Original Research Article

Effect of phosphorus solubilizing bacteria in combination with poultry manure on soil health and sugarbeet productivity under arid climate

Abstract

Sustainable agricultural productivity is under threat due to lack of interest in the use of bio-organic fertilizers to alleviate drawbacks of conventional agricultural practices. Phosphorus solubilizing bacteria plays vital role in improving phosphorus availability to plants through slowly release from inorganic and organic soil phosphorus budgets by solubilization and mineralization. Furthermore, phosphorus solubilizing bacteria in combination with poultry manure has 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) were used using completely randomized design (CRD) for 55 days 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 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; PSB_0=No inoculation; PSB_2=*Bacillus* spp. PSB_2, PSB_3=*Bacillus* spp. PSB_3, PSB_5=*Bacillus* spp. PSB_5, PSB_6=*Bacillus* spp. PSB_6 in combination with poultry manure; PM_0= without poultry manure and PM_1= with poultry manure (20 t ha^{-1}) on growth and yield traits of sugarbeet, and rhizosphere soil health indicators. Results regarding pot experiment showed that phosphorus solubilizing bacterial strains PSB_2, PSB_3, PSB_5 and PSB_6 had great potential to improve growth traits of sugarbeet crop and screened as efficient phosphorus solubilizing bacterial strains. Results of field experiment revealed that the combined application of efficient phosphorus solubilizing bacterial strains (PSB_2 and PSB_5) with poultry manure (20 t ha^{-1}) 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 (PSB_2 and PSB_5) with poultry manure (20 t ha^{-1}) 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.

Introduction

Sugarbeet (*Beta vulgaris* L.) is one of the most important sugar crop 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 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 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 vital role in improving 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 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 increased soil microbial activity (Agbede *et al.,* 2008). Additionally, soil amendment of poultry manure also increases sugarbeet yield and roots 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^{-1} 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.

Methods

Location and Soil

The current research work was carried out at 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 the 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^{-1}), organic matter content (0.64%), soil available nitrogen (62.8 mg kg^{-1}), soil available phosphorus (6.80 mg kg^{-1}) and soil available potassium (205 mg kg^{-1}).

Climate

The site of 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 crop period (October-May), was collected from Automatic Meteorological Station (AMS) at Layyah District, South Punjab-Pakistan (Table 1).

Table 1: Meteorological conditions of the growing years conducted at 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)

Experimental design and treatments

A pot experiment consisting of seven strains of PSB (PSB_0, PSB_1, PSB_2, PSB_3 PSB_4, PSB_5, PSB_6 and PSB_7) was laid 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 were consisted of poultry manure (PM_0= without poultry manure and PM_1= with poultry manure at 20 t ha^{-1}), and four efficient strains of PSB (PSB_2, PSB_3, PSB_5 and PSB_6).

Seed Source

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

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 and 0.48 ppm Mn, 353 ppm Zn and 10.9 ppm B.

Preparation and inoculation of phosphorus solubilizing bacteria (PSB)

The seven strains of PSB being a pure culture preserved in glycerol stock were collected from Department of Bioinformatics and Biotechnology, Government College University, Faisalabad. Using sterilized tryptone soya broth media, inoculum of each separate strain of PSB was prepared. For seed inoculation, the seeds of 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.

Crop husbandry

In present field study, land was kept fallow before the sowing of sugarbeet crop. Poultry manure spread 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 strains of PSB by dipping seeds in the prepared inoculum for about two hours and inoculated seeds were dried under shade and stored at 25° C temperature. On October 10, 2019, the ridges were made with the help of tractor mounted ridger at the distance of 40 cm, and sugarbeet seeds were sown manually at the distance of 15 cm. The recommended dose of synthetic fertilizers (120:80:60 kg ha^{-1} 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 bunds of about 0.5 m between the treatments in order to control water movement among the treatments and prevent from edge effects. Earthing up of ridges was carried out on regular basis during the field experimentation. Weeds were controlled with manual hoeing within the treatment plots. All the experimental plots were treated uniformly except under study. Sugarbeet crop was harvested on May 02, 2020.

