

Original Article

# Synergistic Impact of Phosphorus-Solubilizing Bacteria and Poultry Manure on Soil Fertility and Sugarbeet Yield in a Changing Climate

<sup>1</sup>MUHAMMAD WAQAS, <sup>2</sup>ADEEBA AMJAD, <sup>3</sup>SANAULLAHC

<sup>1</sup>Department of Soil and Environmental Sciences, MNS University of Agriculture, Multan Pakistan.

<sup>2</sup>Department of Botany, University of Agriculture, Faisalabad, Pakistan.

<sup>3</sup>Department of Entomology, MNS University of Agriculture, Multan, Pakistan.

**ABSTRACT:** Sustainable agricultural productivity is under threat due to a lack of interest in the use of bio-organic fertilizers to alleviate the drawbacks of conventional agricultural practices. Phosphorus solubilizing bacteria play a vital role in improving phosphorus availability to plants through the slow release from inorganic and organic soil phosphorus budgets by solubilization and mineralization. Furthermore, phosphorus solubilizing bacteria in combination with poultry manure have great potential to improve phosphorus uptake, growth and development of the plants. Firstly, a pot experiment consisted of seven strains of Phosphorus Solubilizing Bacteria (PSB) with one control (un-inoculated) using a Completely Randomized Design (CRD) for a 55-day screening trial to screen out the efficient phosphorus solubilizing bacterial strains. Secondly, a field experiment was performed in a Randomized Complete Block Design (RCBD) at the Research Farm of College of Agriculture, Bahauddin Zakariya University, Bahadur Sub Campus Layyah, South Punjab, Pakistan, during 2019-2020 to explore the effect of screened efficient phosphorus solubilizing bacterial strains; PSB0=No inoculation; PSB2=*Bacillus* spp. PSB2, PSB3=*Bacillus* spp. PSB3, PSB5=*Bacillus* spp. PSB5, PSB6=*Bacillus* spp. PSB6 in combination with poultry manure; PM0= without poultry manure, and PM1= with poultry manure (20 t ha<sup>-1</sup>) on growth and yield traits of sugarbeet, and rhizosphere soil health indicators. Results regarding the pot experiment showed that phosphorus solubilizing bacterial strains PSB2, PSB3, PSB5 and PSB6 had great potential to improve growth traits of the sugarbeet crop and were screened as efficient phosphorus solubilizing bacterial strains. Results of the field experiment revealed that the combined application of efficient phosphorus solubilizing bacterial strains (PSB2 and PSB5) with poultry manure (20 t ha<sup>-1</sup>) produced the highest growth and yield traits of sugarbeet crop, and rhizosphere soil health indicators. Our findings suggest that the combined application of efficient phosphorus solubilizing bacterial strains (PSB2 and PSB5) with poultry manure (20 t ha<sup>-1</sup>) is a promising and viable option to achieve the maximum sugarbeet productivity under changing environmental scenarios.

**KEYWORDS:** Sugarbeet, Phosphorus solubilizing bacteria, Poultry manure, *Bacillus* spp.

## 1. INTRODUCTION

Sugarbeet (*Beta vulgaris* L.) is one of the most important sugar crops, which accounts for about 30-40% of world sugar production after sugarcane (Iqbal and Saleem, 2015; Ijaz et al., 2023). Sugarbeet is a short-duration crop containing higher concentrations of sucrose (14-20%) as compared to long-duration sugarcane that contains low sucrose content (10-12%) (Ahmad et al., 2012; Mubarak et al., 2016), which is an important aspect for the farmers to shift from sugarcane to sugarbeet cultivation. Sugarbeet productivity is on a decreasing trend due to imbalanced fertilization especially low phosphorus use efficiency, which is associated with its least mobility in the soil solution (Khan et al., 2022). Furthermore, a gradual decline in soil health has been observed due to the excessive use of synthetic agro-chemicals, which leads to the degradation of soil and reduced sustainable crop production (Uphoff and Dazzo, 2016; Bitew and Alemayehu, 2017; Ahmad et al., 2021). The permanent and long-term use of synthetic agro-chemicals is also a major source of diseases in humans and animals because they pollute groundwater reserves by leaching through the root zone (Ijaz et al., 2021). However, organic materials have the capacity to enhance and sustain the soil fertility status on a long-term basis as they contain a sustained amount of plant macro and micro nutrients (Chauhan et al., 2012; Ahmad et al., 2022). Hence, farmers are getting a keen interest in soil amendments of natural fertilizers like poultry manure and phosphorus solubilizing bacteria (PSB) to sustain soil health for improving crop production (Ning et al., 2017; Ijaz et al., 2021; Hui et al., 2017).

Sustainable agricultural practices are adopted to alleviate the detrimental impacts of excessive use of synthetic fertilizers and intensive farming practices under climate change scenarios (Reddy et al., 2020; Ahemad et al., 2009). In this context, the use of phosphorus solubilizing bacteria can play a vital role in improving the bioavailability of soil phosphorus for plant growth and development (Rezakhani et al., 2019; Ahemad, 2012). It mobilizes insoluble forms of phosphorous (P) present in the minerals by lowering soil pH due to the release of various low molecular weight organic acids from PSB (Liu et al., 2020). It also increases the availability of N, K, Fe and Zn, which in turn improves the growth and development of crop plants (Rezakhani et

al., 2022; Bargaz et al., 2018; Etesami and Jeong, 2021; Reddy et al., 2020; Hussain et al., 2023). Poultry manure is a major organic source of macronutrients (N, P and K) and traces of micronutrients (Fe, Mn, B and Zn), which are crucial for the proper growth and development of crop plants (Ahmad et al., 2021; Ahmad et al., 2022). It improves the soil fertility status, soil organic matter, soil porosity, soil water holding capacity and increases soil microbial activity (Agbede et al., 2008). Additionally, soil amendment of poultry manure also increases sugarbeet yield and root sugar content (Ghaly et al., 2020; Ouzounidou et al., 2010). Several studies also revealed that soil amendment of poultry manure at the rate of 10-20 t ha<sup>-1</sup> resulted in significantly increased sugarbeet productivity and soil health due to improved soil physio-chemical properties and sustained availability of macro and micronutrients (Ghaly et al., 2020; Ahmad et al., 2022).

