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Enhancing tomato plant growth and soil health through application of biochar derived from *Microcystis aeruginosa* (Cyanobacteria)

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Abstract:
This research investigated the potential of biochar derived from the cyanobacterium *Microcystis aeruginosa* as a soil amendment for improving tomato plant growth and soil health. *M. aeruginosa* was cultivated in a semi-continuous culture system, and its biomass was converted into biochar through pyrolysis. The biochar was then applied to soil planted with tomato seedlings. The results demonstrated that biochar application significantly enhanced various tomato plant growth parameters, including germination rate, root length, shoot length, leaf length, number of leaves per plant, and water use efficiency. Furthermore, biochar application led to increased chlorophyll a, b and total chlorophyll content in the tomato plants. The study also revealed positive impacts of biochar on soil properties. Biochar application improved soil pH by shifting it from acidic to neutral and increased soil moisture content. Soil microbial populations, including both bacteria and fungi, exhibited significant growth after biochar amendment. Additionally, the activity of soil enzymes, such as soil acid phosphatase and soil dehydrogenase, was enhanced by biochar application. Moreover, biochar application resulted in increased soil content of essential plant nutrients, including nitrogen (N), phosphorus (P), and potassium (K). These findings suggest that biochar derived from *M. aeruginosa* holds promise as a sustainable soil amendment for promoting tomato plant growth and improving overall soil health.

Keywords: *Pyrolysis; semi-continuous culture; biomass; chlorophyll; soil acid phosphatase; soil dehydrogenase.*

1. INTRODUCTION

In the pursuit of sustainable agricultural practices, the application of biochar has received a lot of attention due to its ability to improve soil health and plant development. Biochar, a carbon-rich substance derived from the pyrolysis of organic materials, has been extensively acknowledged for its capacity to improve soil structure, increase water retention, and enhanced nutrient availability [1]. Numerous studies have uncovered the unique properties of biochar, including its high mineral content, broad surface area, multiple surface groups, and stable carbon concentration. The widespread distribution, rapid growth rate, and high CO_2 fixation efficiency of cyanobacterial biomass make them an attractive feedstock [2]. Enhancing cyanobacterial biomass is a unique method of reducing the greenhouse impact by sequestering atmospheric carbon dioxide [3]. In the context of soil health by improving soil structure, increasing water retention, enhancing nutrient availability, and reducing greenhouse gas emissions, it is recognized that there is potential for improving the conversion of biomass to biochar using thermochemical processes as pyrolysis, hydrothermal carbonization, or torrefaction [4].

Since biochar comprises distinct physical and chemical properties, it is acknowledged as a multipurpose material for soil amendments [5]. Biochar are often produce from waste biomass, including wood, algae and agricultural straw [6-8]. The one with the most notable benefits has emerged as algal biochar. A variety of algal feedstocks have recently been used to produce biochar [9].

One particularly intriguing and underexplored feedstock is Microcystis aeruginosa, a species of cyanobacteria commonly found in freshwater environments. *M. aeruginosa* is renowned for forming harmful algal blooms (HABs), which create toxins that impair aquatic life, water quality, and human health. These blooms are exacerbated by nutrient pollution and climate change, posing substantial ecological and economic issues [10]. The proliferation of *Microcystis* blooms is largely driven by anthropogenic activities, including agricultural runoff and wastewater discharge, which introduce high levels of nutrients like nitrogen and phosphorus into water bodies [11]. Managing and mitigating the impacts of *Microcystis* blooms is a significant challenge for water resource management. In addition to providing a way to control harmful algal blooms, using this problematic biomass for the production of biochar offers a unique way to recycle waste into useful agricultural inputs.

The use of biochar derived from *M. aeruginosa* (MA-biochar) presents dual benefits. First, it offers a sustainable solution for managing harmful cyanobacterial blooms, turning an environmental liability into a beneficial product. Second, MA-biochar has the potential to improve soil health and enhance plant growth, thereby contributing to agricultural productivity and sustainability. However, empirical research on the effectiveness of MA-biochar in agricultural applications remains sparse, particularly regarding its impact on specific crops such as tomatoes (*Solanum lycopersicum*). Tomatoes are one of the most widely cultivated and economically important crops globally [12]. Tomato plants are extremely sensitive to the quality of the soil and the availability of nutrients in terms of growth and output [13]. Therefore, finding innovative and effective soil amendments to enhance tomato cultivation is crucial for ensuring food security and sustainable agricultural practices.

