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Influence of indole acetic acid, arginine and mango fruit waste biochar on nutrients, chlorophyll contents and antioxidants of Fenugreek in salt affected soil

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Salinity stress disrupts water uptake and nutrient absorption, causing reduced photosynthesis, stunted growth, and decreased crop yields in plants. The use of indole acetic acid (IAA), arginine (AN), and mango fruit waste biochar (MFWB) can be effective methods to overcome this problem. Indole acetic acid (IAA) is a natural auxin hormone that aids cell elongation and division, thereby increasing plant height and branching. L-arginine, an amino acid, is crucial for plant defense mechanisms, forming proline, polyamines, and nitric oxide, which regulate biological activities and prevent oxidative damage. Mango fruit waste biochar enhances soil fertility and water retention, thereby enhancing fruit development and yield. This study investigates the effects of combining IAA and AN as amendments to fenugreek, with and without MFWB. Four treatments (control, 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA) study were conducted in four replications using a completely randomized design. Results demonstrate that the 250 mg/L AN + 2mM IAA with MFWB treatment led to a significant rise in fenugreek plant length (30.26%), plant fresh weight (36.37%), and plant dry weight (15.78%) over the control under salinity stress. There was a notable increase in chlorophyll a (5.13%), chlorophyll b (14.06%), total chlorophyll (7.79%), and shoot N, P, K from the control under salinity stress also showing the potential of 250 mg/L AN + 2mM IAA with MFWB. In conclusion, applying 250 mg/L AN + 2mM IAA with MFWB is a strategy for alleviating salinity stress in fenugreeks.

Keywords Salinity stress, Arginine, Mango fruit waste Biochar, Chlorophyll content, Antioxidants

In semi-arid and arid areas of the world, stress induced by soil salinity caused a significant decline in the growth and yield of crops[1]. It currently affects 7% of the earth's land surface, affecting approximately 1 billion hectares[2]. Salinity is the primary cause of the daily loss of 2000 hectares of arable land[3]. Under salinity stress conditions, plants experienced osmotic stress, ionic imbalance, and oxidative damage. Such conditions resulted in a significant decline in nutrients and water uptake. This decrease in nutrients and water uptake ultimately decreases photosynthesis in plants[4,5] and can reduce crop yield by up to 10–25%[6]. Further increases in salinity stress intensity can also adversely affect crop growth, which can eventually cause 50% losses in yield under irrigated farming systems[7]. Examining biochemical and molecular interventions is crucial to addressing such adverse impacts of salinity stress. Among different technologies, using L-arginine, an amino acid, is becoming the center of attention for scientists.

L-arginine (AN) is an amino acid, an essential precursor of polyamines (PAs), a vital cell signaling molecule, and nitric oxide (NO)[8]. Its positive effects are linked to the formation of proline, polyamines, or nitric oxide[9]. Furthermore, PAs regulate various plant biological activities, particularly managing cell defense systems

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to prevent oxidative damage[10]. Previous researches have shown that L-arginine can improve crop growth by mitigating salinity stress. Foliar application of L-arginine improves the photosynthetic pigments forma under salinity stress. This improvement in photosynthesis leads to improvement in growth and yield in different crops, i.e., canola, sunflower, mung bean, and lupine[11–13].

Balance application of L-arginine also increased the activity of antioxidants (catalase, ascorbate peroxidase, and guaiacol peroxidase). Such improvement in the activity of antioxidants caused a decline in the oxidative stress caused by salinity stress[13]. Regulation of polyamine levels due to enhancement in proline is also an allied factor that improves the plant's growth under salinity stress due to applying L-arginine as an amendment[13,14].

On the other hand, due to its significant biological activity, indole acetic Acid (IAA) is a notable auxin[15]. Its components include cellular conditions like expanding osmotic content, permeability, decreasing wall pressure, inducing cell wall construction, and triggering RNA and protein production to facilitate cell elongation[16]. Moreover, IAA improves fruit and flower development by inhibiting or delaying leaf shedding and increasing embryonic activity[17].

Mango peels are a rich source of nutrients such as carotenoids, cellulose, hemicellulose, pectin, and polyphenols[18]. Waste mango peels can be utilized to create biochar, an eco-friendly solution that can absorb harmful wastewater metals[19]. Surprisingly, limited research has been done on the efficacy of mango fruit waste biochar in reducing salinity stress[20].

Fenugreek (*Trigonella foenum-graecum*) is a potent herb utilized in cooking and medicine due to its fragrant leaves and seeds[21]. Fenugreek seeds are essential for medicinal and nutritional purposes since they are rich in bioactive substances and nutritional components such as proteins, carbs, fixed oil, alkaloids, phenolics, flavonoids, volatile oils, minerals, and vitamins[22]. Thus, the pharmaceutical sector uses it as a source of steroidal diosgenin, an antioxidant, antidiabetic, anti-lithogenic, antibacterial, and immunological enhancer, and also treats hypocholesterolemia Excessive soil salt or irrigation water can negatively affect the physiology and productivity of fenugreek plants[23].

