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# Combined Application of Inorganic and Organic Phosphorous with Inoculation of Phosphorus Solubilizing Bacteria Improved Productivity, Grain Quality and Net Economic Returns of Pearl Millet (*Pennisetum glaucum* [L.] R. Br.)

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**Abstract:** Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is a climate-smart cereal crop for environments prone to drought and heat stresses. Pearl millet is cultivated in Pakistan on marginal soils with phosphorus (P) deficiency, which significantly decreases its productivity. Moreover, P fixation in the country's calcareous soils is another major constraint which requires attention. P solubilizing bacteria (PSB) have the potential to improve P availability in the soil. However, the potential of PSB in improving P availability in soil and pearl millet yield has been rarely tested in Pakistan. Therefore, this 2-year field study explored the role of combined application of organic and inorganic P sources along with PSB (i.e., *Bacillus* sp. MN54) inoculation to improve yield-related traits, P use efficiency (PUE), net economic returns and grain quality of pearl millet grown under semi-arid climatic conditions. Phosphorus was applied through inorganic sources, organic sources (farmyard manure) and 50% inorganic sources + 50% organic sources with or without PSB inoculation. In control treatment, pearl millet was grown without P application. The individual and combined application of P from different sources and PSB inoculation significantly improved yield-related traits and PUE of pearl millet. The highest grain yield was observed with combined (50% inorganic + 50% organic) application of P with PSB inoculation. The same treatments resulted in higher iron, zinc, protein and P contents in the grains during both years. Likewise, P application through organic and inorganic sources combined with PSB inoculation improved soil bulk density, fertility and microbial population during both years. The highest economic returns and benefit–cost ratio was recorded for combined P application (50% inorganic + 50% organic) and PSB inoculation. In crux, the combined application of organic and inorganic P fertilizers along with PSB (*Bacillus* sp. MN54) inoculation seemed a feasible approach to enhance productivity, grain quality and net economic returns of pearl millet. Therefore, it is recommended that P should be applied through both organic and inorganic sources combined with PSB inoculation to improve P availability and productivity of pearl millet in Pakistan. The current study has explored the potential of combined P application through organic and inorganic sources along with PSB inoculation. Future studies should focus on the determination of mobilized P with the application of PSB.

**Keywords:** pearl millet; phosphorus solubilizing bacteria; farmyard manure; yield; grain protein contents



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## 1. Introduction

Pearl millet (*Pennisetum glaucum* L.) can be cultivated on a wide range of soil types in tropical and sub-tropical climates. Currently, it is grown in Pakistan, China, India and other south Asian countries. Pearl millet is a rich source of protein, vitamins, energy, minerals and antioxidants such as phenolic acids, carotenoids and phenols [1]. It is also a rich source of phytochemicals and minerals [2,3]. The high nutritional content of pearl millet makes it an effective tool in the fight against hunger and malnutrition [4]. It is a popular crop due to its dual nature, i.e., it can be grown for fodder and grain purposes. It is also a drought-tolerant crop, requires water [5], is pest (insect and disease) resistant and provides effective crop insurance in poor countries [6].

However, grain yield of pearl millet is very low in Pakistan as compared to advanced countries [7,8]. This might be attributed to less use of chemical fertilizer, depleted soil fertility, unavailability of high yielding cultivars and little research on agronomical aspects [9]. Crop productivity and soil health is reduced due to minimum use of organic fertilizer and total reliance on inorganic fertilizer [10]. Low use of organic fertilizer reduces total microbes and organic carbon in soil [11]. It is reported that most of the Pakistani soils have <1% carbon [12]. Slow and irregular nutrient delivery is a major drawback of organic fertilizers, which severely limits their use [13]. Another justification for the sparing use of organic fertilizer is the dependence of nutrient release on the environment and microorganisms. Therefore, it is essential to apply both chemical and organic fertilizers at the same time. This would be a better strategy for nutrient management and soil environmental protection than using either one alone [14].

Utilizing macro- and micronutrients in various cropping systems is crucial for crop development, growth and production [15,16]. Phosphorus (P), one of the macronutrients, is essential for plant development and metabolism. The range of P concentration in plants is between 0.05 and 0.5% by dry weight [16]. In addition to its crucial function in metabolism, P is necessary for development, the production of nuclei, the consumption of starch and sugar, cell division and photosynthesis [17]. After nitrogen, it is rated as one of the two biggest global limiting variables for crop growth and production [18]. Since P is rapidly absorbed or fixed by free lime in some calcareous soils, P nutrition is a key limiting factor for crop productivity on such soils [19]. Phosphorus applied in the form of animal manures, biosolids, compost and mineral fertilizers accumulates in the soil and plants use this to increase biomass and grain yield [20].

Therefore, there is an urgent need to apply organic manures and crop residues to enhance the physiochemical characteristics and overall soil health [21]. Both organic and inorganic fertilizers are used in balanced amounts in integrated nutrient management (INM) to increase crop microbial activity and grain production [22]. Furthermore, the use of chemical fertilizer with manure is crucial since organic manure has no chemical or analytical value [23]. Soil microorganisms and enzyme activity, as well as nutrient availability, may be increased by the strategic application of organic and inorganic fertilizers [24]. A combination of organic and inorganic fertilizers in appropriate proportion is used in INM to achieve an optimal level of microbial activity and increase grain yield [22]. Therefore, farmyard manure and phosphorus solubilizing bacteria (PSB) are applied to soil for sustainable agriculture [25].

