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Chitosan-biochar composites as eco-friendly seed coatings for sustainable agriculture

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ABSTRACT

The synergistic potential of chitosan (CS) and biochar (BC) composites for seed coating remains underexplored. This study aimed to develop and evaluate novel eco-friendly wheat seed coatings based on CS-BC composites to enhance germination, seedling vigor, and soil health under controlled conditions, addressing a critical gap in sustainable seed technology. Coatings were prepared by dissolving chitosan in acetic acid and blending with biochar at specific weight ratios (BC-CS 1:1, 2:1, 4:1). Wheat seeds (Barakatli-95) were dip-coated, dried, and sown in both soil-perlite mixtures and Petri dishes. Germination parameters (germination energy – GE, germination percentage – GP, germination index – GI) and soil organic carbon (OC) were evaluated over 14 days. Results demonstrated that BC-CS composites significantly outperformed both control and CS-only treatments. The BC-CS 4:1 formulation yielded the highest improvements: GE increased by 30% (87.8% vs. 67.6%), GP by 20% (89.5% vs. 74.8%), and GI by 66% (18.6 vs. 11.2) compared to the control. Soil OC content also increased by 12%. This synergistic enhancement is attributed to biochar improving soil structure and nutrient/water retention, while chitosan boosts seed vigor and provides antimicrobial protection. We conclude that BC-CS 4:1 is an optimal ratio for significantly boosting wheat seed performance. While promising, these findings are based on controlled lab conditions; field validation under diverse stresses is essential. These coatings offer substantial practical value by potentially reducing synthetic agrochemical reliance and enhancing climate resilience through improved soil health. This study presents the novel application of a CS-BC composite as a seed coating, demonstrating significant synergistic benefits for sustainable agriculture.

Keywords: climate change, sustainable agriculture, seed coating, chitosan, biochar, seed germination, soil health, eco-friendly farming.

INTRODUCTION

Global environmental challenges – such as climate change, soil degradation, water scarcity, and pollution—have intensified the urgency to develop sustainable agricultural practices that ensure food security under increasingly unpredictable conditions. Among these, improving seed germination and seedling establishment under abiotic stress has become a focal point in agricultural research and innovation (Pedrini et al., 2020).

Seed coating technologies, particularly film coating, have gained widespread acceptance due to their ability to protect seeds, deliver active compounds, and improve handling and planting efficiency (Halmer, 2000).

Film coatings consist of thin polymeric layers applied directly onto seeds and are often used to incorporate fertilizers, pesticides, biostimulants, or microorganisms. Studies have demonstrated that such coatings can significantly enhance germination rates and seedling growth, especially in stress-prone environments (Afzal et al., 2009). Bio-based seed coatings, as sustainable alternatives to synthetic polymers, are gaining attention for their agronomic benefits and reduced environmental impact. Coatings made from vermicompost and soy flour have improved nitrogen uptake and seedling vigor across various crops (Amirkhani et al., 2016). Similarly, bioplastic-based coatings not only enhanced seedling development but also reduced dust formation during seed handling (Accinelli et al., 2018). Chitosan, a naturally derived biopolymer from chitin, is widely known for its biodegradability, antimicrobial activity, and ability to induce plant defense mechanisms Khankishiyeva, et al. 2023). Research shows that chitosan coatings increase chlorophyll content, enhance nutrient uptake, and improve growth parameters under abiotic stress such as drought (Kocięcka and Liberacki, 2021). In soybean, chitosan seed treatments improved germination and vegetative growth, leading to higher yields (Khan et al., 2002). Moreover, when chitosan was combined with nanomaterials such as magnetite nanoparticles, it significantly boosted germination rates and stress resistance in wheat (Parfenova et al., 2020).

Biochar is a solid, porous, and carbon-rich material produced by pyrolysis of organic biomass under limited oxygen conditions. It has garnered attention not only as a carbon sequestration strategy but also as a soil amendment to enhance agricultural productivity. By improving soil structure, increasing nutrient retention, and enhancing water-holding capacity, biochar can play a vital role in climate-resilient agriculture (Gao et al., 2019). Numerous studies have demonstrated the beneficial effects of biochar on crop performance under both optimal and stress conditions. Chen et al. (2025) reported that biochar applications improved plant growth characteristics and yield even under stressful environments. In tomatoes, biochar application mitigated drought-induced damage by improving water uptake and enhancing physiological traits such as chlorophyll content and biomass accumulation (Zhang et al., 2023).

