



Full Length Article

Poultry Feathers Composted by UV-Irradiated *Bacillus subtilis* Improved Chromium Stress Tolerance in Cotton (*Gossypium hirsutum*)

Naila Moazzam¹, Saqib Mahmood^{1*}, Sadia Javed², Rohina Bashir¹ and Nusrat Perveen¹

¹Department of Botany, Government College University, Faisalabad, Pakistan

²Department of Biochemistry, Government College University, Faisalabad, Pakistan

*For correspondence: saqibmahmood@gcuf.edu.pk; +92-334-6621512

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Abstract

UV irradiation is known to enhance the activity of keratinases and catalase in microbes. This study aimed to evaluate the decomposing capabilities of wild and UV-irradiated *Bacillus subtilis* strains. Therefore, wild and UV-irradiated strains (0, 60, 150 and 240 min) were allowed to degrade poultry feathers for 0, 30 and 60 days. The biodegraded products were used as supplements (0 and 3 g/kg soil) for cotton. The best-performing biocompost was chosen based on the correlation between its physical and chemical properties. Selected biocompost (60 days matured by 150 min of UV-irradiated *B. subtilis*) was applied as a rooting medium to stressed cotton (control and 50 mg K₂Cr₂O₇/kg soil). Chromium (Cr) stress negatively affected growth, fiber and oil quality in untreated and feather-supplemented plants. *B. subtilis*-degraded biocomposts improved germination rate, index and percentage; leaf numbers, branches, chlorophyll a/b ratio, carotenoids, soluble proteins, amino acids and ascorbic acids; fiber elasticity, fiber uniformity index (FUI) and boll weight; and reduced coefficient of variation (CVt), fiber micronaire, malondialdehyde (MDA) and H₂O₂. Biocompost prepared from UV-irradiated *B. subtilis* strain (150 min) was better in terms of carbon/ nitrogen (C/N) ratio of compost, shoot fresh weight, germination percentage, shoot length, dry weight, chlorophyll a/b ratio, carotenoids, boll weight, seed number and unsaturated fatty acids. In summary, to grow cotton in Cr-stressed areas, feathers degraded by *B. subtilis* can serve as a beneficial, eco-friendly biocompost. UV irradiation can additionally enhance the efficiency of *B. subtilis*.

Keywords: Biocompost; *Bacillus subtilis*; Poultry feathers; Cotton; Chromium, Phenolic metabolism

Introduction

The agricultural system faces many challenges worldwide. Soil contamination and the overuse of chemical fertilizers are two of the most alarming issues. Cr-contaminated soil reduces plant height, biomass and photosynthetic pigments, with an increase in oxidative damage markers in plants (Khalofah 2024). The overuse of chemical fertilizers contributes to soil damage and the accumulation of heavy metals. Biofertilizers are eco-friendly and cost-effective alternatives to expensive chemical fertilizers (Khairi and Joody 2025). Many organic sources are used as renewable materials in the ecosystem, including poultry waste.

The production of poultry waste is continually increasing due to the high demand for poultry products at both commercial and household levels (Ojha *et al.* 2020). Annual poultry feather production reaches millions of tons, resulting in an additional global challenge (Zhang *et al.* 2023). Poultry feathers are rich in beneficial nitrogenous

compounds. Unfortunately, these components are inaccessible to plants if applied directly without decomposition. The natural degradation of poultry feathers is a slow process, transforming this valuable resource into a source of environmental pollution. Traditionally, feathers are degraded through costly chemical and physical methods. To make this process eco-friendly and economical, searching for microbes that can break down feathers is a worthwhile pursuit.

The potential of *B. licheniformis* to degrade keratin-rich poultry feathers has been recognized before. However, turning feathers into useful fertilizer takes a long time and requires a lengthy wait. To speed up this process, Cui *et al.* (2023) used UV irradiations and found increased keratinase activity in UV-treated *B. licheniformis*.

Cotton provides raw materials to the textile, chemical, oil, cosmetics, pharmaceutical and livestock industries. Heavy metals and poor nutrient composition of soil are negatively affecting cotton yield and fiber quality

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worldwide (Avşar and Karademir 2022; Beegum *et al.* 2023). Khalofah (2024) has reported devastation of cotton growth and physiology in Cr-stressed environments associated with higher accumulation of peroxidation products. Therefore, finding a solution for improved cotton growth in Cr-stress may increase the production of cotton, helping to meet the multiple needs of a rising population.

Previously, the direct application of UV-irradiated *B. subtilis* as a plant growth regulator was found to be successful in inducing stress tolerance in maize and mung beans (Shahzad *et al.* 2021). Detailed metabolic studies of stressed plants supplemented with biocomposts prepared by wild/UV-irradiated *B. subtilis* are limited in the literature. Information about UV-irradiated *B. subtilis* for the degradation of feathers, as well as the potential of prepared biocompost for Cr stress tolerance in plants, is scarce in the literature. The aims of the present study were to: (1) prepare UV-irradiated strains for feather decomposition; (2) select the most efficient biocompost with lower C/N and better seedling growth; and (3) compare biocomposts prepared from wild and UV-irradiated *B. subtilis* for Cr stress tolerance, fatty acid (GC-MS) and phenolic (HPLC) profiles of cotton.

Materials and Methods

Bacterial handling

Inoculum preparation: Growth medium (peptone 0.5 g, yeast 0.3 g, NaCl 0.5 g, distilled water 100 mL) with a pH of 8 was autoclaved (15 min at 121°C). *B. subtilis* spores were shifted in this medium and incubated for two days (at 30°C) on a shaker (Xue *et al.* 2005).