These PSBs might be utilized as biofertilizers to boost plant growth and productivity [26,27]. Numerous studies have shown that inoculating seeds with *Bacillus* spp. may enhance the solubilization of fixed P in the soil, leading to increased crop yields and growth-stimulating chemicals that contribute differentially to the productivity and development of different crops [28,29]. The use of PSBs enhanced soil P availability by 30% [30]. A significant increase in the yield of sunflower (*Helianthus annuus* L.) and rice (*Oryza sativa* L.) has been recorded when various inorganic P fertilizes and different PSBs were applied together [31,32]. Similarly, simultaneous application of PSB and rock phosphate significantly improved P nutrition in both cereal and legume crops [33,34].

Khan et al. [28] reported that, if fixed P in the soil becomes bioavailable, no additional P would be required for almost 100 years. The use of PSB in the soil is an environmentally friendly alternative to the use of fixed P applied through mineral fertilizers. In the soil, PSB inoculation secretes protons, and organic and phenolic acids which acidify soil and increase P availability [32]. The benefits of PSB inoculation are not consistent in various soil and climatic condition [27]. Moreover, inoculation of *Bacillus* sp. MN54 with farmyard manure in pearl millet crop was not reported previously in current agro-climatic conditions. Therefore, we assume that PSB inoculation with farmyard manure would be a sustainable fertilizer for improving the productivity of pearl millet.

Phosphorus application in combination with PSB inoculation has been tested in various crops. However, there is currently no information available on the effects of combining organic and inorganic sources with PSB strains on pearl millet production. Therefore, the primary goals of this research were to evaluate how the PSB combined with organic and inorganic sources affects pearl millet growth, yield, PUE and net returns under a semi-arid climate. It was hypothesized that the combined application of PSB and different sources of P would increase the growth, grain yield, quality, productivity and net economic returns of pearl millet, along with improved soil fertility.

## 2. Materials and Methods

### *2.1. Experimental Site*

Field studies were conducted at the Agronomic Research Farm, Bahauddin Zakariya University (BZU) Multan (30.2° N, 71.43° E, and 122 m above sea level), Pakistan during 2020 and 2021. Weather data during crop cycle of both years is given in Figure 1. Before sowing for the experiment in each study year, soil physical and chemical properties were determined through standard procedures and obtained results are summarized in Table 1. The soil of the experimental site belonged to the Sindhlianwali soil series (fine silty, mixed, hyperthermic, sodichaplocambids in USDA classification).

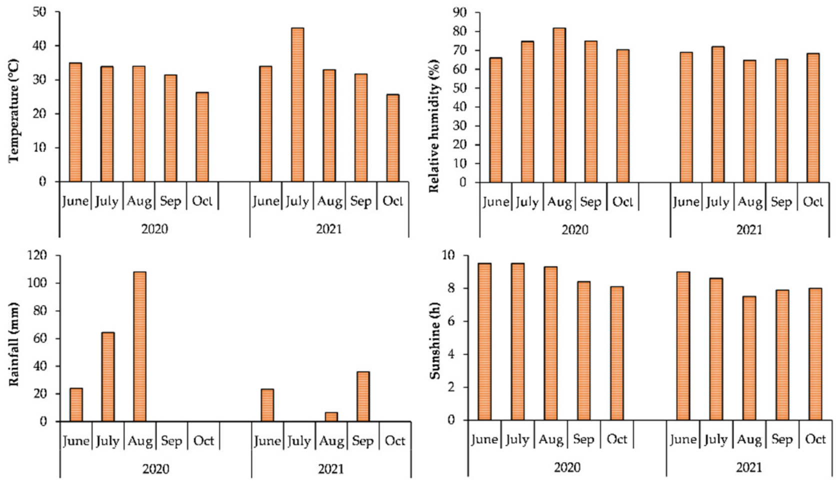


**Figure 1.** Weather data of the experimental site during 2020 and 2021. Source: Pakistan central cotton committee (PCCC), Multan.

**Table 1.** Soil physical and chemical properties of experiment location prior to the initiation of experiment in each year.

| Soil Properties | 2020 | 2021 |
|---|---|---|
| Soil texture | Sandy loam | Sandy loam |
| EC | 2.22 dS $m^{-1}$ | 2.23 dS $m^{-1}$ |
| pH | 8.3 | 8.32 |
| Organic matter | 0.49% | 0.53% |
| Available phosphorus | 6.40 mg $kg^{-1}$ | 6.33 mg $kg^{-1}$ |
| Total nitrogen | 0.03% | 0.03% |
| Available potassium | 110 mg $kg^{-1}$ | 105 mg $kg^{-1}$ |

### *2.2. Treatment Details*

The experiment was laid out according to randomized complete block design (RCBD) with factorial arrangements. There were two factors (PSBs and P sources) and three replications. The PSBs had two levels, i.e., $B_1$ = no PSB inoculation and $B_2$ = PSB inoculation. The P factor had four levels, i.e., $P_1$ = no P application, $P_2$ = P application through inorganic sources, $P_3$ = P application through organic sources (farmyard manure) and $P_4$ = P application by 50% inorganic sources + 50% organic sources. The bacterial strain (*Bacillus* sp. MN54) was used as PSB inoculant. The PSB strain *Bacillus* sp. MN54 (Accession no. KT375574) used in this study was obtained from the Soil and Environmental Microbiology Laboratory, University of Agriculture, Faisalabad, Pakistan (where it was identified for the first time). The optical density of inoculum was adjusted to $10^9$ cfu per mL before seed inoculation. This strain has already been used for enhancing growth and yield of various crops [35].