Different studies conducted and have shown that the application of PSB and organic materials resulted in increased bioavailability of soil phosphorus and improved crop productivity of sugarbeet (Din et al., 2019), rice (Rasul et al., 2019), cotton (Qureschi et al., 2012) and sunflower (Ekin, 2010). However, limited research work has been conducted to evaluate the combined effect of PSB and poultry manure on rhizosphere nutrients availability and sugarbeet productivity. It is hypothesized that the combined application of PSB and poultry manure may improve rhizosphere soil nutrition and sugarbeet productivity. Therefore, the present research work was conducted to study the combined effect of efficient PSB and poultry manure on the rhizosphere soil nutrition and sugarbeet productivity under arid climatic conditions.

## 2. METHODS

### 2.1. LOCATION AND SOIL

The current research work was carried out at the Research Farm, College of Agriculture, Bahauddin Zakariya University, Bahadur Sub Campus, Layyah (31.30° N latitude and 71.41° E longitude) during the year 2019-2020. The experimental field had sandy loam texture with 15% clay (<0.002mm), 35% silt (0.002-0.05mm) and 50% sand (0.05-2.00 mm). The experimental site lies at an elevation of about 182 m above sea level. The soil was analyzed in order to determine the physio-chemical characteristics which showed that it contained saturation (36%), pH (8.00), electrical conductivity (3.88 dS m<sup>-1</sup>), organic matter content (0.64%), soil available nitrogen (62.8 mg kg<sup>-1</sup>), soil available phosphorus (6.80 mg kg<sup>-1</sup>) and soil available potassium (205 mg kg<sup>-1</sup>).

### 2.2. CLIMATE

The site of the present research work receives maximum rainfall during the monsoon months (July to September) as it is located in the sub-tropical region of Punjab, Pakistan. Weather data comprised of the maximum and minimum temperatures, rainfall and relative humidity during the crop period (October-May), was collected from the Automatic Meteorological Station (AMS) at Layyah District, South Punjab, Pakistan (Table 1).

**TABLE 1** Meteorological conditions of the growing years conducted at the research station of bahauddin zakariya university, bahadur sub campus layyah.

Month	Mini T (°C)	Maxi T (°C)	RH (%)	RF (mm)
November	12.9	28.5	75.6	0.00
December	6.96	22.7	85.7	0.23
January	6.66	20.3	79.8	0.93
February	8.68	20.2	80.6	0.21
March	12.7	25.2	74.9	1.00
April	20.3	35.9	72.9	1.04
May	24.4	40.9	58.1	0.21

Mini T = Minimum temperature (°C); Maxi T = Maximum temperature (°C); Relative humidity (%); RF = Rainfall (mm)

### 2.3. EXPERIMENTAL DESIGN AND TREATMENTS

A pot experiment consisting of seven strains of PSB (PSB0, PSB1, PSB2, PSB3, PSB4, PSB5, PSB6 and PSB7) was laid out to screen out the most efficient strains of PSB using Completely Randomized Design (CRD) with three replications. Secondly, a field experiment was carried out in a randomized complete block design (RCBD) under factorial arrangement with three replications. Treatments of the field experiment consisted of poultry manure (PM0= without poultry manure and PM1= with poultry manure at 20 t ha<sup>-1</sup>), and four efficient strains of PSB (PSB2, PSB3, PSB5 and PSB6).

### 2.4. SEED SOURCE

Seed of sugarbeet cultivar "California" was collected from Layyah Sugar Mills Limited, Punjab, Pakistan. It is characterized as a high-yielding and fertilizer responsive cultivar of sugarbeet. Additionally, it is being cultivated by many farmers in the Layyah District, which is attributed to its good yield potential.

## 2.5. POULTRY MANURE

Poultry manure was collected from Hussain and Sons Poultry Farm, and it was analyzed for knowing its chemical composition. Nutritional composition was included of 1.13% N, 0.14 ppm P, 0.08 ppm K, 232 ppm Fe, 0.48 ppm Mn, 353 ppm Zn and 10.9 ppm B.

## 2.6. PREPARATION AND INOCULATION OF PHOSPHORUS SOLUBILIZING BACTERIA (PSB)

The seven strains of PSB, being a pure culture preserved in glycerol stock, were collected from the Department of Bioinformatics and Biotechnology, Government College University, Faisalabad. Using sterilized tryptone soya broth media, an inoculum of each separate strain of PSB was prepared. For seed inoculation, the seeds of the sugarbeet cultivar "California" were inoculated with each selected PSB strain. Inoculation of sugarbeet seeds was done by dipping seeds in the prepared inoculum for about two hours. Later on, inoculated seeds of sugarbeet were dried under shade and stored at 25° C temperature till their sowing.

## 2.7. CROP HUSBANDRY

In the present field study, land was kept fallow before the sowing of the sugarbeet crop. Poultry manure was spread on the soil surface, and the soil was ploughed in order to thoroughly mix poultry manure into the soil prior to 30 days of sugar beet sowing. After 30 days, the soil was ploughed twice, followed by planking in order to facilitate proper seedbed preparation. The seeds were inoculated with each strain of PSB by dipping seeds in the prepared inoculum for about two hours, and the inoculated seeds were dried in the shade and stored at 25° C temperature. On October 10, 2019, the ridges were made with the help of a tractor-mounted ridger at a distance of 40 cm, and sugarbeet seeds were sown manually at a distance of 15 cm. The recommended dose of synthetic fertilizers (120:80:60 kg ha<sup>-1</sup> NPK) was applied to sugarbeet crop. About 13 subsequent irrigations were applied on critical crop growth stages based on the crop water requirements. The treatment plots were separated by creating bands of about 0.5 m between the treatments in order to control water movement among the treatments and prevent edge effects. Earthing up of ridges was carried out on a regular basis during the field experimentation. Weeds were controlled with manual hoeing within the treatment plots. All the experimental plots were treated uniformly except for the one under study. Sugarbeet crop was harvested on May 02, 2020.

