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Improvement in Soil Characteristics of Sandy Loam Soil and Grain Quality of Spring Maize by Using Phosphorus Solublizing Bacteria

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Abstract: Unavailability of balanced nutrients in nutrient-deficient soils is the key reason in reduced yields of spring maize. After application to soil, most of the phosphorus (80–90%) is lost in the environment because of runoff losses and chemically bonding. So, this makes the phosphorus unavailable for plant use. However, soil microorganisms may provide a biological rescue system which is able to solubilize the soil-bound phosphorus (*p*). Keeping this in view, the present study is designed to meet the following objectives; (1) to improve physico-chemical properties of soil (e.g., soil water retention, soil enzyme activities), and (2) to improve growth and yield of spring maize (cv. Hybrid YSM-112) through the inoculation of phosphorus solubilization bacteria (PSB). A pot experiment was carried out with the following treatments; T1: control (uninoculated control, CT), T2: inoculation with PSB (*Enterobacter sakazakii* J129), T3: recommend level of NPK fertilizers (RNPK), T4: PSB + RNPK fertilizers, T5: rock phosphate (RP), T6: PSB + RP. Results showed that the addition of PSB together with RNPK improved the yield and yield-related characteristics of spring maize grown in sandy soil. Moreover, it also enhanced dry mater characteristics and maize grain quality. Soil fertility in the context of P-solubilization, soil organic acids, soil organic matter, enzyme activities, PSB colony, and rhizosphere moisture contents were significantly improved with PSB inoculation together with recommended dose of NPK fertilizers (RNPK) compared to PSB alone, rock phosphate (RP) alone, or PSB together with rock phosphate and control treatment. Maize digestibility attributes such as DM, CP, CF, EE (by 35%, 20%, 33%, and 28% respectively) and grain quality such as NPK, Mg, Ca, Fe, Mn, Cu, and Zn (by 88%, 92%, 71%, 68%, 78%, 90%, 83, 69%, 92%, 48%, and 90% respectively)

were improved compared to control. In conclusion, improvement in maize crop yield and soil characteristics are more prominent and significant when RNPK is supplemented and inoculated. The present study suggests that PSB, together with RNPK, would improve the maize plant growth and soil fertility in sandy soil.

Keywords: maize grain yield; phosphobacteria; phosphorus uptake; water retention; sandy soil

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1. Introduction

Pakistan has a 1.3-million-ha area under maize with an annual production of about 6.13 million tonnes. It contributes 2.7% and 0.5% share in value addition and GDP, respectively [1]. Maize growth and development are critically influenced by the unavailability of phosphorus due to complex reactions with soil [2]. It is a significant constituent of almost all biochemical processes such as respiration, photosynthesis, signal transduction, macromolecule biosynthesis, and energy transfer [3]. Phosphorus has a contribution of about 0.2% in dry matter production, and the world's 30–40% crop yield is associated with phosphorus availability [4]. However, phosphorus is incorporated in the form of soluble fertilizers, 1% of which becomes the constituents of the plant body and rest of the portion is converted into insoluble complexes by entering into the immobile pools through precipitation reactions with highly reactive Al^{3+} and Fe^{3+} in acidic and Ca^{2+} in calcareous soils [5]. Moreover, about 80% P in soil is precipitated with these metal ions, and about 20% of P is recovered from the applied source [6]. To overcome the above problems of P deficiency, the satisfactory amount is applied in order to combat P deficiency in plants [7].

Most of the soils in Pakistan are calcareous as well as alkaline in nature with pH > 7.0 [8]. Therefore, the fixation of phosphorus in soils is a severe problem [9]. On the basis of a single crop, about 15–20% of phosphorus is absorbed by the plants from the applied phosphorus fertilizer [10]. The mineralization process is mediated by the enzymes especially phosphatases [11] and phytases, which are released by the soil microbes [12]. Moreover, phosphatases (e.g., acid and alkaline phosphatases) released from the cell (exo-enzymes) use organic phosphorus as a substrate and transform into inorganic forms of phosphorus [13]. However, negative or positive activities of microorganisms have direct or indirect effects on the soil health [14]. Rhizospheric microorganisms improve the soil quality by mediating the soil processes viz. release and storage of water and nutrients, nutrients mobilization, root mineralization, organic matter decomposition, nitrogen fixation, sulfur reduction, nitrification, and exudation of soluble compounds [15].

The micro-organisms which have the ability to solubilize P in the soil have to be eco-friendly and economical in order to meet the nutrients requirement of a crop [12]. Consequently, these microorganisms, used solely [16] or in combination with other rhizosphere microbes [17], have shown substantial measurable effects on plants in conventional agronomic soils and increased the growth and productivity of many crops [18].

The prevalence of low P availability all over the world, together with low levels of its application and considerable rise in the price of phosphatic fertilizer over the past few years, have encouraged the agricultural scientist to develop techniques targeting at using P-compounds of low solubility and decreasing the dependence of farmers on synthetic phosphatic fertilizer [19]. Insoluble phosphate compounds such as rock phosphate (RP) may be effective as a P source, but its low solubility limits its direct use as a source of P [20]; therefore, RP needs to be processed to make it soluble and phytoavailable [5].

Phosphate dissolution rates can be greatly accelerated in soil in the presence of organic acids such as malate, citrate, and oxalat, leading to 10- to 1000-fold higher soil solution P concentrations depending upon soil type and concentration of organic acids released either as the result of decomposition of organic wastes or released by microorganisms (PSBs) [21] or plant roots in the rhizosphere [22].

Delvasto, et al. [23] reported that phosphorus solubilizing microorganisms may solubilize P from RP through acidification, chelation, exchange reaction, and polymeric substances formation. Phosphate solubilizing bacteria are used as inoculants, and they increased P uptake and crop yield [24]. Rock phosphate inoculated with phosphate solubilizing bacteria can mobilize the insoluble form of P through the release of organic acids such as citric, oxalic, gluconic, lactic, succinic, and propionic acids and, among these, citric acid showed the maximum reduction in pH [16]. Release of these acids creates a localized high acidity in the immediate vicinity of RP and some organic acids even lead to complexion of Ca^{2+} [25]. Savini, et al. [26] also reported that RP, when used in combination with organic wastes P from RP, can be increased due to the release of organic acids which may reduce the pH of soil in addition to chelation of Ca^{+2} and Mg^{+2} ions and eventually improves the availability of P to plants.

Keeping the above discussion in view, the main objective the present study was to evaluate the effect of phospho-bacteria to solubilize plant-available phosphate from rock phosphate in maize (*Zea mays* L.) production under calcareous to assess the effectiveness of P-solubilizing bacteria in soil amended with rock phosphate for the uptake of N, P and K.

2. Materials and Methods

Two experiments were performed during the spring season 2018 at the research area of COMSATS University Islamabad, Vehari Campus (CUIV) (32.08° N latitude, 72.67° E longitude) under the sub-tropical climate of the Punjab, Pakistan. The top soil layer (0–15 cm) was collected from the Cholistan Desert, Bahawalpur, Punjab-Pakistan. The soil was taken back to CUIV and then cleaned, air-dried, and passed through the sieve (2 mm). A subsample of this large sample is used for the experiment and used for soil physico-chemical properties (Table 1).

Table 1. Physico-chemical properties of soil used in the study.

