# Role of Organic Matter and Phosphate Solubilizing Bacteria in Phosphorus Availability and Wheat Growth in Gypsum Soils

**Abd Al Karim Jaafar[1], Suleiman Salim[1], Vishnu D. Rajput[2*], Iman Ahmad[1], Tatiana Minkina[2], Avnish Chauhan[3]**

[1]Soil Science Department, Faculty of Agricultural Engineering, Damascus University, Damascus, Syria.
[2]Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don, Russia.
[3]Department of Environment Science, Graphic Era Hill University, Bharu Wala Grant, Uttarakhand, India.

*Corresponding author: rajput.vishnu@gmail.com

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**Abstract**

Phosphorus is one of the most abundant mineral elements found in the Earth's crust in both organic and inorganic forms. Although present in high concentrations, only 0.1% of total phosphorus is available for agriculture due to its poor solubility and availability. Thus, the present work aimed to study the effects of mixed organic fertilizers and Phosphate Solubilizing bacteria (PSB) (i.e., *Bacillus megaterium*, *Bacillus polymyxa,* and *Bacillus subtilis)* to enhance the efficiency of wheat crop phosphorus availability in gypsiferous soil. The experiment was conducted in the field conditions using seven different fertilizers and PSB combinations. The wheat grain content of total phosphorus increased significantly in mineral fertilizer + organic fertilizer + PSB treatments by 45%, and in organic fertilizer + PSB by 41%, as compared to the control variant. There was also an increase in the total phosphorus content of the wheat straw, as well as in the weight of wheat grains. The treatments, i.e., T7 (mineral + organic fertilizer + PSB), T4 (mineral + organic fertilizer), and T6 (organic fertilizer + PSB) were significantly superior to the rest of the treatments in the case of P enrichment and wheat growth. The percentage increase in the weight of 1000 grains compared to the control was 29%, 24%, and 22%, respectively, for T7, T4, and T6 treatment. The results indicated that the use of PSB with mineral and organic fertilizers could improve the P availability to plants for sustainable crop cultivation in gypsum rich soils.

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## Introduction

The presence of gypsum (approx. 2%) in the soils benefits crop growth; however, between 2-5% gypsum in soil has little or no negative effect, especially if it is in the powder form (Kuttah and Sato, 2015). The high percentage of gypsum in soil causes a significant decrease in the productivity of crops due to an imbalance in the ratio of (K/Ca) and (Mg/Ca) (Ekholm *et al.,* 2024). Gypsum rich soils suffer with various disturbance such as low ability to retain water (Moret-Fernández and Herrero, 2015), weak structure, rough texture, low plasticity and cation exchange capacity, and an imbalance in nutrients (Abdolvand and Sadeghiamirshahidi, 2024). Gypsum also affects organic matter, nitrogen, and phosphorus which causes a decrease in agricultural crop yields and a reduce in economic returns (Niaz *et al.,* 2022; Verma *et al.,* 2023). Phosphorus is one of the most abundant mineral elements, however, only 0.1% of total phosphorus is available for agricultural crops due to its poor solubility (Ibrahim *et al.,* 2022).

In soil, P is fixed with other mineral elements such as Ca, Al, and Fe to form calcium phosphate, aluminum phosphate, and iron phosphate, thus becoming unavailable to plants. The surface layer of gypsum soil contains a low amount of P available to plants, and when the pH is higher than 7, phosphorus exists mainly in the form of $HPO_4$, which is inaccessible to plants (Van Alphen and Romero, 1971). The efficiency of utilization of phosphate fertilizers is low, which does not exceed 25-30% due to the exposure of phosphorus to chemical fixation in the soil (Chen *et al.,* 2022; Cuesta *et al.,* 2024).

Therefore, to address the problems of gypsum rich soils, especially those related to their fertility, attention is turning to investigate a cheap and economically beneficial alternative to secure the nutritional needs of the plant, including organic fertilizers and biofertilizers such as Phosphate Solubilizing Bacteria (PSB). Organic matter contributes to increasing the soil's organic carbon content and ensuring sufficient nutrition of N, P, and K for crops (Janzen, 2006; Wood *et al.,* 2018). It is stated that applying organic fertilizers along with mineral fertilizers contributes to proper nutrition of the wheat crop, maintaining the soil fertility (Khan and Khalil, 2014), and also leads to a higher percentage of mineral elements in the grains (Saravanan *et al.,* 2013). The use of PSB as biofertilizers plays a vital role in maintaining the status and structure of soil nutrients, able to convert insoluble phosphorus into soluble forms that are bioavailable for absorption by plant roots (Ingle and Padole, 2017; Khan *et al.,* 2021; Li *et al.,* 2023).

