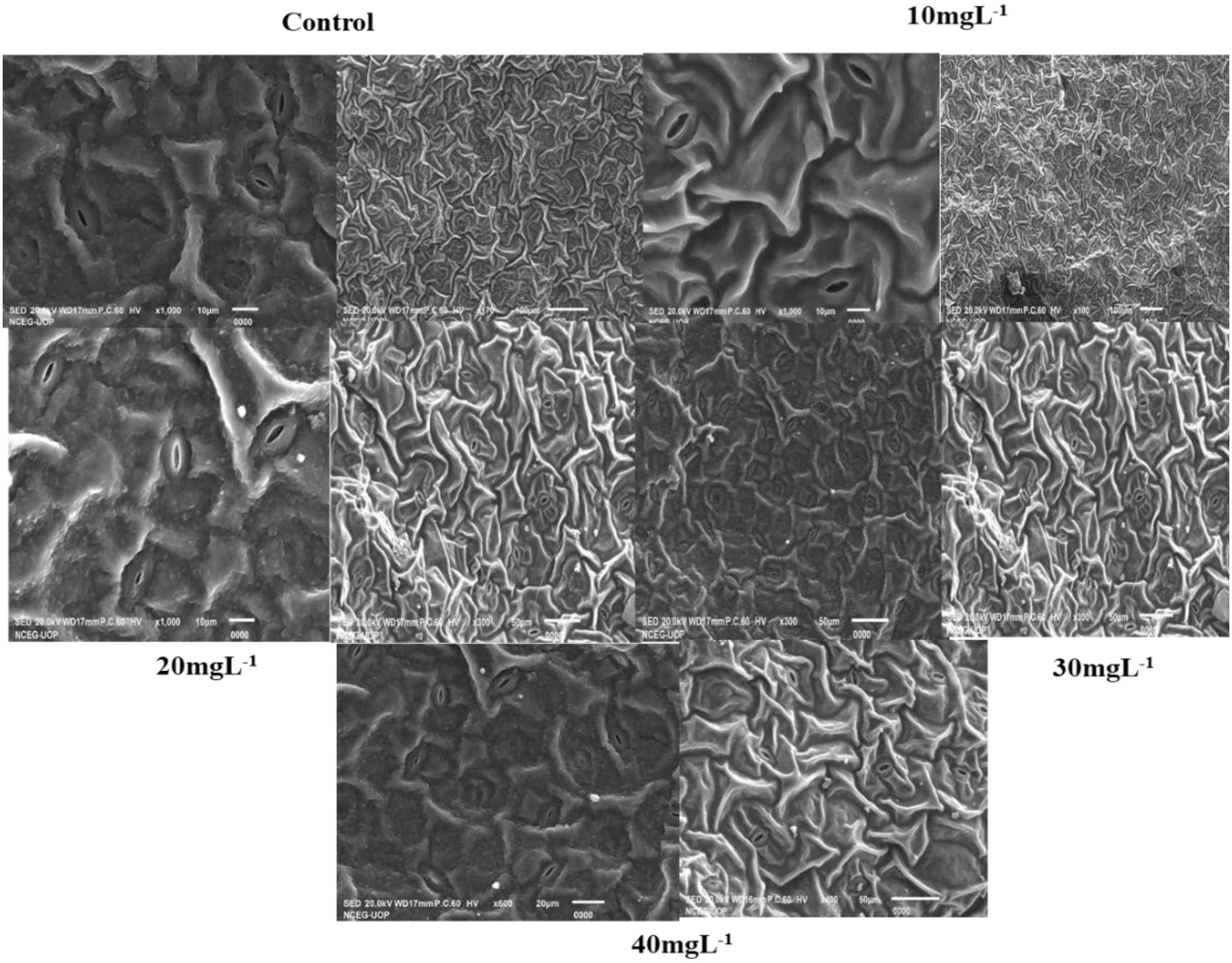

Figure 11: SEM results for different Concentrations of CNP in broccoli plant. The white spots in the pictures are showing the presence of CNP.