## 3. MEASUREMENTS AND ANALYTICAL PROCEDURES

### 3.1. GROWTH AND YIELD TRAITS OF SUGARBEET

In the current experiment, different growth and yield parameters of the sugarbeet crop were recorded. At maturity, leaf length was determined with the help of a measuring tape from randomly tagged ten plants from each experimental plot and their mean was estimated. To determine the number of leaves of plant-1, ten plants were selected randomly from each replication, and their mean was computed. Leaf weight (LW) was determined in grams (g) by taking leaves of ten selected plants with the help of a digital scale (0.01-g precision), and their mean was calculated (Khan et al., 2020). Similarly, a digital scale (0.01-g precision) was used to determine the root weight per plant and their mean was estimated. Chlorophyll contents in the leaves were measured by using a chlorophyll meter (SPAD-502; Minolta, Tokyo, Japan) from randomly selected ten plants and their average was calculated as the leaf chlorophyll content. After harvest, ten plant roots were tagged from each experimental plot to record circumference (cm) with the help of measuring tape and then root diameter (RD) was estimated using the following equation (1) given by Ahmad et al. (2016).

$$\text{Root diameter (cm)} = (\text{Circumference (cm)}) / 3.142 \quad (1)$$

After harvesting, tagged sugarbeet plants were taken, their roots and tops were separated and weighed to estimate the root yield, leaf yield and biological yield and converted into tons ha<sup>-1</sup>.

### 3.2. DETERMINATION OF RHIZOSPHERE SOIL HEALTH INDICATORS

In the present research work, five soil samples to the depth of 0-30 cm from each experimental plot were collected using soil auger and their composite soil sample was prepared to reduce the error factor associated with improper sampling. Composite soil samples were dried and sieved (2-mm mesh). Standard procedures, i.e. Walkley and Black (1934), sodium bicarbonate (Olsen, 1954), alkaline potassium permanganate (Subbaiah and Asija, 1956) and ammonium acetate (Nelson and Heidel, 1952) were used to determine the soil organic matter content, N, P and K, respectively.

### 3.3. STATISTICAL ANALYSIS

The experimental data, including the main and interactive effects of different efficient phosphorus solubilizing bacterial strains and poultry manure on the growth and yield components of sugarbeet and soil health indicators, were subjected to an analysis of variance technique (Steel et al., 1997). The treatment means were compared and separated with the help of the least significant difference (LSD) test, which was considered at a  $p \leq 5\%$  level of probability. Using readxl, cor and corplot packages of R-software, the association between studied growth and yield traits of sugarbeet was also assessed.

## 4. RESULTS AND DISCUSSION

### 4.1. SCREENING OF EFFICIENT PHOSPHORUS SOLUBILIZING BACTERIAL (PSB) STRAINS

Different strains of phosphorus solubilizing bacteria showed significant effects on the dry weight of shoot and root, number of leaves per plant, length of shoot and root of sugarbeet under a pot experiment (Table 2). Phosphorus solubilizing bacterial strains PSB2, PSB3, PSB5 and PSB6 showed promising results, and sugarbeet crop produced the highest dry weight of shoot and root, number of leaves per plant, length of shoot and root with the inoculation of PSB2, PSB3, PSB5 and PSB6 as compared to control treatment and other PSB strains (PSB0, PSB1, PSB4, and PSB7). Furthermore, the performance of other PSB strains (PSB0, PSB1, PSB4, and PSB7) was also good as compared to the control treatment (Table 2). Thus, the present pot research work showed that the performance of PSB2, PSB3, PSB5 and PSB6 was most prominent, as we clearly observed improvement in dry weight of shoot and root, number of leaves per plant, and length of shoot and root of sugarbeet crop in comparison to other PSB strains (PSB0, PSB1, PSB4, and PSB7). Therefore, these four PSB strains (PSB2, PSB3, PSB5 and PSB6) were screened as efficient strains of phosphorus solubilizing bacteria.

**TABLE 2** Effect of different strains of phosphorus solubilizing bacteria on dry weight of shoot and root, number of leaves per plant, and length of shoot and root of sugarbeet under a pot experiment

PSB Strains	Shoot dry weight (g)	Root dry weight (g)	Number of leaves plant <sup>-1</sup>	Shoot length (cm)	Root length (cm)
PSB <sub>0</sub>	0.321 c	0.026 c	4.27 c	16.6 c	5.15 c
PSB <sub>1</sub>	0.336 b	0.029 bc	5.04 b	17.4 b	5.89 b
PSB <sub>2</sub>	0.392 a	0.036 a	7.25 a	19.6 a	8.13 a
PSB <sub>3</sub>	0.395 a	0.034 ab	7.05 a	19.4 a	7.97 a
PSB <sub>4</sub>	0.325 bc	0.026 c	4.51 bc	16.8 c	5.43 c
PSB <sub>5</sub>	0.403 a	0.037 a	7.27 a	19.5 a	8.26 a
PSB <sub>6</sub>	0.394 a	0.034 ab	6.97 a	19.4 a	8.00 a
PSB <sub>7</sub>	0.319 c	0.025 c	5.04 b	17.4 b	5.92 b
HSD (p<0.01)	0.014	0.031	0.602	0.441	0.361

Means sharing the same case letter did not significantly differ for a particular parameter at p<0.01

### 4.2. GROWTH TRAITS OF THE SUGAR BEET CROP

Number of leaves per plant, leaf length, leaf weight per plant, total chlorophyll content and leaf yield of sugarbeet crop showed significantly different responses with the seed inoculation of different efficient strains of phosphorus solubilizing bacteria (PSB0, PSB1, PSB2, PSB3, PSB4), and with the soil amendment of poultry manure (PM0 and PM1). Similarly, the interactive effect of different efficient strains of phosphorus solubilizing bacteria with poultry manure was also significant for the number of leaves per plant, leaf length, leaf weight per plant, total chlorophyll content and leaf yield of sugar beet crop (Table 3). Results regarding the interactive effects of phosphorus solubilizing bacteria with poultry manure are presented in Table 3. Sugar beet crop showed higher number of leaves per plant (33.5-33.8%), leaf length (32.5-32.8%), leaf weight per plant (23-29%), root length (23-29%), total chlorophyll content (22-23%) and leaf yield (61.6-62.9%) with seed inoculation of PSB3, and PSB4 in combination with poultry manure (PM1), respectively in comparison to control treatment (Table 3).

