



## Article

# Deciphering the Potential of Bioactivated Rock Phosphate and Di-Ammonium Phosphate on Agronomic Performance, Nutritional Quality and Productivity of Wheat (*Triticum aestivum* L.)

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**Abstract:** Wheat is one of the leading staple crops in many countries. Phosphorus (P) plays an important role for wheat growth and yield as it takes part in many metabolic pathways. Even for soluble phosphatic fertilizers, most of the Pakistani soils, being alkaline and calcareous in nature, show phosphorus use efficiency (PUE) not more than 10–25%. The major issue is the unavailability of P due to fixation and precipitation reactions with soil particles. Composting of rock-phosphate with animal and poultry manures supplied with bio-stimulated phosphate solubilizing bacteria (PSB) not only enhances the RP solubilization but also serves as a potent source of P for plants. Composted/bio-activated rock-phosphate (B-RP), prepared by group of three bacterial strains i.e., *Pseudomonas* sp. (E11), *Bacillus* sp. (MN54) and *Enterobacter* sp. (MN17) aided with molasses (5%) and urea (10%), was tested alone and in various combinations with di-ammonium phosphate (DAP). In this pot trial, the combined application of B-RP and DAP was found superior to the sole application of B-RP. Even the combination of B-RP and DAP sharing equal amount of recommended P showed better results as compared to the sole application of DAP, giving improved shoot biomass (25%), total P-uptake (67%), recovery efficiency of P (75%), dry matter (29%), crude protein (29%), and other yield, physiological and nutritional quality parameters of wheat. So, it could be concluded that integrated use of B-RP and DAP with equal proportion of recommended P could serve as a better management practice for not only improving quantity but also the quality of the wheat grain.

**Keywords:** bio-activated rock-phosphate; di-ammonium phosphate; phosphate solubilizing bacteria; nutritional quality; phosphorus up-take; wheat

## 1. Introduction

Wheat is one of the leading food crops around the globe. It meets the demand of ever-increasing population, being a staple food in many countries including Pakistan and Saudi Arabia [1,2]. It satisfies the dietary demand of approximately 1/3rd population of the world [3] and accounts for 1.7% gross domestic product (GDP) of Pakistan [4]. Phosphorus (P) is an important macro nutrient for plants as it is vital for energy transformation during photosynthesis and respiration and is a constitutional element for hereditary materials i.e., DNA and RNA [5]. Its deficiency causes stunted plant growth and ultimately severe

yield losses [6]. The fertility status of Pakistani soils is following a drastic decreasing trend due to intensive, extensive, and exhaustive cultivation of crops and nutrient mining [7]. Worldwide, almost 70% of soils (acidic or alkaline) are deficient in P [8]. The calcium activity in calcareous soils results in P-fixation as P becomes unavailable/fixed on complex formations with calcium and magnesium. However, it forms complex compounds with aluminum and iron in acidic soils [9]. The major portion of Pakistani soils (80–90%) is regarded as P deficient. A very small fraction (almost 5–20%) of applied P-fertilizers is exploited by plants and a major part becomes unavailable for plants on formation of insoluble P-complexes [10], resulting in very low phosphorus use efficiency (PUE). The nutrients are supplied to soil through addition of either organic manures or chemical fertilizers or both [11]. Di-ammonium phosphate (DAP), the major P-fertilizer used in Pakistan, is a costly input for farmers [5,12]. Its use efficiency hardly exceeds 20%, therefore requiring new generation technologies for improving phosphorus use efficiency (PUE) and increasing agricultural production with minimum dependence on inorganic fertilizers for better socio-economic status of the country, particularly of farmer's community [13,14]. A natural mineral P-source is rock phosphate (RP). It is a cheaper P-source, having extraordinary more utilization potential than soluble DAP, and hence could be taken as an alternative to these expensive and costly soluble P-fertilizers. However, the major disadvantage regarding its application as P-fertilizer is its low solubility, which can be resolved by developing some technologies which could enhance its solubility [15].

There are about 6.9 million tons of RP deposits in Hazara division, Khyber Pakhtun Khwa, Pakistan [16]. The partial acidulation of RP with organic materials may enhance its solubility [17,18]. Manures or organic residues, through amelioration of soil physical and chemical properties and better nutrient uptake, improve plant growth and increase crop yields [19,20]. Poultry manure is relatively rich in plant nutrients as compared to other manures. It maintains soil fertility and improves soil structure [21,22]. Animal manure, likewise, poultry manure, could also bring about partial acidulation and solubilization of RP in addition to improving soil characteristics [23]. The problem of the bulky nature of these organic materials could be sorted out through enrichment with limiting nutrients like addition of RP to these organic sources. Moreover, composting of these RP-enriched manures could transform these into soil applicable forms [24,25]. Further, the rate of composting can be accelerated by using P-solubilizing microbes known as phosphate solubilizing bacteria (PSB), which also convert RP into soluble form known as biological solubilization [26], hence, it shortens the time required to prepare soil applicable composted-RP [27]. At the same time, P comes into a more available form through different types of microbiologically produced organic and mineral acids [28,29]. The absence of readily available carbon (C) and nitrogen (N) sources results in the failure of these PSB to grow and multiply and bring detectable P solubilization. So, bio-stimulation of these PSB with molasses and urea (as C and N sources, respectively) not only enhances the biodegradation of complex organic sources but also accelerates the rate of solubilization of RP [30,31].