### 2.3. Crop Husbandry

Before sowing of crop, pre-soaking, locally called '*rouni*', irrigation of ~100 mm depth was applied during both years. When moisture level reached a workable level, field was cultivated to create a fine seedbed. Pearl millet was sown on 19 June 2020 and 21 June 2021 with row × row and plant × plant distance of 45 and 15 cm, respectively. Pearl millet variety 'MP-24' purchased from Mercury Seeds Private Ltd., Sahiwal, Pakistan was used in the study. Seed rate was kept at 10 kg $ha^{-1}$ and net plot size was 1.8 m × 4 m. The bacteria were cultured in a 250 mL Erlenmeyer flask which contained tryptic soy broth medium. The optimum density of 0.5 was maintained using spectrophotometer at 600 nm. The optimum population ($10^8$–$10^9$ CFU $mL^{-1}$) of bacteria was obtained for seed inoculation. The Arabic gum was used as adherent for seed inoculation. After inoculation, seeds were sown with a manual drill. Diammonium phosphate (DAP) containing 46% P and 18% N was used as source of inorganic fertilizer, while farm manure (FM) was used as organic source. Inorganic P was applied at 90 kg $ha^{-1}$. The FM contained 2.75% nitrogen, 0.89% $P_2O_5$ and 1.21% $K_2O$. Nitrogen was applied at a rate of 100 kg $ha^{-1}$ in three intervals, while whole amount of FM and P were applied at the time of sowing. Thinning was done 20 days after sowing to maintain plant to plant distance. Six irrigations were applied to avoid moisture stress. Weeds were controlled manually. Finally, crop was harvested when pearl millet reached harvest maturity on 5 October 2020 and 8 October 2021 during the 1st and 2nd year, respectively.

### 2.4. Data Recorded

#### 2.4.1. Soil Nutrient Analysis

Three random soil samples were taken using core sampler at the depth of 15 cm from each plot after crop harvest to record soil physical properties during each year of the experiment. The samples were air dried and then analyzed for soil properties, such as available phosphorus (mg $kg^{-1}$) (Olsen, spectrophotometer), C-organic (mg $kg^{-1}$), [36] (spectrophotometer), and total nitrogen (mg $kg^{-1}$) (Kjeldahl Method, spectrophotometer). Soil bulk density (BD, g $cm^{-3}$) was recorded using the formula given by Walkley & Black [37] as:

$$\mathrm{BD} = \frac{\mathrm{m}}{\mathrm{v}}$$

Here, BD = bulk density, m = mass of oven-dried soil sample and v = volume of soil with pore space

The soil porosity (%) was recorded following Blake's [38] formula given below:

$$\text{Total porosity} = 1 - (\mathrm{BD}/\mathrm{PD})$$

Here, BD = bulk density and PD = particle density

#### 2.4.2. Soil Microbial Population

The PSBs were isolated from the experimental site using the agar pour plate technique. Ten grams of soil samples were disseminated in ninety milliliters of distilled water and vigorously mixed. A $10^{-2}$ dilution was created by adding a 1-mL aliquot to 9 mL of distilled water and stirred for 10 s. Using the same technique, serial dilutions up to $10^{-7}$ were made. A 0.1-mL aliquot of each serial dilution was placed in Pikovskayas agar medium (50 °C). This was followed by a seven-day incubation at 27–30 °C. The composition of the medium was 0.5 g $(NH_4)2SO_4$, 5 g $Ca_3PO_4$, 0.2 g KCl, 0.2 g NaCl 10 g glucose, 0.5 g yeast extract, 0.1 g $MgSO_4.7H_2O$, 20 g agar and 1000 mL water [39]. Colonies which have transparent halos were termed PSB colonies [40,41]. This formula was used: CFU $g^{-1}$ = number of colonies multiplied by the dilution factor [42]. Bergey's manual was utilized to identify P-solubilizing microorganisms [43].

#### 2.4.3. Yield Related Parameters

Ten random plants were selected to record data of plant height at maturity (cm) and number of grains per ear. Plant height was measured with the help of measuring tape. Ten different samples were used to compute grains per ear. The 1000-grain weight from each plot was recorded by counting seeds and weighing them on an electric weight balance. The plants were sundried in the field for 4 days after harvesting to record biological yield (tons $ha^{-1}$). After recording biological yield, ears were manually threshed, grains were separated and weighed using a digital balance to record grain yield. Harvest index was counted as ratio of grain yield to biological yield expressed as a percentage.

#### 2.4.4. Grain Nutrient Analysis

Grain protein was determined by formula: Protein (%) = N content (%) × 6.25. The N content was measured according to Krieg & Holt [44]. Grain iron, zinc and P contents were measured with the wet digestion Di-acid ($HNO_3$+ $HClO_4$) method. After digestion, Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Tokyo, Japan) was used for analyzing grain iron and zinc contents. Phosphorus contents in the seeds were determined calorimetrically. A wet oxidation procedure was used for determination of nitrogen according to Parkinson and Allen [45].

#### 2.4.5. Phosphorus Use Efficiency (PUE)

After measuring P content in grains, PUE in grain was calculated by using the following formula given by AOAC [46]:

$$\text{PUE} = \frac{\text{Grain yield of P fertilized plots} - \text{Grain yield of P unfertilized plots}}{Quantity\ of\ P\ applied}$$

### *2.5. Statistical and Economic Analysis*

Data collected in both experiments were analyzed by Fisher's analysis of variance (two-way ANOVA) technique and treatment means were compared at 95% probability level by least significance difference (LSD) test [47]. As the year effect was significant, data were analyzed separately for both years. Similarly, graphical presentation of data relating to nutrient dynamics, and allometric traits was performed using MS-Excel Program 2013 along with ± standard error (S.E.). Economic analysis was carried out to test the economic efficiency of pearl millet grown with various PSB combined with different P sources.