Soil Properties	Values	
	Pre-Sowing	Post-Harvest
Physical properties		
Sand (g kg^{-1})	470 ± 3.1	
Silt (g kg^{-1})	240 ± 2.3	
Clay (g kg^{-1})	290 ± 1.6	
Textural class		Sandy clay loam
Chemical properties		
Saturation percentage	39.20 ± 1.20	41.73 ± 1.15
pH	7.73 ± 0.03	7.01 ± 0.05
ECe (µS cm^{-1})	16.39 ± 24.1	18.79 ± 29.78
Soil organic matter (g kg^{-1})	8.43 ± 0.68	8.99 ± 0.39
Total soil N (mg kg^{-1})	4.12 ± 9.17	4.72 ± 8.34
NaHCO$_3$ extractable-P (mg kg^{-1} soil)	7.59 ± 0.12	8.70 ± 0.29 Potash
SAR (m mol$_c$ L^{-1})$^{1/2}$	4.56 ± 0.23	4.52 ± 0.30
ESP (m mol$_c$ L^{-1}/100 g)	0.43 ± 0.09	0.41 ± 0.08
Calcium carbonate (%)	6.23 ± 0.15	6.76 ± 0.21
Rock Phosphate characteristics		
P concentration before PSB inoculation (P mg L^{-1})	40.23 ± 4.1	
P concentration after PSB inoculation (P mg L^{-1})	482.5 ± 13.6	

Values are mean of four replicates followed by (±) standard error of means; SAR: sodium adsorption ratio; ESP: exchangeable sodium percentage.

2.1. Experiment 1: Maize Growth

2.1.1. Experimental Site and Design

The experiment was laid out in CRD design, having four replications with six treatments viz., T$_1$: control (CT; un-inoculated), T$_2$: phosphorus solubilization bacteria (PSB), T$_3$: recommend nitrogen

phosphorus potash level (RNPK), T$_4$: PSB + RNPK, T$_5$: rock phosphate (RP; @ 2.5g kg^{-1} of soil), T$_6$: RP + PSB. Soil was sandy clay loam having following physico-chemical characteristics (Table 1). According to our best of knowledge, this is the first ever study about the application of PSB to sandy soil.

2.1.2. Crop Husbandry

Hybrid maize seeds (YSM-112) were inoculated with PSB and sown in the specified treatments. Nitrogen fertilizer was applied in three splits in the form of urea (250 kg nitrogen ha^{-1}, 46% nitrogen), i.e., 1/3rd at the time of sowing, 1/3rd when the crop was at knee height, and 1/3rd was at tasseling stage. The whole of the phosphorus and potash fertilizers were applied in the form of di-ammonium phosphate (300 kg P$_2$O$_5$; 46% phosphorus and 18% nitrogen) and murate of potash (K$_2$O; 50%). The growth period of the crop started from 7 March 2018 and ended on 10 June 2018. The phosphorus solubilization bacteria strain (*Enterobacter sakazakii* J129) was taken from the Soil Microbiology Laboratory, Institute of Soil and Environmental Science, University of Agriculture Faisalabad, Pakistan.

2.1.3. Determination of Macro and Micro-Nutrients from Grain and Dry Matter

After drying of the plants, maize straw and grains were ground in a Wiley mill, with sieves of 0.25 mm (60 mesh). Then the material was crushed, sieved, and digested by the di-acid mixture (HNO$_3$:HCLO$_3$ = 2:1) then analyzed for macro- and microelement analysis (atomic absorption spectrophotometer; PerkinElmer, Singapore) [27]. Nitrogen content was determined by the method of Nessler, phosphorus was determined using molecular absorption spectrophotometer (calorimetry) at a wavelength of 725 nm and potassium by flame photometry. Moisture, crude protein (CP, %), ether extract (EE, %), ash (%), crude fiber (CF, %), neutral detergent fiber (NDF, %), acid detergent fiber (ADF, %), acid detergent lignin (ADL, %), non-structural carbohydrates (NSC, %), were measured by following the methods of AOAC [28]. NSC were determined as a difference.

2.1.4. Determination of Soil Enzymes Activities after Crop Harvesting

The rhizosphere soil samples were collected after the crop harvesting, and the samples were stored at 4 °C. The soil enzymes activities were performed within a week of soil sample collection to avoid any change in soil characteristics, i.e., phosphomonoestrase [29], urease [30], acid phosphate [31], dehydrogenase [32], β-glucosidase [33], and catalase [33], while the other parameters like bulk density, total porosity, chitinase, PSB colony [34], soil organic carbon [35], and rhizosheath.

For urease activity measurement, the soil (1 g) was incubated into the 0.5 mL urease solution (into a flask), and then 4 mL borate buffer (pH 10.0) was added for approx. 2 h at 37 ± 2 °C. After the end of the incubation period, 6 mL KCl (1 M) was added into the flask and remained for 30 min for reaction (A mixture). The ammonium contents were assessed from the mixture (A + NaOH + sodium dichloroisocyanurate) at 690 nm. Alkaline phosphate (ALP) and β-glucosidase (βGS) were determined by adding 1 g soil into nitropheny phosphate (pH 4.0) and p-nitrophenyl-β-ᴅ-glucopyranoside, respectively. Both are incubated at 37 °C for one 1 h and then added tris pH (12.0) to stop the reaction of β-glucosidase activities. The βGS and ALP activities were measured at 464 and 505 nm, respectively. Similarly, the activities of catalase (CAT) were incubated by mixing 5 g soil into 25 mL H$_2$O$_2$ (3%) for 30 min at 4 °C. After the end of incubation, 25 mL H$_2$SO$_4$ (1 M) was added and filtered the solution. Then the 20 mL H$_2$SO$_4$ (0.5 M) was added into 5 mL filtrate, and the solution was titrated as KMnO$_4$ (5 mM) again to determine the unreacted H$_2$O$_2$. The PSB colonies were determined using Pikovskaya's agar plates.

2.1.5. Selected Soil Physio-Chemical Properties (Pre- and Post-harvest)

The texture of sandy soil (pre-harvest) was measured by the pipette method [36] while the pH and EC of soil (pre-and post-harvest) were measured by shaking the soil–water suspension for 1 h (1:2.5, 25 °C). Similarly, CEC (pre- and post-harvest) was determined by mixing the two buffer (sodium acetate, pH 8.2; and ammonium acetate, pH 7.0) [37]. The OC contents (pre- and post-harvest) were

determined by wet oxidation methods [37]. The available phosphorus (P) was measured from the soil extract by using the sodium carbonate [38]. The potassium (k) was noted from the soil sample by using the sodium acetate and then used the atomic absorption spectrophotometer [39]. Similarly, the soil porosity was measured by the Kay method [40]. The soil aggregation percentage (SAS) was determined by dry and wet sieving [41]. The BD was carried out by the method followed by Chaturvedi and Sankar [42].

2.2. Experiment 2: Incubation of PSB Inoculated Sandy Soil

The PSB was inoculated with the same treatments as was applied in Experiment 1. The treatments were incubated for 8 months and simulated the cholistan desert environment by the applying water as average rainfall at cholistan (166 mm annum^{-1}) to create its possible field application after the completion of the study. During the incubation period, the temperature was at 28 ± 2 °C (average cholistan temperature). The experiment was laid out in CRD with factorial arrangements replicated four times. The experiment set up was carried out at COMSATS University Islamabad, Vehari Campus. The soil physico-chemical properties and water retention were recorded using the standard procedures. The purpose of this experiment was to suggest the farmers inoculate the PSBs into sandy soil before the onset of rainfall showers.