UV-irradiation: Ten mL of growth medium with *B. subtilis* spores (1×10^7 spores/mL) was exposed to UV-B radiation using a germicidal lamp of F875-UV-B 20W (Phillips) as a source. Samples were withdrawn after every 30 min (from 30 to 240 min) of exposure at 20 cm. The mutant spores were grown for 48 h at 30°C. Mutants that yield the highest growth of colonies were selected by colony restrictor selection using Triton X-100. Followed by Log kills mutant dose selection by kill/survival curve and calculation of colony-forming units (Belavin and Dedkov 1988; Khattab and Bazaraa 2005).

Biocomposting

Biocompost preparation: Poultry feathers were collected from local sources, cleaned, dried, chopped and preserved. Selected strains of *B. subtilis* irradiated by UV (0, 60, 150 and 240 min) were grown in a nutrient broth medium following the method of Xue *et al.* (2005). Prepared sporulation medium was applied to feathers in covered plastic bins. To ensure aerobic conditions, holes were drilled in the bins. The composting bins were turned and mixed twice a week. Moisture content was maintained between 30

to 40%. After 30 and 60 days of decomposition, biocomposts were picked and subjected to the following studies.

Physiochemical analysis of biocomposts

The odor, color and appearance were noted physically. All samples were passed through 2 and 5 mm sieves to determine particle size. Organic carbon was assayed using muffle furnace loss on ignition (LOI) at 550°C for 2 h by the method of Walkley and Black (1934). For nitrogen estimation, the Kjeldahl digestion-based method was used (Bremner and Mulvaney 1982). Potassium and phosphorus were determined by flame photometry.

Screening of biosompost: Surface-sterilized cotton seeds (FH Lalazar) were supplemented with soil and composts (0 and 3 g/kg soil). The experiment was planned in a Completely Randomized Design (CRD) with five replicates and seven treatments. Soil, feathers, growth medium-aided feathers (BC), BC1 (feathers degraded with wild *B. subtilis*), BC2 (feathers degraded with 60 min UV-irradiated *B. subtilis*), BC3 (feathers degraded with 150 min UV-irradiated *B. subtilis*) and BC4 (feathers degraded with 240 min UV-irradiated *B. subtilis*). twelve-day-old seedlings were harvested for morphological attributes. The correlation of these attributes with the physicochemical properties of the supplements was used as a criterion to select biocompost for the final experiment with Cr stress.

Treatment application: Based on the results of the above experiment (Fig. 1-2), feathers degraded with wild (BC1) and 150 min UV-irradiated *B. subtilis* (BC3) were selected to use in the final pot experiment. Where soil and feathers were used as reference supplements, the four above-mentioned treatments, comprising two Cr levels (control and 50 mg $K_2Cr_2O_7$ kg⁻¹) and five replications in a Completely Randomized Design (CRD), formed the final experimental plan. Three harvests were taken: first, after 12 days of germination (seedling stage), second, after 6 weeks of germination (vegetative stage) and third, at the full maturity stage.

Seedling characteristics: Daily based emergence of seedlings was noted in each treatment. The mean rate of germination, coefficient of variation (CVt) and germination percentage.

Photosynthetic pigments: To determine photosynthetic pigments, fresh samples (0.1 g) were extracted with 1.5 mL of acetone (80%). After centrifuging for 10 min, the supernatant was used to measure absorbance at 645, 663 and 480 nm and calculations were performed using the formulas proposed by Arnon (1949).

Soluble proteins: Buffer extraction was performed using a fresh leaf sample (0.5 g) in 10 mL of chilled 50 mM potassium phosphate buffer (pH 7.5). The extract was centrifuged at 10000 rpm at 4°C for 20 min. One hundred μ L of the buffer extract was reacted with 3 mL Bradford's reagent for total soluble protein determination, followed by 15 min of incubation and reading of absorbance at 595 nm (Bradford 1976).

