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RESEARCH ARTICLE

Evaluating the effects of biochar and plant growth-promoting bacteria on growth parameters of mustard and rice

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Abstract

Soil degradation is a major concern, causing a decline in crop productivity and making sustainable agricultural practices essential for humankind. Biochar and plant growth-promoting bacteria (PGPB) are currently applied as affordable and environmentally safe alternatives. Biochar, a porous, carbon-rich by-product of biomass pyrolysis, was applied at 3 % (w/w) alone (control) and in combination with microbial inoculants, including a bacterial consortium. A 15-day pot experiment was conducted under controlled conditions to evaluate the effects of biochar and plant growth-promoting bacteria (PGPB) on plant growth. This study investigates the effects of biochar and PGPB (three *Pseudomonas* spp. and one *Diaphorobacter* spp.) on rice (*Oryza sativa* L.) and mustard (*Brassica juncea* L.) when applied individually and in combination. The results were evaluated based on plant growth-promoting criteria namely root and shoot length and chlorophyll content. It was observed that biochar had a positive impact on plant growth parameters when applied individually, however in combination with bacterial inoculants, the results were significantly improved. The consortium treatment showed higher values for root length, shoot length and chlorophyll content compared with individual treatments. This study highlights the major scope and potential of utilising plant growth promoting bacteria and biochar to achieve sustainable increases in plant growth and yield. However, some treatments showed variability in results, which could be attributed to the compatibility between biochar and microbial inoculants. This underscores the need for further optimisation studies on the interaction between biochar and bacterial inoculants.

Keywords: biochar; *Brassica juncea*; consortium; *Oryza sativa*; *Pseudomonas* spp.; sustainability

Introduction

The rapid increase in world's population has posed a significant challenge for modern-day agriculture to enhance the productivity while maintaining environmental sustainability (1, 2). Arable land is becoming increasingly degraded because of intensive farming, the overuse of chemical fertilisers and climate change (3). There is an urgent need for sustainable agricultural solutions that improve soil health and increase crop productivity. Biochar has emerged as one of the most viable soil amendments because of its ability to improve soil characteristics, sequester carbon and promote crop development (3, 4).

Biochar is a porous carbon-rich material, produced by the pyrolysis of organic biomass under low oxygen conditions (5). A range of feedstocks such as agricultural waste, wood chips and manure are used for biocharproduction (4). The quality of the final biochar, including its pH, nutritional content, cation exchange capacity (CEC), surface area and porosity, depends on the type of feedstock utilised, along with the temperature and duration of pyrolysis (6, 7).

A high-quality biochar with these characteristics is well-suited for restoring degraded soils, strengthening soil structure and boosting the retention of water and nutrients. Over the past decade, the research has increased showing promising results on the impact of biochar on plant growth (1, 2). It acts as a reservoir, making water and nutrients more accessible to plants due to its porous structure (8, 9). It also contributes to soil fertility and plant nutrition by providing a habitat for beneficial microorganisms such as mycorrhizal fungi and nitrogen-fixing bacteria (10). In addition to improving soil physicochemical properties, biochar can enhance the survival, colonisation and activity of plant growth-promoting bacteria (PGPB). Its porous structure provides protected microhabitats that shield microbial cells from environmental stresses while retaining moisture and nutrients required for microbial growth. Biochar also facilitates microbial attachment and biofilm formation on its surface, thereby improving the persistence and functional activity of beneficial microorganisms in the rhizosphere. As a result, biochar can enhance the effectiveness of PGPB-mediated processes such as, nutrient solubilisation, phytohormone production, nitrogen fixation and stress alleviation, ultimately contributing to improved plant growth and productivity (10).

The ability of plants to absorb light and perform photosynthesis is controlled by shoots, while the uptake of nutrients and water is regulated by roots. Chlorophyll concentration in plants is a useful indicator of plant health and nutritional status and its concentration is directly correlated with the photosynthetic ability of

plants. Therefore, the response of a plant to biochar can be assessed by evaluating three factors: root growth, shoot development and chlorophyll content (1).

Although beneficial for plants, poor-quality biochar can have negative effects on plant growth. For example, biochar may contain phytotoxic compounds if the feedstock contains pollutants or if pyrolysis process is inefficient. In addition, the quantity and the type of biochar as well as the type of soil can influence on plant growth (2). Although many positive results have been obtained through various studies conducted on biochar, further research is required to identify the optimal biochar properties, dosage and application techniques for different crop and soil types to fully understand the effects of biochar on plant yield and soil health (3, 11, 12).

This study aims to evaluate the effects of biochar on plant growth parameters, particularly root length, shoot length and chlorophyll content. It contributes to a more detailed understanding of how biochar influences plant development. The outcomes may have applications for farmers and soil scientists interested in improving soil health and promoting sustainable agriculture. It may be particularly helpful in low-input agricultural systems, where it can be used as a substitute for synthetic fertilisers to enhance plant growth and yield consistently.

Materials and Methods

Materials

The alluvial soil without any added fertiliser obtained from a local nursery in Navi Mumbai was used. Soil was characterised by Bombay Test House, Navi Mumbai and was found to have a pH of 7.8. The nitrogen (N), phosphorus (P) and potassium (K) contents were, 1.16, 0.15 and 0.02 % respectively. The biochar was obtained from Kanha Shanti Vanam in Telangana. It was produced through pyrolysis, a controlled thermal decomposition of biomass under limited oxygen conditions. Prior to pyrolysis, the biomass was air-dried and its moisture content was maintained below 20 % to ensure efficient carbonisation and reduce smoke and ash formation. Dry woody biomass was cut into small, uniform pieces to allow even heat transfer during pyrolysis. The material was heated in a low-oxygen environment, enabling the conversion of biomass into stable carbon-rich biochar. After completion of pyrolysis (approximately 600 °C), the biochar was allowed to cool, quenched, crushed and sieved to obtain a uniform particle size suitable for soil application. The biochar was analysed by Bombay Test House, Navi Mumbai and the following parameters were recorded: N (0.19 %), electrical conductivity (635 us/cm), organic carbon (1.03), pH (7.28), P (0.66 %), total N 0.34 %) and K (0.69 %). Seeds of rice (*Oryza sativa* cv. Swarna) and mustard (*Brassica juncea* cv. Pusa) were procured from a local shop in Varanasi, Uttar Pradesh, India. The varieties selected are commonly cultivated in the middle Gangetic region.