**TABLE 3** Effect of seed inoculation of different efficient strains of phosphorus solubilizing bacteria, and poultry manure on growth traits of sugarbeet crop

Treatments	Number of leaves plant <sup>-1</sup>		Leaf length (cm)		Leaf weight (g plant <sup>-1</sup> )		Chlorophyll content (SPAD)		Leaf yield (t ha <sup>-1</sup> )	
	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>
PSB <sub>0</sub>	28.4 f	36.2 c	29.5 f	37.4 c	302 f	311 b	43.1 g	50.9 c	15.4 f	23.3 c
PSB <sub>2</sub>	35.7 d	38.0 a	36.8 cd	39.2 a	310 bc	313 a	50.4 cd	52.7 a	22.6 cd	25.1 a
PSB <sub>3</sub>	32.5 e	36.5 bc	33.6 e	37.7 bc	307 d	311 ab	47.1 e	51.4 bc	19.4 e	23.6 bc
PSB <sub>5</sub>	34.4 d	37.9 ab	35.5 d	39.1 ab	309 c	313 a	49.1 d	52.6 ab	21.4 d	24.9 ab
PSB <sub>6</sub>	29.7 f	36.1 c	30.9 f	37.3 c	304 e	311 b	44.4 f	50.9 c	16.6 f	23.2 c
HSD (p<0.05)	1.470		1.480		1.482		1.329		1.346	

Values sharing with the same case letter did not differ significantly at p<0.05 for a particular attribute; \* = Significant at p<0.05; \*\* = Significant at p<0.01; PM<sub>0</sub> = without poultry manure; PM<sub>1</sub> = with poultry manure.

### 4.3. YIELD TRAITS OF THE SUGAR BEET CROP

Different efficient strains of phosphorus solubilizing bacteria (PSB<sub>0</sub>, PSB<sub>2</sub>, PSB<sub>3</sub>, PSB<sub>5</sub>, PSB<sub>6</sub>), and with the soil amendment of poultry manure (PM<sub>0</sub> and PM<sub>1</sub>) had significant effects on root diameter, root weight, root length, root yield and biological yield of sugar beet crop. Furthermore, the interactive effect of different efficient strains of phosphorus solubilizing bacteria with

poultry manure was also significant for root diameter, root weight, root length, root yield and biological yield of sugar beet crop (Table 4). Results regarding the interactive effects of phosphorus solubilizing bacteria with poultry manure are presented in Table 3. Sugar beet crop showed higher number of root diameter (23-29%), root weight (23-29%), root length (23-29%), root yield (23-29%) and biological yield (24.3-23.9%) with seed inoculation of PSB<sub>3</sub>, and PSB<sub>4</sub> in combination with poultry manure (PM<sub>1</sub>), respectively in comparison to control treatment (Table 4).

**TABLE 4** Effect of seed inoculation of different efficient strains of phosphorus solubilizing bacteria and poultry manure on yield traits of sugarbeet crop

Treatments	Root diameter (cm)		Root weight (g plant <sup>-1</sup> )		Root length (cm)		Root yield (t ha <sup>-1</sup> )		Biological yield (t ha <sup>-1</sup> )	
	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>
PSB <sub>0</sub>	10.8 f	18.6 c	972 g	981 bc	19.3 b	27.3 ab	64.4 g	72.3 c	79.8 f	95.5 c
PSB <sub>2</sub>	18.0 c	20.5 a	980 cd	983 a	33.4 ab	29.0 a	71.7 cd	74.2 a	94.3 cd	99.2 a
PSB <sub>3</sub>	14.8 e	19.1 bc	976 e	981 bc	23.4 ab	27.6 ab	68.5 e	72.7 bc	87.8 e	96.4 bc
PSB <sub>5</sub>	16.8 d	20.4 ab	979 d	983 a	25.5 ab	28.9 ab	70.5 d	74.0 ab	91.8 d	98.9 ab
PSB <sub>6</sub>	12.1 f	18.6 c	974 f	981 bc	20.7 b	27.3 ab	65.7 f	72.3 c	82.4 f	95.5 c
HSD (p≤0.05)	1.358		1.302		10.55		1.342		2.683	

Values sharing with the same case letter did not differ significantly at p≤0.05 for a particular attribute; \*= Significant at p≤0.05; \*\*= Significant at p≤0.01; PM<sub>0</sub>= without poultry manure; PM<sub>1</sub>= with poultry manure.

