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The Effect of Carbon Dots Obtained from Different Biological Sources on Corn Seed Germination

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ABSTRACT
In the study, the effect of carbon dots (CDs) obtained from different biological sources, such as *Spirulina* and banana peel, which serve as carbon and nitrogen sources, on corn seed germination was investigated using the green hydrothermal synthesis method. The synthesized CDs were thoroughly characterized using various analytical techniques. The surface morphology, wettability, and moisture retention of the seeds after CD application were evaluated using appropriate analytical methods. The synthesized carbon dots exhibited a narrow particle size distribution ranging from 10 to 15 nm and a high antioxidant activity value of 87.72%. Application of CDs to the seeds enhanced their surface properties, improving water retention and accelerating germination, with the most pronounced effect observed at a concentration of 0.1 mg mL^{-1}. At this concentration, root and shoot lengths increased nearly threefold compared to the control. Notably, SB-CD treatment exhibited a clear dose-dependent hormetic pattern, in which low concentrations stimulated germination and seedling growth whereas higher concentrations progressively inhibited development. These results demonstrate the potential of naturally derived SB-CDs as sustainable nanomaterials for agricultural biotechnology, offering promising applications in seed treatment, crop enhancement, and eco-friendly agricultural product development.

Keywords: *Spirulina*, Banana peel waste, Carbon dots, Biomass, Hydrothermal synthesis, Seed germination

1. Introduction

Carbon dots (CDs), which have high adsorption and emission capacity due to their multiple functional groups (–OH, –COOH, –NH_2) and carbon content, are environmentally friendly, water-soluble, easy to synthesize, cost-effective, have a large surface volume, biocompatible, photoluminescent with high durability and photostability, and smaller than 10 nm nanomaterials (Milenkovićet al. 2021; Shi et al. 2022; Ramasubburayan et al. 2024). In addition, CDs are a nanocomponent that significantly increases plant yield due to their water solubility, optical properties, low toxicity, and small size. In studies on agricultural production, it has been found that CDs used in sufficient quantities have a positive effect on seed germination (Zheng et al. 2017; Su et al. 2018). Moreover, it has been determined that CD applications increase biomass by encouraging root and stem elongation in plants (Wang et al. 2018) and support plant growth by exhibiting positive effects, such as increasing resistance to plant diseases and enhancing carbon fixation (Li et al. 2018). Recently, CDs have been utilized to reduce the use of chemical pesticides in agriculture (Wahyudi et al. 2023), minimize plant nutrient loss during fertilization, and increase yield by ensuring the efficient use of water and nutrients (Kang & Lee 2019).

There are generally two approaches to obtaining CDs. CDs are prepared by top-down (arc discharge, laser etching, oxidation) or bottom-up (hydrothermal synthesis, microwave, pyrolysis) approaches. The preferred hydrothermal synthesis method in the bottom-up approach, where cheap, renewable, and widely available biomass is used as a carbon source, is an environmentally friendly method where chemical and straightforward reagents are not used at all or rarely (Boruah et al. 2020; Kurian & Paul 2021; Lin et al. 2021). Biomass-based CDs play a crucial role in plant growth because they provide abundant carbon and nitrogen sources that plants require (Dong et al. 2024). An et al. (2021) reported that CDs obtained from peanut bran by hydrothermal synthesis were effective in lettuce growth. Agnol et al. (2021) utilized CDs obtained from *Spirulina* in their study on lentil seeds and found that they had a growth-supporting effect on the seed. *Spirulina*, one of the abundant microalgae biomass found in nature that can be grown even in adverse conditions, contains high protein (~65%, dry weight) and high nitrogen (9.6%, dry weight) (Du et al. 2013; Jafarian & Tavasoli 2018; Hepburn et al. 2019; Vasudev et al. 2020; Li et al. 2022), and also contains minerals such as P, K and Mg that promote the growth of vegetable crops (Binda et al. 2020). Due to these properties, *Spirulina* is one of the preferred species as a renewable, fertilizer-like raw material for the production of CDs (Jafarian & Tavasoli 2018).

One of the CD studies on photosynthesis, a fundamental physiological process for plant survival and growth, was also conducted by Tan et al. (2023). In the study, it was determined that the nanofertilizer obtained by synthesizing nitrogen-doped CDs significantly increased the chlorophyll A content of the plant, electronic conduction rate, light energy conversion efficiency, and Rubisco activity. Obtaining more detailed information about nanomaterials is necessary to improve the efficiency of nitrogen fertilizers used in plant growth, development, and yield in agricultural applications, and to increase product yield and quality, thereby helping to eliminate deficiencies in this regard (Mu & Chen 2012; Pan et al. 2024; Li et al. 2025). In addition, as a result of the increase in ultraviolet-B (UV-B) radiation on the Earth's surface, the ROS concentration increases and antioxidant enzyme activity changes. As a result, the amount of photosynthesis decreases. CDs increase the rate of photosynthesis by enhancing the accumulation of flavonoids in plants whose growth is negatively affected, by mimicking antioxidants, or by improving antioxidant enzyme activities (Wang et al. 2022).

A large number of reliable precursors have been used as starting materials for the synthesis of high-quality organic CDs from various natural biomass or biological wastes. Since the toxicity of aromatic hydrocarbon precursors severely limits their widespread use, the use of non-toxic, cost-effective, and biocompatible precursors, such as vegetables, fruits, milk, and even waste, has become popular in the production of CDs (Huang et al. 2018). However, since these natural materials typically contain a wide variety of surface functional heteroatom groups, high-fluorescent CDs are obtained from them (Ahn et al. 2019; Alex et al. 2020). Microalgae raw materials, which can be obtained from natural aquatic blooms or large-scale cultivation under laboratory conditions, are highly suitable precursor organisms for preparing heteroatom-doped CDs, especially by the hydrothermal synthesis method (Zhang et al. 2017). It has been determined that the CDs obtained through hydrothermal synthesis can bind to nitrogenase and alter its secondary structure, enhancing electron transfer in the biocatalytic process and ultimately improving nitrogenase activity for nitrogen fixation (Wang et al. 2018). Studies have shown that the stable complex of nitrogen, which is abundant in banana peels, enhances the surface functionalities of CDs during the third stage of the hydrothermal synthesis process, due to its improved solubility and optical properties (Edison et al. 2016). Another study successfully synthesized hydrophilic nitrogen-doped CDs from dwarf banana peels via hydrothermal methods. This study confirmed that the resulting CDs possessed rich hydrophilic groups and had high potential biological applications (Atchudan et al. 2020).

