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Carbon nanodots as modulators of growth, physiological and biochemical responses in forage pea (*Pisum sativum* L.)

Erkan KARATAŞ[1], Büşra ÖZDEMİRCİ[2]
[1,2]*Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum, Türkiye*
**erkan.karatas@erzurum.edu.tr, [2]busra.yazicilar21@erzurum.edu.tr*

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Yem bezelyesinde (*Pisum sativum* L.) karbon nanonoktaların büyüme, fizyolojik ve biyokimyasal yanıtlar üzerine etkileri

Abstract: Forage pea (*Pisum sativum* L.) is an important cool-season legume forage crop, and improving its early vegetative performance is of practical interest. In this study, we investigated the dose-dependent effects of carbon nanodots (CDs) on growth, photosynthetic pigments, and oxidative stress-related biomarkers in forage pea seedlings. We grew seedlings in soil and applied CDs exogenously via foliar spray (20 mL per pot) at 100, 200, and 300 ppm three times at 3-day intervals, with measurements made 14 days after the first application. Each treatment consisted of three pots, with four seedlings per pot. Root and leaf lengths, chlorophyll a, chlorophyll b, total chlorophyll, proline, hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) contents were determined. CDs treatment resulted in statistically significant differences in root and leaf length. Root length increased from 5.14 cm in the control to 8.50 cm at 300 ppm, whereas leaf length showed the highest numerical value at 100 ppm (1.80 cm) compared with 1.34 cm in the control. Root H_2O_2 content increased relative to the control (1.555 µmol g^{-1} FW), with the highest level observed at 200 ppm, while leaf H_2O_2 decreased with increasing CD concentration from the control value of 1.810 µmol g^{-1} FW. MDA responses were tissue- and concentration-dependent, with the highest root MDA content recorded at 300 ppm (0.275 nmol g^{-1} FW) and the highest leaf MDA content at 200 ppm (0.505 nmol g^{-1} FW). Proline accumulation also varied between tissues, reaching 0.811 mg g^{-1} FW in roots at 200 ppm, while leaf proline showed a minimum of 0.352 mg g^{-1} FW at 100 ppm. Statistically significant differences in MDA were detected in both root and leaf tissues, whereas significant differences in proline were detected in leaves ($p<0.05$). Chlorophyll accumulation increased particularly at 300 ppm, where chlorophyll a and total chlorophyll reached 3.050 and 5.521 mg L^{-1}, respectively. Overall, CDs induced concentration-dependent and tissue-specific changes in growth, pigment accumulation, and redox-related responses in forage pea, underscoring the importance of dose optimization for the agricultural use of carbon nanodots.

Key words: Carbon nanodots, chlorophyll, oxidative stress, *Pisum sativum*, proline

Özet: Yem bezelyesi (*Pisum sativum* L.), önemli bir serin iklim baklagil yem bitkisi olup, erken vejetatif gelişiminin iyileştirilmesi pratik açıdan önem taşımaktadır. Bu çalışmada, karbon nanonoktaların (CD'ler) yem bezelyesi fidelerinde büyüme, fotosentetik pigmentler ve oksidatif stresle ilişkili biyobelirteçler üzerindeki konsantrasyona bağlı etkileri araştırılmıştır. Fideler toprakta yetiştirilmiş ve 14 gün boyunca 100, 200 ve 300 ppm konsantrasyonlarında CD'ler ile eksojen olarak yapraktan püskürtme yoluyla (saksı başına 20 mL) 3 gün arayla üç kez uygulanmış ve ölçümler ilk uygulamadan 14 gün sonra yapılmıştır. Her uygulama, saksı başına dört fide olacak şekilde üç saksıdan oluşturulmuştur. Kök ve yaprak uzunlukları ile klorofil a, klorofil b, toplam klorofil, prolin, hidrojen peroksit (H_2O_2) ve malondialdehit (MDA) içerikleri belirlenmiştir. CD uygulaması, kök ve yaprak uzunluklarında istatistiksel olarak anlamlı farklılıklara neden olmuştur. Kök uzunluğu kontrolde 5,14 cm iken 300 ppm uygulamasında 8,50 cm'ye yükselmiş; yaprak uzunluğu ise kontrol grubundaki 1,34 cm'ye kıyasla sayısal olarak en yüksek değerine 100 ppm uygulamasında (1,80 cm) ulaşmıştır. Kök H_2O_2 içeriği kontrol grubuna (1,555 µmol g^{-1} T.A.) kıyasla genel olarak artmış ve en yüksek değer 200 ppm uygulamasında gözlenmiştir. Buna karşılık, yaprak H_2O_2 içeriği, kontroldeki 1,810 µmol g^{-1} T.A. değerinden artan CD konsantrasyonlarına bağlı olarak azalmıştır. MDA yanıtları doku ve konsantrasyona bağlı olarak değişmiş; köklerde en yüksek MDA içeriği 300 ppm uygulamasında (0,275 nmol g^{-1} T.A.), yapraklarda ise 200 ppm uygulamasında (0,505 nmol g^{-1} T.A.) tespit edilmiştir. Prolin birikimi de dokular arasında farklılık göstermiştir; köklerde en yüksek prolin içeriği 200 ppm uygulamasında (0,811 mg g^{-1} T.A.) gözlenirken, yapraklarda en düşük prolin içeriği 100 ppm uygulamasında (0,352 mg g^{-1} T.A.) kaydedilmiştir. MDA açısından hem kök hem de yaprak dokularında, prolin açısından ise yaprak dokularında istatistiksel olarak anlamlı farklılıklar ortaya çıkmıştır ($p\leq 0,05$). Klorofil birikimi özellikle 300 ppm uygulamasında artmış; klorofil a ve toplam klorofil içerikleri sırasıyla 3,050 ve 5,521 mg L^{-1} ile en yüksek değerlerine ulaşmıştır. Sonuç olarak, CD uygulamalarının yem bezelyesi fidelerinde büyüme, fotosentetik pigment birikimi ve oksidatif stresle ilişkili yanıtlar üzerinde konsantrasyona ve dokuya bağlı değişikliklere neden olduğu ve karbon nanonoktaların potansiyel tarımsal uygulamalarda uygun konsantrasyonlarının belirlenmesinin önemli olduğu kanısına varılmıştır.