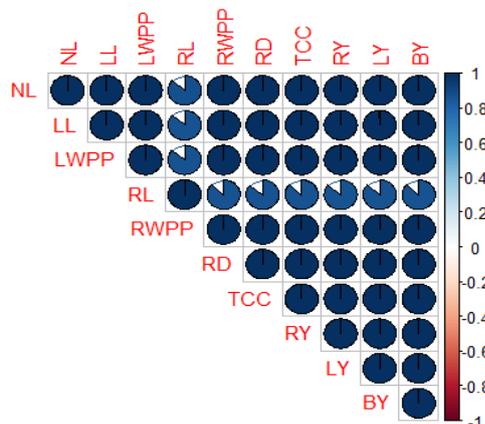
**4.4. RHIZOSPHERE SOIL HEALTH INDICATORS**

The main effects of different efficient strains of phosphorus solubilizing bacteria (PSB<sub>0</sub>, PSB<sub>2</sub>, PSB<sub>3</sub>, PSB<sub>5</sub>, PSB<sub>6</sub>), and the soil amendment of poultry manure (PM<sub>0</sub> and PM<sub>1</sub>) were found to be significant for rhizosphere soil available phosphorus, nitrogen, and potassium. Similarly, the interactive effects of different efficient strains of phosphorus solubilizing bacteria with poultry manure were also found to be significant for rhizosphere soil available phosphorus, nitrogen, and potassium (Table 5). Field results pertaining to the interactive effects of phosphorus solubilizing bacteria with poultry manure are presented in Table 4. Research findings showed 8.61% and 8.45% increase in rhizosphere soil available nitrogen, 15.47% and 15.17% in phosphorus, and 4.47% and 4.47% potassium with the combined application of seed inoculation of PSB<sub>6</sub> and PSB<sub>5</sub>, respectively, in comparison to the control treatment (Table 5).

**TABLE 5** Effect of seed inoculation of different efficient strains of phosphorus solubilizing bacteria and poultry manure on rhizosphere soil health indicators

Treatments	Nitrogen (mg kg <sup>-1</sup> )		Phosphorus (mg kg <sup>-1</sup> )		Potassium (mg kg <sup>-1</sup> )		Soil organic matter (%)	
	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>	PM <sub>0</sub>	PM <sub>1</sub>
PSB <sub>0</sub>	63.9 f	67.6 c	6.72 g	6.97 f	201 f	209 c	0.65	0.64
PSB <sub>2</sub>	67.1 cd	69.4 a	7.62 b	7.76 a	208 cd	210 a	0.65	0.65
PSB <sub>3</sub>	63.8 e	67.8 bc	7.39 d	7.51 c	205 e	209 bc	0.64	0.65
PSB <sub>5</sub>	65.7 d	69.3 a	7.52 c	7.74 a	207 d	210 a	0.65	0.66
PSB <sub>6</sub>	62.6 f	67.5 c	7.21 e	7.33 d	202 f	209 c	0.65	0.65
HSD (p≤0.05)	1.712		0.05		1.478		NS	

Values sharing with the same case letter did not differ significantly at p≤0.05 for a particular attribute; \*= Significant at p≤0.05; \*\*= Significant at p≤0.01; NS= Non-significant; PM<sub>0</sub>= without poultry manure; PM<sub>1</sub>= with poultry manure.



**FIGURE 1** Correlation map

The correlation map showing the effect of phosphorous solubilizing bacteria and poultry manure on different growth, morphological and yield traits of sugar beet crop. The areas of circles show the absolute value of corresponding correlation coefficients tested at \*0.01 significance level. The number of leaves (NL), root length (RL), leaf length (LL) and leaf weight per plant (LWPP) are showing weak correlation with each other. While root weight per plant (RWPP), root diameter (RD), total chlorophyll contents (TCC), root yield (RY), leaf yield (LY) and biological yield (BY) show a positive and strong correlation with each other.

In the current research work, the pot experimental results showed that different phosphorus solubilizing bacterial strains had significant effects on the dry weight of shoot and root, number of leaves per plant and length of shoot and root of sugarbeet crop (Table 2). There was a linear increasing trend with the inoculation of different strains of phosphorus solubilizing bacteria. However, PSB<sub>2</sub>, PSB<sub>3</sub>, PSB<sub>5</sub> and PSB<sub>6</sub> showed maximum dry weight of shoot and root, number of leaves per plant and length of shoot and root of sugarbeet crop as compared to other strains of phosphorus solubilizing bacteria (PSB<sub>0</sub>, PSB<sub>1</sub>, PSB<sub>4</sub>, and PSB<sub>7</sub>). These findings clearly suggest that different strains of PSB enhanced phosphorus mobilization which resulted in the promotion of growth of roots, shoots, and the number of leaves per plant of the sugarbeet crop (Aallam *et al.*, 2022). Based on our findings, we hypothesized that PSB<sub>2</sub>, PSB<sub>3</sub>, PSB<sub>5</sub> and PSB<sub>6</sub> were considered and screened as efficient strains of phosphorus solubilizing bacteria.

In our field study, the sugar beet crop produced the highest growth traits with seed inoculation of efficient bacterial strains PSB<sub>2</sub> and PSB<sub>5</sub> in combination with poultry manure (20 t ha<sup>-1</sup>) as compared to the control treatment and other PSB strains. Higher growth traits might be because higher rhizosphere soil available phosphorus due to lowering of pH with the production of low molecular weight organic acids and phosphate solubilization by inoculation of phosphorous solubilizing bacteria (Rezakhani *et al.*, 2019; Rezakhani *et al.*, 2022). Phosphorus is a major growth-limiting nutrient required for crop plants due to its vital role in the energy transformation in the photosynthesis and respiration processes, and being a structural part of nucleic acids and membranes (Khan *et al.*, 2014; Hadir *et al.*, 2021). Hence, higher availability leads to photosynthesis and respiration processes and ultimately improved growth traits of the sugarbeet crop. On the other hand, PSB stimulates the release of the growth regulators, i.e. indoleacetic acid, gibberellins, and cytokinins (Pathan *et al.*, 2018), and biological nitrogen fixation (Liu *et al.*, 2020), which might be a reason behind the improvement in growth traits of the sugarbeet crop.

