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Enhanced Seed quality and physio-biochemical parameters in lentil through Biochar and Humic Acid- Based Seed priming

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Abstract

Lentil (*Lens culinaris* Medik.) is a nutritionally valuable pulse crop that enhances food security and soil fertility through biological nitrogen fixation. However, its productivity is often constrained by poor germination and uneven seedling establishment. This study investigated biochar and humic acid as sustainable seed-priming agents to improve lentil seed performance. After preliminary optimization, the most effective concentrations of both agents were evaluated under controlled laboratory conditions. Germination percentage, seedling length, vigour indices, chlorophyll content, and oxidative stress response (DAB assay) were assessed. Both treatments significantly enhanced seed and seedling traits compared with unprimed controls ($p < 0.05$). Biochar priming increased germination by 14.7%, seedling length by 26.3%, and vigour index by 32.5% over control, alongside higher chlorophyll content and reduced hydrogen peroxide accumulation, indicating improved physiological efficiency and stress mitigation. Humic acid also improved performance, though to a lesser degree. The results demonstrate

that biochar and humic acid priming enhance seed vigour and metabolic activity by promoting stress regulation and efficient energy utilization. Overall, biochar emerged as the superior and eco-friendly option for improving germination and early growth in lentil, offering a practical approach for sustainable pulse production and climate-resilient agriculture.

Key words: Seed priming, biochar, humic acid, physio-biochemical and early seedling vigour.

Introduction

Lentil is a significant cool-season legume crop in India and ranks alongside chickpea terms of productivity and production [1]. Due to its short duration and early maturity, lentil fits well into various crop rotation systems and is commonly followed by summer crops such as mung bean (Vigna radiata), urad bean (Vigna mungo), or fodder crops like sorghum and maize, which effectively utilize residual soil moisture and nutrients [2]. In 2022, the worldwide production of lentils amounted to 6.65 million tons, representing a 17% rise from the previous year's 5.65 million tons. Lentil productivity is 1074 kg/ha in world and 870 kg/ha in India [3]. Lentil plays a significant role in improving soil health through its ability to fix atmospheric nitrogen in symbiosis with rhizobium bacteria, thereby enriching soil fertility and reducing the need for synthetic fertilizers [3]. Its extensive root system enhances soil structure by improving air circulation and water absorption, which is beneficial for subsequent crops [4]. Additionally, lentil residues play a role in enriching the soil's organic matter, promoting microbial activity, and aiding in the efficient cycling of nutrients. As a consequence, lentil is commonly included in crop rotations because of its positive influence on soil sustainability and productivity. Lentils are a food source that is rich in vitamins and minerals with notable concentrations of protein (20-30%) per 100 g of dry lentils, The food contains a low amount of carbohydrates that are easily digested (20%) a small quantity of Fat, Iron, Zinc and Vitamins. Lentil seed have 24-28% proteins, 30-40% minerals and 22% vitamin. It is predominantly cultivated in dry regions and frequently faces abiotic stresses (such as drought and salinity) during the early stages of plant growth. The rising levels of non-living environmental stress pose a significant threat to the global pulse production, as they are highly susceptible to these changes [5]. To address such stress-induced reductions in germination and seedling establishment, seed priming techniques have gained attention for improving seed vigour and stress

tolerance. Among these, biochar and humic acid have emerged as promising eco-friendly priming agents known to enhance water uptake, enzyme activation, and antioxidative metabolism in various legumes, including chickpea and soybean [6,7]. However, limited information is available on their comparative effects and optimal concentrations for lentil seed priming. Therefore, this study aimed to evaluate the influence of biochar and humic acid priming on germination behaviour, seedling vigour, and oxidative stress modulation in lentil, thereby addressing a critical research gap in sustainable pulse production systems

It is mainly cultivated in temperate and subtropical areas, although it can also thrive in certain tropical highland regions. Major lentil-producing countries such as India, Canada, Australia, and turkey represent diverse Agro-climatic zones, ranging from subtropical plains to temperate uplands, indicating the crop's broad adaptability (3). It is moderately susceptible to different environmental factors, which can greatly impact its growth and productivity. The crop is highly susceptible to high temperatures during the flowering and pod-filling stages, which can result in flower abortion and a decrease in seed production, ultimately reducing the overall yield [6]. It shows progresses through key stages: germination, seedling, vegetative growth, flowering, pod development, and maturity. Germination occurs within 4–7 days, and the full cycle completes in 90–110 days. Flowering is sensitive to heat and drought, affecting pod formation and final seed quality [7]. Seed sprouting is postponed or hindered avoidance of different non-living factors. Therefore, it is of utmost importance to find solutions for the challenges faced by early plants. The techniques for enhancing germination and quality of seedling are seed priming.

The chemicals used in seed priming have demonstrated positive effects, there are worries about their impact on the environment. This concern has led to a growing interest in natural and sustainable alternatives that can provide similar benefits without ecological risks. The urgent need for environmentally friendly compounds such as humic acid (H.A) [8,9] and biochar (B.C) is evident for sustainable agriculture. Biochar improves lentil growth by enhancing soil fertility, structure, water retention, and nutrient availability, especially in nutrient-deficient soils [10]. Humic acid enhances lentil growth by improving soil structure, water retention, and nutrient uptake, especially in nutrient-deficient

soils [11,12]. Both these organic amendments have shown potential in mitigating abiotic stress effects, promoting early seedling development, and improving crop performance in various legumes. However, their comparative influence on lentil seed quality and physiological responses under controlled priming conditions remains insufficiently explored [13]. Hence, the objective of the research was examining impact of chemical priming using biochar, humic acid on the quality of primed lentil seeds. Specifically, the study aimed to identify the most effective priming agent and concentration for enhancing germination, seed vigour, and stress tolerance in lentil, while maintaining environmental sustainability. This concise focus directly links the research objective to the broader context of improving lentil productivity through eco-friendly seed enhancement techniques.

Material and Methods

The experiment was conducted at laboratory at Amity Institute of Organic Agriculture, Amity University, Noida. IPL – 316 variety of lentil was taken from ICAR – IARI, New Delhi and this variety of lentil was used in the experiment (ambient condition). The laboratory conditions during experimentation were maintained at an ambient temperature of 25 ± 2 °C, relative humidity of 60–70%, and light intensity of approximately 120 µmol m^{-2} s^{-1} under a 12-hour photoperiod to ensure reproducibility of the results. The lentil variety IPL–316 was specifically chosen because it is moderately tolerant to abiotic stresses such as drought and salinity, making it suitable for evaluating the effectiveness of seed priming treatments aimed at enhancing stress resilience. After standardization (Table 1) from different combinations of biochar and humic acid at various durations and concentrations for seed priming (Fig. 2, 3). The selected concentrations of biochar (4%) and humic acid (1%) were determined based on preliminary standardization experiments that identified these levels as most effective in improving germination and seedling vigour compared to other tested concentrations (Table. 1). For each treatment, seeds were surface-sterilized with 1% sodium hypochlorite for 2 minutes, rinsed thoroughly with distilled water, and then soaked in the respective priming solutions for 18 hours at room temperature with continuous gentle agitation. After priming, the seeds were air-dried back to their original moisture content. Seeds were sown in sterilized germination trays using moistened filter paper under controlled laboratory conditions. Sowing was done at uniform spacing (2 cm × 2 cm) with three

replications per treatment. Regular monitoring of moisture and temperature was maintained throughout germination. Samples for physiological and biochemical analyses were collected on the seventh day after germination to ensure uniform developmental stage. (Fig. 6).