Anahtar Kelimeler: Karbon nanonoktaları, klorofil, oksidatif stres, *Pisum sativum*, prolin

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1. Introduction

Forage pea (*Pisum sativum* L.) is an important cool-season legume widely cultivated for hay, silage, grazing, and green manure because of its high forage quality, digestibility, and contribution to soil fertility (Sarıkaya et al., 2023). In addition to its nutritional value for livestock, forage pea is agriculturally relevant as a rotational crop that can improve soil nitrogen status and support sustainable forage (Zhang et al., 2025). Accordingly, strategies that enhance its early

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growth performance and physiological vigor are of considerable practical interest.

Plants, being immobile, must endure a wide range of abiotic stresses such as drought, salinity, extreme temperatures, and heavy metals that can severely restrict growth and development and ultimately reduce crop productivity (Alam et al., 2025; Zhu, 2016). Abiotic stress disrupts essential physiological and biochemical processes by promoting reactive oxygen species (ROS) accumulation, nutrient depletion, osmotic imbalance, reductions in leaf water content and chlorophyll levels, impairment of photosynthesis, and damage to cellular membranes and organelles, which collectively compromise plant function and may lead to mortality under severe conditions (Chauhan et al., 2023; Zhang et al., 2022). To survive in harsh environments, plants have evolved complex sensing and signaling networks (e.g., membrane receptor proteins, ion channels, calcium- and hormone-mediated cascades) that activate transcription factors and stress-responsive genes, thereby triggering protective responses such as stomatal regulation, osmoprotectant accumulation (e.g., proline), and morphological adjustments including altered root architecture and leaf traits (Bharti et al., 2025; Liu et al., 2022; Rai et al., 2024). However, rapid or extreme environmental changes can overwhelm intrinsic defense systems, motivating the development of sustainable technologies that enhance plant performance without excessive reliance on conventional agrochemical inputs (Ali et al., 2024).

Although agrochemicals including fertilizers, pesticides, and plant growth regulators remain important for sustaining agricultural productivity under stress, their extensive use can generate ecological and health concerns, including residue accumulation and negative impacts on non-target organisms and soil microbial communities (Elumalai et al., 2025; Steiner et al., 2024; Wan et al., 2025). In response, nanotechnology has emerged as a promising toolset to improve agricultural sustainability by enhancing input efficiency, supporting seed germination and early vigor, enabling biosensing, and improving tolerance to adverse environmental conditions (Feng et al., 2026; Hussain et al., 2026; Zhang et al., 2024). Nonetheless, several metal- or metal oxide-based nanomaterials may present limitations related to aggregation/dissolution behavior, ion release, and relatively higher phytotoxicity risks, which has increased interest in carbon-based alternatives (Cartwright et al., 2020; Javed et al., 2024).

Carbon dots (CDs; also referred to as carbon quantum dots in some contexts) are typically <10 nm in size and display strong photoluminescence, photochemical stability, good water solubility, and high biocompatibility, which has led to extensive use in biosensing and photocatalysis and, more recently, growing interest in agricultural applications (Huang et al., 2026; Li et al., 2023). CDs can be delivered to plants through multiple routes, including foliar spray, soil drenching, seed coating, and vacuum infiltration (Guirguis et al., 2023). Importantly, CDs have been proposed to enhance photosynthesis by improving light capture and utilization by photosystems. Because CDs can exhibit both down- and up-conversion behaviors and can be engineered to tune light absorption/emission characteristics, they have been explored as effective light promoters that support photosynthetic processes and plant growth (Li et al., 2020; Şentürk & Altınkaya, 2026). In addition to optical effects, CDs possess abundant surface functional groups and reactive electronic features that may allow them to act as free radical scavengers by donating electrons or protons, thereby contributing to antioxidant defense and ROS homeostasis under stress conditions (Chen et al., 2025). Accordingly, CDs have been investigated as multifunctional nano-enabled inputs, including as nanoantioxidants, nanofertilizer-like growth promoters (particularly for element-doped CDs), and even as carriers for biomolecules such as small interfering RNA (siRNA) in plants (Schwartz et al., 2020; Zia et al., 2024).