Biochar's impact on nutrient dynamics is another key factor in its agronomic value. In a study on pumpkin plants under drought stress, the application of 20 t ha^{-1} of biochar significantly increased nutrient absorption and chlorophyll levels (Langeroodi et al., 2019). Similarly, Hossain et al. (2020) found that biochar-amended soils promoted higher levels of nitrogen, phosphorus, and magnesium uptake in plants. In comparison to traditional chemical fertilizers, biochar application not only increased the organic matter content and macronutrient availability but also led to overall soil quality improvement (Luo et al., 2025). These benefits contribute to more sustainable and resilient cropping systems by reducing dependency on chemical inputs while enhancing yield potential. In seed coating applications, biochar improves germination and root development, especially under saline and drought conditions (Zhang et al., 2023).

However, the effectiveness of biochar-based coatings depends on the type of binder used. While polyvinyl acetate (PVAc) may negatively affect germination, more eco-friendly alternatives such as inactivated yeast offer improved compatibility (Thomas et al., 2024). Despite extensive research on chitosan and biochar individually, their combined application in seed coating remains underexplored. Integrating the antimicrobial and physiological benefits of chitosan with the structural and sorptive advantages of biochar may lead to synergistic effects, enhancing germination, stress tolerance, and early plant development under adverse conditions. The objective of this study is to develop a novel bio-based seed coating formulation by combining chitosan and biochar and to evaluate its effectiveness in enhancing wheat seed germination and early growth under abiotic stress. This study aims to:
- determine the optimal chitosan-to-biochar ratio for maximum germination,
- evaluate their combined effect on seedling vigor and stress resistance,
- propose a scalable, eco-friendly formulation for agricultural applications.

Through this approach, the research intends to fill a key knowledge gap in sustainable seed coating technologies and provide a foundation for future agronomic innovations.

MATERIALS AND METHODS

Materials

Biochar synthesis was carried out with slight modifications based on the method reported by

Williams et al. [Williams et al. (2016)]. The synthesis process was conducted at the Research Institute of Crop Husbandry (Azerbaijan). The chitosan (CS) used in the experiments was medium molecular weight, obtained from Sigma-Aldrich (degree of deacetylation > 75%, viscosity 200–800 cps), and was used in powder form without further processing. Barakatli-95 (*Triticum aestivum* L.), a local wheat variety known for its high productivity and resilience, was selected to investigate the impact of chitosan-modified biochar on seed germination and early growth.

Modification of biochar

To prepare the chitosan solution, 2.0 g of chitosan was dissolved in 4 mL of glacial acetic acid and then diluted with 100 mL of deionized water under constant stirring. Subsequently, 2 g, 4 g, and 8 g of biochar were separately added to the solution to form three different suspensions. Each mixture was stirred at 340 rpm at 30 °C for 8 hours [Mehmood, et.al. 2020]. After stirring, the mixtures were filtered, and the solid residues were thoroughly washed with distilled water. Then, each residue was re-suspended and stirred for an additional 30 minutes to ensure homogenization. The resulting suspensions were added to 900 mL of 0.1 N NaOH solution and left for 12 hours, pH reached 5.5–6.0.

At this stage, wheat seeds were introduced into the suspensions and soaked for 2 hours. After soaking, the seeds were removed and dried in an oven at 24 °C for 8 hours. To investigate the influence of biochar content on the seed coating performance, different amounts of biochar were incorporated into the chitosan matrix, as shown in Table 1. Preparation steps for chitosan-modified biochar seed coatings are illustrated in Figure 1.

Seed coating using chitosan–biochar dip method

Wheat seeds Barakatli-95 (*Triticum aestivum* L.) were immersed in each coating formulation for 10 minutes at ambient temperature (~22–25 °C). The seeds were then drained to remove excess liquid and dried in a ventilated oven at a temperature

Table 1. Composition of biochar–chitosan seed coating formulations used in the study

Sample code	Composition label	Biochar (g)	Chitosan (g)
S1	Control	-	-
S2	Chitosan	-	2
S3	BC-CS 1-1	2	2
S4	BC-CS 2-1	4	2
S5	BC-CS 4-1	8	2