In recent years, the excessive use of chemical fertilizers and pesticides has led to serious environmental challenges, including soil pollution, reduced microbial diversity, and the accumulation of toxic residues in agricultural ecosystems. Consequently, there is an urgent need to develop alternative, eco-friendly approaches that improve plant productivity and stress resistance while minimizing chemical inputs. The main objective of this study was to synthesize and characterize SB-CDs and to assess their effects on corn seed germination, vigor, and surface properties. Carbon dots (CDs), particularly those synthesized from renewable biomass such as *Spirulina* and banana peel, offer a sustainable solution due to their biocompatibility, antioxidant potential, and ability to modulate physiological processes in plants. We hypothesize that CDs will exert a biphasic, dose-dependent effect on corn seed germination and growth: low concentrations (stimulatory) will significantly increase root and shoot elongation by enhancing physiological activity, while high concentrations (inhibitory) will suppress growth, potentially due to physical or oxidative stress. Although prior studies suggest that CDs can promote germination, the mechanistic determinants of this biostimulatory effect remain insufficiently explored (Zheng et al. 2017; Agnol et al. 2021). In particular, the potential role of CDs in modulating seed surface wettability, water retention capacity, and ROS-mediated signaling during germination has not yet been systematically investigated. Clarifying this mechanism is crucial to transform CD-based seed priming from empirical observation into mechanistically informed agricultural biotechnology. To test this hypothesis, the current study focuses on determining the optimal concentration of SB-CD preparation by first examining its effects on corn seed germination percentage, root, and shoot elongation. Subsequently, we analyze the effects of the determined optimal SB-CD concentration on key physiological and biochemical parameters of developing seedlings, exploring potential underlying mechanisms and providing a scientific basis for the application of waste-derived CDs in agricultural sustainability.

2. Material and Methods

2.1. Materials

A local variety of corn (*Zea mays* L.) commonly cultivated in Manisa, Türkiye, was used in this study, as corn is an economically significant crop and a widely accepted model species for seed germination and growth studies due to its uniform seed size, rapid germination rate, and sensitivity to growth regulators. The seeds were obtained directly from local farmers to ensure genetic purity and regional relevance. The banana fruits were purchased from a local food market in the Republic of Turkey. *Spirulina* powder (8-12% carbohydrate, 60-67% protein, and 5-7% lipid based on dry weight) was obtained from Algolina LTDA (Istanbul, Turkey). Banana peels were chosen as a carbon precursor because they are an abundant agricultural biowaste rich in carbohydrates, polyphenols, and nitrogenous compounds, which facilitate the formation of nitrogen-doped carbon dots with good photoluminescent and antioxidant properties. *Spirulina* was selected as a carbon source due to its high content of carbohydrates, proteins, and nitrogen-containing biomolecules, which can serve as effective precursors for carbon dot synthesis. Its natural composition enables the production of heteroatom-doped CDs with enhanced optical and functional properties, while also providing a sustainable and environmentally friendly alternative to synthetic precursors. All solvents were of analytical grade and used without further purification. Ultrapure water (≥18.25 MΩ.cm) was used in all experiments.

2.2. Methods

2.2.1. Synthesis of SB-CDs

Fluorescent CDs were synthesized by a simple hydrothermal method using dried banana peel powder and *Spirulina* powder. The banana peels dried in the oven were ground into powder. Banana peel powder was placed in a 100 mL Teflon-lined stainless steel hydrothermal synthesis reactor, along with *Spirulina* powder (in a 1:1 ratio), and 50 mL of ultrapure water was added. *Spirulina*-banana Carbon Dots (SB-CDs) were synthesized in a pressurized oven at 200 °C for 12 hours (mass ratio of liquid to solid/25:1). As a result of the reaction, the reactor was cooled rapidly, and then, the obtained brownish yellow crude product was centrifuged at 10000 rpm/min for 20 minutes. After centrifugation, the supernatant was filtered using filter paper, and filtration was then performed with a syringe filter having a pore size of 0.22 µm. This filtered and fluorescent liquid was transferred to the dialysis membrane (1000 Da) to remove small molecules (Xu et al. 2004). After dialysis against pure water for 24 hours, the obtained SB-CDs solution was stored in a refrigerator and kept at 4 °C until use. For SB-CDs characterization, the solution kept in the refrigerator was freeze-dried in a lyophilizer for 48 hours and weighed for analysis.

2.2.2. Characterization of SB-CDs

The SB-CDs solution was used to test its UV-Vis light absorption spectrum using a UV-Vis spectrophotometer (PerkinElmer Lambda 35) and to operate a fluorescence spectrometer (Olympus IX53) with excitation wavelengths ranging from 300 to 500 nm.To investigate the surface functional groups and compositional differences of *Spirulina* powder, dried banana peel powder, and the SB-CDs synthesized from these two precursors, FTIR spectroscopy was employed. The dried samples were finely ground with potassium bromide (KBr) and compressed into transparent pellets. Spectral data were collected using a PerkinElmer Spectrum 100 FTIR spectrophotometer at a resolution of 4 cm^{-1}. The spectra were recorded over the wavenumber range of 400 to 4000 cm^{-1}, using KBr as the background reference. Peaks were presented with transmittance (%) on the y-axis and wavenumber (cm^{-1}) on the x-axis to facilitate the interpretation of characteristic functional group vibrations.

SB-CDs were analyzed by FEI TALOS F200S TEM 200 kV to confirm their morphology and elemental characteristics.

2.2.3. Antioxidant assay of SB-CDs

This study evaluated the radical scavenging ability of SB-CDs using the DPPH method to determine their antioxidant activity. To establish a reliable dose-response curve and calculate the IC_{50} value, SB-CDs dispersions were tested at five varying concentrations (e.g., 0.05-1.0 mg mL^{-1}). For each concentration, 200 µL of DPPH (100 µM) and 20 µL of SB-CDs dispersion were mixed in a microplate well. The microplates were incubated at 25 °C for 30 min in the dark. 1 mM Butylated hydroxyanisole (BHA) was included as a positive control, which typically yields >95% RSA under these conditions, confirming the test's efficacy. The absorbance was measured at 495 nm using a microplate reader. To correct for any intrinsic absorbance or fluorescence interference by the SB-CDs themselves, a specific blank containing SB-CDs dispersion and solvent (without DPPH) was measured for each concentration and subtracted from the sample absorbance. RSA was calculated using the following equation (Moein et al. 2017; Azarbani & Shiravand 2020).

1mM Butylated hydroxyanisole (BHA) was used as a positive control. RSA was calculated using the equation given below (Azarbani & Shiravand 2020):

$$RSA = \frac{AC - AS}{AC} * 100$$

AC refers to the absorbance of the DPPH solution, and AS refers to the absorbance of the DPPH solution in the presence of SB-CDs. SB-CDs were used directly from the synthesized medium.

2.3. Seed sterilization, priming procedure, and germination conditions

The seeds were surface-sterilized by immersing them in a 5% sodium hypochlorite solution for 10 minutes (Ramakrishna et al. 1991; Miché, L & Balandreau 2001). Subsequently, to eliminate residual chemicals, the seeds were rinsed three times with Milli-Q water. Afterward, they were immersed in 50 mL beakers containing priming solutions at different concentrations (0.05, 0.1, 0.2, 0.5, 1, and 2 mg mL^{-1}) of either Milli-Q or SB-CD solutions. Each beaker contained 50 seeds, with a seed weight/solution volume ratio of 1:4 (1.4 g in 5.6 mL), and each treatment was repeated four times. The beakers were placed on a mechanical shaker (100 rpm) at room temperature (25±1 °C) in a dark environment to ensure complete contact between the seeds and the priming solution.