Despite these promising benefits, the safety and sustainability of CDs in agriculture must be evaluated carefully because nanomaterials can function as a double-edged sword (Djanaguiraman et al., 2024; Wang et al., 2025). Their small size and unique surface properties can facilitate migration and accumulation in environmental compartments and biological tissues, raising concerns related to plant accumulation, potential entry into the food chain, and broader ecological effects, including perturbations of soil microbial communities (Huang et al., 2026; Javed et al., 2024; Li et al., 2020). Moreover, dose is a decisive parameter, and nanoparticle effects on plant growth, photosynthesis, and metabolism vary markedly with material composition, concentration, and exposure conditions. Comparative studies have reported that different nanoparticle types at the same concentration can exert opposite effects on photosynthesis and growth, underscoring that both material composition and dose determine biological outcomes (Wang et al., 2025; Zia et al., 2024). Similarly, while nanocrystals can enhance photocurrent through plasmon resonance, excessive concentrations may compromise quantum yield due to energy transfer losses, illustrating potential risks associated with over-application (Sciortino et al., 2022). Conversely, engineered far-red carbon dots at low concentrations have been reported to convert UV light to photosynthetically active far-red radiation and improve electron transport and biomass accumulation without observed toxicity, highlighting the importance of dose optimization and design (Li et al., 2020; Wang et al., 2025). Collectively, these studies suggest that CDs may promote plant performance within an appropriate concentration window, whereas high concentrations may negatively affect growth and physiology (Djanaguiraman et al., 2024; Wang et al., 2025; Zia et al., 2024).

However, systematic studies that concurrently assess growth outcomes together with potential stress/toxicity markers remain limited, particularly for agronomically relevant crops and under soil-grown conditions, where root–soil interactions can influence uptake, distribution, and organ-specific responses. Therefore, establishing a clear dose–response framework that integrates morphological performance with key physiological and biochemical indicators is essential for translating CD-based strategies into sustainable agricultural practice (Liang et al., 2023; Xu et al., 2024).

In our earlier work, hydrothermally synthesized CDs were physicochemically characterized and shown to possess characteristic nanoscale structural and optical features (Karatas et al., 2025). Building on that foundation, we investigated the effects of exogenous CDs application on early seedling performance in forage pea (*Pisum sativum*

L.) grown in soil. CDs were applied as foliar spray at 100, 200, and 300 ppm, and root and leaf elongation were quantified as primary growth indicators. In parallel, chlorophyll a/b/total chlorophyll were measured to evaluate photosynthetic pigment status, while hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and proline were determined in roots and leaves to probe redox state, oxidative membrane damage, and stress-associated metabolic adjustment. By linking organ-level growth responses with pigment and redox-related biomarkers across a defined concentration range, this work aims to clarify concentration-dependent and tissue-specific CD effects in forage pea and to provide a foundation for future optimization and mechanistic investigations.

2. Materials and Methods

2.1. Plant material and application of carbon nanodots (CDs)

Mature seeds of forage pea (*Pisum sativum* L. cv. Ulubatlı) were obtained from the Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Türkiye. Seeds were surface-sterilized with 2% sodium hypochlorite (NaOCl) for 5 min and rinsed three times with sterile distilled water. The sterilized seeds were sown in well-drained loamy soil in 15×14 cm plastic pots, with four plants per pot. Plants were maintained at 25 ± 1°C under 1500 lx illumination with a 16 h light/8 h dark photoperiod and regularly irrigated. No fertilizer was applied throughout the experimental period. Pots were randomly arranged and their positions were periodically changed.

Carbon dots were synthesized using the hydrothermal method previously reported by Karatas et al. (2025). Briefly, 3.00 g of citric acid was dissolved in 25 mL of water under a magnetic stirrer until a transparent solution was obtained, which was transferred to a Teflon-lined 304 stainless steel autoclave reactor and kept at 180 °C for 6 h. Upon completion, the reactor was allowed to cool naturally to ambient temperature, and the resulting solution was centrifuged at 14,000 rpm for 30 min to remove aggregated structures; residual unreacted precursor was then removed by dialysis through a 1 kDa molecular-weight cut-off membrane against distilled water over a period of 24 h. The purified CDs were freeze-dried to a solid powder and stored until use. The synthesized CDs were characterized by UV-vis absorption spectroscopy (Shimadzu UV-1800, Kyoto, Japan), fluorescence spectroscopy (Agilent Cary Eclipse, Santa Clara, CA, USA), and transmission electron microscopy (TEM; Hitachi HighTech HT7700, Tokyo, Japan), and the same physicochemically characterized CD preparation was used in the present study. A stock dispersion of 1000 ppm was freshly prepared in distilled water immediately before each application, from which working concentrations of 100, 200, and 300 ppm (w/v) were freshly prepared by dilution and applied immediately. Each treatment consisted of three pots, with four plants per pot. After 21 days of growth, CDs were applied exogenously by foliar spraying at a volume of 20 mL per pot, three times at 3-day intervals. Plants receiving the same volume of distilled water without CDs served as the control. Root and leaf lengths were measured 14 days after the first treatment, and plant tissues were collected for biochemical analyses. Biochemical measurements were performed using standard spectrophotometric methods; H_2O_2, MDA, and proline were expressed on a fresh-weight basis, whereas chlorophyll was expressed per volume of extract (mg L^{-1}). The experimental design consisted of four groups shown in Table 1.

