UJMR, Vol. 9 No. 1, June, 2024, pp. 123 - 133 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.2491.014

Received: 11 January 2024 **Accepted:** 25 June 2024

Biofertilizer Production using Phosphate-solubilizing *Pseudomonas* specie Isolated from Rhizosphere Soil: Towards Indigenous Biofertilizer for Enhanced Crop Productivity in Katsina, Nigeria

Baha'uddeen Salisu and Sani Isiya
Department of Microbiology, Umaru Musa Yar'adua University Katsina, Nigeria
Correspondence email: bahauddeen.salisu@umyu.edu.ng

Abstract

The utilization of biofertilizers holds promise as a sustainable approach to enhance soil fertility and crop productivity while reducing reliance on chemical fertilizers. Beyond nitrogen, phosphorus is integral to various aspects of plant metabolism, including cell division, growth, development, sugar breakdown, and nuclear transport. The present study focused on isolating Pseudomonas spp. as phosphate-solubilizing bacteria from the rhizosphere soil to produce biofertilizer. Ten rhizosphere soil samples were collected from agricultural fields in Wagini ward, Batsari Local Government area, Katsina state. The isolation and identification of Pseudomonas species from the soil samples were conducted using standard microbiological techniques, followed by screening for plant growth-promoting traits (phosphate solubilization). Subsequently, selected Pseudomonas species exhibiting robust phosphate solubilization were assessed for their efficacy in biofertilizer production, after which the produced biofertilizer was tested on maize, beans, and millet cultivation. The findings highlighted the potential of indigenous Pseudomonas species from agricultural soil as effective biofertilizer agents. The formulated biofertilizers demonstrated remarkable positive effects on the tested crops' growth compared to those not treated with the Pseudomonas-based biofertilizer after seven days of cultivation under controlled conditions. This study underscores the importance of tacking native microbial resources to develop eco-friendly and cost-effective biofertilizers tailored to local agroecosystems, thereby contributing to Nigeria's sustainable agricultural intensification and food security.

Keywords: Pseudomonas, Biofertilizer, Phosphate-Solubilizing Bacteria, Rhizosphere Soil, Agroecosystems

Keywords: Poultry feeds, contamination, Proteus mirabilis, Escherichia coli, Susceptibility

INTRODUCTION

Chemical pesticides and fertilizers have been crucial for agricultural output since ancient times, but their detrimental effects on the environment, plants, animals, and human health have led to a focus on eco-friendly plant protection (Patel *et al.*, 2014). Biofertilizers, which consist of living microorganisms extracted from plant roots or soil (Aggani, 2013), are gaining popularity as replacements for chemical fertilizers. They reduce crop production costs, enhance growth and yields by increasing nitrogen availability, and promote the production of growth-promoting substances like auxins, cytokinins, and gibberellins (Bhattacharjee and Dey, 2014).

Biofertilizers containing beneficial microorganisms, rather than synthetic chemicals, improve plant growth by supplying essential nutrients while preserving environmental health and soil productivity (Singh *et al.*, 2011; Verma *et al.*, 2017). They also boost productivity per unit area, require less energy, reduce soil and water contamination, enhance soil fertility, and promote antagonism and biological control of plant pathogens (Sujanya and Chandra, 2011; Yasin *et al.*, 2012).

Agricultural productivity in Katsina, Nigeria, is fundamental to the region's economic sustenance. Enhancing crop yield and soil fertility through sustainable practices has become imperative. Exploring biofertilizers derived from indigenous microbial strains offers a promising avenue for sustainable agriculture. Among these microbes, *Pseudomonas* spp. has garnered attention for their potential in biofertilizer production due to their phosphate-solubilizing and plant growth-promoting capabilities (El-Ladan *et al.*, 2018).

The widespread use of chemical fertilizers poses a significant threat to the environment, contaminating air, water, and soil (Savci 2012).

As these harmful chemicals accumulate in groundwater, they contribute to water body eutrophication (Savci 2012). Soil is adversely affected, leading to reduced water retention, fertility depletion, increased salinity, and imbalances in nutrient levels (Savci 2012). In response to these detrimental effects, organic farming has emerged as a viable alternative, addressing the demand for healthy food, long-term sustainability, and environmental concerns (Reddy 2015). While chemical fertilizers are essential to meet global food demand, there's an opportunity for organic farming to flourish in selected crops and niche areas (Macilwain 2004). Widely used to accelerate microbial processes, biofertilizers improve soil fertility by fixing atmospheric nitrogen, solubilizing insoluble phosphates, and producing growth-promoting substances (Mazid and Khan 2015). Tacking the natural biological system, biofertilizers significantly increase soil fertility and, consequently, crop yield (Pandey and Singh 2012). This review underscores the potential of biofertilizers in agriculture, ecology, and remediation, positioning them as a promising tool for sustainable agricultural development.