Current development in sustainability involves a coherent exploitation of soil microbial activities and the usage of less expensive RP. Therefore taking this background into account, composted or bio-activated rock-phosphate (B-RP) was prepared after composting of RP, poultry and animal manures bio-augmented with consortium of phosphate solubilizing bacteria, i.e., *Bacillus* sp. MN54, *Enterobacter* sp. MN17 and *Pseudomonas* sp. E11 following bio-stimulation with molasses (5%) and urea (10%). Therefore, we hypothesized that the use of composted/bioactivated RP in combination with commercial DAP would increase the productivity and nutritional quality of wheat through enhanced agronomic P utilization. The specific objectives of the present study were to evaluate the effects of bioactivated RP in combination with DAP on agronomic performance, nutritional profile and productivity of wheat.

## 2. Materials and Methods

### 2.1. Composted/Bio-Activated Rock Phosphate Preparation and Analysis

For the preparation of composted/bio-activated rock phosphate (B-RP), rock-phosphate (RP), animal manure (AM), and poultry manure (PM) were mixed with 50, 25 and 25% ratios of recommended P, respectively. The PSB including *Bacillus* sp. MN54, *Enterobacter* sp. MN17 and *Pseudomonas* sp. E11 were collected from Soil and Environmental Microbiology Laboratory, Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad (UAF), Pakistan [32–36] were introduced with these mixed P-sources and bio-stimulated with urea and molasses (5 and 10% weight of the applied P-sources, respectively) to enhance the rate of biodegradation for solubilization of RP and preparation of composted/bio-activated rock-phosphate (B-RP). The B-RP was analyzed for total P, carbon (C), nitrogen (N), and carbon to nitrogen ratio (C:N) as mentioned in Table 1. It was used in this pot experiment on P-equivalent basis.

**Table 1.** Physico-chemical analysis of soil, organic sources and bio-activated rock phosphate.

Properties	Soil	Animal Manure	Poultry Manure	Bio-Activated Rock Phosphate	LSD
Sand (%)	46 ± 2.66	-	-	-	
Silt (%)	29 ± 2.66	-	-	-	
Clay (%)	25 ± 1.73	-	-	-	
Texture	Sandy clay loam	-	-	-	
Saturation percentage (%)	31.5 ± 1.68	-	-	-	
Cation exchange capacity (CEC) (Cmol <sub>c</sub> kg <sup>-1</sup> )	13.6 ± 1.22	-	-	-	
pHs	7.819 ± 0.01	-	-	-	
Electrical conductivity (ECe) (dS m <sup>-1</sup> )	1.98 ± 0.11	-	-	-	
Organic matter (%)	0.68 ± 0.16	63.51 ± 3.57 a	69.93 ± 2.72 a	37.90 ± 4.11 b	7.02
Carbon (%)	-	35.28 ± 1.98 a	38.85 ± 1.51 a	21.06 ± 2.29 b	3.91
Ash (%)	-	36.49 ± 3.57 b	30.07 ± 2.72 b	62.10 ± 4.11 a	7.02
Olsen phosphorus (mg kg <sup>-1</sup> )	6.43 ± 1.45	-	-	-	
Total phosphorus (%)	-	0.69 ± 0.08 b	1.25 ± 0.07 b	4.92 ± 0.55 a	0.64
Total nitrogen (%)	0.043 ± 0.02	1.19 ± 0.04 c	1.85 ± 0.12 b	2.77 ± 0.11 a	0.19
Extractable potassium (mg kg <sup>-1</sup> )	120 ± 18.33	-	-	-	
Total potassium (%)	-	0.54 ± 0.10 c	1.10 ± 0.07 b	2.25 ± 0.07 a	0.16
Carbon: Nitrogen (C:N)	-	29.64 ± 0.94 a	21.00 ± 1.99 b	7.60 ± 0.68 c	2.66

All values are average of three replicates ± Standard error. Means sharing similar letters in a column do not differ significantly from one another ( $p \geq 0.05$ ). Least significant difference (LSD) test was used to assess statistical significance by analysis of variance.

### 2.2. Soil Analysis

Whole soil lot was taken from field area of ISES, UAF, Pakistan. From this lot, three representative soil samples were ground, sieved (2 mm sieve) and analyzed for soil characteristics (Table 1). Fourteen kilograms of ground and sieved soil were filled in 0.0136 m<sup>3</sup> polyethylene lined pots with a radius of 12 cm and a height of 30 cm. Soil analyses were based on the methods as stated in [37], otherwise mentioned. Bouyoucos [38] method for textural analysis was used. Weight of saturated and oven dried soil was used for soil saturation percentage. The electrical conductivity (EC) and pH of saturated-soil paste were measured with conductivity meter Jenway 4510 (Cole-Parmer, Staffordshire, United Kingdom) and pH meters PHS-25CW (Bante instruments, Shanghai, China), respectively. For total nitrogen (N), Jackson [39] method was used, while [40] method was used for extractable potassium (K) and cation exchange capacity (CEC) and organic matter (OM) were determined as mentioned by [41]. Sodium bicarbonate solution (0.5 M) at pH 8.5 was used for available/Olsen's P [42].

### 2.3. P-sources Analysis

All the P-sources (AM, PM and B-RP) were analysed for mineral matter/ash, organic matter (OM), carbon (C), nitrogen (N), C:N, total phosphorus (P), and potassium (K) while RP was analysed for total P only before applying to this pot study (Table 1). Ash and OM were determined directly by igniting the P-sources in muffle furnace (at 400–600 °C) for almost 4–6 h till white ash [41]. Organic carbon was estimated by dividing the OM with 1.8 as mentioned by Brake [43]. Organic sources were subjected to Kjeldahl method for N-determination [44]. Samples were digested by following wet digestion method for P and K determination [45]. Phosphorus was analysed at 430 nm absorbance spectrophotometrically in digested samples of all P-sources [46] and K by using flame photometer in the same digested samples [47]. Carbon to nitrogen ratio (C:N) was estimated using the division formula (dividing C with N).