Table 1. Experimental groups and corresponding exogenous CDs treatments

Groups	Treatments
1	Control
2	100 ppm CDs
3	200 ppm CDs
4	300 ppm CDs

2.2. Quantification of chlorophyll content

Three replicate samples of leaf tissue (0.2 g each) were homogenized on ice in the dark in 10 mL of 80% acetone. Chlorophyll a, chlorophyll b, and total chlorophyll (a+b) in the extract were determined spectrophotometrically at 663 and 645 nm according to the Arnon (1949).

2.3. Determination of proline content

Proline content was determined according to the method proposed by Bates et al. (1973). Fresh root and leaf tissues (400 mg each) were analyzed separately. The samples were homogenized in 7.5 mL of 3% aqueous sulfosalicylic acid and centrifuged at 4800 rpm for 15 min at 4 °C. A 2 mL aliquot of the supernatant was mixed with 1 mL of acid-ninhydrin reagent and 1 mL of glacial acetic acid in test tubes. The reaction mixture was incubated at 100 °C for 1 h and then immediately cooled in an ice bath to terminate the reaction. Subsequently, 4 mL of toluene was added, and the mixture was vigorously mixed to extract the chromophore. The absorbance of the upper toluene phase was measured at 520 nm using toluene as a blank. Proline concentration was calculated from a standard curve and expressed on a fresh weight basis.

2.4. Determination of malondialdehyde (MDA) and H_2O_2 content

Plant tissue was ground in liquid nitrogen, and MDA was determined according to the Heath and Packer (1968). 0.2 g of ground plant material was dispersed in 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The sample was boiled at 98 °C for 30 min and was then quickly placed into an ice bath. The sample content was then centrifuged at 3000×g for 10 min, and the absorbance of the supernatant was recorded at 440, 532 and 600 nm (Dhindsa et al., 1981). The calculations were performed based on the formula specified by Hodges et al. (1999). MDA content was expressed as nmol g^{-1} FW.

$$\text{MDA}\left(\frac{nmol}{g}\right) = \frac{[(A_{532}-A_{600})-[(A_{440}-A_{600})x\ 0.0571]]}{157000} x10^6$$

H_2O_2 content was determined according to the method of Sergiev et al. (1997), with KI-based spectrophotometric detection as commonly applied in plant tissues following Velikova et al. (2000). Briefly, 0.4 g of both leaf and root tissue material was homogenized separately in 4 mL of trichloroacetic acid and centrifuged at 4 °C for 15 min at 13.000 rpm. A 2 mL aliquot of extract was mixed with 0.8

mL of KH_2PO_4 (pH 7.0), and 1.6 mL of KI in test tubes. The absorbance of the reaction mixture was measured at 390 nm using a standard curve with H_2O_2 solutions.

2.5. Statistical analysis

A single *P. sativum* variety was used for all analyses. All experiments were conducted in a completely randomized design with three independent biological replicates (three pots per treatment), each of which was analyzed in triplicate (technical replicates) to ensure analytical reproducibility; the four plants within each pot were pooled into one sample before the three technical replicate measurements, and the mean of the technical replicates was calculated and used for statistical evaluation. Data are presented as mean ± standard deviation (SD). Statistical analyses and graphs were performed using GraphPad Prism v11.1 (GraphPad Software, USA). Data were analyzed by two-way analysis of variance (ANOVA), with CDs concentration and tissue (root vs. leaf) as the two factors for root and leaf length, H_2O_2, MDA, and proline, and with CDs concentration and pigment type (chlorophyll a, chlorophyll b, total chlorophyll) as the two factors for the chlorophyll dataset, followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$. Asterisks above the brackets in Figures 2–6 indicate Tukey-adjusted p values for the compared pairs of means.

3. Results

3.1. Effects of CDs on root and leaf length

Figure 1 shows the overall growth status of *P. sativum* seedlings under different CDs treatments, while the quantitative effects on root and leaf length are presented in Figure 2. We demonstrated that CDs application induced statistically significant differences in both root and leaf length of *P. sativum*. Root length in the control plants was 5.140 cm, and increasing CDs concentrations resulted in a gradual enhancement of root growth, with the highest value recorded at 300 ppm (8.5 cm). In contrast, leaf length showed a different pattern: while the control plants exhibited an average leaf length of 1.34 cm, the greatest increase was observed at 100 ppm (1.8 cm). These findings indicate that CDs promote vegetative growth in a dose-dependent manner, particularly enhancing root elongation, while leaf length responds more sensitively at lower concentrations.