Considering the adverse effects of salinity stress, the current study explored the interactive effects of AN, IAA, and MFWB on fenugreek plants. The study covers the knowledge gap regarding using AN, IAA, and MFWB to alleviate salinity stress in fenugreek plants. The study's novelty lies in utilizing the AN, IAA, and MFWB as a combined amendment against salinity stress. It is hypothesized that IAA, AN, and MFWB might effectively mitigate the negative effects of salinity stress on fenugreek plants.

Materials and methods

Experimental site

In 2022, the ResearchSolution experimental area, situated at 30°09'41.6"N 71°36'38.0" E, was conducted as a planned experiment. The physicochemical parameters of soil samples from the area were evaluated by sifting them through a 2-mm mesh screen after air drying. The following features of the soil (Table 1) were determined as follows:

Salinity stress

For the introduction of salinity stress, salt-affected soil was used. This soil was naturally salt-affected and selected based on pre-experimental soil characteristics.

Synthesis of mango fruit waste biochar

The mango fruit waste was collected at the local fruit market (30°11′30.5"N 71°28′46.9" E). After sun drying, the pyrolysis process was conducted at 500 °C with a limited oxygen supply. The biochar was successfully crushed and extracted using a 2 mm sieve. Biochar's properties include pH values of 7.93, EC*e* (dS/m) of 4.39, ash content (%) of 40, volatile matter (%) of 20, fixed carbon (%) of 40, total P (%) of 0.99, total N (%) of 0.57, and total K (%) of 1.11.

Seeds sterilization

We purchased fenugreek seeds (Qasuri Methi) from an authorized seed dealer recognized by the Punjab government in Pakistan. The seeds were thoroughly cleaned and disinfected with a 5% sodium hypochlorite

Soil	Values	Irrigation	Values
pH	8.19	pH	7.29
EC*e* (dS/m)	6.11	EC*e* (µS/cm)	249
Soil organic matter (%)	0.40	Carbonates (meq. /L)	0.00
Total nitrogen (%)	0.02	Bicarbonates (meq. /L)	4.11
Extractable phosphorus (mg/kg)	5.11	Chloride (meq. /L)	0.05
Available potassium (mg/kg)	101	Ca + Mg (meq. /L)	3.19
Silt (%)	45	Sodium (mg/L)	64
Sand (%)	26	Ca + Mg = Calcium + Magnesium CEC = Cation exchange capacity EC*e* = Electrical conductivity	
Clay (%)	29		
Texture	Clay Loam		

Table 1. Characteristics of experimental soil.

solution after three 95% ethanol washes. The seeds were thoroughly cleaned and disinfected with a 5% sodium hypochlorite solution after three 95% ethanol washes. Ten seeds were sown in each pot containing five kilograms of soil. After germination, five seedlings were kept in each pot by trimming.

Experimental design and treatment plan
The treatments include, i.e., control, indole acetic acid (2mM IAA), arginine (250 mg/L AN), and 250 mg/L AN + 2 mM IAA. All the treatments were applied with and without MFWB. The study utilized a completely randomized design (CRD), with four replications for each treatment.

Fertilizers
During seeding, 0.050 g of P_2O_5 (or 0.31 kg of superphosphate per 5 kg of soil) and 0.03 g of nitrogen (or 0.074 g of urea per 5 kg of soil) were added to each pot. Moreover, a single NPK (20:20:20) fertilizer spray was applied at a rate of 5 g/L of water twenty days after the initial planting.

Irrigation
The pots field capacity (FC) was maintained at 60% using a moisture meter (YIERYI 4 in 1; Shenzhen, Guangdong Province, China)[24].

Harvesting and data collection
The data was collected 45 days after the plants' seeding. After harvesting, the plant's fresh and dry weights were measured using a digital balance. The samples were oven-dried for 72 h at 65 °C to calculate the dry weights of plant.

Chlorophyll contents and carotenoids
The study measured chlorophyll concentrations in fresh leaves using Arnon's standard methods[25]. An 80% acetone solution was used for the extraction process, and the final absorbance was measured at 663, 645, and 480 nm[26].

Antioxidants
The superoxide dismutase (SOD) activity was measured using the nitro blue tetrazolium (NBT) reduction inhibition at 560 nm[27]. Peroxidase (POD) activity was measured at 420 nm with standard procedure[28]. The decrease in absorbance at 240 nm due to H_2O_2 breakdown determined CAT activity[29]. The oxidation of ascorbate in the presence of H_2O_2 at 290 nm ascorbate peroxidase (APX) activity was measured[30].

Nutrient analysis
A mixture of nitrogen sulfuric corrosive and phosphorus and potassium di-acidic was utilized during the initial phase of digestion[31,32]. The nitrogen-fixing rate was determined using a modified version of the miniature Kjeldahl's technique[32]. The phosphorus fixation at 420 nm was determined using a spectrophotometer's yellow color method[32]. The potassium level was measured using a flame photometer.

Statistical analysis
The data was analyzed using conventional statistical methods[33]. The study's characteristics were evaluated using Excel 365, OriginPro software, and MS Office through a two-way ANOVA and Normality Test. The study utilized Tukey's test, Pearson correlation, Convex hull cluster plots, and cluster plots to create paired comparison graphs at $p < 0.05$ using OriginPro software[34].