Measurements and analytical procedures

Growth and yield traits of sugarbeet

In current experiment, different growth and yield parameters of the sugarbeet crop were recorded. At maturity, leaf length was determined with the help of measuring tape from randomly tagged ten plants from each experimental plot and their mean was estimated. To determine the number of leaves 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 digital scale (0.01-g precision) and their mean was calculated (Khan *et al*., 2020). Similarly, 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 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 following equation (1) given by Ahmad *et al.* (2016).

$$\text{Root diamter (cm)} = \frac{\text{Circumference (cm)}}{3.142} \qquad (1)$$

After harvesting, tagged sugarbeet plants were taken, their roots and tops were separated and weighed to estimate the root yield and leaf yield and biological yield and converted in tones ha^{-1}.

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 augar 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.

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 was subjected to analysis of variance technique (Steel *et al.,* 1997). The treatment means were compared and separated with the help of least significant difference (LSD) test, which was considered at $p \leq 5\%$ level of probability. Using readxl, cor and corrplot packages of R-software, the association between studied growth and yield traits of sugarbeet were also assessed.

Results and discussion

Screening of efficient phosphorus solubilizing bacterial (PSB) strains

Different strains of phosphorus solubilizing bacteria showed significant effects on dry weight of shoot and root, number of leaves per plant, length of shoot and root of sugarbeet under pot experiment (Table 2). Phosphorus solubilizing bacterial strains PSB_2, PSB_3, PSB_5 and PSB_6 showed promising results and sugarbeet crop produced highest dry weight of shoot and root, number of leaves per plant, length of shoot and root with the inoculation of PSB_2, PSB_3, PSB_5 and PSB_6 as compared to control treatment and other PSB strains (PSB_0, PSB_1, PSB_4, and PSB_7). Furthermore, the performance of other PSB strains (PSB_0, PSB_1, PSB_4, and PSB_7) was also good as compared to the control treatment (Table 2). Thus, present pot research work showed that the performance of PSB_2, PSB_3, PSB_5 and PSB_6 had 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 (PSB_0, PSB_1, PSB_4, and PSB_7).

Therefore, these four PSB strains (PSB_2, PSB_3, PSB_5 and PSB_6) 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, and number of leaves per plant, and length of shoot and root of sugarbeet under pot experiment

PSB Strains	Shoot dry weight (g)	Root dry weight (g)	Number of leaves plant^{-1}	Shoot length (cm)	Root length (cm)
PSB_0	0.321 c	0.026 c	4.27 c	16.6 c	5.15 c
PSB_1	0.336 b	0.029 bc	5.04 b	17.4 b	5.89 b
PSB_2	0.392 a	0.036 a	7.25 a	19.6 a	8.13 a
PSB_3	0.395 a	0.034 ab	7.05 a	19.4 a	7.97 a
PSB_4	0.325 bc	0.026 c	4.51 bc	16.8 c	5.43 c
PSB_5	0.403 a	0.037 a	7.27 a	19.5 a	8.26 a
PSB_6	0.394 a	0.034 ab	6.97 a	19.4 a	8.00 a
PSB_7	0.319 c	0.025 c	5.04 b	17.4 b	5.92 b
HSD ($p \leq 0.01$)	0.014	0.031	0.602	0.441	0.361

Means sharing same case letter did not significantly differed for a particular parameter at $p \leq 0.01$

Growth traits of 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 response with the seed inoculation of different efficient strains of phosphorus solubilizing bacteria (PSB_0, PSB_1, PSB_2, PSB_3, PSB_4), and with the soil amendment of poultry manure (PM_0 and PM_1). Similarly, the interactive effect of different efficient strains of phosphorus solubilizing bacteria with poultry manure was also significant for 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 PSB_3, and PSB_4 in combination with poultry manure (PM_1), 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^{-1}	Leaf length (cm)	Leaf weight (g plant^{-1})	Chlorophyll content	Leaf yield (t ha^{-1})

