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Original Research

Optimizing Phosphorus Availability and Maize Yield Using Biochar And *Pseudomonas Aeruginosa* In An Acidic Soil

Hachib Mohammad Tusar[1],Md. Kamal Uddin[*1], Shamim Mia[2,3], Susilawati Kasim[1], Samsuri Bin Abd. Wahid[1], NorAsrina Sairi[4] Komariah[5]

[1] Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia; hachibmdtusar@gmail.com mkuddin@upm.edu.my, samsuriaw@upm.edu.my, susilawati@upm.edu.my

[2] School of Life and Environmental Sciences,The University of Sydney, Sydney-2015, Australia; smia_agr @pstu.ac.bd

[3] Department of Agronomy, Faculty of Agriculture, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh; smia_agr@pstu.ac.bd

[4] Department of Chemistry, Faculty of Science, Universiti Malaya, Kuala Lumpur, Malaysia, asrina@um.edu.my

[5] Department of Soil Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia; komariah@staff.uns.ac.id

Corresponding author: Md Kamal uddin; mkuddin@upm.edu.my

Abstract: Maize plays a vital role in enhancing food security, particularly in regions facing agricultural challenges such as poor soil conditions, erratic rainfall, and limited access to resources. It can be advantageous for smallholder farmers in developing countries, where it can enhance productivity on limited land and under suboptimal soil conditions. One of the potential means for improving crop yield under suboptimal soil conditions such as acidic soils is application of soil amendments. However, the combined effects of functionalized biochar (a pyrogenic carbon) and microbes on phosphorus (P) bioavailability and plant growth performance are still not well understood. This study investigates the optimization of transplanted maize growth in acidic soil through the application of rice husk biochar (RHB) that was oxidized with 10% hydrogen peroxide and inoculated with *Pseudomonas aeruginosa*, a phosphate-solubilizing bacterium. The oxidized biochar's pH was adjusted to 6.2 for enhancing its effectiveness in challenging soil conditions. Soil properties and maize performance were determined using a pot culture. Results showed that the combined use of 10% oxidized RHB and *Pseudomonas aeruginosa* significantly increased P availability and phosphatase enzyme activity by 435% and a 418% respectively. Furthermore, the yield of maize increased by 413.21%, demonstrating the effectiveness of the treatment in improving soil fertility and crop productivity. This improvement in yield might have occurred due to an increase soil pH, P bioavailability, and a reduction in Al toxicity since there were significant positive relationships between yield and soil pH and available P and a negative relationship with available Al concentration. These findings underscore the potential of integrating oxidized biochar and beneficial microbes *Pseudomonas aeruginosa* to enhance crop performance in acidic soils.

Key Words	Transplanted maize; acidic soil, pyrogenic carbon; *Pseudomonas aeruginosa*; phosphorus; yield
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1. INTRODUCTION

Maize (*Zea mays*) has been an integral component of global agri-food systems after its domestication around 9,000 years ago. In recent decades, worldwide maize production has significantly been increased, driven by growing demand, technological innovations, higher yields, and the expansion of cultivated areas (Erenstein *et al.*, 2022). Transplanting involves relocating a plant from one site to another. This method is often used to establish crops when direct seeding is less viable due to unfavorable conditions (Sardar *et al.*, 2020). Additionally, research findings indicate that transplanting cereals can help them adapt to unpredictable weather conditions, enhance their efficiency in using radiation and water, and suppress weed growth (Biswas, 2020). Using transplanting as a strategy in maize cultivation helps achieve optimal plant density, improves crop stands, and ultimately enhances yield. This technique also lowers nutrient requirements and shortens the crop's growth period (Sardar *et al.*, 2020).

Soil pH plays a critical role in agricultural productivity because it directly affects the availability of essential nutrients to plants (Barrow and Hartemink, 2023). In tropical regions, frequent rainfall and high temperatures typically lead to the loss of essential cations, resulting in heavily weathered, acidic soils that poses a significant challenge for maize cultivation (Hasbullah, Ahmed and Ab Majid, 2020).

Low soil organic matter is a major issue in arid and semi-arid regions. The increasing preference for inorganic fertilizers over organic alternatives has greatly contributed to the reduction of soil organic content, leading to adverse effects on soil health and fertility (Hussain*et al.*, 2024). Organic amendments enhance the soil's physical, chemical, and biological properties, leading to improved soil fertility and increased crop yields (Eid *et al.*, 2017).

Biochar, a carbon-rich material produced from waste biomass through pyrolysis, is gaining popularity for its ability to neutralize soil acidity while improving soil fertility and health (Tusar *et al.*, 2023). It has gained worldwide attention due to its broad applications in enhancing soil quality and boosting soil productivity (Wang *et al.*, 2017). When incorporated into soil, biochar can modify the soil's texture, pore size distribution, and bulk density, leading to enhanced aeration and increased water-holding capacity (Hussain, Garg and Ravi, 2020). Biochar has an liming properties (Feigl *et al.*, 2012). It enhances the cation exchange capacity of soil by raising its pH, modifying the availability of nutrients, and preventing nutrient loss through leaching (Fidel *et al.*, 2017). Acidic soils frequently have limited phosphorus availability because phosphorus (P) tends to bind to soil particles, making it inaccessible for plant absorption (Muchoka, 2021).

The natural solubilization of mineral phosphates is a key process carried out by various microorganisms, collectively referred to as phosphate-solubilizing microorganisms (PSM). Among these, bacteria are the most common and effective agents in dissolving mineral phosphates in natural environments compared to other types of microorganisms (Paul and Sinha, 2017). For ensuring agricultural safety and sustainability, plant-growth-promoting rhizobacteria (PGPR) such as *Pseudomonas spp*. offer an excellent alternative to synthetic fertilizers. These eco-friendly and cost-effective biological applications serve as viable bioinoculants without posing a threat to soil biota (Das Mohapatra, Sahoo and Tuteja, 2024).

Phosphorus is a vital nutrient for plant growth and development, fulfilling multiple key functions in plant nutrition (Malhotra *et al.*, 2018). Plants require phosphorus for various essential processes including growth, sugar and starch utilization, photosynthesis, nucleus formation, and cell division. A lack of P can impede overall plant growth and delay crop maturation (Gurmu, 2023). Acid soils that bind significant amounts of phosphorus are typically medium- to fine-textured (Sanchez and Uehara, 1980). Phosphorus is a highly reactive element that readily forms insoluble complexes with cations such as aluminum (Al) and iron (Fe) in acidic soils (Oburger, Jones and Wenzel, 2011). Research has demonstrated that phosphate-solubilizing microorganisms and different bacterial strains can greatly enhance phosphorus release from biochar, thereby increasing phosphorus availability in the soil and promoting plant growth (Rossati, Figueiredo and Mendes, 2023). Oxidized biochar creates a stable environment for the colonization and activity of phosphate-solubilizing bacteria (PSB), improving their survival and longevity in the soil (Ouyang *et al.*, 2023).

Limited research has been conducted comparing the effects of rice husk biochar and *Pseudomonas aeruginosa* on transplanted maize specifically in acidic soils. Our study provides a tailored solution by leveraging the soil-amending properties of biochar alongside the biological action of *Pseudomonas aeruginosa*. Unlike conventional biochar applications, our work employs oxidized rice husk biochar treated with 10% hydrogen peroxide. This specific functionalization increases the surface properties and enhances the biochar's ability to adsorb and subsequently desorb phosphorus. While phosphate-solubilizing bacteria have been studied independently, our research uniquely demonstrates the combine effects of *Pseudomonas aeruginosa* with oxidized biochar. This combination would not only boost P solubilization but also enhance microbial activity, resulting in improved nutrient cycling and better soil health metrics such as pH balance and reduced aluminum toxicity. By using locally available rice husk biochar and a well-characterized bacterial strain, our approach is both cost-effective and environmentally sustainable. This holistic approach might demonstrate the long-term benefits of the treatments on soil health, going beyond the immediate yield improvements typically emphasized in other studies. Thus, our research is novel and impactful in overcoming the dual challenges of nutrient scarcity and soil acidity with significant implications for agriculture.