Experimental Set up

A column experiment (PVC; plastic column) was carried out to determine the influence of PSB or without PSB on the sandy soil water retention and replicated four times. For this purpose, the PSB colonies or RP or RNPK or mixture of all these were mixed well (plastic tub; v. 30 L) into measured soil 500 g soil (sieved and sir dried soil) and left for 15 days. Then the soil and PSB mixture was filled into a plastic column (25 cm long; 12.5 cm diameter) and an un-inoculated plastic column acted as the control. The headspace (3 cm) of each column was left above the treated or non-PSB-treated soils for the addition of water. All the PVC columns were covered at the bottom with cheesecloth to stop the sandy soil leaching during the experiment. The columns were placed on the wooden rack at the height of 500 m and allowed to settle and established their colonies; plastic bottles were placed to collect the leachates. The distilled water was added to each PVC cylinder until 50% FC was achieved and then left for equilibrium approximately 24 h, after which leaching process was started. The leaching process was started by adding water, and this process lasted overnight. Leachates were collected in the plastic bottles at the bottom of cylinders and then, volume was recorded. To determine the aging of DBCs on the sandy soil water retention, the leaching process was performed five times at the start of this experiment, and after this, it was carried out after 15, 30, 45, 60, 75, and 90 days (crop duration). The retained water (RW) was calculated by using the following equation as described by Novak and D. Rehrah [43]

$$RW\% = (\text{volume of added water} - \text{volume of leachates}) \times 100.$$

2.3. Experiment 3: Influence of fresh PSB Inoculation on Sandy Soil Water Retention

Experimental Set up: In Pakistan, PSB is mostly applied directly to soil without any incubation. So, this study is conducted to evaluate the effects of fresh PSB application on the sandy soil water retention. A similar experimental set up was used as was discussed in Section 2.2.

2.4. Data Analysis

The data of experiment was statistically analyzed by using the Statistix software package (version 8.1; Analytical Software, Tallahassee, FL, USA). The two-way factorial analysis of variance was used for the data of Experiment 1 where the plant and soil attributes were analyzed. One-way ANOVA was used for the analysis of data of Experiment 2 where the PSB or RC or RNPK or their treatments were analyzed. The statistical data are represented either in the form of tables or figures. The probability

level for the data was 5% [44], and all the graphs were constructed by using Origin Pro software (version 2018).

3. Results

3.1. Experiment 1: Physiological and Yield Related Attributes in Response to PSB

PSB application together with RNPK significantly ($p < 0.05$) improved the physiological- and yield-related attributes compared to sole application of RNPK or RP or a combination of these two (Table 2). Inoculation, together with PSB and RNPK fertilization, increased the physiological parameters such as shoot length (34%), root length (62%), cob diameter (29%), fresh biomass (32%), and dry biomass (54%) (Table 1).

Table 2. Influence of phosphor bacteria on the physiological characteristics of spring maize.

Physiological Characteristics	Shoot Length (cm)	Root Length (cm)	Cob Diameter (cm)	Fresh Biomass (g Plant^{-1})	Dry Biomass (g Plant^{-1})
CT	124.78e	20.85d	2.84c	379.91e	302.44e
PSB	136.14c	21.55c	3.32ab	401.66d	341.66c
RNPK	161.32a	28.43b	3.54a	451.23b	431.28b
PSB + RNPK	167.48a	33.70a	3.66a	501.08a	466.22a
RP	131.81cd	20.48cd	3.05bc	388.64e	333.89d
PSB + RP	147.05b	25.15b	3.29ab	428.78c	414.78b
LSD $p < 0.05$	7.62	4.31	0.31	10.47	15.11
PSB * RNPK	*	*	*	**	**
PSB * RP	*	*	*	*	*
RNPK * RP	**	*	*	**	**

Mean values within each column sharing the same letters are not significantly differed from each other ($p = 0.05$). The values after ± show the standard error in the data.

The yield-related attributes such as kernel rows cob^{-1} (12%), kernel cob^{-1} (50%), and 100 kernel weight (55%) and biological yield (34%) increased, as compared to control (Table 2). Similarly, maize grain yield increased by 37% and by 10% over the control and sole NPK application, respectively. Moreover, the maize grain yield was decreased by 35% and by 18% compared to the sole application of RP and PSB. So, PSB application, along with RNPK, can improve the yield compared to the sole application of RNPK and other sources in the alkaline soil of tropical areas.

3.2. Phosphorus Solublization in Response to PSB

The phosphorous solubilization (PS) was increased in PSB together with the application of RNPK and also on the PSB-PR as compared to the sole application of RNPK or RP or control at the end of soil sampling duration (50 days; Figure 1A). Similarly, the PS was improved with the passage of time (days) in all treatments, but the maximum was observed in PSB + RNPK treatments, followed by PSB + RP, and PSB that was statistically similar to RNPK. Figure 1A indicates that after 10 days from seedling emergence, the maximum PS was noted in the RNPK (by 4.5%, 8.3%, and 11.4%, respectively), compared to PSB + RNPK, PSB + RP, or PSB, but after 20 days, the gap between RNPK and PSB + RNPK was reduced to 2.2%. Moreover, after 30 and 40 days from crop emergence, the PS was increase in PSB + RNPK (by 8% and 19.4% higher vs. RNPK) as compared to all other treatments i.e., RNPK > PSB + PR > PSB. As far as after 50 days, the PS was recorded as the following order PSB + RNPK > RNPK > PSB + PR > PSB > control. The data exhibited the positive impacts of PSB on the long term availability of P over non-inoculant soil (Figure 1A).
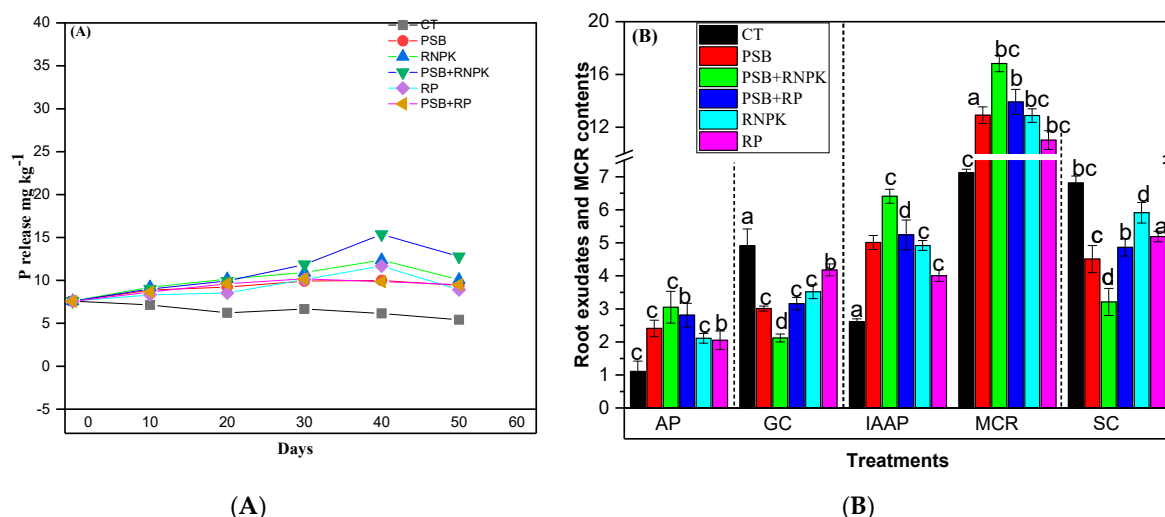
(**A**)　　　　　　　　　　　　　　　　　　　　　　　(**B**)

Figure 1. Effect of phosphorus solubilization bacteria (PSB) on the phosphorus (P) release with the passage of time (days; 10 days to 50 days after plant emergence; (**A**) and some root exudates (AP, GC, IAAP, SC) and moisture contents of rhizoseath (MCR; %) (**B**). All the bars (B) having similar betters among the other bars are not signifincalty similar according to DMR test ($p \leq 0.05$) while the error bars show the standard errors of the mean values. AP, auxin production (mg mL^{-1}); GC, glucose contents (μg g^{-1}); IAAP indole acetic acid production (μg mL^{-1}); and SC score contents (μg g^{-1}). CT, control; PSB, phosphorus solublization bacteria; PSB + RNPK (PSB + recommended nitrogen, phosphorus and potash), PSB + RP (PSB + rock phosphate).