The interactive effect of seed inoculation with an efficient strain of phosphorous solubilizing bacteria (PSB<sub>2</sub> and PSB<sub>5</sub>) with poultry manure (20 t ha<sup>-1</sup>) also showed higher yield components of the sugarbeet crop. Phosphorous solubilizing bacteria are involved in the mobilization of insoluble forms of phosphorous present in the soil minerals and increase phosphorus availability in the rhizosphere, which might be the reason behind the higher yield components of sugarbeet (Vives-Peris *et al.*, 2020; Sashidhar and Podile, 2010). Poultry manure is a rich source of macro and micro nutrients (Ahmad *et al.*, 2021), and their availability might be improved in the rhizosphere, resulting in higher yield components of the sugarbeet crop. Furthermore, PSB is involved in the production of growth regulators (Pathan *et al.*, 2018) that might be a reason to improve the yield components of the sugarbeet crop. Our research findings also proved several previous studies that have shown higher and sustainable sugarbeet crop yield was recorded with the seed inoculation of phosphorous solubilizing bacteria in combination with poultry manure (Nacoon *et al.*, 2020; Tahir *et al.*, 2018; Din *et al.*, 2019).

Poultry manure directly adds macronutrients (N, P and K) and also improves soil porosity and nutrient-holding capacity, which in turn increases soil nutrient budgets (Ahmad *et al.*, 2021; Add Reference). On the other hand, PSB inoculation improves the bioavailability of soil nutrients due to its imperative role in lowering soil pH. Our research findings also showed that combined application of efficient phosphorus solubilizing bacterial strains PSB<sub>2</sub> and PSB<sub>5</sub> with 20 t ha<sup>-1</sup> PM also showed higher rhizosphere soil available N, P and K. These results were closely associated with more mobilization of phosphorus due to phosphorus solubilizing bacterial strain PSB<sub>2</sub> and PSB<sub>5</sub>, and slowly release of phosphorus, nitrogen, and potassium from the decomposition of PM (Curvelo *et al.*, 2018). Our findings proved that phosphorus solubilizing bacteria enhance phosphorus availability due to their imperative role in increasing phosphatase and organic phosphate mineralization activity in the soil (Iqbal *et al.*, 2016). Higher rhizosphere N availability might be correlated with the vital function of PSB in the stimulation of biological nitrogen fixation in the rhizosphere (Liu *et al.*, 2020). Additionally, phosphorus solubilizing bacteria decrease the soil pH due to the production of organic acids, mainly gluconic and keto gluconic acids, which might be a reason for the improvement in rhizosphere soil available N, P and K (Ning *et al.*, 2017). Our results showed that phosphorus solubilizing bacteria with poultry manure could further increase the bioavailability of N, P and K in the soil solution.

## 5. CONCLUSION

In conclusion, our results indicated that phosphorus solubilizing bacterial strains vary in their performance, and PSB<sub>2</sub>, PSB<sub>3</sub>, PSB<sub>5</sub> and PSB<sub>6</sub> had great potential to improve growth traits of the sugarbeet crop. Furthermore, field experimental results revealed that the combined application of efficient phosphorus solubilizing bacterial strains PSB<sub>2</sub> or PSB<sub>5</sub> with poultry manure (20 t ha<sup>-1</sup>) produced the highest growth and yield traits of sugarbeet crop, and rhizosphere soil health indicators. Hence, the combined application of efficient phosphorus solubilizing bacterial strains PSB<sub>2</sub> or PSB<sub>5</sub> with poultry manure (20 t ha<sup>-1</sup>) is a promising and sustainable option to achieve the maximum sugarbeet productivity and profitability under changing

environmental scenarios. Further studies may evaluate the effect of these findings on growth, and yield promotion of sugar beet crop, and soil health indicators under future climate in order to ensure sustainable sugarbeet production under future climate change scenarios.