2. MATERIALS AND METHODS

2.1 Experimental Site

The pot trial was conducted in the new glasshouse, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor. The experimental site was located at 2°98′36.6″ N (north) latitude and 101°73′81.9″ E (east) longitudes with an elevation of 56.8 m from sea level at the west coast of Peninsular Malaysia. The local climate was hot, humid tropic, and the average minimum temperature was around 25 °C, and the average maximum temperature was 36 °C, relative humidity was 79% during the whole experiment.

2.2 Rice Husk Biochar Collection, Activation and Characterization

In this pot experiment, rice husk biochar (RHB) was utilized, produced from locally sourced feedstock in Malaysia. The biochar was collected from Sendi Enterprise (Sungai Burong, Selangor, Malaysia) and was produced by a pyrolysis process at 300 °C. To measure the pH of the rice husk biochar, a 1:2.5 ratio of air-dried biochar to distilled water was used, using a pH meter following the method by (Ahmedna, Marshall and Rao, 2000). The biochar was oxidized using 5%, 10%, and 15% H_2O_2 and pH was adjusted $\approx$ 6.2. Total elemental composition was determined using a CHN analyzer (LECO, Tru Spec Micro CHNS Analyzer, Germany). For elemental composition, 1 mg of biochar samples was weighed into tin cups and the mass was recorded. The total elemental composition was determined using a CHN analyzer (LECO, Tru spec® Micro). All samples were measured in triplicate.

Surface functionality was characterized using Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR, model Spectrum- 100, Perkelmer). The surface area and micro-pore volume were determined by the Brunauer-Emmett-Teller method (Brunauer, Emmett and Teller, 1938) which is the most well-known technique for accurate determination of the surface area of carbonous materials. Briefly, the material is typically ground or sieved to ensure a uniform particle size and then degassed to remove any adsorbed gases or contaminants. The biochar samples were degassed at 300 °C for 24 h under vacuum. By analyzing the amount of gas adsorbed as a function of pressure, specific surface area and micro-pore volume was calculated using mathematical model, Brunauer-Emmett-Teller (BET) equation. The micro-pore volume was calculated using additional methods like the BJH method, which analyzes the distribution of pore sizes within the material (Barrett, Joyner and Halenda, 1951) . The physico-chemical properties of the oxidized RHB are shown below (Table 1).

Table 1. Physical and chemical properties of oxidized RHB

Treatments	Elemental composition (%)							Specific Surface Area (SSA)	
	C	H	N	S	O	C:H	O: C	BET Surface area (m^2/g)	Pore diameter Å
CBC	31.18±0.66a	2.11±0.05a	2.07±0.05b	1.78±0.07b	61.74±0.21c	14.78±0.05b	1.98±0.05c	278.52±0.92a	15.82±0.12d
5% OBC	24.67±0.83b	1.17±0.03b	3.66±0.05a	1.75±0.03b	66.81±0.33b	21.09±0.10a	2.71±0.08b	235.16±1.63b	16.97±0.03c
10% OBC	25.05±0.53b	1.15±0.01b	4.01±0.01a	0.98±0.02c	65.04±1.66bc	21.87±0.30a	2.60±0.01b	203.78±1.58d	38.09±0.16a
15% OBC	18.29±0.56c	1.16±0.01b	3.19±0.68ab	2.26±0.04a	71.56±0.83a	15.83±0.37b	3.92±0.08a	216.3±1.11c	24.95±0.01b
P value	<0.0001*	<0.0001*	0.0185*	<0.0001*	0.0006*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

C= Carbon, H= Hydrogen, N= Nitrogen, S= Sulpher, O= Oxygen

2.3 Soil Collection and Preparation

Surface soil (0-20 cm depth) was collected from the Bungor soil series (Typic Paleudult; Order: Ultisol) at Taman Pertanian, Universiti Putra Malaysia, Puchong, Selangor (2°58′59.7″ N latitude; 101°38′47.5″ E longitude). The soil sample was air-dried, ground, and sieved to less than 2 mm before undergoing chemical characterization and subsequent treatment. The properties of the soil, including texture, pH, cation exchange capacity (CEC), and exchangeable cations, are detailed in Table 2.

Table 2: The physical and chemical properties of the initial soil

Properties	Soil
Textural Class	Sand- 69.27%, Silt- 2.28% Clay-28.44% (Sandyclay loam)
pH	4.40±0.01
CEC (mmol/kg)	7.11± 0.03
Available P (mg kg)	1.70± 0.05
Total C (%)	1.03± 0.02
Total N (%)	0.03± 0.02
Total S (%)	0.01±0.02
Exchangeable K (mmol/ kg)	0.06±0.01
Exchangeable Ca (mmol/ kg)	0.19±0.01
Exchangeable Mg (mmol/kg)	0.32± 0.02
Exchangeable Al (mmol/kg)	0.78± 0.04

2.4 Pot trial

A two-factor experiment was conducted following a randomized complete block design (RCBD) with three replicates. The treatment included- a) biochar application-fresh and oxidized biochar with one control and b) microbial inoculation- *Pseudomonas aeruginosa*(Agkt 1) and with a control. Treatments were administered in plastic containers (38 cm in height, 32 cm in diameter, and 30 cm in depth) filled with 20 kg of soil. Each container had three holes drilled at the bottom to allow leachate to drain out. Moisture content was monitored and maintained using a portable moisture meter (FieldScout TDR 150 Soil Moisture Meter). Biochar was mixed into the top 15 cm of the soil two weeks before maize seeds were sown. Four seedlings were transplanted at a depth of 2 cm in each pot, and thinned to one healthy seedling per pot after 7 days of emergence. N-P-K fertilizer was applied in each pot recommended by (Kashiani, 2012), application rates of: urea (4.87g/pot), triple superphosphate(3.49g/pot), and muriate of potash (2.64g/pot). The full dose of P and K fertilizer were applied as a basal dose one day before the seeds were sown. N fertilizer was applied in three equal splits on the10^{th},40^{th} and 65^{th} day safter sowing (DAS). In this experiment, the maize variety F1 hybrid sweet corn, a common variety used by Malaysian farmers and obtained from a local market, was used as the test crop. Plant management included manual weeding and pesticide application as needed to maintain experimental conditions. The experiment was conducted from January 2024 to April 2024 to assess the effects of bacterial inoculation on soil and plant health.

2.5 Preparation and application of bacterial inoculums

The bacterial culture of *Pseudomonas aeruginosa* (10^{-5}cfu/ml) was utilized for soil microbial treatment. Bacterial strain was collected from the laboratory of microbiology, department of land management, faculty of Agriculture, Universiti Putra Malaysia. The strains were initially sub-cultured in 100 ml Erlenmeyer flasks

containing LB (Luria-Bertani) broth. These cultures were then shaken continuously for 24 hours at 180 rpm and 28°C, following the method described by (Jensen, 1951). Bacterial concentration were measured using serial dillution techniques. Approximately 20 ml of the bacterial suspension was applied in two equal splits: one at the time of sowing (0 DAS - days after sowing) and the other at 20 DAS.

2.6 Soil Analysis

2.6.1 Determination of exchangeable K, Ca, Mg and CEC in soil

Soil cation exchange capacity (CEC) was examined using the ammonium acetate shaking method (Rowell, 2014) at pH 7. Five grams of soil sample was placed into a Falcon tube. Subsequently, 50 ml of 1M ammonium acetate (NH_4OAc buffered at pH 7) was added to each tube, followed by shaking at 180 rpm for 30 minutes to facilitate cation exchange. Upon completion of shaking, the samples were centrifuged at 4000 rpm for 10 minutes to separate the soil particles from the solution. The filtered solution was kept for determining the concentrations of potassium (K), calcium (Ca), and magnesium (Mg) using an Atomic Absorption Spectrophotometer (AAS). Following the initial analysis, the soil in each tube, now saturated with ammonium ions, passed through a three times washing step with 50 ml of 95% ethyl alcohol to remove excess ammonium acetate. The washed soil was then mixed with 50 ml of 0.1N potassium sulfate (K_2SO_4) to facilitate the replacement of exchangeable ammonium ions by potassium ions. Subsequently, the process of centrifugation, filtration, and analysis of ammonium was performed using a segmented flow analyzer (AA 500).

2.6.2 Determination of Inorganic nitrogen in soil

Soil inorganic N (NH_4^+-N and NO_3 –N) was analyzed by extracting with 2M KCl (soil: solution, 1:5) (Nelson, 1982). Briefly, 10 g of soil was taken into a falcon tube, and 50 ml of 2M KCI was added while the suspension was shaken for 1 hour. Ammonium and nitrate were analyzed using segmented flow analyzer (AA 500) as discussed before (Nelson, 1982).