For economic analysis, production costs (seedbed preparation, land rent, sowing, irrigation, inputs and harvesting of crop) were calculated. Gross income was subtracted from all expenses to compute net income. For benefit–cost ratio (BCR), gross income was divided by cost incurred in crop production [48]. Only significant interactions were reported and interpreted in the manuscript.

## 3. Results

### *3.1. Yield and Related Traits*

Different phosphorus (P) sources, inoculation of P-solubilizing bacteria (PSB) and their interaction (P × PSB) had significant effect on all yield-related traits of pearl millet during both years. Number of grains per ear and 1000-grain weight were significantly affected by different P sources and PSB inoculation (Table 2). Combined application of 50% inorganic + 50% organic P with PSB inoculation significantly improved number of grains per ear and 1000-grain weight compared to the control treatment.

**Table 2.** Effects of phosphorus application through organic and inorganic sources and inoculation of PSB on yield-related traits of pearl millet during 2020 and 2021.

| Phosphorus Sources | Number of Grains Per Ear | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | No-PSB | PSB | Means | No-PSB | PSB | Means |
| No P | 3117 h | 3199 f | 3158 D | 3161 g | 3248 e | 3204 D |
| IP (DAP) | 3216 e | 3285 b | 3250 B | 3259 d | 3350 b | 3304 B |
| OP (FM) | 3163 g | 3258 d | 3210 C | 3206 f | 3309 c | 3257 C |
| 50% IP + 50% OP | 3277 c | 3349 a | 3312 A | 3308 c | 3402 a | 3355 A |
| Means | 3193 B | 3273 A | | 3233 B | 3327 A | |
| LSD at 5% for P = 3.01, PSB = 2.13 and P × PSB = 4.25 | | | | LSD at 5% for P = 4.54, PSB = 3.21 and P × PSB = 6.42 | | |
| | 1000-grain weight (g) | | | | | |
| No P | 6.02 f | 6.15 e | 6.09 D | 6.04 e | 6.18 d | 6.11 D |
| IP (DAP) | 6.26 c | 6.33 b | 6.30 B | 6.27 c | 6.34 b | 6.31 B |
| OP (FM) | 6.16 e | 6.23 d | 6.20 C | 6.18 d | 6.25 c | 6.22 C |
| 50% IP + 50% OP | 6.33 b | 6.41 a | 6.37 A | 6.35 b | 6.45 a | 6.40 A |
| Means | 6.19 B | 6.28 A | | 6.21 B | 6.31 A | |
| LSD at 5% for P = 0.02, PSB = 0.01 and P × PSB = 0.02 | | | | LSD at 5% for P = 0.01, PSB = 0.01 and P × PSB = 0.02 | | |

Means followed by the same letter within a column or row are statistically similar at $P > 0.05$. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, and PSB = inoculation of P solubilizing bacteria.

Grain and biological yields were significantly altered by individual and interactive effects of different P sources and PSB inoculation during both years (Table 3). Combined application of 50% inorganic + 50% organic P significantly improved grain and biological yields. Higher grain and biological yields were observed with PSB inoculation than no inoculation with PSB. Similarly, combined application of 50% inorganic + 50% organic P with PSB inoculation significantly improved grain and biological yields compared to control. Moreover, harvest index was significantly altered by different P sources and PSB inoculation, while their interaction was non-significant during 2nd year (Table 4).

**Table 3.** Effects of phosphorus application through organic and inorganic sources and inoculation of PSB on grain- and biological yield of pearl millet during 2020 and 2021.

| Phosphorus Sources | Grain Yield (t ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | No-PSB | PSB | Means | No-PSB | PSB | Means |
| No P | 2.96 h | 3.11 g | 3.04 D | 2.95 f | 3.18 e | 3.07 D |
| IP (DAP) | 3.29 e | 3.49 b | 3.39 B | 3.23 d | 3.46 b | 3.34 B |
| OP (FM) | 3.15 f | 3.32 d | 3.24 C | 3.16 e | 3.28 c | 3.22 C |
| 50% IP + 50% OP | 3.38 c | 3.62 a | 3.50 A | 3.29 c | 3.68 a | 3.49 A |
| Means | 3.20 B | 3.38 A | | 3.16 B | 3.40 A | |

**Table 3.** *Cont.*

| Phosphorus Sources | Grain Yield (t ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | No-PSB | PSB | Means | No-PSB | PSB | Means |
| LSD at 5% for P = 0.02, PSB = 0.01 and P × PSB = 0.02 | | | | LSD at 5% for P = 0.03, PSB = 0.02 and P × PSB = 0.04 | | |
| | Biological yield (t ha$^{-1}$) | | | | | |
| No P | 10.51 g | 14.24 d | 12.38 D | 11.13 g | 14.64 d | 12.89 D |
| IP (DAP) | 13.36 e | 16.50 b | 14.93 B | 13.83 e | 17.40 b | 15.61 B |
| OP (FM) | 11.90 f | 15.21 c | 13.56 C | 12.47 f | 15.37 c | 13.92 C |
| 50% IP + 50% OP | 14.40 d | 18.33 a | 16.37 A | 14.31 de | 18.74 a | 16.53 A |
| Means | 12.54 B | 16.07 A | | 12.94 B | 16.54 A | |
| LSD at 5% for P = 0.25, PSB = 0.18 and P × PSB = 0.36 | | | | LSD at 5% for P = 0.42, PSB = 0.29 and P × PSB = 0.59 | | |

Means followed by the same letter within a column or row are statistically similar at P > 0.05. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, and PSB = inoculation of P solubilizing bacteria.