After 20 hours of priming, the seeds were gently rinsed with Milli-Q water and placed in sterile Petri dishes (90 mm × 15 mm) containing 10 mL of Milli-Q moistened filter paper. The dishes were wrapped in moist paper towels and incubated in a dark environment at 27±0.5 °C for 3 days (80% relative humidity). A seed was considered germinated when the emerging radicle

reached 2 mm in length (ISTA, 2020). Germination rates were recorded every 6 hours until all seeds had germinated, spanning approximately 36 hours.
After three days, the germinated corn seeds were transferred to growth chambers under simulated daylight conditions (12-hour photoperiod) in a controlled environment (Nüve test chamber TK 120). The lighting system provided a cool white spectrum (6400 K) and the light intensity was maintained at 150±10 μmol m^{-2} s^{-1} (PPFD), measured at the seedling level using a quantum sensor (LI-COR, model LI-190). The chamber was maintained at 80% relative humidity and 28±1 °C. All experiments, including priming and subsequent growth assays, were performed with four biological replicates.

2.3.1. Wettability characteristics

Contact angle, TGA, and SEM analysis were performed to evaluate the wettability properties of the seeds. Contact angle measurement analysis was performed to examine the surface wettability of the corn seed surface after it was soaked in pure water and a carbon dot-containing solution. Contact angle analysis is a technique used to determine the wettability of a surface and its hydrophilic (water-loving) or hydrophobic (water-repellent) properties by measuring the angle formed at the point where a liquid drop contacts a solid surface. In general, a small contact angle (<90°) indicates that the surface wets water well, i.e., it is hydrophilic, while a large contact angle (>90°) indicates a hydrophobic surface (Yuan & Lee 2013).

TGA is an analysis that records the change in mass of a substance as a function of temperature in a controlled environment using a precision balance (Skoog et al. 1996). The water holding capacity of the seed was calculated by determining the free water (moisture) and intracellular bound water (hydration water) amounts of corn seeds that were kept in pure water and carbon dot solution by adjusting the analysis temperature from 25 °C to 200 °C at four heating rates (5, 10, 15, and 20 °C/min) under a nitrogen atmosphere (50 mL/min). The morphological and topological changes in the corn seed after priming with SB-CDs were analyzed using SEM.

2.4. Statistical analysis

Data are presented as the mean ± SD. All data were analyzed using GraphPad Prism (version 8, GraphPad Software, San Diego, California, USA). To determine whether there was a statistically significant difference in root and shoot lengths across the different SB-CD priming concentrations, a One-Way Analysis of Variance (ANOVA) was performed. Tukey's Multiple Comparisons Test was applied post-hoc to identify specific differences between all concentration pairs. Assumptions of normality and variance homogeneity were not formally tested (e.g., Shapiro–Wilk or Levene tests). However, visual inspection of residual plots and the distribution of group variances did not indicate severe deviation from ANOVA assumptions; therefore, one-way ANOVA was applied. Data are presented as mean ± Standard Deviation (SD). Differences were considered statistically significant at $P<0.05$.

3. Results and Discussion

3.1. Structural characterization of SB-CDs by UV-Visible and fluorescence spectroscopy

UV-Vis absorption and fluorescence spectroscopy were used to investigate the photoluminescence properties of SB-CDs. When observed under visible and UV light (excitation at 360 nm), the SB-CD solution, which was prepared at 200 °C for 12 hours, displayed distinct deep yellow and intense fluorescent blue emissions, respectively (Figure 1).

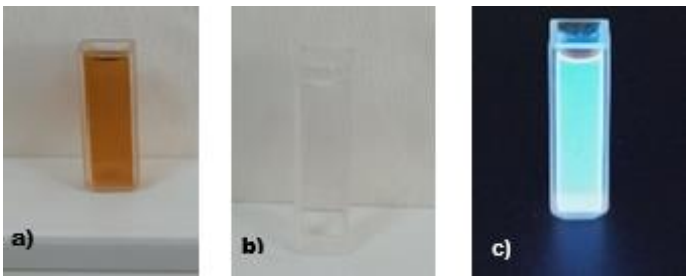


Figure 1- Fluorescent photo of not diluted SB-CDs under sunlight (a), 100x diluted SB-CDs under sunlight (b), and 100x diluted SB-CDs under UV-365 nm (c)

SB-CDs exhibited strong optical absorption in the UV-Vis region (240-500 nm). As shown in the UV-Vis absorption spectrum (Figure 2a), the SB-CDs showed a distinct peak around 272 nm and a tail extending into the visible range. The peak around 270 nm, detected by UV-Vis spectroscopy, is attributed to the π-π* transitions of C=C bonds, as well as C=O and C−N transitions, and functional groups in the environment. This result is consistent with previously published scientific studies by Agnol et al. (2021).

The excitation fluorescence of SB-CDs was observed within an excitation wavelength range from 215 nm to 240 nm. SB-CDs were excited at 225 nm, and emission was observed at 406 and 457 nm (Figure 2b). It was observed that the photoluminescence emission (Em) peak shifted to higher wavelengths, especially in the excitation wavelength range of 300-350 nm. The SB-CDs in the experiment exhibited excellent fluorescence intensity, which can be utilized in other applications involving fluorescence.

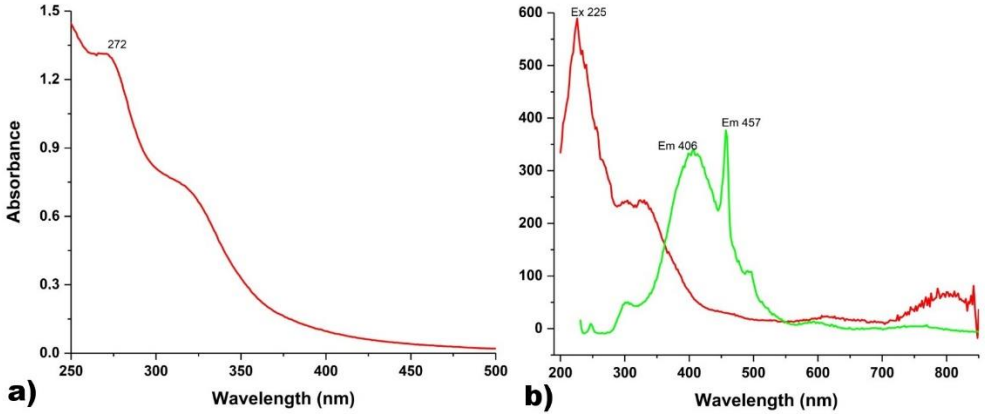


Figure 2- UV-Vis absorption spectra of SB-CDs (a) and Fluorescence spectra of SB-CDs (excitation:225 nm and emission:457 nm) (b)

3.2. Characterization of Spirulina, banana peel, and SB-CDs by FTIR

FTIR analysis was performed to examine the changes and formations in functional groups resulting from the formation of SB-CDs. FTIR spectra of *Spirulina* powder, banana peel powder, and SB-CDs are shown in Figure 3. In addition to the similarities between the FTIR spectra of *Spirulina* and banana peel, intense peaks were observed in both biomaterials. The determined spectrum peaks correspond to the functional groups of lipids, flavonoids, carbohydrates, glycosides, alkaloids, and terpenoids in the structure (Genc et al. 2020). In the FTIR spectra of SB-CDs obtained by the hydrothermal synthesis method, a decrease in the number of peaks and differences in their intensities were observed.