Methodology

This research is in continuation of previous work on 50 cadmium-tolerant bacterial isolates (13, 14). These isolates were further screened for multi-heavy-metal tolerance (multi-HMT) against 5 heavy metals namely arsenic (As), zinc (Zn), lead (Pb), chromium (Cr), cobalt (Co).

Selection of bacterial cultures

Four bacterial isolates were selected for the plant growth experiment based on a multi-step screening process as follows:

Firstly, quantitative phosphate solubilisation, indole-3-acetic acid (IAA) production and ammonia production values of the selected isolates were included in the isolate selection criteria to clarify the basis for selecting cultures used in the plant growth study. One isolate that showed highest combined quantitative performance across all three plant growth promoting (PGP) traits was selected.

Secondly, these isolates were screened for positivity for at least one indirect PGP trait out of 4 namely amylase, cellulase, pectinase or caseinase activity.

Thirdly all the isolates were assessed for biocompatibility before their use in a consortium.

Lastly, all the isolates were required to be sensitive to antibiotics namely penicillin, streptomycin, ampicillin, tetracycline, vancomycin.

Among the selected isolates, CDW14 showed the highest IAA production (39 µg mL^{-1}), CDS20 showed the highest phosphate solubilisation (99 µg mL^{-1}) and CDW6 showed the highest ammonia production (1 µmol mL^{-1}), whereas CDS12 was selected based on its combined quantitative PGP performance (IAA: 1 µg mL^{-1}, phosphate solubilisation: 72.7 µg mL^{-1} and ammonia production: 0.69 µmol mL^{-1}).

The selected bacterial isolates (CDW6, CDW14, CDS12 and CDS20) were identified using 16S rRNA gene sequencing and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). The 16S rRNA sequencing was performed at HiMedia Laboratories Pvt. Ltd., Navi Mumbai, India. MALDI-TOF MS analysis was conducted at Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, Gujarat, India, using a Bruker MALDI Biotyper system (Run ID: 221221–1455–211).

Plant treatment groups

(A) Control groups: C1 and C2 were used as controls, C1 contained only autoclaved soil, while C2 contained biochar, bacterial cultures and autoclaved soil.

(B) Bacterial culture + Biochar: Treatments T1–T5 consisted of biochar, bacterial cultures and autoclaved soil. T1–T4 contained individual bacterial isolates, whereas T5 consisted of a consortium of the four isolates along with biochar.

(C) Bacterial culture only: Treatments T6–T10 included bacterial cultures and autoclaved soil without biochar. T6–T9 contained individual bacterial isolates, while T10 comprised a consortium of all four isolates without biochar. All treatments were maintained in triplicate.

Surface sterilisation of seeds

Seeds were first soaked in water to separate and remove non-viable seeds. The remaining seeds were then thoroughly rinsed with clean water. Subsequently, they were surface-sterilised using 1 % sodium hypochlorite (NaOCl) solution and rinsed three times with sterile distilled water to remove any residual disinfectant. The seeds were placed in a sterile petri dish at an equal distance from each other and moistened with sterile distilled water to ensure uniform germination. They were then covered with another sterile filter paper moistened with sterile distilled water. The Petri plates were covered with lids and incubated under in dark conditions at room temperature for 24 hr to allow germination (15).

Bacterial culture, consortium and soil preparation

Each selected bacterial isolate was inoculated individually into nutrient broth and incubated at 28 ± 2 °C for 24 hr on a rotary shaker at 100 rpm. After incubation, the cultures were adjusted to a cell density of 10^5 CFU mL^{-1} and 1mL of bacterial culture was added to 20 g of soil for the T1–T4 and T5–T9 treatments. Equal volumes of each isolate were then combined to prepare a microbial consortium, which was subsequently used for T5 and T10 treatments (16, 17).

The soil was sterilised by autoclaving at 121 °C for 20 min. The cooled soil was half-filled in sterile plastic cups. Surface-sterilised germinated mustard and rice seeds (procured from local agricultural stores in Varanasi, Uttar Pradesh, India) were sown (4 seeds per cup) at depths of 0.5 cm and 2 cm in the soil, respectively. After adding 2 mL of bacterial suspension (OD_{600} = 0.5) to the rhizospheric region of the seeds, they were covered with a layer of soil. To assess the effect of biochar (3 %), it was first mixed uniformly with respective bacterial suspension (single bacterial isolate or consortium) and incubated for 24 hr under aseptic conditions. This was followed by placing the biochar mixture over the seeds. The seeds were then covered with a 2–3 cm layer of soil. Precautions were taken to allow water drainage and maintained 100 % water saturation. All experimental setups were done in triplicate and the plant growth parameters were recorded on the 15th day (18).

Evaluation of plant growth parameters

Root length was measured from the tip of the primary root to the base of the hypocotyl. Shoot length was measured from the tip of the uppermost leaf to the hypocotyl region. The mean root and shoot lengths were expressed in centimetres. Total chlorophyll content was estimated spectrophotometrically (19–20) and calculated using the following equation.

$$\text{Total chlorophyll (mg g}^{-1}) = \{[(20.2 \times A_{645}) + (8.02 \times A_{663})] \times V\} / (1000 \times W)$$

Where,

A645 and A663 represent the absorbance values measured at 645 and 663 nm, respectively,

V represents the final volume of the extract (mL) and

W represents the fresh weight of the tissue sample (g).

Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine statistically significant differences among the treatment groups for each growth parameter. The parameters (root length, shoot length, chlorophyll content) for rice and mustard were analysed independently. The mean values were compared across treatments with significance set at $p < 0.05$. Treatments were considered significant if the p - value was less than 0.05 and non-significant if they were above this threshold.

Results

Based on MALDI-TOF MS analysis, isolate CDW6 (T1) was identified as *Pseudomonas aeruginosa* ATCC 27853 THL, showing a high-quality match (+++)* with a matched pattern score of 2.27 and NCBI Taxonomy ID 287. Similarly, isolate CDW14 (T2) was identified as *P.aeruginosa* ATCC 27853 THL, showing a high-quality match (+++)* with a matched pattern score of 2.42 and NCBI Taxonomy ID 287. Isolate CDS12 (T3) was identified as *Diaphorobacter nitroreducens* strain Ruchi_574 based on 16S rRNA gene sequencing. The partial 16S rRNA sequence (1442 bp) was deposited in the NCBI GenBank database (Accession number OR855996.1). Isolate CDS20 (T4) was identified as *P.aeruginosa* strain Ruchi_576 based on 16S rRNA gene sequencing and the partial 16S rRNA sequence (1455 bp) was deposited in GenBank (Accession number OR855998.1).

*+++ indicates a high-confidence MALDI-TOF MS identification (score range 2.00–3.00) based on the Bruker Biotyper reference database.

Effect of single isolate and consortium on root length of rice (*Oryza sativa* L.)

The biochar-amended control group (C2) showed higher root length compared to the control group (C1) without biochar. Although, an exception to this trend was observed in treatment T3, where root length (4.83 cm) was lower than that in T8 (9.8 cm), despite T3 (*P. aeruginosa* ATCC27853 THL) being amended with biochar (Fig. 1). The highest root length was observed in T1 (13.17 cm), whereas T3 showed the lowest root length (4.83 cm) (Fig. 1). Among the treatments, biochar-amended soils with consortium showed longer root lengths compared to those without biochar. When compared with their respective control groups, most treatments showed improved root length performance. However, exceptions were observed in T3 (biochar-amended) and T10 (non-biochar), which showed lower root lengths than their respective controls. On comparing C1 with the biochar treatment groups, all treatments except T3 showed increased root growth. In contrast, when C2 was compared with other treatment groups, only T7 and T8 exhibited greater root lengths (7.7 % and 20 % respectively) while all remaining treatments recorded lower values (Fig. 1).

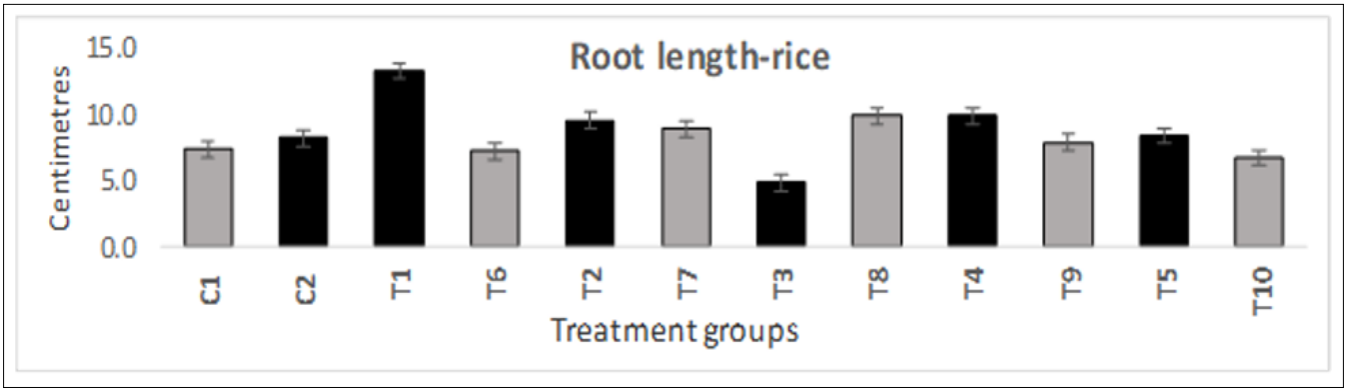


Fig. 1. Root length (cm) of rice plants under different treatment groups.

C1: control (no biochar, no bacterial inoculation); C2: biochar only; T1–T10: different combinations of biochar and microbial inoculants (PGPR isolates or consortia); T1, T2, T3, T4: bacterial inoculation with biochar; T6, T7, T8, T9: bacterial inoculation without biochar; T5: consortium with biochar; T10: consortium without biochar.

Effect of single isolate and consortium on root length of mustard (*Brassica juncea* L.)

For mustard, variable results were observed for root length across different treatments. Treatment T3 showed a higher root length (2.83 cm) compared with T8 (1.50 cm), while T2 and T7 showed similar root lengths of 1.50 cm (Fig. 2). Treatments T1 and T4 showed shorter root lengths than T6 and T9. Among the treatments with a consortium, T5 produced a higher root length (3.50 cm) compared with T10 (1.17 cm).

The treatment groups (T6–T10) generally showed higher root lengths compared with the control (C1), except for T10, which showed a lower value. The highest root length among the treatment groups was observed in T9 (3.83 cm) (Fig. 2). When C2 was compared with the other biochar treatment groups, T1, T2 and T4 showed shorter root lengths, whereas T3 and T5 showed longer root lengths. Similarly, when C2 compared with all the other treatments with biochar, only T9 showed a higher root length value while the remaining treatments recorded lower values.

Effect of single isolate and consortium on shoot length of rice (*Oryza sativa* L.)