### 2.4. Climatic Conditions and Growth Period

Faisalabad has a semi-arid climate, according to Köppen–Geiger classification, with very hot and humid summers and dry cool winters. The average maximum and minimum temperatures in June are 40.5 °C (104.9 °F) and 26.9 °C (80.4 °F). In January the average minimum and maximum are 19.4 °C (66.9 °F) and 4.1 °C (39.4 °F). Wheat (*Triticum aestivum* L.) variety Galaxy-2103 were sown on 25th November and harvested on 30th April. This variety was developed through three way hybridization crossing between Punjab-96, Wattan and MH-97 by Wheat Reaserach Institute (WRI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Galaxy-2013 is a semi-erect plant with a height of 105–115 cm and a yellow straw colour. Its flag leaf is semi-erect, and its auricle is white in colour.

### 2.5. Experiment Description

The P-sources (RP, AM, PM, B-RP, DAP and combinations of B-RP with DAP) were applied to the experimental pots on the P-equivalent basis. During combined application of B-RP and DAP, these sources were mixed with different ratios (B-RP:DAP as 75:25, 50:50 and 25:75% of recommended P). Fertilizers and manures were introduced to the pots (N:P:K at the rate of 120:90:60 kg ha<sup>-1</sup>). Urea was applied as main N-source in 3 splits (1/3rd at sowing and each remaining 1/3rd after an interval of 30 and 45 days after sowing). All the P-sources (RP, AM, PM, B-RP, DAP and combinations of BRP with DAP) were applied at sowing time. Muriate of potash (MOP), a potassium source, was also applied at sowing of wheat seeds. All pots as well as control were supplied with K and N (there was no phosphatic fertilizer in control). Six seeds of 'Galaxy-2013' (a wheat variety) per pot were sown for germination and pots were irrigated with canal water. The seeds were very kindly provided by WRI, AARI, Faisalabad. Later on, only three plants (per pot) were sustained for maturity. A total of 9 treatments were applied at the time of sowing and each treatment was replicated 3 times thus making 27 experimental pots comprising of control 00% P (T<sub>1</sub>), RP 100% P (T<sub>2</sub>), AM 100% P (T<sub>3</sub>), PM 100% P (T<sub>4</sub>), B-RP 100% P (T<sub>5</sub>), B-RP 75% P+DAP 25% P (T<sub>6</sub>), B-RP 50% P+DAP 50% P (T<sub>7</sub>), B-RP 25% P+DAP 75% P (T<sub>8</sub>) and DAP 100% P (T<sub>9</sub>). Weeds were eradicated manually and mixed in the same pot. For all treatments alike agronomic practices were performed; the only difference among the treatments was the P-source. Treatments were repeated thrice in completely randomized design (CRD). At maturity, plants were harvested.

### 2.6. Plant Sampling and Analysis

Plant growth parameters including spike length, plant height, fertile tillers, grain weight (100 grains) and yield (biological, grain and straw yields) were observed for wheat plants. Spike length and plant height (at maturity) were measured by using a meter rod. Fertile tillers per pot were counted. After harvesting grain weight (100 grains) and yield were recorded. For physiological measurement, the fully expanded top second leaf of each pot was selected. SPAD-502 (Konica-Minolta, Osaka, Japan) meter was used for the measurement of chlorophyll contents. CIRAS-3 portable photosynthesis system (PP system,

Amesbury, USA) was used for the measurement of gaseous exchange measurement like plant photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs). Chemical parameters (N, P, and K in grains and straw) and nutritional quality parameters (dry matter, crude protein, fat/oil, fiber and mineral matter/ash in grains) were also determined to evaluate the effect of combined application of B-RP and DAP. All these chemical and nutritional quality parameters were determined according to the methods of Association of Official Analytical Chemists, [48]. Total P uptake and PUE were deduced from the formulae given below;

$$\text{Total P-uptake} = \text{P-uptake (Grains)} + \text{P-uptake (Straw)} \quad (1)$$

and

$$\text{PU} = \frac{\text{Grains or straw weight (oven dried) / Yield}}{100} \times \text{P}(\%) \quad (2)$$

where PU = Phosphorus uptake

$$\text{PUE} (\%) = \frac{\text{Total P uptake by fertilized plant} - \text{Total P uptake by unfertilized plant}}{\text{Amount of fertilizer applied}} \times 100 \quad (3)$$

where PUE = phosphorus use efficiency.

### 2.7. Statistical Data Analysis

The collected data were analyzed statistically by using analysis of variance (ANOVA) [49] and treatment means were compared using Tukey's Honest significant difference (HSD) test at 5% probability. Standard errors (SE) were calculated to check variability of population (replicate) mean from sample mean through Microsoft Excel 2016 (Microsoft, Redmond, Washington, DC, USA). Pearson correlation among different plant growth attributes was determined through Minitab version 17 (Minitab LLC, Pannsylvania, United States), while regression lines were drawn by using XLStat 2018 (Addinsoft Inc., New York, NY, USA).