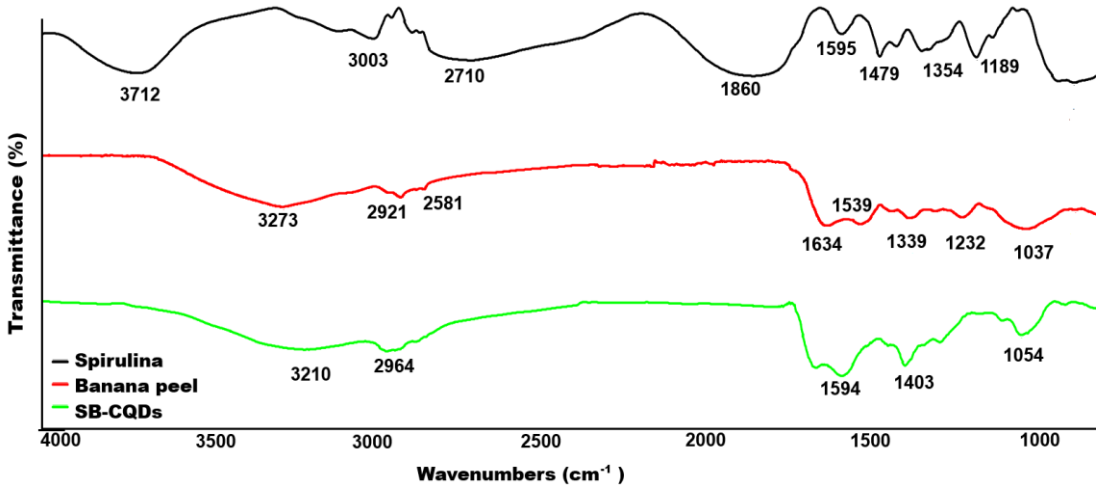


Figure 3- FTIR spectra of *Spirulina*, Banana peel and SB-CDs

FTIR analysis revealed the presence of hydroxyl groups (–OH) in *Spirulina*, as indicated by the characteristic peaks of water, alcohol, phenol, or carboxylic acid at 3712 cm−1 (Wang et al. 2022). FTIR spectroscopy of *Spirulina* revealed the presence of the following bands: C–H stretching (3003 cm^{-1}, indicating stretching vibrations involving aromatic or double bonds), aldehyde C–H stretching (2710 cm^{-1}, corresponding to C–H stretching vibrations of –CHO groups), asymmetric C=O stretching (1860 cm^{-1}, indicating stretching vibrations of double carbonyl groups), –C=C stretching or N–H bending (1595 cm^{-1}, indicating amide groups formed due to the rich protein content of *Spirulina*), –CH_2 bending (1449 cm^{-1}, methylene groups), –C–N stretching (1354 cm^{-1}, amine groups) and –C–O–C stretching (1189 cm^{-1}, ether or ester groups) (Coates 2000; Stuart 2004; Barth 2007). The FTIR spectrum of *Spirulina* reveals that it contains functional groups typical of proteins (amide bands), lipids (CH2), carbohydrates, and phenolic structures. This confirms that it is a rich source of functional groups for carbon dots of biological origin.

Similarly, the peaks seen in the banana peel FTIR spectrum were determined as: 3273 cm^{-1} (O–H stretching, from the lignocellulosic structure), 2927 and 2851 cm^{-1} aliphatic C–H stretching (from methyl/methylene groups), 1712 cm^{-1}, –C=O stretching (carboxylic acid or ester), 1624 cm^{-1}: aromatic C=C, 1449–1232 cm^{-1}: C–H bending, C–N stretching, 1189 cm^{-1} C–O–C (cellulose and hemicellulose structure), 1037 cm^{-1}: C–O stretching (polysaccharides), respectively (Coates 2000; Stuart 2004; Barth 2007). The banana peel FTIR profile exhibits functional groups characteristic of lignocellulosic compounds, including cellulose, lignin, and hemicellulose. These natural carbon sources are likely to provide oxygenated functional groups to the SB-CDs.

In the production of SB-CDs, large structures in the raw materials (cellulose, protein, etc.) were transformed into smaller surface groups as a result of the partial disintegration of the structures by hydrothermal processes. The functional groups present in SB-CDs (O–H, C=O, C=C, and C–O–C) are located on the surface of the carbon dots and influence their water solubility, biological interactions, and photoluminescence properties. *Spirulina* proteins and the carbon skeleton of banana peel combined to enable the production of functionalized SB-CDs with both oxygenated and nitrogenous groups.

3.3. Characterization of SB-CDs by TEM

The high-resolution TEM of SB-CDs is presented in Figure 4. The SB-CDs, which are observed to have regular shapes despite varying sizes, are shown in the TEM image, indicating that the diameter size of the carbon dots ranges from 10 to 15 nm.