2.6.3 Determination of available phosphorus in soil

Available phosphorus in the soil was determined using the Bray II method (Bray and Kurtz, 1945). Two grams of air-dried soil (2.00 mm) were weighed into a 20 ml plastic vial and reacted with 14 ml of extracting solution (0.03 NH_4F and 0.1M HCl), then sealed with parafilm. The soil suspension was shaken for 45 seconds using the wrist inversion technique. The extract was filtered through Whatman No. 42 filter paper into a plastic vial. The final product was analyzed using an segmented flow analyzer (AA 500).

2.6.4 Determination of soil microbial population

Ten grams of fresh soil samples were used to determine the total microbial population using the spread dilution plate technique (Parkinson, Gray and Williams, 1971). Serial dilutions from 10^{-2} to 10^{-7} were prepared by sequentially transferring 1.0 ml of the sample into each test tube containing 9 ml of sterile distilled water (SDW). The samples were spread over the respective media using a sterilized bent glass rod. The plates were incubated at 28 ± 2°C for 24-48 hours for bacteria. The colonies formed were counted, and populations were calculated as colony-forming units (CFU) per ml solution.

2.6.5 Determination of soil phosphatase activity

Soil phosphatase enzyme activities were determined by using testing kids from Beijing solarbio Science and Technology Co. Ltd (China). The catalog number of soil alkaline phosphatase (S-AKP/ALP) Activity Assay kit was BC0280 (Guan and Wang, 2023). To prepare the soil samples, 0.1 g of soil was placed in a vial. Then, 0.05 ml of toluene was added, and the mixture was shaken for 15 minutes. Next, 0.4 ml of reagent 1 was added to the vial, and the mixture was incubated at 37°C for 24 hours. After incubation, 1 ml of reagent 2 was added, and the mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was carefully collected for further analysis. To prepare the supernatant solution, 50 µl of the collected supernatant was transferred to a new vial, and 100 µl of reagent 3 and 20 µl of reagent 4 were added. For the blank solution, 50 µl of reagent 1, 100 µl of reagent 3, and 20 µl of reagent 4 were mixed in a separate vial. For the standard solution, 50 µl of the supplied standard solution was combined with 100 µl of reagent 3 and 20 µl of reagent 4. Additionally, 830 µl of distilled water was added to the supernatant, blank, and standard solutions. All solutions were allowed to stand at room temperature for 30 minutes. After this incubation period, the absorbance of each solution was measured at 660 nm using a spectrophotometer.

2.7 Plant performance analysis

We examined the treatment effect of alkaline biochar on plant performance by measuring plant height, stem diameter, cob length, cob diameter, and yield using a measuring tape, vernier caliper scale, and weighing balance. After harvest, the plant parts (stem, leaves, and corn) were placed into envelopes and dried in an oven at 60 °C for 72 hours (Lija, Haruna and Kasim, 2014). The plant biomass was then recorded.

2.7.1 Measurement of SPAD value by using SPAD-502 meter

Leaf greenness, serving as an indicator of chlorophyll content, was evaluated using a portable chlorophyll meter (SPAD-502, Konica Minolta, Inc., Tokyo, Japan). To ensure precision and consistency, SPAD readings were taken from fully matured leaves of each plant, with an average of three measurements per leaf (Yuan *et al.*, 2016).

2.7.2 Root measurement

After harvesting the plant from the pots were enclosed in a plastic bag immediately to prevent the dehydration, washed carefully with tap water and separated into shoot and root to the root growth. After root being washed, the root was prepared for the determination of the root length by using measuring tape.

2.7.3 Plant Nutrient Analysis

The dried and ground plant material (0.25 g) was used for digestion. The single wet digestion technique (Cottenie, 1980) was conducted to extract the macro elements from the plant tissues. The wet digestion technique is a widely used method for determining plant nutrient concentrations by breaking down organic material using strong acids. To begin, plant samples are first dried at 60-70°C, ground into fine powder, and then weighed (0.25 g) into digestion tubes. A mixture of concentrated sulfuric acid (H_2SO_4) was added to the sample. The sample was then pre-digested for over night. After that 2ml 30% H_2O_2 was added and heated on a hotplate or digestion block at 285°C until the solution becomes clear, indicating complete breakdown of organic matter. Perchloric acid ($HClO_4$) may also be used for tougher samples to ensure full digestion. Once digestion is complete, the solution is cooled, diluted to a 100 ml volume with distilled water, and filtered to remove undissolved particles. The resulting clear solution was analyzed for nutrient concentrations.

N and P concentrations were analyzed using a segmented flow analyzer (AA 500). Additionally, K, Ca, and Mg concentrations were determined using atomic absorption spectroscopy (AAS, PerkinElmer). The plant nutrient uptake was calculated using the following formula(Rabileh *et al.*, 2015).

Uptake (mg /plant) = Total nutrient concentration (%) × biomass (g),

where the nutrient concentration was found using AAS, and the biomass was the plant'srespective dry weight.

2.7.4 Percent Relative Data

The relative data of the values were expressed as percentages, relative to control for eachelement recommended by (Ashraf and Waheed, 1990), the formula are as follows where the treatment value were the biochar and microbes amendment and the control value was without amendment.

Relative data (%) = (Treatment value- control value/control value) × 100

2.8 Statistical Analysis

All data were analyzed using the two way analysis of variance (ANOVA) procedure, and means were separated by Tukey's Honestly Significant Difference (HSD) test at a 5% level of significance using Statistical Analysis System, JMP software (SAS incorporation).

3. RESULTS

3.1 Soil pH

Rice Husk Biochar (RHB), microbes, and their combination significantly increased soil pH at 30 and 65 days after sowing (DAS) ($P<0.05$, Figure 1). At 30 DAS, pH ranges from 4.5 to 4.89. The application of biochar increased soil pH, with 10% oxidized biochar (T2) showing a significant difference ($p<0.01$) compared to the control (T1), resulting in an increase of pH 0.12 units. Microbial treatment at T3 showed a significant increased ($p<0.01$) in soil pH compared to the control. Among all treatments, the combination of 10% oxidized biochar and *Pseudomonas aeruginosa* (T4) demonstrated the highest soil pH (4.89), significantly different ($p=0.0128$) from the control T1 (4.5).

At 65 DAS, pH ranges from 4.5 to 4.85. The combine effect of 10% oxidized biochar and *Pseudomonas aeruginosa* (T4) resulted in the highest soil pH (4.85) that 0.35 units higher than control and significantly different ($p<0.01$) from the control T1 (4.5).

At 85 DAS, lowest pH value (4.33) was found at T1 and highest value (4.49) at combine treatment (T4). 10% oxidized biochar and *Pseudomonas aeruginosa* (T4) increased soil pH by 0.16 units that was significantly different ($p= 0.01$) from the control treatment.

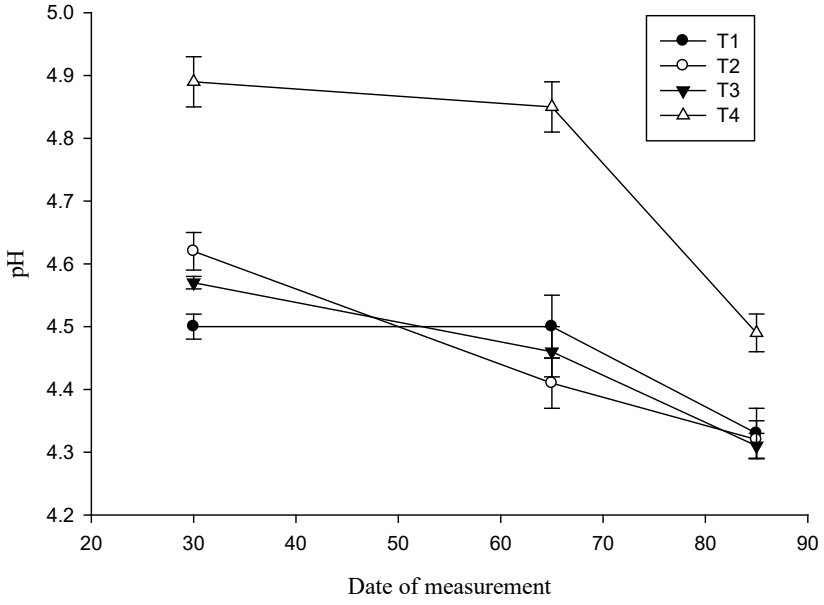

Figure 1. pH of different biochar amendments measured at different date

T1= No microbes and no biochar, T2= No microbes and 10% oxidized biochar, T3= *Pseudomonas aeruginosa* and no biochar, T4= *Pseudomonas aeruginosa* and 10% oxidized biochar,Error bar represents standard error of mean

3.2 Post-harvest soil nutrient status

The application of treatments significantly influenced the availability of P in the soil (Table 3). The highest available P was found in combine treatment T4 (7.92 mg/kg), which was significantly greater than the control

treatment T1(1.48 mg/kg). Biochar treatment (T2) (4.74 mg/kg) and microbes treatment T3 (2.74) mg/kg) also showed significant increases. Statistical analysis confirmed that both biochar (p= <0.0001), microbial treatments (p= 0.0002) and their combination (p= 0.0233) significantly increased available P levels by 220%, 85% and 435% respectively.