## 3. Results

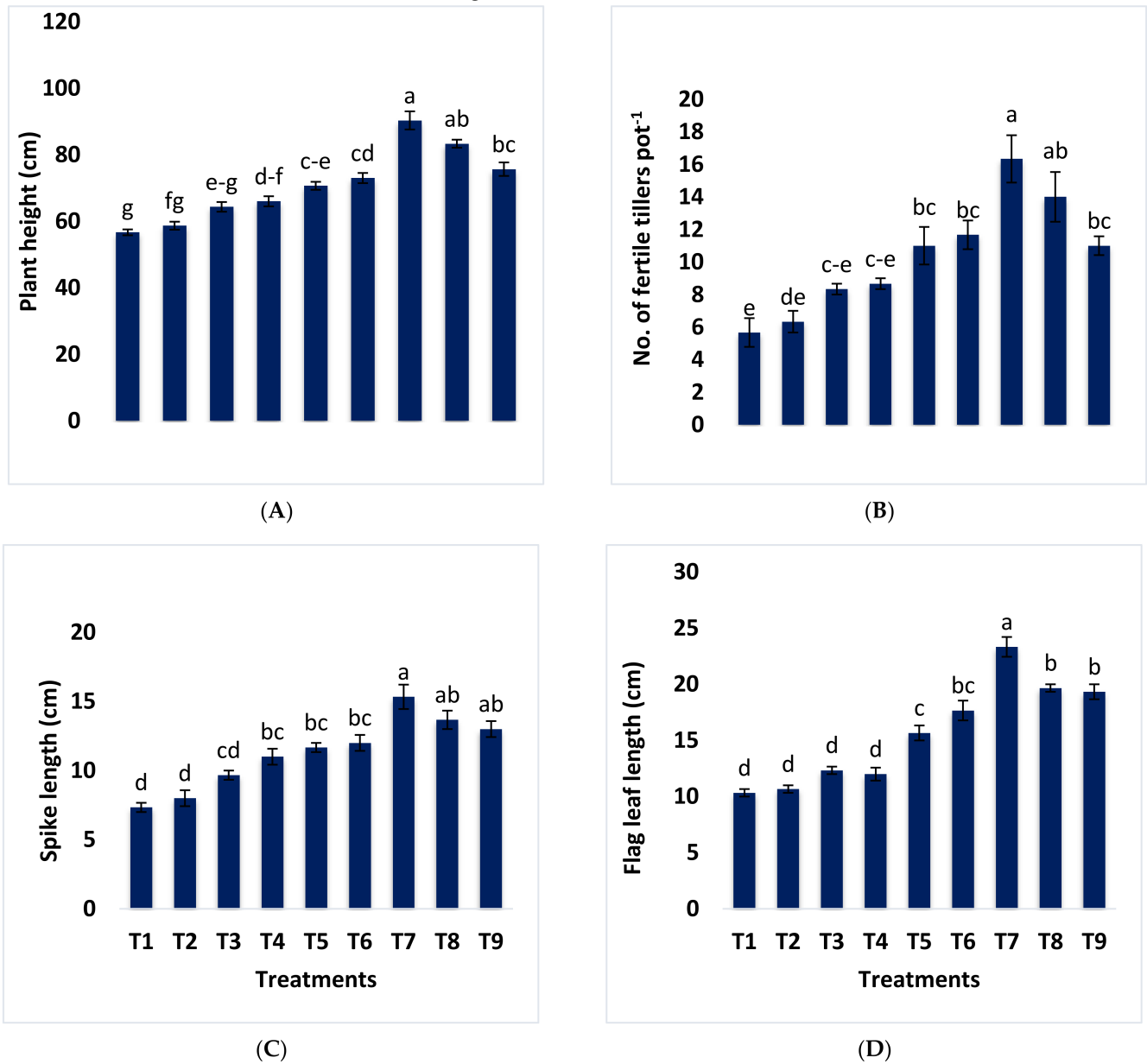
### 3.1. Effect of Different Combinations of B-RP and DAP on Growth and Yield Parameters of Wheat

Sole application of B-RP resulted in improved growth and yield contributing parameters as compared to that of control and RP treatments, albeit maximum improvement in all these parameters was observed where B-RP and DAP were used in combination with equal P-share i.e., T<sub>7</sub> treatment having 50% of recommended P from each source as presented in Figures 1 and 2.