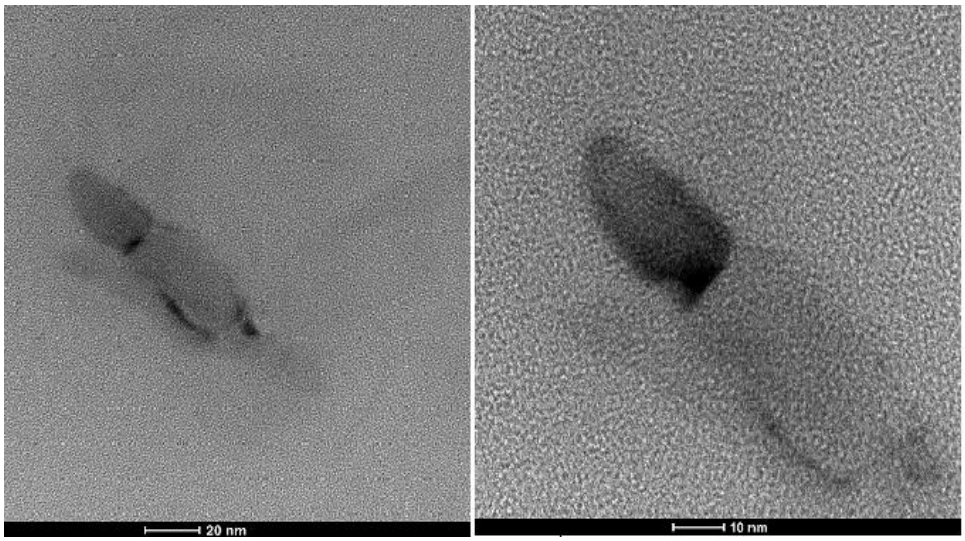


Figure 4- TEM images of SB-CDs at various resolutions

In the study, it was observed that SB-CDs obtained from *Spirulina* and banana peel were in the 10-15 nm size range, with some dots being rod-like or ellipsoidal, while others were more amorphous. Spherical morphology is the most commonly reported shape for SB-CDs. However, depending on the synthesis method and the precursor used, different morphologies, such as ellipsoidal, rod-like, or amorphous, can also be observed (Liu et al. 2016; Saraswat et al. 2024). Qin et al. (2024) and Zhang et al. (2022) reported that SB-CDs synthesized from *Spirulina* by the hydrothermal method had an average size of 1-4 nm and a spherical morphology of 20-50 nm, respectively. Sul et al. (2023) reported that SB-CDs synthesized from banana peel were spherical with a size distribution in the proton range (<10 nm). The hydrothermal carbonization time, temperature, pressure, and precursor concentration significantly affect the size and morphology of the final SB-CDs. Longer reaction times or higher temperatures can generally lead to the formation of larger particles. However, the use of precursors together or separately, along with different purification steps (e.g., dialysis, centrifugation, filtration), and the possibility of carbon dots to exhibit aggregation (coalescence) can lead to an enrichment of the size ranges of SB-CDs (Ullal et al. 2024).

3.4. Antioxidant activity of SB-CDs

Following the hydrothermal synthesis, the solution containing SB-CDs was filtered and its antioxidant activity was evaluated using the DPPH method. The concentrations of SB-CD solutions used for the antioxidant activity test were determined as 5, 10, 25, 50, and 100 µL mL^{-1} (Table 1). BHA, used as a positive control, showed 97.43% antioxidant activity. The antioxidant activity of SB-CDs increased directly proportional to the increasing concentration, from 12.89% to 87.72%.

Table 1- Radical scavenging activity (%) and standard deviation of SB-CDs

Sample concentration (µL mL^{-1})	*Radical Scavenging Activity (ROS) %*
5	12.89 ± 0.005
10	37.10 ± 0.001
25	69.87 ± 0.003
50	86.04 ± 0.007
75	87.27 ± 0.005
100	**87.72 ± 0.004**

The high value of the antioxidant activity of SB-CDs, 87.72%, can be associated with the quenching of DPPH radicals by H transfer from the hydroxyl groups on the surface of the SB-CDs. It has been determined that other functional groups, such as carboxyl on the surface of the carbon dots, also have a supporting effect on antioxidant activity (Son et al. 2021; Kuchaiyaphum et al. 2024).

Spirulina and banana peel have become a popular source for carbon dot synthesis in recent years due to their rich nutritional content and bioactive properties. These biomaterials, rich in flavonoids, phycobiliproteins, phenolic acids, carotenoids, and polysaccharides, nourish antioxidant activity. Bijoy & Sangeetha (2024) determined that SB-CDs synthesized from *Spirulina* using the hydrothermal method exhibit potent antioxidant activity. In this study, conducted using the DPPH free radical scavenging assay, a radical scavenging capacity of more than 80% was obtained. Similarly, Ismail et al. (2021) determined both antioxidant and biocompatible properties of nitrogen-doped carbon dots obtained from *Spirulina*. In the study, a significant increase in antioxidant activity was observed with increasing carbon dot concentration. Similar to our findings, Meng et al. (2025) also observed that banana peel–derived carbon dots displayed high antioxidant activity, supporting our interpretation that biomass origin contributes to redox-active surface groups. Compared to the studies in the literature, the results obtained from this study clearly demonstrate that carbon dots obtained from the combination of sustainable biomasses, *Spirulina* and banana peel, exhibit a significant increase in antioxidant activity. Similar enhancements in antioxidant enzyme activities have been reported for biomass-derived CDs, supporting their role in ROS homeostasis (Wang et al. 2025). The strong antioxidant capacity of SB-CDs suggests that their biological activity during seed germination may be mediated through ROS scavenging mechanisms. During early germination, moderate ROS levels act as signaling molecules for cell expansion and root elongation; however, excessive ROS can cause oxidative damage. The hydroxyl and carboxyl surface groups of SB-CDs may act as electron donors to neutralize ROS, maintaining redox homeostasis and thus promoting balanced cell division and elongation. This mechanism may explain the stimulatory effect observed at low concentrations and the inhibitory effects at higher doses (Li et al. 2023). These strong antioxidant properties indicate that SB-CDs can play a significant role in protecting seeds from oxidative stress during germination and early seedling growth. This antioxidant effect is agriculturally valuable, as it may help improve seed vigor and stress tolerance under adverse environmental conditions such as drought, salinity, or UV stress, contributing to enhanced crop establishment in the field.

3.5. The influence of SB-CDs on corn seed germination

Corn seeds primed with SB-CDs at different concentrations (0.05–2 mg mL^{-1}) were evaluated for germination after 3 days of dark incubation followed by 3 days under light/dark conditions. The highest germination performance and seedling vigor were observed in seeds treated with 0.1 mg mL^{-1} SB-CDs ($P<0.05$). While the mean root and shoot lengths in the control group were 3.0 ± 0.2 cm and 1.0 ± 0.1 cm, respectively, these values increased to 7.5 ± 0.5 cm and 8.0 ± 0.5 cm for the 0.1 mg mL^{-1} SB-CD-treated seeds.

At higher concentrations (≥ 0.5 mg mL^{-1}), both root and shoot elongation gradually declined, indicating a dose-dependent (hormetic) effect. Figure 5 illustrates the morphological appearance of germinated seeds, and Table 2 summarizes the mean ± SD values and significance groups for each concentration.