Several studies have shown that the addition of PSB to agricultural fields enhanced the growth of various crops, viz., maize, rice, wheat, barley, soybeans, and sesame (Chouyia *et al.,* 2020; Kusale *et al.,* 2021; Martins da Costa *et al.,* 2015). Considering the importance of the wheat crop, the present work was aimed to investigate the impact of organic matter and PSB on wheat crop phosphorus availability in gypsiferous soil, the combination of various fertilizers and PSB was tested to increase the efficiency of phosphorus utilization by wheat, and its productivity in gypsum rich soils under field conditions.

## Materials and Methods

### Study area

The present work was conducted in Raqqa Governorate, northern Syria, on gypsum soils, Beer Al-Hasham area. The experiment area was 30 km west of Raqqa Governorate at the intersection of longitude 38°52'58.77"E and latitude 36° 0'5.16"N, and 280 m above sea level with average precipitation. This region has rainfall: less than 200 mm annually.

### Experiment design

The experiment was conducted in the field within 100 $m^2$ (10 × 10 m) plots (taking into account leaving gaps between the plots of about 1 m), with 8 treatments. The treatments were as follows:

T0: Control without any addition.

T1: Mineral fertilizers: 138 $kg\ h^{-1}$ N, 50 $kg\ h^{-1}$ ($P_2O_5$), 140 $kg\ h^{-1}$ $K_2O$. (According to the fertilizer recommendations of the Ministry of Agriculture) (Al-Zoubi *et al.,* 2013)

T2: Organic fertilizer (Sheep manure): ($20\ tons\ h^{-1}$).

T3: Phosphate solubilizing bacteria (PSB): 5 $liters\ h^{-1}$.

T4: Mineral and organic fertilizer.

T5: Mineral fertilizers + PSB.

T6: Organic fertilizer + PSB.

T7: Mineral fertilizers + organic fertilizer + PSB.

**Soil samples, physical-hydro-physical, chemical and fertility Analyses**

Random soil samples were sampled from the surface layer of 0–25 cm, then it was mixed homogeneously to obtain a composite sample, dried and sieved on a sieve with holes diameter of 2 mm, and stored for physical, chemical and fertility analyzes, and it performed immediately for mechanical analysis, i.e., bulk density; particle density; total porosity; pH; EC; NPK. The results were parented in tables 1 and 2.

**Table 1. Physical properties in the soil of experimental research area**

| **Mechanical composition** (gm $kg^{-1}$) | | | **Texture** | **ρb** | **ρs** | **F** | **FC** |
|---|---|---|---|---|---|---|---|
| sand | silt | clay | Loam | (g $cm^{-1}$) | | (%) | |
| 470 | 350 | 180 | | 1.31 | 2.51 | 47.81 | 21 |

**Mechanical analysis**: by pipette method (Vieillefon, 1979), and the texture was determined based on the American triangle of texture (USDA, 2017).

- **Bulk density (ρb)**: by the field cylinder method (Blake and Hartge, 1986a).

- **Particle density (ρs)**: using the pycnometer method (Blake and Hartge, 1986b).

- **Total porosity (f)**: through the relationship between the apparent and real densities (Danielson and Sutherland, 1986).

$$f = (1 - \frac{\rho b}{\rho s}) \times 100$$

**Field capacitance (FC)**: *In-situ* water is added to a field soil to wet the soil profile to the desired depth. After the water has redistributed into the drier underlying soil and drainage from the initially wetted zone becomes negligible, the water content is taken as *In-situ* FC (Cassel and Nielsen, 1986).

**Soil reaction (pH)**: Soil reaction was determined by using a pH meter on a soil-water suspension (1:2.5) (Thomas, 1996).

**Salinity ($EC_{1:5}$)**: Analyzed by measuring electrical conductivity in a soil-water extract (1:5) (Rhoades, 1996).

**Calcium carbonate ($CaCO_3$)**: By the calcimeter method (Horváth *et al.,* 2005).

**Gypsum ($CaSO_4.2H_2$)**: By the acetone method (Richards, 1954).

**Organic matter (OM)**: By estimating organic carbon using the wet oxidation method with potassium dichromate, then calculating the organic matter according to the following law (Nelson and Sommers, 1996):

**Total nitrogen (TN) in the soil**: Evaluated by digesting the sample using the Kjeldahl method using concentrated sulfuric acid (Bremner, 1996).

**Mineral nitrogen (available)**: Mineral nitrogen was extracted using the Kjeldahl method by extraction with 1 standard solution of potassium chloride (Bremner and Breitenbeck, 1983).