In this study, *M. aeruginosa* was cultured in semi-continuous culture technique for the production of biomass. Then, biomass was converted into biochar by traditional pyrolysis. The tomato plant was characterized by growth parameters, leaf parameters, germination and pigments while potted soil was characterized by pH, moisture content, available phosphorus, exchangeable potassium, available nitrogen, microbial population, acid phosphatase activity and dehydrogenase activity.

2. MATERIAL AND METHODS

2.1 Test organism and cultivation

M. aeruginosa was obtained from algal culture laboratory of the Department of Botany at Mizoram University, India. The culture was maintained in 500 ml Erlenmeyer flask containing 250 ml liquid Chu-10 medium at pH 7.5. Cultures were maintained at 25°C and under 10:14 light:dark period using cool white fluorescent tubes. 1.0 g (fresh weight) biomass was added to 20-l plastic jar with 10 l culture medium connected with air bubble pump for aeration carried out in semi-continuous culture techniques.

2.2 Pyrolysis experiment

The biomass of *M. aeruginosa* was converted into biochar by traditional way through slow pyrolysis which gives a high char yield [14]. The biomass was dried in direct sunlight,

crushed and sieved to get the particle size ranging from 200 µm to 300 µm. Traditional pyrolysis was carried out in a ceramic pot (8 cm x 8 cm), a batch of 10 g of dried biomass was kept inside the ceramic pot and then sealed with lids completely so that oxygen flow was completely blocked. The pot was surrounded by tin sheet 30 cm away from the ceramic, the space was filled with wood and plant debris and then burned and subjected to pyrolysis around 500 to 700°C. The conversion of the starting material took place in a period of 40–60 min. At the end of pyrolysis, heating was halted and the ceramic was cooled. Soon after, the lid of the ceramic was then cautiously opened, and the solid, black residue (biochar) was collected.

2.3 Application of biochar for tomato plant growth and soil health

2.3.1 Experimental layout

The pot experiment was conducted to investigate the growth of tomato plants and soil amelioration as a result of addition of biochar produced from *M. aeruginosa*. The pot trial experiments was carried out in randomized complete block design with a total number of 12 pots with 4 treatments and 3 replications conducted for a period of 30 days. Treatment T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar and T3: soil with 5% biochar as shown in Table 1.

Table 1. Details of treatments

Treatment Number	Treatment details
T0	Control (10% coco peat)
T1	10% coco peat + 2% FYM
T2	10% coco peat + 2% Biochar
T3	10% coco peat + 5% Biochar

2.3.2 Characteristics of tomato plant

The analysis of tomato plants growing in different treatments of soil was conducted at the end of experiments. Plant height and root length was measured by using ruler. Germination was determined by counting the number of germinated seeds and expressed in terms of percentage. The water use efficiency was determined by the ratio between biomass yield and water consumed. Extraction and estimation of chlorophyll "a" and "b", total chlorophyll and carotenoid were determined according to the method of [15], and calculated by using the following formula:

$$\text{Chlorophyll a (mg/g f.wt.)} = (10.1 \times A_{662} - 1.01 \times A_{644}) \times 0.2$$

$$\textit{Chlorophyll b (mg/g f.wt.)} = (16.4 \times A_{644} - 2.57 \times A_{662}) \times 0.2$$

$$\textit{Total chlorophyll} = \textit{chlorophyll a} + \textit{chlorophyll b}$$

$$\textit{Carotenoid (mg/g f.wt.)} = (A_{470} - 1.28 \times \textit{Ch a}) + (56.7 \times \textit{Ch b})/256 \times 0.906$$

Where, *Ch a* is chlorophyll a, and *Ch b* is chlorophyll b.

2.3.3 Determination of soil

pH

Soil pH was determined by the method given by [16]. 20 g of soil were mixed with the right amount of distilled water and the solution was agitated firmly for 5 min to make a 1:5 soil-water suspension. The mixture was allowed to stand for 1 h and then the pH was measured.

Moisture content

Soil moisture content was determined by the method given by [17]. In moisture boxes with known weights, 20 g of soil samples were collected and then oven dried at 105°C for 24 h to obtained constant mass. The soil moisture content was calculated by the following formula:

$$\textit{Soil moisture content (\%)} = (W_1 - W_2)/W_1 \times 100$$

Where, W_1 is initial weight, and W_2 is final weight.