In this study, inorganic nitrogen (N) levels ranges from 16.97 to 30.54 mg/kg presented in Table 3. Biochar treatment (T2) significantly increased inorganic N. The combine application of biochar and microbes was not significant .

Table 3 represented the significant effect of biochar, microbes and their interaction on soil CEC. Soil CEC ranges from 5.25 to10.19 mmol/kg. Biochar effect was not significantly different but microbes effect (p= 0.0081) and biochar microbes combine effect (p= 0.0142) were significant. Exchangeable potassium levels ranged from 0.09 to 0.16 mmol/kg. Biochar (p=0.0001), microbes (p=0.0175) and their combine effect (p=0.0175) had a significant positive effect on exchangeable K. Combine treatments of biochar and microbes (T4) increased K level 78% compared to control. Biochar, microbes and their combination influenced exchangeable calcium levels. The highest values were observed in combine treatment T4 (0.13 mmol/kg) and lowest value (0.09 mmol/kg) was found in control treatment (T1) and total increment was 44%.

In this study Table 3 showed exchangeable magnesium (Mg) values that was highest in 10% oxidized RHB treatments, T2 (0.15 mmol/kg) which indicates significantly increased exchangeable Mg levels (P = 0.007). Microbial treatments effect was not significant but combine effect was significant (p= 0.0200). In this study, exchangeable aluminum ranged from 0.36 to 0.45 mmol/kg. The control treatment (0.45 mmol/kg) demonstrated the highest levels of exchangeable aluminum (Al). Microbes treatment T3 and combine treatment T4 significantly reduced exchangeable Al 25% and 10% respectively.

Table 3. Effect of oxidized RHB and microbes on changes in nutrients of the post harvest soil

Treatments	Available P mg/kg	Inorganic N mg/kg	CEC (mmol/kg)	Exchangeable K (mmol/kg)	Exchangeable ca (mmol/kg)	Exchangeable Mg (mmol/kg)	Exchangeable Al (mmol/kg)
T1	1.48± 0.18c	16.97± 0.82a	5.25±0.14b	0.09±0.003b	0.09±0.01b	0.06±0.02b	0.45±0.01a
T2	4.75±0.63b	30.87±7.56a	9.82±1.11a	0.12±0.008b	0.08±0.01b	0.15±0.03a	0.39±0.01b
T3	2.74±0.63c	20.16±0.83a	11.79±1.45a	0.0.09±0.006b	0.10±0.01b	0.12±0.01a	0.36±0.003c
T4	7.92±0.18a	30.54± 4.09a	10.19±0.73a	0.16±0.01a	0.13±0.01a	0.14±0.01a	0.41±0.01b
Biochar	*P*= < 0.0001*	*P*= 0.0234*	*P*= 0.1717	*P*= 0.0001*	*P*= 0.0606	*P*= 0.007*	*P*= 0.4996
Microbes	*P*= 0.0002*	*P*= 0.7502	*P*= 0.0081*	*P*= 0.0175*	*P*= 0.0066*	*P*= 0.0827	*P*= 0.0006*
BC*M	*p*= 0.0233*	*p*= 0.6961	*p*= 0.0142*	*p*= 0.0175*	*p*= 0.0285*	*p*= 0.0200*	*p*= < 0.0001*

T1= No microbes and no biochar, T2= No microbes and 10% oxidized biochar, T3= *Pseudomonas aeruginosa* and no biochar, T4= *Pseudomonas aeruginosa* and 10% oxidized biochar.

3.3 RHB and Microbes effect on transplanted maize nutrient concentration

Table 4 and 5 demonstrated the effect of oxidized Rice Husk Biochar (RHB), microbes, and their interactions on nutrient concentration and nutrient uptake in maize. The nutrient concentration of maize plants is presented in Table 4. P levels ranged from 0.13% to 0.51%, with the highest concentration observed in treatment T4 (10% oxidized biochar combined with *P. aeruginosa*), which was significantly higher than the control. This combination increased P concentration by 292% compared to the control. Biochar (p=0.0013), microbes (p=0.0001), and their combination (p=0.0040) all showed significant differences from the control. N concentration ranged from 0.05% to 0.13%, with the highest value in T4, representing a 160% increase. Biochar, microbes, and their combined effect were all statistically significant. K concentration varied from 0.78% to 1.45%, with the lowest value in the control (T1) and the highest in T2 (10% oxidized biochar without microbes). Again, biochar, microbes, and their combination significantly affected K levels. Ca concentrations ranged from 0.01% to 0.11%, with the highest value in T1. Biochar had a significant effect on Ca concentration, but the influence of microbes and their combination was not significant compared to the control. Lastly, magnesium (Mg) concentrations ranged from 0.01% (in the control) to 0.017% (in T3 with *P. aeruginosa* but no biochar). Biochar, microbes, and their combination did not show significant effects on Mg concentration compared to the control.

Table 4 : Acidic RHB and microbes effect on maize plant nutrient concentration

Treatments	% P concentration	%N concentration	% K concentration	%Ca concentration	%Mgconcentration
T1	0.13±0.02c	0.05±0.01b	0.78±0.08a	0.11±0.01a	0.01±0.01a
T2	0.34±0.02b	0.12±0.01a	1.49±0.18	0.07±0.01b	0.014±0.001a
T3	0.47±0.02a	0.13±0.02a	1.27±0.2a	0.01±0.01c	0.017±0.001a
T4	0.51±0.02a	0.13±0.01a	1.45±0.05a	0.01±0.002c	0.016±0.001a
Biochar	p= 0.0013*	0.0284*	0.0341*	0.0046*	0.6605
Microbes	p=0. 0001*	0.0118*	0.1792	<0.0001*	0.2898
BC*M	P= 0.0040*	0.0284*	0.1284	0.0066*	0.5453

Means within the same column followed by the different letters are significantly different at p≤ 0.05; (Turkey's HSD test). T1= No microbes and no biochar, T2= No microbes and 10% oxidized biochar, T3= *Pseudomonas aeruginosa* and no biochar, T4= *Pseudomonas aeruginosa* and 10% oxidized biochar.

3.4 RHB and Microbes effect on transplanted maize total nutrient uptake

The effect of oxidized Rice Husk Biochar (RHB), microbes, and their combination on phosphorus (P) uptake in maize represented on Table 5 . It was found that P uptake ranges from 189.42 to 460.83 mg/plant. P uptake significantly increased with the application of biochar, microbes, and their combination. On average, across microbial treatments, 10% oxidized biochar (T2) increased P uptake by 78%, which was significantly different (p=0.0060) from the control (T1). Microbial treatment and combine treatment (10% oxidized biochar and *Pseudomonas aeruginosa*) at T3 and T4 also exhibited a significant increase in P uptake 133% and 143% respectively compared to control treatment (T1).