3.2. Effects of CDs on H_2O_2 accumulation

As shown in Figure 3, CDs application induced distinct effects on H_2O_2 accumulation in the roots and leaves of forage pea. In root tissues, CDs treatments generally increased H_2O_2 levels compared with the control (1.555 µmol g^{-1} FW), with the highest cellular damage observed at



Figure 1. Growth status of *P. sativum* L. cv. Ulubatlı treated with CDs. The letters refer to the applied treatment. a) Control, b) 100 ppm CDs, c) 200 ppm CDs, d) 300 ppm CDs

200 ppm. In contrast, leaf tissues exhibited a decreasing trend in H_2O_2 accumulation relative to the control (1.810 µmol g^{-1} FW), with higher CDs concentrations resulting in lower H_2O_2 levels; the lowest accumulation was recorded at 300 ppm. Overall, these findings indicate that CDs induce tissue-specific oxidative responses, enhancing oxidative stress in roots while exerting a stress-mitigating effect in leaves, particularly at higher concentrations.

3.3. Effects of CDs on MDA accumulation

Figure 4 presents the changes in MDA content in root and leaf tissues following CDs treatment. In root tissues, the MDA level in the control group was 0.267 nmol g^{-1} FW, and CD treatments produced only minor fluctuations around this value. However, the highest MDA content was recorded at 300 ppm (0.275 nmol g^{-1} FW), indicating increased cellular damage at this concentration, whereas the lowest MDA level was observed at 200 ppm (0.244 nmol g^{-1} FW).

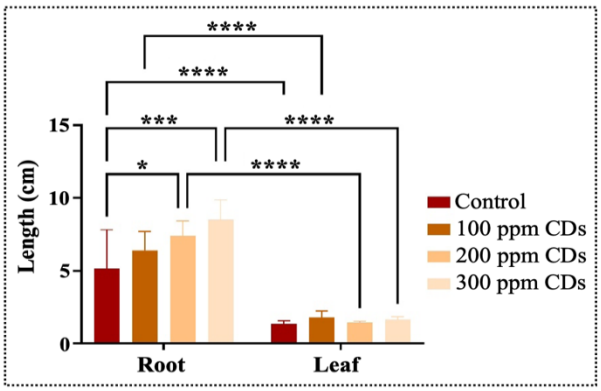


Figure 2. Effects of CDs on root and leaf lenght of *P. sativum* L. cv. Ulubatlı. Data are presented as mean ± SD. Asterisks indicate statistical significance (Tukey's post hoc multiple comparisons test following two-way ANOVA): $p < 0.05$ (*), *statistically significant; $p < 0.01$ (**), **highly significant; $p < 0.001$ (***)**, very

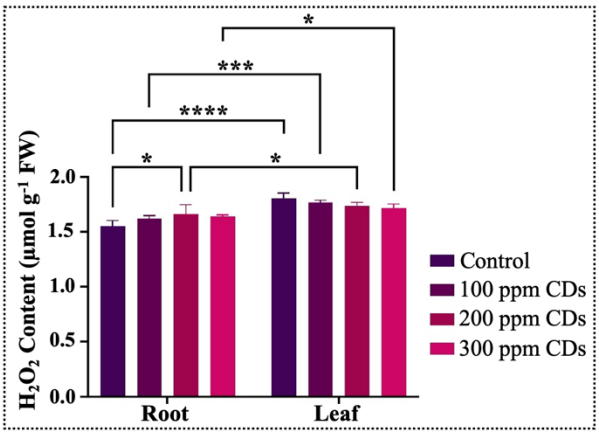


Figure 3. Effect of CDs on H_2O_2 levels in *P. sativum* L. cv. Ulubatlı. Data are presented as mean ± SD. Asterisks indicate statistical significance (Tukey's post hoc multiple comparisons test following two-way ANOVA): $p < 0.05$ (*), *statistically significant; $p < 0.01$ (**), **highly significant; $p < 0.001$ (***)**, very highly significant; and $p < 0.0001$ (****), extremely highly significant. FW: fresh weight.

highly significant; and $p < 0.0001$ (****), extremely highly significant.

In leaf tissues, CD applications generally reduced MDA accumulation compared with the control (0.331 nmol g^{-1} FW). The lowest MDA content occurred at 100 ppm (0.169 nmol g^{-1} FW), while higher concentrations, particularly 200 ppm (0.505 nmol g^{-1} FW), led to a notable increase in MDA levels. Additionally, statistically significant differences were detected in both root and leaf tissues at the $p<0.05$ level.

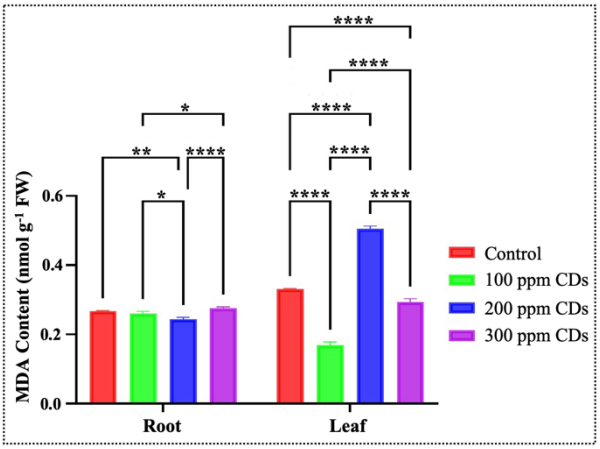


Figure 4. Effect of CDs on MDA levels in *P. sativum* L. cv. Ulubatlı. Data are presented as mean ± SD. Asterisks indicate statistical significance (Tukey's post hoc multiple comparisons test following two-way ANOVA): $p < 0.05$ (*), *statistically significant; $p < 0.01$ (**), **highly significant; $p < 0.001$ (***)**, very highly significant; and $p < 0.0001$ (****), extremely highly significant. FW: fresh weight.