Available nitrogen

Available nitrogen of the soil sample was determined by the method given by [18]. The available nitrogen content was calculated by using the following formula:

$$\textit{Soil available nitrogen (Kg/ha)} = (X - Y) \times 0.00028/20 \times 2240000$$

Where, X is volume of N/50 H_2SO_4 taken in ml, and Y is volume of N/50 NaOH used in ml.

Available phosphorus

Soil available phosphorus content was determined by ammonium molybdate method given by [19]. The phosphorus content was calculated by the following formula:

$$\textit{Phosphorus content (Kg/ha)} = R \times V/v \times 1/S \times ((2.44 \times 10\text{^}6))/10^6$$

Where, V is total volume of extractant (ml), v is volume of aliquot taken for analysis (ml), S is weight of sample (g), and R is weight of phosphorus in the aliquot in µg (from standard graph).

Exchangeable potassium

Potassium content of the sample was determined by the flame photometer method as described by [20]. The potassium concentration in the extract was determined by using flame photometer.

$$\textit{Potassium content (Kg/ha)} = R \times V/W \times 224 \times 10^6/10^6$$

Soil microbial population

The soil microbial population was determined by serial dilution and plate counting method. The number of colonies for each dilution was recorded and the number of colony-forming units per gram of soil (CFU/g) was calculated [21].

Soil acid phosphatase activity

Soil acid phosphatase activity was determined by using the protocol described by [22]. The enzyme activity was expressed in terms of µg p-NP/ml/h.

Soil dehydrogenase activity

Soil dehydrogenase activity was determined by the method given by [23]. The enzyme activity was expressed in terms of µgTPF/ml/h.

2.4 Statistical analysis

The statistical analysis was carried out using Microsoft excel 2010, Washington, USA. Experimental data were analyzed by one-way analysis of variance (ANOVA). A P value of <0.05 was considered statistically significant. The results were expressed as mean ±standard deviation of the mean.

3. RESULTS AND DISCUSSION

The highest plant height was observed in T2 treatment with 13.60 ±0.88 cm and lowest by T0 treatment with 10.40 ±0.50 cm. The root length of tomato plant was found highest in T2 treatment with 23.53±2.77 cm and the lowest in T0 treatment with 11.03 ±0.77 cm. Plants from T1 treatment shows 4.73±0.20 cm internode length while T0 treatment shows 3.76±0.28 cm (Fig 1 A). The increased height in T2 treatment (2% biochar) indicates that the biochar provided a more conducive environment for the tomato plants, possibly by enhancing soil fertility and moisture conditions. Root length is a crucial indicator of a plant's ability to access water and nutrients. The T2 treatment resulted in the highest root length (23.53±2.77 cm), more than double the root length observed in the T0 treatment (11.03±0.77 cm). This significant enhancement in root growth suggests that MA-biochar improves the rhizosphere conditions, facilitating better root development. These findings are consistent with previous studies that have demonstrated similar growth-promoting effects of biochar on various crops [12]. T2 treatment shows 3.46±0.12 cm of leaf length on tomato plant while T0 shows 2.43 ±0.12 cm. T2 shows 2.13±0.12 cm leaf width while T0 shows 1.50±0.08 cm (Fig 2). The leaf numbers on tomato plant were 18.66±1.24 on T2 treatment while 11.66±0.47 on T0 (Fig 1 B & C). These findings may have an impact on farming methods as they indicate that adopting biochar treatment may produce stronger tomato plants with higher leaf area and density, which may improve photosynthesis and, consequently, increase crop yield. The addition of biochar on soil has significantly effect on germination of tomato plants. Tomato plants on T2 treatment shows 93.33±4.71%, which is significantly higher as compared to soil without biochar T0 treatment (60.00±8.16%). The significant difference was shown in Fig 1 D. The significant improvement in germination rates observed in the T2 treatment with biochar is consistent with several studies that have demonstrated biochar's positive effects on seed germination. For instance, [24] reported

that biochar addition to soil can improve seed germination and early seedling growth by enhancing soil moisture retention.