Results
Growth attributes
The application of 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments without MFWB resulted in increases in plant length 10.80%, 4.23%, and 18.36%, plant fresh weight 45.99%, 26.20%, and 74.60%, and plant dry weight 45.25%, 32.94%, and 59.62% over the control, respectively. Under MFWB, adding 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments showed 22.73%, 7.98%, and 30.26% increase in plant length, 21.17%, 11.61%, and 36.37% in plant fresh weight, and 10.22%, 4.67%, and 15.78% in plant dry weight related to the control (Fig. 1).

Chlorophyll contents and carotenoids
In no MFWB, applying 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments exhibit a 5.45%, 4.44%, and 6.82% increase in chlorophyll a, 10.90%, 5.77%, and 16.03% in chlorophyll b, 6.92%, 4.80%, and 9.31% in total chlorophyll, and 8.03%, 4.38%, and 12.41% increase in carotenoids from the control. Adding 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments under MFWB, led to a 2.60%, 1.14%, and 5.13% increase in chlorophyll a, 9.37%, 4.69%, and 14.06% chlorophyll b, 4.62%, 2.19%, and 7.79% total chlorophyll, and 8.02%, 4.94%, and 14.20% increase in carotenoids then the control (Fig. 2).

Antioxidants
A significant 8.42%, 4.30%, and 13.50% decrease in POD, 12.05%, 5.68%, and 19.23% SOD, 4.35%, 2.86%, and 7.46% CAT, and 8.20%, 4.76%, and 11.86% decrease in APx with 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments under no MFWB than the control. With MFWB these 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments resulted in an 11.25%, 5.49%, and 19.72% decrease in POD, 12.18%,

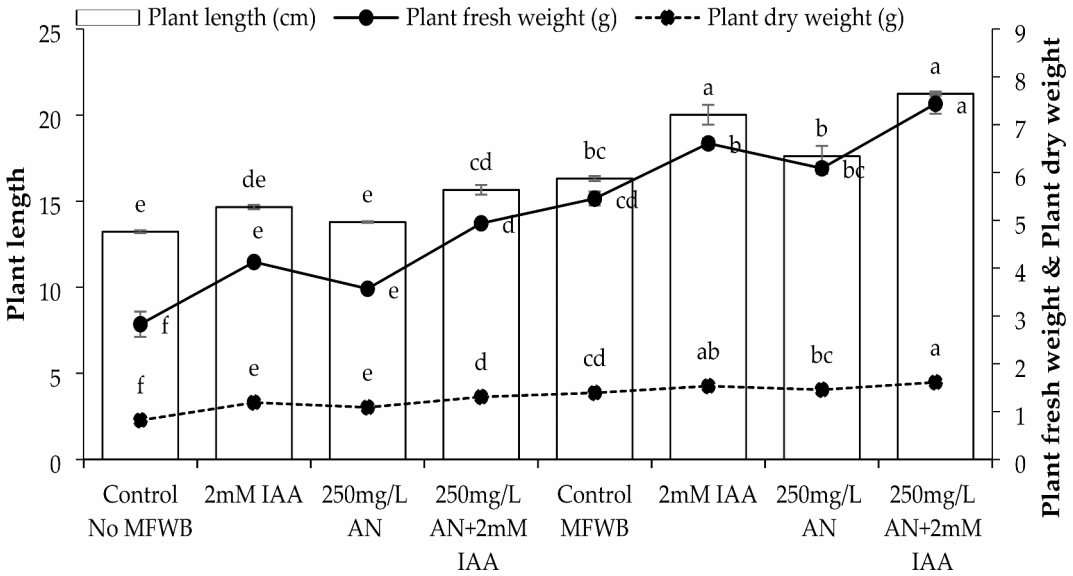

Fig. 1. The impacts of arginine (AN) and indole acetic acid (IAA) on fenugreek plant length, plant fresh, and dry weight grown with and without MFWB. Significant differences were detected at ($p < 0.05$) using the Tukey test; different letters on the bars represent the means of four replicates.