Phosphate-solubilizing bacteria, known as plant growth-promoting rhizobacteria (PGPR), can enhance plant P availability and reduce reliance on expensive chemical fertilizers (Lugtenberg and Kamilova, 2009; Oliveira *et al*., 2009; Singh *et al*., 2011). Bacteria from genera such as *Pseudomonas, Bacillus, Rhizobium, Agrobacterium, Burkholderia, Achromobacter, Micrococcus, Aerobacter, Enterobacter, Flavobacterium, Paenibacillus, Rahnella, Escherichia, and Erwinia* have been isolated from soils (Rodríguez and Fraga, 1999). *Pseudomonas* isolates exhibit additional plant probiotic traits such as antagonism to fungal pathogens, systemic resistance, biodegradation of pollutants, and biocontrol and biofertilization properties in agricultural fields (Haas and Défago, 2005; Picard and Bosco, 2008; Ramette *et al*., 2011; De Souza *et al*., 2003). Their excellent rhizosphere colonization abilities make them attractive for biofertilizer production, which is crucial for sustainable agriculture. *Pseudomonas* produce plant growth-promoting substances, supporting crop development, soil fertility, and reducing environmental impact (Browne *et al*., 2009). Previous studies have investigated the production of biofertilizers using *Pseudomonas* species from agricultural soil, focusing on isolation, characterization, growth conditions optimization, nutrient content analysis, and impact on plant growth and soil health (Kannaiyan *et al*., 2004; Mishra *et al*., 2013; Rajasekaran *et al*., 2012; Gomare *et al*., 2013). However, challenges such as maintaining consistent bacterial species, optimizing production conditions, ensuring viability during storage, and addressing contamination issues hinder regulatory approval and adoption.

This study aimed to delve into the isolation and screening of biofertilizer-producing *Pseudomonas* spp. from agricultural soil in Katsina. Through a systematic approach, this research endeavors to identify and characterize these microbial strains for their biofertilizer potential. The ultimate goal is to contribute to developing indigenous biofertilizers optimized for the region's agricultural needs, fostering sustainable and resilient farming practices in Katsina, Nigeria.

MATERIALS AND METHODS

Reagents and Media

Nutrient agar medium for slant preparation contained peptone (5gm), beef extract (3gm), NaCl (5gm), agar (18gm), and distilled water (1000ml). Pikovaskya agar media for *Bacillus* and *Pseudomonas* spp included glucose, yeast extract, ammonium sulfate $(NH_4)_2SO_4$, magnesium sulfate ($MgSO_4$), calcium phosphate $Ca_3(PO_4)_2$, sodium chloride (NaCl), potassium chloride (KCL), manganese sulfate ($MnSO_4$), ferrous sulfate ($FeSO_4$), agar, and distilled water. Pikovskaya broth for phosphate solubilizing bacteria (PSB) production comprised glucose ($C_6H_{12}O_6$), yeast extract, ammonium sulfate $(NH_4)_2SO_4$, magnesium sulfate ($MgSO_4$), calcium phosphate $Ca_3(PO_4)_2$, sodium chloride (NaCl), potassium chloride (KCl) manganese sulfate ($MnSO_4$), ferrous sulfate ($FeSO_4$), and distilled water. Biochemical tests involved methyl red, Voges Proskauer, indole, and catalase. Gram staining used methylene blue, Gram's iodine, and safranin, while additional reagents included Kovac's reagent, alpha-naphthol, and hydrogen sulfide.

Soil sample collection and processing

1kg of soil sample was collected (about 10cm long) from Wagini ward (western Wagini agricultural farmlands), Batsari local government area, Katsina State, Nigeria, and was put inside a sterile polyethylene bag and brought to the Microbiology laboratory of Umaru Musa Yar'adua University, Katsina, Katsina State, Nigeria.

Soil samples were subjected to serial dilution by initially extracting 1 gram of composite (mixture of soil samples) soil, which was then diluted with 10 ml of distilled water in a test tube to create a stock solution. Subsequent dilutions, ranging from 10^{-1} to 10^{-9}, were achieved by transferring 1 ml of the stock solution into 9 ml of sterilized distilled water using pipettes. Effective sterilization was carefully maintained throughout the experiment and was conducted within a laminar airflow environment.

Identification of bacterial colony and external morphology study

The bacterial colony identification and external morphology were investigated using the spread plate method. Nutrient agar medium was prepared, and 100 ml of it was autoclaved and poured into four sterilized Petri plates. Serial dilutions (10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}) were made, and 0.1 ml of culture from each dilution was spread on the Petri plates. The plates were then incubated anaerobically at 37°C for 24 hours to promote bacterial growth. After incubation, the Petri dishes were removed, and the external morphologies of the bacterial colonies were examined.

Distinct colonies on nutrient agar plates were identified, marked, and then individually transferred to six (total number of Petri dishes used for inoculation) Petri dishes of nutrient agar using an inoculating needle. These plates were labeled according to the chosen colonies from the Petri plates and placed in a 37°C incubator overnight. Following incubation, pure cultures of different bacterial species developed in the test tubes, and specific species were selected and purified.

Gram staining of the bacterial species from the pure culture

The distinct colonies from test tubes were cultured separately and subjected to gram staining in a laminar airflow hood. Each colony was marked, smeared, and heat-fixed using six slides previously washed with ethanol. Following the Christian Gram staining technique, staining involved applying methylene blue on each of four slides, then a 30seconds wait, followed by distilled water wash, followed by applying iodine (as a mordant) for 1 minute, followed by 95% alcohol wash, followed by distilled water rinse for 10seconds, followed by addition of safranin then 30seconds wait, then another distilled water wash, and finally, air drying (Collee *et al.*, 1996).

Bacterial strain screening for phosphate solubilization

Following the gram staining and microscopic examination of bacterial species obtained from pure culture plates, the species identified as phosphate-solubilizing bacteria, such as *Bacillus* spp. strain and *Pseudomonas* species strain based on morphological features were subsequently confirmed through their ability to thrive on Pikovskaya agar media, a crucial test for phosphate-solubilizing bacteria in both *Bacillus spp.* and *Pseudomonas* spp. Using an inoculating needle, bacterial colonies which turned out to be *Pseudomonas* were only extracted from the pure culture plates (since it is the target species) and inoculated onto a test tube containing 4ml sterile distilled water (until the sterile water turned milky in color) before inoculation on PVK agar media plates, then incubated at 37°C for 48hrs. A sterile syringe was used to inoculate the milky suspension of the *Pseudomonas* isolates onto four Pikovskaya (PVK) agar media plates. The first plate was done by extracting 1ml of the suspension and inoculating it onto the Pikovskaya (PVK) agar media plate as a suspension, and the same goes for all the remaining 3 plates. After 48 hours of incubation, distinct halozone formations were displayed, affirming their classification as phosphate-solubilizing bacteria. These colonies were cultivated in nutrient agar media for further investigation, and various biochemical tests were conducted.

