

SYNTHESIS OF LIQUID BASED BIOFERTILIZER AND ITS IMPACT ON GROWTH PARAMETERS OF *ABELMOSCHUS ESCULENTUS* L.

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Abstract

Phosphate biofertilizers are one of the effective methods for the improvement of agriculture and food security in the developing countries. Thus, the main objective of the current research was the isolation, identification of phosphate solubilizing bacteria, optimization of culture conditions, formulation of the liquid biofertilizer, and evaluation of its impact on the growth of *Abelmoschus esculentus* L. Soil samples collected from Charsadda district of Pakistan have been studied. *Bacillus subtilis* was isolated and identified. The isolates displayed holozonal activity on Pikovskaya agar media. The most efficient of them was *B. subtilis* 2 (PSI 128.57%). Highest growth of *B. subtilis* was observed at 35°C and pH 7. Biofertilizer had a significant impact on *Abelmoschus esculentus* seed germination. The germination percentage in 30% biofertilizer showed the highest root length, shoot length, fresh and dry weight. Urea fertilizer was less effective than biofertilizer. Liquid biofertilizers containing higher concentrations of *B. subtilis* greatly improve plant growth and soil phosphate availability.

Introduction

Abelmoschus esculentus (L.) Moench is a member of Malvaceae, known as lady's finger and okra contain nutrients, vitamins and minerals. It is important due to its antioxidant property and also for diabetic treatment (Kwok *et al.* 2025).

Biofertilizer is one of the most promising environmentally friendly alternatives to chemical fertilizer. Phosphate solubilizing bacteria (PSB) are particularly powerful among these microbes because they can transform insoluble inorganic phosphate into a soluble form that plants can use (Enriquer-Leon *et al.* 2025). *Bacillus* and *Pseudomonas* produce phosphatases and organic acids, which lower the soil pH and allow plants to receive phosphorus that is bound to calcium, iron, or aluminum compounds (Puri *et al.* 2020). The PSB inoculation increase soil phosphorus availability up to 40%, improving crop growth and yield. The PSB-mediated biofertilizers reduces the requirement for chemical phosphorus fertilizers, which can lower environmental pollution (Amy *et al.* 2022). Liquid biofertilizers have greater microbial populations, a longer shelf life, and are easy to apply to fields (Allouzi *et al.* 2022). Recent research suggests that the combined application of bio-fertilizers along with decreased amounts of inorganic fertilizers can not only sustain but also increase agricultural productivity without causing any negative effects on the environment (Kumar *et al.* 2024).

The present study was undertaken to isolate and identify phosphate solubilizing bacteria from the soil samples of Charsadda, to formulate biofertilizers and to evaluate their effects on the growth parameters of *A. esculentus*.

Materials and Methods

Soil samples were collected from three different places of Khyber Pakhtunkhwa. A stock solution (10^{-1}) and then serial dilutions of 10^{-1} to 10^{-9} were made to isolate bacteria. The morphological and biochemical analysis i.e., catalase, indole, citrate, oxidase, coagulase, and carbohydrate fermentation was done to identify the bacteria following the procedures described by Ohabughiro *et al.* (2024). Gram staining of isolated bacteria was carried out using the conventional Christian Gram method, and phosphate solubilization was carried out on Pikovskaya (PVK) agar medium (Roychowdhury *et al.* 2015).

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For qualitative phosphate solubilization, each isolate was evaluated using Pikovskaya agar medium. Streaking the bacterial cultures on PVK plates, the plates were then incubated for 7 days at 28 ± 2 °C. The phosphate solubilization was indicated by the appearance of a clear halo zone around the colonies (Roychowdhury *et al.* 2015).

The PSB isolates were added in PVK broth and incubated for 48 hrs at 30, 35, and 40°C. Phosphate solubilization was measured through optical density (Sagervanshi *et al.* 2012). Liquid formulations of PSB contained Tween 80 (2%), molasses (1%), and starch, gum arabic, and carboxymethyl cellulose (CMC) (0.5%); and polyvinylpyrrolidone (PVP), glycerol, and dimethyl sulfoxide (DMSO) (Kumari *et al.* 2023).

The germination of *Abelmoschus esculentus* seeds under four treatments i.e., control (0%), biofertilizer (10, 20, and 30%), and urea (10, 20, and 30%), was studied using a Completely Randomized Design (CRD). In the pot experiment seven treatments (control, 10, 20, 30 urea, and 10, 20, 30% biofertilizer) were replicated three times applying CRD. Plant height, root length, leaf area, and fresh/dry biomass were measured after 14 days. ANOVA was used to analyze the data in accordance with Embrandiri *et al.* (2013).