Key agronomic details

The field study was carried out under open field conditions at the experimental farm of Amity Institute of Organic Agriculture, Amity University, Noida (28.38°N, 77.12°E; 200 m above mean sea level). The soil was sandy loam in texture, with pH 7.6, organic carbon 0.48%, and available N, P, and K of 232, 21.5, and 228 kg ha^{-1}, respectively. Primed and control seeds were sown manually in 3 m × 2 m plots at a spacing of 30 cm between rows and 10 cm between plants. Each treatment was replicated three times in a randomized complete block design (RCBD). Recommended agronomic practices for lentil cultivation were followed uniformly, including basal application of organic manure, timely weeding, and irrigation as required. No chemical fertilizers or pesticides were applied during the experiment. Data on germination, growth, and physiological parameters were recorded at regular intervals, and samples for biochemical analysis were collected at 30 days after sowing (DAS) from randomly selected plants within each plot to ensure representative sampling.

Table 1. Different concentrations of biochar and humic acid standardization and after standardization.

Treatment	HA concentration (%)	BC concentration (%)	Duration (h)	Criteria used for selection	Selected for final experiment
T1	Control	Control		Untreated baseline	Included as absolute control
T2	1	1	18	Improved germination (>90%) and vigour index	Selected (18 h) for final study
T3	2	2	18	Slight improvement, minor root inhibition at 24 h	Not selected

| T4 | 3 | 3 | 18 | No significant benefit over 1%; seed coat darkening | Not selected |
| T5 | 4 | 4 | 18 | Reduced germination and delayed emergence | Selected (4%) only for comparison |

Germination Test (ISTA, 2024)

The seeds surface was sterilized with (1%) sodium hypochlorite. For the germination test, Petri plates were prepared using a double layer of filter paper to provide a uniform and moist base for seed placement. A total of 50 dry seeds were carefully arranged on each plate with temperature of 20ºC. The respective treatments were applied at regular intervals to ensure consistent exposure. The treatments were applied after 18 hours of chemical priming using a 1:1 ratio of solution to water to ensure uniform absorption and activation before germination evaluation. Observations were recorded initially on the 5th day and subsequently on the 10th day. These assessments allowed for the classification of seeds into normal, abnormal, and diseased categories based on their germination and seedling development characteristics. For the calculation of standard germination percentage, we used percentage of normal seedlings.

Seed Vigour Index

The 50 lentil seeds were placed on a double layer of moist filter paper in sterilized Petri plates and incubated at a constant temperature of 20°C in a controlled laboratory environment, following standard germination test procedures [15]. Moisture levels were regularly maintained with distilled water to avoid desiccation or waterlogging. Only healthy seedlings with well-developed roots and shoots were considered. On the 10th day, seedlings from each replication were randomly selected to assess seedling vigour by measuring root length, shoot length, and total seedling length using a precise centimetre-scale.

Vigour index I - Germination (%) × Total seedling length

Vigour index II - Germination (%) × Seedling dry weight

Root and shoot

On the 10th day, seedling root and shoot measurements were conducted to evaluate seedling growth and vigour. A measuring scale was used to measure the length of both the root and the shoot of each seedling. This assessment helped determine the overall seedling development under different treatments. By measuring these parameters, valuable insights into the effectiveness of the treatments on early seedling growth were obtained.

Chlorophyll content

The fresh seedling sample (1 gm) was carefully weighed and macerated with 3 ml of 80% acetone then the mixture was transferred into an Eppendorf tube and centrifuged at 10,000 rpm for 10 minutes. After centrifugation, the sample was collected, and absorbance readings were taken using a spectrophotometer at different wavelength viz., 470nm, 645nm, 652nm and 663nm to quantify the pigment content [12]. Here, V represents the final volume of the extract in milliliters (ml), and W denotes the fresh weight of the sample in grams (g). Chlorophyll content was expressed as milligrams per gram (mg g^{-1}) of fresh weight

$$Chl\ a = 12.9(Ab663) - 2.69\ (Ab645)\ x\ V/1000\ x\ W$$

$$Chl\ b = 22.9\ (Ab645) - 4.68\ (Ab663)\ x\ V/1000\ x\ W$$

$$Total\ Chl = Chl\ a + Chl\ b$$

Histochemical analysis of H_2O_2 by 3,3'diaminobenzidine (DAB)

The DAB staining solution was formulated based on the in-situ hydrogen peroxide detection techniques described by [16]. To prepare the DAB (3,3′-diaminobenzidine) staining solution with H_2O_2, 50 mg of DAB was dissolved in 45 mL of sterile distilled water to obtain a final concentration of 1 mg/mL. The pH was adjusted to approximately 3.0 using 0.2 M HCl to facilitate proper dissolution. Tween 20 was

added to achieve a final concentration of 0.05% (v/v) to enhance tissue permeability, followed by 2.5 mL of 200 mM Na_2HPO_4 for buffering and pH stabilization. The prepared solution was used for in-situ detection of hydrogen peroxide accumulation following the standard DAB staining protocol described by [16].

The DAB staining solution was formulated based on the in-situ hydrogen peroxide detection techniques described by [16]. To prepare the DAB (3,3′-diaminobenzidine) staining solution with H_2O_2, begin by adding 50 mg of DAB to 45 mL of sterile distilled water in a 50 mL Falcon tube, which will give you a final concentration of 1 mg/mL. Include a small magnetic stir bar to ensure everything mixes well. Since DAB needs acidic conditions to dissolve properly, slowly adjust the pH to about 3.0 using 0.2 M HCl while stirring. Once the DAB is completely dissolved, cover the tube with aluminium foil to protect it from light, as DAB is very sensitive to it. After that, add 25 μL of Tween 20 to achieve a final concentration of 0.05% (v/v), which helps with tissue penetration. Then, add 2.5 mL of 200 mM Na_2HPO_4 to buffer the solution and raise the pH to a suitable level for staining.