A trend similar to root length was observed for shoot length in rice across treatments, where groups amended with biochar showed greater shoot elongation than those without biochar. The highest shoot length was observed in T5 (18.0 cm), followed closely by T10 (17.2 cm) and T4 (17.5 cm), indicating a positive effect of microbial consortium and biochar application (Fig. 3).

When compared with C2 (biochar-amended control), T1–T5 (biochar-amended treatments) showed increased shoot length, except T1 and T3 (Fig. 3). Similarly, in the non-biochar control group C1, all treatments showed improved shoot length with the exception of T7, which was comparable to the control.

When C1was compared with biochar-amended treatments, all treatment groups demonstrated higher shoot length values. However when the biochar control (C2) was compared with other treatment groups, only T10 exhibited a higher shoot length, while the remaining treatments recorded slightly lower values (Fig. 3).

Effect of single isolate and consortium on shoot length of mustard (*Brassica juncea* L.)

It was observed that treatments with biochar showed longer shoot lengths compared with treatments without biochar. Although exceptions to this trend were observed in treatments T1 and T2, which showed shoot lengths of 1.90 cm and 3.17 cm, respectively; both values were lower than T6 (4.10 cm) and T7 (4.00 cm) (Fig. 4). The biochar-amended control group showed higher shoot length than the control without biochar. Among the treatments with a consortium, a similar trend was observed, with T5 (3.00 cm) showing a 66.7 % increase in shoot length compared with T10 (1.83 cm) in the presence of biochar (Fig. 4).

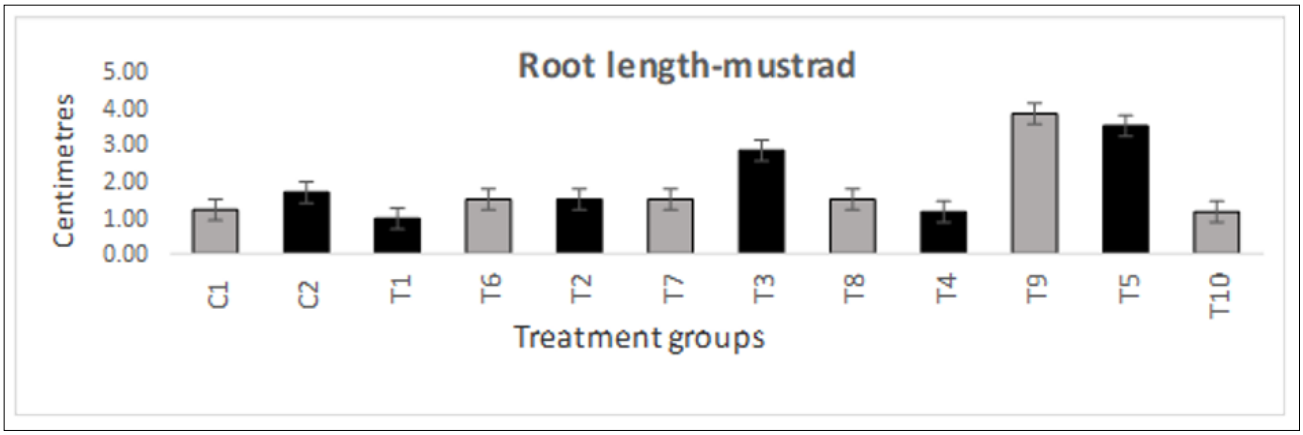


Fig. 2. Root length (cm) of mustard plants under different treatment groups.

C1: control (no biochar, no bacterial inoculation); C2: biochar only; T1–T10: different combinations of biochar and microbial inoculants (PGPR isolates or consortia); T1, T2, T3, T4: bacterial inoculation with biochar**;** T6, T7, T8, T9: bacterial inoculation without biochar; T5: consortium with biochar; T10: consortium without biochar.

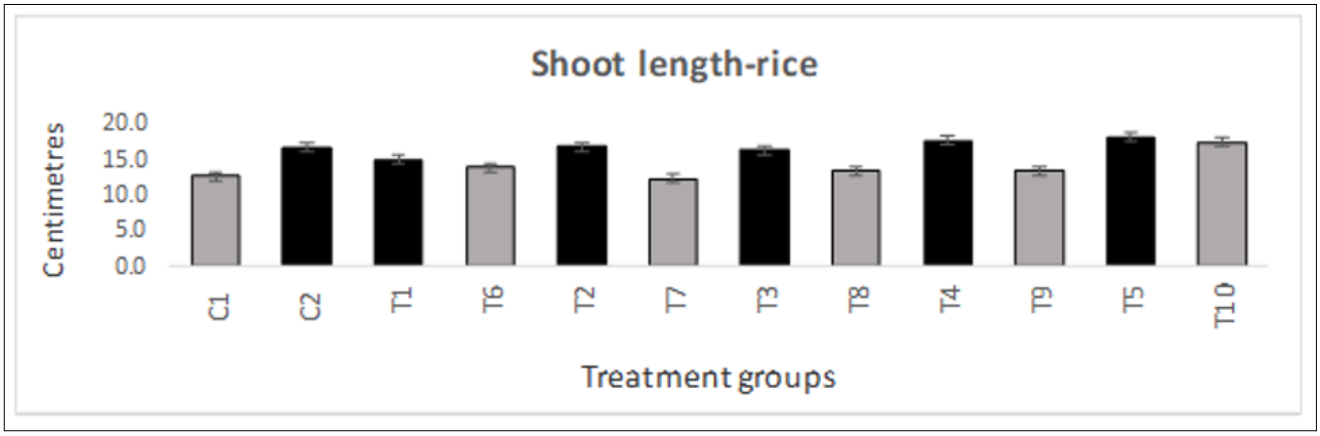


Fig. 3. Shoot length (cm) of rice plants under different treatment groups.

C1: control (no biochar, no bacterial inoculation); C2: biochar only; T1–T10: different combinations of biochar and microbial inoculants (PGPR isolates or consortia); T1, T2, T3, T4: bacterial inoculation with biochar**;** T6, T7, T8, T9: bacterial inoculation without biochar; T5: consortium with biochar; T10: consortium without biochar.