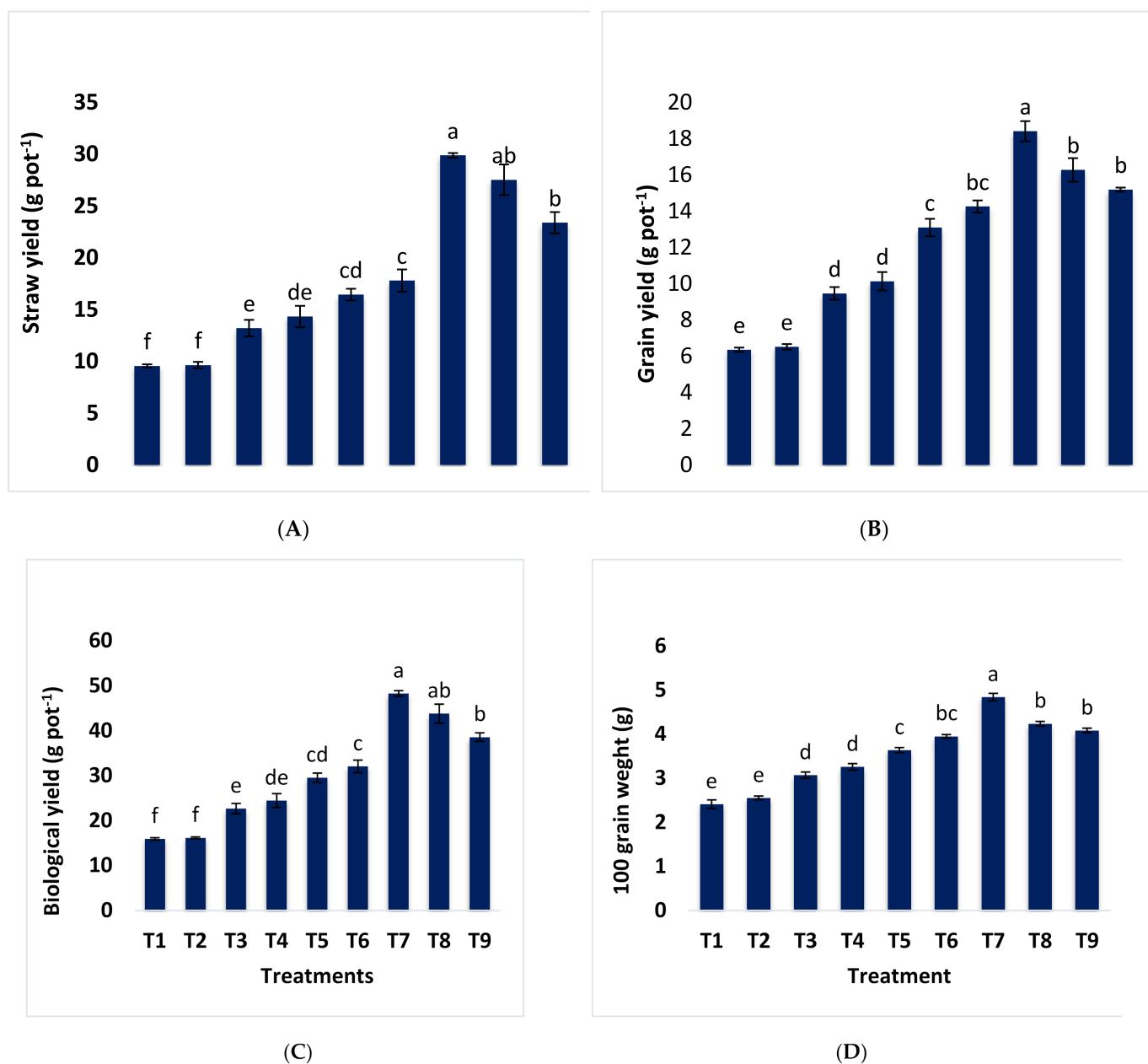
Data in Figure 1A revealed that plant height of the leading T<sub>7</sub> treatment (90.3 cm) was at par (83.3 cm) with T<sub>8</sub> treatment (B-RP + DAP with 25:75 P ratio). These two treatments increased plant height by 19.4 and 10.1%, respectively over the treatment of sole DAP (at 100% P). All the treatments (T<sub>6</sub>, T<sub>7</sub> and T<sub>8</sub>) applying B-RP in combination with DAP in different proportions showed compatible results with sole application of DAP (T<sub>9</sub>). However, maximum value (16.3) for number of fertile tillers per pot was counted for T<sub>7</sub> treatment and it was found non-significant to T<sub>8</sub> i.e., B-RP + DAP sharing 25 and 75% P, respectively, with 14.0 tillers (Figure 1B). These treatments were followed by T<sub>6</sub> {B-RP (75% P) + DAP (25% P)}, T<sub>9</sub> {DAP (100% P)}, and T<sub>5</sub> {RP 100% P} treatments with 11.7, 11.0 and 11.0 tillers per pot, respectively. Similarly, maximum spike length (15.3 cm) was acquired by the same treatment using half P from B-RP and half from DAP i.e., T<sub>7</sub> (B-RP + DAP having 50% P from each source). Spike length of pots furnished with B-RP + DAP (in 25:75% P ratio) and DAP (100% P) were 13.7 and 13.0 cm, respectively and these were found at par with T<sub>7</sub> treatment. Moreover, T<sub>7</sub> treatment increased spike length by 58.5 and 39.4% over treatments of sole application of AM (T<sub>3</sub>) and PM (T<sub>4</sub>), respectively. As evidenced from Figure 1D, maximum flag leaf length (23.3 cm) was measured in plants treated with B-RP and DAP used at equal rates (T<sub>7</sub>). Minimum (10.3 cm) of it was measured in control was very close to the treatments of sole application of each of rock-P, AM and



PM with flag leaf length of 10.7, 12.3 and 12.0 cm, respectively. The outstanding treatment T<sub>7</sub> produced estimated increase of 94.4, 89.2 and 20.7% over sole applications of AM, PI M and DAP, respectively.



**Figure 1.** Effect of different combinations of B-RP and DAP on plant height (A), no. of tillers (B), spike length (C) and flag leaf length (D) of wheat. Bars sharing similar letters do not differ significantly at  $P < 0.05$ ; The standard error (SE) represents variation of population mean from sample mean ( $n = 3$ ); T1: Control (0% P), T2: RP (100% P), T3: AM (100% P), T4: PM (100% P), T5: B-RP (100% P), T6: B-RP (75% P) DAP (25% P), T7: B-RP (50% P) + DAP (50% P), T8: B-RP (25% P) + DAP (75% P), T9: DAP (100% P). RP: Rock phosphate, AM: Animal manure, PM: Poultry manure, B-RP: Bio-activated rock phosphate, DAP: Diammonium phosphate.