Results and Discussion

Soil samples have a pH range from 6.7 to 6.9 and moderate electrical conductivity and contains organic matter, sand, clay, and silt made up the sandy loam soil texture (Table 1) Yadav *et al.* (2013) highlighted such soil characteristics as ideal for PSB growth. Microbial density was decreased by serially diluting soil samples (10^{-1} to 10^{-9}), resulting in well-isolated colonies on nutrient agar medium. Distinct colonies exhibited diverse morphologies - circular, punctiform, raised, and flat varying in color from yellowish to greenish and off-white. The results confirm that *Bacillus subtilis* are dominant phosphate solubilizers in agricultural soils, verifying findings by Roychowdhury *et al.* (2015). *Bacillus subtilis* showed creamy white, circular, smooth, and shiny colonies with rod-shaped, motile, and Gram-positive cells. Biochemical tests confirmed positive catalase, citrate utilization, carbohydrate fermentation, and negative indole, coagulase, and oxidase tests was consistent with the findings of Giresha *et al.* (2024).

Table 1. Physical and chemical characteristics of soil samples.

Sample No.	Soil pH	Organic matter (%)	Electrical conductivity (mS/cm)	Sand (%)	Clay (%)	Silt (%)
1	6.8	4.38	327	58.20	11.65	29.30
2	6.9	4.41	334	59.10	11.87	30.01
3	6.7	4.21	312	58.03	11.44	28.90

Bacillus subtilis capacity to solubilize phosphate was verified on Pikovskaya agar medium by the formation of a transparent halo zone. *Bacillus subtilis* isolate 2 had the highest efficiency (128.57%), followed by isolates 5 and 4, respectively (Table 2). The Phosphate Solubilization Index (PSI) ranged from 88.89 to 128.57% which is consistency with findings of Roychowdhury *et al.* (2015), who linked halo zone size to solubilization activity. Ouattara *et al.* 2019 observed similar phosphate solubilization efficiency (20-200%) among cocoa rhizosphere isolates. Bacterial growth optical density is 1.6 and solubilization peaked at 35°C, which also had the maximum phosphate solubilization efficiency. The OD dropped to 0.8 and 1.1 at 30 and 40°C, respectively, suggesting reasonable efficiency at less than ideal temperatures. Sagervanshi *et al.* (2012)

demonstrate that *B. subtilis* is mesophilic, with 35°C being the ideal temperature for enzymatic phosphate release (Table 3). Reduced efficiency was noted at pH 5 (OD = 0.7) and pH 6 (OD = 0.9); however maximum phosphate solubilization happened at pH 7 (OD₆₁₀ = 1.4). The results of Mengesha and Legesse (2024), who showed that PSB growth was best at pH 6-7, are supported by the fact that neutral pH offered the ideal conditions for bacterial metabolism and enzymatic activity.

Table 2. Halozone diameter and phosphate solubilization index(PSI) of *Bacillus subtilis*.

Isolate No.	Colony dia. (mm)	Halo zone dia. (cm)	PSI (%)
1	0.9	1.9	111.11
2	0.7	1.6	128.57
3	0.9	1.7	88.89
4	0.8	1.7	112.50
5	0.7	1.5	114.29

Table 3. Effect of temperature and pH on efficiency of phosphate solubilization.

Parameter	Level	Optical density (OD) at 610 nm	Growth	Efficiency of solubilization
Temperature (°C)	30	0.8	Moderate	Moderate
	35	1.6	Excellent	Very high
	40	1.1	Good	High
pH Level	5	0.7	Moderate	Moderate
	6	0.9	Good	High
	7	1.4	Excellent	Very high

In one week, the *B. subtilis* strains were successfully grown on a mass scale in nutrient broth with aeration. This mixture was then combined with the sterilized carrier materials (molasses, gum arabic, and carboxymethyl cellulose), at a ratio of 2:1 carrier to media. The formulations that showed greater stability contained glycerol, DMSO, and PVP, whereas molasses proved to be an efficient carbon carrier material. As suggested by Jaiswal *et al.* (2023), bacterial stability increased with the use of glycerol as a stabilizing agent, and 4°C preservation extended microbial viability beyond 16 months.