3.3. Influence of PSB Inoculation on Root Exudate and Moisture Contents of Rhizoseath

PSB together with RNPK or PSB alone reduced the root exudates (GC and SC) and increased the AP, IAAP, and MCR of the sandy soil, as in the case of our study (Figure 1B). The figure exhibits that AP (mg mL^{-1}) and IAAP (μg mL^{-1}) were increased by 44.5% and 23.2% (PSB + RNPK vs. RNPK), 21% and 21.8% (PSB + RNPK vs. PSB), and 63.6% and 59.3% (PSB + RNPK vs. CT). Similarly, GC (μg g^{-1}) and SC (μg g^{-1}) were increased in the CT treatments compared to sole or combined application of PSB + RNPK (by 57% and 53%) or PSB + RP (by 36% and 29%) or PSB (by 39% and 34%) or RNP (by 28% and 13%), or RP (24% and 15%), respectively (Figure 1B). Moreover, the GC and SC concentrations were also increased in the sole application of RNPK (by 60% and 84%), or PSB (by 42% and 40%), or RP (by 97% and 63%) over the PSB together applied with RNPK. The higher concentration of GC and SC in control soil indicates that the plants are under stress, as seen in our study. Rhizoseath moisture contents (MCR) are very important for bacterial growth and colony formation. The higher MCR (%) was recorded in PSB applied together with RNPK over all other treatments such as RNPK (by 23%) or PSB + RP (by 17%) or PSB (by 23%) or control (by 58%). However, PSB akine application also increased the MSC over other treatments, i.e., RNPK (by 6%), or RP (by 15), or control (by 45%). The increased in MCR in our study improved the growth of PSB colonies (Table 3).

3.4. Soil Physio-Chemical Properties and Bacterial Colonization in Response to PSB

The data in Table 3 indicate that the soil physico-chemical properties and bacterial colonization improved with the addition of PSB along with RNPK. The values of SBD, SP, ACC act. SOC, Rhiz. from 1.40 to 1.53, 0.44 to 0.51, 145.89 to 332.48, 7.61 to 8.94, 1.06 to 3.45, respectively. The most promising increase of these properties was recorded in PSB, together with RNPK, to RNPK alone (by 3.5%, 19%, 7%, and 32%, respectively) compared to sole RNPK application. Moreover, the PSB alone application increased the SPD (by 2%), SP (by 4%), ACC act. (by 13%), SOC (by 2%), and Rhiz. (by 15%) over the RNPK, respectively. Interestingly, the values of Ch. Act and PSB colonies were higher in PSB together with RNPK over RNPK (negative, by 71%) or PSB (positive, by 58%) or PSB + RP (positive, by 68%) treatments.

Table 3. Influence of phosphor bacteria on the yield and yield-related attributes of spring maize.

Yield and Yield Attributes	Kernel Rows Cob^{-1}	Kernels Cob^{-1}	100-Kernel Weight (g)	Kernel Yield Plant^{-1} (g)	Biological Yield Plant^{-1} (g)
CT	15.7d	311.35e	11.00e	118.93e	241.64d
PSB	16.1c	348.81d	14.99c	138.79d	282.06c
RNPK	17.4ab	442.18bc	16.92ab	149.56b	301.23.69b
PSB + RNPK	17.6a	466.71a	17.12a	164.12a	322.84a
RP	17.1b	411.24c	13.01d	121.04e	236.88d
PSB + RP	16.9ab	428.74bc	15.11c	143.11cd	296.19bc
LSD $p < 0.05$	0.32	11.05	1.91	5.41	15.01
PSB * RNPK	NS	NS	*	**	***
PSB * RP	NS	NS	*	*	**
RNPK * RP	*	*	*	**	**

Mean values within each column sharing the same letters are not significantly differed from each other ($p = 0.05$). The values are the means of four replications. * = statistically significant; ** = statistically highly significant; NS = non-significant.

3.5. Effects of PSB Inoculation on Organic Acid in Rhizosphere

The current study indicates that PSB inoculation increases the organic acids in the rhizosphere compared to non-inoculant control (Figure 2). Moreover, the maximum organic acid was produced in the PSB addition together with RNPK in the sandy soil as compared to control and all other treatments RP alone, or PSB alone, or PSB together with RP. The data in Figure 2 show that AC by 155%, CA by 153%, MA by 129%, OXA by 39%, and SA by 137% was increased over the non-inoculant control. Moreover, when we compared the PSB treatments, PSB alone and PSB together with RP decreased the organic acid concentrations by 22% and 17% (AC), 19% and 25% (CA), 74% and 91% (MA), 25% and 33% (OXA), and 26% and 63% (SA) as compared to PSB together with RNPK, respectively (Figure 2).
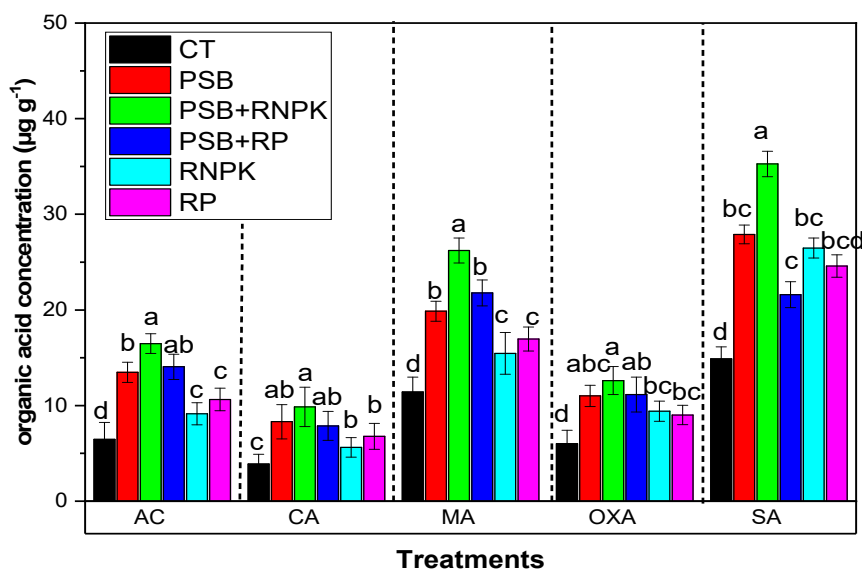

Figure 2. Effect of phosphorus solubilization bacteria (PSB) on the organic acids in the rizosphere. All the bars having similar betters among the other bars are not significantly similar according to the DMR test ($p \leq 0.05$), while the error bars show the standard errors of the mean values. AP, auxin production (mg mL^{-1}); GC, glucose contents (μg g^{-1}); IAAP indole acetic acid production (μg mL^{-1}); and SC score contents (μg g^{-1}). CT, control; PSB, phosphorus solubilization bacteria; PSB + RNPK (PSB + recommended nitrogen, phosphorus and potash), PSB + RP (PSB + rock phosphate).

