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Impact of gibberellic acid GA3, quantum dot biochar, and rhizosphere bacteria on fenugreek plant growth and stress responses under lead stress

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Lead (Pb) is a stress that can cause problems with several aspects of a plant's metabolism, potentially impeding the plant's ability to grow and develop. The use of gibberellic acid (GA3), quantum dot biochar (QDBC), and rhizobacteria (RB) can be effective methods to overcome this problem. Gibberellic acid is a crucial plant hormone that regulates plant growth, cell division, tissue differentiation, flowering, photosynthesis, and transpiration rate. It also significantly impacts crop resilience to stress, affecting plant morphology, enzymatic activity, and physiology. Biochar, a soil supplement, enhances plant development soil health, and reduces stress effects. Due to its large surface area and porosity, it increases soil water-holding capacity, nutrient retention, and microbial activity. Quantum dots, semiconductor nanoparticles, have been proposed as a potential method to alleviate plant stress by acting as antioxidants, reducing oxidative stress, and controlling nutrient and growth regulators. Rhizobacteria, soil bacteria in plant roots, stimulate plant growth, nutrient absorption, and harvesting capacity. They produce phytohormones, increase mineral and nitrogen accessibility, and can induce systemic resistance (ISR), affecting plant defense. This study investigates the effects of combining GA3, QDBC, and RB as amendments to fenugreek, both with 500 Pb stress and without Pb stress. Treatments (control, 0.25 GA3mg/L-QDBC, 0.5 GA3mg/L-QDBC, 0.25 GA3mg/L-QDBC + RB, and 0.5 GA3mg/L-QDBC + RB) were applied in six replications using a completely randomized design. Results demonstrate that the combination of 0.5 GA3mg/L-QDBC + RB with 500 Pb stress led to significant enhancements in fenugreek shoot fresh weight (15.62%), root fresh weight (73.53%), shoot dry weight (24.00%), and root dry weight (36.53%) compared to the control. Additionally, there were notable improvements in chlorophyll a (57.23%), chlorophyll b (19.21%), and total chlorophyll (36.23%) compared to the control under Pb stress, also showing the potential of 0.5 GA3mg/L-QDBC + RB with 500 Pb stress. The study suggests that combining 0.5 GA3mg/L-QDBC + RB with 500 Pb stress can effectively mitigate Pb stress in fenugreek.

Keywords Gibberellic acid, Quantum dot biochar, Rhizobacteria, Lead stress, Chlorophyll, Antioxidants

Lead (Pb) can cause problems with several aspects of a plant's metabolism, potentially impeding the plant's ability to grow and develop. Redox balance, respiration, permeability of cell membranes, photosynthesis, protein synthesis, cell division[1], germination of seeds, development of seedlings, cell wall growth, and ultrastructural alterations are all affected. Phenotypic changes caused by plant lead stress can result in reduced root length, slowed growth, blackened roots, yellowing leaves, changed enzyme activity, and disturbed water balance[2]. When exposed to lead stress, plants frequently produce excessively reactive oxygen species (ROS), which oxidizes a

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range of biomolecules and eventually causes cell death. Plants thus developed an efficient antioxidant system to control the quantity of ROS during Pb poisoning[3,4]. Besides that toxicity of Pb has potential to decrease the yield of crops up to 39%[5]. GA3, QDBC, and Rhizobacteria can decrease the stress of Pb from plants.

A plant hormone known as gibberellic acid (GA3) is essential to several physiological functions in plants[6]. Plant height, leaf growth, dry matter accumulation, cell division, tissue differentiation, flowering, photosynthesis, and transpiration rate are all regulated by it. Furthermore, GA3, a diterpenoid molecule, has been shown to have a major impact on different crops' ability to withstand stress[7]. It influences plants' morphology, enzymatic activity, and physiology, helping them adapt to various environmental conditions.

A soil supplement called biochar has several advantages for plant development, soil health, and reducing the effects of stress[8,9]. It is a great substance for increasing soil water-holding capacity, nutrient retention, and microbial activity because of its large surface area and porosity. Biochar raises soil fertility by absorbing and holding onto nutrients, promoting microbial activity, and enhancing soil structure[10,11]. Quantum dots are semiconductor nanoparticles that possess distinct optical and electrical characteristics. They have been presented as a potential method to alleviate plant stress. By serving as antioxidants, lowering oxidative stress, and a vehicle for nutrients and plant growth regulators, quantum dots can improve plant development and reduce stress[12]. The potential of quantum dots biochar as a cutting-edge technology to reduce stress on plant growth and development, perhaps enhancing the growth by controlling antioxidants[13].

Plant development-stimulating rhizobacteria are naturally occurring soil bacteria that live in plant roots and benefit plants by improving growth, boosting nutrient absorption, and enhancing harvesting capacity via various methods[14,15]. Certain rhizobacteria produce phytohormones that directly alter plant physiology, while other rhizobacteria increase the accessibility of minerals and nitrogen to promote development. Induced systemic resistance (ISR), which results in anatomical or physiological alterations linked to plant defense, is another ability of some other PGPR.

Fenugreek is a hypocholesterolemia, antidiabetic, antineoplastic, antitumor, antioxidative, anti-inflammatory, antiulcerogenic, antihypertriglyceridemic, and antipyretic drug[16,17]; it has been utilized for millennia in traditional medicine. Vitamins, amino acids, fatty acids, polysaccharides, fibers, fixed oils, flavonoids[18], saponins, alkaloids (trigonelline and choline), and fibers have all been linked to the medicinal activity of fenugreek. However, Pb induced toxicity can minimize its growth and yield. It can minimize the plant height, biomass production, root length, chlorophyll content, leaf number and soluble sugars in fenugreek. High exposure of Pb stress also resulted in damage which enhanced the hydrogen peroxide and malondialdehyde levels in fenugreek plants[19].