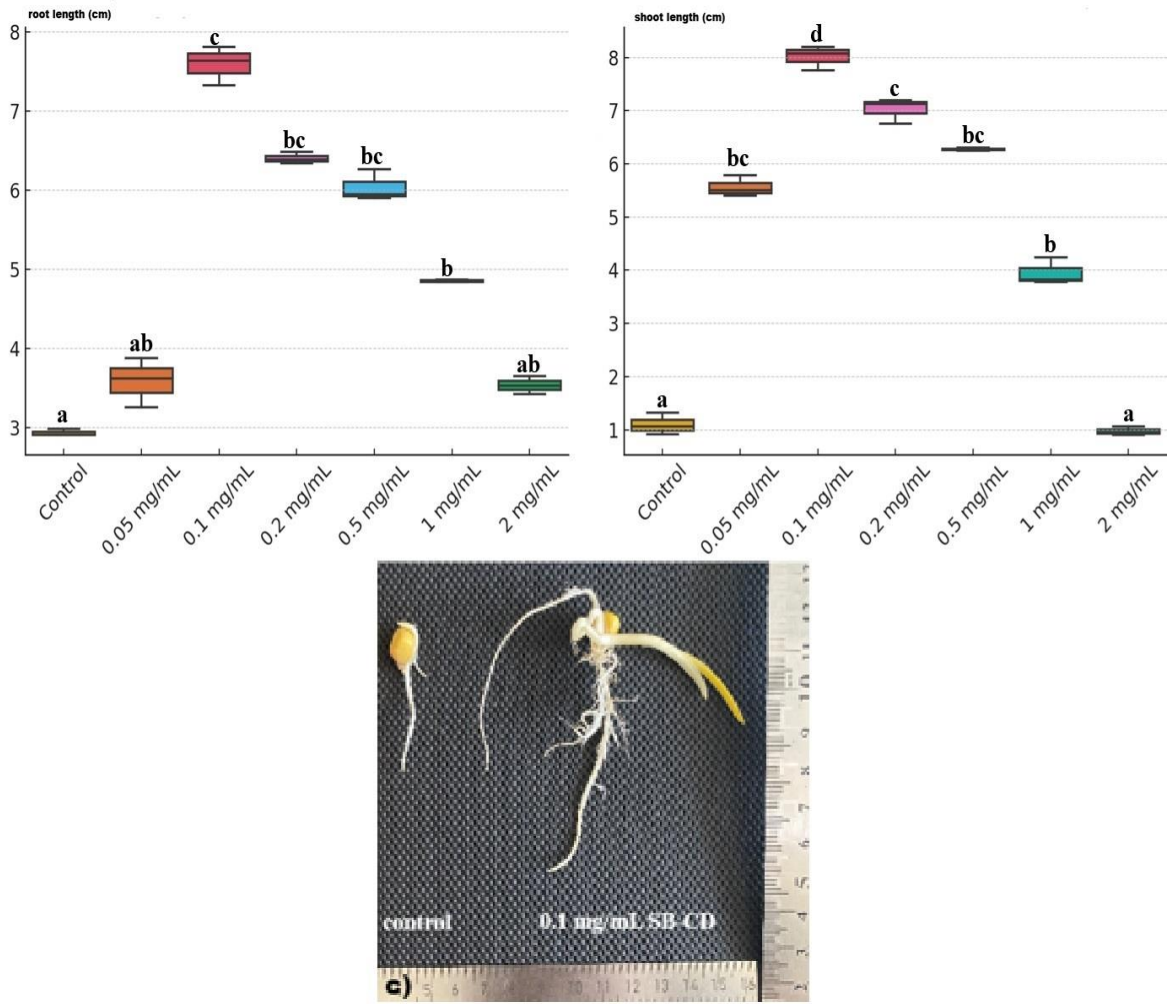


Figure 5- (a) Root and (b) shoot length of corn seeds after SB-CD application at different concentrations. Different letters indicate statistically significant differences between treatments at P<0.05 (Tukey's multiple comparison test). (c) Representative photograph of germinated corn seeds after priming with SB-CD solution (0.1 mg mL^{-1}) and Milli-Q water

Table 2- Effect of different SB-CD concentrations on root and shoot length of corn seeds (mean ± SD, n=3). Different letters within each column indicate statistically significant differences at P<0.05 (Tukey test).

Concentration (mg mL^{-1})	*Root length (cm)*	*Shoot length (cm)*	*Significance group (root/shoot)*
0 (control)	3.0 ± 0.2	1.0 ± 0.1	a/a
0.05	3.5 ± 0.3	5.5 ± 0.4	ab/bc
0.1	7.5 ± 0.5	8.0 ± 0.5	c/d
0.2	6.5 ± 0.4	7.0 ± 0.3	bc/c
0.5	6.0 ± 0.3	6.5 ± 0.2	bc/bc
1.0	5.0 ± 0.4	4.0 ± 0.3	b/b
2.0	3.5 ± 0.3	1.0 ± 0.2	ab/a

According to the experimental results, especially low-concentration carbon dot applications showed a biostimulant effect on corn seeds. This growth in the root and shoot compared to the control group may have occurred as a result of SB-CDs supporting antioxidant defense and reducing cellular stress. In addition, especially low doses of SB-CDs can increase the permeability of the seed coat, facilitate water absorption, and promote cell division and elongation by showing hormone-like properties (Gong et al. 2021). The observed pattern of enhanced germination and growth at low SB-CD concentrations followed by inhibition at higher doses reflects a hormetic dose–response behavior. Such biphasic responses are common in nanomaterial–plant interactions, where low concentrations can stimulate physiological activity by improving water uptake and antioxidant defense, while high concentrations may generate excess ROS or disrupt ion balance, leading to growth suppression (Jampilek & Kráľová 2017; Liang et al. 2021). This hormetic model provides a mechanistic explanation for the dose-dependent behavior observed in this study. The observed improvement in seed germination and seedling vigor at low SB-CD concentrations suggests that SB-CDs can be employed as nanobiostimulants to promote early-stage crop growth. This property could be particularly beneficial in agricultural systems aiming to improve seed performance under limited water or nutrient availability. The hormetic response observed here also highlights the importance of dose optimization for safe and effective agricultural applications of

nanomaterials. Studies have shown that high concentrations (>0.1 mg mL^{-1}) of SB-CD applications cause oxidative stress by increasing ROS production, cell membrane damage, and osmotic stress through the disruption of ion balance due to excessive surface charge (Jampilek & Kráľová 2017; Liang et al. 2021). The effect of SB-CDs in biological systems is directly related not only to their presence but also to their concentration. Determining the optimum dose enables the safe and effective use of carbon-based nanomaterials in agricultural applications. Comparable findings have been reported in the literature: Agnol et al. (2021) synthesized SB-CDs (~10 nm) from *Spirulina* and observed enhanced lentil seed growth at 0.05–0.1 mg mL^{-1}; Tan et al. (2021) and Wang et al. (2021) demonstrated that N-doped or photoluminescent SB-CDs improved photosynthetic efficiency and root–shoot development in rice and maize; and Abinaya et al. (2024) found that low concentrations of N-doped SB-CDs promoted germination and yield in green lentil plants. Collectively, these studies support existing findings that sustainable, biomass-derived SB-CDs can act as potent nanobiostimulants in seed germination when applied at appropriate concentrations. Similar studies on the effect of SB-CDs on plant growth are shown in Table 3.

Table 3- Comparative summary of biomass-derived carbon dots and their effects on plant growth

Source Biomass	***Target Plant***	***CDs Concentration (mg mL^{-1})***	***Optimum Concentration (mg mL^{-1})***	***Observed Effect***	***Mechanistic Explanation / Key Finding***	***Authors***
Macrocystis	Mung bean seeds	2.5–10	2.5–7.5	Increased germination and seedling vigor	Improved photosynthesis via N-doped groups	Sankaranarayanan et al. (2019)
Spirulina	Lentil seeds	0.05–0.5	0.05–0.1	Enhanced root and shoot growth	Nitrogen-doped surface improved nutrient uptake	Agnol et al. (2021)
Piper betle leaves	Strawberry plants	0.05–1.0	0.1–0.5	Increased fruit yield	Nanofertilizer-like nutrient delivery	Abu Salha et al. (2023)
Algae (Se-CDs)	Tomato leaves	0.25–0.5	0.05–0.2	Enhanced Se uptake and photosynthesis	Selenium doping improved ROS balance	Li et al. (2023)
Soybean, walnut, laver	Pakchoi seedlings	0.01–0.05	0.1	Improved biomass and chlorophyll content	Enhanced light utilization efficiency	Cheng et al. (2023)
Betel leaves	Pepper and lettuce seeds	0.05–0.5	0.05–0.2	Promoted root elongation	Improved irrigation efficiency and ion transport	Salha et al. (2025)
***Spirulina*–Banana peel**	**Corn seeds**	**0.05–2**	**0.05–0.1**	**Enhanced germination, root–shoot elongation, and water retention**	**ROS modulation and improved surface hydrophilicity**	**This study**

