

Effect of Phosphate Solubilizing *Bacillus* on the Growth of Sorghum (*Sorghum bicolor* [L.] Moench)

¹S. A. Muhammad, ²Z. M Bello, ²S. Muhammad, ²A. A. Aliero, ³R. Adamou and ¹A. D. Ibrahim

¹Department of Microbiology, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto

²Department of Plant Sciences, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto

³Universite Abdou Moumouni, Niamey, Niger Republic

[*Corresponding author: Email address: subeeab2@gmail.com]

ABSTRACT

The presence of phosphate solubilizing bacteria (PSB) in the soil is essential for increasing the availability of nutrients, especially phosphorus, which is necessary for plant growth. The development of sorghum (*Sorghum bicolor* [L.] Moench) was examined in this work in relation to the inoculum of phosphate solubilizing bacillus from three previously obtained PSB. Sorghum plants were injected with the *Bacillus* strains under carefully monitored circumstances for 28 days, and several growth metrics were evaluated and compared to control groups, including shoot length, shoot fresh and dry weights, and root fresh and dry weights. Selected *Bacillus* species were tested for their ability to solubilize phosphate in agar and broth medium supplemented with tri calcium phosphate (TCP). Using 16S RNA amplicon sequencing, the three isolates (DN1, K1, and K3) were further characterized. With percentage similarity values of 98.65%, 85.37%, and 99.67%, respectively, isolates DN1, K1, and K3 were determined to be *Bacillus subtilis*, *Lysinibacillus sphaericus*, and *Bacillus cereus*, respectively. According to the findings, *Bacillus subtilis* increased shoot length by 36.39%, *Bacillus cereus* by 32.34%, and *Lysinibacillus sphaericus* by 31.27%. The results suggest that utilizing PSB could be an eco-friendly method to enhance sorghum yield and contribute to food security.

Keywords: Phosphate solubilizing bacteria, Sorghum; National Botanical Research Institute, Phosphate; *Bacillus*.

INTRODUCTION

Phosphate-solubilizing bacteria (PSB) play a significant role in enhancing soil fertility by making phosphorus (P) available to plants and improving the soil micro-environment. These bacteria secrete low molecular weight organic acids that chelate cations bound to phosphorus or release protons to transform it into soluble forms, thereby increasing plant phosphorus uptake (Chung *et al.*, 2005; Perez *et al.*, 2007; Yadav *et al.*, 2015). In addition to solubilizing phosphorus, PSB produces phytohormones like indole-3-acetic acid (IAA) (Bahadur *et al.*, 2017), antibacterial compounds, siderophores for iron sequestration (Linu *et al.*, 2019), and cyanogenic compounds (Shabanamol *et al.*, 2018), all of which promote plant growth and suppress phytopathogens. The use of PSB as biofertilizers presents a sustainable alternative to synthetic fertilizers, which are expensive and pose environmental risks (Narayanan *et al.*, 2012). Phosphorus deficiency in plants often leads to stunted growth, while its scarcity in humans can cause bone abnormalities (Schlemmer, 2014). PSB helps alleviate these challenges by improving phosphorus availability in soils where natural reserves are immobilized by calcium in alkaline soils or by aluminium and iron oxides in acidic soils.

Crops like sweet sorghum, which is nutrient-dense and rich in carbohydrates (Dar *et al.*, 2018; Awika and Rooney, 2004), could greatly benefit from PSB applications. Sorghum has higher energy output compared to many staple crops and contains essential minerals, vitamins, and micronutrients required for growth and development. Enhancing sorghum production aligns with global sustainable development goals (SDGs), particularly poverty eradication and food security by 2050 (Popova and Mihaylova, 2019). Despite the potential of

rock phosphate and rich total phosphorus reserves, poor solubility and rising costs of phosphorus fertilizers necessitate alternative solutions for sustainable agriculture. This study investigates the role of phosphate-solubilizing bacteria in improving phosphorus availability and promoting the growth and nutritional quality of sorghum.