**Available phosphorus**: was determined by the Olsen method (Olsen, 1954).

**Total phosphorus**: Estimated by digestion with aqua regia, color development using ammonium molybdate vanadate, and phosphorus measurement using a flame photometer device at a wavelength of 410 nm (Olsen and Sommers, 1982).

**Available potassium**: Determined by extraction with ammonium acetate and measuring the potassium concentration using a flame device. Photometer (Helmke and Sparks, 1996).

**Cation exchange capacity**: It was calculated using the sodium acetate method, then replacing it with an ammonium acetate solution, then measuring the sodium concentration in the solution resulting from the displacement using a flame Photometer Flam (Chapman, 1965).

The chemical properties of soils and their fertility were presented in Table 2.

**Table 2. Chemical properties and fertility status in the soil**

| pH | EC | $CaCO_3$ | Gypse | OM | TN | TP | N | P | K | CEC |
|---|---|---|---|---|---|---|---|---|---|---|
| | (dS. $m^{-1}$) | (%) | | | | | (gm $kg^{-1}$) | | | (cmol $kg^{-1}$) |
| 7.72 | 0.45 | 4 | 21 | 0.74 | 0.03 | 0.05 | 11 | 7.60 | 112 | 16 |

## Application of organic, mineral, and biofertilizers and their determination

Fermented sheep waste, which is abundant in this region, was chosen as organic fertilizer. The collected sheep waste was dried and sifted on a sieve with hole diameter of 0.5 mm *(Duan et al.,* 2024), and chemical analyzes were carried out for NPK totals, C and organic matter shown in table 3. Organic fertilizer was added at a rate 20 $ton\ h^{-1}$. Mineral fertilizers were applied according to the fertilizer recommendation by the Ministry of Agriculture, and as per the results of the soil analysis. Nitrogen fertilizers at a rate of 138 $kg\ h^{-1}$ in the form of urea (46% N), in three doses the first at planting and the second at planting were applied. First application was on 10/12/2023, (20%), 3rd weeks after sowing (in the 2-3 leaf stage). Second application was on 20/1/2024, (40%) at tillering stage (when there are 3-5 tillers per plant visible). Third application was on 25/3/2024, (40%) 10- 15 days before heading (flowering stage).

**Table 3. Chemical properties of organic fertilizer (sheep manure)**

| pH | EC | C | OM | N | P | K | (C/N) |
|---|---|---|---|---|---|---|---|
| | (dS. $m^{-1}$) | (%) | | | | | |
| 6.94 | 2.11 | 33.65 | 58 | 2.72 | 1.16 | 2.38 | 12.37 |

Phosphate fertilizer in the form of triple super phosphate (TSP) at a rate of 50 $kg\ h^{-1}$ ($P_2O_5$ 46%) before planting were added. Potassium fertilizer in the form of potassium sulfate (50% $K_2O$) at a rate of 140 $kg\ h^{-1}$ were considered for this experiment.

Biofertilizer (commercial of Indian origin- indiaMART PSB) was added in liquid form at a rate of 5 liters $h^{-1}$ (containing three types of bacteria: *Bacillus megaterium*, *Bacillus polymyxa* and *Bacillus subtilis*) ($10^7$ cells). Half a liter of liquid biofertilizer was dissolved in 1 cubic meter of irrigation water, and since the surface area of each plot is 100 $m^2$, so the volume of biofertilizer added to each plot was 100 liters. This volume was mixed with the fermented sheep waste as a carrier material (organic fertilizer) and then spread on the soil in the plot. It was mixed well and homogeneously before planting.

The pH on a suspension (1:5) (organic matter: water by volume), the EC on an extract (1:5) (organic matter: water by volume), the organic matter by incineration at a temperature of 450 °C, and the nitrogen by digesting the organic matter with a mixture of acid concentrated sulfur with selenium, then total nitrogen was determined by the Kjeldahl method, total phosphorus and potassium were digested using the same previous solution, further phosphorus was determined by the colorimetric method and the potassium was determined using a flame photometer device (Tandon, 1993).

**Plant growth condition, cultivation, sampling and analysis**

Wheat seeds, (class Sham7), were planted at a rate of $150\ kg\ h^{-1}$ seeds per plot on 20/11/2023. The aforementioned class is characterized as a high-yielding durum wheat ($7\ tons\ h^{-1}$), resistant to yellow rust and leaf rust, and its grains have good technological specifications. The plots were irrigated by sprinkler irrigation at a rate of $4200\ m^3h^{-1}$. The harvesting process took place on 06/20/2024, where the plants were cut near the surface of the soil, the grains were separated from the straw, then they were dried in the oven at a temperature of 70 °C for 24 hours until the weight was constant, and the weight of the straw and the weight of the grains were calculated. Grinding both grains and straw, and performed chemical analyzes on them.