Biofertilizer Production

Starter Cultures for Phosphate-Solubilizing Bacteria

Following the screening of phosphate solubilizing bacterial species, Pseudomonas spp. from pure culture plates were subsequently inoculated into a liquid broth. This broth served as the production medium and the starter culture for cell growth. The production medium is essential for increasing the viable bacterial cell count, as the bacteria specifically thrives and proliferates in this medium. Therefore, in the case of phosphate solubilization, only *Pseudomonas* spp. were cultivated in Pikovskaya production medium since it is the main aim and only target of the research.

Figure 1 below shows some types of production media of bacterial species. The media at the right is a cetrimide agar base (with glycerin added inside, which is a selective agent that inhibits the growth of most bacteria and results in the release of phosphorus and nitrogen from the bacterial cell wall other than *Pseudomonas aeruginosa*), which is mainly used for the cultivation of *Pseudomonas* species isolated from soil, pus, etcetera. Hence, it is a selective media. The medium in the middle is a Pikovskaya (PVK) agar media that detects phosphate solubilizes soil microorganisms. The media at the left is a nutrient agar media for cultivating non-fastidious microorganisms.

Figure 1: Media used for biofertilizer production

Preparation of carrier material for the biofertilizer production

4kg of black charcoal made at home was crushed into powder using a pestle and mortar. It was then sealed in a sterile polythene bag and brought into the Microbiology laboratory at Umaru Musa Yar'adua University, Katsina. After it was brought into the laboratory, the powdered charcoal was transferred into a sterile beaker for sterilization. The charcoal was sterilized following the standard sterilization method in Microbiology, 121°C for 15 minutes.

The procedure maintained sterility throughout and was conducted within a laminar airflow environment.

Inoculum preparation with carrier material (Mixing)

The *Pseudomonas* cell cultures were retrieved from storage. The cell cultures were combined with sterilized carrier material in separate beakers. The mixing of the carrier material and the production media was on a ratio of 2:1, which is equivalent to a 30:60 ratio, with 1 part of the production media mixed with 2 parts of the sterilized carrier material. The process was manually carried out and under aseptic conditions.

Biofertilizer storage and testing on crops

After mixing the sterilized carrier material and production media finally became a biofertilizer. The produced biofertilizer was then sealed in a sterile polyethylene bag and stored in a cool place for 19 hours before usage. After 19 hours, 2.5kg of the produced biofertilizer was then put in an 11cm-wide container with an open end, where sterile water was poured onto it until it finally became a bit thick suspension.

The effectiveness of the produced biofertilizer was tested in a laboratory for 7 days, where, on the first day, 9 seeds of maize were first inserted into the biofertilizer produced for 35 seconds and then removed. The seeds were then air-dried for 45 seconds before planting on plates. The same goes for beans. For millet, the concentration varies, as about 40 seeds were inserted into the biofertilizer produced for 35 seconds and then air dried for 45 seconds.

RESULTS

Cultural characteristics of the bacterial isolates from the various dilutions of the soil

Table 1 shows the characteristics of the bacterial species based on exterior strain analysis and color, as observed on Nutrient Agar media. The macroscopic characteristics in distinguishing the isolates include colony elevation, margin, shape, transparency, size, and color. A total of 24 isolates were obtained from the various dilutions (10^{-2} to 10^{-8}) of the composite soil sample. These isolates appeared opaque or translucent in three different shapes (circular, punctiform, and rhizoid) with three distinct elevation types (raised, flat, and punctiform), three different margin patterns (curly, undulate, and entire), and six different colors (brownish, greenish, offwhite, clear white, or shade).

In the 10^{-2} plate, 7 distinct colonies were found to have the same elevation, margin, shape, transparency, size, and color. In the 10^{-4} plate, 5 colonies were found to have the same elevation, margin, shape, transparency, size, and color, while 4 colonies were found to have the same elevation, margin, shape, transparency, size, and color. In the 10^{-6} plate, 2 colonies have been found to have the same elevation, margin, shape, transparency, size, and color, while 3 colonies were found to have the same elevation, margin, shape, transparency, size, and color as well. Talking about the 10^{-8} plate, 1 colony has been found to be raised in elevation, entire in margin, circular in shape, opaque in transparency, small in size, and greenish shade in color, while 2 colonies have been found to have the same elevation, margin, shape, transparency, size, and color as well.

Table 1: Exterior strain analysis and color of the isolates on nutrient agar

Plate	No. of Isolates	Elevation	Margin	Shape	Transparency	Size	Color
10^{-2}	7	Raised	Undulate	Punctiform	Opaque	Small	Brownish
10^{-4}	5	Flat	Entire	Circular	Translucent	Moderate	Greenish
	4	Punctiform	Entire	Circular	Opaque	Medium	Offwhite
10^{-6}	2	Flat	Undulate	Rhizoid	Opaque	Medium	Offwhite
	3	Raised	Curly	Punctiform	Opaque	Small	Clear White
10^{-8}	1	Raised	Entire	Circular	Opaque	Small	Greenish Shade
	2	Punctiform	Entire	Circular	Opaque	Medium	Offwhite

Microscopic and biochemical characteristics of the isolates

From the cultures shown in Table 1 above, seven different colony types were identified and subcultured in fresh nutrient agar (NA) for gram staining and biochemical characterisation. The typical appearance of the pure culture on NA is shown in Figure 2. On the other hand, the microscopic and biochemical characteristics of the isolates are summarized in Table 2. Of the 7 groups of the colonies obtained in nutrient agar plates, 5 colonies were found to retain the secondary dye (safranin) during Gram staining, which was later confirmed to be *Pseudomonas* (gram-negative bacteria) based on the biochemical characteristics. In contrast, the remaining 2 colonies did not retain the secondary dye (safranin) during the Gram staining, which was later confirmed to be *Bacillus* (gram-positive bacteria) based on the biochemical characteristics.