The water use efficiency was found highest in T2 (0.43±0.01 g/L) while lowest in T0 with 0.15±0.01 g/L (Fig 2 A). The significant improvement in water use efficiency with biochar application has profound implications for sustainable agriculture, particularly in regions where water resources are limited. Enhancing water use efficiency means that crops can produce more biomass with less water, contributing to both increased agricultural productivity and conservation of water resources. This is especially critical in the context of climate change, which is expected to exacerbate water scarcity in many agricultural regions. The chlorophyll "a", chlorophyll "b", total chlorophyll and carotenoid contents was found highest in tomato plants of T2 treatment with 2.099 ±0.05 mg/g f. wt., 1.796 ±0.19 mg/g f. wt., and 3.896 ±0.22 mg/g f. wt., 0.482 ±0.02 mg/g f. wt. respectively (Fig 2 B). The significant increase in chlorophyll "a" and "b" content in the T2 treatment

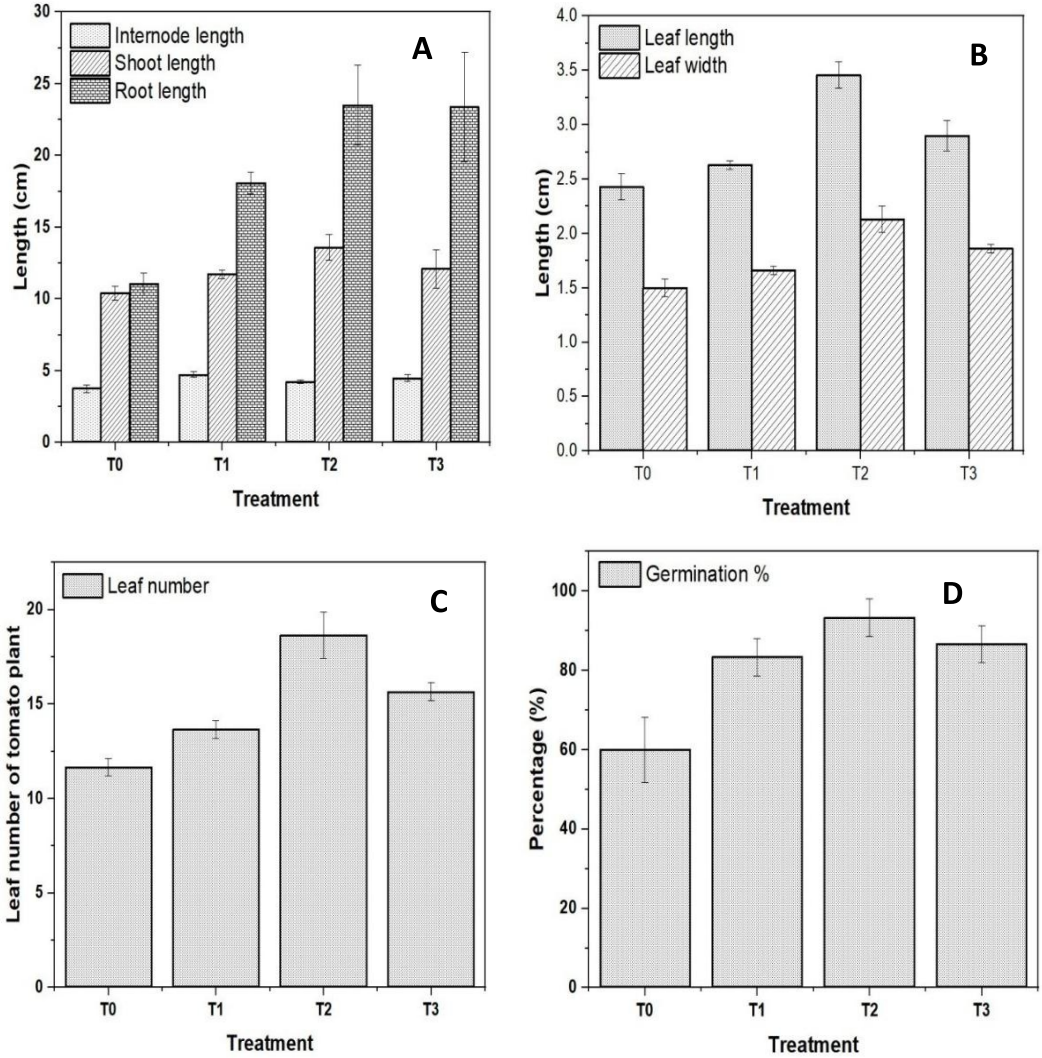


Figure 1. Effect of different treatments of soil on various parameters of tomato plant. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on shoot, root and internode length of tomato plant. (B) Effect of biochar on leaf length and leaf width of tomato plant. (C) Effect of biochar on leaf number of tomato plant. (D) Effect of biochar on germination of tomato plant.

indicates enhanced photosynthetic capacity in biochar soil-treated plants. Higher chlorophyll levels are often associated with improved photosynthetic efficiency, leading to better growth and higher biomass production. The significant increase in carotenoid content in the T2 treatment suggests that biochar treatment on soil not only improves photosynthetic efficiency but also enhances the plant's resilience to environmental stresses.