The plant samples were dried in a drying oven at a temperature of 70 °C until the weight was constant, and the grains were ground using a special grinder (Hanson, 1993). Total phosphorus in grains was estimated by digesting samples with a mixture of concentrated sulfuric acid with selenium and salicylic acid, using vanadate and molybdate, forming a complex yellow-colored compound in an acidic medium with phosphorus, and measuring the color intensity on a spectrophotometer at a wavelength of 430 nanometers (Reuter and Robinson, 1997).

**Statistical analysis**

The experiment adopted a Randomized Complete Block Design (R.C.B.D.), with three replicates for each treatment. The data were analyzed statistically using the statistical analysis program SPSS, version 26 (Pallant, 2020). The analysis of variance (ANOVA) test (F-test) was used to determine whether there were significant differences between the means of the measured elements. The results were compared and the means were arranged according to the least significant difference (LSD) test at the 5%.

**Results and Discussion**

**Physical, chemical properties and fertility status of studied soils and organic matter content**

It is noted from the Tables (1 and 2) that the studied soil has a lubricating consistency according to the American texture triangle (USDA, 2017), and is slightly alkaline (USDA, 2017). It is non-salty and non-calcareous (Bashour and Sayegh, 2007), with a high content of gypsum (Richards, 1954), and a low content of organic matter (FAO, 1980). It has a moderate content of mineral nitrogen, a weak content of potassium (Bashour and Sayegh, 2007), and low availability of phosphorus (Olsen, 1954) and moderate CEC content (Metson, 1957).

**Effects of phosphorus-dissolving bacteria on the average soil content of available phosphorus**

The results at the end of the agricultural season (Figure 1) showed an increase in the soil content of available phosphorus, as treatments T7 (mineral, organic fertilizer and PSB), T4 (mineral and organic fertilizer), T6 (organic fertilizer and PSB), and T2 (organic fertilizer and PSB) significantly outperformed compared to the rest treatments.

Treatments and the percentage increase compared to the control were 151%, 115%, 111%, and 84%, respectively, as the phosphorus level increased from weak to moderate in these treatments. While there were no significant differences between the rest of the treatments, and the level of available phosphorus in the soil remained weak even after adding mineral fertilizer. The order of the treatments according to the soil content of available phosphorus was as follows: T7>T4>T6>T2>T1>T5>T3>T0 (Figure 1). The results clearly showed the role of PSB in increasing the soil content of available phosphorus in treatments to which organic fertilizer (fermented sheep waste) and PSB were added. This is due to the organic fertilizer's high total phosphorus content and the ability of these bacteria to decompose organic matter and secrete organic acids (such as lactic acid and formic acid), converting insoluble phosphorus into soluble forms. This trend is similar with earlier findings (Khan *et al.,* 2021). The role of organic matter is shown to increase the soil content of available phosphorus (Khan and Khalil, 2014). Earlier research also showed that PSB enhanced phosphorus content of calcareous soil (Adnan *et al.,* 2022). The input of organic fertilizers with mineral fertilizers contributes to the proper nutrition of wheat plants and maintains soil fertility.