3.4. Effects of CDs on proline accumulation

As illustrated in Figure 5, CDs application elicited distinct tissue-specific responses in proline accumulation in forage pea. In root tissues, the proline level in the control group was 0.677 mg g^{-1} FW, and CDs treatments generally caused limited variation around this value. However, the highest proline accumulation was recorded at 200 ppm (0.811 mg g^{-1} FW), indicating a dose-dependent increase at this concentration. In leaf tissues, CDs applications tended to

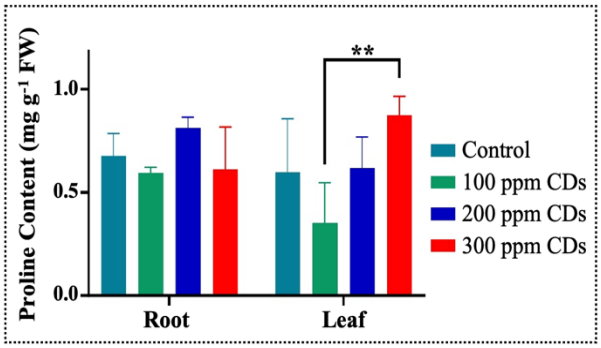


Figure 5. Effect of CDs on proline levels in *P. sativum* L. cv. Ulubatlı. Data are presented as mean ± SD. Asterisks indicate statistical significance (Tukey's post hoc multiple comparisons test following two-way ANOVA): $p < 0.05$ (*), *statistically significant; $p < 0.01$ (**), **highly significant; $p < 0.001$ (***)**, very highly significant; and $p < 0.0001$ (****), extremely highly significant. FW: fresh weight.

enhance proline accumulation compared with the control (0.598 mg g^{-1} FW). Nevertheless, the lowest proline content was observed at the 100 ppm dose (0.352 mg g^{-1} FW), whereas higher concentrations resulted in increased proline levels. Moreover, statistically significant differences were detected in leaf tissues at the $p \leq 0.05$ level.

Overall, these findings suggest that CDs modulate proline metabolism in a tissue- and dose-dependent manner. The pronounced increase in root proline content at 200 ppm indicates that CDs enhance stress-related proline accumulation in root tissues. In contrast, the suppressive effect at low doses and the stimulatory effect at higher doses in leaves point to a dose-dependent adaptive response. Collectively, the results indicate that CDs regulate proline accumulation differentially in roots and leaves as part of the plant's metabolic adjustment to stress.

3.5. Chlorophyll a, b, and total chlorophyll responses of *P. sativum* to CDs treatment

Figure 6 shows the effects of CDs treatment on chlorophyll a, chlorophyll b, and total chlorophyll contents in *P. sativum*. CDs application exerted a notable influence on chlorophyll dynamics in *P. sativum*, indicating that CDs influenced photosynthetic pigment accumulation.

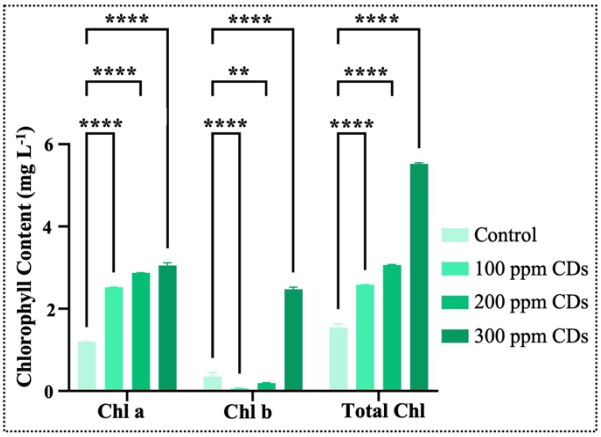


Figure 6. The effect of CDs on chlorophyll accumulation in *P. sativum* L. cv. Ulubatlı. Data are presented as mean ± SD. Asterisks indicate statistical significance: $p < 0.05$ (*), *statistically significant; $p < 0.01$ (**), **highly significant; $p < 0.001$ (***)**, very highly significant; and $p < 0.0001$ (****), extremely highly significant.

Chlorophyll a content in the control plants was 1.191 ± 0.007 mg L^{-1}, while increasing CDs concentrations resulted in a clear dose-dependent enhancement, reaching 2.521 ± 0.003 mg L^{-1} at 100 ppm, 2.868 ± 0.003 mg L^{-1} at 200 ppm, and a maximum of 3.050 ± 0.061 mg L^{-1} at 300 ppm.

Chlorophyll b exhibited a contrasting pattern at lower doses: although the control value was 0.353 ± 0.095 mg L^{-1}, treatments with 100 and 200 ppm (0.062 ± 0.005 and 0.191 ± 0.010 mg L^{-1}, respectively) led to reductions in chlorophyll b. However, a substantial increase was observed at 300 ppm (2.471 ± 0.051 mg L^{-1}), highlighting the stimulatory impact of higher CDs concentration on pigment accumulation.