The addition of biochar on soil increases soil pH around 7 which were conducted during the experiment. T3 treatment shows highest increase with 9.78±0.16% increase in soil pH. T1 treatment shows lowest increase with 3.73 ±0.53% at the end of experiment as shown in Fig. 3 A. Biochar typically contains a significant amount of ash, which includes alkaline substances such as calcium carbonate, potassium carbonate, and magnesium carbonate. When biochar is incorporated into the soil, these alkaline compounds can neutralize soil acidity, leading to an increase in pH. The variation in pH increase among different biochar treatments (T2 to T3) could be attributed to the varying quantities of biochar applied, with higher application rates likely contributing more alkaline substances to the soil. The findings of this study are consistent with numerous reports in the literature that highlight the pH-modifying effects of biochar. For instance, studies have shown that biochar can increase soil pH in a range of soil types and environmental conditions, leading to improved soil fertility and plant growth [25]. The biochar addition treatment on soil T3 shows highest increase with 54.43 ±0.04% of soil moisture content as compared to control (without biochar addition) as lowest with 25.94 ±0.09% as shown in Fig 3 B. [26] found that biochar application increased soil water retention and reduced soil bulk density, leading to improved moisture availability for crops.

Both addition of biochar treatment T2 (19.50 ±0.36%) and T3 (9.80 ±0.39%) on soil increases soil fungal population while both treatment without biochar T0 (2.37 ±3.47%) and T1 (14.07 ±0.27%) decreases soil fungal population (Fig 4 A). T0 treatment shows highest increase in bacterial population with 24.79 ±0.05% while the lowest increase was shown by T1 treatment with 1.06 ±3.54% (Fig 4 B). Increase of soil fungal population in biochar treatment shows that biochar modify soil environment by altering pH, increasing nutrient availability and improving soil structure, all of which promote fungal proliferation. Biochar can enhance bacterial growth by providing a habitat and increasing nutrient availability, it can also inhibit certain bacterial groups by altering the soil chemical environment or by promoting competitive fungal populations.

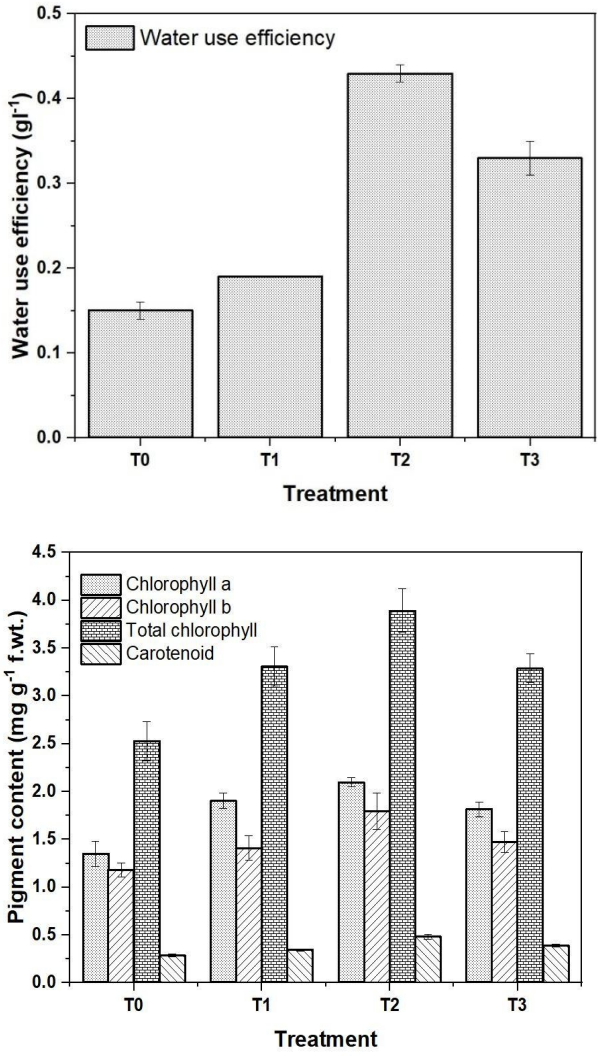


Figure 2. Effect of different treatments of soil on various parameters of tomato plant. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on water use efficiency of tomato plant. (B) Effect of biochar on pigment content of tomato plant.