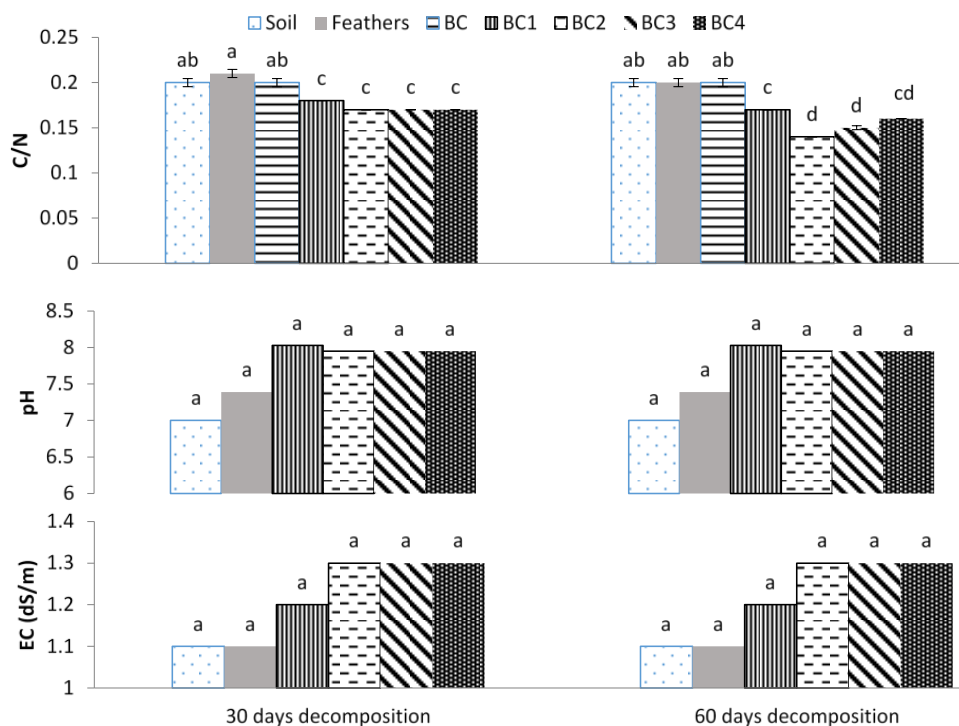


Fig. 1: Chemical comparison of soil, untreated poultry feathers and growth medium-aided feathers (BC) with biocomposts matured by wild *B. subtilis* (BC1), 60 min UV irradiated *B. subtilis* (BC2), 150 min UV irradiated *B. subtilis* (BC3) and 240 min UV irradiated *B. subtilis* (BC4). Different letters on each column represent a significant difference of means (LSD)

Total free amino acids: For the determination of total free amino acids, 1 mL of buffer extract was allowed to react with 10% pyridine and 2% ninhydrin, followed by heating in a water bath for 20 min. Finally, absorbance was recorded at 570 nm (Dickinson and Hamilton 1966).

Total soluble sugars: For the determination of total soluble sugars, plant tissue (0.5 g) was ground in 96% ethanol, vortexed and kept at 4°C. Thereafter, in 0.2 mL of each extract, 3 mL of fresh Anthrone reagent was poured in, followed by boiling in a water bath at 95°C (15 min). Absorbance was noted at 625 nm (Yoshida *et al.* 1971).

Ascorbic acid: Ascorbic acid was extracted (0.5 g sample in 10 mL of 6% TCA) and 4 mL each was allowed to react with 2 mL of 2% dinitrophenyl hydrazine. One drop of 10% thiourea (in ethanol) was also added. After an incubation in a water bath (15 min), cooling and addition of 5 mL of 80% sulfuric acid (v/v), the absorbance was recorded at 530 nm (Mukherjee and Choudhuri 1983).

Oxidative stress parameters: Hydrogen peroxide was assayed from a fresh sample ground with 5 mL of 0.1% TCA in cold conditions and followed by centrifugation (at 12000 × g for 15 min). Supernatant (0.5 mL) was reacted with 0.5 phosphate buffer (pH 7.0, 10 mM) and absorbance was recorded at 390 nm (Velikova *et al.* 2000). The MDA content was determined from a fresh sample (0.5 g). It was extracted with 5 mL of 5% TCA and centrifugation (at 10,000 × g) for 15 min was performed. The supernatant (1 mL) and 4 mL of TBA (0.5% in 20% TCA) were mixed. It

was followed by incubation at 95°C for 30 min. Two absorbances were noted at 532 and 600 nm (Heath and Packer 1968).

Spectrophotometric phenolics analysis: The assay of total phenolics was performed using 0.5 g of sample ground in 10 mL of acetone (80%), followed by centrifugation for 10 min. The reaction mixture (10 µL supernatant + 2 mL distilled water + 5 mL 20% Na₂CO₃ + 1 mL Folin-Ciocalteu reagent) was prepared and the volume was adjusted to 10 mL. After shaking and incubation (at 25°C for 30 min), the absorbance was recorded at 750 nm (Julkunen-Tiitto 1985).

Flavonoid content: Total flavonoids were determined from ethanolic (80%) extraction. The 4 mL extract was combined with water in a 1:1 ratio and 0.3 mL of 5% NaNO₂. Thereafter, it was incubated for five min and 0.3 mL of 10% AlCl₃ was added to it. Afterwards, 2 mL of 1 M sodium hydroxide was diluted to 10 mL with distilled water and the absorbance was measured at 510 nm (Ribarova *et al.* 2005).

HPLC-phenolic profile: Crude extract was prepared using methanol and preserved. Acidic hydrolysis and saponification were performed as proposed by Stalikas (2007). Hence, 2 N HCl was used for one h. Saponification using NaOH was performed overnight at room temperature. An aqueous suspension was made with double-distilled water (pH 2) and heated to 100°C for 3 hr. Filtration from a 0.451 µm syringe membrane filter (Type Millipore) was