3.6. Soil Enzymes Activities in Response PSB Inoculation

All the treatments significantly increased the soil enzymes activities over control (Table 4). The measured activities of phosphomonoestrase (PHE), urease (UE), catalase (CL), acid phosphate (APH, dehydrogenase (DHG), β-glucosidase (βGS) were in the ranges from 42% to 63%, 20% to 43%, 40% to 71%, 24% to 52%, 31% to 67%, and 36% to 46%, respectively, compared to control (Table 5). The most promising increase in soil enzymes activities was seen in the PSB together with RNPK ($p < 0.05$). The PSB together with RNPK proved the most effective treatment towards the increase of PHE (by 63%), UE (by 43%), CL (by 71%), APH (by 52%), DHG (by 67%) and βGS (by 46%) over the control, as in our study (Table 5). Moreover, the PSB + RNPK treatments were also significantly differed from RNPK alone or PSB alone or PSB together with RP treatments. The interaction pf PSB * RNPK, PSB * RP, and RNPK * RP were significant for all soil enzymes except the βGS treatments ($p < 0.05$).

Table 4. Effects of PSB inoculation on soil physic-chemical properties and bacterial colonization.

Treatments	SBD	SP	ACC act.	Ch. Act.	PSB-Cloy	SOC	Rhiz
CT	1.53e	0.51e	145.89e	Negative	$3.45 \times 104d$	7.61d	1.06d
PSB	1.43b	0.44a	305.68b	Positive	$6.82 \times 105b$	8.42b	2.01b
RNPK	1.46c	0.46b	268.89c	Negative	$5.56 \times 104b$	8.30b	2.36b
PSB + RNPK	1.41a	0.44a	332.48a	Positive	$7.55 \times 105a$	8.94a	3.45a
RP	1.49d	0.49d	221.26d	Negative	$4.32 \times 104c$	8.08c	1.92c
PSB + RP	1.43b	0.47c	296.68b	Positive	$5.42 \times 105b$	8.33b	2.45b
LSD $p < 0.05$	0.018	0.001	15.29		1.40×105	0.21	0.41
PSB * RNPK	NS	NS	*		*	***	***
PSB * RP	NS	NS	**		*	**	**
RNPK * RP	NS	NS	*		*	*	*

Mean values within each column sharing the same letters are not significantly differed from each other ($p = 0.05$). The values are the means of four replications. * = statistically significant; ** = statistically highly significant; *** = highly highly significant; NS = non-significant; SBD, soil bulk density (Mg m^{-3}); SP, soil porosity (m^3 m^{-3}); ACC act. (deaminase activity (n mole αketobutyrate g^{-1} biomass h^{-1}); Ch. Act., chitinase activity (indicator); phosphorus solubilization bacteria, PSB colonization (cfu g^{-1} soil); SOC, soil organic carbon (mg kg^{-1}), Rhiz., rhizosheath (g plant^{-1}).

Table 5. Effects of PSB inoculation on soil enzyme activities after crop harvesting.

Treatments	PHE	UE	CL	APH	DHG	βGS
CT	0.44e	1.44d	0.15d	14.69e	0.09e	2.01d
PSB	0.84c	1.81	0.28bc	21.47	0.15d	3.21c
RNPK	0.91b	2.22b	0.44b	28.79b	0.22b	3.51b
PSB + RNPK	1.11a	2.51a	0.51a	30.51a	0.27a	3.66ab
RP	0.76d	1.54d	0.25c	19.48d	0.13d	3.15c
PSB + RP	0.96b	2.31c	0.33bc	25.01c	0.18c	3.26c
LSD $p < 0.05$	0.06	0.21	0.09	1.51	0.02	0.06
PSB * RNPK	***	***	**	***	***	*
PSB * RP	**	**	**	***	**	NS
RNPK * RP	*	**	*	**	*	NS

Mean values within each column sharing the same letters are not significantly differed from each other ($p = 0.05$). The values are the means of four replications. * = statistically significant; ** = statistically highly significant; *** = statistically highly highly significant; NS = non-significant; PHE, phosphomonoestrase (mole PNF g^{-1} ha^{-1}); UE, urease (µgN-NH4 kg^{-1} h^{-1}); CL, catalase (vol of KMNO4 g^{-1} soil), APH, acid phosphate (µg p-NPg^{-1} 24h^{-1}); DHG, dehydrogenase (µg TFP g^{-1} h^{-1}); βGS, β-glucosidase (mol PNFg^{-1} h^{-1}).

3.7. Effect of PSB Inoculated and Non-Inoculated on Dry Matter Characteristics

Often in Pakistan, the maize stem is used as green fodder for animals after removing the corn from the stem. So, the dry matter (DM) analysis is necessary for this scenario. The PSB alone or together with RNPK or RP are significant after the maize feeding characteristics over the control treatments ($p < 0.05$). DM, CP, EE, ash, and CF were higher in PSB together with RNPK by 36%, 21%, 33%, 28%,

and 12% compared to control, while the NDF, ADF, ADL, and NSC had higher values than the all other PSB alone or together with SNPK or RP (Table 4). The interactive effect of PSB * RNPK was significant in almost all DM values, while the PSB * RP and RNPK * RP were found non-significant in most of the cases.

3.8. Impacts of PSB Inoculation on Maize Grain Quality

Grain quality is one of the vital parameters of any food product. The concentrations of all macro elements (NPK, Mg, Ca, g kg^{-1}) were found in the ranges from 7.61 to 14.32, 2.47 to 4.42, 2.91 to 5.12, 1.61 to 3.06, and 1.91 to 3.51, respectively, while micronutrients (Fe, Mn, Cu, and Zn; mg kg^{-1}) were in the ranges from 10.82 to 18.26, 2.56 to 4.92, 1.09 to 1.61, and 12.91 to 24.56, respectively (Table 6). Surprisingly, the concentration of all micro- and macronutrients increased over the application of PSB together with RNPK compared to PSB or RNPK alone. However, the increment in values were variable among the different treatments of PSB (with RP or alone). Moreover, the PSB together with RNPK was found to be the most promising treatment in enhancing the N by 88%, P by 79%, K by 76%, Mg by 90%, Ca by 84%, Fe by 69%, Mn by 92%, Cu by 48%, and Zn by 90%, respectively, in maize grain compared to control. In addition, the PSB alone or together with RP significantly improved the macro- (NPK, Mn, Ca) and micronutrients (Fe, Mn, Cu, and Zn) over the control treatments (Table 7).

Table 6. Effects of PSB inoculation on the grain quality of maize.

Treatments	Macronutrients					Micronutrients			
	N	**P**	**K**	**Mg**	**Ca**	**Fe**	**Mn**	**Cu**	**Zn**
CT	7.61e	2.47e	2.91f	1.61e	1.91d	10.82e	2.56c	1.09c	12.91e
PSB	12.01bc	3.01c	3.14d	1.96c	2.64c	16.22d	3.81b	1.29a	15.89d
RNPK	13.01b	3.51b	4.41b	2.15b	2.07b	17.51b	4.41ab	1.51a	21.76b
PSB + RNPK	14.32a	4.42a	5.12a	3.06a	3.51a	18.26a	4.92a	1.61a	24.56a
RP	10.78d	2.64d	3.01e	1.78d	2.84c	15.61d	3.66b	1.16b	15.31d
PSB + RP	11.41bc	3.21c	3.86c	2.01c	3.16b	16.91c	4.01b	1.44a	17.89c
LSD	1.12	0.31	0.21	0.11	0.22	0.61	0.45	0.21	1.63
PSB * RNPK	***	***	***	**	*	*	*	*	*
PSB * RP	*	**	**	*	NS	*	NS	NS	*
RNPK * RP	**	**	***	**	*	*	NS	NS	*

Mean values within each column sharing the same letters are not significantly differed from each other (*p* = 0.05). The values are the means of four replications. * = statistically significant; ** = statistically highly significant; NS = non-significant; micro-nutrient (NPK Mg Ca (gkg^{-1})); (micro-nutrients (mg kg^{-1})).