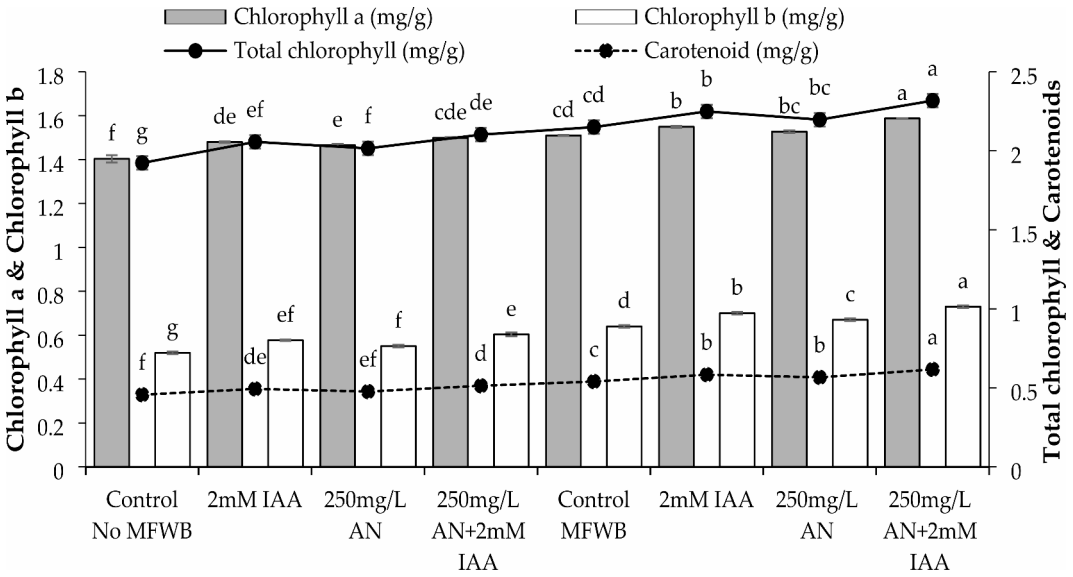

Fig. 2. The impacts of arginine (AN) and indole acetic acid (IAA) on fenugreek on chlorophyll a, b, total, and carotenoids grown with and without MFWB. Significant differences were detected at ($p < 0.05$) using the Tukey test; different letters on the bars represent the means of four replicates.

6.76%, and 19.46% SOD, 6.45%, 4.76%, and 10.00% CAT, and 9.62%, 5.56%, and 16.33% decrease in APx compared to the control (Fig. 3).

Shoot N, P, and K

Without MFWB, applying 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments showed 8.82%, 5.39%, and 11.76% increases in shoot N, 4.17%, 1.67%, and 5.83% shoot P, and 2.00%, 0.67%, and 3.33% in shoot K from the control. Applying MFWB with 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments caused a significant increase in 6.33%, 2.53%, and 10.13% shoot N, 3.85%, 1.54%, and 5.39% shoot P, and 3.19%, 1.91%, and 5.10% shoot K were recorded over the control (Fig. 4).

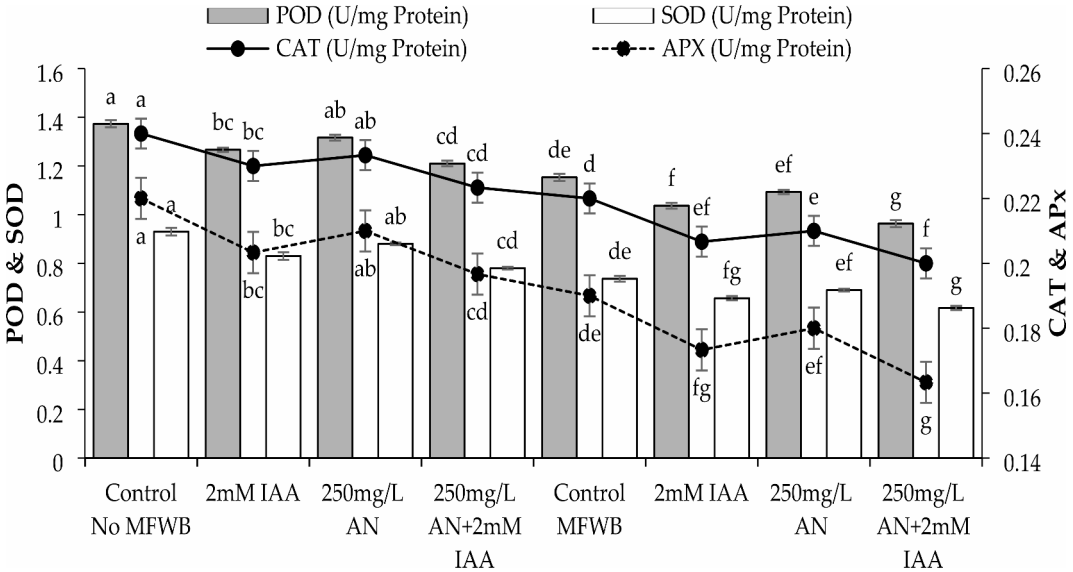

Fig. 3. The impacts of arginine (AN) and indole acetic acid (IAA) on fenugreek on POD, SOD, CAT, and APx grown with and without MFWB. Significant differences were detected at ($p < 0.05$) using the Tukey test; different letters on the bars represent the means of four replicates.