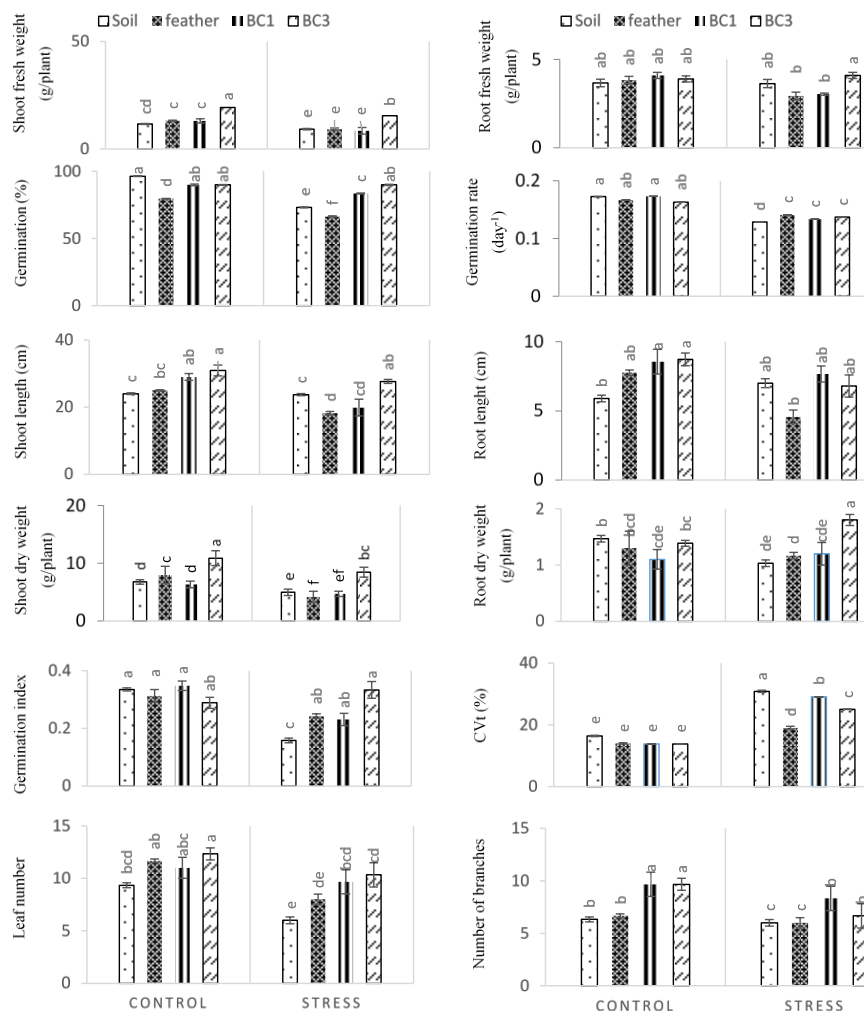


Fig. 2: Morphological attributes of cotton supplemented (0 and 3 g/kg soil) with untreated and 60 days decomposed feathers by wild *B. subtilis* (BC1) and 150 min UV irradiated *B. subtilis* (BC3), growing under Cr stress (control and 50 mg $K_2Cr_2O_7$ kg⁻¹ soil). Different letters on each column represent a significant difference of means (LSD)

followed by sonication (15 min in a Microclean 109 bath). Profiling was performed using an HPLC system (LC-10AT, SCTL, Shimadzu, Japan). The elution rate was 1 mL/min (60 min) and the gradient comprised phase A (H_2O_2 : AA-94:6, pH 2.27) and phase B (ACN 100%).

GC-MS-based Fatty acid profile: For the analysis of fatty acid methyl esters, the IUPAC (1987) standard method 2.301 was followed. The Perkin Elmer gas chromatograph model Clarus 500, having a fitting with aRt-2340 NB (RESTEK, Corp., 800-356-1638, USA) methyl-lignocerate-coated (film thickness 0.20 μ M), polar capillary column (60 m x 0.25 mm) and an FID detector, was used. Nitrogen was used as a carrier (67.4 Psi) for the application. Samples were converted into fatty acid methyl esters of triglycerides. Glycosides' saponification and esterification were as per standard methods. An oil sample (0.2 g), 100 mL and a pellet of KOH were mixed with 30 mL of methanol for 25 min. The cooled reaction mixture was shifted to a separating funnel and a small amount of n-

hexane was added. With stirring and rotation of the separating funnel, the upper layer of hexane was separated and washed three times with 10 mL of 24 deionized water. This hexane solution was dried over anhydrous sodium sulfate, filtered and used for GC analysis. The dry and solvent-free methyl esters were preserved in a sealed sample tube in a deep freezer and used for further analysis.

Yield parameters

Yield parameters were measured at the time of final harvesting, which occurred at maturity. The number of bolls per plant was counted. Completely open bolls were chosen and weighed to estimate the boll weight, number of seeds per cotton boll and the number of branches was also noted. Mean length of fiber was evaluated as Upper Half Mean Length (UHML). Fiber strength against breakage was determined as a force that can break a fiber bundle (cN dtex⁻¹). Fiber uniformity was taken as a ratio as a %age

between the average fiber length and the value of UHML. The percentage of fibers (by weight) having a length less than 12.7 mm was taken as the short fiber index. Micronaire was taken as the air permeability of the fiber after compressing to a constant mass.

Statistical analysis

Data collected was subjected to analysis of variance technique (ANOVA) and correlation by using the computer software Costat CoHort 6.4. The means were compared by least significant difference (LSD).

Results

Selection of the best-performing biocomposts

Physiochemical properties: The odor of all biocomposts varied with time during composting. Initially, the smell was extremely pungent. With time, the pungency decreased. All samples were passed through 2 and 5-mm sieves. Untreated feathers were unable to pass through any sieve due to their larger particle size. Overall, 30-day-degraded poultry feathers passed only through a 5mm sieve, while 60-day-degraded samples passed through both sieves due to their powdery appearance. Sixty days after decomposing, feathers were brownish and darker in the shade. The texture of untreated feathers was soft and fluffy, whereas decomposed feather samples were compact and amorphous.