Table 7. Effects of PSB inoculation on maize dry matter characteristics.

Treatment	DM	CP	EE	Ash	CF	NDF	ADF	ADL	NSC
CT	81.56e	6.51e	3.01e	1.34d	3.44c	17.61a	4.81a	1.68a	70.36a
PSB	94.56c	7.11c	3.41c	1.51c	3.61b	17.51ab	4.61bc	1.44b	66.01c
RNPK	96.01ab	7.51ab	3.88b	1.65ab	3.76a	17.26b	4.36d	1.28b	63.42d
PSB + RNPK	97.45a	7.84a	4.01a	1.71a	3.84a	17.15c	4.16e	1.19c	61.89e
RP	90.12d	6.91d	3.14d	1.41cd	3.68b	17.44ab	4.68b	1.61a	69.01b
PSB + RP	95.01ab	7.71b	3.94ab	1.58b	3.81a	17.32b	4.52c	1.36b	65.15c
LSD	1.41	0.35	0.15	0.13	0.11	0.18	0.14	0.1	0.23
PSB * RNPK	*	*	*	**	**	*	*	NS	*
PSB * RP	NS	NS	*	*	NS	NS	NS	NS	NS
RNPK * RP	NS	NS	*	NS	*	NS	NS	NS	NS

Mean values within each column sharing the same letters are not significantly differed from each other (*p* = 0.05). The values are the means of four replications. * = statistically significant; ** = statistically highly significant; NS = non-significant; DM, dry matter (%); CP, crude protein (%); EE, extractable ether (%); ash (%), CF, crude fiber (%); NDF, neutral detergent fiber (%); ADF, acid detergent fiber (%); ADL, acid detergent lignin (%); NSC, non-structural carbohydrate (%).

3.9. Incubation Study of PSB Inoculated Sandy Soil

Impacts of Incubation on the Sandy Soil Water Retention and Selected Soil Properties

Using the PVC column experiment, the current study indicates the effect of PSB on soil water retention in the sandy soils. The data was collected up to 90 days after crop emergence, which was the actual crop water requirement duration to reach its maturity. Hence, the column experiment study had some inspiration for farmers for its field application. The incubation of PSB alone or together with RNPK or RP significantly increased the water retention (Figure 3A). In addition, the incubation also improved the soil properties (Table 8) compared to control. Our data results indicate that inoculation of PSB in sandy soil over some period (8 months) increased the water retention in PSB + RNPK by 205%, PSB + RP by 163%, RNPK by 138%, PSB by 120%, and RP by 78% compared to control. Moreover, the sole application of RNPK and RP into sandy soil decreased water retention by 29% and 73% compare to PSB + RNPK. Similarly, the selected soil properties, i.e., pH (by 14%), CE (by 127%), CEC (by 85%), SAS (by 148%), SBD (by 14%), and SP (by 24%) were improved in PSB together with RNPK over the control treatments. In addition, other soil properties were also improved by the sole application of PSB or PNPK compared to control (Table 8). Our study concludes that the aging of PBS increases the water retention of sandy soil and hence helps the farmers in increasing their crop productivity. The mixing of PSB before the onset of rainfall helps to increase soil water holding capacity.

Table 8. Effects of PSB inoculation on the selected soil properties after incubation (8 months).

Treatments	pH	EC	OM	CEC	SAS	SBD	SP
		dsm^{-1}	%	meq/100	%	(Mg m^{-3})	(m^3 m^{-3})
CT	7.53a	0.51d	0.25	4.96d	6.61d	1.46a	0.51a
PSB	6.82c	0.84c	0.31	8.51b	14.36bc	1.34bc	0.47c
RNPK	6.66d	0.91b	0.39	8.73b	13.66c	1.38bc	0.47c
PSB + RNPK	6.51e	1.16a	0.41	9.17a	16.44a	1.26d	0.39e
RP	6.96b	0.76c	0.28	7.11c	14.62bc	1.41b	0.49b
PSB + RP	6.80c	1.04b	0.38	8.982b	14.88bc	1.31c	0.44d
LSD (0.05)	0.11	0.16	NS	0.51	1.25	0.08	0.01

Mean values within each column sharing the same letters are not significantly differed from each other ($p = 0.05$). The values are the means of four replications. EC, electrical conductivity; OM, organic matter; CEC, cation exchange capacity; SAS, soil aggregation stability; SBD, soil bulk density; SP, soil porosity.
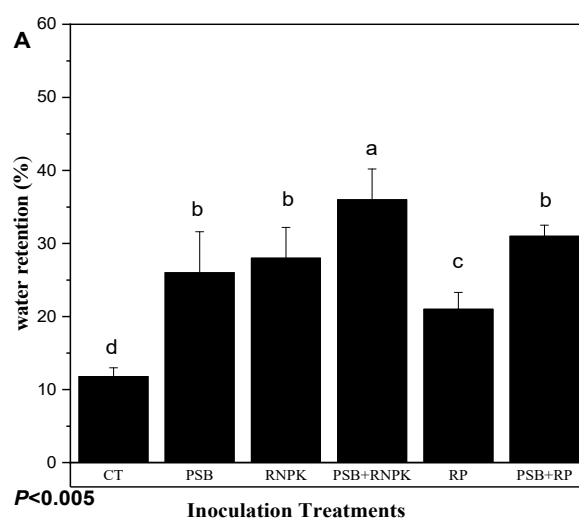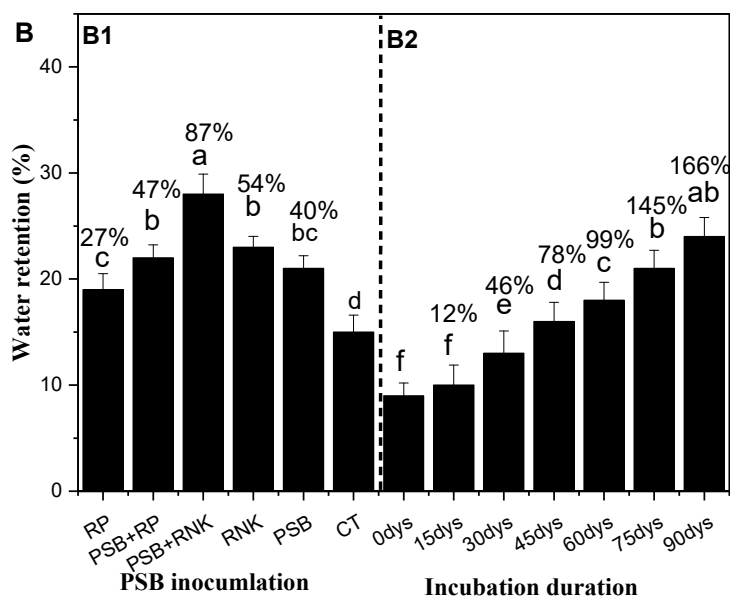

Figure 3. *Cont.*

Figure 3. Effect of phosphorus solubilization bacteria (PSB) incubation on the selected soil properties (Table 7) and water retention (**A**) and effect of PSB fresh inoculation on the soil water retention (**B**; (Figure **B**1,**B**2)). All the bars (**A**,**B**) having similar letters among the other bars are not significantly similar according to the DMR test ($p \leq 0.05$), while the error bars show the standard errors of the mean values. CT, control; PSB, phosphorus solubilization bacteria; PSB + RNPK (PSB + recommended nitrogen, phosphorus, and potash), PSB + RP (PSB + rock phosphate).