When C2 (biochar-amended control)) was compared with treatments T1–T5, all treatments showed reduced shoot length. In contrast, when C1 was compared with treatments without biochar, T6 and T7 showed greater shoot lengths than the control, while the remaining treatments showed lower values. When C1 was compared with biochar-treated groups, all treatments except T1 and T4 showed higher shoot lengths. Similarly, when C2 was compared with biochar treated groups, only T8, T9 and T10 showed higher shoot length, whereas the remaining treatments showed lower values (Fig. 4).

Effect of single isolate and consortium on chlorophyll content in rice (*Oryza sativa* L.)

Chlorophyll content was found to be higher in biochar amended soil compared with non-biochar amended soil. The C2 group showed higher chlorophyll content (1.17 mg g^{-1}) than the non-biochar control (C1), (1.00 mg g^{-1}) (Fig. 5).

In the biochar treatment group, plants treated with all isolates showed greater chlorophyll content than the C2 control. The highest chlorophyll content was observed in T5 (2.50 mg g^{-1}), followed closely by T4 (2.39 mg g^{-1}) (Fig. 5). Similarly, among non-biochar treatment groups, all isolates produced higher chlorophyll content than the C1 control. The maximum chlorophyll content was observed in T10 (1.44 mg g^{-1}), with T9 showing a comparable value of 1.408 mg g^{-1}. When comparing the biochar control (C2) with treatment groups without biochar, T6, T7, T8, T9 and T10 showed slightly higher chlorophyll levels; however, they remained lower than those observed in the biochar-amended treatments (T1–T5) containing bacterial inoculants (Fig. 5).

Effect of single isolate and consortium on chlorophyll content in mustard (*Brassica juncea* L.)

Chlorophyll production in the biochar-amended control group (C2) was higher than that in the control group without biochar (C1), with concentrations of 1.41 mg g^{-1} and 1.01 mg g^{-1}, respectively (Fig. 6). T1, T3 and T5 showed higher chlorophyll concentrations (2.14 mg g^{-1}, 1.52 mg g^{-1} and 2.07 mg g^{-1}, respectively) compared with the control (C2). Similar chlorophyll content was observed in T4 and the control C1 (1.41 mg g^{-1}), while T2 showed a slightly lower value (1.33 mg g^{-1}). Among the treatment groups without biochar, all plants treated with isolates showed higher chlorophyll levels than the control group C1. In non-biochar-amended soil, the highest chlorophyll concentration was observed in T10 (1.96 mg g^{-1}), followed by T6 (1.76 mg g^{-1}). When comparing treatment groups with biochar to those without biochar, none of the treatments without biochar produced higher chlorophyll concentrations than their corresponding biochar-amended treatments (Fig. 6).

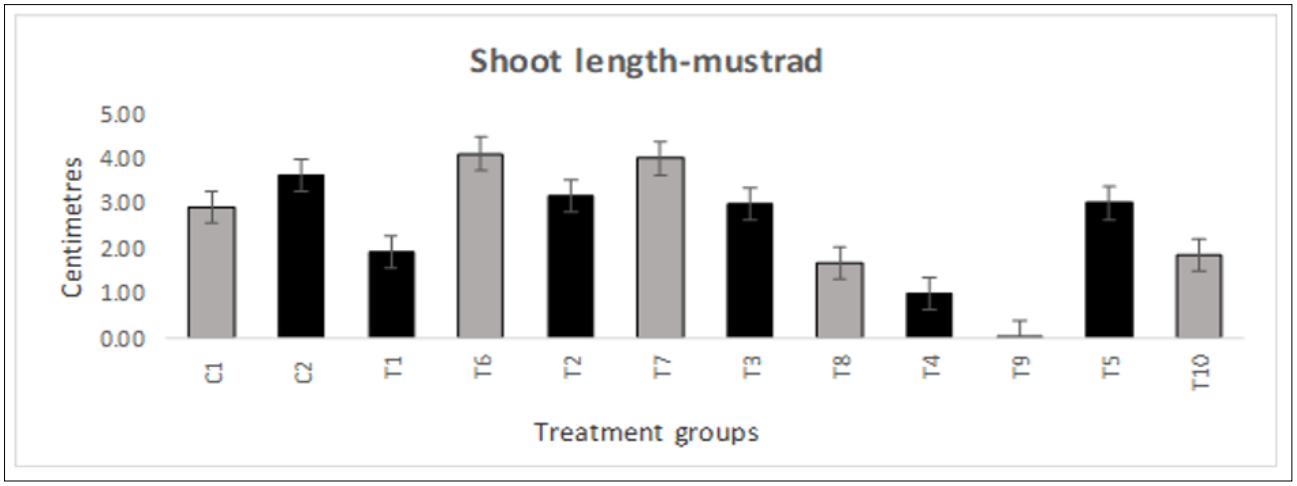


Fig. 4. Shoot length (cm) of mustard plants under different treatment groups.

C1: control (no biochar, no bacterial inoculation); C2: biochar only; T1–T10: different combinations of biochar and microbial inoculants (PGPR isolates or consortia); T1, T2, T3, T4: bacterial inoculation with biochar; T6, T7, T8, T9: bacterial inoculation without biochar; T5: consortium with biochar; T10: consortium without biochar.