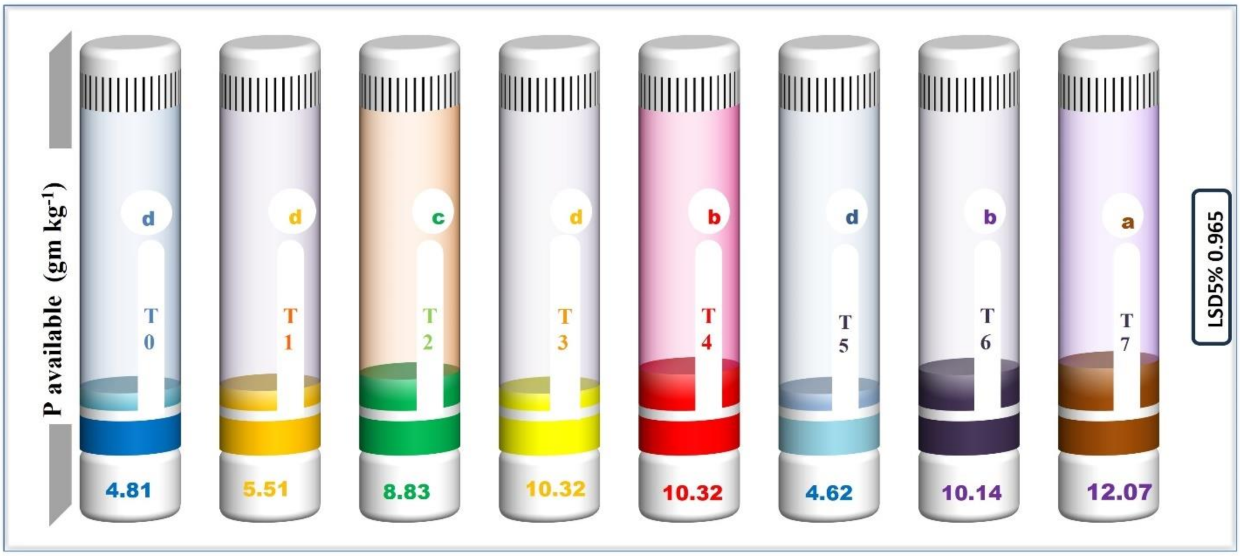


**Figure 1. Average soil content of available phosphorus under the different treatments studied**

**Average plant height of wheat (Sham 7) under the influence of different fertilization treatments**

The results showed an increase in the average height of wheat plants (Figure 2), where treatments T7 (mineral, organic fertilizer and PSB), T6 (organic fertilizer and PSB), T4 (mineral and organic fertilizer) and T2 (organic fertilizer) significantly performed well compared to the control, and height was noted 11.40% (T7), 11.11% (T6), 10.36% (T4), and 10.33% (T2). Then, in terms of significant differences, they were followed by treatments T5 (mineral fertilizer and PSB) and T1 (mineral fertilizer), where the percentage increase in wheat plant height compared to the control was 6.23% and 5.36%, respectively, without significant differences between them, while no significant differences were recorded between the T3 treatment (PSB) and the control. The order of treatments according to plant height was as follows: T7 > T6 > T4 > T2 > T5 > T1 > T3 > T0. The increase in wheat plant height in treatments to which organic fertilizer and PSB were added is attributed to the role of organic matter in enriching the soil with fertility elements and increasing the activity of microorganisms, which improved the vegetative growth of plants (Oldfield *et al.,* 2018).

Organic matter, along with PSB, also plays an important role in increasing the availability of phosphorus in the soil (Kumar and Rai, 2020); thus, it is absorbed by plants, which stimulates its role in basic biological processes within the plant (photosynthesis, respiration), cell formation and division (Havlin *et al.,* 2016), and improves productivity (Ingle and Padole, 2017).

It should be mentioned that the conspicuous superiority of the organic fertilization treatments with phosphate-solubilizing bacteria (T7, T6) indicates the important role of the organic matter beneficial microbe's interaction in enhancing soil quality and promoting plant growth. The findings further indicate that organic fertilizers coupled with PSB might be a viable alternative to enhance wheat productivity and minimize dependence on mineral fertilizers alone.