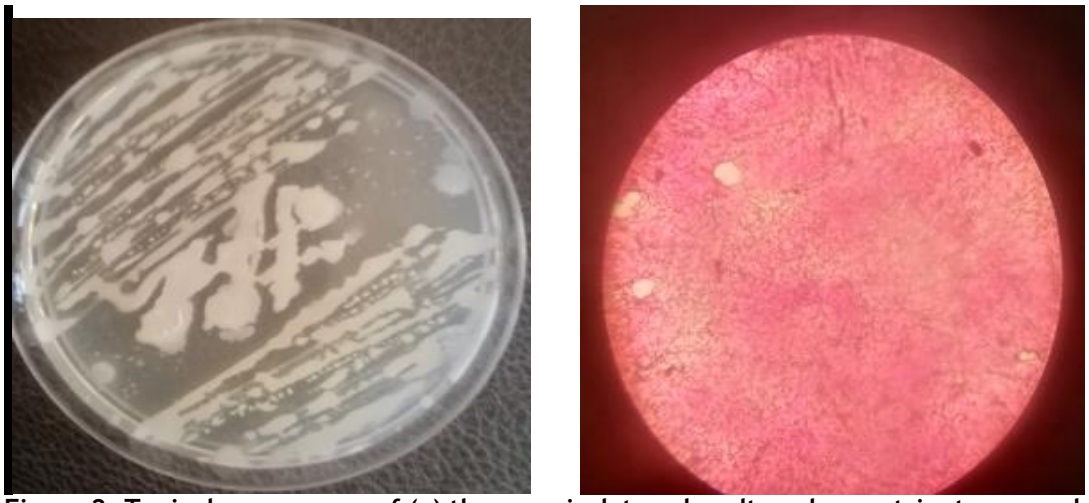


Figure 2: Typical appearance of (a) the pure isolate sub-cultured on nutrient agar and (b) Gram-stained *Pseudomonas* isolates.

Table 2: Microscopic and biochemical features of the isolates

Isolate number	Microscopic features	Biochemical characteristics				Identified bacteria
		I	MR	Cat	VP	
1	Gram-negative and rod-shaped	-ve	+ve	+ve	-ve	*Pseudomonas* species
2	Gram-negative, Onk-colored, rod-shaped	-ve	+ve	+ve	-ve	*Pseudomonas* species
3	Gram-positive, rod-shaped in chains	+ve	+ve	+ve	+ve	*Bacillus* species
4	Gram-negative, rod-shaped	-ve	+ve	+ve	-ve	*Pseudomonas* species
5	Gram-positive, rod-shaped, dispersed, and long sequence forming	+ve	+ve	+ve	+ve	*Bacillus* species
6	Gram-negative, rod-shaped	-ve	+ve	+ve	-ve	*Pseudomonas* species
7	Gram-negative, rod-shaped	-ve	+ve	+ve	-ve	*Pseudomonas* species

Key: -ve = negative, +ve = positive, I = Indole test, MR = Methyl red test, Cat = catalase test, VP = Voges-Proskauer test

Screening of the *Pseudomonas* isolates for phosphate solubilization

The pattern of halo zones in the Pikovskaya agar media plate produced by the *Pseudomonas* species when incubated at 37°C after 48 hours is shown in the Plate.

Figure 3: Pattern of halo zone produced by the *Pseudomonas* species on Pikovskaya agar media

Effect of *Pseudomonas*-based biofertilizer on the tested crops

Figure 4 below shows a bar chart representation of the effect of *Pseudomonas*-based biofertilizer on maize, beans, and millet at 7 days of growth under laboratory-controlled conditions. It depicts the growth of maize, beans, and millet in the laboratory for seven days.

Maize 1, bean 1, and millet 1 are the treatment groups mixed with the *Pseudomonas*-based biofertilizer, and maize 2, bean 2, and millet 2 are the negative controls that have not been mixed with the *Pseudomonas*-based biofertilizer.

On the first day of cultivation, there was no growth for both maize 1&2, and so was for beans 1&2 and millet 1&2. The same goes for the second cultivation day for the maize, beans, and millet.

Growth starts to show up on the third day of cultivation, i.e., day 3, where maize 1 was found to reach a height of up to 2cm and maize 2 reached a height of 1.71cm. Pertaining beans, beans 1 reached a height of 1.7cm, and beans 2 reached a height of 1.3cm. Talking about millet, millet 1 was found to reach a height of up to 2cm, and millet 2 reached a height of 1.86cm.

On the fourth day of cultivation, maize 1 was found to reach a height of up to 4.7cm, and maize 2 reached a height of 3.97cm. Pertaining beans, beans 1 reached a height of 2.2cm, and beans 2 reached a height of 2.0cm. Talking about millet, millet 1 was found to reach a height of up to 4.72, and millet 2 reached a height of 4.1cm.