							(SPAD)			
PSB Strains	**PM$_0$**	**PM$_1$**	**PM$_0$**	**PM$_1$**	**PM$_0$**	**PM$_1$**	**PM$_0$**	**PM$_1$**	**PM$_0$**	**PM$_1$**
PSB$_0$	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$_2$	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$_3$	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$_5$	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$_6$	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 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$_0$= without poultry manure; PM$_1$= with poultry manure

Yield traits of sugar beet crop

Different efficient strains of phosphorus solubilizing bacteria (PSB$_0$, PSB$_2$, PSB$_3$, PSB$_5$, PSB$_6$), and with the soil amendment of poultry manure (PM$_0$ and PM$_1$) 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 were 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 4. 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$_3$, and PSB$_4$ in combination with poultry manure (PM$_1$), 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^{-1})**		**Root length (cm)**		**Root yield (t ha^{-1})**		**Biological yield (t ha^{-1})**	
PSB Strains	**PM$_0$**	**PM$_1$**	**PM$_0$**	**PM$_1$**	**PM$_0$**	**PM$_1$**	**PM$_0$**	**PM$_1$**	**PM$_0$**	**PM$_1$**
PSB$_0$	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$_2$	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$_3$	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_5	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_6	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 ($p \leq 0.05$)	1.358		1.302		10.55		1.342		2.683	

Values sharing with same case letter did not differ significantly at $p \leq 0.05$ for a particular attribute; *= Significant at $p \leq 0.05$; *= Significant at $p \leq 0.01$; PM_0= without poultry manure; PM_1= with poultry manure

Rhizosphere soil health indicators

The main effects of different efficient strains of phosphorus solubilizing bacteria (PSB_0, PSB_2, PSB_3, PSB_5, PSB_6), and the soil amendment of poultry manure (PM_0 and PM_1) were found 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 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 5. 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_6, and PSB_5, respectively in comparison to 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 ($mg\ kg^{-1}$)**		**Phosphorus ($mg\ kg^{-1}$)**		**Potassium ($mg\ kg^{-1}$)**		**Soil organic matter (%)**	
PSB Strains	**PM_0**	**PM_1**	**PM_0**	**PM_1**	**PM_0**	**PM_1**	**PM_0**	**PM_1**
PSB_0	63.9 f	67.6 c	6.72 g	6.97 f	201 f	209 c	0.65	0.64
PSB_2	67.1 cd	69.4 a	7.62 b	7.76 a	208 cd	210 a	0.65	0.65
PSB_3	63.8 e	67.8 bc	7.39 d	7.51 c	205 e	209 bc	0.64	0.65
PSB_5	65.7 d	69.3 a	7.52 c	7.74 a	207 d	210 a	0.65	0.66
PSB_6	62.6 f	67.5 c	7.21 e	7.33 d	202 f	209 c	0.65	0.65
HSD ($p \leq 0.05$)	1.712		0.05		1.478		NS	

Values sharing with same case letter did not differ significantly at $p \leq 0.05$ for a particular attribute; *= Significant at $p \leq 0.05$; *= Significant at $p \leq 0.01$; NS= Non-significant; PM_0= without poultry manure; PM_1= with poultry manure

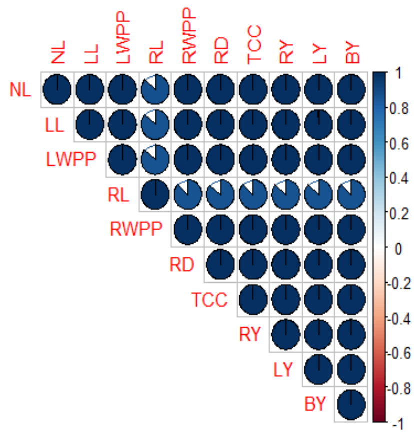

Fig. 1. Correlation map showing the effect of phosphorous solubilizing bacteria and poultry manure on different growth, morphological and yield traits of sugar beet crop.