Biocomposts that were decomposed for 60 days showed a lower C/N ratio. The lowest C/N was noted in BC3 and BC2, followed by BC1. The ratio of N: P: K of BC4 (15:10.12), BC3 (24:10.12), BC1 (20:10.10) and feather (9:08:10) was also different. All samples showed non-significant differences for pH, K/Na and EC (Fig. 1).

Correlation of chemical properties with seedling establishment

The correlation between the physicochemical properties of biocomposts and seedling growth varied significantly (Table 1). Overall, the dominant trend was of positive correlation for the majority of attributes. The C/N ratio of composts was negatively correlated with NPK, germination percentage, dry weights (roots and shoots), shoot length, mean germination rate, moisture, EC, pH, N and K/Na ratio. At the same time, a positive correlation was observed between this ratio and root length. There was a negative correlation of CVt and all attributes except the germination percentage and C/N ratio (Table 1).

Efficacy of selected biocomposts for Cr stress tolerance in cotton

The stress (Cr) reduced shoot fresh weight, germination percentage, germination index, root dry weight, leaf

number, mean germination rate and branches with increased CVt. Untreated feathers decreased germination percentage, shoot length and dry weight (Fig. 2). Both selected biocomposts (BC1 & BC3) improved the mean germination rate, germination percentage, germination index, leaf number and number of branches. BC3 was superior for shoot fresh weight, germination percentage, shoot length and dry weight.

Among biochemical attributes, stress reduced the chlorophyll a/b ratio, amino acids, soluble proteins and ascorbic acid, while increasing phenolics, MDA and H₂O₂. Both biocomposts exhibited a positive decrease in H₂O₂ and MDA levels under stress. A marked rise was observed in the chlorophyll a/b ratio, proteins and amino acids in plants supplemented with both biocomposts. BC3 maintained its superiority for the chlorophyll a/b ratio and carotenoids (Fig. 3).

For yield attributes, the adverse effect of stress on cotton was expressed in terms of decreased fiber strength, uniformity, elasticity, seed number per boll and boll weight, as well as increased micronaire. A reduction in micronaire and an increase in elasticity, boll weight and FUI were observed in plants grown with both biocomposts. BC3 was superior for boll weight and seed number (Fig. 4). Both factors (stress and biocompost treatment) differed for their effect upon the pattern of phenolic profile (Fig. 5).

Cottonseed oil contained myristate, palmitate, laurate, behenic acid, arachidonic, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), linoleate and oleate (C16:0, C12:0, C22:0, C20:4(r6), C22:6(r3), C20:5(r3), C18:2cis(r6) and 18:1cis(r9) respectively). Cr stress increased the production of saturated fatty acids, thereby reducing the ratio of unsaturated to saturated fatty acids. Untreated feathers as a supplement showed a lower value of this ratio, while both composts improved this ratio (Fig. 6).

Discussion

Microbes convert organic waste into a useful biocompost, consuming carbon and producing nitrogen (Kong *et al.* 2024). Therefore, the ratio between carbon and nitrogen is considered a reliable indicator of maturity in biocompost. Before proposing the application of any explored bio-fertilizer, as well as phytochemical analysis, C/N and germination characters are measured as genuine maturity indices. (Ajaweed *et al.* 2022; Carvalho *et al.* 2024; Kong *et al.* 2024). In the current project, biocompost with a powdery appearance, brown color and low C/N ratio supported better cotton growth (Table 1; Fig. 2), in line with the existing literature. It was also one of the principal objectives of the current study.

Generally, microbial degradation is associated with enzymatic efficiency. Previously, UV radiation has been reported to increase protease (Almahasheer *et al.* 2022), catalase (Idris *et al.* 2024) and keratinase (Cui *et al.* 2023) in different species of *Bacillus*. Currently, the powdery appearance and lower C/N of feathers degraded by UV-

Table 1: Correlation matrix between physicochemical properties of root supplements and cotton seedling growth characters, including mean germination rate (MR), germination percentage (%), coefficient of variation (CVt), root dry weight (RDW), root length (RL), shoot length (SL), shoot dry weight (SDW) and moisture content (Moist.), NPK, EC, pH, N and K/Na. Where, ns indicates non-significant correlation and * presents significance at the 0.01 level

Variables	NPK	RDW	SDW	RL	SL	%	MR	C/N	Moisture	EC
NPK	1.0	0.5	0.2	0.6	0.8	-0.1	0.5	-0.2	-0.1	-0.1
RDW	0.6	1.0	0.6	-0.3	0.9	0.6	0.1	-0.5	-0.3	0.4
SDW	0.2	0.6	1.0	-0.4	0.4	0.3	0.4	-0.4	-0.2	-0.3
RL	0.6	-0.3	-0.4	1.0	0.1	-0.5	0.5	0.1	0.4	-0.2
SL	0.8	0.9	0.4	0.1	1.0	0.2	0.3	-0.3	-0.3	0.2
%	-0.1	0.6	0.3	-0.5	0.2	1.0	-0.5	-0.6	0.1	0.8
MR	0.5	0.1	0.5	0.5	0.3	-0.5	1.0	-0.2	0.2	-0.5
C/N	-0.2	-0.5	-0.4	0.1	-0.3	-0.6	-0.2	1.0	-0.7	-0.6
Moisture	-0.1	-0.3	-0.2	0.4	-0.2	0.1	0.2	-0.7	1.0	0.5
EC	-0.05	0.4	-0.2	-0.2	0.2	0.7	-0.5	-0.6	0.4	1.0
pH	0.4	0.3	0.4	0.2	0.5	0.0	0.6	-0.8	0.7	0.3
N	0.1	0.3	-0.1	0.0	0.3	0.3	0.2	-0.7	0.7	0.7
K/Na	0.2	0.3	0.4	0.2	0.3	0.2	0.6	-0.9	0.8	0.3