3.10. Fresh Inoculation Study and Water Retention

The PSB inoculation treatments, duration, and their interactive effects significantly affected the sandy soil water retention (Figure 3A,B). The study results exhibit that PSB together RNPK is the most effective treatment over all other treatments towards the increase of water retention. The incubation duration of fresh PSB inoculants also increases soil water retention (Figure 3B). This phenomenon of our study indicated that a fresh application of PSB to sandy soil also increased the sandy soil water retention over time (days) and supported early maize growth and development for a certain period of time. So, among all treatments, the interactive effects (Figure 3B, B1 and B2)) show that PSB + RNPK and the 75 or 90 days of fresh incubation is helpful to farmers. So, the water retention ability of the treatments are in the following order: PSB + RNPK > PSB + RP > RNPK ≥ RNPK > control. However, the incubation duration (days) also increased the water retention of fresh PSB inoculation in the following order: 90 days ≥ 75 days > 60 days > 45 days > 30 days > 15 days ≥ 0 day.

4. Discussion

Inoculation of PSB together with fertilizer application markedly improved the physiological and yield related parameters compared to an inoculated control. Among all treatments, PSB + RNPK proved the most effective treatment towards the increase of spring maize grain yield [45] compared to inoculation alone, RNPK alone [46] or PR alone and CT. In the present study, RNPK together with PSB resulted in maximum shoot length and fresh and dry biomass of shoot. This increase might be due to releases of higher chlorophyll activities (Supplementary Figures S1–S3) and more photosynthate availability that increased the growth of the plants [47]. These findings are also in agreement with Saxena, et al. [48], who reported that longer root length could be improved by PSB with the chemical fertilizer. P-solubilizing bacteria excrete hormones that induce longer root growth, which leads to an enhance uptake of nutrients [49]. Similarly, maximum maize fresh and dry biomass in PSB together with RNPK might be due to the availability of nutrients, especially P, by the inducing role of PSB. The use of PSB ensures the availability of nutrients in plants which result in maximum growth [15]. Such a synergistic effect of PSB application together with RNPK enhances the fresh and dry biomass

compared to RP alone or PSB alone or CT [50]. The physiological attributes were improved in PSB together with RNPK treatments, which might due to the increase in the retention of assimilates that are essential for the cob production in the stem of the plant [51–53]. In addition, the application of PSB together with RNPK enhanced the availability and uptake of nutrients from the soil and, ultimately, better 100-grain weight was achieved. The study results related to grain yield was in accordance with those of Afzal and Bano [52] and Young, et al. [54] that PSB together with RNPK increased the yield components up to 30–50% as compared to RP alone or PSB alone or CT.

P-mobilization/solubilization was improved in the PSB treatments as compared to RNPK alone or PSB alone or CT, while the maximum P-solubilization was recorded in PSB together with RNPK. The study results are in accordance with those of Kaur and Reddy [46] who stated that application of fertilizer enriched with PSBs in the rhizosphere enhanced P-availability and increased the rice crop yield. Our study results indicate that highest yield was recorded in the inoculation treatments together with RNPK. Moreover, phosphorus (P) solubilization was increased with the passage of time when the inoculation of PSB was done along with RNPK compared to the control and other treatments, i.e., RNPK, PSB + RP, RP and PSB. The P solubilization in the soil increased with days and maximum P was recorded after 40 days of seedling emergence, and, afterward, this phenomenon was slowed down. This might be due to more accumulation of P in the soil which reduces the P solublization in the soil. After 45 days, the silking and tasseling process starts and the plant utilizes the photosynthates instead of soil P reservoirs. Similarly, the study results show that maximum P solubilization was when PSB was applied together with RNPK, and that was due to the availability of initial substrate resources to PSB. These study results conclude that the crop reaped the benefits imparted by the PSBs to the sandy soil in terms of available P contents and further improved the photosynthesis activity (see Supplementary Figure S1) and respiration (see Supplementary Figures S2 and S3) of the maize plant [55]. The soil P-availability showed in the form P uptake by the maize plant in various treatments. Swarnalakshmi, et al. [56] noted that PSB application together with chemical fertilizers enhanced P-availability and P-uptake compared to PSB alone or RNPK alone in wheat crops. Our study results confirmed the hypothesis that PSB amendment to sandy soil had strongly influenced the plant growth, physiology, yield, and yield-related attributes compared to other treatments. These positive effects were related to improved sandy soil structural stability (bulk density, porosity, soil organic carbon), higher nutrients retention capacity (CEC, PSB-colony, and rhizosheath) and superior production of organic acids (Figure 2; Table 9). As in the case of our study, PSB increased plant biomass but the activities of PSB towards P-solubilization in P-deficient soils could be different to different plant species and the prevailing environmental conditions [57,58]. One more phenomenon, i.e., production of organic acids by PSBs, enhances the P-mobilization/solubilization [59] in the sandy soil and finally enables the plant to uptake mineral nutrients [60]. In addition, the carboxylic groups of the acids increase the chelation of the cations, which further bind to phosphate and convert them into soluble forms [61,62]. From the results of the current study, it may be reaffirmed that P-solubilization by the different PSBs is involved in the production of organic acids [63].

Our results exhibit that the PSB inoculation on the maize root differentially affects dry matter characteristics (Table 6). More pronounced effects on DM, CP, CF, and EE were noted in the PSB addition together with RNPK to sandy soil and increased digestion in the animals [62]. These results are in agreement with the results of, who stated that PSB increases the nitrogen contents in dry matter characteristics. Moreover, the CP contents are co-related with P-solubilization [64] but even more improved when PSB is involved. Moreover, the PSB triggers the growth of already-available microbes, and then the P-uptake and P-transporter gene expression in maize. In addition, it has been noted that P-transporter gene expression was increased in the presence of PSB [65].

Table 9. Correlations coefficients (r) of different growth, yield, physiological, and nutrients contents related traits in maize as effected by PSB alone and in mixture with different doses of chemical fertilizers and rock phosphate (* $p < 0.1$, ** $p < 0.01$ n = 4).