That's why the current study aims to explore the potential of gibberellic acid (GA3), quantum dots biochar (QDBC), and rhizobacteria to mitigate the effects of Pb stress on fenugreek plants. This research evaluates how an individual and combined use of gibberellic acid (GA3), quantum dots biochar (QDBC), and rhizobacteria potentially enhances fenugreek growth under Pb stress. By addressing this research gap and proposing an environmentally sustainable approach to mitigate the detrimental effects of Pb stress on fenugreek cultivation, this study contributes to the broader objective of preserving ecosystems and crops.

Material and methods

Experimental site

The Department of Botany at Islamia University Bahawalpur, Rahim Yar Khan Campus, conducted a pot experiment in the Botanical Garden to examine the impact of GA3 QDBC and RB on the growth of fenugreek (Short day plant). A composite soil test involving six samples for the pre-exploratory soil examination. Table 1 displays the characteristics of the soil before the experiment.

Soil	Values	References
pH	8.41	[20]
EC*e* (dS/m)	3.35	[21]
Soil organic matter (%)	0.38	[22]
Extractable potassium (µg/g)	111	[23]
Extractable sodium (µg/g)	146	[24]
Total nitrogen (%)	0.0028	[25]
Available phosphorus (µg/g)	5.81	[26]
Texture	Clay Loam	[27]
Irrigation	Values	References
pH	7.04	[28]
EC (µS/cm)	464	
Chloride (meq./L)	*0.01*	
Bicarbonates (meq./L)	5.26	
Sodium (mg/L)	104	
Ca + Mg (meq./L)	3.15	
Carbonates (meq./L)	0.05	

Table 1. Soil and irrigation parameters before experimentation.

Zn-quantum dots

A three-neck flask equipped with a stir bar was used in the acid-based synthesis of zinc (Zn) quantum dots. To this flask, 0.5 g of zinc acetate dihydrate (Sigma-Aldrich ACS reagent, ≥ 98%; Source Batch: MKCS5674, Batch Number: 0,000,210,790; Product Number: 383,058–500 G), 10 mL of oleic acid (Product Number: O1008-VAR; Sigma-Aldrich ≥ 99% (GC); Source Batch: 0,000,224,594; and Batch Number: 0,000,228,437), and 10 mL of 1-octadecene (Product Number: 74,738; Batch Number: BCCH3599; Sigma-Aldrich; analytical standard, ≥ 99.0% (GC); Brand: SIAL) was added and stirred for 10 min at room temperature. After that, the mixture was heated for 30 min under nitrogen at 120 °C until the solution was clear. In a separate flask, simultaneously, 3.8 g of sodium hydroxide (Sigma-Aldrich; reagent grade, ≥ 98%, pellets (anhydrous); MDL Number: MFCD00003548; Batch Number: SLBT5620; Product Number: S5881) was dissolved in 40 mL of methanol with vigorous stirring. The zinc-oleic acid mixture was mixed with this sodium hydroxide solution in a three-neck flask, and the solution was heated for two hours at 120 °C in nitrogen. After cooling, the flask was filled with 20 milliliters of hexane, precipitating quantum dots. The quantum dots were separated after being centrifuged for ten minutes at 5000 rpm. After that, they were washed with methanol and ethanol to remove any impurities. A solution of Zn quantum dots was produced when the resulting quantum dots were resuspended in toluene.

Biochar

We collected vegetable and fruit waste from the local market to make biochar (30°11′29.8′′N 71°28′48.8′′E). The waste material was first sun-dried before being chopped up and pyrolyzed at 325 °C under partially aerobic conditions. After the pyrolysis process, the material was allowed to cool, then crushed and ground to a 2 mm particle size. The biochar produced was then kept in plastic jars until it could be used to make quantum dots biochar.

Zn-quantum dots biochar

In an independent vessel, the quantum dots solution was combined with biochar, and the resulting mixture underwent continuous stirring for 24 h to facilitate the binding of quantum dots to the biochar, forming what is referred to as QDB. Following this period, the quantum dots biochar mixture underwent multiple washes with ethanol to eliminate any quantum dots not bound to the biochar. Subsequently, the quantum dots biochar blend was dried in a vacuum oven at 60 °C for 24 h.

Isolation, incubation, and purification of PGPR isolates

To isolation, *Bacillus altitudinis*, 1.0 g of homogenized soil from the rhizosphere, underwent serial dilutions ranging from 10^{-1} to 10^{-7}. To facilitate the isolation of ACC deaminase-producing *Bacillus altitudinis*, a DF minimal salt medium supplemented with ACC (nitrogen source) was prepared[29]. The Petri dishes containing the isolated samples were then incubated at 25 °C for 48 h. In the subsequent purification step, 55 isolates were selected and subjected to repeated streaking on DF media to obtain pure strains.

Sowing and sterilization of seeds

The fenugreek (*Trigonella corniculata*) seeds employed in this study were obtained from a certified seed supplier sanctioned by the Punjab government in Pakistan. Before planting, the seeds underwent neutering through immersion in a 5% sodium hypochlorite solution for 5 min. Subsequently, they underwent three rinses with 95% ethanol and then underwent three additional rinses with sterilized deionized water to remove any remaining sterilizing agents. Now, the seeds are ready for sowing.

Sowing and thinning of seeds

On October 15, 2023 (Temperature 31–34 ˚C, humidity 41-64%), sowing took place, with 10 seeds planted in each pot containing 5 kg of soil. After germination, thinning was conducted, reducing the number of seedlings to two per pot.