*The areas of circles show the absolute value of corresponding correlation coefficients tested at *0.01 significance level. 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) are showing positively 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_2, PSB_3, PSB_5 and PSB_6 showed maximum dry weight of shoot and root, number of leaves per plant and length of shoot and root of sugarbeet crop as compared other strains of phosphorus solubilizing bacteria (PSB_0, PSB_1, PSB_4, and PSB_7). These findings clearly suggests that different strains of PSB enhanced phosphorus mobilization which resulted in promotion of growth of roots, and shoots, and number of leaves per plant of sugarbeet crop (Aallam *et al.,* 2022). Based on our findings, we hypothesized that PSB_2, PSB_3, PSB_5 and PSB_6 were considered and screened as efficient strains of phosphorus solubilizing bacteria.

In our field study, sugar beet crop produced highest growth traits with seed inoculation of efficient bacterial strains PSB_2 and PSB_5 in combination with poultry manure (20 t ha^{-1}) as compared to 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 lead to photosynthesis and respiration processes and ultimately improved growth traits of 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), that might be a reason behind the improvement in growth traits of sugarbeet crop.

Interactive effect of seed inoculation with efficient strain of phosphorous solubilizing bacteria (PSB_2 and PSB_5) with poultry manure (20 t ha^{-1}) also showed higher yield components of sugarbeet crop. Phosphorous solubilizing bacteria is involved in the mobilization of insoluble forms of phosphorous present in the soil minerals and increases phosphorus availability in the rhizosphere that might be reason behind the higher yield components of sugarbeet (Vives-Peris *et al*., 2020; Sashidhar and Podile, 2010). Poultry manure is rich source of macro and micro nutrients (Ahmad *et al.,* 2021) and their availability might be improved in the rhizosphere which resulted in higher yield components of sugarbeet crop. Furthermore, PSB is involved in the production of growth regulators (Pathan *et al*., 2018) that might be reason to improve yield components of sugarbeet crop. Our research findings also proved several previous studies that have showed 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 nutrients holding capacity which in turn increases soil nutrients budgets (Ahmad *et al.,* 2021; Add Reference). On the other hand, PSB inoculation improves 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_2 and PSB_5 with 20 t ha^{-1} 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_2 and PSB_5, and slowly release of phosphorus, nitrogen, and potassium from the decomposition of PM (Curvelo *et al*., 2018). Our findings proved that phosphorus solubilizing bacteria enhances phosphorus availability due to its 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 vital function of PSB in the stimulation of biological nitrogen fixation in the rhizosphere (Liu *et al*., 2020). Additionally, phosphorus solubilizing bacteria decreases the soil pH due to the production of organic acids mainly gluconic and keto gluconic acids that might be a reason of 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.

Conclusion

In conclusion, our results indicated that phosphorus solubilizing bacterial strains vary in their performance and PSB_2, PSB_3, PSB_5 and PSB_6 had great potential to improve growth traits of sugarbeet crop. Furthermore, field experimental results revealed that the combined application of efficient phosphorus solubilizing bacterial strains PSB_2 or PSB_5 with poultry manure (20 t ha^{-1}) 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_2 or PSB_5 with poultry manure (20 t ha^{-1}) 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

Agbede, T.M., S.O. Ojeniyi and A.J. Adeyemo. (2008). Effect of poultry manure on soil physical and chemical properties, growth and grain yield of sorghum in southwest, Nigeria. American-Eurasian. J. Sust. Agric. 2:72-77.

Ahemad, M. and Khan, M.S., (2009). 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*, *12*:pp.217-226.

Ahemad, M., (2012). Implications of bacterial resistance against heavy metals in bioremediation: a review. *Journal of Institute of Integrative Omics and Applied Biotechnology (IIOAB)*, *3*(3).

Ahmad S, Zubair M, Iqbal N, Cheema NM, Mahmood K (2012). Evaluation of sugar beet hybrid varieties under Thal-Kumbi soil series of Pakistan. International Journal of Agriculture and Biology 14: 605–608.

Ahmad, I., B. Ahmad, S. Ali, H.Q. Fang, T. Liu, M. Kamran, Y. Wu, M.U. Rahman and S. Faisal. (2016). Organic and Inorganic Fertilization Influenced on Yield and Quality of Sugar Beet Genotypes, Russian Agricultural Sciences. 42 (3-4): 218–223.