Statistical Analysis

For every treatment, we used 50 seedlings across three separate replications (n = 3). We calculated the standard errors (SEs) of the arithmetic means for each treatment. The data were analyzed through analysis of variance (ANOVA) and pairwise comparisons among treatments. We determined the means using Tukey's test at a significance level of p = 0.05, utilizing SPSS 16.0 from Chicago, IL, USA. **Before conducting ANOVA, data were examined for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. When necessary, percentage data were arcsine-transformed to meet ANOVA assumptions.**

Result

Germination Percentage, Shoot length and Root length

Seed priming with 1% humic acid resulted in the highest germination percentage (77%), which was significantly higher than the 2% and 3% treatments (both 63%). The control and 4% humic acid

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treatments recorded similar germination rates of around 70% (Fig. 1A). In contrast, biochar priming at 4% produced the significantly highest germination (90%), followed by 3% (80%), 1% (76%), and 2% (70%), while the control showed the lowest germination (63%) (Fig. 1A). After standardization, the 4% biochar treatment maintained the highest germination (86%), followed by 1% humic acid (80%), hydropriming (76%), and the control (68%) (Fig. 4). These contrasting responses between humic acid and biochar suggest a differential physiological influence on seed germination. The 1% humic acid treatment promoted optimal germination, while higher concentrations (2–4%) reduced performance. This decline at elevated concentrations could be attributed to osmotic or ionic imbalances induced by excessive humic compounds, which may hinder water uptake during imbibition or interfere with nutrient and hormonal signaling. In contrast, the positive response to 4% biochar likely reflects improved nutrient retention, aeration, and modulation of the seed microenvironment that enhanced germination efficiency.

Among the different humic acid treatments, 1% humic acid produced the significantly longest shoot length (7.2 cm) compared to the control (5.0 cm). The 2% treatment resulted in a shoot length of 4.3 cm, while the 3% and 4% concentrations recorded 3.5 cm and 2.4 cm, respectively (Fig. 1B). In the case of biochar priming, the 4% concentration resulted in the highest shoot length (9.1 cm), followed by 2% (8.8 cm), 3% (8.1 cm), and 1% (7.9 cm). The control exhibited the lowest shoot length (4.6 cm) (Fig. 1B). Humic acid priming treatments showed that seeds treated with 1% humic acid exhibited the longest root length, measuring 5.86 cm, which was significantly higher compared to the control treatment (4.4 cm). However, when the concentration of humic acid was increased to 3% (2.8 cm) and 4% (2.9 cm), the root length decreased. This reduction in root elongation at higher humic acid concentrations may be linked to possible nutrient imbalances, increased osmotic potential, or direct inhibitory effects on root meristem activity. Excess humic acid might alter ion homeostasis, restrict cell expansion, or introduce phenolic compounds that suppress elongation growth. The lowest root length was recorded at the 2% concentration (2.2 cm) (Fig. 1C).
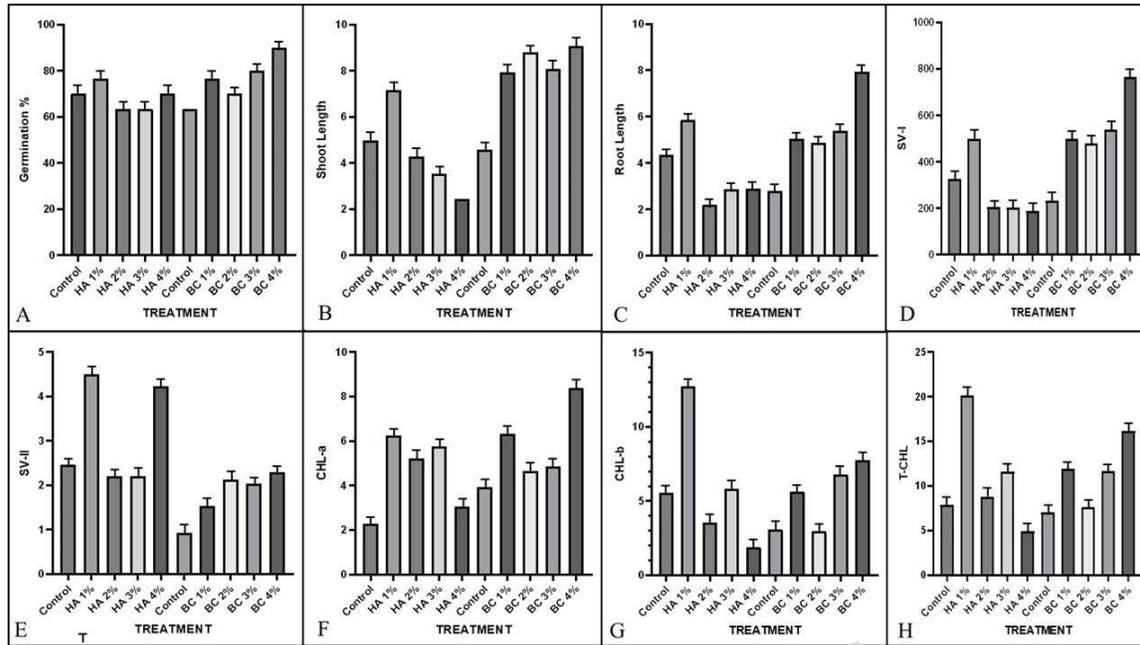
Fig. 1. Effect of the different priming agents (H.A and B.C) on the seed quality parameters; (A) germination percentage; (B) shoot length; (C) root length; (D) seed vigour index-I; (E) seed Vigour index-II; (F) chlorophyll a; (G) chlorophyll b; (H) total chlorophyll.
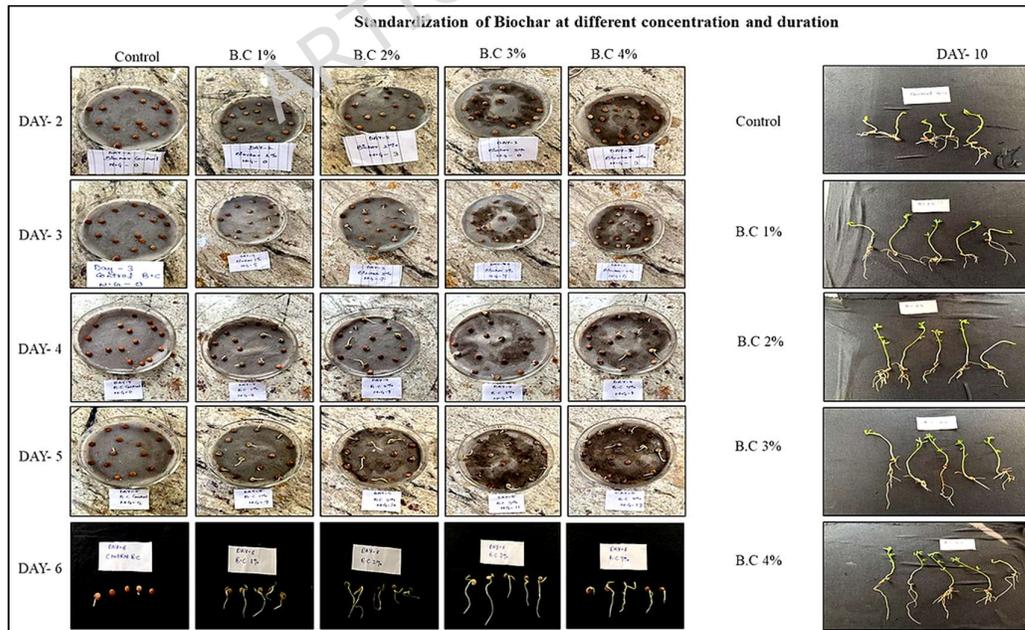
Fig. 2. Visualization of seedling at first and final count in different treatments and concentration of biochar
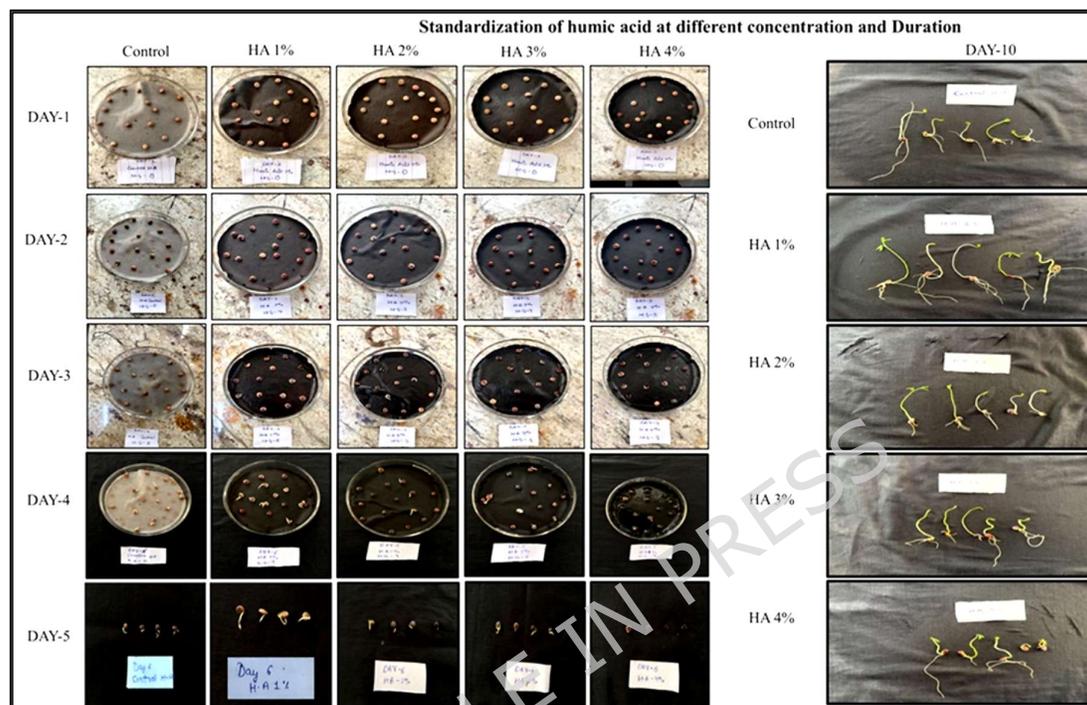