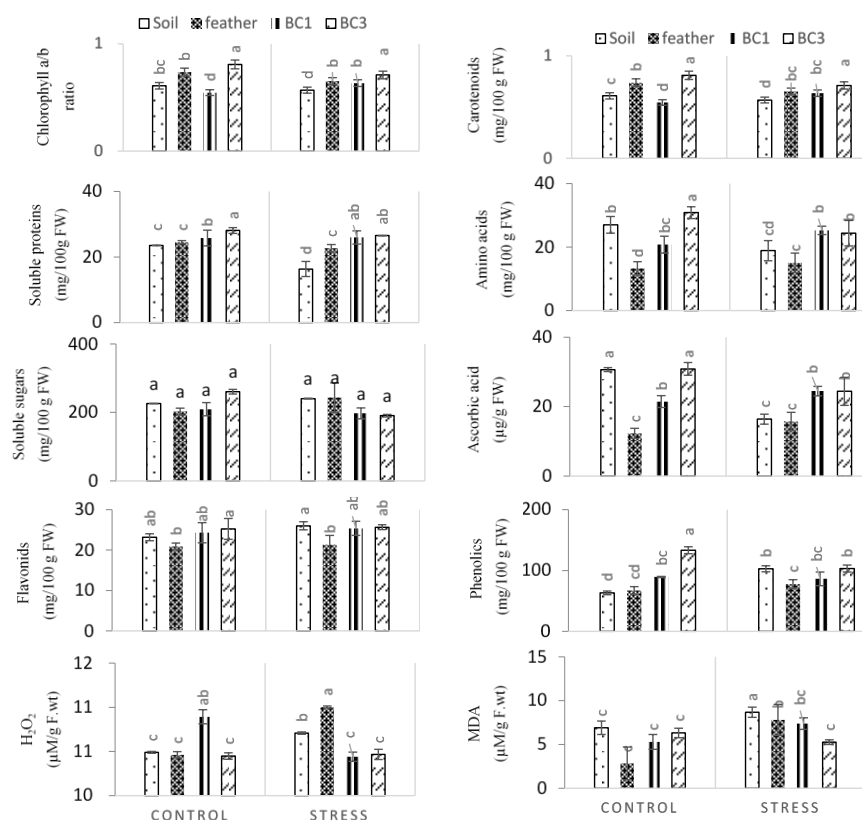


Fig. 3: Biochemical attributes of cotton supplemented (0 and 3 g/kg soil) with untreated and 60 days decomposed feathers by wild *B. subtilis* (BC1) and 150 min UV irradiated *B. subtilis* (BC3), growing under Cr stress (control and (50 mg $K_2Cr_2O_7 \cdot kg^{-1}$ soil). Different letters on each column represent a significant difference in means (LSD)

irradiated strains may be linked to their enzymatic efficiency. This was also confirmed by the negative correlation of C/N and most of the germination characters and chemical properties of composts. The photosynthetic efficiency of a plant determines its metabolic efficiency. The chlorophyll a/b ratio is one of the authentic indicators of this efficiency. This ratio is associated with the ratio of PSII cores to light-harvesting complex II (LHCII) of plants (Shah *et al.* 2024;

Fatima *et al.* 2024). Cr stress decreased this ratio in cotton plants growing without any biocompost. The improvement of this ratio in biocompost-treated plants demonstrated that they are good supplements.

Plants experience a deterrent effect in Cr stress, which causes damage to plant metabolites, including Vitamin C, proteins and free amino acids. The pool of primary metabolites is considered an indicator of a plant's potential

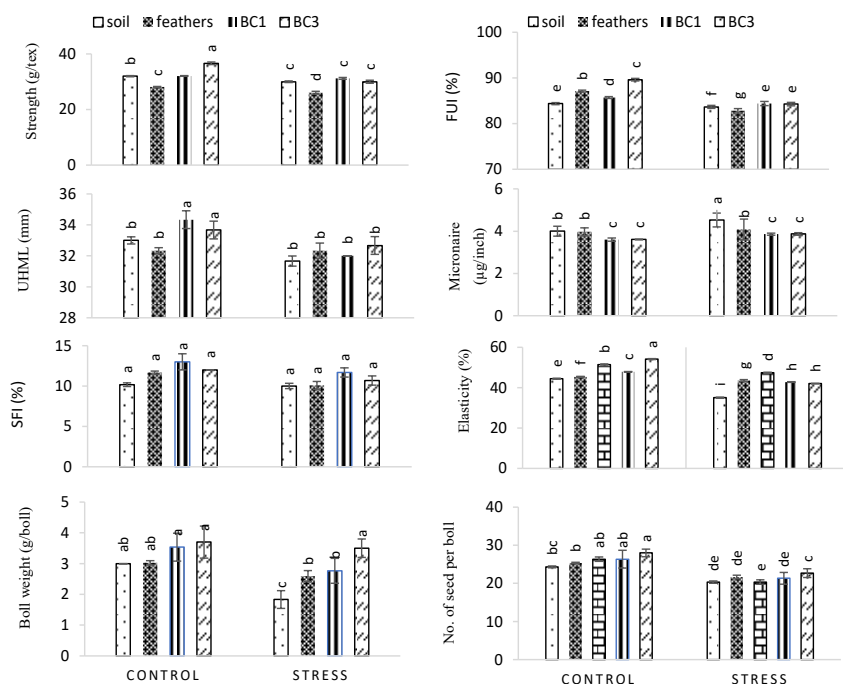


Fig. 4: Yield of cotton growing under chromium stress (control and 50 mg $K_2Cr_2O_7 kg^{-1}$ soil) supplemented (0 and 3 g/kg soil) with untreated feathers, and feathers decomposed for 60 days by wild *B. subtilis* (BC1) and 150 min UV irradiated *B. subtilis* (BC3). The % elasticity is at 5% extension. Different letters on each column represent a significant difference in means (LSD)