Ahmad, S.; Ghaffar, A.; Rahman, M.H.U.; Hussain, I.; Iqbal, R.; Haider, G.; Khan, M.A.; Ikram, R.M.; Hussnain, H.; Bashir, M.S. (2021). Effect of Application of Biochar, Poultry and Farmyard

Manures in Combination with Synthetic Fertilizers on Soil Fertility and Cotton Productivity under Arid Environment. *Comm. Soil Sci. Plant Anal. 52*, 2018–2031.

Ahmad, S.; Hussain, I.; Ghaffar, A.; Rahman, M.H.U.; Saleem, M.Z.; Yonas, M.W.; Hussnain, H.; Ikram, R.M.; Arslan, M. (2022). Organic amendments and conservation tillage improve cotton productivity and soil health indices under arid climate. *Sci. Rep. 12*, 14072.

Bargaz, A., Lyamlouli, K., Chtouki, M., Zeroual, Y. and Dhiba, D., (2018). Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. *Frontiers in microbiology*, *9*, p.1606.

Bitew, Y. and M. Alemayehu. (2017). Impact of crop production inputs on soil health: a review. Asian Journal Plant Science 16:109-131.

Chauhan, P.S., A. Singh, R.P. Singh, and M.H. Ibrahim. (2012). Environmental impacts of Organic fertilizer usage in agriculture. Organic fertilizers: types, production and environmental impact. Nova Science Publisher, Hauppauge 63-84.

Curvelo, C.R.S.; L.H.B. Diniz; A.I.A. Pereira and L.L. Ferreira (2018). Influence of fertilizer type on beet production and post-harvest quality characteristic. Agric. Sci., 9: 557-565.

Diacono, M. and Montemurro, F., (2019). 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*, *19*(1), pp.63-70.

Din, M., Nelofer, R., Salman, M., Khan, F.H., Khan, A., Ahmad, M., Jalil, F., Din, J.U. and Khan, M., (2019). Production of nitrogen fixing Azotobacter (SR-4) and phosphorus solubilizing Aspergillus niger and their evaluation on Lagenaria siceraria and Abelmoschus esculentus. *Biotechnology Reports*, *22*, p.e00323

Ekin, Z. (2010). Performance of phosphate solubilizing bacteria for improving growth and yield of sunflower (*Helianthus annuus* L.) in the presence of phosphorus fertilizer. *Afr. J. Biotechnol.* 9: 3794–3800.

Enticknap, J.J., H. Nonogaki, A.R. Place, and R.T. Hill. (2006). Microbial diversity associated with odor modification for production of fertilizers from chicken litter. Applied and Environmental Microbiology *72*:4105-4114.

Etesami, H. and Jeong, B.R., (2021). Contribution of arbuscular mycorrhizal fungi, phosphate–solubilizing bacteria, and silicon to P uptake by plant: a review. *Frontiers in Plant Science*, *12*: p.1355.

Ghaly, F.A., Sarhan, H.M., Abdel-Hamied, A.S. and Mansour, T.M.A., (2020). 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*, *11*:pp.43-48.

Hasanen, G.H.P; I.H. Elsokkary; M.Z. Kamel and A.M. Abd Elsamea (2013). Influence of nitrogen and organic fertilization on growth, yield and quality of sugar beet grown in calcareous soil. J. Plant Production, Mansoura Univ., 4: 733-743.

Hui, L.I., W.T. Feng, X.H. HE, Z.H.U. Ping, H.J. GAO, S.U.N. Nan, and M.G. XU. (2017). 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 16:937-946.

Hussain, I., M. Ijaz, S. Ul-Allah, A. Sattar, A. Sher, A. Nawaz, A. Ghaffar, M.H.U. Rahman, S. Ahmad, I. Rasheed, O. Nasif and M.J. Ansari. (2023). 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.*

Ijaz, M., Hussain, I., Tahir, M., Shahid, M., Ul-Allah, S., Zafar, M., Rasheed, I. and Nawaz, A., (2021). Alternatives to Synthetic Fertilizers. In *Agricultural Waste* (pp. 253-273). Apple Academic Press.