Fig. 3. Visualization of seedling at first and final count in different treatments and concentration of humic acid.

Average shoot length (ASL), Average root length (ARL), and Total seedling length (TSL)

After standardization, seed priming with 4% biochar produced the highest average shoot length (ASL) of 8.5 cm, followed by 1% humic acid at 8.0 cm and hydropriming at 6.7 cm, while the control recorded the lowest value at 4.3 cm (Fig. 4). For average root length (ARL), 4% biochar again showed the best performance (6.6 cm), followed by 1% humic acid (5.3 cm), hydropriming (4.6 cm), and the control (2.7 cm). In terms of total seedling length (TSL), 4% biochar remained the most effective treatment with a value of 7.6 cm, followed by 1% humic acid (6.6 cm), hydropriming (4.6 cm), and the control (2.7 cm) (Fig. 4).

Seed vigour index -I&II

Humic acid at 1% concentration resulted in the significantly highest Seed Vigour Index-I (SVI-I) of 500%, indicating a strong positive effect on early seedling growth. The control treatment showed moderate vigour at 326%, whereas higher concentrations of humic acid 2% (205%), 3% (202%), and 4% (187%) exhibited comparatively lower SVI-I values (Fig. 1D). In biochar treatments, seed priming with 4% biochar recorded the highest SVI-I at 765%, followed by 3% (540%), 1% (499%), and 2% (478%), while the control displayed the lowest value at 233% (Fig. 1D). These results indicate a concentration-dependent response, likely due to enhanced nutrient availability, improved water retention, and better physiological conditions under biochar treatment. After standardization, the 4% biochar treatment maintained the highest SVI-I (652%), followed by 1% humic acid (531%), hydropriming (429%), and the control (240%) (Fig. 5E).

Among the humic acid treatments, the 1% concentration recorded the highest Seed Vigour Index-II (SVI-II) of 4.3%, followed by the 3% treatment with 4.1%, both showing a strong potential for enhancing seedling vigour. The control recorded a lower SVI-II of 2.5%, while the 2% treatment showed an even lower value of 2.0% (Fig. 1E). In biochar priming, the 4% concentration produced the significantly highest SVI-II at 2.3%, followed by the 2% (2.1%) and 3% (2.0%) treatments. The 1% biochar treatment showed a comparatively lower value of 1.5%, while the control recorded the lowest SVI-II of 0.9% (Fig. 1E). After standardization, the 4% biochar treatment maintained the highest SVI-II (2.2%), followed by 1% humic acid (2.0%), whereas hydropriming and the control recorded lower values of 1.5% and 1.0%, respectively (Fig. 5F).