Fertilizer

Fertiliser was applied to fenugreek plants to address soil nutrient deficiency. Nitrogen and phosphorus were incorporated at 25 kg per acre and 12 kg per acre, respectively. Urea was the nitrogen source, while single superphosphate supplied the phosphorus. Potassium supplementation was provided at 12 kg per acre using potassium sulfate. These measures were necessary to meet the nutritional needs of the plants.

Irrigation

Irrigation regulation for each pot was meticulously controlled using a soil moisture gauge (ADVANCED™; 4 in 1 Soil Meter; China). Regular monitoring ensured moisture levels were consistently maintained within the specified threshold, as indicated by the device's scale. The term 'wet' on the scale corresponded to approximately 70% of the soil's field capacity.

Harvesting and data collection procedure

After 60 days post-planting, the plants were harvested to collect data. Fresh shoots and roots were immediately weighed after harvesting. Following this, the samples were dried in an oven set at 65 °C for 72 h to achieve uniform weight and determine the dry mass of both the shoots and roots.

Chlorophyll contents, carotenoids, anthocyanin, and lycopene

The study utilized Arnon's method[30] to determine the levels of chlorophyll in freshly harvested leaves. An 80% acetone solution was used for the extraction process. Absorbance readings were taken at different wavelengths:

663 645, 480, 537, and 503 nm wavelength for the final computation of chlorophyll contents[30], carotenoids[31], anthocyanin[32], and lycopene[33].

Antioxidants

Superoxide dismutase (SOD) activity was determined by assessing the reduction inhibition of nitro blue tetrazolium (NBT) at 560 nm was noted[34]. Peroxidase (POD) activity was measured at 420 nm with standard procedure[35]. Catalase (CAT) activity was determined by observing the breakdown of hydrogen peroxide (H_2O_2) and reduction in absorbance at 240 nm due to H_2O_2 decomposition[36]. Ascorbate peroxidase (APX) activity was assessed by monitoring ascorbate oxidation in the presence of H_2O_2 at 290 nm[37]. MDA was determined by reacting with the sample extract with thiobarbituric acid (TBA) to form a colored complex. MDA concentration was calculated at 532 nm[38]. It was determined by using the following formula:

$$MDA\,level\ (nmol) = \frac{\Delta\ (A532nm - A600nm)}{1.56 \times\ 10.5}$$

$$\text{MDA was determined by using an absorption coefficient of } 156\ mmol^{-1}\ \text{cm}^{-1}$$

Electrolyte leakage (EL)

Before EL testing, the deionized water was rinsed leaves to remove contaminants. 1 cm steel cylinders are used to obtain 1 g leaf samples for the 20 ml deionized water test tubes. These tubes were incubated at 25 °C for 24 h to diffuse electrolytes from leaf tissues into water. After incubation, an EC meter measured the water solution's electrical conductivity (EC1), then heated the tubes to 120 °C in a water bath for 20 min to measure the second electrical conductivity (EC2)[39].

$$\text{Electrolyte Leakage}\ (\%) = \left(\frac{\text{EC1}}{\text{EC2}}\right) \times\ 100$$

Statistical analysis

The data collected underwent analysis employing standard statistical procedures[40]. Standard statistical procedures were used for data analysis, including two-way ANOVA for treatment significance and the Tukey test for paired comparisons ($p < 0.05$). OriginPro software performs convex hull, hierarchical cluster plot, and Pearson correlation analysis[41].

Results

Growth attributes

The 0.25 GA3mg/L-QDBC and 0.5 GA3mg/L-QDBC treatments under no Pb stress exhibit a rise in shoot fresh weight (23.03% and 26.32%), root fresh weight (7.69% and 14.84%), shoot dry weight (3.81% and 14.29%), and root dry weight (8.00% and 16.50%) from the control. In contrast to the control + Rb, applying 0.25 GA3mg/L-QDBC + RB and 0.5 GA3mg/L-QDBC + RB treatments with no Pb stress increased shoot fresh weight (16.28% and 30.23%), root fresh weight (20.43% and 30.11%), shoot dry weight (19.05% and 38.10%), and root dry weight (19.52% and 25.06%) respectively. A significant rise in shoot fresh weight (14.53% and 17.95%), root fresh weight (32.58% and 77.30%), shoot dry weight (28.57% and 28.57%), and root dry weight (27.50% and 45.83%) was observed with 0.25 GA3mg/L-QDBC, 0.5 GA3mg/L-QDBC treatments under 500 Pb stress compared to the control. Adding 0.25 GA3mg/L-QDBC + RB and 0.5 GA3mg/L-QDBC + RB with 500 Pb stress showed a rise in shoot fresh weight (12.50 and 15.62%), root fresh weight (60.78% and 73.53%), shoot dry weight (20.00% and 24.00%), and root dry weight (31.91% and 36.53%) than the control + Rb (Table 2).

The values represent the means of six replicates at $p \leq 0.05$ (Tukey test). Significant changes are indicated by using different letters with mean values.

Chlorophyll contents and carotenoids

Under no Pb stress, adding 0.25 GA3mg/L-QDBC and 0.5 GA3mg/L-QDBC treatments showed a significant increase in chlorophyll a (25.27% and 33.13%), chlorophyll b (5.73% and 14.13%), total chlorophyll (15.90% and 24.02%), and carotenoids (6.56% and 10.49%) over the control. Applying 0.25 GA3mg/L-QDBC + RB and 0.5 GA3mg/L-QDBC + RB treatments with no Pb stress increased in chlorophyll a (30.94% and 40.38%), chlorophyll b (19.53% and 23.40%), total chlorophyll (25.66% and 32.51%), and carotenoids (12.62% and 19.28%) than the control + Rb. Under 500 Pb stress, a significant rise in chlorophyll a (37.33% and 58.04%), chlorophyll b (11.17% and 15.62%), total chlorophyll (22.36% and 33.77%), and carotenoids (19.58% and 25.46%) were recorded above the control with 0.25 GA3mg/L-QDBC and 0.5 GA3mg/L-QDBC treatments. Applying 0.25 GA3mg/L-QDBC + RB and 0.5 GA3mg/L-QDBC + RB with 500 Pb stress compared to the control + Rb rise in chlorophyll a (44.50% and 57.23%), chlorophyll b (13.85% and 19.21%), total chlorophyll (27.57% and 36.23%), and carotenoids (13.04% and 19.72%) were observed (Table 3).