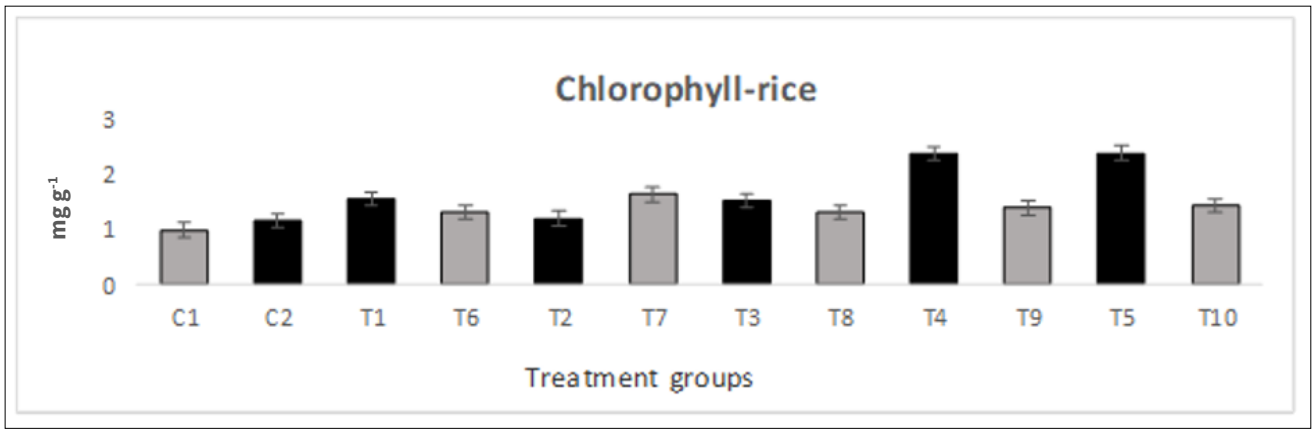


Fig. 5. Chlorophyll content (mg g^{-1}) of rice plants under different treatment groups.

C1: control (no biochar, no bacterial inoculation); C2: biochar only; T1–T10: different combinations of biochar and microbial inoculants (PGPR isolates or consortia); T1, T2, T3, T4: bacterial inoculation with biochar**;** T6, T7, T8, T9: bacterial inoculation without biochar; T5: consortium with biochar; T10: consortium without biochar.

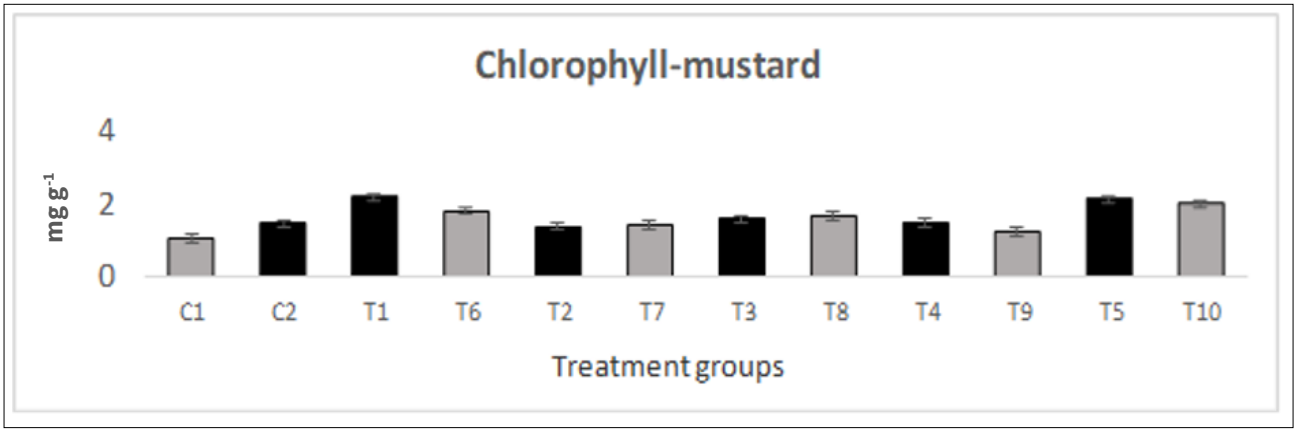


Fig. 6. Chlorophyll content (mg g^{-1}) of mustard plants under different treatment groups.

C1: control (no biochar, no bacterial inoculation); C2: biochar only; T1–T10: different combinations of biochar and microbial inoculants (PGPR isolates or consortia); T1, T2, T3, T4: bacterial inoculation with biochar; T6, T7, T8, T9: bacterial inoculation without biochar; T5: consortium with biochar; T10: consortium without biochar.

Statistical analysis

The one-way ANOVA results showed clear variation in treatment effects across different growth parameters. Shoot length in rice showed statistically significant differences among treatments ($p < 0.0001$), whereas root length and chlorophyll content did not show significant variation. Both shoot length and root length in mustard showed strong statistical significance ($p < 0.0001$ and $p = 0.0014$ respectively), whereas chlorophyll content similar to rice, remained statistically non-significant.

Discussion

Biochar creates a stable microhabitat, regulates soil pH and reduces nutrient depletion (21, 22), while simultaneously improving soil structure, moisture retention, nutrient availability and microbial colonisation, making it suitable for PGPB (23). The PGPB enhances plant growth through various mechanisms like nutrient solubilisation, phytohormone production and alleviation of stress (24, 25). The combination of biochar and PGPB holds great potential, as biochar enhances microbial survival and activity, which may amplify its impact on plant growth and physiology (26). Individual bacterial strains have been shown to improve traits such as root length, shoot length and chlorophyll content (27); however, when combined as microbial consortia, they can achieve wider and more consistent results by integrating complementary functions such as phytohormone production, nutrient solubilisation and stress alleviation (28, 29).

Biochar acts as a strong, porous matrix that promotes microbial colonisation and sustains microbial activity, thereby further enhancing this synergy (30, 31). Biochar–PGPB combinations offer promising potential for sustainable crop improvement (12, 32). Of the four bacterial isolates selected for this study, three were identified as *Pseudomonas* spp. and one as *Diaphorobacter* spp.