**Table 4.** Effects of phosphorus application through organic and inorganic sources and inoculation of PSB on harvest index and P content in grains of pearl millet during 2020 and 2021.

| Phosphorus Sources | Harvest Index (%) | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | No-PSB | PSB | Means | No-PSB | PSB | Means |
| No P | 28.21 a | 21.82 e | 25.01 A | 26.54 | 21.76 | 24.15 A |
| IP (DAP) | 24.65 c | 21.18 f | 22.92 C | 23.34 | 19.89 | 21.62 C |
| OP (FM) | 26.49 b | 21.83 e | 24.16 B | 25.32 | 21.32 | 23.32 B |
| 50% IP + 50% OP | 23.50 d | 19.73 g | 21.61 D | 23.02 | 19.69 | 21.36 C |
| Means | 25.71 A | 21.14 B | | 24.56 A | 20.67 B | |
| LSD at 5% for P = 0.39, B = 0.28 and P × B = 0.56 | | | | LSD at 5% for P = 0.72, B = 0.51 and P × B = 1.02 | | |
| | Phosphorus in Grain (%) | | | | | |
| No P | 0.81 | 0.96 | 0.89 C | 0.86 f | 0.94 de | 0.90 D |
| IP (DAP) | 0.96 | 1.09 | 1.02 B | 0.96 d | 1.06 b | 1.01 B |
| OP (FM) | 0.87 | 0.94 | 0.90 C | 0.93 e | 0.99 c | 0.96 C |
| 50% IP+ 50% OP | 1.10 | 1.26 | 1.18 A | 1.06 b | 1.17 a | 1.11 A |
| Means | 0.93 B | 1.06 A | | 0.95 B | 1.04 A | |
| LSD at 5% for P = 0.04, B = 0.03 and P × B = NS | | | | LSD at 5% for P = 0.02, B = 0.01 and P × B = 0.03 | | |

Means followed by the same letter within a column or row are statistically similar at P > 0.05. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, PSB = inoculation of P solubilizing bacteria, and NS = non-significant.

### *3.2. Grain Quality and Phosphorus Use Efficiency*

Various P sources, PSB inoculation and their interaction had significant effect on P uptake in grain and phosphorus use efficiency (PUE) during each year (Tables 4 and 5) The highest P uptake and PUE were observed in combined application of inorganic and organic P with PSB inoculation, while the lowest PUE was noted for the application of organic P with no PSB inoculation. The application of different P sources with PSB inoculation significantly improved protein, iron (Fe) and zinc (Zn) concentration in pearl millet seeds (Tables 5 and 6). The P sources by PSB inoculation interaction had significant effect on all quality parameters; but proved non-significant for protein during 1st year. The highest values for protein, Fe and Zn contents were observed in combined application of 50% organic and 50% inorganic fertilizer with PSB inoculation, while the lowest values were recorded for no P application by no PSB inoculation interaction.

**Table 5.** Effects of phosphorus application through organic and inorganic sources and inoculation of PSB on PUE and grain quality of pearl millet during 2020 and 2021.

| Phosphorus Sources | PUE | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | **No-PSB** | **PSB** | **Means** | **No-PSB** | **PSB** | **Means** |
| No P | - | - | - | - | - | - |
| IP (DAP) | 3.67 d | 4.29 c | 3.98 B | 3.04 c | 3.07 c | 3.06 B |
| OP (FM) | 2.11 e | 2.37 e | 2.24 C | 2.26 d | 1.04 e | 1.65 C |
| 50% IP + 50% OP | 4.67 b | 5.67 a | 5.17 A | 3.78 b | 5.63 a | 4.71 A |
| Means | 2.61 B | 3.08 A | | 2.27 $^{NS}$ | 2.44 | |
| LSD at 5% for P = 0.22, PSB = 0.16 and P × PSB = 0.31 | | | LSD at 5% for P = 0.12, PSB = NS and P × PSB = 0.28 | | | |
| | **Protein (g/100 g)** | | | | | |
| No P | 8.23 | 9.12 | 8.68 D | 8.57 f | 9.36 cd | 8.97 C |
| IP (DAP) | 8.73 | 9.55 | 9.14 B | 9.22 e | 9.38 bc | 9.30 B |
| OP (FM) | 8.41 | 9.35 | 8.88 C | 8.53 f | 9.24 de | 8.89 C |
| 50% IP + 50% OP | 9.42 | 10.40 | 9.91 A | 9.51 b | 10.19 a | 9.85 A |
| Means | 8.70 B | 9.60 A | | 8.96 B | 9.55 A | |
| LSD at 5% for P = 0.14, PSB = 0.09 and P × PSB = NS | | | LSD at 5% for P = 0.09, PSB = 0.07 and P × PSB = 0.13 | | | |

Means followed by the same letter within a column or row are statistically similar P > 0.05. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, and PSB = inoculation of P solubilizing bacteria.