The values represent the means of six replicates at $p \leq 0.05$ (Tukey test). Significant changes are indicated by using different letters with mean values.

Anthocyanin, lycopene, electrolyte leakage, and MDA

In comparison to the control, the 0.25 GA3mg/L-QDBC and 0.5 GA3mg/L-QDBC treatments under no Pb stress led to a significant increase in anthocyanin (15.90% and 24.36%) caused a decrease in lycopene (2.61% and 4.22%), electrolyte leakage (16.23% and 27.26%), and MDA (27.03% and 54.10%) respectively. Adding 0.25 GA3mg/L-QDBC + RB and 0.5 GA3mg/L-QDBC + RB treatments showed an increase in anthocyanin (23.22%

Pb Levels	Treatments	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
0 mg/kg Pb	Control	50.67 e	7.00 cd	3.03 e	0.67 cde
	0.25GA3-QDBC	62.33 c	7.27 bcd	3.27 d	0.72 c
	0.5GA3-QDBC	64.00 bc	8.00 bc	3.48 c	0.78 b
	Rhizobacteria	57.33 d	7.00 cd	3.10 de	0.69 cd
	0.25GA3-QDBC + Rhizobacteria	66.67 b	8.33 b	3.73 b	0.83 ab
	0.5GA3-QDBC + Rhizobacteria	74.67 a	9.67 a	4.03 a	0.87 a
500 mg/kg Pb	Control	39.00 i	4.67 f	1.48 i	0.40 h
	0.25GA3-QDBC	44.67 gh	6.00 de	1.97 g	0.51 g
	0.5GA3-QDBC	46.00 fg	6.00 de	2.63 f	0.58 f
	Rhizobacteria	42.67 h	5.00 ef	1.70 h	0.47 g
	0.25GA3-QDBC + Rhizobacteria	48.00 ef	6.00 de	2.73 f	0.62 ef
	0.5GA3-QDBC + Rhizobacteria	49.33 e	6.20 de	2.95 e	0.64 de

Table 2. Effect of gibberellic acid (GA3), quantum dot biochar (QDBC), and rhizobacteria (RB) on shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of fenugreek grown under 0 pb and 500 pb stress.

Pb Levels	Treatments	Chlorophyll a (mg/ g FW)	Chlorophyll b (mg/ g FW)	Total Chlorophyll (mg/ g FW)	Carotenoids (mg/ g FW)
0 mg/kg Pb	Control	0.80 de	0.73 de	1.53 cd	0.50 c
	0.25GA3-QDBC	1.00 c	0.78 c	1.77 b	0.53 bc
	0.5GA3-QDBC	1.06 bc	0.84 b	1.90 b	0.55 b
	Rhizobacteria	0.88 d	0.76 cd	1.63 c	0.51 c
	0.25GA3-QDBC + Rhizobacteria	1.15 ab	0.90 a	2.05 a	0.57 ab
	0.5GA3-QDBC + Rhizobacteria	1.23 a	0.93 a	2.16 a	0.61 a
500 mg/kg Pb	Control	0.42 i	0.56 j	0.98 i	0.30 g
	0.25GA3-QDBC	0.58 h	0.63 hi	1.20 gh	0.36 ef
	0.5GA3-QDBC	0.66 gh	0.65 gh	1.31 fg	0.38 def
	Rhizobacteria	0.48 i	0.60 ij	1.08 hi	0.35 f
	0.25GA3-QDBC + Rhizobacteria	0.70 fg	0.68 fg	1.37 ef	0.39 de
	0.5GA3-QDBC + Rhizobacteria	0.76 ef	0.71 ef	1.47 de	0.42 d

Table 3. Effect of gibberellic acid (GA3), quantum dot biochar (QDBC), and rhizobacteria (RB) on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoids (D) of fenugreek grown under 0 pb and 500 pb stress.

and 38.18%) and a decrease in lycopene (5.04% and 7.00%), electrolyte leakage (29.14% and 37.65%), and MDA (61.69% and 90.07%) with no Pb stress than the control + Rb. Under 500 Pb stress, the rise in anthocyanin (34.34% and 45.40%), a reduction in lycopene (2.67% and 3.80%), electrolyte leakage (5.38% and 12.70%), and MDA (22.50% and 35.43%) were recorded with 0.25 GA3mg/L-QDBC and 0.5 GA3mg/L-QDBC treatments from the control. Applying 0.25 GA3mg/L-QDBC + RB and 0.5 GA3mg/L-QDBC + RB caused an increase in anthocyanin (24.63% and 29.45%) and a decrease in lycopene (2.74% and 3.04%), electrolyte leakage (14.96% and 16.68%), and MDA (35.65% and 50.31%) over the control + Rb (Table 4).

The values represent the means of six replicates at $p \leq 0.05$ (Tukey test). Significant changes are indicated by using different letters with mean values.