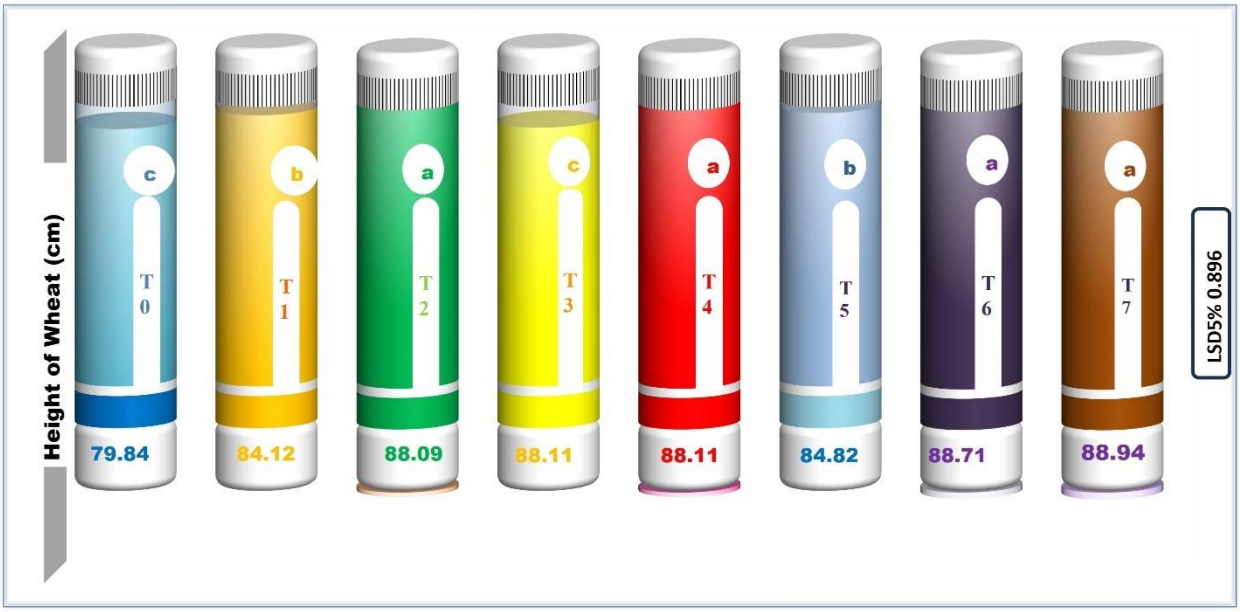


**Figure 2. Average height of wheat under the influence of the studied treatments**

**Effect on the average grain content of total phosphorus under different fertilization treatments** (%)

The results showed an increase in the average grain content of total phosphorus (%) (Figure 3), where treatments T7 (mineral, organic fertilizer and PSB) and T6 (organic fertilizer and PSB) significantly outperformed, and the percentage increase was in the grain content of phosphorus compared to the control (45% and 41%), respectively (Figure 3). In terms of significant differences, it was followed by treatments T2 (mineral and organic fertilizer) and T4 (organic fertilizer), where the percentage increase in the seeds' total phosphorus content compared to the control was 31% and 30.67%, respectively, without significant differences between them. The rest of the treatments were ranked third, outperforming compared to the control without any significant differences between them. The order of treatments according to plant height was as follows: T7 > T6 > T4 > T2 > T5 > T1 > T3 > T0. These results are consistent with noted by (Egamberdiyeva et al., 2004), that the total phosphorus content of cotton plants increased significantly compared to the control as a result of inoculating the plant with PSB.

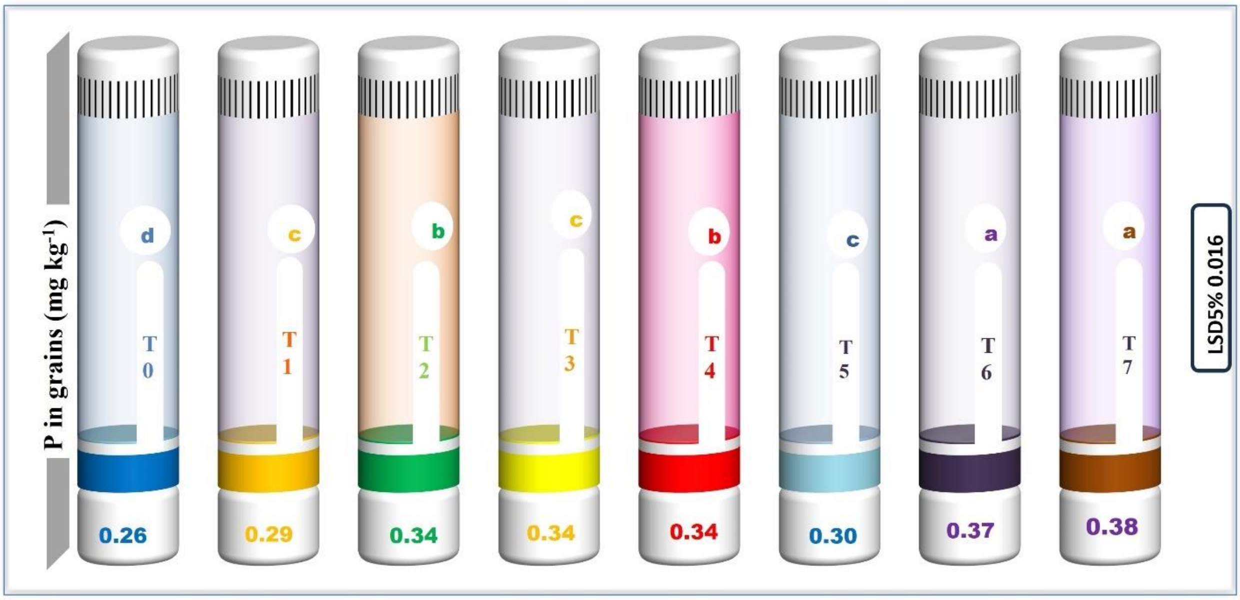


**Figure 3. Average grain content of total phosphorus under different fertilization treatments (mg kg$^{-1}$)**

**Effect on the average straw content of total phosphorus under different fertilization treatments (%)**

The results showed an increase in the average total phosphorus content of wheat straw in treatments containing both organic fertilizer and PSB (Figure 4). The treatments T7, T6, T4 and T2 were significantly better to the rest of other treatments without any significant differences between them, followed by treatments T5 and T1, where the percentage increase in wheat plant height compared to the control was 18% and 15%, respectively, without significant differences between them. While PSB and T3 treatment were slightly superior to the control treatment, and the difference in the total phosphorus content of straw did not exceed by 3%, there were no significant differences between them. The order of the treatments according to the straw's total phosphorus content was as follows: T7>T6>T4>T2>T5>T1>T3>T0. The increase in straw total P content was attributed to the increase in soil P content available in treatments containing organic matter and PSB (Kumar and Rai, 2020); Consequently, plant roots have a greater ability to absorb, transfer, and accumulate phosphorus in the vegetative parts.