MATERIALS AND METHODS

Sample Collection and Preparation

Ten (10) bacterial isolates were collected from the Usmanu Danfodiyo University Sokoto Microbiology laboratory from a stock culture of previously isolated samples (Bello *et al.* 2024). Rock Phosphate was purchased from Malcomines Minor Material Ltd, Jos, Plateau State. Seeds of Sorghum (*Sorghum bicolor* [L.] Moench) were purchased from FADAMA III FARMS of Sokoto State, Nigeria. Rock Phosphate was crushed with a pestle and mortar and then sieved until a fine powder was obtained. It was then autoclaved at 121°C for 15 minutes and kept for further inoculation in greenhouse trials. Sorghum seeds were planted in seedling trays for further use three weeks after plantation.

Purification of Bacteria

Ten (10) bacterial isolates were sub-cultured in Nutrient Agar (NA) until a pure isolate was obtained. The media was prepared according to the manufacturer's instructions (Cowan and Steel, 1993).

Morphological and Biochemical Identification of Bacteria

To more precisely identify the colonies, the pure cultures of the various colonies were subjected to Gram staining. The Christian Gram technique was followed by the

adoption of the standard gram staining methods (Collee *et al.*, 1996).

With a little modification, the colony morphology of isolates was investigated using NBRIP (National Botanical Research Institute) Phosphate agar plates as described by Aneja (2003).

Citrate Test

To ascertain whether the organisms could use citrate as a source of carbon, the Baron and Finegold (1990) method was applied to conduct the Citrate Test.

Catalase Test

The Catalase Test was run to see if PSB could hydrolyze the 3% H₂O₂ solution that had been made according to Cowan and Steel, (1993) method to produce oxygen (O₂).

Urease Test

Using Cowan and Steel's approach, the urease test was performed to identify bacteria that could hydrolyze urea to create carbon dioxide and ammonia. The purpose of the starch hydrolysis test was to ascertain whether microbes could use starch as a source of carbon (Oliveira *et al.*, 2009).

Methyl Red-Vogues Proskauers Test

To ascertain which fermentation pathway uses glucose, the Methyl Red-Vogues Proskauers Test was performed in accordance with Oyeleke and Manga, (2008).

Indole Test

The ability of an organism to make indole through the breakdown of the amino acid tryptophan was assessed using the Cheesebrough, (2006) method, which is known as the Indole Test.

Phosphorus Solubilizing Capacity and Phosphate Solubilizing Index of Isolates on NBRIP Agar

On the National Botanical Research Institute Phosphate (NBRIP) medium, the isolates' ability to solubilize insoluble phosphates was investigated. Agar plates of NBRIP medium containing tri-calcium phosphate (10g [Ca₃(PO₄)₂] as an insoluble phosphate source, 5g; MgCl₂·6H₂O, 5g; MgSO₄·7H₂O, KCl; 0.25g, and (NH₄)₂SO₄; 0.1g in 1 liter of distilled water) were spotted with each isolated single colonial form of bacterial culture (Nautiyal, 1999). Halo formation occurs when insoluble phosphates are soluble in isolates.

The diameter of the colonies and that of the halo zone in plate assays was measured using a calliper after seven days of incubation at 37°C. The solubilisation index (SI) was calculated as the ratio of the total diameter to the colony diameter (Edi-Premono *et al.*, 2007).

$$\text{Phosphate Solubilization Index (SI)} = \frac{B}{A}$$

Where; A = Colony diameter

B = Total diameter (colony + halo zone)

Assessment of P-Solubilization in Liquid Medium

Sterilized liquid NBRIP medium (20 ml) was used to cultivate bacterial strains that displayed a zone of inhibition on the medium. The strains were incubated for two days at 30°C with constant shaking at 150 rpm. A 500 ml flask filled with 200 ml of sterilised liquid NBRIP medium was filled with the bacterial suspension (1 × 10⁸ CFU ml⁻¹), and was cultured for seven days at 30°C with constant shaking. The control was an uninoculated, sterilized media. At day Two, five, and seven days of inoculation, an aliquot (10 ml) of each culture and control was obtained, and the pH of the was measured using a pH meter equipped with a glass electrode (Murphy and Riley, 1962).