Similarly, nitrogen (N) uptake was significantly increased by the application of biochar, microbes, but not significant on their combination (Table 5). N uptake was ranges from 36.98 to 150.72 mg/plant. Biochar treatment at T2 and microbes treatment at T3 represented in a 71% and 135% increment of N uptake compared to the control (T1). Among all treatments, the highest N uptake (150.72 mg/plant) was observed with the combined application of 10% oxidized biochar and *Pseudomonas*

aeruginosa (T4). This study represented a significant increase in potassium (K) uptake due to the application of biochar and microbes, as shown in Table 4. Total K ranges from 441.42 to 1401 mg/plant. The biochar treatment at T2, microbes treatment at T3 and biochar microbes combine treatment at T4 represented K uptake increment 609.57mg/plant, 662.55 mg/plant and 959.58 mg/plant compared to control treatment T1. This study exhibited that the biochar and the combine application of biochar and microbes did not showed significantly different at calcium (Ca) uptake, as shown in Table 5. Highest Ca uptake (48.45 mg/plant) was found at biochar treatment T2 that was not significantly different with control treatment (T1) and lowest Ca uptake was found at combine treatment T4 (7.89 mg/plant). Table 5 exhibited significant influenced on biochar, microbes and their combination for Mg uptake. Mg uptake ranges from 6.17 to 13.87. Biochar treatment T2, microbes treatment T3 and combine treatment T4 showed 124%, 113% and 117% increment of Mg uptake that are significantly different with the control treatment (T1).

Table 5. Oxidized RHB and microbes effect on transplanted maize plants total nutrient uptake

Treatments	Total P mg/plant	Total N mg/plant	K (mg/plant)	Ca(mg/plant)	Mg (mg/plant)
T1	189.42±3.83c	36.98±5.73b	441.42±23.22c	46.46±2.75a	6.17±0.91b
T2	336.26±29.15a	63.18±18.42b	1050.99±82.82b	48.45±9.15a	13.87±0.77a
T3	441.87±18.98ab	86.95±18.60b	1103.97±91.63ab	8.36±1.28b	13.16±2.83ab
T4	460.83±33.33a	150.72±6.17	1401±44.50	7.89±2.41	13.39±0.64a
Biochar	*P*= 0.0060*	*P*= 0.0113*	*P*= 0.0001*	*P*= 0.8820	*P*= 0.0352*
Microbes	*P*= < 0.0001*	*P*= 0.0011*	*P*= < 0.0001*	*P*= < 0.0001*	*P*= 0.0720*
BC*M	*p*= 0.0212*	*p*= 0.2091	*p*= 0.0471	*p*= 0.8115	*p*= 0.0440*

Means within the same column followed by the different letters are significantly different at p≤ 0.05; (Turkey's HSD test). T1= No microbes and no biochar, T2= No microbes and 10% oxidized biochar, T3= *Pseudomonas aeruginosa* and no biochar, T4= *Pseudomonas aeruginosa* and 10% oxidized biochar.

3.5 Oxidized RHB and *P. aeruginosa* combine effect on SPAD value

The SPAD value was influenced by the application of biochar, microbe and the combination of biochar with phosphate-solubilizing bacteria (Table 6). At 30 DAS, SPAD value ranges from 32.13 to 40.8. The combination of biochar and microbes (p=0.0031) showed a significant effect compared to the control T1. At 45 DAS, the highest SPAD value was found at microbes treatment T3 (46.2) and lowest value was found at biochar treatment T2 (40.63). Application of biochar, microbes and biochar microbes combination effects were not significant at 45 DAS. By 65 DAS highest SPAD value (49.03) was observed at *Pseudomonas aeruginosa* and 10% oxidized biochar treatment T4. Lowest SPAD value (42.26) was found at control treatment T1. Biochar and combine effects of biochar microbes was not significant at 65 DAS but microbes effect was significant (p= 0.0058).

Table 6. Effect of RHB and microbes on SPAD value (mean±SE)

Treatments	30 DAS	45 DAS	65 DAS
T1	33.06 ± 0.47b	44.5± 1.67a	42.26±0.77b
T2	32.13 ± 0.89b	40.63 ± 0.81a	42.5±1.79b
T3	32.9 ± 1.49b	46.2± 2.05a	46.1±1.64ab
T4	40.8±1.09a	45.06±0.64a	49.03±1.06
Biochar (BC)	*p*=0.0109*	*p*=0.1165	*p*=0.2816
Microbes(M)	*p*=0.0038*	*p*=0.0629	*p*=0.0058*
BC×M	*p*= 0.0031*	*p*= 0.3642	*p*= 0.3642

Means within the same column followed by the different letters are significantly different at p≤ 0.05; (Turkey's HSD test). T1= No microbes and no biochar, T2= No microbes and 10% oxidized biochar, T3= *Pseudomonas aeruginosa* and no biochar, T4= *Pseudomonas aeruginosa* and 10% oxidized biochar.

3.6 Combine effect of oxidized RHB and *P. aeruginosa* on soil phosphatase activity

Application of oxidized RHB and phosphate solubilizing bacteria *Pseudomonas aeruginosa* in soil significantly improved soil phosphatase activity Figure 2. Among all treatments, highest enzyme value was found at10% oxidized RHB and *Pseudomonas aeruginosa* treatment, T4 (2.14 U/g soil) and lowest value was found at control treatment, T1 (0.98 U/g soil). Biochar, microbes and their combination showed significantly increment of 100%, 59% and 418% phosphatase activity compared to the control. Inoculation of bacteria *Pseudomonas aeruginosa* on soil phosphatase activity was better than non-inoculated.

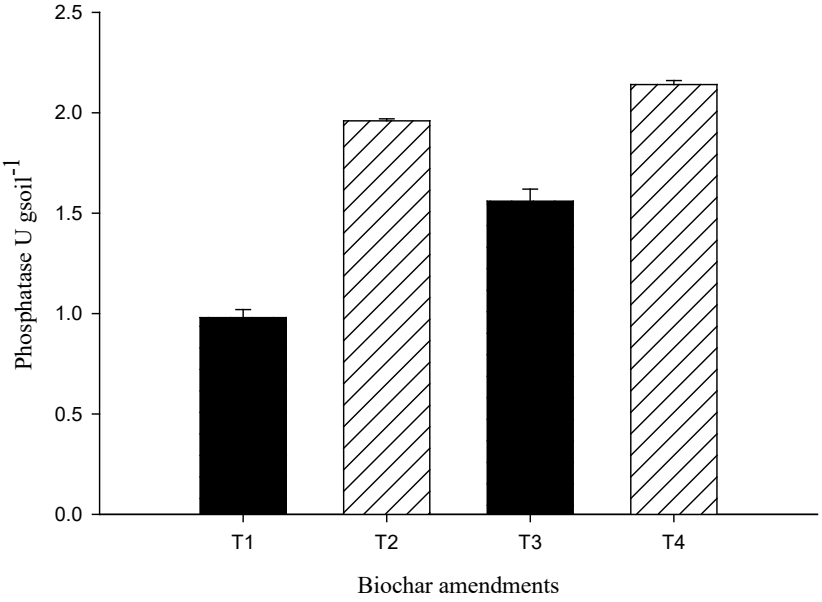

Figure 2 RHB and microbes effect on soil phosphatase activity

T1= No microbes and no biochar, T2= No microbes and 10% oxidized biochar, T3= *Pseudomonas aeruginosa* and no biochar, T4= *Pseudomonas aeruginosa* and 10% oxidized biochar,Error bar represents standard error of mean.

3.7 Effect of oxidized RHB and *P. aeruginosa* on soil microbial populations

The influence of phosphate solubilizing and N2-fixing bacteria and oxidized RHB biochar on microbial population was presented in Table 7. No significant different was found in this study among biochar (p=0.4092), microbes (p=0.8368) and their combination treatments (p=0.5760) compared to control (T1).

Table 7. Oxidized RHB and Microbes effect on soil microbial populations

Treatments	Microbial Populaion (cfu/ml)
T1	$6.3\times10^{-5} \pm 1.01\times10^{-5}$ a
T2	$3.94\times10^{-5} \pm 1.78\times10^{-5}$a
T3	$5.7\times10^{-5} \pm 5.29\times10^{-6}$a
T4	$5.2\times10^{-5} \pm 2.45\times10^{-5}$a
Biochar (BC)	*p*=0.4092
Microbes(M)	*p*=0.8368
BC×M	*p*= 0.5760

Means within the same column followed by the different letters are significantly different at p≤ 0.05; (Turkey's HSD test). T1= No microbes and no biochar, T2= No microbes and 10% oxidized biochar, T3= *Pseudomonas aeruginosa* and no biochar, T4= *Pseudomonas aeruginosa* and 10% oxidized biochar.