## REFERENCES

- [1] "T. M. Agbede, S. O. Ojeniyi and A. J. Adeyemo, "Effect of Poultry Manure on Soil Physical and Chemical Properties, Growth and Grain Yield of Sorghum in Southwest Nigeria," *American-Eurasian Journal of Sustainable Agriculture*, vol. 2, no. 1, pp. 72-77, 2008.
- [2] M. Ahemad and M. S. Khan, "Effect of insecticide-tolerant and plant growth-promoting Mesorhizobium on the performance of chickpea grown in insecticide stressed alluvial soils," *Journal of Crop Science and Biotechnology*, vol. 12, no. 4, pp. 217–226, Dec. 2009, doi: <https://doi.org/10.1007/s12892-009-0130-8>.
- [3] M. Ahemad, "Implications of bacterial resistance against heavy metals in bioremediation: a review," *Journal of Institute of Integrative Omics and Applied Biotechnology (IIOAB)*, vol. 3, no. 3, 2012.
- [4] S. Ahmad et al., "Evaluation of sugar beet hybrid varieties under Thal-Kumbi soil series of Pakistan," *International Journal of Agriculture and Biology*, vol. 14, pp. 605–608, 2012.
- [5] I. Ahmad et al., "Organic and inorganic fertilization influenced on yield and quality of sugar beet genotypes," *Russian Agricultural Sciences*, vol. 42, no. 3–4, pp. 218–223, May 2016, doi: <https://doi.org/10.3103/s1068367416030022>.
- [6] Ahmad, S.; Ghaffar, A.; Rahman, M.H.U.; Hussain, I.; Iqbal, R.; Haider, G.; Khan, M.A.; Ikram, R.M.; S. Ahmad et al., "Effect of Application of Biochar, Poultry and Farmyard Manures in Combination with Synthetic Fertilizers on Soil Fertility and Cotton Productivity under Arid Environment," *Communications in Soil Science and Plant Analysis*, vol. 52, no. 17, pp. 2018–2031, Apr. 2021, doi: <https://doi.org/10.1080/00103624.2021.1908324>.
- [7] S. Ahmad et al., "Organic amendments and conservation tillage improve cotton productivity and soil health indices under arid climate," *Scientific Reports*, vol. 12, no. 1, p. 14072, Aug. 2022, doi: <https://doi.org/10.1038/s41598-022-18157-0>.
- [8] A. Bargaz, K. Lyamlouli, M. Chtouki, Y. Zeroual, and D. Dhiba, "Soil Microbial Resources for Improving Fertilizers Efficiency in an Integrated Plant Nutrient Management System," *Frontiers in Microbiology*, vol. 9, Jul. 2018, doi: <https://doi.org/10.3389/fmicb.2018.01606>.
- [9] Y. Bitew and M. Alemayehu, "Impact of Crop Production Inputs on Soil Health: A Review," *Asian Journal of Plant Sciences*, vol. 16, no. 3, pp. 109–131, Jun. 2017, doi: <https://doi.org/10.3923/ajps.2017.109.131>.
- [10] P.S.Chauhan et al., Environmental impacts of Organic fertilizer usage in agriculture, Organic fertilizers: types, production and environmental impact, Nova Science Publisher, Hauppauge, pp. 63-84, 2012.
- [11] C. R. da S. Curvêlo, L. H. B. Diniz, A. I. de A. Pereira, and L. L. Ferreira, "Influence of Fertilizer Type on Beet Production and Post-Harvest Quality Characteristic," *Agricultural Sciences*, vol. 09, no. 05, pp. 557–565, 2018, doi: <https://doi.org/10.4236/as.2018.95038>.
- [12] M. Diacono and F. Montemurro, "Olive Pomace Compost in Organic Emmer Crop: Yield, Soil Properties, and Heavy Metals' Fate in Plant and Soil," *Journal of Soil Science and Plant Nutrition*, vol. 19, no. 1, pp. 63–70, Feb. 2019, doi: <https://doi.org/10.1007/s42729-019-0010-3>.
- [13] M. Din et al., "Production of nitrogen fixing Azotobacter (SR-4) and phosphorus solubilizing Aspergillus niger and their evaluation on Lagenaria siceraria and Abelmoschus esculentus," *Biotechnology Reports*, vol. 22, p. e00323, Jun. 2019, doi: <https://doi.org/10.1016/j.btre.2019.e00323>.
- [14] Z. Ekin, "Performance of phosphate solubilizing bacteria for improving growth and yield of sunflower (*Helianthus annuus* L.) in the presence of phosphorus fertilizer," *African Journal of Biotechnology*, vol. 9, no. 25, pp. 3794–3800, 2010, doi: <https://doi.org/10.4314/ajb.v9i25>.
- [15] J. J. Enticknap, H. Nonogaki, A. R. Place, and R. T. Hill, "Microbial Diversity Associated with Odor Modification for Production of Fertilizers from Chicken Litter," *Applied and Environmental Microbiology*, vol. 72, no. 6, pp. 4105–4114, Jun. 2006, doi: <https://doi.org/10.1128/aem.02694-05>.
- [16] H. Etesami, B. R. Jeong, and B. R. Glick, "Contribution of Arbuscular Mycorrhizal Fungi, Phosphate–Solubilizing Bacteria, and Silicon to P Uptake by Plant," *Frontiers in Plant Science*, vol. 12, Jul. 2021, doi: <https://doi.org/10.3389/fpls.2021.699618>.
- [17] F. Ghaly, H. Sarhan, A. Abdel-Hamied, and T. Mansour, "Effect of different Sources and Rates of Organic Fertilization on Sugar Beet (*Beta vulgaris* var. *Saccharifera* L.) Yields and its Quality Grown under Newly Reclaimed Sandy Soils," *Journal of Soil Sciences and Agricultural Engineering*, vol. 11, no. 1, pp. 43–48, Jan. 2020, doi: <https://doi.org/10.21608/jssae.2020.79170>.
- [18] G.H.P. Hasanen, "Influence of nitrogen and organic fertilization on growth, yield and quality of sugar beet grown in calcareous soil," *Journal of Plant Production*, vol. 4, pp. 733–743, 2013.
- [19] H. LI et al., "Chemical fertilizers could be completely replaced by manure to maintain high maize yield and soil organic carbon (SOC) when SOC reaches a threshold in the Northeast China Plain," *Journal of Integrative Agriculture*, vol. 16, no. 4, pp. 937–946, Apr. 2017, doi: [https://doi.org/10.1016/s2095-3119\(16\)61559-9](https://doi.org/10.1016/s2095-3119(16)61559-9).
- [20] I. Hussain et al., "Optimum Zinc Fertilization and Sowing Date Improved Growth, Yield Components, and Grain Zn Contents of Bread Wheat Under Different Tillage Systems," *Journal of Soil Science and Plant Nutrition*, vol. 23, no. 2, pp. 2344–2353, Feb. 2023, doi: <https://doi.org/10.1007/s42729-023-01185-8>.