Molecular Characterisation of Isolates

The selected isolates were characterised genotypically by cloning and sequencing the 16S rRNA. Briefly, the genomic DNA from a pure culture of each isolate was extracted and purified for PCR amplification of the 16S rRNA sequence using Pfu Ultra II Fusion HS DNA polymerase (Agilent, CA, USA) and the 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525r (5'-AAGGAGGTGWTCCARCC-3') primers. The amplification was conducted in a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, CA, USA) with the following program: 95 °C for 5 min; 30 cycles at 72°C for the 30s, 55°C for 30s, and 72°C for 90s; and 72 °C for 7 min. The amplified 16S rRNA genes were cleaned using PCR clean up and Gel extraction kit (Thermo Fisher, MA, USA), sequenced (ABI 3730xl; Applied Biosystems) and were subjected to a BLAST search (Altschul *et al.*, 1990).

Physiochemical Properties of the Soil used for Plantation

The physiochemical properties of the soil used for the plantation were measured before and after the plantation. The following parameters were measured; the pH of the soil suspension was determined using a pH meter in accordance with AOAC (1990). Phosphorus Content (PO₃⁴) was performed using the method of Juo (1979). Electrical Conductivity (EC) was measured according to the method of Rayment and Higginson (1992). Nitrogen content (N) was determined by the Macro-Kjeldahl digestion method of Juo (1979). Cation Exchange Capacity (CEC); Calcium (Ca²⁺) and magnesium (Mg²⁺) were determined by EDTA titration while potassium (K⁺) and sodium (Na⁺) were determined by flame photometry. Effective CEC was thus calculated by the sum of exchangeable bases (Ca, Mg, K, Na).

Greenhouse Experiment

The effects of bacterial treatments on sorghum growth were assessed in a greenhouse experiment. The Usmanu Danfodiyo University Botanical Garden in Sokoto served as the site of the experiment. After surface-sterilizing the seeds with 1% sodium hypochlorite (NaOCl) (Hariprasad and Niranjana, 2009), the sorghum seeds were raised in seedling trays until they reached three weeks of age (Pathak *et al.*, 2017). After three

weeks, the seedlings were moved to medium-sized pots that measured 18 cm in height and 18 cm in diameter (18×18) (Pathak *et al.*, 2017) containing Five (5) kg of autoclaved sandy loam soil. Two days were given to the sorghum seedlings for growth before the bacterium treatment was applied. The strains that shown effective P solubilizing qualities were used to expose the plants to various feeding conditions. With seven treatments and three duplicates of each treatment, the experiment was set out in a completely randomised manner, resulting in twenty-one (21) pots: Control (C), DN1(Isolate DN1 alone), K1(Isolate K1 alone), K3(Isolate K3 alone), DN1+RP (Isolate DN1 plus Rock phosphate), K1+RP (Isolate K1 plus Rock phosphate), and K3+RP (Isolate K3 plus Rock phosphate). Isolates were initially streaked on Nutrient Agar (NA) plates and incubated at 37 °C for 24 hours in order to prepare the bacterial treatment. To achieve a minimum final cell concentration of 108 CFU/ml at (OD = 0.5), single colonies on NA plates were selected using an inoculating loop and transferred to 10 ml of sterile distilled water in test tubes. The diluted cell suspension was used to treat sorghum plants in the form of soil drenching. The application of bacterial suspension (5 mls) (DN1, K1 and K3) each was conducted four times at a 7-day interval (Pathak *et al.*, 2017) with some modifications. DN1+RP, K1+RP, K3+RP treatments were supplemented with 4g/kg Jos RP i.e each kilogram of soil consists of 4g of Rock phosphate making 20 g in 5 kg soil (Sane and Mehta, 2015). All the pots were kept in the greenhouse for 28 days. After completion of the trial, the plants were washed thoroughly with water, and several morphological parameters such as the length of roots and shoots, dry weights and fresh weights of plants, etc., were recorded for comparative evaluation in different combinations (Sane and Mehta, 2015).