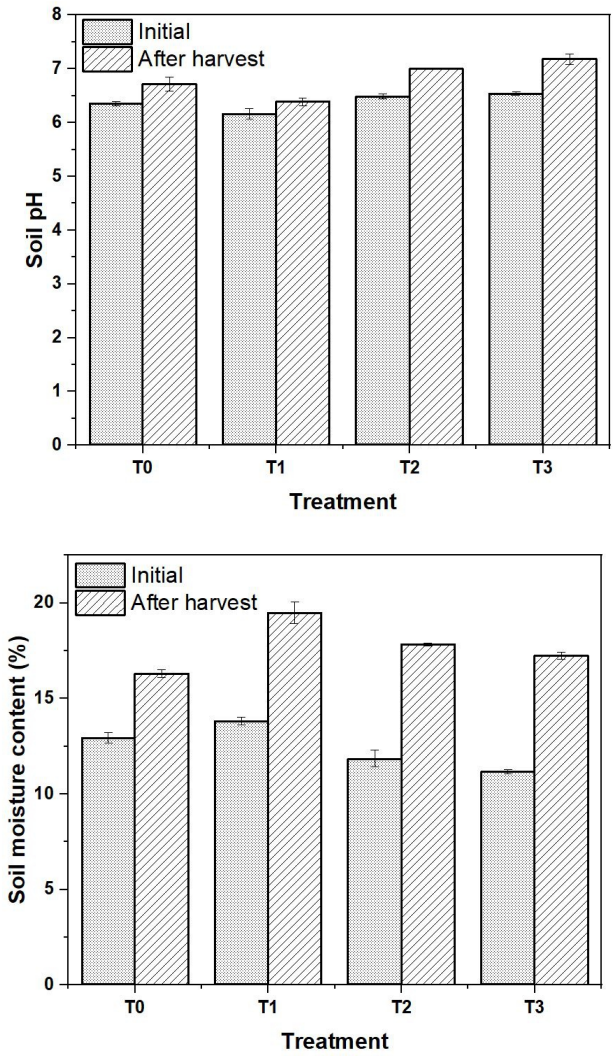
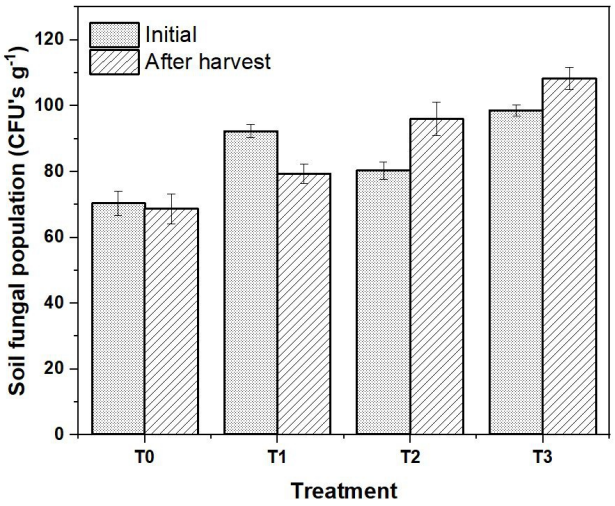


Figure 3. Effect of different treatments on soil parameters at initial and after harvest of experiment. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on soil pH. (B) Effect of biochar on soil moisture content.

The soil available nitrogen increases at the end of experiment with 11.64±0.12% by T2 treatment while decrease in soil available nitrogen was observed in T0 with 0.64±5.46% (Fig 5 A). Biochar itself contain a small amount of nitrogen, which can be slowly released into the soil as the biochar decomposes. This slow release provides a sustained source of nitrogen over time, contributing to the overall increase in soil available nitrogen. T2 shows increase in soil available phosphorus with 32.30±0.22% while T1 shows decrease in soil available phosphorus at the end of experiment with 5.51±1.44% (Fig 5 B). The significant increase in soil phosphorus in the T2 treatment reflects the direct contribution of phosphorus from the applied biochar. T3 shows 6.04±0.24% increase in soil exchangeable potassium followed by T2 with 4.66±0.16% while T0 shows 0.42±2.79% (Fig 5 C). The negligible change in soil exchangeable potassium observed in the T0 treatment suggests that without the addition of biochar, soil potassium levels remain relatively static or are subject to depletion. In many soils, potassium can become fixed or bound to soil particles, particularly in soils with high clay content. This fixation reduces the availability of potassium for plant uptake. The low increase in soil potassium in the T0 treatment could be due to such fixation processes.