**Figure 2.** Effect of different combinations of B-RP and DAP on straw yield (A), grain yield (B), biological yield (C) and 100 grain weight (D) of wheat. Bars sharing similar letters do not differ significantly at  $P < 0.05$ ; The standard error (SE) represents variation of population mean from sample mean ( $n = 3$ ); T1: Control (0% P), T2: RP (100% P), T3: AM (100% P), T4: PM (100% P), T5: B-RP (100% P), T6: B-RP (75% P) + DAP (25% P), T7: B-RP (50% P) + DAP (50% P), T8: B-RP (25% P) + DAP (75% P), T9: DAP (100% P). RP: Rock phosphate, AM: Animal manure, PM: Poultry manure, B-RP: Bio-activated rock phosphate, DAP: Diammonium phosphate.

The combination of B-RP + DAP (in 50:50 ratio) produced 27.8, 108.6, 126.29, 209.8 and 212.8% increase in straw yield in comparison to that yielded by the treatments sole application of each of DAP, PM, AM, RP and control, respectively. Sole application of B-RP though produced more, yet its combination with DAP resulted in better straw production. As the combinations of B-RP + DAP in 75:25, 25:75 and 50:50 ratios generated 8.3, 67.4 and 81.8% more straw yield, respectively, relative to that produced by sole B-RP. Similarly, this prominent treatment (T<sub>7</sub>) which excelled among all the treatments, again resulted in 21.2, 81.6, 94.5, 182.9, and 189.3% increase in grain yield over that of sole applications of DAP, PM, AM, RP and control, respectively (Figure 2A,B). Relatively, maximum shoot

biomass ( $48.28 \text{ g pot}^{-1}$ ) was yielded by  $T_7$  treatment (Figure 2C). The following treatments were B-RP + DAP (in 25:75 ratio) and sole DAP which yielded  $43.78$  and  $38.56 \text{ g pot}^{-1}$ , respectively. The leading treatment ( $T_7$ ) resulted in 25.2, 97.5 and 113.1% more increases in shoot biomass as compared to that yielded by the treatments of sole applications of DAP, PM and AM, respectively. It also increased yield by 203.5 and 199.0% as compared to that of control and sole RP, respectively. The combination B-RP+DAP, with equal share of P from each source ( $T_7$ ), indicated an increase of 18.7% in 100 grains weight over that of DAP (100% P). Again, it could also be drawn from these results that the combined application of B-RP and DAP as B-RP:DAP at 75:25, 50:50 and 25:75 ratios increased 8.5, 33.0 and 24.7% 100 grains weight, respectively, over that of B-RP applied alone as evidenced from (Figure 2D).