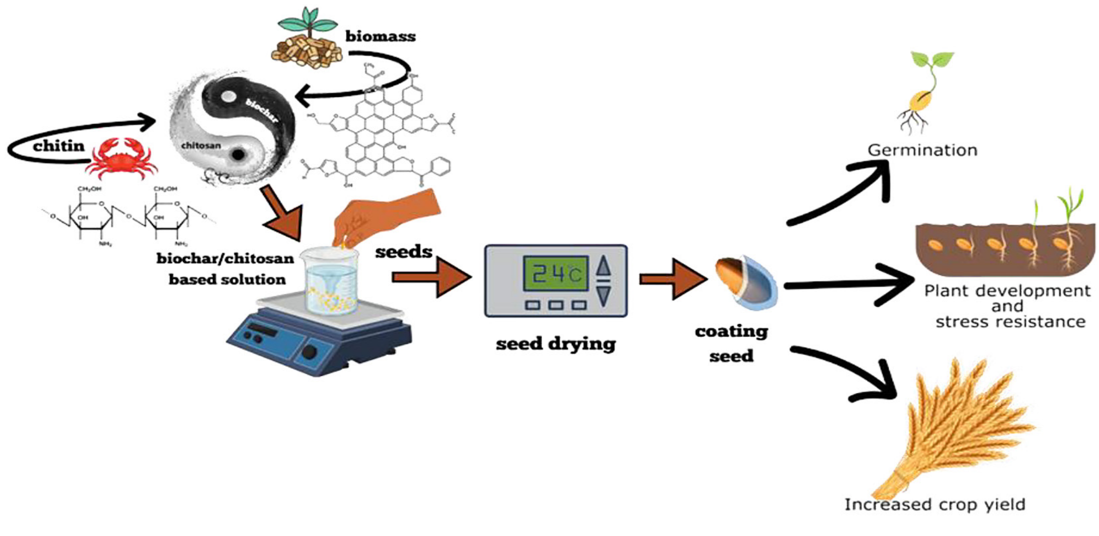

Figure 1. Schematic representation of the biochar/chitosan-based seed coating process and its effects on plant growth and crop yield

not exceeding 40 °C to preserve seed viability (Accinelli et al., 2018; Langeroodi et al., 2019). For all treatments, including control (uncoated) and chitosan-only groups, coated seeds were stored in a controlled growth chamber at 24 ± 1 °C and 65 ± 5% relative humidity. Germination tests were conducted over a 7-day period using standard procedures in Petri dishes lined with moistened filter paper (ISTA, 2020 protocol).

Storage of control seeds

The control seeds of each species were stored at −20 °C with a moisture content of 5–6%, following the long-term storage conditions of the seed gene bank. Prior to storage, seeds were dehydrated to 5–6% moisture by placing them over silica gel in closed plastic boxes at a 1:5 (seed:silica gel) ratio at room temperature. The seeds were weighed before dehydration, and the silica gel was used to ensure effective moisture reduction (Riseh et al., 2024). All samples were stored in a growth chamber maintained at 24 °C and 65% humidity. Germination was observed over a period of 7 days. Key parameters assessed included germination percentage, germination energy, and vigor index, which were calculated to evaluate the effectiveness of the treatments.

Germination tests

Germination tests were carried out over a ten-day period at a constant temperature of 25 °C under uniform illumination (2400 lux). Twenty-five seeds were placed evenly on glass Petri dishes lined with moistened tissue paper, arranged in a 5 × 5 grid to ensure full surface coverage. Dedicated dishes and lids were used throughout the experiment to maintain consistent conditions. Prior to germination, the seeds underwent cold stratification for 48 hours at 4 °C in light-protected conditions. Following this, the dishes were covered to maintain humidity and placed under a controlled photoperiod (16 hours light: 8 hours dark). The moisture content of the substrate was continuously monitored during the germination period. In the early stages, particular attention was given to assessing the effect of the coating's thickening agent on seed germination rate.

After 10 days, germinated seedlings were evaluated based on several morphological and physiological parameters, including root length, shoot length, fresh biomass of sprouts, and chlorophyll content. Chlorophyll content was measured using an OPTI-SCIENCES CCM-300 chlorophyll meter (Hudson, NH, USA).

Germination potential was assessed for both treated and untreated (control) seeds in accordance with the International Rules of Seed Testing (ISTA, 2023) and the guidelines of the Association of Official Seed Analysts (AOSA). A germination percentage (GP) of ≥ 80% is considered acceptable for certified wheat seeds. Since commercial seed lots may contain non-viable seeds, using a larger sample size improves the accuracy of germination calculations.