The synergistic effect of organic matter and microbial activity is highlighted by straw's noticeably higher phosphorus content from combined organic-PSB treatments (T7, T6). Improved phosphorus solubilization through organic acid production during decomposition and sustained phosphorus release through PSB-mediated mineralization processes are probably the causes of this increased P accumulation. These results highlight the integrated organic-biofertilizer systems could maximize phosphorus use efficiency in wheat production, especially in low-fertility soils where phosphorus availability is usually limiting.

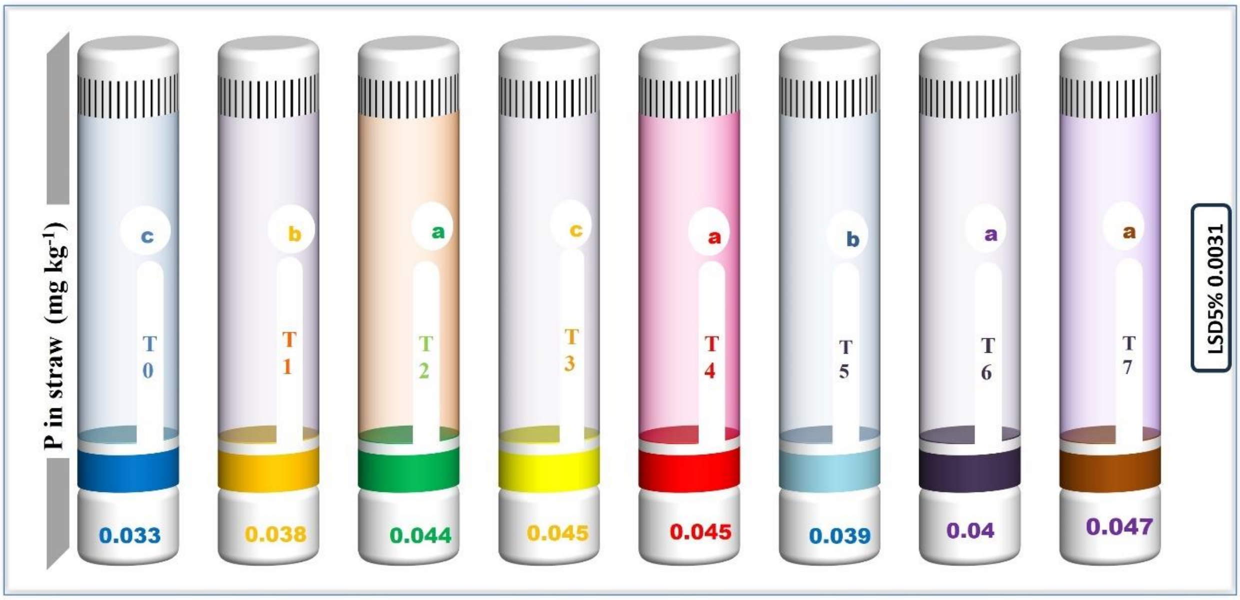


**Figure 4. Average straw content of total phosphorus Under different fertilization treatments (mg kg$^{-1}$)**

**Effects of phosphorus-dissolving bacteria on the average weight of 1000 grains under different fertilization treatments**

The results showed a statistically significant increase in average 1000-grain weight for certain fertilization treatments (Figure 5). Treatments T7, T4, and T6 had the best effects on grain weight, which were 29%, 24%, and 22% increases compared to T0. Treatments T1, T2, and T5 made small or moderate, however, statistically significant, increases in the average 1000-grain weight (11-12% increase over T0), as well as no significant difference among them.

This increase in 1000 grain weight was likely due to the soil in which P was more available because of the interaction of organic matter with PSB. The importance of P to the grain filling process is well documented, as the translocation of assimilates (i.e., carbohydrates and sugars) from the leaves (photosynthetic tissue) to sink organs (grains) is critical in improving yields (Chouyia *et al.,* 2020; Kusale *et al.,* 2021; Nithyapriya *et al.,* 2021).