Table 4 shows the percentage of germination of seeds of *A. esculentus* on the petri dish experiment. One-way ANOVA, which showed that the germination percentage data were statistically different at $p < 0.001$. Control treatment had the lowest average germination percentage ($36.67 \pm 5.77\%$). The highest germination rate was $83.33 \pm 5.77\%$ at 30% concentration followed by 20 and 10%. The urea fertilizer treatment showed the maximum germination at 30% concentration, which was followed by 20 and 10%, respectively. The biofertilizer at 30% concentration was substantially higher than the control and urea fertilizer concentrations. Bakonyi *et al.* (2013) demonstrated that biofertilizers significantly enhance seed germination in petri dish experiments in maize (37%) and okra (70-83%), respectively.

In terms of *A. esculentus* growth metrics and biomass output, biofertilizers performed noticeably better after 14 days. Biofertilizer was 30% higher than that of urea and control fertilizer ($p < 0.001$), as shown in Table 5. With root length, shoot length, and fresh biomass measurements, respectively, the control treatment had the lowest values for all growth metrics. Compared to the urea and control treatments, the application of biofertilizer greatly increased seedling growth with a highly significant effect ($p < 0.001$). As the concentration of biofertilizer increased, growth parameters gradually increased. The root and shoot lengths at the maximum urea concentration (30%) were 11.00 ± 1.00 and 11.33 ± 0.58 cm, respectively.

Table 4. Effect of fertilizer treatments on germination percentage of *Abelmoschus esculentus* seeds.

Treatment	Concentration (%)	% Germination (R1, R2, R3)	Mean \pm SD (%)
Control	0	40, 30, 40	36.67 ± 5.77
Biofertilizer	10	70, 80, 60	70.00 ± 10.00
	20	70, 80, 80	76.67 ± 5.77
	30	80, 90, 80	83.33 ± 5.77
Urea fertilizer	10	60, 60, 50	56.67 ± 5.77
	20	60, 70, 60	63.33 ± 5.77
	30	70, 80, 70	73.33 ± 5.77

Table 5. Effect of biofertilizer and urea on vegetative growth of *Abelmoschus esculentus* after 14 days.

Treatment	Conc. (%)	Root length (cm)	Shoot length (cm)	Leaf length (cm)	Fresh biomass (g)	Dry biomass (g)	Significance (ANOVA)
Control	0	7.33 ± 0.58	8.00 ± 1.00	1.03 ± 0.06	1.07 ± 0.06	0.23 ± 0.06	-
Biofertilizer	10	12.00 ± 1.00	12.33 ± 0.58	1.27 ± 0.06	1.50 ± 0.10	0.30 ± 0.10	$p < 0.05$
	20	15.50 ± 0.50	13.50 ± 0.50	1.40 ± 0.10	1.97 ± 0.06	0.40 ± 0.10	$p < 0.01$
	30	16.00 ± 1.00	16.00 ± 1.00	1.80 ± 0.10	2.60 ± 0.10	0.63 ± 0.06	$p < 0.001$
Urea	10	10.17 ± 1.04	9.33 ± 0.58	1.10 ± 0.10	1.23 ± 0.06	0.37 ± 0.06	$p < 0.05$
	20	10.00 ± 1.00	10.50 ± 0.50	1.17 ± 0.06	1.30 ± 0.10	0.33 ± 0.06	$p < 0.05$
	30	11.00 ± 1.00	11.33 ± 0.58	1.23 ± 0.06	1.40 ± 0.10	0.43 ± 0.12	$p < 0.01$

Bacillus subtilis obtained from soil, which possess the capability to dissolve large amounts of phosphates with good growth at optimum temperature and pH level. The prepared liquid biofertilizer remained viable for a long period of time without degradation, and showed enhanced results on seed germination, seedling growth, and biomass production in *A. esculentus* in comparison with chemical fertilizer. Thus, this study clearly reflects that the prepared biofertilizer is an effective alternative to chemical fertilizers for improving crop productivity.

The comprehensive analysis confirms that *B. subtilis*, an effective phosphate solubilizer that succeeds at optimal pH (7) and temperature (35°C), was isolated and identified. The developed liquid biofertilizer formulation showed high shelf stability, effective phosphate solubilization, and a significant improvement in *A. esculentus* seed germination and growth. The study validates the potential of PSB-based biofertilizers as ecofriendly alternatives to chemical fertilizers for sustainable crop production.

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