**Table 6.** Effects of phosphorus application through organic and inorganic sources and inoculation of phosphorus solubilizing bacteria on grain quality of pearl millet during 2020 and 2021.

| Phosphorus Sources | Iron (mg kg$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | **No-PSB** | **PSB** | **Means** | **No-PSB** | **PSB** | **Means** |
| No P | 38.09 f | 42.86 e | 40.48 D | 38.36 f | 43.21 e | 40.78 D |
| IP (DAP) | 46.62 d | 54.18 ab | 50.40 B | 46.86 d | 54.89 ab | 50.88 B |
| OP (FM) | 41.08 e | 52.19 bc | 46.63 C | 41.35 e | 52.63 bc | 46.99 C |
| 50% IP + 50% OP | 50.16 c | 55.88 a | 53.02 A | 50.36 c | 56.29 a | 53.33 A |
| Means | 43.99 B | 51.27 A | | 44.23 B | 51.75 A | |
| LSD at 5% for P = 1.67, B = 1.18 and P × B = 2.36 | | | LSD at 5% for P = 1.81, B = 1.28 and P × B = 2.56 | | | |
| | **Zinc (mg kg$^{-1}$)** | | | | | |
| No P | 22.1 f | 27.3 d | 24.7 D | 24.5 e | 29.3 c | 26.9 D |
| IP (DAP) | 28.3 c | 31.2 b | 29.7 B | 30.0 c | 31.5 b | 30.8 B |
| OP (FM) | 25.7 e | 28.0 cd | 26.9 C | 27.7 d | 30.0 c | 28.9 C |
| 50% IP+ 50% OP | 30.9 b | 34.5 a | 32.7 A | 31.5 b | 36.5 a | 34.0 A |
| Means | 26.8 B | 30.2 A | | 28.5 B | 31.8 A | |
| LSD at 5% for P = 0.58, B = 0.41 and P × B = 0.83 | | | LSD at 5% for P = 0.69, B = 0.49 and P × B = 0.97 | | | |

Means followed by the same letter within a column or row are statistically similar at P > 0.05. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, and PSB = inoculation of P solubilizing bacteria.

### *3.3. Soil-Related Parameters*

Different P sources had significant effect on soil porosity and bulk density, while PSB inoculation and interaction (P × PSB) had non-significant effect on soil porosity and bulk density during both years (Table 7). The highest soil porosity was recorded in control treatment, which was at par with inorganic P application, while the lowest value was recorded for the application of organic P. The highest bulk density was recorded in organic P followed by combined application of organic and inorganic P, whereas the lowest bulk density was recorded for control treatment.

**Table 7.** Effects of phosphorus application through organic and inorganic sources and inoculation of PSB on soil porosity and soil bulk density after pearl millet harvest during 2020 and 2021.

| Phosphorus Sources | Soil Porosity (%) | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | No-PSB | PSB | Means | No-PSB | PSB | Means |
| No P | 34.33 | 34.36 | 34.35 A | 34.42 | 34.45 | 34.44 A |
| IP (DAP) | 34.71 | 35.17 | 34.94 A | 34.79 | 35.26 | 35.03 A |
| OP (FM) | 30.47 | 30.29 | 30.38 C | 30.56 | 30.39 | 30.47 C |
| 50% IP + 50% OP | 32.74 | 32.38 | 32.56 B | 32.83 | 32.47 | 32.65 B |
| Means | 33.06 | 33.05 | | 33.15 | 33.14 | |
| LSD at 5% for P = 0.68, PSB = 0.48 and P × PSB = NS | | | | LSD at 5% for P = 0.68, PSB = 0.48 and P × PSB = NS | | |
| | Bulk density (g $cm^{-3}$) | | | | | |
| No P | 1.36 | 1.35 | 1.35 C | 1.43 | 1.42 | 1.42 C |
| IP (DAP) | 1.35 | 1.35 | 1.35 C | 1.42 | 1.42 | 1.42 C |
| OP (FM) | 1.46 | 1.48 | 1.47 A | 1.53 | 1.55 | 1.54 A |
| 50% IP + 50% OP | 1.43 | 1.44 | 1.44 B | 1.50 | 1.51 | 1.51 B |
| Means | 1.40 | 1.40 | | 1.47 | 1.47 | |
| LSD at 5% for P = 0.02, PSB = NS and P × PSB = NS | | | | LSD at 5% for P = 0.02, PSB = NS and P × PSB = NS | | |

Means followed by the same letter within a column or row are statistically similar P > 0.05. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, and PSB = inoculation of P solubilizing bacteria.

The individual and interactive effect of P sources and PSB inoculation had significant effect on organic carbon (Table 8). Different P sources had significant effect on total nitrogen, while PSB inoculation and interaction among P sources and PSB inoculation had non-significant effect (Table 8). The interaction among P sources and PSB inoculation indicated that the highest organic carbon was observed in control treatment followed by organic P application, while the lowest value was noted for inorganic P application. The highest soil nitrogen was observed in organic P application, while the lowest was recorded in control treatment.

Individual and interactive effects of various P sources and PSB had significant effect on total available P and microbial population during both years (Table 9). Combined application of 50% inorganic + 50% organic P with PSB inoculation resulted in the higher total available P and soil microbial population than control.