Total chlorophyll content (calculated as the sum of chlorophyll a and chlorophyll b) followed a consistent upward trend with rising CDs levels — 1.545 ± 0.088 mg L^{-1} in the control, 2.583 ± 0.006 mg L^{-1} at 100 ppm, and 3.059 ± 0.012 mg L^{-1} at 200 ppm — indicating increased pigment accumulation. The highest total chlorophyll concentration, 5.521 ± 0.026 mg L^{-1}, was recorded at 300 ppm, which is consistent with enhanced chlorophyll accumulation; because photosynthetic efficiency itself was not measured, no direct conclusion is drawn on photosynthetic performance in *P. sativum*.

4. Discussion

Carbon nanodots/carbon quantum dots have recently emerged as nano-enabled biostimulant candidates capable of modulating plant growth and stress physiology in a concentration-dependent manner, with reported benefits on germination, biomass accumulation, photosynthesis, and antioxidant responses across multiple crop species (Huang et al., 2026; Li et al., 2023; Zia et al., 2024). In the present study, exogenous CDs application (100–300 ppm) produced clear dose- and tissue-specific responses in forage pea seedlings grown in soil, affecting root/leaf elongation as well as redox- and metabolism-linked biomarkers (H_2O_2, MDA, proline) and photosynthetic pigment accumulation.

A major outcome of the study was the stimulatory effect of CDs on seedling growth. Root elongation increased progressively with increasing concentration and showed its strongest response at 300 ppm. In contrast, leaf elongation reached its highest value at 100 ppm rather than at the highest concentration. This divergence suggests that roots and leaves differ in their sensitivity to CDs exposure. Roots are the first organs to encounter externally applied nanomaterials in soil-based systems. Therefore, they may experience stronger local interactions, greater exposure, or a broader tolerance window. Leaves, in contrast, may respond positively at lower concentration but may not benefit further when exposure increases. This pattern supports the idea that the biological effect of CDs depends not only on concentration but also on organ-specific uptake, transport, and redox capacity. Notably, pea-related studies have also shown that CDs can improve germination and early growth under appropriate treatment conditions, while recent dose-response analyses in other crop systems have similarly emphasized the importance of concentration optimization (Liang et al., 2023; Wang et al., 2025).

The observed growth stimulation is consistent with the proposed role of CDs as nano-enabled plant growth modulators (Huang et al., 2026). Two mechanistic pathways have been proposed in the literature for such effects: a photosynthesis-related pathway, in which the optical properties of CDs are hypothesized to influence light utilization and photochemical processes, and a redox-related pathway, in which the surface chemistry of CDs may facilitate electron/proton transfer and influence ROS-related processes (Cheng et al., 2025). Neither pathway was directly tested in the present study, which measured growth, chlorophyll content, and redox-related biomarkers (H2O2, MDA, proline) rather than photosynthetic performance (e.g., gas exchange, chlorophyll fluorescence) or electron transport; these mechanisms therefore remain hypotheses for future work (Chen et al., 2025; Cheng et al., 2025).

The chlorophyll results strengthen this interpretation. Chlorophyll a and total chlorophyll increased most strongly at 300 ppm, indicating that higher CDs concentration promoted pigment accumulation. This suggests that CDs may enhance photosynthesis-related potential, at least at the pigment level. Increased chlorophyll content is often associated in the literature with improved light-harvesting capacity and photosynthetic efficiency, although photosynthetic performance itself was not measured here. In the present study, the strongest root growth and the highest total chlorophyll were observed at the same concentration, which is compatible with the view that enhanced pigment status may contribute to growth stimulation under CDs treatment, although this association does not establish causation.

At the biochemical level, the responses were more complex and revealed an important redox component. Root H_2O_2 generally increased under CDs treatments, whereas leaf H_2O_2 decreased, particularly at higher concentration. This opposite pattern between roots and leaves is biologically meaningful. H_2O_2 is widely recognized as both a stress marker and a signaling molecule. In roots, the increase in H_2O_2 may reflect an early oxidative signal triggered by direct contact with CDs. This does not necessarily indicate irreversible damage. Controlled increases in H_2O_2 can activate downstream defense and adaptation pathways. In leaves, the reduction in H_2O_2 suggests that CDs treatment may have improved ROS control, possibly by enhancing antioxidant activity or reducing net oxidative burden in aerial tissues.

The MDA data further support a concentration-sensitive redox adjustment rather than a simple uniformly beneficial response. In roots, MDA remained relatively stable, with only limited changes across concentrations. This suggests that although root H_2O_2 increased, oxidative membrane damage did not increase proportionally. In other words, root tissues may have tolerated the oxidative shift without severe membrane injury. In leaves, however, the response was biphasic. At 100 ppm, MDA decreased markedly, indicating improved membrane stability. At 200 ppm, MDA increased sharply, suggesting that this concentration may represent a stress threshold in leaf tissue. Notably, the partial decline in the leaf growth-promoting effect at 200 and 300 ppm relative to 100 ppm coincides with this sharp increase in leaf MDA at 200 ppm, suggesting that above 100 ppm the onset of oxidative membrane damage in leaves may begin to offset the growth-promoting action of CDs — consistent with the narrow effective window discussed below. This finding is important because it shows that a treatment can stimulate some parameters while simultaneously increasing oxidative damage markers in a specific organ. Therefore, CDs action should not be

interpreted as universally beneficial across all concentrations.