The soil acid phosphatase activity was significantly increase by T2 treatment with 33.29±0.02% as compared to T1 treatment with 1.34±1.36% (Fig 6 A). The T2 treatment likely provided a rich source of organic matter and nutrients that stimulated microbial activity. Soil microorganisms, particularly fungi and bacteria, produce acid phosphatase enzymes to mineralize organic phosphorus compounds into inorganic forms that plants can absorb. The significant increase in enzyme activity in the T2 treatment



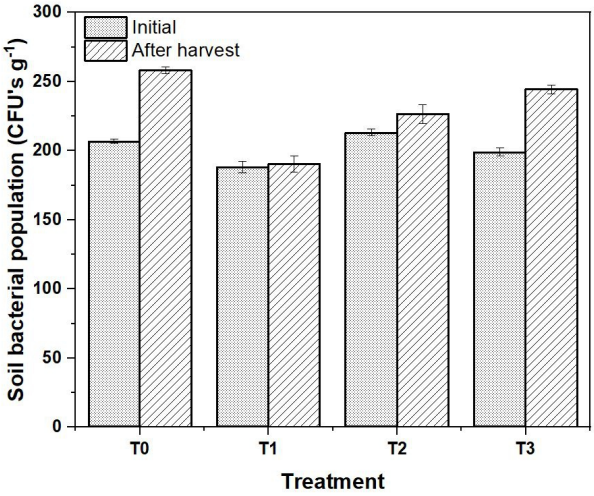
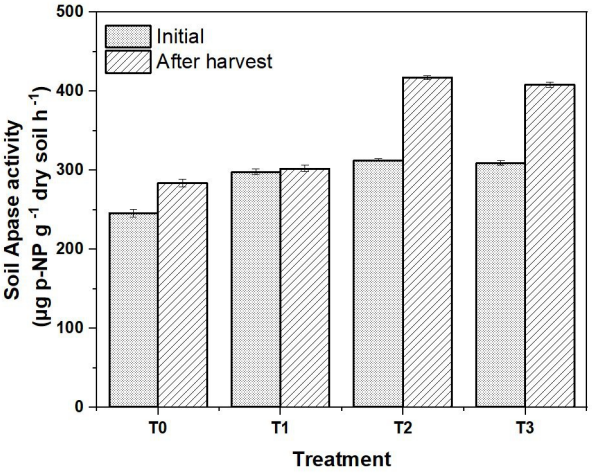
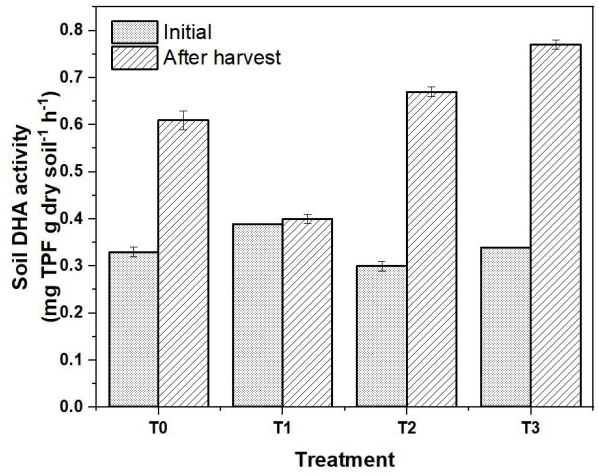
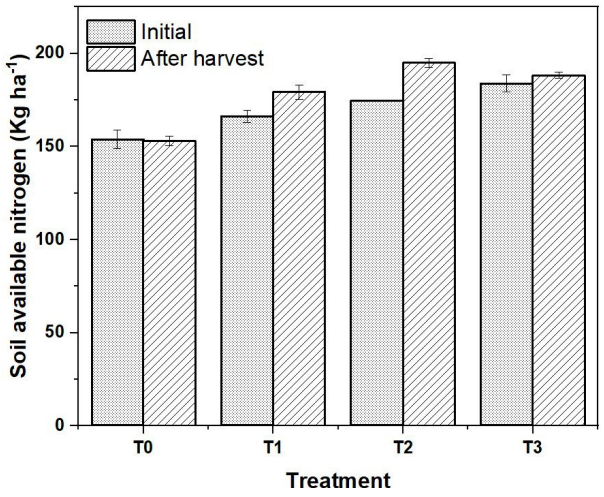