to survive in abiotic stress through metabolic adjustments (Al-Huqail *et al.* 2020; Dawood *et al.* 2025). In the current project, a better pool of these metabolites in plants treated with selected biocompost proved its potential to metabolically adjust cotton plants growing under Cr stress.

A higher accumulation of oxidation products (H_2O_2 and MDA) in cotton growing under Cr stress has been reported earlier (Khalofah *et al.* 2024). In the current study, Cr stress negatively affected cotton growth, germination characters, metabolites and fiber quality, associated with higher damage indicators (MDA and H_2O_2). Untreated feathers with poor maturity indices showed a negative or negligible effect on seedling growth and oxidation products (Ajaweed *et al.* 2022; Carvalho *et al.* 2024; Kong *et al.* 2024). Biocompost prepared with the use of UV-irradiated strain showed better accumulation of the primary metabolites mentioned above and a reduction in oxidation products (Fig. 3).

The quality of textile products is directly related to the quality parameters of cotton fibers. The higher the tensile strength and elasticity of the fiber, the better the textile product. The values of UHML and FUI represent the uniformity of the fiber (Yasar and Karademir 2021). In the present study, Cr stress decreased fiber strength, UHML, FUI, elasticity, seed number per boll and boll weight. Meanwhile, an increase was noted in micronaire. A positive effect was expressed in terms of a decline in micronaire

level and a rise in the values of FUI, elasticity and boll weight. A comparatively better performance was achieved by the biocompost prepared from UV-irradiated species. (Fig. 5). Lower C/N level of the same biocompost may be associated with better performance (Fig. 4).

The medicinal potential and stress tolerance of a plant are proportionately linked with phenolic accumulation. Chlorogenic acid, gallic acid, Hydroxybenzoic acid (HB acid), caffeic acid, sinapic acid, ferulic acid, p-coumaric acid and benzoic acid were determined in all samples. Both factors (stress and biocomposts) differed for the phenolic profile in cotton. Cr stress increased the total phenolic content. A significant component of cotton oil was a renowned phenol with medicinal value, ranging from the treatment of neurodegenerative problems, diabetes, inflammation, oxidation, cardiovascular issues, skin problems, organ injuries and tumors (Nguyen *et al.* 2024). A better pool of medicinally important phenolics (Fig. 5), particularly p-coumaric, gallic, ferulic, coumarins and vanillic acid (Sun and Shahrajabian 2023), was maintained in plants grown under stress and biocompost prepared by using a UV-irradiated (150 min) strain (Fig. 5).

A higher unsaturated-to-saturated fatty acid ratio generally indicates a better nutritional value of the oil. Myristate, palmitate, laurate, behenic acid, arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid, linoleate and oleate were determined in the oil harvested from cotton currently. Cr stress not only reduced the number of seeds