### 3.2. Effect of Different Combinations of B-RP and DAP on Physiological Parameters of Wheat

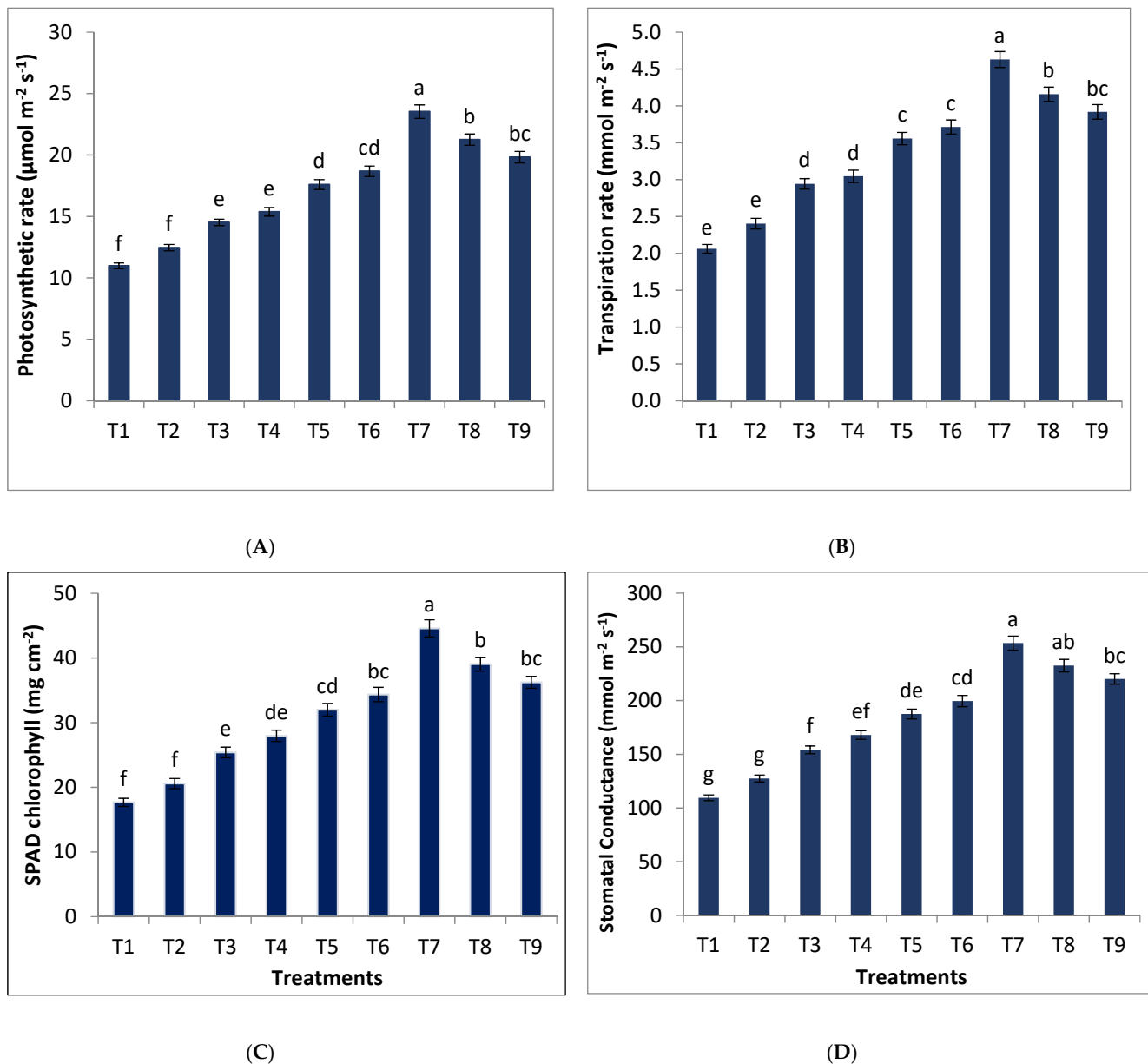
Plants treated with B-RP and DAP at equal rates ( $T_7$ ) showed highest increase in photosynthetic rate ( $23.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). Minimum ( $11.0 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) of it was measured in control which was very close to the treatments of sole application of each of rock-P, AM and PI M with photosynthetic rate of 12.5, 14.5 and  $15.4 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , respectively. The outstanding treatment  $T_7$  produced estimated increase of 62.1, 53.0 and 18.7% over sole applications of AM, PI M and DAP, respectively (Figure 3A). Almost parallel trends were monitored for evaluating the effect of combined application of B-RP and DAP regarding transpiration rate. Combinations (B-RP + DAP at 50:50 and 25:75 ratios) were found either superior or equivalent to the sole application of DAP. Transpiration rate in  $T_7$  treatment (B-RP + DAP at 50:50 ratio) increased up to 18.1% over that of sole DAP application as shown in Figure 3B. Sole application of B-RP and its all combinations with DAP were statistically at equality over the sole application of DAP. Similarly, highest gas exchange characteristics i.e., stomatal conductance ( $254 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) was attained by using half P from B-RP and half from DAP ( $T_7$ ). Stomatal conductance of pots furnished with B-RP + DAP (in 25:75% P ratio) and DAP (100% P) were 233 and  $220 \text{ mmol m}^{-2} \text{ s}^{-1}$ , respectively and these were found at nearly same level with  $T_7$  treatment. In addition,  $T_7$  treatment increased stomatal conductance by 64.5 and 50.9% over treatments of individual application of AM ( $T_3$ ) and PM ( $T_4$ ), respectively. Chlorophyll contents were found minimum in the control ( $17.7 \text{ mg cm}^{-2}$ ) and almost equal to sole RP ( $20.6 \text{ mg cm}^{-2}$ ) treatments. Utmost contents of chlorophyll ( $44.6 \text{ mg cm}^{-2}$ ) were found in combination of B-RP + DAP at 50:50 ratio ( $T_7$ ) which revealed 23.0% increase over DAP applied at the rate of 100% P. The B-RP + DAP at 25:75 and 75:25 ratios statistically equivalent over sole DAP (100% P). It is observed from treatments of B-RP in different combinations with DAP performed better than the treatment with the sole application.

### 3.3. Effect of Different Combinations of B-RP and DAP on Chemical Parameters of Wheat

For N-content in wheat grains following treatments of B-RP + DAP at 50:50 and 25:75 ratios ( $T_7$  and  $T_8$ ) were superior to sole DAP treatment while B-RP + DAP at 75:25% P, respectively, and B-RP at 100% P produced competitive results with sole DAP. The combinations of B-RP with DAP performed much better than the sole application of B-RP. The treatments comprising of B-RP + DAP with 75:25, 25:75 and 50:50 P-ratios were found with 20.1, 29.8, and 42.2% higher grain N-concentration, respectively, over that of B-RP (with 100% P) as shown in Table 2. For straw N-concentration,  $T_7$  produced 21.7, 78.3, 84.4, 185.7 and 228.9% more N-concentration in comparison to the treatment applied with sole DAP, PM, AM, RP and control, respectively. The combined application of B-RP with DAP in 50:50 P ratio showed 31.1 and 36.1%, 103.4 and 162.1%, 143.8 and 196% increases in grain and straw P-concentration over DAP, PM and AM (each applied as sole P-source with 100% P), respectively (Table 2). Similarly, the results indicated superiority of combined application of B-RP and DAP over sole application of B-RP as well as DAP for improving K in wheat grain and straw (Table 2). As for grain K-content, treatments B-RP + DAP (P in 75:25 ratio), B-RP + DAP (P in 25:75 ratio) and B-RP + DAP (P in 50:50 ratio) showed 2.7 and



4.6% (non-significant), 14.1 and 17.0% (significant), 26.4 and 29.5% (significant) increases in K-content over sole DAP and B-RP treatments, respectively. Likewise, for straw K-content, sole application of DAP was inferior to the treatments of combined application of B-RP and DAP. Hence, treatments T<sub>6</sub> (B-RP+DAP having P in 75:25 ratio), B-RP + DAP having P in 25:75 ratio) and T<sub>7</sub> (B-RP + DAP having P in 50:50 ratio) showed 2.3, 20.0 and 28.2% increases, respectively, over that of DAP applied as sole P source while these resulted in 8.4, 27.2 and 35.8% increases, respectively, over that of B-RP applied as sole P-source.