DISCUSSION

The application of carbon Nanoparticles (CNP) has been shown to enhance vegetative growth and increase the rate of photosynthesis in plants (Wu *et al*., 2022), which likely contributed to the observed increase in plant height in this study. These findings align with Mohamed *et al*. (2021), who reported a similar increase in plant height in broccoli treated with CNP Concentrations of 3, 5, and 7 g/L, with height increasing with higher Concentrations. The enhancement of chlorophyll content in leaves, coupled with increased plant height and number of leaves per plant, also led to a significant increase in stem diameter. This is consistent with the results of Amin *et al*., (2025) and Lopez *et al*. (2020), who observed a similar increase in stem diameter in plants treated with CNP at Concentrations of 10, 100, 250, and 500 mg/L compared to untreated controls. A reduction in the number of days to head initiation was noted with increasing CNP Concentrations, although no significant differences were observed among treatments with 20 mg/L, 30 mg/L, and 40 mg/L CNP. The accelerated growth of broccoli plants treated with CNP may have facilitated earlier head initiation. This observation is supported by Hermes *et al*. (2020), who reported similar improvements in vegetative traits across various plant species treated with different CNP Concentrations. The application of CNP also boosted chlorophyll content, photosynthetic rate (Wu *et al*., 2022), and overall plant growth (Hermes *et al*., 2020), resulting in the largest head diameters in treatments with higher CNP levels. A comparable increase in head diameter was reported by Mohamed *et al*. (2021) when broccoli was treated with CNP Concentrations of 3 g/L, 5 g/L, and 7 g/L. Plants treated with CNP exhibited improvements in nearly all physical and chemical parameters, including head diameter, number of leaves, chlorophyll content, and stem diameter. This enhancement in yield components likely contributed to the increased yield per plant. These results corroborate the findings of Mohamed *et al*. (2021), who noted the maximum head weight at a CNP Concentrations of 5 g/L when treating plants with 3 g/L, 5 g/L, and 7 g/L. The chlorophyll "a" content initially increased with CNP application up to 30 mg/L and then declined, a trend consistent with Vicas *et al*. (2019), who reported a similar increase in chlorophyll content in broccoli treated with nano-selenium. A parallel increase in chlorophyll "b" was also noted by Vicas *et al*. (2019) and Amin *et al*., (2025) under similar nano-selenium treatments. Carotenoids, a vital plant pigment and precursor to vitamin A, are synthesized in plastids as a byproduct of photosynthesis. The increased photosynthetic rate following CNP application (Wu *et al*., 2022) likely enhanced carotenoid synthesis. This is supported by Ghasempour *et al*. (2019), who also observed elevated carotenoid levels with CNP application. Total soluble solids (TSS) typically increase with maturity. The reduced number of days to head initiation and ultimate maturity due to CNP application may have contributed to higher TSS in treated plants. These findings are in line with Abd Al-Shammari *et al*. (2023), who reported increased TSS in broccoli treated with copper Nanoparticles. The application of CNP at different growth stages enhanced carotenoid content, a precursor to vitamin C, potentially leading to increased ascorbic acid levels. This is consistent with Abd Al-Shammari *et al*. (2023), who documented a rise in vitamin C content with copper nanoparticle application. For the detection of CNP in vegetative parts (head and leaves), scanning electron microscopy (SEM) was conducted at the National Centre of Excellence, University of Peshawar. SEM images revealed CNP attached to the leaves and heads of treated plants, appearing as white particles, whereas no such materials were detected in control plants. Plants treated with 40 mg/L exhibited a higher density of particles, with CNP Concentrations increasing with dosage. Notably, no CNP were detected in plants treated at the early four leaves stage, while greater CNP attachment was observed in plant parts treated at the head stage.

CONCLUSION

The study demonstrated that carbon nanoparticles (CNP) and their application stage significantly influenced broccoli growth and quality parameters. The highest chlorophyll a (4.84 mg/g), chlorophyll b (4.43 mg/g), and yield per plant (90.23 g) were observed with 30 mgL^{-1} CNP application. Maximum head diameter (22.99 cm), stem diameter (1.92 mm), and number of leaves (21.89) were recorded at 40 mgL^{-1}, followed closely by 30 mgL^{-1}. Total soluble solids (TSS) peaked at 7.60 °Brix with 20 mgL^{-1}, followed by 7.28 °Brix at 30 mgL^{-1}, while carotenoids were highest (13.51 mg/g) at 10 mgL^{-1}. Control plants showed delayed head initiation compared to 40 mgL^{-1}. Growth stage 2 (8-leaf stage) yielded the highest TSS, chlorophyll b, yield, plant height, stem diameter, and leaf number. Interactions between 30 mgL^{-1} and 40 mgL^{-1} CNP at growth stage 2 produced superior yield and quality, though results varied.

Authors' Contributions

Sabir Khan conceptualized the study and prepared the original draft. Badshah Islam provided supervision, oversaw project administration, and contributed to reviewing and editing the manuscript. Wasim Khan developed the methodology and curated the data. Sheema BiBi conducted the formal analysis and participated in the investigation. Safia Gul assisted with reviewing and editing the manuscript and handled visualization. Muhammad Ismail managed the software and performed validation. Muhammad Ishaq Khan supplied resources and provided supervision. Noor ul Wahab and Muhammad Waseem contributed to the investigation and data curation. Fathma Gul performed formal analysis and provided resources. Salman Khan and Shahid Zaman contributed to conceptualization.

Conflict of 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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