On the fifth day of cultivation, maize 1 was found to reach a height of up to 6.1cm, and maize 2 reached a height of 5.4cm. Pertaining beans, beans 1 reached a height of 4.3cm, and beans 2 reached a height of 3.88cm. Talking about millet, millet 1 was found to reach a height of up to 6.38, and millet 2 reached a height of 6.0cm.

On the sixth day of cultivation, maize 1 was found to reach a height of up to 8.2cm, and maize 2 reached a height of 7.29cm. Pertaining beans, beans 1 reached a height of 6.7cm, and beans 2 reached a height of 6.5. Talking about millet, millet 1 was found to reach a height of up to 8.49, and millet 2 reached a height of 8.1cm.

On the seventh day of cultivation, maize 1 was found to reach a height of up to 11.7cm, and maize 2 reached a height of 10.4cm. Pertaining beans, beans 1 was found to reach a height of 8.89cm, and beans 2 reached a height of 7.9cm. Talking about millet, millet 1 was found to reach a height of up to 12.43, and millet 2 reached a height of 11.3cm.

Based on the above raw data of the cultivated maize, beans, and millet given, it is good to recognize the effect of *Pseudomonas*-based biofertilizer in giving faster growth of height in maize, beans, and millet treated with the *Pseudomonas*-based biofertilizer against the maize, beans, and millet which were not treated with the *Pseudomonas*-based biofertilizer prior to planting. Below is the bar chart representing the effect of *Pseudomonas*-based biofertilizer among the tested crops: maize, beans, and millet grown at 7 days. In the bar chart presented, the length of the crops was plotted on the (Y-axis), measured in centimeters against the time taken for their growth measured in days, which was plotted on the (X-axis).

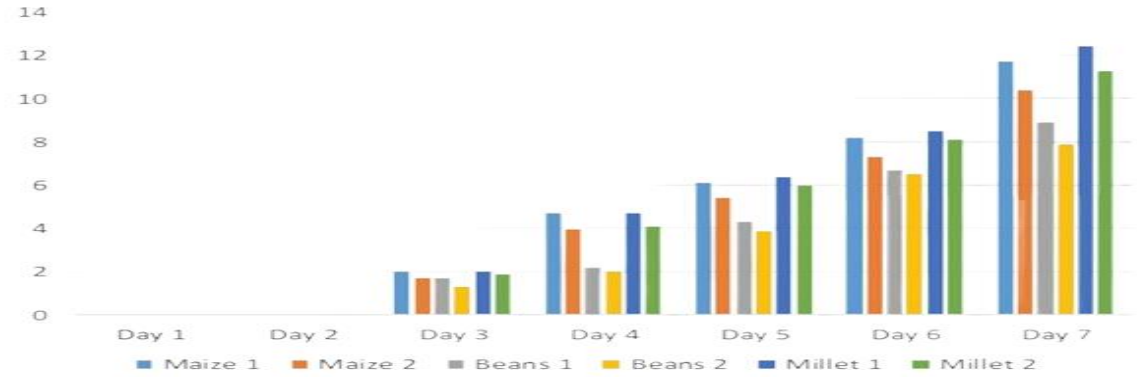


Figure 4: Effect of *Pseudomonas*-based biofertilizer on the growth of the tested crops
Key: Maize 1: Maize with biofertilizer Maize 2: Maize without biofertilizer Beans 1: Beans with biofertilizer Beans 2: Beans without biofertilizer Millet 1: Millet with biofertilizer Millet 2: Millet without biofertilizer

DISCUSSION

Biofertilizers can convert nutritionally vital elements from an unusable to a usable form; hence, they consist of live microorganisms. These microorganisms rely on organic matter for their growth and activity in the soil, supplying valuable nutrients to plants, where they come in the form of live formulations of beneficial microorganisms that, when applied to seeds, roots, or soil, enhance nutrient availability through biological activity, and this process contributes to the development of microflora, promoting overall soil health (Rajendra *et al.*, 1998). Phosphorus, a crucial macronutrient often found in an unavailable form in the soil, can be made soluble by certain soil bacteria, such as *Bacillus* spp. and *Pseudomonas* spp., and these phosphobacteria release organic acids that lower pH, leading to the dissolution of bound phosphorous forms, and when applied through seeds or soil, these bacteria enhance phosphorus uptake by crops, addressing its low mobility and solubility in soil, and these phosphate-solubilizing bacteria play a vital role in improving plantain phosphate uptake in various ways (Rajendra *et al.*, 1998; Rajasekaran *et al.*, 2012).

In soil, inorganic phosphorus exists as phosphate anions adhering to diverse soil particles (such as Fe and Al oxides, silicates, and Ca carbonates) or, based on pH levels, forms less soluble precipitates like Ca-P in neutral to alkaline soils and Fe-P, and Al-P in acidic soils (Richardson 2001). Tricalcium phosphate is commonly used to assess microbial P solubilization potential, but bacteria solubilizing phosphate from Ca3(PO4)2 may not dissolve more resistant compounds like Fe-P or Al-P (Collavino *et al.* 2010).

Survival, tolerance, competition with native rhizospheric microorganisms, and successful root colonization are essential for establishing inoculated bacteria in the rhizosphere. The inability of Plant Growth-Promoting Rhizobacteria (PGPR) to achieve desired effects in the field post-seed inoculation is frequently linked to their limited root-colonizing capability (Ahmad *et al.* 2011).