Statistical Analysis

The significant effect of PSB isolates on the sorghum growth-promoting potential was determined by using ANOVA table in a completely randomised design (CRD). F values and means were made by using the Turkey mean separation model at P=0.01 probability levels.

RESULTS AND DISCUSSION

In this study, 10 isolates were screened for phosphate solubilization. All the isolates were found to be Gram-positive rods during purification on NA (Table 1). This conforms to several other studies highlighting the presence of Gram-positive bacteria as effective PSM (Hanif *et al.*, 2015).

Using the NBRIP agar method, the phosphate solubilization index (PSI) of every organism examined was greater than 1. PSI values varied from 1.19 to 3.85 (Table 2). DN3, G1, and G2 isolates had lower PSIs while having more colonies and higher zone development. Three isolates had smaller colonies and halo zones, but their PSIs were higher—3.85, 3.00, and 2.40, respectively. These results were consistent with those of Haile *et al.* (2021), whose isolates K-10-1 and K-10-41

had colony diameters of 0.2 and 0.4, respectively, and halo zone diameters of 0.53 and 0.25, respectively, but a higher PSI of 3.12. Variations in the colour of isolates were observed. Isolate DN1, DN3, G1, G2, G3, K1 and K3 were all observed as Milk, M1 and M2 were White, and finally, M3 was pink on NBRIP agar. Earlier studies (Sharon *et al.*, 2016) describe PSB variations in colony size, shape, and colour.

Table 1: Cultural and morphological characteristics of the presumed phosphate solubilising bacteria isolated from sorghum rhizosphere.

Isolate	Gram stain
DN1	+ rods
DN3	+ rods
G1	+ rods
G2	+ rods
G3	+ rods
K1	+ rods
K3	+ rods
M1	+ rods
M2	+ rods
M3	+ rods

DN: Dundaye, G: Gidan doki, K: Kwanni/Konni/Nkonni, M: Makera

Table 2: The diameter of the colony and halo zone indicating phosphate solubilization by the presumed P solubilising bacteria

Isolate	Colony diameter (mm)	Halo diameter (mm)	SI	Colour
DN1	0.20	0.57	3.85	Milk
DN3	0.83	0.73	1.88	Milk
G1	1.00	0.67	1.67	Milk
G2	1.27	0.83	1.65	Milk
G3	0.63	0.27	1.43	Milk
K1	0.20	0.40	3.00	Milk
K3	0.57	0.80	2.40	Milk
M1	0.47	0.20	1.43	White
M2	0.67	0.13	1.19	White
M3	0.43	0.13	1.30	Pink

SI= Colony diameter+ Halo diameter/ Colony diameter

DN: Dundaye, G: Gidan doki, K: Kwanni/Konni/Nkonni

M: Makera

The colony diameter and halo zone formation by some of the selected PSB isolates, notably DN1 and K3. The colony diameter is denoted as (C) which indicates the growth rate and metabolic activity of the organism, and the Halo zone as (H) which demonstrates the organism's ability to produce enzymes, as indicated on the plate (Plate 1).



Plate 1: Bacterial P-solubilising potential based on colony diameter and Halo zone formation: (a) P-solubilizing bacteria DN1, (b) P-solubilizing bacteria K3. The ring insert indicates colony diameter (C) and Halo zone diameter (H)

As shown in Figure 1, the pH reduction in NBRIP liquid media during phosphate solubilization by the isolates which depends on a drop in pH of the media from neutral to acidic due to the liberation of acids during P solubilisation was supported by the findings of Carillo *et al.* (2002) who mentioned that one mechanism by which phosphate can be released from the tricalcium complex is by decreasing the pH of the surrounding medium through the secretion of organic acids.