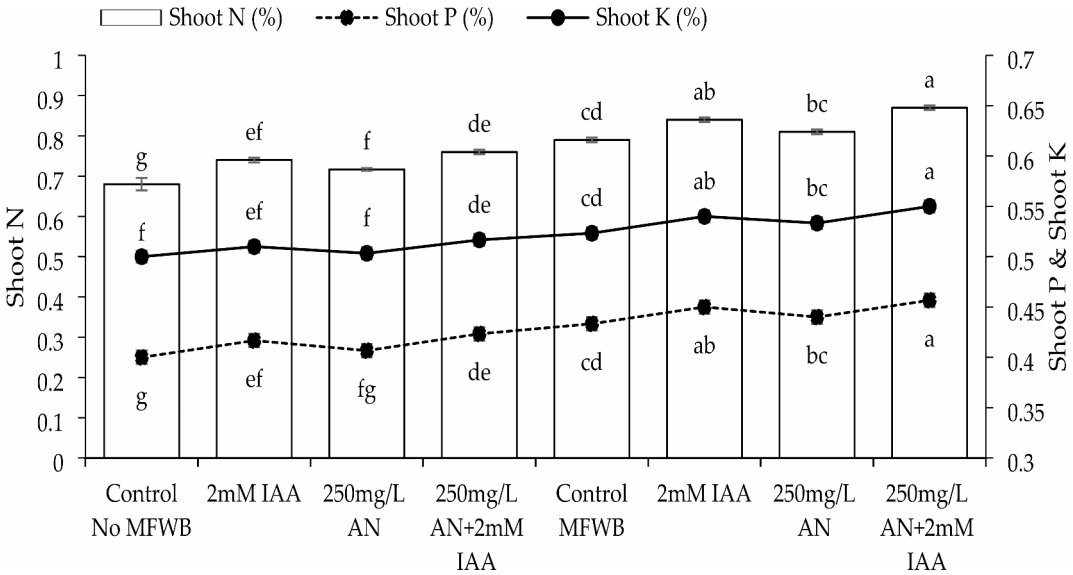

Fig. 4. The impacts of arginine (AN) and indole acetic acid (IAA) on fenugreek on shoot N, P, and K grown with and without MFWB. Significant differences were detected at ($p < 0.05$) using the Tukey test; different letters on the bars represent the means of four replicates.

Root N, P, and K

In comparison to the control, adding 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments led to a 5.22%, 2.41%, and 7.23% increase in root N, 1.59%, 0.00%, and 1.59% root P, and 4.17%, 1.39%, and 6.95% in root K respectively without MFWB. Under MFWB, applying 2mM IAA, 250 mg/L AN, and 250 mg/L AN + 2mM IAA treatments exhibit a 4.78%, 2.57%, and 8.09% increase in root N, 1.03%, 0.00%, and 2.57% root P, and 3.85%, 2.57%, and 7.69% in root K related to the control (Fig. 5).

Convex hull and hierarchical cluster analysis

The no MFWB group generally occupies a region in the lower-left quadrant, with most of the scores having negative values for PC 1 and slightly varying around zero for PC 2. These scores range from − 6.78038 to -0.22547 for PC 1 and from 1.27617 to -0.52929 for PC 2. In contrast, the MFWB group is predominantly located in the

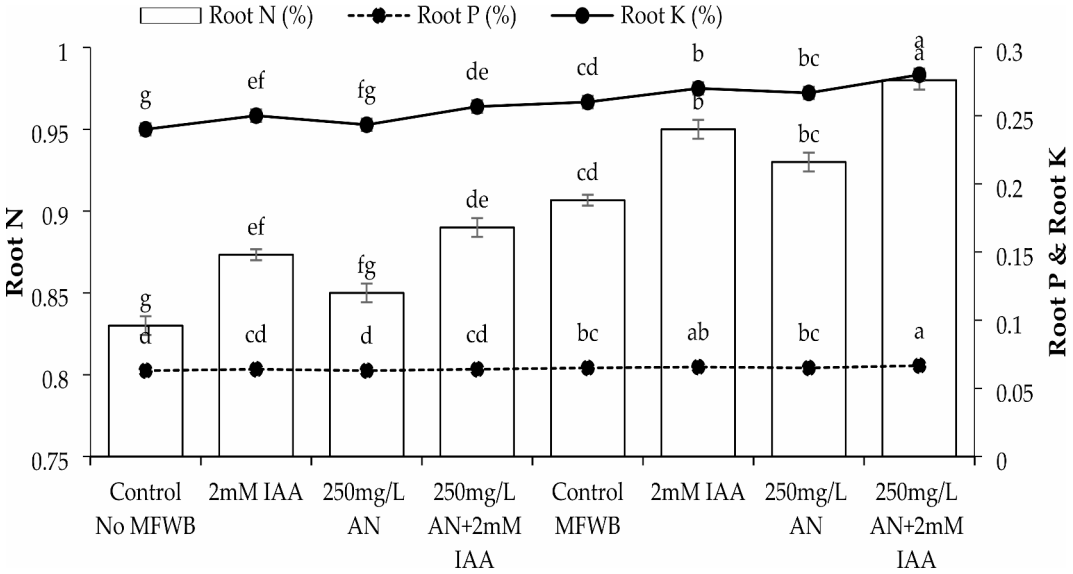

Fig. 5. The impacts of arginine (AN) and indole acetic acid (IAA) on fenugreek on root N, P, and K grown with and without MFWB. Significant differences were detected at ($p < 0.05$) using the Tukey test; different letters on the bars represent the means of four replicates.

upper-right quadrant, with positive values for PC 1 and small variations around zero for PC 2. The scores for this group range from 0.25487 to 7.0377 for PC 1 and from −0.22218 to 0.70554 for PC 2 (Fig. 6A).

In the control group, the PC1 scores ranged from −6.78038 to 1.22615, with corresponding PC2 scores between 1.27617 and −0.11368. The 2mM IAA treatment showed a shift in the PC1 scores from −2.89667 to 4.76242 and PC2 scores from −0.09423 to 0.23959. The 250 mg/L AN treatment had PC1 scores ranging from −4.75699 to 3.002, with PC2 scores between −0.50035 and 0.00709. Finally, the combination treatment of 250 mg/L AN and 2mM IAA showed the most positive PC1 scores, ranging from −1.66636 to 7.0377, and PC2 scores from −0.52929 to 0.70554 (Fig. 6B).