3.6. Surface wettability characteristics

In the contact angle findings performed to evaluate surface wettability and hydrophobic/hydrophilic character, the average contact angle of corn seeds soaked in pure water for 4 hours was determined to be 95.1°. This value indicates that the surface has hydrophobic properties. In contrast, the contact angle of seeds soaked in an aqueous dispersion containing SB-CDs at a concentration of 0.1 mg mL^{-1} for the same period decreased to 56.1°. This significant decrease indicates that the seed surface has become more hydrophilic (Figure 6). The improved wettability of seed surfaces treated with SB-CDs, evidenced by the reduced contact angle, facilitates faster and more uniform water uptake. This hydration promotes enzymatic activation and mobilization of stored nutrients necessary for germination. Additionally, the hydrophilic functional groups on SB-CDs can interact with seed coat proteins, modifying surface tension and enabling better diffusion of oxygen and signaling molecules essential for embryo activation (Li et al. 2023). Therefore, the physicochemical changes induced by SB-CDs contribute synergistically to the biochemical events underlying enhanced germination. Contact angle, TGA, and SEM analyses were performed at a carbon dot concentration of 0.1 mg/mL.

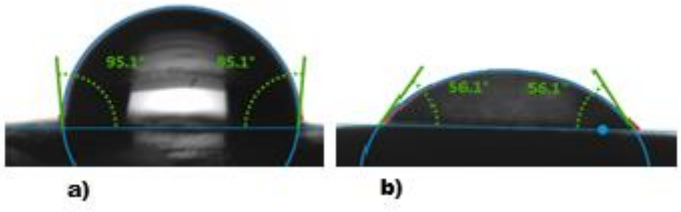


Figure 6- Contact angle measurement of corn seeds soaked in Milli-Q water (a) and 0.1 mg mL^{-1} SB-CDs solution (b)

The hydrophilic functional groups (such as carboxyl, hydroxyl, and amine groups) on the surface of SB-CDs may have adsorbed onto the seed surface, causing an increase in surface energy. This situation allowed water droplets to spread more on the surface, thereby decreasing the contact angle (Parvate et al. 2020). These physicochemical interactions of SB-CDs with the surface represent a positive change that can enhance the water uptake potential of the seeds and, consequently, increase their germination

efficiency. It can be said that the surface of corn seeds treated with SB-CDs becomes more wettable (hydrophilic). This can positively impact the germination process by enhancing the seed's water uptake capacity. Thus, it is understood that SB-CDs can provide potential contributions to agricultural applications by creating physicochemical changes on the seed surface.

According to the TGA analysis results, it was determined that the water retention capacity of corn seeds kept in 0.1 mg/mL SB-CD solution increased compared to the control group. In the TGA analysis, water loss was evaluated in the temperature range of up to 200 °C, and the difference shows that corn seeds treated with SB-CDs retained more water, i.e., had a higher water retention capacity (Figure 7).

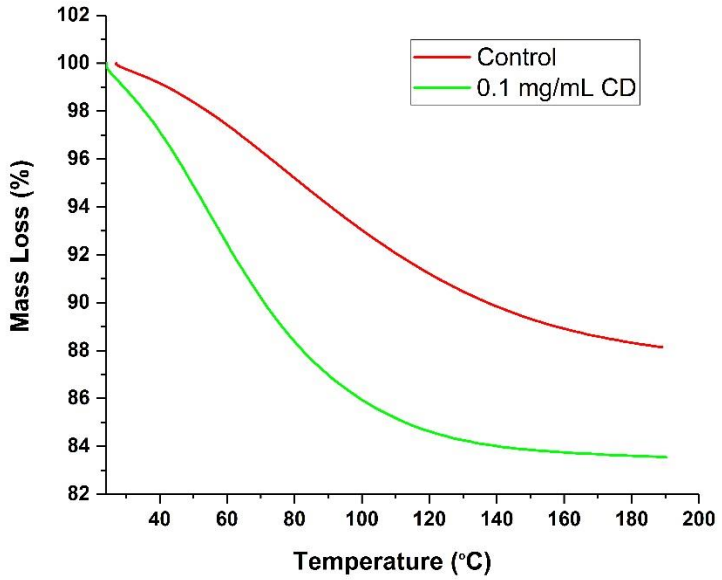


Figure 7- Thermogravimetric analysis of corn seeds that were kept in Milli-Q water (control) and 0.1 mg mL^{-1} SB-CDs solution

CDs have a large number of functional groups, including hydroxyl (–OH), carboxyl (–COOH), and amine (–NH_2). These groups increase hydrophilic interactions by forming hydrogen bonds with water molecules. When CDs are adsorbed on the seed surface, they can better hold water molecules thanks to these hydrophilic groups. Meng et al. (2019) demonstrated that the surface functional groups of CDs enhance water retention capacity. It is also known that CDs can penetrate plant cells and change cell wall permeability. This allows water to be retained more effectively within the cell. Meng et al. (2019) and Liang et al. (2023) reported that CDs slow down water loss by affecting intracellular water Dynamics.

In TGA analysis, water loss up to 200 °C provides direct information about the water retention capacity of the seed. These findings show that SB-CDs increase the seed's ability to bind water, with this effect occurring at both the surface and cellular levels, allowing seeds to better adapt to humid environments. This feature can provide a significant advantage, especially in developing drought-resistant seeds or seed storage technologies. Enhanced seed surface wettability and water retention indicate that SB-CDs can improve water-use efficiency, which is a crucial trait for crops grown under semi-arid or water-limited conditions. By increasing hydrophilicity and promoting rapid hydration, SB-CD treatment could be integrated into seed-priming technologies to improve germination uniformity and field emergence, ultimately supporting sustainable agricultural production.

Morphological changes observed in SEM images of corn seeds after priming in carbon dot solution showed significant differences, especially in terms of surface structure and starch vesicles. It was observed that the surface morphology of the seeds soaked in pure water was generally regular, compact, smooth, and had a natural seed coat (testa) structure. It was also determined that microcracks were few or nonexistent, and the pore structure was limited. In addition, in the control group, starch granules appeared compact, regular in shape (mostly polygonal or round), and tightly packed. The granules were stable, and the surrounding cell membranes were intact. The granules were observed to swell less than the SB-CD-treated group (Figure 8a,c).