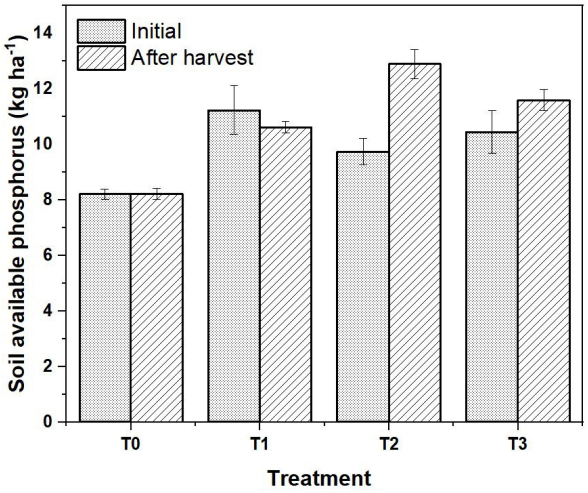
Figure 4. Effect of different treatments on soil microbial populations at initial and after harvest of experiment. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on fungal population of soil. (B) Effect of biochar on bacterial population of soil.

suggests enhanced microbial activity and abundance, leading to higher enzyme production. Biochar addition treatment T3 shows significantly increase in soil dehydrogenase activity at the end of experiment with 126.47 ±0.02% as compared to treatment with farm yard manure (2.56 ±1.00%) as shown in Fig 6 B. Biochar serves as a carbon substrate, providing a source of energy for soil microorganisms. Microorganisms utilize carbon compounds released from biochar through physical and biochemical processes, stimulating microbial metabolism and enzyme production, including dehydrogenase. The availability of carbon substrates from biochar likely contributed to the observed increase in dehydrogenase activity in the T3 treatment.









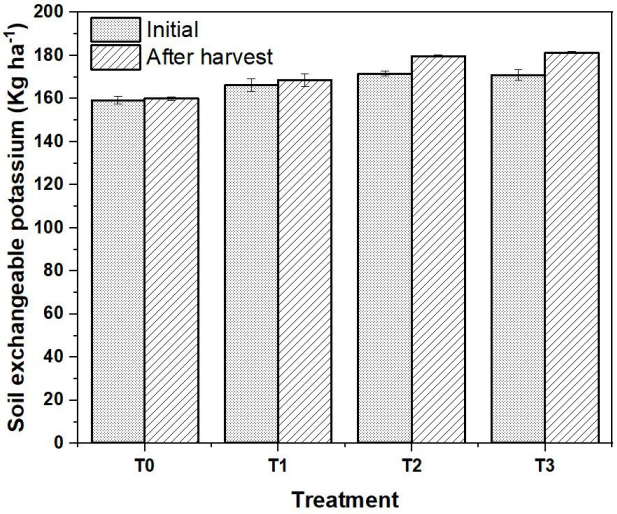


Figure 5. Effect of different treatments on soil nutrients at initial and after harvest of experiment. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on soil available nitrogen. (B) Effect of biochar on soil available phosphorus. (C) Effect of biochar on soil exchangeable potassium.

Figure 6. Effect of different treatments on soil enzyme activity at initial and after harvest of experiment. T0: soil without biochar (control), T1: soil with 2% farm yard manure, T2: soil with 2% biochar, T3: soil with 5% biochar. (A) Effect of biochar on soil acid phosphatase activity. (B) Effect of biochar on soil dehydrogenase activity.

4. CONCLUSION

In conclusion, the findings collectively underscore the significant potential of biochar as valuable soil amendments for enhancing various aspects of soil health, plant growth, and nutrient dynamics. From improving water use efficiency and photosynthetic efficiency to enhancing soil pH, moisture content, and nutrient availability, these organic amendments offer promising avenues for sustainable soil management and agricultural productivity. Future research should continue to explore optimization strategies and long-term impacts across diverse agricultural systems to fully harness the benefits of these amendments for enhancing crop productivity and sustainability.

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