**Table 8.** Effects of phosphorus application through organic and inorganic sources and inoculation of PSB on soil organic carbon and soil total nitrogen after pearl millet harvest during 2020 and 2021.

| Phosphorus Sources | Soil Organic Carbon (%) | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | No-PSB | PSB | Means | No-PSB | PSB | Means |
| No P | 0.62 ab | 0.67 a | 0.64 A | 0.69 ab | 0.74 a | 0.71 A |
| IP (DAP) | 0.39 c | 0.59 ab | 0.49 C | 0.46 c | 0.66 ab | 0.56 C |
| OP (FM) | 0.61 ab | 0.61 ab | 0.61 AB | 0.68 ab | 0.68 ab | 0.68 AB |
| 50% IP + 50% OP | 0.58 ab | 0.54 b | 0.56 B | 0.65 ab | 0.61 b | 0.63 B |
| Means | 0.55 B | 0.60 A | | 0.62 B | 0.67 A | |
| LSD at 5% for P = 0.07, PSB = 0.05 and P × PSB = 0.09 | | | | LSD at 5% for P = 0.07, PSB = 0.05 and P × PSB = 0.09 | | |
| | Soil total nitrogen (%) | | | | | |
| No P | 0.02 | 0.03 | 0.03 B | 0.03 | 0.03 | 0.03 C |
| IP (DAP) | 0.02 | 0.04 | 0.03 B | 0.03 | 0.04 | 0.03 C |
| OP (FM) | 0.06 | 0.06 | 0.06 A | 0.05 | 0.06 | 0.06 A |
| 50% IP + 50% OP | 0.04 | 0.05 | 0.05 A | 0.04 | 0.05 | 0.05 B |
| Means | 0.04 | 0.05 | | 0.04 | 0.05 | |
| LSD at 5% for P = 0.02, PSB = NS and P × PSB = NS | | | | LSD at 5% for P = 8.05, PSB = NS and P × PSB = NS | | |

Means followed by the same letter within a column or row are statistically similar at P > 0.05. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, and PSB = inoculation of P solubilizing bacteria.

**Table 9.** Effects of phosphorus application through organic and inorganic sources and inoculation of PSB on soil total phosphorus and microbial population during 2020 and 2021.

| Phosphorus Sources | Total Available Phosphorus (mg kg$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 2020 | | | 2021 | | |
| | No-PSB | PSB | Means | No-PSB | PSB | Means |
| No P | 17.74 f | 19.27 e | 18.51 D | 17.22 g | 19.13 f | 18.18 D |
| IP (DAP) | 22.38 c | 23.62 b | 23.00 B | 21.56 d | 23.28 b | 22.42 B |
| OP (FM) | 20.48 d | 22.77 c | 21.63 C | 19.54 e | 22.85 c | 21.20 C |
| 50% IP + 50% OP | 23.64 b | 24.85 a | 24.25 A | 22.87 c | 24.66 a | 23.76 A |
| Means | 21.06 B | 22.63 A | | 20.30 B | 22.48 A | |
| LSD at 5% for P = 0.38, PSB = 0.27 and P × PSB = 0.54 | | | | LSD at 5% for P = 0.16, PSB = 0.11 and P × PSB = 0.23 | | |
| | Soil microbial population (10$^3$ CFU g$^{-1}$) | | | | | |
| No P | 40.33 f | 124.3 d | 82.33 D | 54.67 e | 154.0 c | 104.3 C |
| IP (DAP) | 42.00 f | 130.7 c | 86.33 C | 50.00 f | 156.0 c | 103.0 C |
| OP (FM) | 52.00 e | 145.3 b | 98.67 B | 62.33 d | 173.0 b | 117.7 B |
| 50% IP + 50% OP | 51.00 e | 157.0 a | 104.0 A | 63.00 d | 185.0 a | 124.0 A |
| Means | 46.33 B | 139.33 A | | 57.50 B | 167.0 A | |
| LSD at 5% for P = 1.56, PSB = 1.11 and P × PSB = 2.21 | | | | LSD at 5% for P = 2.61, PSB = 1.84 and P × PSB = 3.69 | | |

Means followed by the same letter within a column or row are statistically similar at P > 0.05. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, and PSB = inoculation of P solubilizing bacteria.

### 3.4. Economic Analysis

Individual and interactive effects of different P sources and PSB inoculation substantially improved the net economic benefits and benefit-cost ratio (BCR) of pearl millet during both years of study (Table 10). The highest net economic benefits and BCR were recorded in combined application of P (50% organic + 50% inorganic) with PSB inoculation, while control treatment resulted in the lowest economic benefits and BCR (Table 10).

**Table 10.** Effects of phosphorus application through organic and inorganic sources and inoculation of PSB on economic returns and benefit cost ratio during 2020 and 2021.

| Treatments | | 2020 | | | | 2021 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total Cost (USD ha$^{-1}$) | Gross Income (USD ha$^{-1}$) | Net Income (USD ha$^{-1}$) | BCR | Total Cost (USD ha$^{-1}$) | Gross Income (USD ha$^{-1}$) | Net Income (USD ha$^{-1}$) | BCR |
| No-PSB | No P | 721.6 | 1471.6 | 749.6 | 2.0 | 737.9 | 1786.5 | 1048.6 | 2.4 |
| | IP (DAP) | 758.6 | 1672.6 | 914.0 | 2.2 | 774.2 | 1997.7 | 1223.5 | 2.6 |
| | OP (FM) | 737.9 | 1581.9 | 844.0 | 2.1 | 737.9 | 1925.3 | 1187.4 | 2.6 |
| | 50% IP + 50% OP | 736.9 | 1733.1 | 996.6 | 2.4 | 784.7 | 2044.4 | 1259.7 | 2.6 |
| PSB | No P | 732.7 | 1613.9 | 881.3 | 2.2 | 748.9 | 1998.2 | 1249.3 | 2.7 |
| | IP (DAP) | 769.7 | 1825.3 | 1055.7 | 2.4 | 785.9 | 2212.8 | 1427.6 | 2.8 |
| | OP (FM) | 748.9 | 1724.4 | 975.5 | 2.3 | 748.9 | 2065.0 | 1316.1 | 2.8 |
| | 50% IP + 50% OP | 747.6 | 1917.3 | 1169.7 | 2.6 | 795.7 | 2365.1 | 1569.4 | 3.0 |

1 USD = 227 Pakistani Rupees. Here: P = phosphorus, IP = inorganic P, DAP = di-ammonium phosphate, OP = organic P, FM = farm manure, No-PSB = no inoculation of P solubilizing bacteria, and PSB = inoculation of P solubilizing bacteria.