3.8 Effect of oxidized RHB and *P. aeruginosa* on plant growth characters and yield

In this experiment, Maize plant height, stem diameter, root length, dry biomass, number of grain and yield were influenced by the addition of biochar, microbes and their combination, as presented on Table 8. Plant height ranges from 162.33 (microbes treatment,T3) to 183.33 cm (combine treatment T4). Among all treatments highest plant height (183.33 cm) was found at *Pseudomonas aeruginosa* and 10% oxidized biochar treatment (T4) and was significantly different with microbial treatment (T3) but not significant with others treatment. Stem diameter was ranges from 1.36 to 1.49 cm. Highest stem diameter was found at combine treatment T4 (1.49 cm) and lowest stem diameter at control (1.36 cm). The effect of biochar, microbes and their combination on stem diameter was not significant in this experiment.

Root length was significantly influenced by biochar (p=0.0041), microbes (p=0.0590) and the combination with biochar and microbes (p=0.0463). The longest roots were observed in treatment T2 (10% RHB biochar and no microbes) with an average length of 63.66 cm. The shortest roots were observed in the control treatment T1 (36.33 cm). Among all treatments, biochar treatments and biochar microbes combine treatment significantly increased root length 75% and 73% respectively compared to the control treatment T1. In this study, dry biomass was significantly influenced by biochar (p=0.0294) and microbes (p=0.0260), although their interaction was not significant (p=0.7354). The highest dry biomass was recorded in treatment T4 (89.93 g/pot) and lowest dry biomass was in control treatment, T1 (45.42 g/pot). The total increment of dry biomass was 98% that was found by combination of biochar and microbes treatment (T4).

However, the maize yield exhibited significant different (refer to Table 8) with the biochar (p=< 0.0001), microbes (p=< 0.0001) and combined application of oxidized RHB and *Pseudomonas aeruginosa* (p= 0.0081) compared to untreated soil (T1). Highest yield was found at T4 (42.85 g/pot) and lowest yield was found at control treatment (10.37g/pot). 10% Oxidized RHB treatment (T2), microbes treatment (T3) and combination of biochar and microbes treatment (T4) showed yield increment 52% , 51% and 313% respectively compare

Table 8. Effect of oxidized RHB and *Pseudomonas aeruginosa* on transplanted maize plant growth characters and yield

Treatments	Plant height (cm)	Stem diameter (cm)	Root length (cm)	Dry biomass (g/pot)	No.of grain/cob	Yield g/pot
T1	168±3.05ab	1.36± 0.06a	36.33±2.40b	45.42±1.20b	79±3.5b	10.37±0.73c
T2	165.66±1.85ab	1.37±0.04a	63.66±1.45a	65.10±12.61ab	165±21.5a	21.47±1.32b
T3	162.33±1.85b	1.37± 0.03a	56±7.57a	64.45±6.89ab	159±11.5a	21.18±0.99b
T4	183.33± 1.85a	1.49±0.09a	63±3.05a	89.93±8.14a	204±12a	42.85±2.42a
Biochar	*p*= 0.0680	*p*= 0.3445	*p*= 0.0041*	*p*= 0.0294*	*p*= 0.0087*	*p*= < 0.0001*
Microbes	*P*= 0.2122	*P*= 0.3217	*P*= 0.0590	*P*= 0.0260*	*P*= 0.0120*	*P*= < 0.0001*
BC*M	*P*= 0.0299*	*P*= 0.4200	*P*= 0.0463*	*P*= 0.7354	*P*= 0.2135	*P*= 0.0081*

Means within the same column followed by the different letters are significantly different at p≤ 0.05; (Turkey's HSD test). T1= No microbes and no biochar, T2= No microbes and 10% oxidized biochar, T3= *Pseudomonas aeruginosa* and no biochar, T4= *Pseudomonas aeruginosa* and 10% oxidized biochar.

These improvement in yield might have occurred due to an increase soil pH, P bioavailability (r^2= 0.74), and a reduction in Al toxicity (r^2= 0.68) (Figure 3).

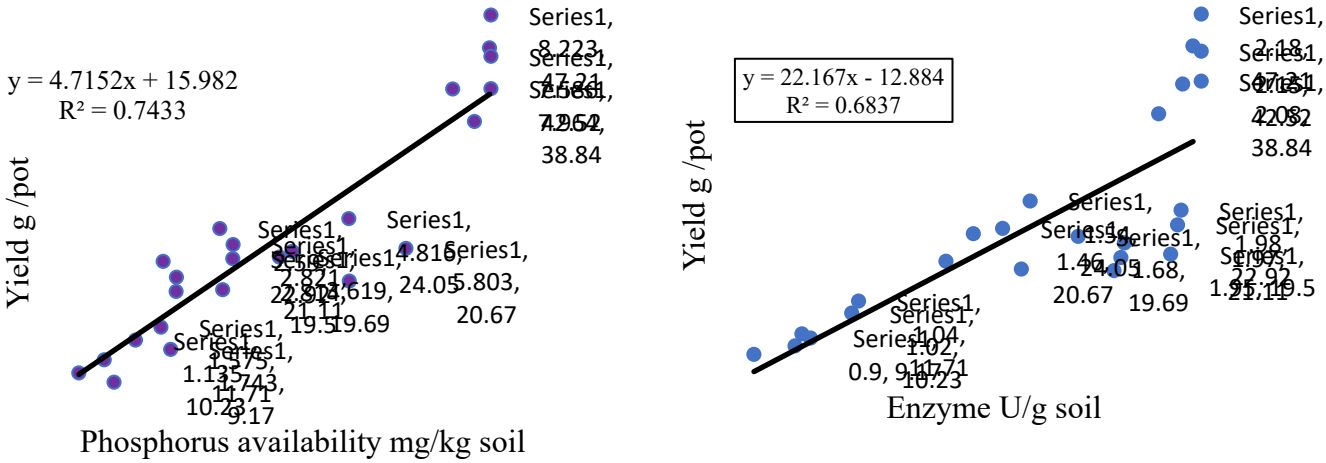

Figure 3. Regression analysis to show the relationship between the change of soil available Pand enzyme against grain yield.

The biplot (Figure 4) displayed the first two principal components (PC1 and PC2), which together account for a significant portion of the variability in the data: 54.8% for Component 1 and 16.2% for Component 2. Variables such as Inorganic N mg/plant, Enzyme (U/g), and CEC have arrows pointing toward the right, indicating a positive association with Component 1. Variables like Ex Al mmol/kg and Microbial population have arrows pointing toward the left, suggesting a negative association with Component 1. Variables such as Average pH and AVP (phosphorus availability) have arrows pointing upward, showing a positive association with Component 2.