Ijaz, M., Ul-Allah, S., Sattar, A., Sher, A., Hussain, I., and Nawaz, A. (2023). 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*.

Iqbal MA, Saleem AM (2015). Sugar beet potential to beat sugarcane as a sugar crop in Pakistan. AmericanEurasian Journal of Agricultural and Environmental Sciences 15: 36–44.

Liu, J., Qi, W., Li, Q., Wang, S. G., Song, C., and Yuan, X. Z. (2020). Exogenous phosphorus-solubilizing bacteria changed the rhizosphere microbial community indirectly. *3 Biotech* 10:164.

Mubarak, M.U., M. Zahir, S. Ahmad and A. Wakeel. (2016). Sugar beet yield and industrial sugar contents improved by potassium fertilization under scarce and adequate moisture conditions. J.Integrat. Agric., 15:2620-2626.

Nacoon, S., Jogloy, S., Riddech, N., Mongkolthanaruk, W., Kuyper, T. K., and Boonlue, S. (2020). Interaction between phosphate solubilizing bacteria and arbuscular mycorrhizal fungi on growth promotion and tuber inulin content of *Helianthus tuberosus* L. *Sci. Rep.* 10:4916.

Ning, C.C., P.D. Gao, B.Q. Wang, W.P. Lin, N.H. Jiang and K.Z. Cai. (2017). Impacts of chemical fertilizer reduction and organic amendments supplementation on soil nutrient, enzyme activity and heavy metal content. Journal of integrative agriculture *16*:1819-1831.

Olsen SR (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, Washington.

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*: pp.21-38.

Qureschi, M. A., Ahmad, Z. A., Akhtar, N., Iqbal, A., Mujeeb, F., and Shakir, M. A. (2012). Role on phosphate solubilizing bacteria (PSB) in enhancing availability and Promoting cotton growth. *J. Anim. Plant Sci.* 22: 204–210.

Rasul, M., Yasmin, S., Suleman, M., Zaheer, A., Reitz, T., Tarkka, M. T., et al. (2019). Glucose dehydrogenase gene containing phosphobacteria for biofortification of Phosphorus with growth promotion of rice. *Microbiol. Res*. 223, 1–12.

Reddy, G.C., Goyal, R.K., Puranik, S., Waghmar, V., Vikram, K.V. and Sruthy, K.S., (2020). Biofertilizers toward sustainable agricultural development. In *Plant microbe symbiosis* (pp. 115-128). Springer, Cham.

Rezakhani, L., Motesharezadeh, B., Tehrani, M.M., Etesami, H. and Hosseini, H.M., (2019). 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*, *173:* pp.504-513.

Rezakhani, L., Motesharezadeh, B., Tehrani, M.M., Etesami, H. and Hosseini, H.M., (2022). 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*, pp.1-22.

Sashidhar, B., and Podile, A. R. (2010). Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. *J. Appl. Microbiol.* 109: 1–12.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. (1997). Principles and Procedures of Statistics: A Biometric Approach, Third edition McGraw Hill Book Co. Inc., New York, USA.

Subbaiah VV, Asija GK (1956) A rapid procedure for utilization of available nitrogen in soil. Curr Sci 26:258–260.

Tahir M, Khalid U, Ijaz M, Shah GM, Naeem MA, Shahid M, Mahmood K, Ahmad N, Kareem F (2018). 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. Braz J Microbiol 49:15-24.

Uphoff, N. and F.B. Dazzo. (2016). Making rice production more environmentally-friendly. Environments 3(2):12.

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., and Pérez-Clemente, R. M. (2020). Root exudates: from plant to rhizosphere and beyond. *Plant Cell. Rep.* 39, 3–17.

Walkley A, Black IA (1934) An examination of the digestion method for determining soil organic matter and a proposed modifcation of the chromic acid titration method. Soil Sci 1:29–38.

Nelson LB, Heidel H (1952). Soil analysis methods as used in the Iowa. State College Soil Testing Laboratory.