Analyzing root colonization in natural soil using bacteria isolates doubly labeled with a selective marker (tetracycline resistance) and the gap reporter gene reveals insights for efficient PGPR strain selection. Results indicate that many phosphate-mobilizing *Pseudomonas* isolates successfully established themselves in the rhizosphere of maize, wheat, and soybean roots, often surpassing colonization densities of 10^6 CFU g^{-1}. These values align with reported root colonization levels for pseudomonads in natural soil. For instance, P. putida strain GR7.4lux achieved an average of 4.8 log10 CFU g^{-1} on soybean roots (Beauchamp and Kloepper 2003). In maize roots, P. fluorescens ANP15 and P. aeruginosa 7NSK2 reached 6.7 and 6.8 log10CFU g−1, respectively (Devliegher *et al.* 1995). Notably, the root-colonizing potential of a single isolate like LF-MB1 is comparable to fluorescent pseudomonad counts detected in maize rhizospheres (5.4 to 5.8 log10CFU g−1) (Picard *et al.* 2000).

Bacterial plant growth promotion involves a complex phenomenon, achieved through various probiotic traits, such as antagonism against phytopathogenic fungi (Haas and Defago 2005). The microbial production of extracellular proteases and volatile cyanhidric acid contributes to the biocontrol of root pathogens (Ramette *et al.* 2003; Haas and Defago 2005; Hayat *et al.* 2010). Recent reports indicate that the inorganic phosphate solubilization potential of pseudomonads is often combined with the production of other metabolites participating in the biological control of soil-borne phytopathogens (Vassilev *et al.* 2006; Jha *et al.* 2009).

The effectiveness of a biofertilizer on maize, beans, and millet was tested by measuring the height of the plants with and without the biofertilizer over seven days. The results showed that the biofertilizer significantly enhanced the growth of all three crops, with the most notable improvement seen in maize, followed by millet and then beans.

In maize, the biofertilizer-treated were 3.93cm taller than the untreated after seven days, with a consistent height difference observed throughout the growth period. Similarly, in millet, the biofertilizer-treated were 2.56cm taller than the untreated after seven days, while in beans, the biofertilizer-treated were 2.21cm taller than the untreated after seven days.

These findings suggest that the produced biofertilizer effectively promotes plant growth and height, with the most significant impact seen in maize. This biofertilizer has the potential to improve crop yields and enhance agricultural productivity, making it a valuable tool for sustainable agriculture.

CONCLUSION

Using *Pseudomonas*-based biofertilizers in agricultural soil has demonstrated remarkable effectiveness across diverse crops such as maize, beans, and millet. The positive outcomes underscore the potential of tacking *Pseudomonas* for sustainable and enhanced crop productivity. As we navigate the ever-evolving landscape of agricultural practices, integrating biofertilizers into mainstream approaches holds promise for fostering eco-friendly, efficient, and resilient farming systems, paving the way for a more sustainable future in agriculture.

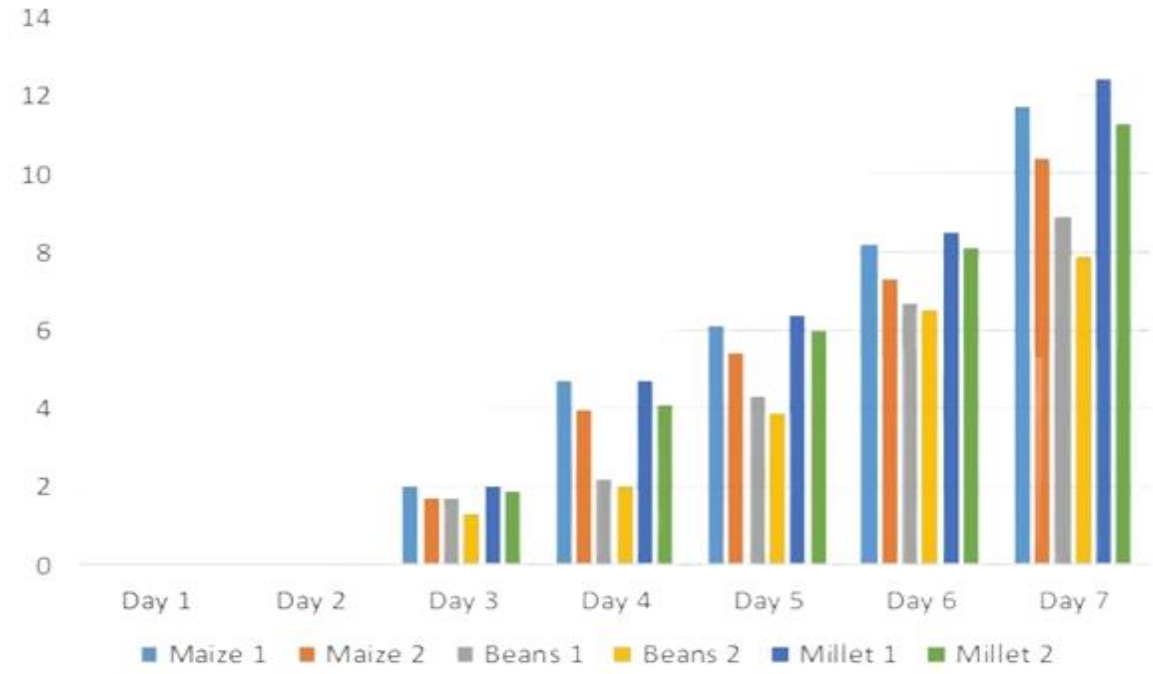
Based on the results obtained, significant height differences existed between the crops treated with the Pseudomonas-based biofertilizer over those not treated with the biofertilizer before planting.