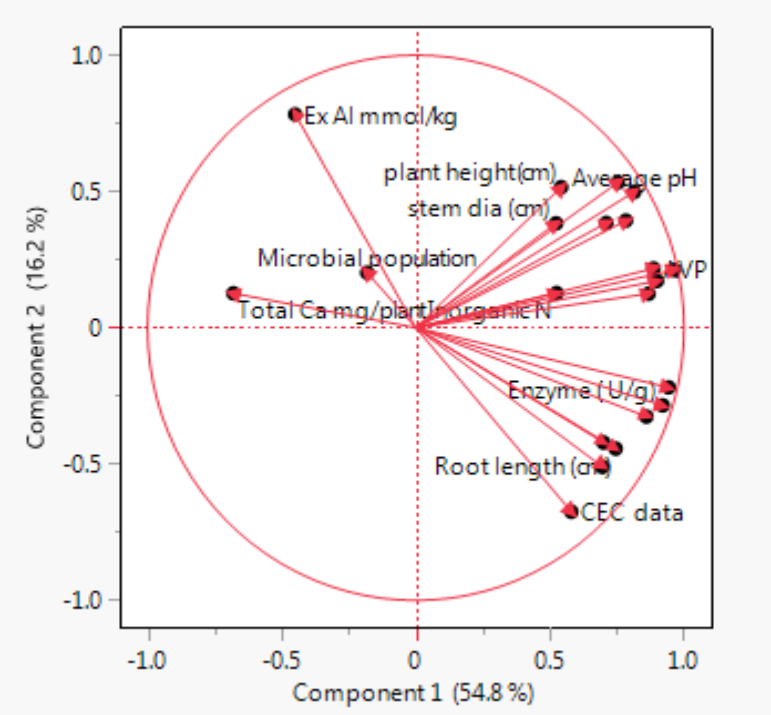

Figure 4: Analysis of principal component of different variables

3.8 Correlation between soil parameters, plant parameters and nutrient uptake

Pearson's correlation analysis was conducted to know the relationship among the soil nutrients, plant parameters, plant nutrient uptake, and yield (Table 6.9). Grain yield was positively correlated with soil pH (r = 0.89), Soil available P(r = 0.93), exchangeable K, Ca, Mg (r = 0.84, 0.80 and 0.63 respectively), enzyme (r = 0.82), P uptake (r = 0.73), N uptake (r = 0.88), Root length (r= 0.64) and dry biomass(r= 0.81). Furthermore, available phosphorus was positively correlated with soil pH (r = 0.84), Enzyme was positively correlated with available P (r = 0.67), pH (r = 0.60) and CEC (r = 0.76) dry biomass was positively correlated with pH (r = 0.72), available P (r = 0.77) P, N , K, Mg uptake (r =0.70, 0.86, 0.82, and 0.70 respectively).

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Table 9. The relationship between transplanted maize soil parameters, plant parameters and nutrient absorption

Parameters	Av pH	Av SPAD	AVP	Inorganic N	CEC data	Ex K mmol/kg	Ex Ca mmol/kg	Ex Mg mmol/kg	Ex Al mmol/kg	Microbial population	Enzyme (U/g)	Total P mg/plant	Total N mg/plant	Total K mg/ plant	Total Ca mg/plant	Total Mg mg/ plant	plant height(cm)	stem dia (cm)	Root length (cm)	Dry biomaass g/plant	yield gm/pot
Average pH	1.00																				
Average SPAD	0.74	1.00																			
AVP	0.84	0.54	1.00																		
Inorganic N	0.38	0.32	0.47	1.00																	
CEC data	0.18	0.18	0.38	-0.03	1.00																
Ex K mmol/kg	0.85	0.57	0.93	0.24	0.21	1.00															
Ex Ca mmol/kg	0.86	0.78	0.72	0.36	0.12	0.78	1.00														
Ex Mg mmol/kg	0.37	0.17	0.69	0.37	0.66	0.52	0.14	1.00													
Ex Al mmol/kg	0.02	-0.09	-0.14	-0.09	-0.83	0.02	-0.06	-0.49	1.00												
Microbial population	-0.07	-0.02	-0.09	-0.34	-0.14	0.03	0.10	-0.46	0.22	1.00											
Enzyme (U/g)	0.60	0.67	0.67	0.40	0.76	0.53	0.58	0.68	-0.70	-0.19	1.00										
Total P mg/plant	0.50	0.61	0.61	0.27	0.74	0.52	0.55	0.58	-0.70	0.05	0.94	1.00									
Total N mg/plant	0.80	0.70	0.82	0.56	0.37	0.72	0.77	0.43	-0.23	0.14	0.76	0.77	1.00								
Total K mg/ plant	0.62	0.55	0.82	0.51	0.64	0.68	0.58	0.75	-0.54	-0.05	0.90	0.91	0.87	1.00							
Total Ca mg/plant	-0.52	-0.76	-0.35	-0.19	-0.60	-0.26	-0.53	-0.27	0.55	0.04	-0.83	-0.78	-0.60	-0.60	1.00						
Total Mg mg/ plant	0.26	0.23	0.49	0.34	0.60	0.37	0.21	0.70	-0.61	-0.30	0.71	0.75	0.55	0.79	-0.38	1.00					
plant height(cm)	0.73	0.51	0.60	0.19	0.08	0.61	0.67	0.19	0.15	-0.24	0.34	0.25	0.46	0.30	-0.37	0.16	1.00				
stem dia (cm)	0.58	0.48	0.49	0.55	0.02	0.47	0.55	0.39	0.03	-0.41	0.38	0.18	0.36	0.33	-0.25	0.10	0.42	1.00			
Root length (cm)	0.44	0.12	0.68	0.46	0.67	0.48	0.22	0.86	-0.56	-0.34	0.70	0.62	0.60	0.80	-0.30	0.79	0.16	0.27	1.00		
Dry biomass g/plant	0.72	0.59	0.77	0.53	0.31	0.74	0.76	0.46	-0.28	-0.08	0.69	0.70	0.86	0.82	-0.41	0.70	0.44	0.46	0.65	1.00	
yield g/pot	0.89	0.76	0.93	0.57	0.40	0.84	0.80	0.63	-0.24	-0.18	0.82	0.73	0.88	0.86	-0.61	0.53	0.61	0.58	0.64	0.81	1.00

Av.P: available P; Ex. K: exchangeable K; Ex. Ca: exchangeable Ca; Ex. Mg: exchangeable Mg; Ex Al: ex-changeable Al;

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4.1 DISCUSSION

4.1 Soil properties

Biochar and P solubilizing bacteria can potentially affect soil properties. In our study, rice husk biochar, microbes, and their combination significantly increased soil pH at all three measured dates (30, 65 and 85 days after sowing, DAS). At 30 DAS, 65 DAS and 85 DAS, the combination of 10% oxidized biochar and *P. aeruginosa* demonstrated an increase in soil pH that ranged from 4.5 to 4.89, 4.5 to 4.85 and 4.33 to 4.49 and the increments were 0.39, 0.35 and 0.16 unit respectively compared to control treatment. An increases in pH contributed to the reduction of Al concentration. Compared to the control treatment, biochar decreased Al^{3+} content by helping to mitigate Al toxicity in acidic soils(Xia *et al.*, 2023). Biochar application reported to increase soil pH. in acidic soil (Tusar et al., 2023). Similarly, individual studies also reported pH increase. For instance, water hyacinth biochar increased soil pH by 0.72 units at a 1% application rate and by 0.86 units at a 2% application rate, while simultaneously decreasing exchangeable acidity (Lewoyehu *et al.*, 2024).

Oxidized biochars exhibited substantially higher Al^{3+} binding capacity compared to unmodified primary biochars result in pH increased 2.5 to 3.5 (Qian and Chen, 2014) . The addition of biochars significantly enhanced the soil pH buffering capacity (pHbuff), with the extent of improvement influenced by the acid buffering capacity of the biochar applied. The primary mechanism driving the increase in pHbuff within the pH range of 4.0–7.0 was the release of cations, attributed to the protonation of carboxyl groups on the biochar surfaces and the dissolution of carbonates. This mechanism accounted for over 67% of the observed pHbuff increase (Shi et al., 2017).

Our result was in line with the study by (Lewoyehu *et al.*, 2024). *Pseudomonas aeruginosa* an environmentally-derived pathogen known for its antibiotic resistance, is capable of producing metabolites such as ammonia, which can raise soil pH by forming ammonium, thereby neutralizing soil acidity (Laborda *et al.*, 2021). Combining oxidized biochar with *Pseudomonas aeruginosa* can boost microbial activity in the soil, support plant growth, enhance soil structure, and facilitate nutrient release, leading to improved soil health and better pH buffering (Ouyang *et al.*, 2023).

The effect of biochar, microbes and their combination on soil CEC was demonstrated on Table 3. Soil CEC ranges from 5.25 to 10.19 mmol/kg. In this study, combine effect of biochar and *Pseudomonas aeruginosa* was significantly different with control treatment T1 on soil CEC. The CEC is one of the most important soil chemical properties that can be changed through biochar application.The functional groups on oxidized biochar can indeed interact with soil organic matter, potentially increased the effective CEC (Kharel et al., 2019).The aged biochars exhibit an increase in negative surface charge and a reduction in their isoelectric point, attributed to

the carboxylation of their surfaces during the aging process. The development of carboxylate groups on biochar surfaces enhances both the negative surface charge and the cation exchange capacity (CEC) (Lawrinenko et.ai., 2016). The application of biochar enhancedCEC by promoting the availability of basic cations like Ca^{2+} and Mg^{2+}, which are crucial for soil fertility. Studies have shown that applying biochar to soil improved in soil pH reaching 1.49 units and larger increases in CEC (Haile *et al.*, 2024). Microbially induced soils amended with biochar has been demonstrated to enhance soil properties, including cation exchange capacity (CEC), through the activity of microorganisms (Shukla and Sharma, 2024). The combine application of biochar and *Pseudomonas aeruginosa* substantially increases microbial populations. It enhance microbial activity results in the release of byproducts that improve soil structure and increase nutrient availability, thereby further boosting soil CEC (Olajumoke *et al.*, 2024).