Several studies have demonstrated the plant growth-promoting ability of *Pseudomonas* spp. For example, *Pseudomonas fluorescens* strains 9704 and SLU99 have been shown to improve root length, shoot length and chlorophyll content in soybean, tomato and potato plants (33, 34). *P. aeruginosa* PAR increased the plant biomass and chlorophyll pigment levels in tomato plants under fluoride stress (35). *Pseudomonas* sp. Y1 increased root length by reducing the effect of salt stress, whereas strains CM11, WCS417 and RRLJ 008 under abiotic stress enhanced root branching, shoot biomass and disease resistance (36–38). Strains B2, B4 and *P. putida* B5 improved the chlorophyll pigment production and antioxidant activity in *Sorghum bicolor* L. under salt stress conditions (28, 39). Fluorescent *Pseudomonas* strains Pf-1 promoted plant growth and improved its resistance to stress through phytohormone production and nutrient mobilisation (40).

The genus *Diaphorobacter* was first reported in 2003, including species such as *D. nitroreducens*, which belongs to the family Comamonadaceae (41). A related species, *Diaphorobacter ruginosibacter* was later isolated from root nodules of soybean (42). *Diaphorobacter* species are gram-negative, motile rods with a single polar flagellum. They are capable of growing under both aerobic and anaerobic conditions and can utilise diverse carbon and nitrogen sources (43). Their denitrification capabilities make them relevant to nitrogen cycling in soil environments and affect nitrogen availability and microbial interactions (44). They are also capable of degrading polyhydroxybutyrate (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) polymers, contributing to organic matter turnover and the transformation of biodegradable carbon substrates. These properties are useful in biochar-amended soils, where biochar provides porous habitat and stabilises microbial populations, allowing *Diaphorobacter* to persist and remain metabolically active (45). The combination of biochar and *Diaphorobacter* can therefore improve nutrient dynamics, microbial activity and overall soil fertility, resulting in increased plant growth (46). However, the genus remains largely unexplored and suffers from a paucity of literature. A few studies have reported its PGP capabilities compared to well-characterised genera such as *Pseudomonas* or *Bacillus*. Its strain-specific characteristics and inoculation conditions also remain poorly understood. In this context, the present study provides novel insights into the role of *Diaphorobacter* in biochar-soil systems and its potential contribution to plant growth promotion through improved nutrient cycling and microbial activity. In this study, treatment groups T3 and T8 consisted of *D. nitroreducens* with and without biochar amendments which has not been previously reported to exhibit PGP traits. During this study, improvements in root and shoot length, chlorophyll content in both rice (*O. sativa*) and mustard (*B. juncea*) were observed in the presence of this isolate highlighting its potential as a PGP bacterium. In contrast, previous studies have reported *P. aeruginosa* to exhibit PGP traits such as phosphate solubilisation (PS), IAA production and ammonia production (AP), along with beneficial effects on plant

growth parameters (35, 47–49). Thus, while the PGP role of *P. aeruginosa* is well established, the findings of this study also highlight the novel PGP capability of *D. nitroreducens*. Notably, *D. nitroreducens* showed beneficial effects under both biochar-amended and non-biochar conditions, increasing mustard root length and chlorophyll content by 69.5 % and 8.2 %, respectively, in biochar-amended treatments and rice root length and chlorophyll content by 34.3 % and 33.2 %, respectively, in non-biochar treatments.

In this study, clear differences were observed in plant growth parameters across various microbial treatments. Biochar-treated groups showed increased root length with longer roots observed in biochar-treated rice and mustard compared with non-biochar treated groups. Previously, similar observations have been made by various other researchers. For example, a 14–18 % increase in root length was observed due to alterations in rhizosphere microbial communities following biochar application in sandy maize soil (50, 51).

Soybean showed improved root biomass due to increase in root porosity and nutrient dynamics after the addition of 1 % biochar (52). Another study on maize confirmed that the combination of biochar with PGPB (*Lysinibacillus fusiformis* strain 31MZR.) performed better than individual application of PGPB (53). Yet, in another study on tomato seedlings, immobilisation of *Pseudomonas* sp. on cotton straw-derived biochar resulted in a 3.75-fold increase in root length, chlorophyll content and phosphorus availability (29). In cucumber subjected to water stress, treatment with biochar along with P. fluorescens resulted in a 29 % increase in root length, a 10 % increase in shoot length and a 5 % increase in chlorophyll content compared with individual treatments.

The increase in root length is generally because of biochar's effect on soil structure and the microbial communities (18). In addition to improving soil physical properties, biochar may enhance the activity and persistence of plant growth-promoting bacteria capable of producing phytohormones such as IAA. Increased IAA availability can stimulate root elongation and root system development, thereby improving the uptake of water and nutrients. Enhanced root growth may subsequently contribute to improved shoot development through greater resource acquisition and allocation to above-ground tissues. Although biochar-treated groups showed better results than the non-biochar-treated groups, a few deviations from this trend were observed. For example, treatment T3 (with biochar) showed lower root length than its non-biochar counterpart in rice. Similarly, in mustard the highest root length was observed in the non-biochar treatment T9. This could be due to the variation in biochar effectiveness based on the microbial compatibility and characteristics of biochar material. In addition, biochar may adsorb microbial signalling molecules and other bioactive compounds, potentially affecting plant–microbe communication and bacterial colonisation efficiency. The observed results may also reflect strain-specific interactions with the biochar matrix, where certain isolates establish and function more effectively than others, resulting in variable plant growth responses among treatments. (21, 23, 30, 51). The trend of biochar-amended treatments showing better results compared with the treatments without biochar was also seen in shoot length.