The clustering begins with closely related variables such as total chlorophyll and shoot N, which both show a similarity of 0.15883, indicating they are highly correlated. Similarly, chlorophyll b and root N cluster together with a similarity of 0.24297, suggesting a moderate correlation. As the analysis progresses, other variables, such as Carotenoid and Plant fresh weight cluster with similarity values of 0.30563 and 0.31393, respectively. POD and SOD enzymes, both involved in antioxidant activity, cluster with a similarity of 0.35869, reflecting their related functional roles. Further down the hierarchy, APX clusters with a larger group at a higher similarity value of 0.79284, highlighting its distinction from the other antioxidant enzymes. Plant dry weight and chlorophyll a show nearly perfect similarity (0.99292), indicating a strong correlation between these variables. Notably, Root P shows the highest level of dissimilarity within the dataset, clustering at a late stage with a similarity of 3.74785. The final clusters, 31 and 32, with similarity indices of 96.25215 and 98.60956, respectively, indicate the culmination of the hierarchical process, grouping the least similar variables (Fig. 6C).

Pearson correlation analysis

Plant length strongly correlates positively with plant fresh weight ($r = 0.9632$) and plant dry weight ($r = 0.90724$). It also shows high positive correlations with chlorophyll a ($r = 0.92879$), chlorophyll b ($r = 0.973$), total chlorophyll ($r = 0.96336$), and Carotenoid content ($r = 0.9713$). These findings suggest that as plant length increases, there is a corresponding increase in biomass and pigment concentration, which are important indicators of plant health and vigor. Plant fresh weight is very strongly correlated with plant dry weight ($r = 0.97325$) and similarly with chlorophyll a ($r = 0.97179$), Chlorophyll b ($r = 0.99389$), total chlorophyll ($r = 0.99411$), and carotenoid ($r = 0.99155$), indicating that larger plants, as measured by fresh weight, also tend to have higher pigment concentrations. Antioxidant enzyme activities, such as POD (Peroxidase), SOD (Superoxide Dismutase), CAT (Catalase), and APX (Ascorbate Peroxidase), are negatively correlated with plant length and pigment concentrations. For instance, POD shows strong negative correlations with plant length (r = -0.97588), plant fresh weight (r = -0.99565), and total chlorophyll (r = -0.99376), indicating that higher enzyme activities are associated with lower biomass and pigment content. Shoot and root nutrients (N, P, K) also demonstrate various correlations with plant metrics. Shoot N and Root N have strong positive correlations with plant length and weight, such as Root N with Plant fresh weight ($r = 0.99282$). Shoot P and root P show lower correlations, but still maintain positive relationships with plant length and weight (Fig. 7).