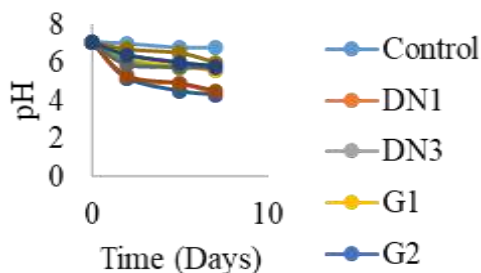


Figure 1: Reduction of pH in NBRIP liquid media during phosphate solubilization by the isolates.

DN: Dundaye, G: Gidan doki,

The biochemical result (Table 4) showed the organisms as *Bacillus* spp. DN1 as *Bacillus subtilis*, K1 as *Lysinibacillus sphaericus* and K3 as *Bacillus cereus*. *Bacillus* spp are amongst the most predominant phosphorus solubilizing bacteria (Wani *et al.*, 2007). The findings of Ahmad *et al.* (2008), observed solubilization of Phosphorus was higher in *Bacillus* spp, where 80% of the *Bacillus* isolates exhibited phosphate-solubilizing capabilities, indicating a significant potential for enhancing phosphorus availability in soils.

Table 4: Morphological and biochemical identification of the phosphate solubilizing bacillus

Biochemical test	<i>Bacillus subtilis</i>	<i>Lysinibacillus sphaericus</i>	<i>Bacillus cereus</i>
Citrate	+	+	+
Catalase	+	+	+
Starch hydrolysis	+	+	+
Urease	-	-	-
Indole	-	-	-
MR	-	-	-
VP	+	+	+
Oxidase	+	-	-

The isolate DN1 displayed close homology to *Bacillus subtilis* with 98%, K1 to *Lysinibacillus sphaericus* with 85.14%, and K3 to *Bacillus cereus* with 99% (Table 5). This agrees with previous research as reported by Wang *et al.* (2017) that among Gram-positive PSB *Bacillus* is the most predominant specie.

Table 5: The BLAST results correspond to the similarity between the sequence queried and the biological sequences within the NCBI database.

Sample ID	Predicted Organism	Percentage Similarity	Accession Number
K1	<i>Lysinibacillus sphaericus</i>	85.37%	EU880531.1
DN1	<i>Bacillus subtilis</i>	98.65%	MF616407.1
K3	<i>Bacillus cereus</i>	99.67%	MK202350.1

DN1; *Bacillus subtilis*, K1; *Lysinibacillus sphaericus*, K3; *Bacillus cereus*

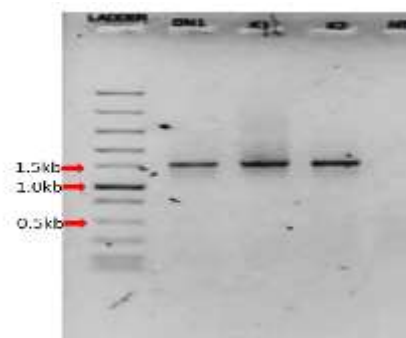


Figure 2: An agarose gel indicating the amplification of the 16S target region of the isolates DN1, K1 and K3.

The three isolates were subjected to inoculation on sorghum plants in greenhouse pot trials. The bacterial treatment inoculated every week over 28 days positively impacted the shoot length of the sorghum plants, as presented in Table 6. Similarly, Vikram (2007) reported that PSB significantly increased the shoot growth and grain yield of Sorghum under a greenhouse experiment compared to plants grown without bacterial cultures, or RP. Wang *et al.* (2022), also reported the growth of wheat was enhanced by *Bacillus safensis* and *Falsibacillus pallidus* soil treatments. There was no significant difference between DN1 and DN1+RP, K3 and K3+RP treatments at $p=0.01$. This demonstrates that the organisms were not positively impacted by the rock phosphate that was utilized as a phosphate source. In contrast to well-developed technologies and processes that chemically convert RP into P mineral fertilizers, RP, which stands as a natural P source primarily used in the production of P fertilizers for agriculture, still lacks the technologies enabling a higher P solubilization (Elhaissofi *et al.*, 2022).