For maize, on the day growth was first seen, the maize with the biofertilizer reached a height of 2cm, whereas the one without the biofertilizer reached a height of 1.71cm. It should be noted that the produced biofertilizer is effective as a height difference of 0.29 existed between the two maize. On the fourth day, the maize with the biofertilizer reached a height of 4.7cm, whereas the one without the biofertilizer reached a height of 3.97cm, obtaining a height difference of 0.73cm between the two maize. On the fifth day, the maize with the biofertilizer reached a height of 6.1cm, whereas the one without the biofertilizer reached a height of 5.4cm, giving a height difference of 0.7cm. On the sixth day, the maize with the biofertilizer reached a height of 8.2cm, whereas the one without the biofertilizer reached a height of 7.29cm, leaving a height difference of 0.91cm. On the seventh day, the maize with the biofertilizer reached a height of 11.7cm, whereas the one without the biofertilizer reached a height of 10.4cm, having a height difference of 1.3cm.

For beans, on the day growth was first seen, the beans with the biofertilizer were found to reach a height of 1.7cm, whereas the ones without the biofertilizer reached a height of 1.3cm. It should be noted that the produced biofertilizer is effective as a height difference of 0.4cm existed between the two bean samples. On the fourth day, the beans with the biofertilizer reached a height of 2.2cm, whereas the ones without the biofertilizer reached a height of 2cm, obtaining a height difference of 0.2cm between the two bean samples. On the fifth day, the beans with the biofertilizer reached a height of 4.3cm, whereas the ones without the biofertilizer reached a height of 3.88cm, giving a height difference of 0.42cm. On the sixth day, the beans with the biofertilizer reached a height of 6.7cm, whereas the ones without the biofertilizer reached a height of 6.5cm, leaving a height difference of 0.2cm. On the seventh day, the beans with the biofertilizer reached a height of 8.89cm, whereas the one without the biofertilizer reached a height of 7.9cm, having a height difference of 0.99cm.

For millet, on the day growth was first seen, the millet with the biofertilizer reached a height of 2cm, whereas the one without the biofertilizer reached a height of 1.86cm. It should be noted that the produced biofertilizer is effective, as a height difference of 0.14 existed between the two millet samples. On the fourth day, the millet with the biofertilizer reached a height of 4.72cm, whereas the one without the biofertilizer reached a height of 4.1cm, obtaining a height difference of 0.62 cm between the two maize. On the fifth day, the millet with the biofertilizer reached a height of 6.38cm, whereas the one without the biofertilizer reached a height of 6.0cm, giving a height difference of 0.38cm. On the sixth day, the millet with the biofertilizer reached a height of 8.49cm, whereas the one without the biofertilizer reached a height of 8.2cm, leaving a height difference of 0.29cm. On the seventh day, the millet with the biofertilizer reached a height of 12.43cm, whereas the one without the biofertilizer reached a height of 11.3cm, having a height difference of 1.13cm.

From the results obtained, it can be concluded that the produced biofertilizer is more effective on maize, where the maize treated with the biofertilizer prior to planting surfaced the one without the biofertilizer prior to planting with a remarkable height difference of 3.93cm in total after 7days growth in laboratory-controlled conditions. Followed by millet, where the millet with the biofertilizer before planting surfaced the one without the biofertilizer before planting with a remarkable height difference of 2.56cm in total after 7 days of growth in laboratory-controlled conditions. Then followed by beans, where the beans treated with the biofertilizer before planting surfaced the ones without the biofertilizer prior to planting with a height difference of 2.21cm in total after 7 days of growth in laboratory-controlled conditions.



Effect of *Pseudomonas*-based biofertilizer on the growth of the tested crops

Key: Maize 1: Maize with biofertilizer
Maize 2: Maize without biofertilizer
Beans 1: Beans with biofertilizer.
Beans 2: Beans without biofertilizer
Millet 1: Millet with biofertilizer
Millet 2: Millet without biofertilizer

REFERENCES

Aggani, S. L. (2013). Development of bio-fertilizers and its future perspective. *Scholars Academic Journal of Pharmacy*, 2(4), 327-332.

Ahmad, F., Husain, F. M., & Ahmad, I. (2011). Rhizosphere and root colonization by bacterial inoculants and their monitoring methods: a critical area in PGPR research. *Microbes and microbial technology: Agricultural and environmental applications*, 363-391. [Crossref]

Beauchamp CJ, Kloepper JW (2003) Spatial and temporal distribution of a bioluminescent-marked Pseudomonas putida on soybean root. Luminescence 18:346–351. [Crossref]

Bhattacharjee, R., & Dey, U. (2014). Biofertilizer, a way towards organic agriculture: A review. *African Journal of Microbiology Research*, *8*(24), 2332-2343. [Crossref]

Browne, P., Rice, O., Miller, S. H., Burke, J., Dowling, D. N., Morrissey, J. P., & O'Gara, F. (2009). Superior inorganic phosphate solubilization is linked to phylogeny within the Pseudomonas fluorescens complex. *Applied soil ecology*, *43*(1), 131-138. [Crossref]

Chang, C. H., & Yang, S. S. (2009). Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. *Bioresource Technology*, *100*(4), 1648-1658. [Crossref]

Collavino, M. M., Sansberro, P. A., Mroginski, L. A., & Aguilar, O. M. (2010). Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote Phaseolus vulgaris growth. *Biology and fertility of soils*, *46*, 727-738. [Crossref]

Collee, J. G., Fraser, A. G., Marmion, B. P., & Simmons, A. (Eds.). (1996). *Practical medical microbiology* (pp. xiv+-978).

De Souza, J. T., Mazzola, M., & Raaijmakers, J. M. (2003). Conservation of the response regulator gene gacA in Pseudomonas species. *Environmental Microbiology*, *5*(12), 1328-1340. [Crossref]

Devliegher, W., Arif, M., & Verstraete, W. (1995). Survival and plant growth promotion of detergent-adapted Pseudomonas fluorescens ANP15 and Pseudomonas aeruginosa 7NSK2. *Applied and environmental microbiology*, *61*(11), 3865-3871. [Crossref]

El-ladan, Ibrahim & Saminu D, Umar. (2018). Katsina Region: Agricultural Production Systems.