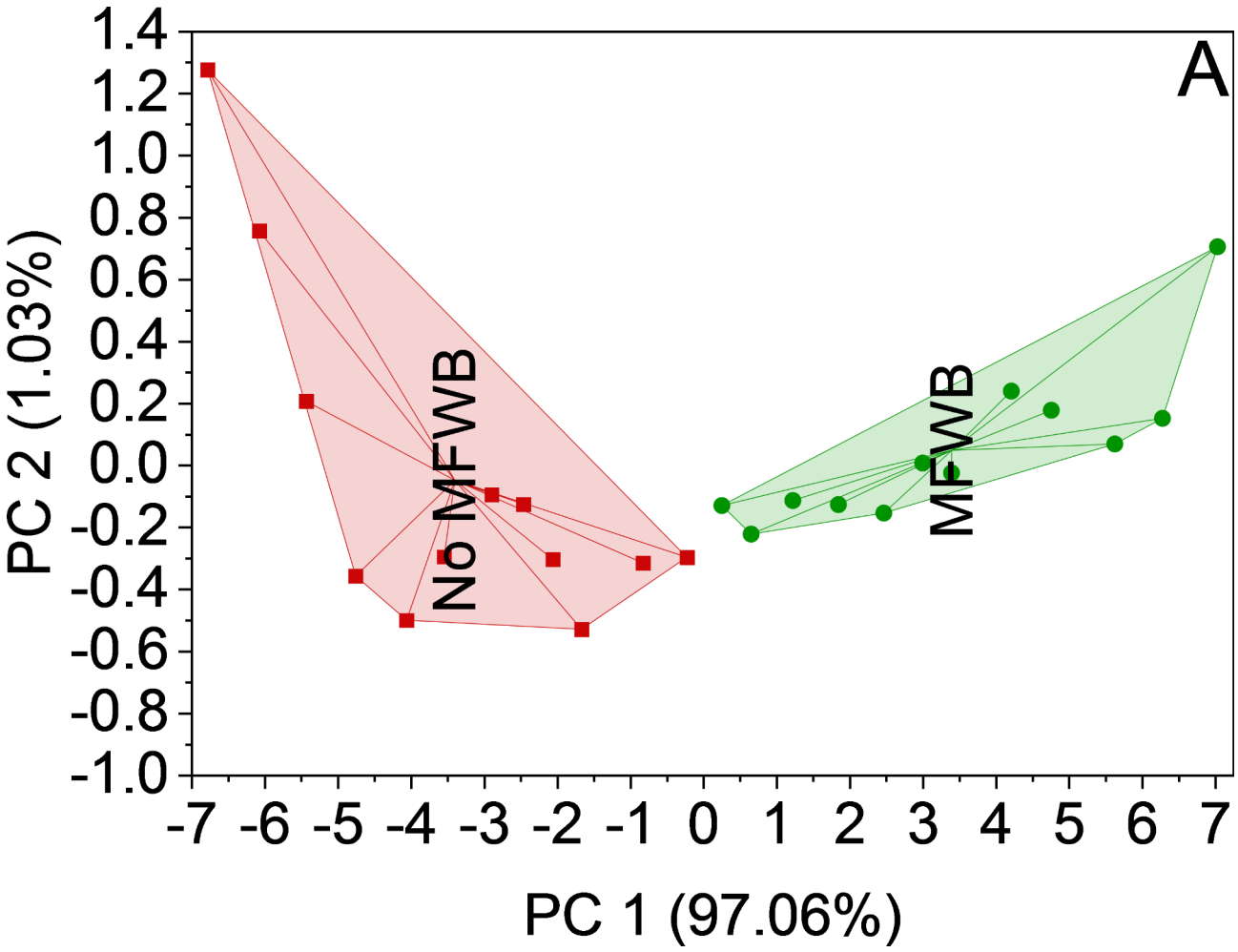

Fig. 6. Cluster plot convex hull for mango fruit waste biochar (MFWB) levels (**A**), treatments (**B**), and hierarchical cluster plot (**C**) for studied attributes.

Discussion

Salinity stress

Salinity stress disrupts plant growth, reducing photosynthesis and crop yields[35]. Excessive salt levels can induce oxidative stress, damaging cellular structures and compromising overall productivity[36]. Furthermore, ionic imbalance in plant cells also causes toxicity that adversely affects chlorophyll contents[37–40] and the electron transport chain. It also inhibits photosystem II, which alters the anatomy of leaves as well as the ultrastructure of chloroplast[14]. The current study also noted similar results where control plants under salinity stress showed a decline in chlorophyll contents and nutrients. Such results justified the disturbance in the chloroplast activity and ionic imbalance in plants caused by the salinity stress, which resulted in poor plant length, fresh weight, and dry weight.

Indole acetic acid (IAA)

Indole acetic acid (IAA), a crucial auxin, encourages cell division and elongation, boosting plant growth[41]. Plant length and biomass are directly related to auxins such as IAA, which also affect cell wall extensibility and aid in redistributing growth hormones[42]. It also promotes cell elongation and division, while polyamines from arginine aid in plant growth and stress resilience[43,44]. Foliar application of IAA minimizes the Na^+ accumulation in the roots and shoots. In addition to that, it also enhances the K^+, Ca^{2+}, and Mg^{2+} uptake in plants, which play a vital role in the improvement of physiological attributes under salinity stress conditions[45]. In the current study, improvement in P and K concentration in shoot and root by application of 2mM application of IAA also validated the effectiveness of its role in improving spinach growth under salinity stress. An improvement in K helps regulate stomatal conductance, which in return helps in the mainatance of stomatal conductance and water uptake in plants[24,46,47].

Arginine

Scientists have found that the application of arginine improves nutrient uptake, especially nitrogen, under salt-stress conditions[11]. It also enhances the catalase antioxidant activity, which minimizes plant oxidative stress[48].