Although biochar priming, particularly at 4%, produced superior Average Shoot Length (ASL), Average Root Length (ARL), and Total Seedling Length (TSL), its SVI-II values were lower than those of 1% humic acid. This apparent discrepancy may be attributed to the higher seedling dry weight contribution in the humic acid treatment, which directly influences SVI-II. Biochar-primed seedlings, while morphologically vigorous, may have retained higher moisture or exhibited slower dry matter accumulation at the early growth stage, leading to relatively lower SVI-II values. Thus, the difference reflects a variation in biomass partitioning rather than a reduction in overall physiological vigour.
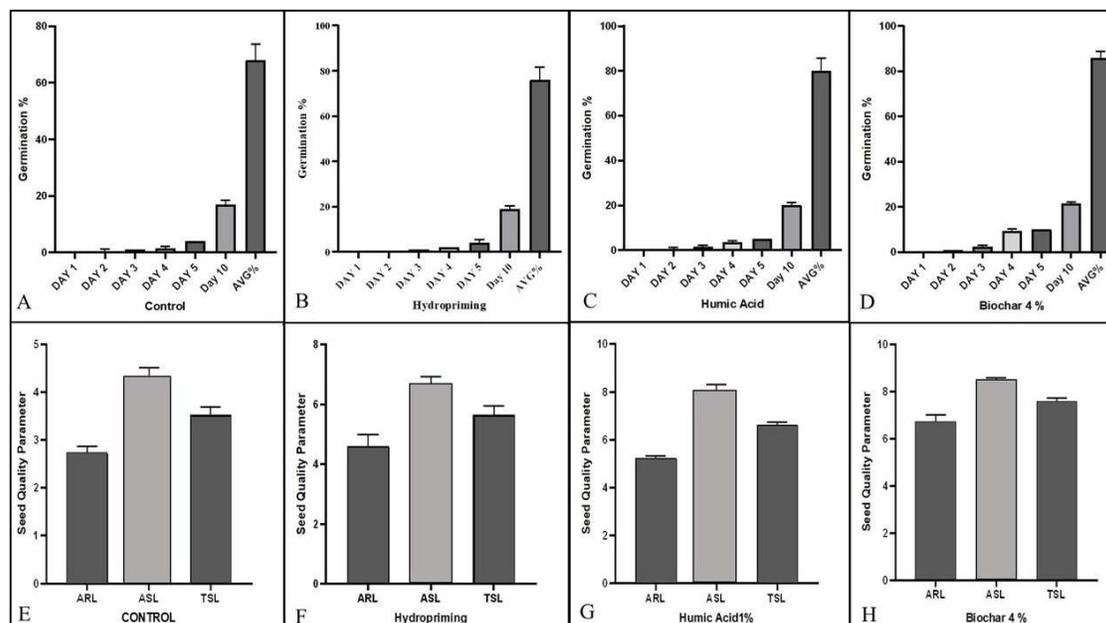
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Fig. 4. Effect of the priming agents (H.A and B.C) on the seed quality parameters after standardization; (A) germination percentage in control; (B) germination percentage in hydropriming; (C) germination percentage after standardization in humic acid 1%; (D) germination percentage in biochar 4% treatment ; (E) seed quality parameters in control (ARL, ASL and TSL); (F) seed quality parameters after hydropriming (ARL, ASL and TSL); (G) seed quality parameters after humic acid 1% (ARL, ASL and TSL); (H) seed quality parameters after biochar 4% (ARL, ASL and TSL).

Chlorophyll-a, Chlorophyll-b, Total-Chlorophyll

Seed priming with 1% humic acid resulted in the significantly highest chlorophyll-a content (6.3%), indicating enhanced photosynthetic efficiency. This was followed by 3% and 2% humic acid treatments, with values of 5.7% and 5.2%, respectively. Lower chlorophyll-a content was observed in the 4% treatment (3.1%) and in the control (2.2%). These findings suggest that humic acid priming, particularly at 1%, effectively increases chlorophyll-a levels, thereby promoting better plant health (Fig. 1F). Among the biochar treatments, the 4% concentration recorded the highest chlorophyll-a content (8.4%), likely due to improved nutrient retention and nitrogen availability. Biochar at 1% also enhanced chlorophyll-a (6.4%), while the 3% and 2% treatments showed moderate values of 4.9% and 4.7%, respectively. The control had the lowest content (3.9%) (Fig. 1F). After standardization, the 4% biochar

treatment maintained the highest chlorophyll-a content (8.0%), followed by 1% humic acid (6.2%), hydropriming (4.8%), and the control (3.9%) (Fig. 5).

Among the humic acid treatments, the 1% concentration recorded the highest chlorophyll-b content (12.7%), followed by 3% (5.8%). The control, 2%, and 4% treatments recorded lower values of 5.6%, 3.5%, and 1.9%, respectively (Fig. 1G). In biochar treatments, 4% biochar resulted in the significantly highest chlorophyll-b content (7.8%), followed by 3% (6.8%), 1% (5.6%), and 2% (3.0%), while the control showed the lowest value (3.1%) (Fig. 1G). After standardization, 4% biochar continued to record the highest chlorophyll-b content (9.1%), followed by 1% humic acid (8.9%), hydropriming (6.9%), and the control (3.1%) (Fig. 5).

Among the humic acid treatments, the 1% concentration produced the highest total chlorophyll content (TCC) of 20.2%, indicating a significant improvement in photosynthetic capacity. This was followed by 3% (11.5%) and 2% (8.8%), while the lowest contents were observed in the control (7.8%) and 4% humic acid treatment (5.0%) (Fig. 1H). In biochar treatments, 4% biochar resulted in the significantly highest total chlorophyll content (16.1%), followed by 1% (11.9%) and 3% (11.7%). The 2% and control treatments recorded the lowest values (7.0% and 7.6%, respectively). After standardization, 4% biochar maintained the highest total chlorophyll content (16.9%), followed by 1% humic acid (15.2%), hydropriming (11.7%), and the control (7.0%) (Fig. 5).