Table 6: The effect of P-solubilising bacterial inoculums on the shoot length of sorghum plant

Treatments	Shoot lengths (cm)			
	Wk1	Wk2	Wk3	Wk4
Control	3.98±0.86 ^A	4.88±0.33 ^A	6.09±0.39 ^{a,B}	8.06±1.74 ^{a,C}
DN1	3.81±0.31 ^A	4.57±0.49 ^A	8.61±0.92 ^{c,d,C}	11.72±0.67 ^{c,D}
K1	2.93±0.49 ^A	4.54±1.03 ^B	8.11±0.19 ^{b,c,d,C}	9.11±0.92 ^{a,b,C}
K3	3.29±0.35 ^A	4.14±1.08 ^A	7.02±0.63 ^{a,b,B}	11.05±0.86 ^{b,c,C}
DN1+R	3.27±0.40 ^A	5.15±1.21 ^B	9.30±0.57 ^{d,C}	11.78±1.66 ^{c,D}
K1+RP	2.61±1.40 ^A	3.61±0.63 ^B	7.44±1.17 ^{b,c,C}	9.11±1.78 ^{b,c,D}
K3+RP	3.70±0.82 ^A	4.45±0.47 ^A	7.95±0.69 ^{b,c,B}	10.56±1.36 ^{b,c,C}

Values are expressed as mean ± standard deviation of triplicate determination; Values with different superscripts (lower case) in the same column are significantly different at p=0.01 between treatments; Values with different superscripts (Upper case) in the same row are significantly different at p=0.01 between duration DN1; *Bacillus subtilis*, K1; *Lysinibacillus sphaericus*, K3; *Bacillus cerues*, DN1+RP; *Bacillus subtilis* plus rock phosphate, K1+RP; *Lysinibacillus sphaericus* plus rock phosphate, K3+RP; *Bacillus cerues* plus rock phosphate

The shoot and root weights showed a significant difference between the treatments except for treatment K1 and K1+RP, as seen in Tables 7 and 8. This may occur because, in the presence of rock phosphate as a phosphate source, the organism was unable to establish a rhizospheric interaction with the roots of the sorghum plant. The results of Mattos *et al.* (2020) which evaluated the effect of PSB inoculation together with RP on two sorghum genotypes cultivated in the greenhouse significantly enhanced root biomass and P content and believed that the inoculation response was dependent on sorghum genotype. The highest shoot dry weight (1.04 g/pot) and root dry weight (0.40 g/pot) were determined in K3 treatments, also presented in Tables 7 and 8. This result confirmed that the PSB application might effectively increase the root weight, as reported by Bashan *et al.* (2004). Similar findings were also observed by Puente *et al.* (2004), who recorded that the inoculation of cactus seedlings with *Bacillus pumilus* and *Bacillus subtilis* individually changed several plant growth parameters.

The positive effect of the PSB treatment on sorghum plants after 28 days of plantation is shown in Plate 2. A significant difference at p=0.01 is observed in DN1, K3, DN1+RP and K3+RP treatments. This is confirmed by our result for the effects of the treatments described in this research.



Plate 2: Effect of the inoculum on sorghum plants after 28 days

Table 7: Effect of P-solubilising *bacillus* on the shoot fresh and dry weights of experimental sorghum plants after 28 days

Treatment	Shoot weight (g)	
	Fresh	Dry
Control	3.16 ± 0.26 ^{a,b,*}	0.55 ± 0.10 ^a
DN1	5.98 ± 0.72 ^{d,*}	1.02 ± 0.07 ^c
K1	2.72 ± 0.19 ^{a,*}	0.48 ± 0.06 ^a
K3	6.29 ± 0.02 ^{d,*}	1.04 ± 0.06 ^c
RP+DN1	5.87 ± 0.43 ^{d,*}	0.88 ± 0.01 ^{b,c}
RP+K1	4.12 ± 1.68 ^{b,c,*}	0.69 ± 0.27 ^{a,b}
RP+K3	4.73 ± 0.63 ^{c,d,*}	0.82 ± 0.13 ^{b,c}