In the seeds soaked in the SB-CD solution, changes were observed on the surface. SB-CDs interacted with the surface, increasing porosity. It was determined that the surface roughness increased after swelling, microcracking, or softening in the cell walls. This can accelerate water absorption. Additionally, it was observed that the starch granules in the SB-CD group were swollen, and their surfaces were irregular. SB-CDs increased cell wall permeability, supporting more water absorption. Parvate et al. (2020) also stated that seeds primed with carbon dots increased their water retention capacity by enhancing cell surface permeability, resulting from the expansion and sometimes disintegration of starch granules (Figure 8b, d).

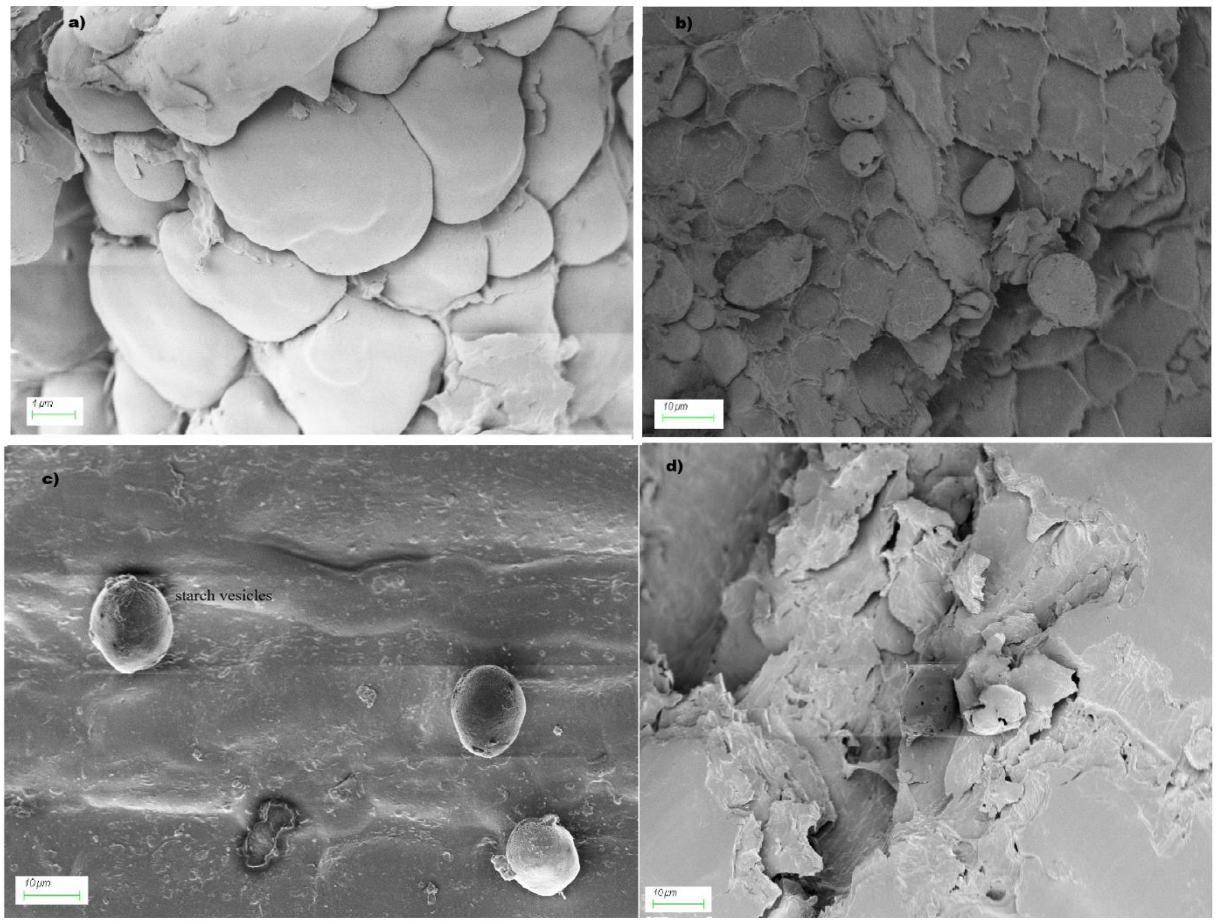


Figure 8- SEM images of corn seeds soaked in Milli-Q water (a: starch vesicles and c: seed surface) and 0.1 mg mL^{-1} SB-CDs solution (b: starch vesicles and d: seed surface)

Overall, the results demonstrate that the synergistic combination of *Spirulina* and banana peel-derived carbon dots can serve as a sustainable and eco-friendly nanobiostimulant for agricultural use. Their antioxidant capacity, enhancement of seed surface hydrophilicity, and promotion of root and shoot elongation collectively contribute to improved seed performance. These findings align with current efforts to develop environmentally compatible nanotechnologies that enhance crop productivity while reducing dependence on chemical fertilizers and pesticides. The stimulatory impact of low-dose SB-CD priming has direct agronomic importance beyond laboratory germination responses. Improved early root and shoot growth is strongly associated with enhanced seed vigor, faster field emergence, and better stand establishment under variable environmental conditions. Enhanced surface hydration and water-retention capacity, as observed in this study, may provide a practical advantage in rain-fed or drought-prone agricultural systems where rapid and uniform emergence is critical for yield. To improve crop establishment and resilience under field conditions, large-scale seed-priming methods may be used to enhance SB-CD-mediated improvements in early seed performance. Therefore, SB-CDs represent a promising green approach for seed priming and early plant growth enhancement in modern sustainable agriculture.

4. Conclusions

SB-CDs were successfully synthesized from *Spirulina* and banana peel using a green hydrothermal process, resulting in carbon dots (10–15 nm) enriched with hydroxyl, carboxyl, and amine groups that enhanced antioxidant and biological performance. The synthesized SB-CDs exhibited a high free radical scavenging activity of 87.72%, confirming their strong antioxidant potential. Seed priming experiments demonstrated that SB-CDs significantly promoted corn seed germination and early growth in a dose-dependent manner, with an optimum concentration of 0.1 mg mL^{-1}. At this dose, root and shoot elongation increased approximately 2.5–3 times compared to the control ($P<0.05$), whereas higher concentrations caused growth inhibition, indicating a hormetic response pattern. Contact angle and TGA analyses revealed that SB-CD treatment enhanced surface hydrophilicity and water retention capacity, thereby improving seed hydration and germination efficiency. Overall, these results demonstrate that SB-CDs derived from sustainable biomass sources can act as safe, natural, and effective nanobiostimulants, supporting seed vigor and stress tolerance at low concentrations. Determining such optimal nanomaterial doses is critical for the safe and efficient integration of carbon dots into agricultural biotechnology and crop enhancement applications. Future studies should move beyond laboratory germination tests to evaluate the agronomic performance of SB-CD-primed seeds under field conditions and abiotic stress factors (e.g., drought, salinity, or temperature extremes), in order to validate their potential for scalable and sustainable agricultural applications.

Author Declarations

Authors' contributions: Concept: T.Ş., E.A., Design: T.Ş., E.A., Data collection: T.Ş., E.A., Analysis: T.Ş., E.A., Writing: T.Ş., E.A.,

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