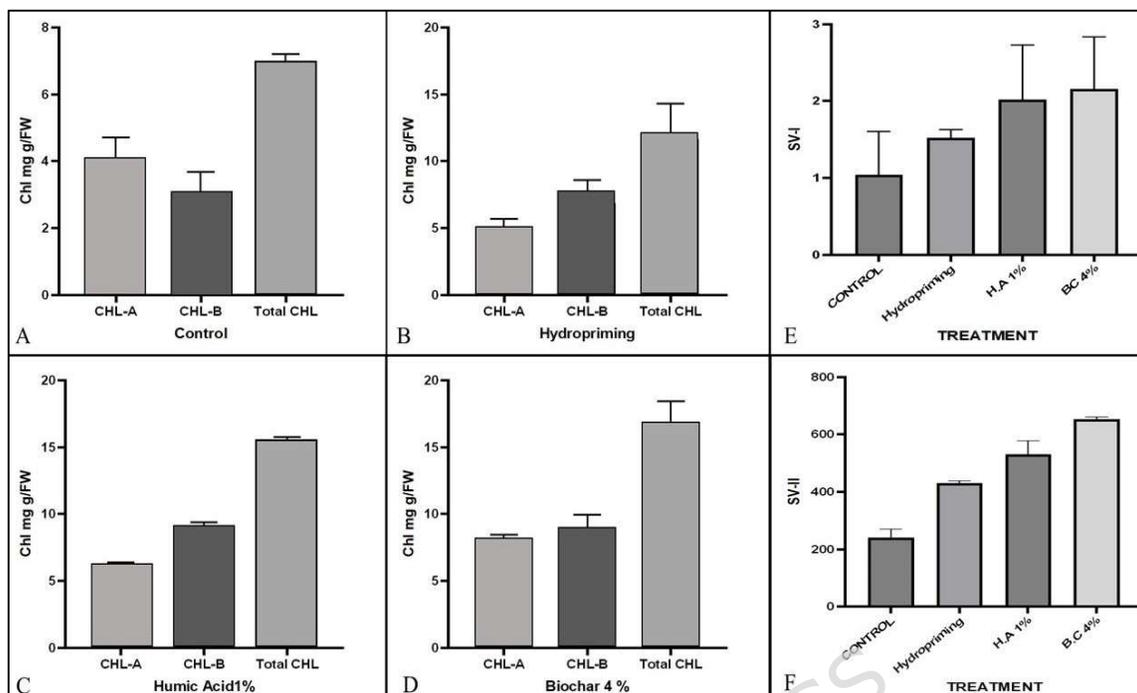**Fig. 5.** Effect of the priming agents (H.A and B.C) on the physio-biochemical parameters after standardization; (A) chlorophyll content in control; (B) chlorophyll content in hydropriming; (C) chlorophyll content in humic acid 1%; (D) chlorophyll content in biochar 4% treatment; (E) seed vigour index-I in all the treatment after standardization; (F) seed vigour index-II in all the treatment after standardization.
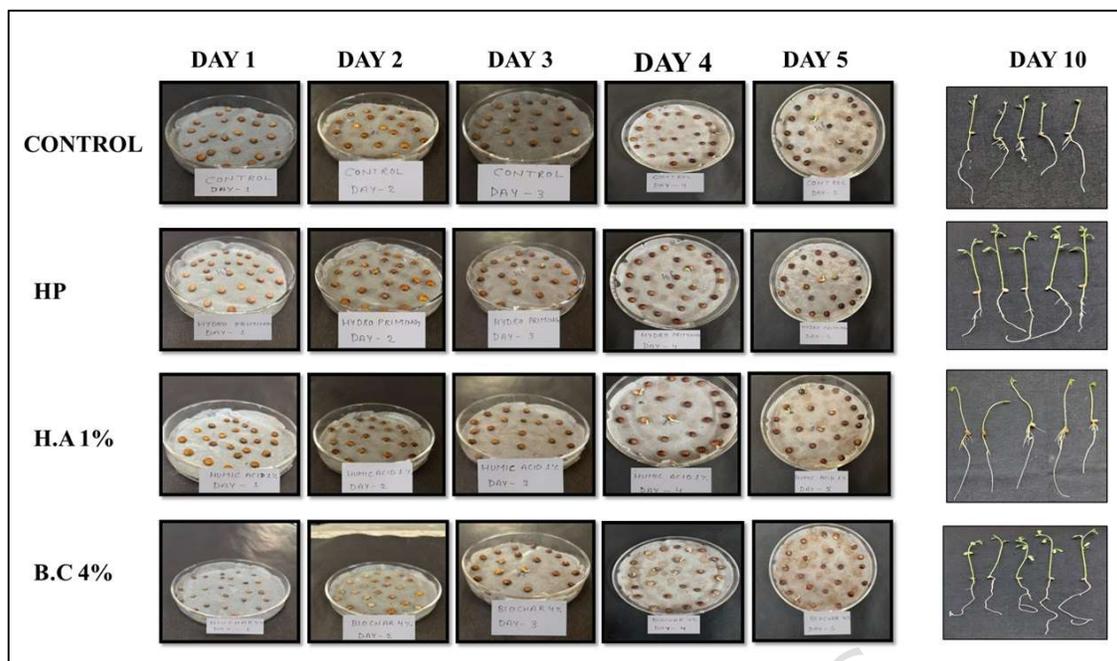
Fig. 6. Visualization of seedling at first and final count in different treatments and concentration of humic acid, biochar 4 %, hydropriming and control

Histochemical analysis of H_2O_2 by 3,3'diaminobenzidine (DAB)

Histochemical staining using DAB revealed distinct differences in H_2O_2 accumulation among the treatments (Fig. 7). The control seedlings showed light brown coloration, indicating a low level of oxidative stress. In contrast, hydroprimed seeds (HP 18 hr) exhibited a slightly deeper brown stain, suggesting moderate H_2O_2 accumulation. Seedlings primed with 1% humic acid (HA 1%) displayed comparatively lighter staining, indicating reduced oxidative stress and better maintenance of cellular redox balance. The 4% biochar (BC 4%) treatment showed the darkest brown coloration, representing the highest accumulation of H_2O_2. Semi-quantitative image analysis of DAB staining intensity using ImageJ confirmed these visual observations, showing that H_2O_2 levels were significantly lower in the 1% HA treatment and highest in the 4% BC treatment ($p < 0.05$). These results indicate that humic acid priming mitigated oxidative stress, whereas higher concentrations of biochar induced greater H_2O_2 accumulation, possibly due to stress responses associated with excessive nutrient availability or osmotic imbalance.
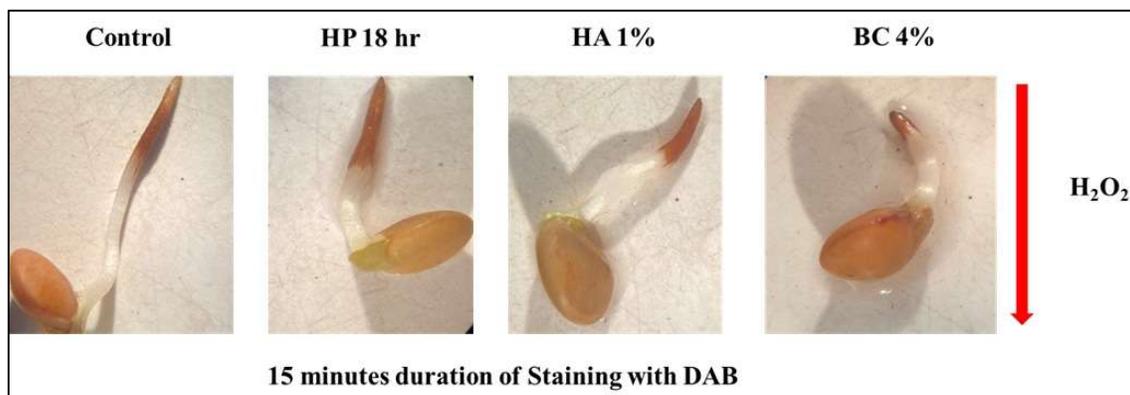
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Fig. 7. Histochemical analysis of H_2O_2 by 3,3'diaminobenzidine (DAB)