The study investigated the impact of the phosphate-solubilizing bacterium *P. aeruginosa*, in combination with oxidized rice husk biochar (RHB), on the soil microbial population. The findings, as presented in Table 7, offer insights into how these amendments interact and their combined effects on soil microbial dynamics. When applied separately or combine, neither the biochar nor the microbial treatment resulted in a significant difference in the microbial population compared to the unamended control. At pH levels below 5.0, *P. aeruginosa* shows a significant reduction in cell count and metabolic activity, suggesting a critical point where its growth is greatly hindered (Wang *et al.*, 2024). Our experimental pH was ranges between 4 to 4.5. It is crucial to recognize that strong acids and bases can influence the functional activity of microorganisms (Teng *et al.*, 2020).

Oxidized RHB and phosphate solubilizing bacteria *Pseudomonas aeruginosa* in soil significantly influenced soil phosphatase activity (Figure 2). Biochar, microbes and their combination showed significantly increment of 100%, 59% and 418% phosphatase activity compared to the control. *Pseudomonas* species, including *P. aeruginosa*, are known to produce phosphatases that catalyze the hydrolysis of organic phosphorus compounds (Chauhan and Dhaked, 2022). The combined use of biochar and *P. aeruginosa* has been demonstrated to substantially enhance microbial populations and enzyme activities, such as phosphatase (Olajumoke *et al.*, 2024). The porous nature of biochar allows for better microbial colonization, which can protect enzymes from degradation, thus sustaining their activity over time (Sun *et al.*, 2024). Phosphatase enzymes generally show greater activity in alkaline pH conditions; therefore, the rise in pH caused by the biochar can boost the activity of these enzymes (Guan and Wang, 2023; Schalk and Perraud, 2023).

The application of treatments significantly influenced the availability of phosphorus (P) in the soil (Table 3). Statistical analysis confirmed that biochar, microbial treatments and their combination significantly influenced available P levels at 220%, 85% and 435% respectively. The possible mechanisms are –a) Enhance soil pH and liming effect.b) Improved P desorptionand c) others activities such as surface functionalization of biochar, reduction of iron and aluminium binding of phosphorus, stimulation of microbial activity, increase CEC, enhance organic matter mineralization, Mitigation of soil compaction etc.Soil amendment can affect the

availability and adsorption ability of phosphorus in soil, and biochar has the best effect on the availability of soil phosphorus (Hong et al., 2018)

Biochar can enhance phosphorus (P) availability in soil by offering a habitat and carbon source for phosphate-solubilizing bacteria (PSB), which are capable of dissolving low-solubility P compounds. Thus, the combined use of biochar and PSB presents an eco-friendly approach to boost PSB activity in soil, thereby increasing P mobilization and improving plant productivity (Siddiqui *et al.*, 2016).

Acidic oxidized biochar can reduce phosphorus fixation and increasing plant phosphorus availability in acidic soils (Zhao *et al.*, 2023). Phosphate-solubilizing bacteria can dissolve insoluble phosphorus compounds in the soil, making the phosphorus available for plant uptake (Tao GuangCan *et al.*, 2008).

Biochar can facilitate the formation of *Pseudomonas aeruginosa* biofilms, improving soil colonization and nutrient solubilization, which may enhance phosphorus availability and microbial activity in specific areas of the soil (Egamberdieva *et al.*, 2018). The interaction of oxidized biochar with soil and microorganisms may lead to the release of phosphorus, thereby increasing the amount of available phosphorus in the soil solution and affecting nutrient dynamics (Liu *et al.*, 2023). The combination of organic material and phosphate-solubilizing bacteria can increase phosphorus availability more effectively than individual (Timofeeva et al., 2023).

4.2 RHB and Microbial Interactions on transplanted Maize growth and yield

In this experiment, maize plant height, stem diameter, root length, dry biomass and yield were influenced by the addition of biochar, microbes and their combination, as presented on Table 8. Among all treatments highest plant height (183.33 cm) was found at *Pseudomonas aeruginosa* and 10% oxidized biochar treatment (T4) and was significantly different with microbial treatment (T3) but not significant with others treatment. The effect of biochar, microbes and their combination on stem diameter was not significant in this experiment. The longest roots were observed in treatment T2 (10% RHB biochar and no microbes) with an average length of 63.66 cm. The shortest roots were observed in the control treatment T1 (36.33 cm). Among all treatments, biochar treatments and biochar microbes combine treatment significantly increased root length 75% and 73% respectively compared to the control treatment T1. In this study, dry biomass was significantly influenced by biochar ($p=0.0294$) and microbes ($p=0.0260$), although their interaction was not significant ($p=0.7354$). The highest dry biomass was recorded in treatment T4 (89.93 g/pot) and lowest dry biomass was in control treatment, T1 (45.42 g/pot). The total increment of dry biomass was 98% that was found by combination of biochar and microbes treatment (T4).

However, the maize yield exhibited significant different (refer to Table 8) with the biochar ($p=< 0.0001$), microbes ($p=< 0.0001$) and combined application of oxidized RHB and *Pseudomonas aeruginosa* ($p= 0.0081$) compared to untreated soil (T1). Highest yield was found at T4 (42.85 g/pot) and lowest yield was found at

control treatment (10.37g/pot). 10% Oxidized RHB treatment (T2), microbes treatment (T3) and combination of biochar and microbes treatment (T4) showed yield increment 52% , 51% and 313% respectively compare to control.

The PCA (Table 9, Figure 4) biplot suggests that certain soil properties and nutrient uptakes (such as soil pH, available P and enzyme activity) have a strong influence on maize yield and growth parameters. Biochar alone increased root length and volume, while microbial inoculation improved stem diameter and dry biomass. Its porosity and adsorption capacity make biochar an effective carrier for plant growth-promoting rhizobacteria (PGPR), enhancing crop growth. (Ajeng *et al.*, 2020). The combination of biochar (5 t/ha) and bacteria (Sb16) was found to enhance soil quality and promote the growth of maize (Abdulrahman *et al.*, 2017). In a greenhouse experiment, applying biochar at 5 tons per hectare along with bacterial inoculation significantly ($P < 0.05$) boosted the growth of maize, improving shoot and root biomass, root length, root volume, plant height, and leaf chlorophyll content. Oxidized rice husk biochar likely helped neutralize harmful substances, promoting healthier plants with increased biomass (Rizwan *et al.*, 2016).

(Ashry and Hassan, 2019) studied the combined effects of biochar and plant growth-promoting endophytic bacteria (PGPE) on pepper plants. The combination significantly improved all growth metrics and yield compared to individual applications. (El-Naggar *et al.*, 2019) discussed various studies where biochar combined with microbial inoculants, such as *Pseudomonas* species, resulted in yield increases ranging from 20-30% due to enhanced nutrient cycling and reduced soil acidity.

The combination of biochar and *P. aeruginosa* led to a notable increased in maize yield, ranging from 15-25% compared to untreated control groups (Rafique *et al.*, 2017). The combined effect of biochar and beneficial microbes likely promoted the growth of a well-developed, branched root system in maize plants. This enhanced the efficiency of nutrient uptake and assimilation, ultimately resulting in a higher grain yield (Sun, Wang and Wu, 2022; Ain and Noraini, 2023). This is a short-term study, and the result may vary for long-term experiments under field conditions. A long-term field study should be carried out to validate the results.

5. CONCLUSION

This study concludes that the combined application of oxidized rice husk biochar (RHB) and *Pseudomonas aeruginosa* effectively enhances soil properties and boosts transplanted maize yield in acidic soils. The use of acidic oxidized RHB and phosphate-solubilizing bacteria significantly improved phosphorus availability by 435% and phosphatase enzyme activity by 418%. Additionally, combination of biochar and microbes treatment showed yield increment 413.21%. These findings highlight the potential of this combined treatment for improving soil fertility and crop productivity in challenging soil conditions.

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