SOD, POD, CAT, and APx

Adding 0.25 GA3mg/L-QDBC and 0.5 GA3mg/L-QDBC showed a decrease in POD (36.53% and 58.20%), SOD (20.36% and 39.58%), CAT (9.49% and 16.89%), and APx (14.25% and 27.05%) under no Pb stress compared to the control. Applying 0.25 GA3mg/L-QDBC + RB and 0.5 GA3mg/L-QDBC + RB treatments with no Pb stress exhibit a decrease in POD (53.57% and 91.11%), SOD (46.03% and 70.37%), CAT (17.03% and 23.04%), and APx (24.21% and 29.76%) than the control + Rb. A significant decrease in POD (15.71% and 24.51%), SOD (9.20% and 15.39%), CAT (5.52% and 8.59%), and APx (7.79% and 11.15%) was observed with 0.25 GA3mg/L-QDBC and 0.5 GA3mg/L-QDBC treatments under 500 Pb stress over the control. The 0.25 GA3mg/L-QDBC + RB and 0.5 GA3mg/L-QDBC + RB treatments with 500 Pb stress resulted in a decrease in POD (23.21% and 29.78%), SOD (18.70% and 26.39%), CAT (8.82% and 13.22%), and APx (11.22% and 22.47%) respectively compared to the control + Rb (Table 5).

The values represent the means of six replicates at $p \leq 0.05$ (Tukey test). Significant changes are indicated by using different letters with mean values.

Pb Levels	Treatments	Anthocyanin (µmol/mL)	Lycopene (µg/ g FW)	Electrolyte Leakage (%)	MDA (nmol/ mg Protein)
0 mg/kg Pb	Control	0.038 fg	333.00 ef	56.52 c	0.94 fg
	0.25GA3-QDBC	0.034 gh	324.55 gh	48.63 d	0.74 hi
	0.5GA3-QDBC	0.032 hi	319.51 h	44.41 e	0.61 i
	Rhizobacteria	0.036 fg	327.85 fg	52.06 d	0.83 gh
	0.25GA3-QDBC + Rhizobacteria	0.029 i	312.10 i	40.31 f	0.43 j
	0.5GA3-QDBC + Rhizobacteria	0.024 j	306.39 j	37.82 f	0.30 j
500 mg/kg Pb	Control	0.059 a	353.98 a	70.11 a	1.80 a
	0.25GA3-QDBC	0.050 bc	344.76 bc	66.53 a	1.47 c
	0.5GA3-QDBC	0.046 cd	341.01 cd	62.21 b	1.33 cd
	Rhizobacteria	0.053 b	347.78 b	68.19 a	1.62 b
	0.25GA3-QDBC + Rhizobacteria	0.043 de	338.52 d	59.31 bc	1.20 de
	0.5GA3-QDBC + Rhizobacteria	0.040 ef	337.51 de	58.44 bc	1.08 ef

Table 4. Effect of gibberellic acid (GA3), quantum dot biochar (QDBC), and rhizobacteria (RB) on anthocyanin (A), lycopene (B), electrolyte leakage (C), and MDA (Malondialdehyde) (D) of fenugreek grown under 0 pb and 500 pb stress.

Pb Levels	Treatments	POD (U/mg Protein)	SOD (U/mg Protein)	CAT (U/mg Protein)	APX (U/mg Protein)
0 mg/kg Pb	Control	1.00 f	0.67 fg	0.21 fg	0.16 f
	0.25GA3-QDBC	0.73 h	0.56 h	0.19 h	0.14 g
	0.5GA3-QDBC	0.63 hi	0.48 i	0.18 i	0.12 h
	Rhizobacteria	0.86 g	0.61 gh	0.20 gh	0.15 fg
	0.25GA3-QDBC + Rhizobacteria	0.56 ij	0.42 ij	0.17 ij	0.12 h
	0.5GA3-QDBC + Rhizobacteria	0.45 j	0.36 j	0.16 j	0.11 h
500 mg/kg Pb	Control	1.47 a	0.95 a	0.25 a	0.23 a
	0.25GA3-QDBC	1.27 bc	0.87 bc	0.24 bc	0.21 bc
	0.5GA3-QDBC	1.18 cd	0.82 cd	0.23 cd	0.20 cd
	Rhizobacteria	1.38 ab	0.91 ab	0.24 ab	0.22 ab
	0.25GA3-QDBC + Rhizobacteria	1.12 de	0.77 de	0.22 de	0.20 d
	0.5GA3-QDBC + Rhizobacteria	1.06 ef	0.72 ef	0.21 ef	0.18 e

Table 5. Effect of gibberellic acid (GA3), quantum dot biochar (QDBC), and rhizobacteria (RB) on peroxidase (POD) (A), superoxide dismutase (SOD) (B), catalase (CAT) (C), and ascorbate peroxidase (APX) (D) of fenugreek grown under 0 pb and 500 pb stress.

Convex hull and hierarchical cluster analysis

The primary principal component (PC1) accounted for 97.08% of the total variance, while the secondary principal component (PC2) explained an additional 1.27%. The dataset includes scores for each sample, categorized into two groups based on their Pb concentration levels: samples labeled as 0 Pb (with no Pb) and samples labeled as 500 Pb (with 500 ppm Pb). The 0 Pb group displayed scores predominantly clustered in the positive region of PC1, with values ranging from 0.17274 to 5.87438 for PC1 and from −0.2271 to 0.9117 for PC2. In contrast, the 500 Pb group exhibited scores primarily situated in the negative region of PC1, with scores ranging from −5.34861 to -0.22006 for PC1 and from −0.61585 to 0.48698 for PC2 (Fig. 1A).