Pearson Correlation Analysis of different treatment on seed quality parameters

The Pearson correlation matrix revealed strong positive associations among most germination and seedling growth parameters (Fig. 8). Highly significant correlations were observed between average germination percentage (AVG%) and shoot length (ASL, $r = 0.98$), root length (ARL, $r = 0.96$), and seed vigour indices (SVI-I, $r = 0.92$; SVI-II, $r = 0.90$). Similarly, a strong interrelationship was noted between root and shoot length ($r = 0.99$), indicating their coordinated development during early seedling growth. Germination rates at different days also showed progressive and significant positive correlations, particularly from Day 3 to Day 10 ($r = 0.97$), suggesting consistent germination patterns across treatments. Biologically, these correlations imply that treatments improving early germination performance also tend to enhance subsequent seedling growth and vigour. The strong positive relationships between SVI, root, and shoot traits highlight that seedling length parameters can serve as reliable indicators of overall seed quality and vigour potential. However, these associations should be interpreted as indicative trends rather than direct causal effects.
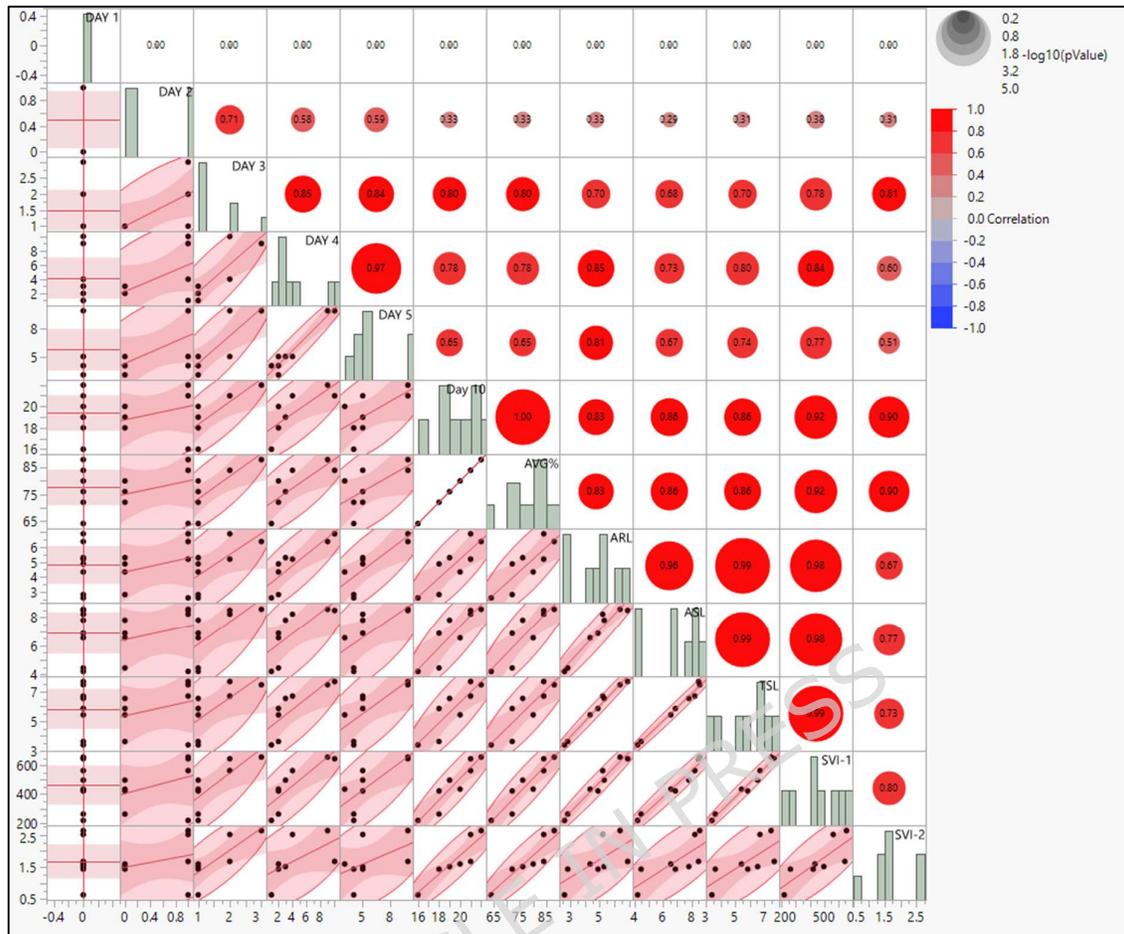
Fig. 8. Diagram of Pearson Correlation Analysis of different treatment on seed quality parameters.

Discussion

Seed germination rates were significantly highest in the 4% biochar treatment compared to 1% humic acid, while the control and hydropriming treatments recorded the lowest germination rates. The superior performance of biochar at higher concentrations can be mechanistically linked to its ability to improve the seed–soil microenvironment by promoting beneficial microbial colonization, enhancing nutrient mineralization, and stabilizing moisture and temperature conditions [17]. These effects collectively stimulate early metabolic reactivation during imbibition, ensuring uniform and rapid germination. Biochar's porous structure enhances oxygen diffusion and water retention, facilitating better enzyme activation and energy metabolism [18]. Conversely, the reduction in germination at higher humic acid concentrations (2–4%) may result from osmotic stress or ionic imbalances that hinder water uptake and

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impair early metabolism. Excessive humic compounds may accumulate around the seed coat, creating a localized hyperosmotic environment that restricts oxygen and water absorption, leading to delayed germination. Similar inhibitory responses at elevated humic concentrations have been reported in wheat and maize, where humic acid beyond the optimal range altered ion homeostasis and enzymatic balance [19]. Thus, while biochar primarily enhances the physical–biological interface of germinating seeds, humic acid acts as a biochemical stimulant whose benefits plateau beyond a certain threshold.

Shoot and root lengths were also markedly higher in the 4% biochar treatment compared to 1% humic acid, hydropriming, and control treatments. Mechanistically, biochar enhances seedling elongation through improved rhizosphere aeration, microbial symbiosis, and nutrient uptake efficiency. The enhanced microbial activity in biochar-treated seeds promotes the synthesis of plant growth–promoting substances such as indole acetic acid (IAA) and gibberellins, which facilitate cell division and elongation [20]. A meta-analysis revealed that biochar increased root biomass by approximately 32% across several crops, largely due to improved nutrient transport and water availability [21]. In our study, biochar likely increased root–shoot coordination by maintaining a balanced carbon–nitrogen ratio, promoting sustained seedling vigor. In contrast, the 1% humic acid treatment improved elongation by stimulating root development through auxin-like and cytokinin-like effects, enhancing membrane permeability and nutrient absorption. However, higher concentrations of humic acid could interfere with enzymatic activation, potentially explaining the reduced elongation. Similar findings in maize and soybean have shown optimal growth at moderate humic levels but suppressed elongation under excess [22]. Hence, biochar and humic acid appear to influence growth through complementary mechanisms biochar primarily through structural and microbial mediation, and humic acid via hormonal and metabolic regulation.