The formulas used to evaluate seed germination and vigor are summarized in Table 2.

Table 2. Calculation formulas for seed germination and vigor parameters

	Formula	Description / Variables
Germination percentage (*GP*)	$GP = \frac{N_g}{N_t} \times 100$	N_g – number of germinated seeds N_t – total number of sown seeds
Germination rate (*GR*)	$GR = \sum_{i=1}^{d} \frac{n_i}{D_i}$	n_i – number of seeds germinated on day i D_i = day i
Coefficient of velocity (*CV*)	$CV = \frac{\sum N_i}{\sum N_i T_i} \times 100$	N_i – number of seeds germinated on day *i* T_i – time (in days) for each count of germinated seeds
Germination energy (*GE*)	$\mathrm{GE} = \left(\frac{N_4}{N_t}\right) \times 100$	N_4 – number of seeds germinated on the 4th day N_t – total number of seeds sown
Germination index (*GI*)	$GRI = \frac{G_1}{1} + \frac{G_2}{2} + \frac{G_3}{3} + \cdots + \frac{G_i}{i},$	G_i – seeds germinated on day *i*
Seed vigor index (*SVI*)	$SVI = (PL + RL) \times GP$	*PL* – shoot length *RL* – root length *GP* – germination percentage

RESULTS AND DISCUSSION

The application of biochar-chitosan (BC-CS) composites led to clear improvements in wheat seed germination and early seedling growth compared to both the untreated control and the 1% chitosan-only treatment. As shown in Table 3, all germination parameters – germination energy, final germination percentage, and germination index—increased steadily with higher BC-CS concentrations, with the 4:1 ratio yielding the most favorable results.

The experimental results clearly demonstrate the positive effect of BC-CS composites on wheat seed germination and early seedling development. As observed visually (Figure 1), germination performance varied significantly across treatments, with marked differences between the control, individual chitosan application, and increasing concentrations of BC-CS composites. Specifically, the BC-CS 4-1 treatment recorded a germination energy of 87.8 ± 1.1%, a germination percentage of 89.5 ± 1.2%, and a germination index of 18.6 ± 0.3%, indicating that a composite formulation incorporating.

The Figure 2. shows variations in shoot and root development among seedlings. S1 and S2 groups exhibit stunted growth and poorly developed root systems, suggesting suboptimal or inhibitory conditions. In contrast, S3, S4, and especially S5 demonstrate progressively improved shoot elongation and enhanced root architecture, indicating favorable germination and early growth conditions. These visual differences highlight the potential impact of varying treatments or environmental conditions on early plant development (Table 4).

In the control group, germination was significantly suppressed. Only a few seeds showed radicle emergence, and visible fungal contamination (mold) developed on the moist surface, indicating a non-sterile and biologically unfavorable environment. The presence of mold likely competed with seeds for moisture and oxygen, impeding germination and early growth. This observation aligns with earlier findings that untreated media

Table 3. Germination parameters of treated and control seeds

Treatment	Germination energy (%)	Final germination (%)	Germination index
Control	67.6 ± 1.6	74.8 ± 1.3	11.2 ± 0.7
1% Chitosan	76.2 ± 1.2	79.8 ± 1.2	16.6 ± 0.5
BC-CS 1-1	82.1 ± 0.9	87.5 ± 1.2	17.2 ± 0.4
BC-CS 2-1	83.1 ± 1.2	84.3 ± 1.1	17.5 ± 0.5
BC-CS 4-1	87.8 ± 1.1	89.5 ± 1.2	18.6 ± 0.3