The control group clusters around lower positive and negative PC 1 scores, with PC 2 scores ranging between −0.2271 and 0.48698. The GA3-QDBC treatments (0.25 GA3-QDBC and 0.5 GA3-QDBC) show a shift towards higher PC 1 values, extending from 1.49021 to 2.75189 for 0.25 GA3-QDBC, and from 2.46049 to 3.08756 for 0.5 GA3-QDBC. When combined with RB (Rice Bran), these treatments (0.25 GA3-QDBC + RB and 0.5 GA3-QDBC + RB) are further separated along the PC 1 axis, with 0.25 GA3-QDBC + RB spanning from 3.32608 to 4.23297, and 0.5 GA3-QDBC + RB ranging from 4.52083 to 5.87438. On the negative side of PC 1, the Control group's scores extend from −5.34861 to -4.3177. The negative scores of the GA3-QDBC treatments and their combinations with RB demonstrate a mirrored but more compact distribution. The 0.25 GA3-QDBC scores range from −2.97916 to -2.41176, while the 0.5 GA3-QDBC scores range from −1.99473 to -1.55112. The addition of RB to these treatments (0.25 GA3-QDBC + RB and 0.5 GA3-QDBC + RB) results in even more negative scores, with 0.25 GA3-QDBC + RB spanning from −1.40348 to -1.01151, and 0.5 GA3-QDBC + RB from −0.83228 to -0.22006 (Fig. 1B).

In the early stages of clustering, chlorophyll-related variables such as Chl.a and T. Chl were grouped with a high similarity score of 0.06038, indicating a close relationship between these two parameters. Similarly, antioxidant enzyme activities, specifically SOD and CAT, also clustered early with a similarity score of 0.10613, suggesting that these enzymes exhibit similar response patterns. POD activity was later grouped with SOD and CAT at

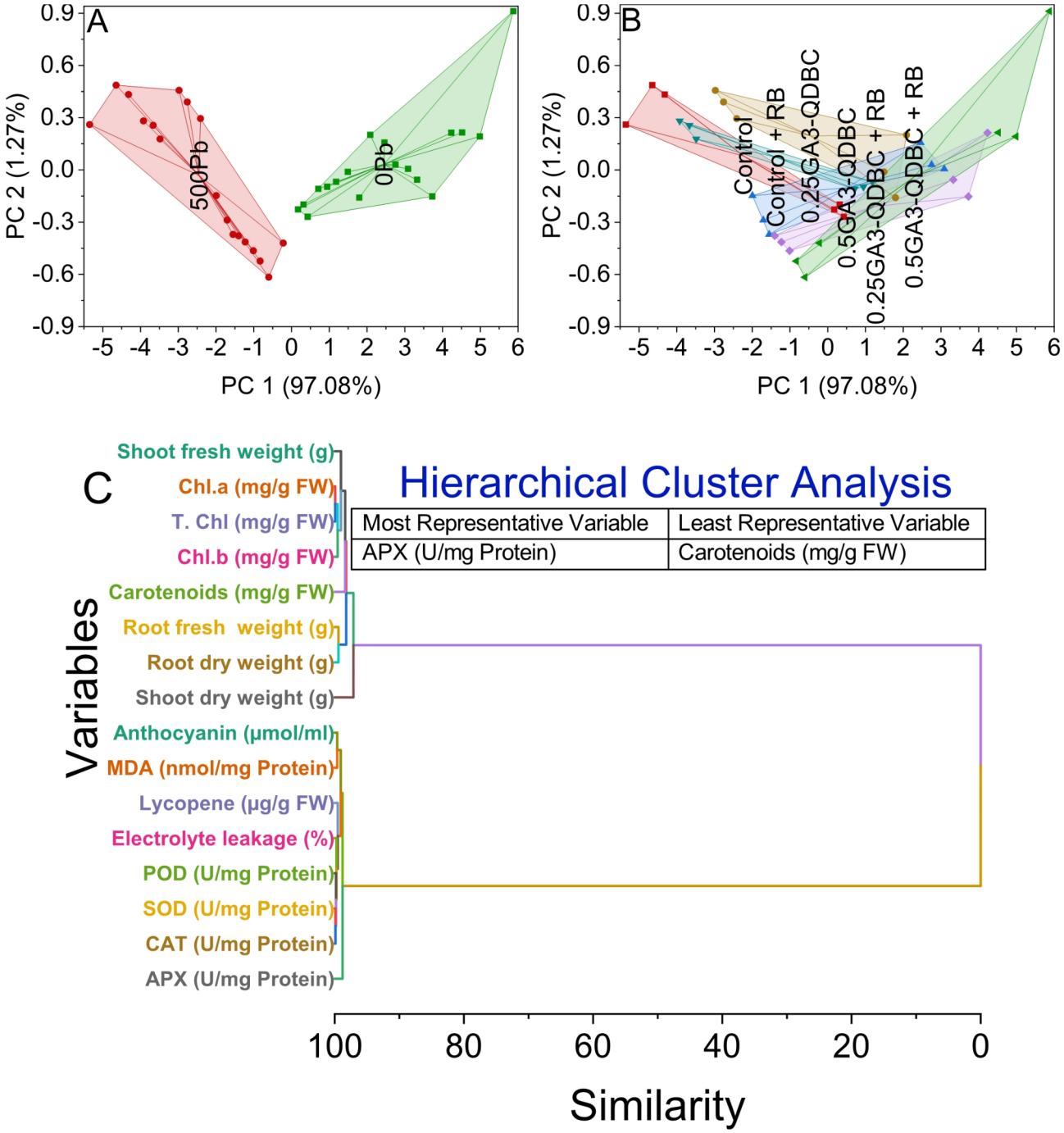

Fig. 1. Cluster plot convex hull for lead (Pb) levels (**A**) treatments (**B**) for the growth attributes, and hierarchical cluster plot (**C**) for all the studied parameters.