For example, the biochar-amended control (C2) showed longer shoot length than the control without biochar (C1), with increases of 25.2 % in mustard and 32 % in rice. Similar results were reported, where addition of biochar alone resulted in a 77 % increase in shoot length of spinach (53, 54). In the biochar-treated group, the consortium treatment showed a 17.4 % increase in mustard and a 9.1 % increase in rice shoot length compared to the control. A previous study supporting these findings reported that *Trichoderma harzianum* L. and *Bacillus subtilis* L. when applied along with biochar to soybean under cadmium stress outperformed all individual treatments (55). Similar to the observations for root length, some deviations were observed in shoot length also. The difference in shoot length in rice and mustard in the presence of biochar amended treatments may be due to the differences in their growth physiology, root architecture and nutrient uptake patterns. Rice is a monocot and a semi-aquatic crop that naturally thrives in soils with high moisture and porosity. The presence of biochar might have improved the aeration, water availability and microbial colonisation, which could have created a favourable environment for the shoot development of rice (31).

In contrast, mustard a dicot crop, has rapid early growth rate and higher nutrient requirements. It may have experienced temporary nutrient immobilisation due to biochar application. During the initial stages of growth, biochar can adsorb available N and P, thereby limiting their availability to the plants. This effect may be more pronounced in mustard because of its shorter growth cycle and dependence on rapid nutrient uptake during early development. Mustard roots are morphologically different from those of rice, which may limit their interaction with biochar and associated bacterial inoculants during the early growth stages, potentially delaying plant growth-promoting effects (31).

Furthermore, microbial colonisation and biochar-microbe interactions often require time to stabilise within soil systems. The delayed establishment of bacterial inoculants in biochar-amended mustard soils may have contributed to lower shoot growth compared with the control. Over time, however, these effects may be reversed as nutrient cycling improves and microbial populations become more active (31).

Similar to root and shoot length, higher chlorophyll content was observed in biochar-amended treatments. The C2 showed a higher chlorophyll content as compared with C1. Among biochar treatments, T1, T3 and T5 as well as non-biochar treatments, T6 and T10 showed similar trends. These findings are in alignment with previous observations reported by researchers; for example, a > 70 % increase in chlorophyll a, chlorophyll b and total chlorophyll in maize was observed when it was treated with biochar and PGPR treatment (26). In another study, silver maple grown in urban soil was inoculated with a combination of biochar and microbial biofertilisers which increased the foliar chlorophyll content (12). The increase in the pigment levels may be attributed to the combined effects of biochar and PGPB, where biochar retains nutrients in the soil and PGPB facilitates processes such as nitrogen fixation and mineralisation (26). The porous structure of biochar helps to retain microbial communities which enhances their long-term functionality (22). Similar to root and shoot length, some deviations were observed, which may be due to differences in microbial interactions. The T4 showed chlorophyll content comparable to the control whereas T2 showed lower pigment production. Although numerical differences in chlorophyll content were observed among treatments, these differences were not statistically significant, which may be attributed to variability among replicates and the short duration of the experiment (15 days). Nevertheless, biochar treated groups consistently showed higher pigment production, with increases of 27.6 % in rice and 7.7 % in

mustard. This further reaffirms the role of biochar as a microbial carrier and soil conditioner, improving nutrient use efficiency, enhancing nitrogen availability in the rhizosphere and promoting chlorophyll accumulation, thereby supporting photosynthetic activity and plant growth.

In recent years, the benefits of co-applying biochar and PGPB have been reported. In 2025, a study from Pakistan observed that the co-application of *Acinetobacter baumannii* MN24 and *Piriformospora indica* with biochar improved root architecture and overall plant productivity (56). Similarly, a meta-analysis conducted in China reported that combined application of biochar and PGPB improved nutrient uptake and increased root and shoot biomass by 84 % and 116 %, respectively, in plants affected by heavy metal stress (57). A study in arsenic and cadmium contaminated paddy fields observed that the combined use of bacterium T3F4 with biochar improved plant growth and reduced metal uptake while increased selenium content in rice (58). These effects were attributed to the ability of biochar to increase cation exchange capacity (CEC), provide protected microbial niches that enhance bacterial survival and activity, and regulate adsorption–desorption dynamics in soil, thereby reducing metal bioavailability and improving nutrient retention. Together, these mechanisms reduce the availability of toxic metals, improve nutrient retention and enhance the effectiveness of beneficial microorganisms, thereby promoting plant growth and nutrient acquisition.

Conclusion

This study demonstrates that combining biochar with plant growth-promoting bacteria can improve plant growth parameters including root length, shoot development and chlorophyll content in rice and mustard. Treatments involving microbial consortia and biochar showed better performance compared with individual isolates or biochar alone. The findings highlight the synergistic effects of biochar as a microbial transporter and soil conditioner. Overall, this strategy represents a promising and sustainable alternative to chemical fertilisers for enhancing agricultural productivity. However, consortium treatments did not always show better results than individual treatments. This may be due to the complexity of interactions among bacterial combinations, biochar and soil properties. The water content in this study was maintained at 100 % (until leakage), which may have influenced microbial activity and nutrient availability. Differences in results may occur under field conditions, where complete saturation is not achieved.

The inclusion of *Diaphorobacter nitroreducens*, a relatively novel and understudied species in agricultural contexts adds novelty to this study and suggests its potential role in biochar-soil systems and plant growth promotion. The monitoring period of this study was 15 days, whereas the complete growth cycles of rice and mustard are considerably longer. Therefore, the results represent early-stage growth responses and long-term studies under greenhouse and field conditions are required to evaluate the effects of biochar–microbe interactions on plant growth throughout the crop life cycle, crop yield, soil health and microbial persistence.

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Authors' contributions

RD carried out data collection, performed experiments, analysed the data and wrote the manuscript. SS was involved in conceptualisation of the project, experimental design, data analysis and provided critical revisions and suggestions to improve the scientific content and clarity of the manuscript. AG contributed expert insights and suggestions to enhance the depth and relevance of the study. DD was involved in the overall planning of the research, provided expert insights and suggestions and critically revised the manuscript to improve its scientific content and clarity. SR contributed to the development of the research hypothesis, provided the biochar and participated in the experimental design. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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