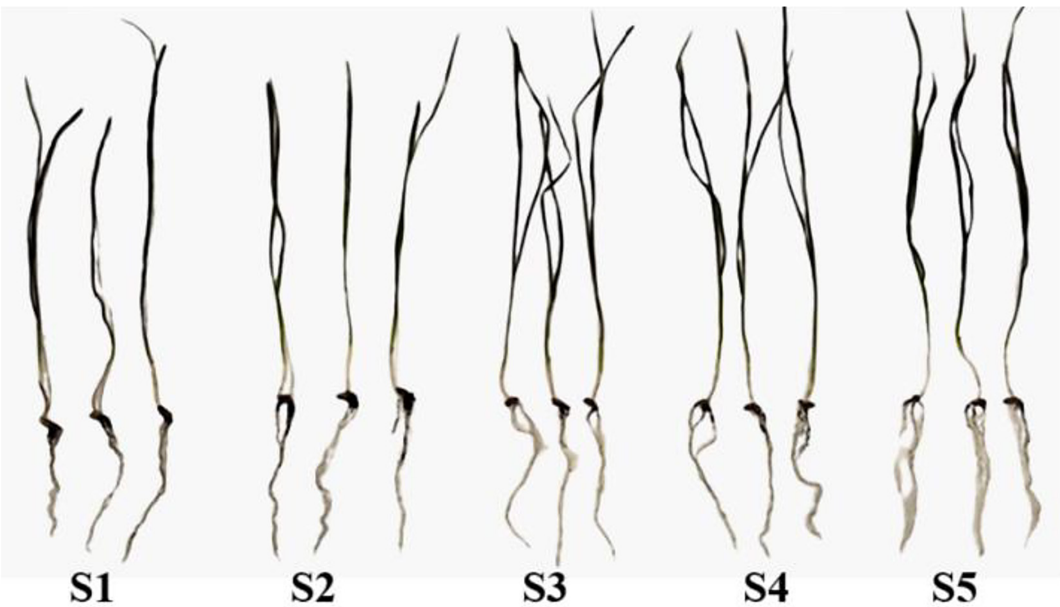

Figure 2. Phenotypic comparison of seedling growth under different treatment conditions (S1–S5)

Table 4. Seedling morphological parameters and mold incidence

Treatment	Plant weight (mg)	Shoot length (cm)	Shoot weight (mg)	Root length (cm)	Root weight (mg)	Moldy seeds (per 100)
Control	65–85	12.0–14.0	32–45	6.5–8.5	18–28	8–9
1% Chitosan	75–95	13.5–16.0	40–55	8.5–10.5	26–36	6–7
BC-CS 1-1	85–105	14.5–17.0	50–62	9.0–11.0	30–38	4–6
BC-CS 2-1	90–110	15.0–18.5	54–65	9.5–11.5	32–40	3–5
BC-CS 4-1	95–115	16.0–20.0	58–70	10.0–12.0	35–42	2–3

often foster microbial growth detrimental to seedling establishment (Ramos et al., 2020) (Figure 3).

Compared to the control group (S1), the 1% chitosan treatment (S2) showed a gentle but noticeable improvement in seedling development. Germination rates increased slightly, and mold incidence appeared lower – likely due to chitosan's natural antimicrobial activity and its role in triggering the plant's innate defense mechanisms, such as the production of protective proteins and reactive oxygen species (El Hadrami and El Hadrami, 2009; Malerba and Cerana, 2016). Still, these improvements were limited, suggesting that chitosan alone may not fully optimize conditions for early seedling growth.

More pronounced effects were seen with BC-CS composite coatings (S3–S5). Seedlings treated with BC-CS 1:1 and 2:1 formulations exhibited gradual enhancements in root and shoot development. Among them, the BC-CS 4:1 treatment (S5) stood out: nearly all seeds germinated, and the resulting seedlings displayed vigorous shoots, robust roots, and no visible mold. These visual results point to a synergistic effect between biochar and chitosan—biochar helps improve aeration, moisture retention, and nutrient dynamics, while chitosan adds antimicrobial protection and primes early growth responses.

The results presented in Figure 4 reveal clear differences in pigment concentrations between

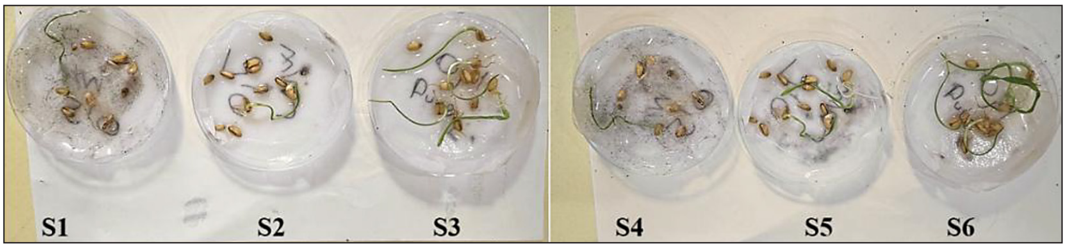

Figure 3. Morphological comparison of wheat seedlings after 7 days of germination under different treatments (S1–S5)