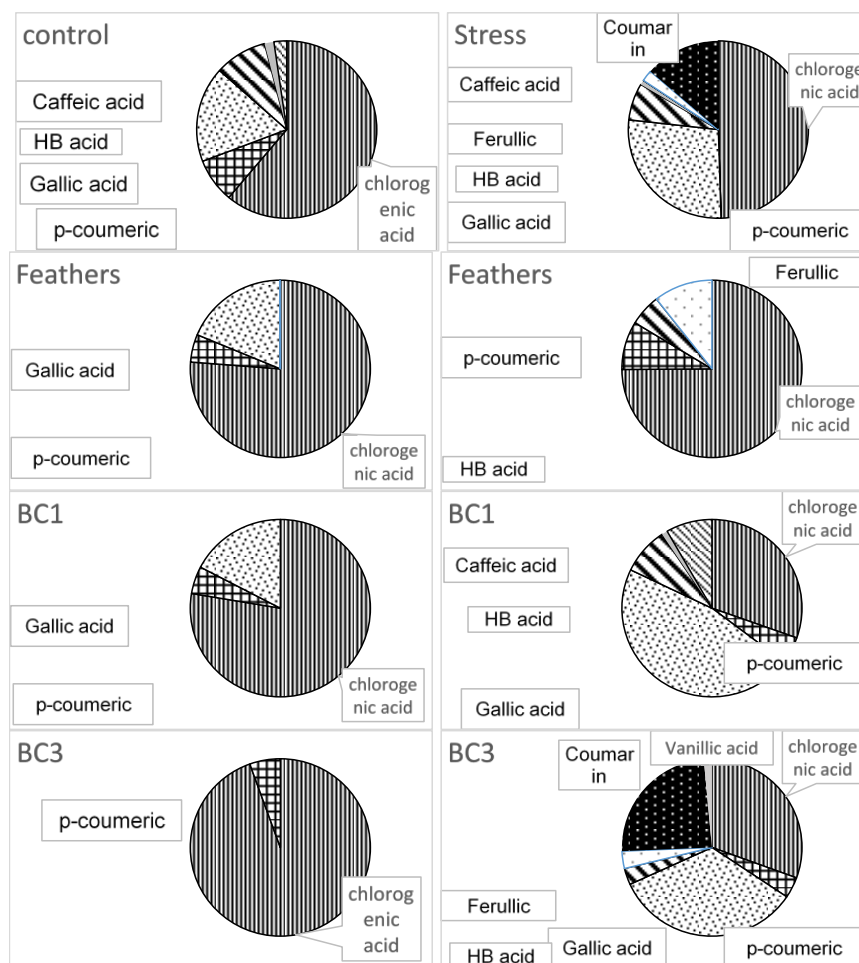


Fig. 5: Phenolic compounds identified (%) in leaves of cotton growing under control (left) and 50 mg K₂Cr₂O₇ kg⁻¹ soil (right) with supplementations (0 and 3 g/kg soil) of untreated feathers, and feathers decomposed for 60 days by wild *B. subtilis* (BC1) and 150 min UV irradiated *B. subtilis* (BC3)

produced per boll but also decreased the nutritive value of its oil. Untreated feathers decreased this ratio, indicating poor nutritive value of the produced oil. Biocomposts showed a better nutritive value of cotton oil compared to plants grown with untreated feathers as root supplementation. Biocompost prepared from UV-irradiated *B. subtilis* demonstrated its superiority in terms of higher accumulation of nutraceutical eicosapentaenoic acid, too (Pettit *et al.* 2013) (Fig. 6). Nutrient supplementation has been previously reported to affect fatty acid profile (Ran *et al.* 2024). In the present study, biocomposts with better NPK and lower C/N (Table 1) levels, compared to feather and soil, demonstrated better performance. This supported our hypothesis that degraded feathers can serve as effective, nutrient-rich biofertilizers.

Conclusion

Poultry feathers can be a poor biofertilizer if applied untreated. *B. subtilis* is a good candidate to degrade it, though it takes a long time. UV irradiated *B. subtilis* strain

(150 min) decomposed feathers into a good biofertilizer in 60 days. It is the first detailed document to optimize and evaluate UV-irradiated *B. subtilis* strains for the metabolic adjustment of cotton growth under Cr stress.

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Author Contributions

All authors contributed equally.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

Any additional information about optimization and

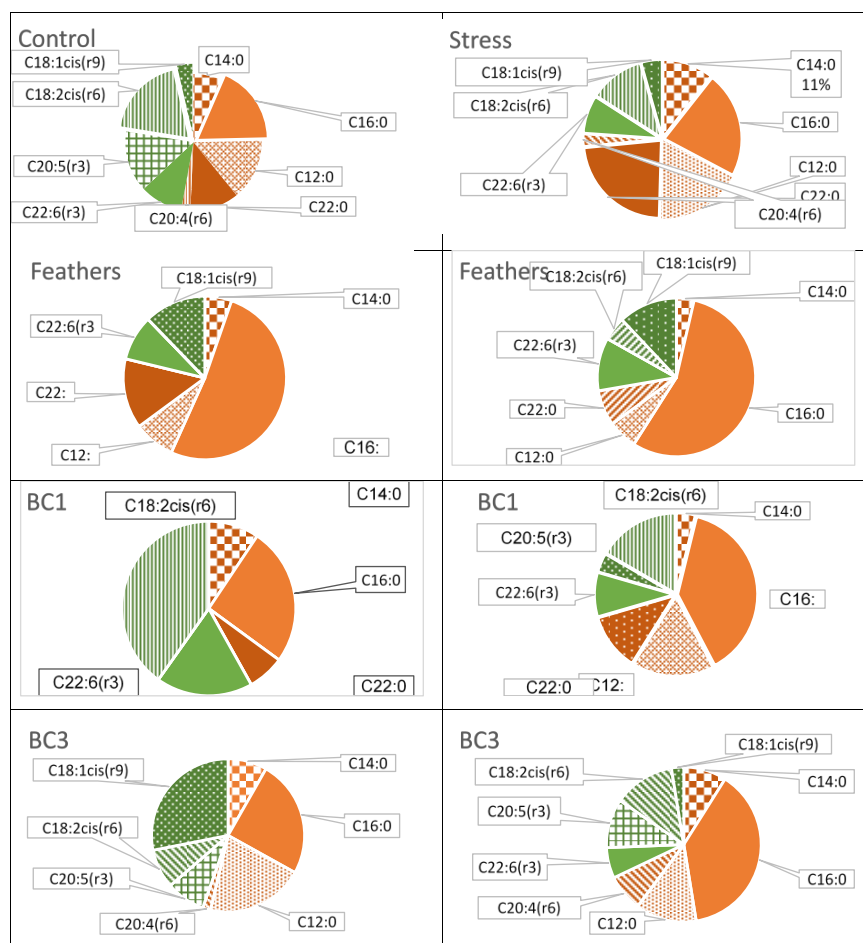


Fig. 6: Fatty acids (%) in oil of cotton growing under control (Left) and 50mg $K_2Cr_2O_7 kg^{-1}$ soil (right) with untreated feathers, and feathers decomposed for 60 days by wild *B. subtilis* (BC1) and 150 min UV irradiated *B. subtilis* (BC3)

screening experiments will be provided on a fair request to the corresponding author.

Ethics Approval

No ethical approval required.

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