	SL	RL	RDW	FBM	DBM	SY	GY	COD	GWT	SBD	SP	SWR	NCG	PCG	KCG	PSBC	SOC	SWRI
SL	1	***0.82	***0.84	***0.91	***0.91	***0.87	***0.93	***0.77	***0.87	***0.73	***0.90	***0.94	***0.95	***0.91	***0.93	***0.84	***0.93	***0.85
RL	***0.82	1	***0.86	***0.87	***0.86	***0.85	***0.86	***0.81	***0.80	**0.60	***0.82	***0.83	***0.84	***0.88	***0.84	***0.79	***0.86	***0.87
RDW	***0.84	***0.86	1	***0.92	***0.91	***0.89	***0.89	***0.80	***0.82	***0.68	***0.89	***0.87	***0.85	***0.88	***0.87	***0.81	***0.85	***0.86
FBM	***0.91	***0.87	***0.92	1	***0.99	***0.97	***0.98	***0.81	***0.90	**0.69	***0.95	***0.98	***0.96	***0.96	***0.91	***0.93	***0.97	***0.92
DBM	***0.91	***0.86	***0.91	***0.99	1	***0.99	***0.98	***0.83	***0.88	**0.68	***0.94	***0.97	***0.96	***0.96	***0.90	***0.92	***0.97	***0.91
SY	***0.87	***0.85	***0.89	***0.97	***0.99	1	***0.94	***0.81	***0.86	**0.68	***0.93	***0.95	***0.94	***0.95	***0.89	***0.91	***0.94	***0.87
GY	***0.93	***0.86	***0.89	***0.98	***0.98	***0.94	1	***0.81	***0.89	**0.67	***0.92	***0.96	***0.97	***0.96	***0.89	***0.91	***0.97	***0.94
COD	***0.77	***0.81	***0.80	***0.81	***0.83	***0.81	***0.81	1	**0.65	**0.64	***0.76	***0.82	***0.83	***0.83	***0.76	***0.79	***0.86	***0.85
GWT	***0.87	***0.80	***0.82	***0.90	***0.88	***0.86	***0.89	**0.65	1	**0.68	***0.84	***0.88	***0.86	***0.87	***0.90	***0.85	***0.86	***0.82
SBD	***0.73	**0.60	**0.68	**0.69	**0.68	**0.68	**0.67	**0.64	**0.68	1	**0.66	***0.71	***0.70	***0.71	**0.69	***0.70	**0.68	**0.61
SP	***0.90	***0.82	***0.89	***0.95	***0.94	***0.93	***0.92	***0.76	***0.84	**0.66	1	***0.93	***0.92	***0.93	***0.90	***0.86	***0.90	***0.85
SWR	***0.94	***0.83	***0.87	***0.98	***0.97	***0.95	***0.96	***0.82	***0.88	***0.71	***0.93	1	***0.99	***0.96	***0.91	***0.96	***0.98	***0.90
NCG	***0.95	***0.84	***0.85	***0.96	***0.96	***0.94	***0.97	***0.83	***0.86	***0.70	***0.92	***0.99	1	***0.96	***0.90	***0.94	***0.98	***0.91
PCG	***0.91	***0.88	***0.88	***0.96	***0.96	***0.95	***0.96	***0.83	***0.87	***0.71	***0.93	***0.96	***0.96	1	***0.87	***0.92	***0.96	***0.93

Table 9. *Cont.*

	SL	RL	RDW	FBM	DBM	SY	GY	COD	GWT	SBD	SP	SWR	NCG	PCG	KCG	PSBC	SOC	SWRI
KCG	***	***	***	***	***	***	***	***	***	**	***	***	***	***	1	***	***	***
	0.93	0.84	0.87	0.91	0.90	0.89	0.89	0.76	0.90	0.69	0.90	0.91	0.90	0.87		0.83	0.88	0.81
PSBC	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	1	***	***
	0.84	0.79	0.81	0.93	0.92	0.91	0.91	0.79	0.85	0.70	0.86	0.96	0.94	0.92	0.83		0.94	0.89
SOC	***	***	***	***	***	***	***	***	***	**	***	***	***	***	***	***	1	***
	0.93	0.86	0.85	0.97	0.97	0.94	0.97	0.86	0.86	0.68	0.90	0.98	0.98	0.96	0.88	0.94		0.93
SWRI	***	***	***	***	***	***	***	***	***	**	***	***	***	***	***	***	***	1
	0.85	0.87	0.86	0.92	0.91	0.87	0.94	0.85	0.82	0.61	0.85	0.90	0.91	0.93	0.81	0.89	0.93	

** = highly significant; *** = highly higly significant; SL = shoot length; RL = root length; RDW = root dry weight; FBM = fresh biomass; DBM = dry biomass; SY = straw yield; GY = grain yield; COD = cob diameter; GWT = 100 grain weight; SBD = soil bulk density; SP = soil porosity; SWR = soil water retention; NCG = nitrogen content in grain; PCG = phosphorus content in grain; PSBC = phosphorus solubilization bacterial colony; SOC = soil organic carbon; SWRI = soil water retention after incubation.

The maize grain is a vital portion for both humans and animals for food and feeding purposes. The more solubilization of nutrients by PSB together with RNPK emphatically enhanced the macro- and micronutrients in the grain over PSB alone or RP alone or CT [48]. Our study also shows that inoculation with microbes strains increases the accumulation of macro- and micronutrients in the kernels of spring maize grown in the sandy soils [62]. Improved maize grain quality might be due to an increased photosynthetic rate (Supplementary Figure S2) and plant growth and, ultimately, the availability of nutrients [66].

This study results conclude that the incubation process (8 months) improved the soil structural (SAS, SBD, SP) and chemical properties (pH, EC, CEC, and OM) by creating favorable niches for microbial activities and, subsequently, enhancing soil water retention and nutrient availability. In addition, this microbial growth promotes the physiology and, ultimately, the yield of maize plants. Our study results are in line with those of Yadav, Jain, Mishra, Khare, Shukla, Karak and Singh [63] who stated that PSB thrived efficiency on readily available subtracts (BC) and up-scaled the mobilization of P due to secretion of organic acids (anions). This readily available subtracts for PSB strengthened the plant–microbe interaction [67] in the sandy soils. Similarly, the use of PSB alone or in combination with NPK fertilizers [46] improved plant growth and yield [45]. As far as water infiltration in the incubation soil is concerned; all the incubation treatments significantly increased the soil water retention over the control (Table 9) but higher water retention was observed in the PSB addition to sandy soil together with RNPK where infiltration of water was much slower and less leachates were collected in the bottle at the bottom. At the start of the experiment, the PSB inoculated treatments took higher water to mist the whole column over the non-inoculant treatments.

The results of the current study indicate that fresh PSB together with RNPK significantly increases soil water retention (Table 9), relative to the RP alone or PSB alone or CT (non-inoculant control). These results are supported by a number of other studies [68,69]. The difference observed in higher water retention in PSB together with RNPK might be due to slow water movement by PSB colonialization in the column (Figure 3A) against different time durations compared to other treatments. Interestingly, it was observed that the water-staying time at head height was higher over other PSB alone and RP alone treatments, resulting in slow water drainage into the column.

In the end, when we compared the impact of fresh PSB and incubated PSB on sandy soil, it was clear that fresh PSB may be hydrophobic and then hydrophilic when PSB is incubated with prolonged contact with moist sandy soil, as was observed in other past studies [63,68,70,71]. The correlation amonf differet studied parameter are given in the Table 9.

5. Conclusions

In conclusion, the inoculation of PSB together with RNPK significantly increase physiology, growth, yield, and yield related characteristics, and the total P-uptake of maize grown in sandy soil. Bacterial strains used in the present study exhibited plant growth-promoting traits i.e., the production of IAA and solubilization of P and Zn. It also improves the sandy soil selected of physico-chemical properties, dry matter, and qualitative characteristics of maize. The digestibility of maize in term of dry matter attributes is also improved by the addition of PSB.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "conceptualization, H.M.R.J., M.A., R.Q. and A.R.; methodology, H.M.R.J. and M.A.; software, A.R.; validation, H.M.R.J. and M.A.; formal analysis, M.A., A.Z. and A.R.; investigation, H.M.R.J.; resources, H.M.R.J.; data curation, H.M.R.J. and M.H.; writing—original draft preparation, H.H.R.J., M.F., M.N., M.A.C. and M.A.; writing—review and editing, S.I.Z.; visualization, S.I.Z.;

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