Values are expressed as mean ± standard deviation of triplicate determination; Values with different superscripts (lower case) in the same column are significantly different at P=0.01 between treatments; Values with superscripts (*) in the same row are significantly different at P=0.01 within treatment

DN1; *Bacillus subtilis*, K1; *Lysinibacillus sphaericus*, K3; *Bacillus cerues*, DN1+RP; *Bacillus subtilis* plus rock phosphate, K1+RP; *Lysinibacillus sphaericus* plus rock phosphate, K3+RP; *Bacillus cerues* plus rock phosphate

Table 8: Effect of P-solubilising *bacillus* on the root fresh and root dry weight of sorghum plants after 28 days

Treatment	Root fresh weight (g)	Root dry weight (g)
Control	0.82±0.29 ^{a,*}	0.20±0.07 ^{a,b,c}
DN	1.25±0.02 ^{b,c,*}	0.31±0.00 ^{c,d}
K1	0.67±0.00 ^{a,*}	0.11±0.01 ^a
K3	1.59±0.19 ^{c,*}	0.40±0.08 ^d
RP+DN1	1.63±0.48 ^{c,*}	0.33±0.10 ^d
RP+K1	0.92±0.19 ^{a,b,*}	0.19±0.08 ^{a,b}
RP+K3	1.48±0.05 ^{c,*}	0.30±0.00 ^{b,c,d}

Values are expressed as mean ± standard deviation of triplicate determination; Values with different superscripts in the same column are significantly different at P=0.01 between treatments; Values with superscripts (*) in the same row are significantly different at P=0.01 within treatment.

DN1; *Bacillus subtilis*, K1; *Lysinibacillus sphaericus*, K3; *Bacillus cerues*, DN1+RP; *Bacillus subtilis* plus rock phosphate, K1+RP; *Lysinibacillus sphaericus* plus rock phosphate, K3+RP; *Bacillus cerues* plus rock phosphate

The treatments, whether applied alone or in combination, had a notable impact on the soil's pH levels. pH increased from 6.74 to 7.04, 7.05, and 7.09 for PSB DN1, K1 and K3, respectively, as shown in Figure 3. This finding agrees with the finding of Liu *et al.* (2021), in which pH increased from 4.53 to 6.03 and 4.47 to 6.12 for *Bacillus thuringiensis* and *Pantoea ananatis*. The low pH observed in this study suggests acidification of the medium promotes inorganic phosphate solubilization (Yadav *et al.*, 2017). One key process that helps with the solubilization of P from mineral phosphates through acidification and metal chelation is the release of organic acids by PSB. However, as noted by Nobahar *et al.* (2017), their secretion is typically insufficient to cause soil acidification.

The soil that has been inoculated with PSB should have a higher pH due to changes in the soil microbial community (Yuan *et al.*, 2017; Teng *et al.*, 2021) and a higher amount of bicarbonate hydroxide HCO_3^- (OH) discharged by the root than H^+ because anions (PO_4^{3-}) are more easily absorbed than cations (Rasool *et al.*, 2021). The plantation-grade sandy loam soil's other physiochemical characteristics either rose or declined. As presented in Table 9, the phosphorus concentration was not significantly impacted and instead was constant across all treatments. Matsumura and Diamon (2018) discovered that the PSB inoculation greatly enhanced the amount of nitrogen and phosphorus that was accessible in the soil and successfully encouraged the growth of willow seedlings. This was not the case in our studies as both the nitrogen and phosphorus content in our soil before and after plantation showed little or no effect which might be due to several environmental and soil-related factors.