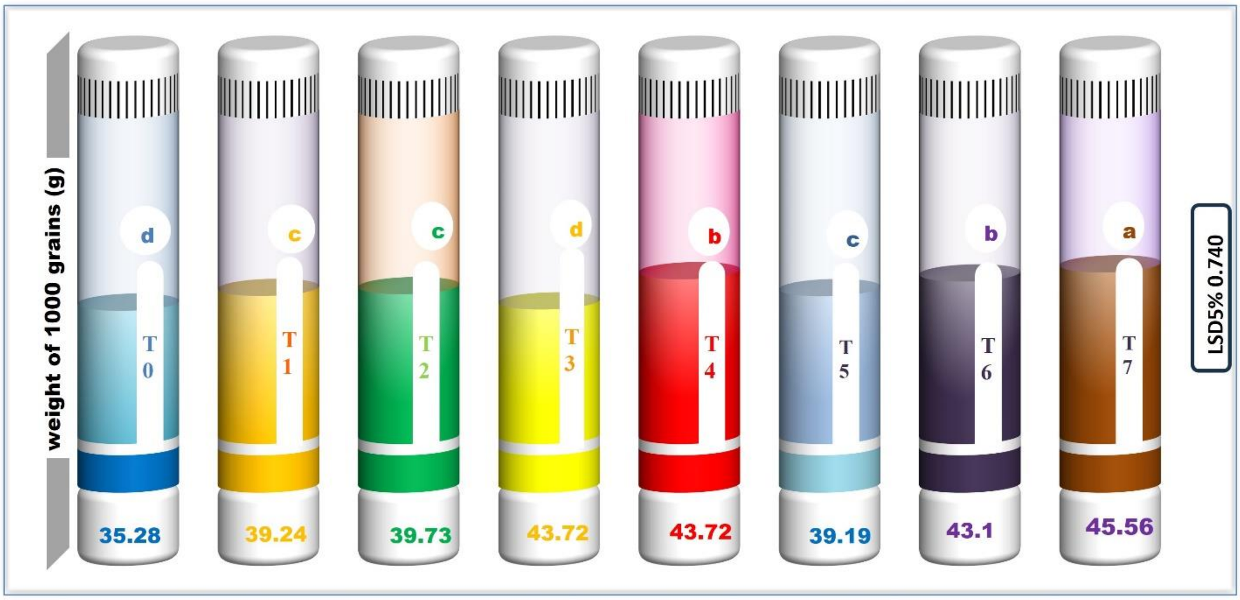


**Figure 5. Average weight of 1000 grains under the influence of different fertilization treatments (g)**

## Pearson correlation coefficient

The Pearson correlation coefficient results (Table 4) showed a strong positive correlation at a significant level (1%) between available phosphorus in the soil and plant height, total phosphorus in grains and straw, and the weight of 1000 grains. This shows the role of the process of mixing organic and mineral fertilizers and PSB in increasing the available phosphorus in the soil and making the plant absorb larger amounts of phosphorus .Which contributes to improving vegetative growth, increasing phosphorus in the plant, and improving productivity (Chinnappa *et al.,* 2025; Richardson *et al.,* 2011; Zhang *et al.,* 2023).

**Table 4. Pearson correlation coefficient between the studied indicators**

| Studied indicators | P Available (gm $kg^{-1}$) | Plant high (cm) | P in grains (%) | P in straw (%) | Weight of 1000 (g) |
|---|---|---|---|---|---|
| **P Available (gm $kg^{-1}$)** | 1 | | | | |
| **Plant high (cm)** | $0.87^{**}$ | 1 | | | |
| **P in grains (%)** | $0.92^{**}$ | $0.925^{**}$ | 1 | | |
| **P in straw (%)** | $0.86^{**}$ | $0.944^{**}$ | $0.92^{**}$ | 1 | |
| **Weight of 1000 (g)** | $0.91^{**}$ | $0.92^{**}$ | $0.90^{**}$ | $0.90^{**}$ | 1 |

$^{(**)}$ Significant level at 1%.

## Conclusions

Integrated input of organic fertilizers with PSB is one of the effective approaches that can be followed to increase soil available phosphorus to enhance crop productivity in gypsum soils. Mineral fertilizers (recommended does: 138 kg $h^{-1}$ N, 50 kg $h^{-1}$ ($P_2O_5$), 140 kg $h^{-1}$ $K_2O$ + organic fertilizer: (20 tons $h^{-1}$) + PSB: 5 liters $h^{-1}$ showed effective amendments to enhance available phosphorus and its uptake by wheat crop grown in the gypsiferous soil. The detailed analysis recommended input of PSB with mineral and biofertilizers to soil P availability at low-cost input and could reduce the cost of cultivation with improved crop yield. However, the effectiveness of PSB with other fertilizers needs to be intensively explored to prepare a sustainable material for gypsum rich soil.

## Conflicts of Interest

The authors declare no conflicts of interest to report regarding the present study.

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