a similarity score of 0.20067, indicating a moderate relationship among the antioxidant enzymes. Electrolyte leakage was clustered with other variables at a similarity score of 0.27487, reflecting its distinct response compared to the chlorophyll content and antioxidant activities. Stress-related indicators, such as Anthocyanin and MDA, were clustered with a similarity score of 0.38642, indicating a correlation between these markers. This clustering suggests that these stress indicators are responding somewhat coordinatedly. Root biomass variables, including root fresh and dry weight, clustered with a similarity score of 0.57616, while shoot biomass variables showed lower similarity scores (0.97192 for shoot fresh weight and 2.89247 for shoot dry weight). This suggests a

distinction between the root and shoots biomass responses, with the shoot dry weight showing the most distinct response. Carotenoids, a variable related to photosynthesis and photoprotection, clustered at a similarity score of 1.60613, indicating a unique response pattern compared to other variables. In the later stages of the analysis, variables such as APX activity clustered with others at a similarity score of 1.2107, and the final clustering of shoot dry weight occurred with a similarity score of 2.89247, highlighting the distinct response profiles of these parameters (Fig. 1C).

Pearson correlation analysis

Shoot fresh weight is highly positively correlated with shoot dry weight ($r=0.95$), root fresh weight ($r=0.93$), and root dry weight ($r=0.9537$). These high positive correlation scores suggest that root biomass and shoot dry weight increase proportionally as shoot biomass increases. Root fresh weight strongly correlates with root dry weight ($r=0.98862$), reflecting the consistent relationship between fresh and dry biomass within the roots. Chl. a shows very high positive correlations with Chl. b ($r=0.98773$) and total chlorophyll (T. Chl.) ($r=0.99881$). Similarly, Chl. b is highly correlated with T. Chl. ($r=0.99418$). These strong correlations indicate a close association among different forms of chlorophyll in the plant. Carotenoids exhibit positive correlations with Chl. a ($r=0.97306$), Chl. b ($r=0.9649$), and T. Chl. ($r=0.97308$), suggesting that as chlorophyll levels increase, carotenoid content also rises. Anthocyanin has a very strong positive correlation with shoot fresh weight ($r=0.99$) and shoot dry weight ($r=0.96673$), indicating that higher anthocyanin content is associated with greater shoot biomass. Lycopene shows strong negative correlations with many parameters, particularly with shoot fresh weight ($r = -0.98612$) and shoot dry weight ($r = -0.95582$). This negative relationship suggests higher lycopene content is linked to lower biomass accumulation. Electrolyte leakage, an indicator of cellular stress, is negatively correlated with shoot fresh weight ($r = -0.98$) and root dry weight ($r = -0.97748$) and also negatively correlated with Chl. a ($r = -0.99211$). These correlations imply that higher stress levels are associated with reduced biomass and chlorophyll content. Antioxidant enzyme activities such as POD, SOD, CAT, and APX are all strongly negatively correlated with chlorophyll content, with scores like POD over Chl. a ($r = -0.99662$) and SOD over Chl. a ($r = -0.99435$). These enzymes are positively correlated with stress indicators, for example, POD over electrolyte leakage ($r=0.99407$), suggesting that as stress increases and chlorophyll content decreases, the activity of these enzymes rises. MDA, a marker of lipid peroxidation, also follows this pattern, being negatively correlated with Chl. a ($r = -0.98305$) and positively correlated with POD ($r=0.98554$), indicating increased oxidative damage under stress conditions (Fig. 2).

Discussion

Chlorophyll content and carotenoids

Several processes can be responsible for increased chlorophyll content and carotenoids seen after applying GA3-QDBC and rhizobacterial in both stressed and non-stressed conditions. First, gibberellic acid (GA3) is known to increase the amounts of chlorophyll[42,43] in plant tissues by stimulating the expression of genes involved in chlorophyll production. Furthermore, GA3 is carried by QDBC, which improves its absorption and transport inside the plant and encourages chlorophyll production. Additionally, QDBC's[44,45] porous nature helps the rhizosphere retain water and nutrients, which encourages root development and nutrient intake and ultimately increases the formation of chlorophyll. Furthermore, GA3 and QDBC are essential for photoprotection and light harvesting[46]; it has been demonstrated that they both boost photosynthetic efficiency, which increases carotenoid accumulation. Rhizobacteria release substances[47] like organic acids and phytohormones that promote plant development, enhancing plant metabolism and nutrient absorption. They help the soil's nutrient cycle, which increases the availability of vital minerals to plants and promotes the production of carotenoids and chlorophyll.

Anthocyanin accumulation

GA3 stimulates the expression of genes related to the synthesis of anthocyanins, which results in a greater build-up of anthocyanin pigments in plant tissues. By improving soil fertility and nutrient availability, QDBC[48] gives plants the resources to produce anthocyanins. The synergistic effects of the combination treatment on gene expression and food absorption increase anthocyanin production[13]. As a component of induced systemic resistance (ISR), rhizobacteria boost the synthesis of secondary metabolites, such as anthocyanins, and plant defense mechanisms. They trigger signaling pathways that increase the anthocyanin content of plant tissues by enhancing the expression of genes encoding the enzymes involved in anthocyanin production.

Antioxidants activity

By scavenging reactive oxygen species (ROS) and enhancing antioxidant enzyme activity, GA3-QDBC treatments diminish oxidative stress and lipid peroxidation. Additionally, they increase the integrity and stability of the membrane[49], which lowers peroxidase activity and electrolyte leakage. Along with GA3's antioxidative qualities, QDBC acts as an antioxidant accumulation and facilitates ROS detoxification[50].

Plant cells are protected from oxidative damage by exopolysaccharides and other substances produced by rhizobacteria that populate the rhizosphere. They improve plant stress tolerance by regulating antioxidant enzyme activities and lipid peroxidation levels[51]. This is demonstrated by decreased lycopene buildup, electrolyte leakage, and peroxidase activity during stressful situations.