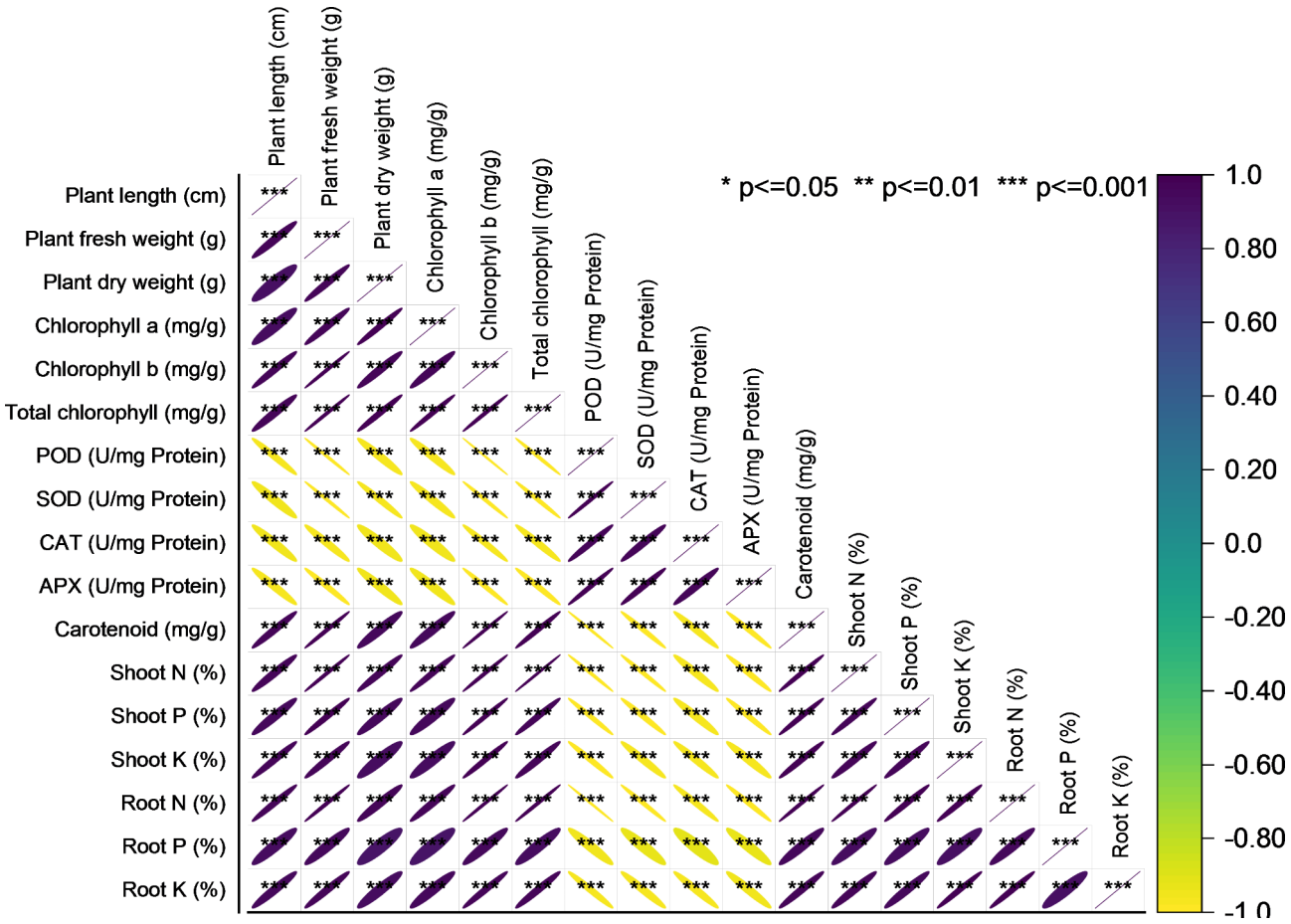

Fig. 7. Pearson correlation analysis for the studied attributes.

In the current study, a hierarchical cluster plot confirmed that the root N concentration improvement was the prime factor for enhancing spinach plant growth under salinity stress. This improvement was more significant when IAA and arginine were applied as a combined amendment. Better uptake of nitrogen. Adequate nitrogen uptake in plants is an allied factor for improving membrane stability, leaf gas exchange, and water relations. Such improvements protect the plants from oxidative damage under stress conditions[49]. In addition to the above, a significant improvement in chlorophyll contents and photosynthetic activity due to the balance uptake of nitrogen also improved plant growth under salinity stress[50].

Biochar

The addition of biochar in soil caused significant improvement in soil organic carbon. This organic carbon is served as food, while the porous structure of biochar provides shelter to the soil microbes. Improving soil microbial proliferation resulted in better cycling of nutrients and their availability to the plants. Furthermore, biochar application causes the stabilization of soil aggregates. It also increases soil water holding capacity, which not only dilutes the salt effects but also helps better uptake of water and nutrients in plants. Such improvement in water uptake plays a key role in minimizing osmotic stress and nutrient deficiency or imbalance when biochar is applied as an amendment in soi[51–53].

Conclusion

In conclusion, adding 250 mg/L AN + 2mM IAA with MFWB can enhance fenugreek growth under salinity stress by improving the nitrogen concentration in the root. This improvement in root N concentration was a key mechanism that worked when 250 mg/L AN + 2mM IAA treatment with MFWB was applied to spinach under salinity stress. Improvement in shoot N and chlorophyll contents were also associated with root N concentration where 250 mg/L AN + 2mM IAA with MFWB was applied as treatment. Although the current study suggests the better root N concentration as the prime mechanism for alleviation of salinity stress by application of 250 mg/L AN + 2mM IAA with MFWB as treatment, more investigations are suggested at the field level under different agroclimatic zones to declare 250 mg/L AN + 2mM IAA with MFWB as best amendment against salinity stress.

Data availability

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

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Conceptualization; M.H.; S.D.; Conducted experiment; M.H.; S.M.; Formal analysis; M.H.; S.M.; Methodology; M.H.; R.K.I.; Writing—original draft; M.H.; S.D.; Writing—review & editing; M.H.; A.A.A.; S.A.A.; R.K.I.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
I declare that manuscript reporting studies do not involve human participants, data, or tissue. So, it is not applicable. Experimental research and field studies on plants (cultivated or wild), including the collection of plant material, must comply with relevant institutional, national, and international guidelines and legislation. I confirmed that all methods were performed according to the relevant guidelines/regulations/legislation. The seeds were purchased from a local certified seed dealer of the Government of Punjab, Pakistan.

Consent for publication
Not Applicable.

Additional information
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