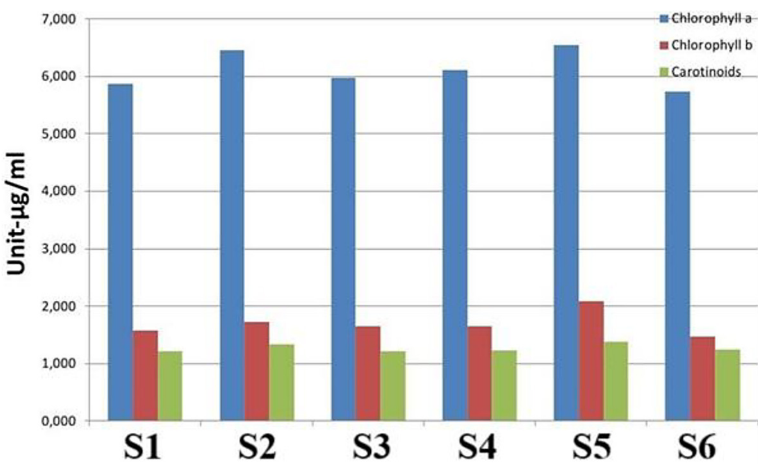

Figure 4. Comparative amounts of chlorophyll-a, chlorophyll-b, and carotenoids across treatments (S1–S6)

treatments. Chlorophyll-a was dominant in all samples, ranging from approximately 5.900 to 6.500 µg/ml, followed by chlorophyll-b (1.700–2.100 µg/ml) and carotenoids (1.200–1.500 µg/ml). These values align with the known prevalence of chlorophyll-a in photosynthetic tissues and its primary role in light harvesting (Lichtenthaler and Wellburn, 1983).

The control group (S1) exhibited the lowest pigment levels, which is consistent with reduced physiological activity likely caused by poor germination and fungal contamination. This observation supports previous findings linking microbial competition and suboptimal seedbed conditions with limited chlorophyll synthesis (Ramos et al., 2020).

The application of 1% chitosan (S2) produced a moderate increase in pigment levels, reflecting a mild stimulatory effect on early plant metabolism. Chitosan is recognized for enhancing plant defense and resilience through systemic resistance, involving antioxidant enzyme activity and improved chloroplast development (El Hadrami and El Hadrami, 2009; Malerba and Cerana, 2016). However, the pigment enhancement remained modest, suggesting that chitosan alone may not adequately mitigate early stress factors.

BC-CS composite treatments (S3–S5) led to significantly higher pigment concentrations. In particular, BC-CS 4:1 (S5) showed the most pronounced increase in chlorophyll-a, chlorophyll-b, and carotenoids. This indicates elevated photosynthetic capacity and healthier seedling physiology. The enhanced results likely stem from a synergistic interaction between biochar and chitosan: biochar improves substrate aeration, water retention, and adsorbs phytotoxins, while chitosan provides antimicrobial action and primes plant immune responses.

In conclusion, the data suggest that BC-CS 4:1 coatings significantly improve early photosynthetic pigment development, supporting robust seedling vigor. This confirms the dual benefits of the biochar–chitosan composite in protecting seeds and promoting physiological growth under potentially adverse conditions.

CONCLUSIONS

This study showed that BC-CS composite coatings significantly improved wheat seed germination, early seedling growth, and pigment levels under controlled conditions. The BC-CS 4:1 formulation yielded the best results across all measured parameters, including germination energy (87.8%), final germination (89.5%), and germination index (18.6). Treated seeds also showed longer shoots and roots, greater biomass, and minimal mold development.

The improved performance of BC-CS 4:1 is attributed to the complementary effects of its components—biochar enhancing moisture retention and soil aeration, and chitosan providing antimicrobial activity and early metabolic stimulation. Together, they created a favorable microenvironment for seedling establishment. These findings highlight the potential of BC-CS coatings as a sustainable alternative to synthetic seed treatments. Future research should focus on validating these results under field conditions and exploring the formulation's compatibility with nutrients or beneficial microbes. In conclusion, BC-CS 4:1 emerges as an effective and practical seed coating for promoting early wheat growth while reducing reliance on chemical inputs.

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