- [21] M. Ijaz et al., "Alternatives to Synthetic Fertilizers," *Agricultural Waste*, pp. 253–273, Mar. 2021, doi: <https://doi.org/10.1201/9781003105046-11>.
- [22] M. Ijaz, S. Ul-Allah, A. Sattar, A. Sher, I. Hussain, and A. Nawaz, "Evaluation of Various Organic Amendment Sources to Improve the Root Yield and Sugar Contents of Sugar Beet Genotypes (*Beta vulgaris* L.) under Arid Environments," *Sustainability*, vol. 15, no. 5, p. 3898, Feb. 2023, doi: <https://doi.org/10.3390/su15053898>.
- [23] MA Iqbal, and AM Saleem, "Sugar beet potential to beat sugarcane as a sugar crop in Pakistan. American Eurasian," *Journal of Agricultural and Environmental Sciences*, vol. 15, no. 1, pp. 36–44, 2015.
- [24] J. Liu, W. Qi, Q. Li, S.-G. Wang, C. Song, and X. Yuan, "Exogenous phosphorus-solubilizing bacteria changed the rhizosphere microbial community indirectly," *3 Biotech*, vol. 10, no. 4, Mar. 2020, doi: <https://doi.org/10.1007/s13205-020-2099-4>.
- [25] M. U. Mubarak, M. Zahir, S. Ahmad, and A. Wakeel, "Sugar beet yield and industrial sugar contents improved by potassium fertilization under scarce and adequate moisture conditions," *Journal of Integrative Agriculture*, vol. 15, no. 11, pp. 2620–2626, Nov. 2016, doi: [https://doi.org/10.1016/s2095-3119\(15\)61252-7](https://doi.org/10.1016/s2095-3119(15)61252-7).
- [26] S. Nacoon, S. Jogloy, N. Riddech, W. Mongkolthananuk, T. W. Kuyper, and S. Boonlue, "Interaction between Phosphate Solubilizing Bacteria and Arbuscular Mycorrhizal Fungi on Growth Promotion and Tuber Inulin Content of *Helianthus tuberosus* L.," *Scientific Reports*, vol. 10, no. 1, Mar. 2020, doi: <https://doi.org/10.1038/s41598-020-61846-x>.
- [27] C. NING, P. GAO, B. WANG, W. LIN, N. JIANG, and K. CAI, "Impacts of chemical fertilizer reduction and organic amendments supplementation on soil nutrient, enzyme activity and heavy metal content," *Journal of Integrative Agriculture*, vol. 16, no. 8, pp. 1819–1831, Aug. 2017, doi: [https://doi.org/10.1016/s2095-3119\(16\)61476-4](https://doi.org/10.1016/s2095-3119(16)61476-4).
- [28] "Olsen, S.R., Cole, C.V. and Watanabe, F.S. (1954) Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA Circular No. 939, US Government Printing Office, Washington DC. - References - Scientific Research Publishing," *Scirp.org*, 2022. <https://www.scirp.org/reference/referencespapers?referenceid=1117235>
- [29] "Ouzounidou, G., Zervakis, G.I. and Gaitis, F. (2010) Raw and Microbiologically Detoxified Olive Mill Waste and Their Impact on Plant Growth. *Terrestrial and Aquatic Environmental Toxicology*, 4, 21-38. - References - Scientific Research Publishing," *Scirp.org*, 2026. <https://www.scirp.org/reference/referencespapers?referenceid=1392706> (accessed Feb. 05, 2026).
- [30] M. A. Qureshi, Z. Ahmad, N. Akhtar, A. Iqbal, Fakhra Mujeeb, and M. A. Shakir, "Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth," *The Journal of Animal and Plant Sciences*, vol. 22, no. 1, pp. 204–210, Jan. 2012.
- [31] M. Rasul et al., "Glucose dehydrogenase gene containing phosphobacteria for biofortification of Phosphorus with growth promotion of rice," *Microbiological Research*, vol. 223–225, pp. 1–12, Jun. 2019, doi: <https://doi.org/10.1016/j.micres.2019.03.004>.
- [32] G. C. Reddy, R. K. Goyal, S. Puranik, V. Waghmar, K. V. Vikram, and K. S. Sruthy, "Biofertilizers Toward Sustainable Agricultural Development," *Plant Microbe Symbiosis*, pp. 115–128, 2020, doi: [https://doi.org/10.1007/978-3-030-36248-5\\_7](https://doi.org/10.1007/978-3-030-36248-5_7).
- [33] L. Rezakhani, B. Motesharezadeh, M. M. Tehrani, H. Etesami, and H. Mirseyed Hosseini, "Phosphate-solubilizing bacteria and silicon synergistically augment phosphorus (P) uptake by wheat (*Triticum aestivum* L.) plant fertilized with soluble or insoluble P source," *Ecotoxicology and Environmental Safety*, vol. 173, pp. 504–513, May 2019, doi: <https://doi.org/10.1016/j.ecoenv.2019.02.060>.
- [34] L. Rezakhani, B. Motesharezadeh, M. M. Tehrani, H. Etesami, and H. M. Hosseini, "The effect of silicon fertilization and phosphate-solubilizing bacteria on chemical forms of silicon and phosphorus uptake by wheat plant in a calcareous soil," *Plant and Soil*, Jan. 2022, doi: <https://doi.org/10.1007/s11104-021-05274-4>.
- [35] B. Sashidhar and A. R. Podile, "Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase," *Journal of Applied Microbiology*, vol. 109, no. 1, pp. 1–12, Jun. 2010, doi: <https://doi.org/10.1111/j.1365-2672.2009.04654.x>.
- [36] "Steel, R., Torrie, J. and Dickey, D. (1997) Principles and Procedures of Statistics. A Biometrical Approach. 3rd Edition, McGraw Hill Book Co., New York. - References - Scientific Research Publishing," *Scirp.org*, 2026. <https://www.scirp.org/reference/referencespapers?referenceid=1377414> (accessed Feb. 05, 2026).
- [37] VV Subbaiah, and GK Asija, "A rapid procedure for utilization of available nitrogen in soil," *Current Science*, vol. 26, pp. 258–260, 1956.
- [38] M. Tahir et al., "Combined application of bio-organic phosphate and phosphorus solubilizing bacteria (*Bacillus* strain MWT 14) improve the performance of bread wheat with low fertilizer input under an arid climate," vol. 49, pp. 15–24, Nov. 2018, doi: <https://doi.org/10.1016/j.bjm.2017.11.005>.
- [39] N. Uphoff and F. Dazzo, "Making Rice Production More Environmentally-Friendly," *Environments*, vol. 3, no. 4, p. 12, May 2016, doi: <https://doi.org/10.3390/environments3020012>.
- [40] V. Vives-Peris, C. de Ollas, A. Gómez-Cadenas, and R. M. Pérez-Clemente, "Root exudates: from plant to rhizosphere and beyond," *Plant Cell Reports*, vol. 39, no. 1, pp. 3–17, Jul. 2019, doi: <https://doi.org/10.1007/s00299-019-02447-5>.
- [41] A. Walkley, IA Black, "An examination of the digestion method for determining soil organic matter and a proposed modification of the chromic acid titration method," *Soil Science*, vol. 1, pp. 29–38, 1934.
- [42] LB. Nelson, Helen Heidel, "Soil analysis methods as used in the Iowa," State College Soil Testing Laboratory, 1952.