## 4. Discussion

This two-year field study revealed that P application from different sources with the inoculation of PSB significantly improved yield-related traits, soil fertility status and net economic returns of pear millet (Tables 3–10). The increase in yield-related traits was higher in combined application of P through organic and inorganic fertilizer coupled with PSB inoculation. Similarly, previous research revealed that organic and inorganic fertilizers used in balance quantity can improve grain yield [22]. The PSB inoculation improved grains per ear, 1000-grain weight, and grain and biological yields (Tables 2 and 3). The increase in grain yield was due to solubilization of fixed P in the soil. Inoculation of PSB in seed or soil increased solubilization of applied and fixed P resulting in better yield [49]. Mehrvarz et al. [50] reported that PSB inoculation develops extended network of roots which help the plants to capture P from wider area. As a result, PSB show significant beneficial outcomes in conventional soils applied alone or combined with other microorganisms [51]. Several studies have shown that inoculation of *Bacillus* spp. can solubilize fixed and applied P, resulting in higher productivity of different crops [28,29]. The FM, being the poor P source [52], cannot improve the plant growth when applied alone. Therefore, low yield was observed in the treatments receiving FM only. Previous studies reported that combined application of FM with mineral fertilizer resulted in the highest yield of sunflower crop due to increased mobilization of fixed P [53].

The results revealed that combined application of 50% organic + 50% inorganic P with PSB inoculation improved P content in grain and PUE (Tables 4 and 5). The highest values for P uptake and PUE were due to higher P availability. Similar results have been reported for mung bean where *Bacillus* inoculant was applied and increased grain yield and P-uptake [54]. The application of PSB increased soil P availability by nearly 30% which resulted in higher P uptake and PUE [30] as observed in this study. The inoculation of PSB significantly improved PUE with the availability of fixed P in the soil. Since P fertilizer is rapidly immobilized in the soil, microbial inoculation is necessary for adequate P availability to plants [55].

The application of P through different sources and PSB inoculation significantly improved grain quality such as protein, Fe and Zn in grains (Tables 5 and 6). The improvement in grain quality was due to availability of more P and favorable soil environment. The PSB inoculation altered P availability in soil which resulted in more P nutrition. The P is the part of nucleic acid which is the key component for protein synthesis. The P regulates enzymatic activities and signaling events through protein phosphorylation and de-phosphorylation pathways [56]. Plants absorb P that has been provided to the soil in the form of animal manures, biosolids, compost and mineral fertilizers to improve biomass and grain quality [20].

The application of FM with inorganic P and PSB inoculation significantly improved soil health, i.e., bulk density and soil fertility (Table 7). The improvement in soil health was due to PSB inoculation and FM application. Therefore, application of organic manures and crop residues is direly needed to improve physiochemical properties and soil health [21]. The PSB have shown the capacity to recover the unproductive and slightly unproductive soils which by improving the soil quality [57]. The combined application of organic + inorganic P with PSB inoculation significantly improved soil organic carbon in soil, while PSB inoculation and P sources' interaction was non-significant for nitrogen (Table 8). Soil microbial population and available P also increased due to PSB inoculation combined with organic and inorganic fertilizers (Table 9). In addition, soil nutrients availability was increased due to increased microbial activity from FM application [58,59]. Increased microbial activity helped to mobilize nutrients. Several factors, such as microorganisms, pH, presence of cations and organic matter, influenced the availability of P [60].

## 5. Conclusions

Our results provide valuable insights indicating that P application through organic and inorganic sources in equal proportion combined with PSB inoculation can improve pearl millet productivity and economic returns compared to the sole application of each P source. Phosphorus application through organic and inorganic sources along with inoculation of PSB *Bacillus* sp. strain MN-54 considerably enhanced grain yield, quality, soil fertility and PUE of pearl millet. Similarly, combined application of 50% inorganic + 50% organic P along with PSB inoculation improved grain yield P uptake. The highest P availability was observed in combined application of inorganic and organic P application, while the lowest P availability and grain yield were observed in sole application of organic P. Therefore, P application through organic and inorganic sources combined with PSB inoculation is a feasible option to increase productivity, economic returns, grain P concentration and PUE of pearl millet. Based on the results, it is recommended that P application in pearl millet should be applied through organic and inorganic sources and combined with PSB inoculation to improve productivity and economic returns. Future studies must focus on the amount of P mobilized by the application of PSB in pearl millet.

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## Abbreviations

| Abbreviation | Description |
|---|---|
| P | Phosphorus |
| PSB | Phosphorus solubilizing bacteria |
| CS | Calcareous soils |
| Fe | Iron |
| Al | Aluminum |
| AS | Acid soils |
| INM | Integrated nutrient management |
| DAP | Di-ammonium phosphate |
| FM | Farmyard manure |
| IP | Inorganic phosphorus |
| OP | Organic phosphorus |
| Zn | Zinc |
| N | Nitrogen |
| PUE | Phosphorus use efficiency |
| LSD | Least significant difference |
| BD | Bulk density |

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