Gomare, K. S., Mese, M., & Shetkar, Y. (2013). Isolation of Azotobacter and cost effective production of biofertilizer. *Indian J. Appl. Res*, *3*(5), 54-56. [Crossref]

Haas, D., & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature reviews microbiology*, *3*(4), 307-319. [Crossref]

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of microbiology*, *60*, 579-598. [Crossref]

Jha, B. K., Gandhi Pragash, M., Cletus, J., Raman, G., & Sakthivel, N. (2009). Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent pseudomonad strains, Pseudomonas aeruginosa, P. plecoglossicida and P. mosselii. *World Journal of Microbiology and Biotechnology*, *25*, 573-581. [Crossref]

Kannaiyan, S., Kumar, K., & Govindarajan, K. (2004). Biofertilizers technology for rice based cropping. *Jodhpur, Scientific. Xviii*.

Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual review of microbiology*, *63*, 541-556. [Crossref]

Macilwain, C. (2004). Organic: is it the future of farming?. *Nature*, *428*(6985), 792-794. [Crossref]

Mazid, M., & Khan, T. A. (2015). Future of bio-fertilizers in Indian agriculture: an overview. *International Journal of Agricultural and Food Research*, *3*(3). [Crossref]

Mishra, D., Rajvir, S., Mishra, U., & Kumar, S. S. (2013). Role of bio-fertilizer in organic agriculture: a review. *Research Journal of Recent Sciences ISSN*, *2277*, 2502.

Naik, P. R., Raman, G., Narayanan, K. B., & Sakthivel, N. (2008). Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. *BMC microbiology*, *8*, 1-14. [Crossref]

Pandey, J., & Singh, A. (2012). Opportunities and constraints in organic farming: an Indian perspective. *Journal of Scientific Research*, *56*(1), 47-72.

Patel, N., Patel, Y., & Mankad, A. (2014). Bio fertilizer: A promising tool for sustainable farming. *Int J Innovative Res Sci Engg Technol*, *3*(9), 56-69.

Picard, C., & Bosco, M. (2008). Genotypic and phenotypic diversity in populations of plant-probiotic Pseudomonas spp. colonizing roots. *Naturwissenschaften*, *95*, 1-16. [Crossref]

Picard, C., Di Cello, F., Ventura, M., Fani, R., & Guckert, A. (2000). Frequency and biodiversity of 2, 4-diacetylphloroglucinol-producing bacteria isolated from the maize rhizosphere at different stages of plant growth. *Applied and Environmental Microbiology*, *66*(3), 948-955. [Crossref]

Rajasekaran, S., Ganesh Shankar, K., Jayakumar, K., Rajesh, M., Bhaaskaran, C., & Sundaramoorthy, P. (2012). Biofertilizers current status of Indian agriculture. *J. Environ. Bioenergy*, *4*(3), 176.

Rajendra Prasad, R. P., Surendra Singh, S. S., & Sharma, S. N. (1998). Interrelationships of fertiliser use and other agricultural inputs for higher crop yields.

Ramette, A., Frapolli, M., Défago, G., & Moënne-Loccoz, Y. (2003). Phylogeny of HCN synthase-encoding hcnBC genes in biocontrol fluorescent pseudomonads and its relationship with host plant species and HCN synthesis ability. *Molecular Plant-Microbe Interactions*, *16*(6), 525-535. [Crossref]

Ramette, A., Frapolli, M., Fischer-Le Saux, M., Gruffaz, C., Meyer, J. M., Défago, G., ... & Moënne-Loccoz, Y. (2011). Pseudomonas protegens sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2, 4-diacetylphloroglucinol and pyoluteorin. *Systematic and applied microbiology*, *34*(3), 180-188. [Crossref]

Reddy, B. S. (2015). "Soil Health: Issues and Concerns - A Review," Working Papers id:7599, eSocialSciences.

https://ideas.repec.org/p/ess/wpaper/id7599.html

Richardson, A. E., & Simpson, R. J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant physiology*, *156*(3), 989-996. **[Crossref]**

Rodríguez, H., & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology advances*, *17*(4-5), 319-339. **[Crossref]**

Savci, S. (2012). An agricultural pollutant: chemical fertilizer. *International Journal of Environmental Science and Development*, *3*(1), 73. **[Crossref]**

Singh, J. S., Pandey, V. C., & Singh, D. P. (2011). Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agriculture, ecosystems & environment*, *140*(3-4), 339-353. **[Crossref]**

Sujanya, S., & Chandra, S. (2011). Effect of part replacement of chemical fertilizers with organic and bio-organic agents in ground nut, Arachis hypogea. *Journal of Algal Biomass Utilization*, *2*(4), 38-41.

Vassilev, N., Vassileva, M., & Nikolaeva, I. (2006). Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. *Applied microbiology and biotechnology*, *71*(2), 137-144. **[Crossref]**

Verma, S., Singh, A., Pradhan, S. S., Singh, R. K., & Singh, J. P. (2017). Bio-efficacy of organic formulations on crop production-a review. *International Journal of Current Microbiology and Applied Sciences*, *6*(5), 648-665. **[Crossref]**

Yasin, M., Ahmad, K., Mussarat, W., & Tanveer, A. (2012). Bio-fertilizers, substitution of synthetic fertilizers in cereals for leveraging agriculture. *Crop Environ*, *3*(1-2), 62-66.