The seed vigour indices (SV-I and SV-II) were highest in 4% biochar, followed by 1% humic acid, indicating superior physiological potential of biochar-treated seeds. The enhancement of seed vigour in biochar treatments can be attributed to increased antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), which mitigate oxidative stress during early

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seedling growth. These enzymes ensure stable redox homeostasis, supporting rapid tissue differentiation and elongation [23]. Biochar's capacity to increase cation exchange and provide a steady nutrient release also contributes to sustained vigour throughout seedling establishment. Studies in rice and maize corroborate these findings, where biochar-coated seeds showed 30–35% higher vigour indices due to enhanced enzymatic activity and better soil structure [24]. In contrast, humic acid improves vigour by stimulating mitochondrial respiration and enhancing the availability of micronutrients such as Fe and Zn, which serve as cofactors for vital enzymes. However, excessive humic acid can oversaturate the enzymatic sites, reducing metabolic efficiency [24]. The contrasting performance underscores the importance of optimal concentration and the interplay between nutrient dynamics and seed metabolism.

In addition to vigour, chlorophyll-a, chlorophyll-b, and total chlorophyll contents were significantly higher in the biochar 4% treatment, with 1% humic acid showing slightly lower but comparable values. This enhancement is mechanistically linked to improved nutrient accessibility particularly nitrogen, magnesium, and iron essential for chlorophyll biosynthesis. Biochar's microstructure improves the retention and gradual release of these nutrients, thereby supporting continuous chlorophyll formation and enhanced photosynthetic efficiency [25,26]. Moreover, biochar increases soil microbial diversity, which facilitates nutrient cycling and production of phytohormones that stimulate chloroplast development. Humic acid also contributes to chlorophyll enhancement by increasing membrane permeability and chelating Fe ions, improving the biosynthetic pathway of chlorophyll. However, its influence is transient compared to biochar's sustained effect. Similar results have been reported in lentil and maize, where biochar application enhanced chlorophyll concentration and photosynthetic rate under stress conditions. Therefore, the synergistic use of both amendments may provide cumulative benefits by combining physical soil improvement and biochemical activation of photosynthesis [27].

The pattern of H_2O_2 accumulation observed in 4% biochar and 1% humic acid treatments reflects an active metabolic state rather than oxidative injury. Moderate ROS production, including H_2O_2, is a well-established signal for breaking dormancy, enhancing mitochondrial respiration, and triggering

antioxidant defense pathways [28]. The DAB staining observed in these treatments likely indicates heightened enzymatic activity associated with germination metabolism rather than cellular damage. Biochar may promote redox regulation by increasing the activities of antioxidant enzymes and improving the cellular balance between ROS production and scavenging [29]. Similarly, humic acid has been reported to modulate the ascorbate–glutathione cycle, ensuring efficient detoxification of ROS while sustaining signaling functions. The contrasting intensity of DAB staining between treatments supports the hypothesis that both biochar and humic acid enhance oxidative metabolism to a beneficial degree. This interpretation aligns with earlier findings in rice and wheat where moderate ROS accumulation was associated with improved seedling vigor and enzymatic efficiency [30,31].

Overall, the combined interpretation of physiological, biochemical, and enzymatic responses indicates that both biochar and humic acid exert concentration-dependent, mechanistically distinct effects on early seedling development. Biochar primarily modulates the physical and microbial environment, enhancing water retention, aeration, and microbial metabolite production, while humic acid acts as a biochemical regulator, influencing ion uptake, enzyme activity, and hormonal balance. When optimally applied, both agents improve germination, vigour, and photosynthetic efficiency while maintaining oxidative homeostasis. These findings are consistent with previous studies demonstrating the integrative benefits of organic amendments on crop resilience and seed performance [32]. Various studies demonstrate that organic compounds like biochar and fulvic acid can play multifaceted roles in improving soil health and mitigating environmental stresses [33, 34].

Conclusion

This study demonstrated that seed priming with 4% biochar and 1% humic acid significantly enhanced seed quality and physio-biochemical parameters under controlled conditions. Among all treatments, 4% biochar consistently produced the highest germination percentage, shoot and root lengths, and seed vigour indices (SVI-I and SVI-II), while 1% humic acid also showed a pronounced stimulatory effect on early seedling growth. These improvements are likely driven by enhanced water uptake, nutrient availability, and modulation of enzymatic and antioxidative activities, which collectively reduced

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oxidative damage during germination and seedling establishment. Mechanistically, biochar improved the physical and microbial environment by enhancing aeration, water retention, and nutrient cycling, while humic acid acted as a biochemical regulator, stimulating enzymatic function, hormonal balance, and ion uptake. Together, these treatments produced a synergistic effect that optimized seed physiological and biochemical performance, supporting their use as sustainable seed-priming agents. The practical significance of this work lies in demonstrating how biochar and humic acid can be used as eco-friendly, low-cost seed treatments to enhance crop establishment, particularly in soils with moderate fertility constraints or under early-season stress. However, the effects of these treatments may vary across crop species, soil types, and environmental conditions; therefore, field-level validation is essential to identify optimal combinations for specific agro-ecological zones. Future research should focus on elucidating the molecular mechanisms underlying these responses. Specifically, studies should examine the expression of genes and enzymatic markers involved in antioxidant defense (e.g., *SOD*, *CAT*, *POD*), hormonal regulation (e.g., *GA*, *IAA*, *ABA* pathways), and nutrient transporters that govern early seedling metabolism. Such molecular-level understanding will strengthen the mechanistic basis of biochar and humic acid priming and support their integration into broader crop management strategies. In summary, the findings highlight biochar (4%) and humic acid (1%) priming as promising sustainable tools for improving seed quality, germination efficiency, and early seedling vigor. With further optimization and validation under field conditions, these techniques could play a pivotal role in enhancing agricultural productivity and resilience in an environmentally sustainable manner.

Statements and Declarations

Ethics approval and consent to participate

This study adhered to all relevant institutional, national, and international guidelines and legislation in India. Plant materials were collected was taken from ICAR – IARI, New Delhi. Permission for the collection of these plant materials was obtained from Amity Institute of Organic Agriculture, Amity University, which does not require special permits for academic research without commercial use. All plant materials used in the study were in compliance with Indian regulations and legislation.

Clinical trial: Not applicable.

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