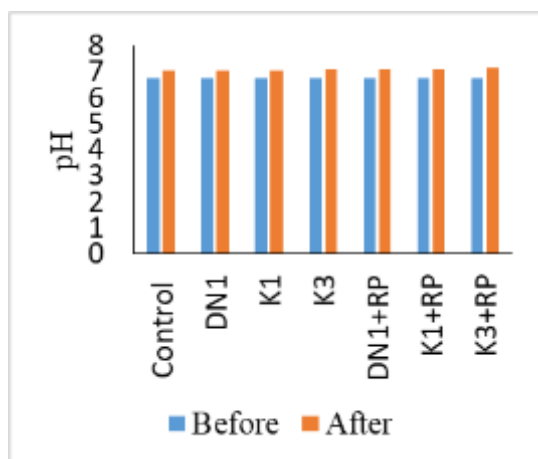


Figure 3: pH of the soil (sandy loam) used before and after plantation in a pot experiment after 28 days

CONCLUSION

This study highlights the significant role of phosphate-solubilizing bacteria (PSB) in promoting the growth of sorghum by enhancing phosphorus availability. The results demonstrate that inoculation with PSB leads to improved plant height, shoot length, root length, and plant biomass, underscoring the potential of these bacteria as biofertilizers in sustainable agriculture. Incorporating PSB into farming practices can reduce reliance on chemical fertilizers, improve soil health, and contribute to environmentally friendly crop production.

RECOMMENDATIONS

Further research is recommended to explore the long-term effects of PSB application in different soil types and environmental conditions to optimize their use in agricultural systems.

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Table 9: Physiochemical parameters of the soil (sandy loam) used before and after plantation of sorghum plants over a period of 28 days.

Treatments	Contro l	DN1		K1		K3		DN1+RP		K1+RP		K3+RP		
PO ₄ mg/kg	0.42	0.41	0.42	0.43	0.42	0.43	0.42	0.43	0.42	0.46	0.42	0.36	0.42	0.43
EC ds/cm	11.34	3.22	11.34	4.91	11.34	8.28	11.34	6.89	11.34	7.22	11.34	4.88	11.34	5.06
N cmol/kg	0.07	0.09	0.07	0.22	0.07	0.09	0.07	0.09	0.07	0.26	0.07	0.09	0.07	0.22
K cmol/kg	0.28	0.05	0.28	0.05	0.28	0.03	0.28	0.05	0.28	0.05	0.28	0.03	0.28	0.03
Ca cmol/kg	0.9	0.9	0.9	1.2	0.9	1.0	0.9	1.0	0.9	1.1	0.9	1.0	0.9	1.0
Mg cmol/kg	2.35	0.8	2.35	0.9	2.35	1.4	2.35	1.4	2.35	1.0	2.35	1.0	2.35	1.5
CEC cmol/kg	4.2	7.8	4.2	10.8	4.2	9.4	4.2	11.0	4.2	10.0	4.2	6.0	4.2	10.0

- PO₄ (mg/kg): This represents the concentration of phosphate (PO₄) in the soil, measured in milligrams per kilogram. It indicates the availability of phosphorus to plants.
- EC (dS/cm): Electrical Conductivity (EC) is measured in deciSiemens per centimeter and reflects the soil's salinity level, which affects plant growth.
- N (cmol/kg): This measures nitrogen (usually in the form of ammonium or nitrate) in centimoles per kilogram of soil, an essential nutrient for plant growth.
- K (cmol/kg): Potassium content in the soil, measured in centimoles per kilogram, a critical macronutrient for plant development.
- Ca (cmol/kg): Calcium content in the soil, measured in centimoles per kilogram, vital for soil structure and plant health.
- Mg (cmol/kg): Magnesium content, also in centimoles per kilogram, necessary for photosynthesis and plant enzyme activation.
- CEC (cmol/kg): Cation Exchange Capacity, which measures the soil's ability to hold and exchange positively charged ions (cations). It is crucial for soil fertility and nutrient availability.

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