GA3-QDBC treatments increase the production of antioxidant enzymes, including SOD, CAT, and APx, which scavenge ROS and shield cellular components from oxidative damage[48]. These effects are shown in treatments. They also improve membrane stability and lower ROS generation, which lowers lipid peroxidation and MDA buildup. By acting as a source of bioavailable nutrients and promoting the activity of antioxidant enzymes, QDBC supports antioxidant defense systems[42,52]. Rhizobacteria cause plants to mount systemic defenses, which include lowering lipid peroxidation and increasing antioxidant enzyme activity. Adjusting MDA content and

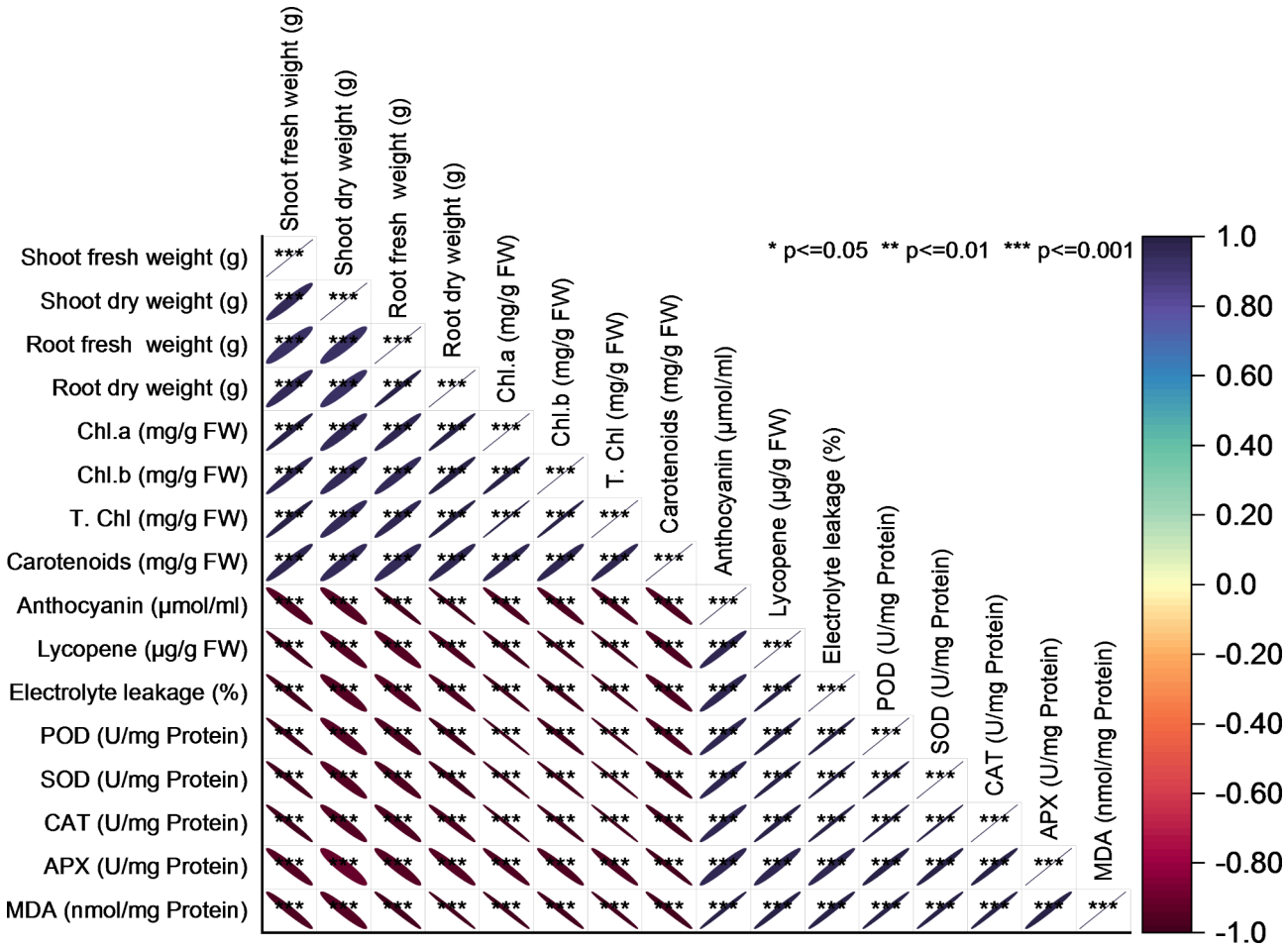

Fig. 2. Pearson correlation analysis for the studied attributes.

ROS levels improves plant stress tolerance by reducing oxidative damage to cellular macromolecules[53]. Through their ability to maintain redox equilibrium in plant cells and encourage ROS scavenging, rhizobacteria also help plants become more resilient to environmental impacts.

Conclusion

In conclusion, using 0.5 GA3-QDBC + RB treatment can reduce the lead effect in fenugreek plants by enhancing growth parameters and stress responses. Treatment 0.5 GA3-QDBC + RB improves yield, chlorophyll content, carotenoids, and anthocyanin levels, suggesting potential synergies. More investigations are recommended at the field level for the declaration of 0.5 GA3-QDBC + RB as the best amendment for mitigating lead stress in fenugreek.

Data availability

All data generated or analyzed during this study are included in this published article.

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Author contributions

M.A.S.; U.Y.; contributed to the conceptualization and design of the study, as well as data collection, analysis, and interpretation. U.Y.; A.E.; contributed to the statistical analysis; M.J.A.; A.A.A.; S.A.A.; interpretation of the data. All authors have reviewed and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

Study protocol must comply with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not Applicable.

Human and animal rights

We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

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