Proline responses followed a similarly organ-dependent pattern. Root proline increased and reached its highest value at 200 ppm, whereas leaf proline was lowest at 100 ppm and increased again at higher concentrations. Proline is commonly associated with osmotic adjustment and stress-related metabolic protection. Its accumulation under CDs treatment may therefore indicate metabolic adaptation to altered redox conditions. The elevated root proline at 200 ppm is consistent with the increased root H_2O_2 at this concentration and suggests that roots activated a compensatory response to maintain cellular balance. In leaves, the lower proline level at 100 ppm coincided with lower MDA, which may indicate a less stressful biochemical environment at this dose. The increase at higher concentration may reflect renewed metabolic demand for protection.

An additional point that strengthens the biological interpretation of the present findings is that the CDs used here originated from a nanomaterial platform that had previously demonstrated biologically favorable behavior in another in vitro context. In our earlier work, CQD-containing chitosan-based systems, evaluated alone and together with Ti_2NT_x MXene, exhibited antibacterial activity, low hemolysis, antioxidant capacity, high fibroblast viability and proliferation, and improved scratch closure (Karatas et al., 2025). Although those findings were obtained in a mammalian wound-healing context and cannot be directly extrapolated to plant biology, they support the view that this CDs platform is not biologically inert. Consistent with that broader bioactive profile, the present study demonstrates that hydrothermally derived CDs also induce measurable biological responses in a plant system by modulating organ growth, pigment status, and oxidative stress-related biomarkers in forage pea.

Taken together, the results reveal that CDs act within an effective but narrow physiological window. At suitable concentrations, they can promote growth and improve pigment status. At the same time, they can shift redox metabolism, and this shift may be beneficial, adaptive, or potentially stressful depending on the organ and concentration. In the present study, 300 ppm appeared favorable for root elongation and chlorophyll accumulation, whereas 100 ppm appeared more favorable for leaf elongation and membrane stability. The intermediate concentration, 200 ppm, produced mixed responses and may represent a transitional zone between stimulation and stress in some tissues.

These findings highlight the importance of dose optimization in agricultural applications of CDs. They also show that growth measurements alone are not sufficient to evaluate nano-enabled treatments. The simultaneous assessment of redox-related markers such as H_2O_2, MDA, and proline provides a more complete understanding of plant responses. In future studies, the mechanistic basis of these responses should be explored further by measuring antioxidant enzyme activities such as SOD, CAT, and APX, as well as photosynthetic performance indicators such as chlorophyll fluorescence. In addition, analyses of CD uptake and translocation would help explain why roots and leaves respond differently under the same external treatment.

5. Conclusions

Key findings: This study shows that exogenous CDs application altered early vegetative growth and redox-associated physiology of forage pea in a dose- and tissue-dependent manner. Root elongation increased progressively with increasing CDs concentration, with the strongest response at 300 ppm, whereas leaf elongation was highest at the lower dose of 100 ppm. Root H_2O_2 was generally elevated relative to the control, whereas leaf H_2O_2 decreased with increasing CDs concentration. Leaf MDA increased at 200 ppm but decreased at 100 ppm, while root MDA remained comparatively stable across concentrations. Root proline peaked at 200 ppm, and chlorophyll a and total chlorophyll increased progressively with concentration, reaching their highest values at 300 ppm. Collectively, these findings indicate that CDs act within a narrow, concentration- and tissue-dependent window, promoting growth and pigment accumulation at some doses while shifting redox status — in some cases toward an apparent stress response — at others.

Limitations: This study has several limitations. Only a single pea cultivar, one application route (foliar spray), and three CD concentrations were tested at one growth stage, so the findings may not generalize to other cultivars, concentrations, or application methods. The mechanisms proposed to explain the observed responses — including possible effects on light utilization, electron transport, and photosynthetic efficiency — were not directly tested, since only chlorophyll content, and not photosynthetic performance itself, was measured. Similarly, redox status was inferred from H_2O_2, MDA, and proline alone, without measurements of antioxidant enzyme activities (e.g., SOD, CAT, APX) or direct evidence of CD uptake and translocation, which would be needed to explain the observed root–leaf divergence. Chlorophyll content was expressed per volume of extract rather than on a fresh-weight basis, unlike the other biomarkers, which should be considered when comparing across studies.

Future perspectives: Future work should extend this dose–response framework to additional application routes, cultivars, and environmental conditions, and should incorporate antioxidant enzyme assays, chlorophyll fluorescence or gas-exchange measurements, and CD uptake/translocation analyses to clarify the mechanistic basis of the tissue-specific responses reported here. Such studies would help define safe and effective application ranges and clarify the potential of carbon nanodots as nano-enabled biostimulants for forage pea and other crops.

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.

Authors' Contribution

Erkan Karatas: Data curation, interpretation, conceptualization, investigation, resources, supervision, validation, visualization, writing, review and editing. Busra Ozdemirci: Data curation, interpretation, conceptualization, investigation, resources, supervision, validation, visualization, writing, review and editing.

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