**Figure 3.** Effect of different combinations of bio-activated rock-phosphate (B-RP) and di-ammonium phosphate (DAP) on photosynthetic rate (A), transpiration rate (B), SPAD chlorophyll (C) and stomatal conductance (D) of wheat. Bars sharing similar letters do not differ significantly at  $P < 0.05$ ; The standard error (SE) represents variation of population mean from sample mean ( $n = 3$ ); T1: Control (0% P), T2: RP (100% P), T3: AM (100% P), T4: PM (100% P), T5: B-RP (100% P), T6: B-RP (75% P) + DAP (25% P), T7: B-RP (50% P) + DAP (50% P), T8: B-RP (25% P) + DAP (75% P), T9: DAP (100% P). RP: Rock phosphate, AM: Animal manure, PM: Poultry manure, B-RP: Bio-activated rock phosphate, DAP: Diammonium phosphate.

**Table 2.** Effect of different combinations of B-RP and DAP on chemical parameters of wheat.

Treatments	Grain			Straw		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
T <sub>1</sub> : Control (0% P)	0.82±0.03 f	0.06 ± 0.01 f	0.65 ± 0.03 e	0.44 ± 0.44 e	0.05 ± 0.01 g	0.93 ± 0.01 d
T <sub>2</sub> : RP (100% P)	0.89 ± 0.04 f	0.07 ± 0.01 f	0.67 ± 0.02 e	0.51 ± 0.04 e	0.05 ± 0.01 g	0.96 ± 0.03 d
T <sub>3</sub> : AM (100% P)	1.18 ± 0.07 e	0.12 ± 0.01 e	0.82 ± 0.01 d	0.79 ± 0.07 d	0.08 ± 0.01 f	1.42 ± 0.027 c
T <sub>4</sub> : PM (100% P)	1.25 ± 0.08 e	0.15 ± 0.01 e	0.84 ± 0.02 d	0.82 ± 0.03 d	0.10 ± 0.01 f	1.42 ± 0.01 c
T <sub>5</sub> : B-RP (100% P)	1.51 ± 0.05 d	0.19 ± 0.01 d	1.13 ± 0.01 c	1.15 ± 0.03 c	0.14 ± 0.01 e	1.70 ± 0.03 b
T <sub>6</sub> : B-RP (75% P) + DAP (25% P)	1.81 ± 0.06 bc	0.20 ± 0.01 cd	1.18 ± 0.01 c	1.22 ± 0.05 bc	0.16 ± 0.01 d	1.84 ± 0.07 b
T <sub>7</sub> : B-RP (50% P) + DAP (50% P)	2.15 ± 0.058 a	0.30 ± 0.02 a	1.47 ± 0.05 a	1.46 ± 0.04 a	0.25 ± 0.01 a	2.30 ± 0.04 a
T <sub>8</sub> : B-RP (25% P) + DAP (75% P)	1.96 ± 0.115 ab	0.26 ± 0.01 b	1.32 ± 0.01 b	1.33 ± 0.08 ab	0.22 ± 0.01 b	2.16 ± 0.11 a
T <sub>9</sub> : DAP (100% P)	1.67 ± 0.09 cd	0.26 ± 0.02 c	1.16 ± 0.04 c	1.20 ± 0.05 bc	0.18 ± 0.01 c	1.80 ± 0.07 b
HSD	0.201	0.024	0.069	0.139	0.016	0.148

All values are average of three replicates. Means sharing similar letters in a column do not differ significantly from one another ( $p \geq 0.05$ ). The standard error (SE) represents variation of population mean from sample mean ( $n = 3$ ); RP: Rock phosphate, AM: Animal manure, PM: Poultry manure, B-RP: Bio-activated rock phosphate, DAP: Diammonium phosphate.

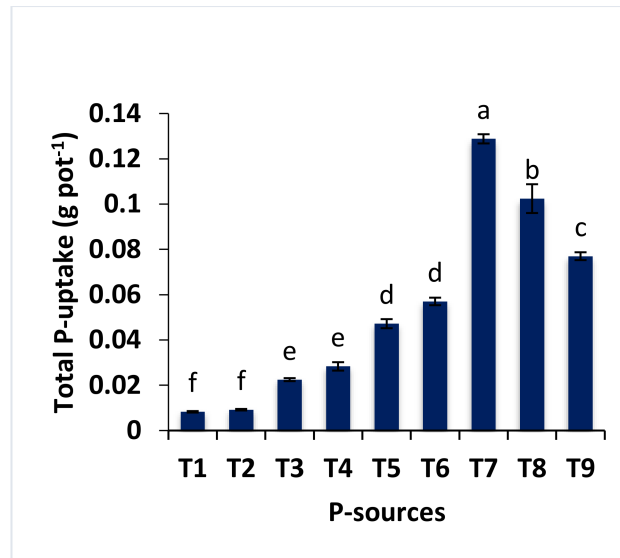
The two leading treatments, T<sub>7</sub> (B-RP + DAP having P in 50:50 ratio) and T<sub>8</sub> (B-RP + DAP having P in 25:75 ratio), brought about 67.3 and 172.9%, 33.0 and 117.0% increases in P uptake of wheat, respectively (Figure 4A), as compared to that of sole application of each of DAP (T<sub>9</sub>) and B-RP (T<sub>5</sub>). Again, for P-recovery efficiency, T<sub>7</sub> (B-RP + DAP in 50:50 P-ratio) was regarded as the best treatment with maximum P-recovery efficiency (33.4%) followed by P-recovery efficiency (26.1%) treatment T<sub>8</sub> (B-RP + DAP in 25:75 P-ratio); these fetched about 75.3 and 36.9% increases in P-recovery efficiency, respectively, over that of sole DAP treatment (T<sub>9</sub>) (Figure 4B).

### 3.4. Effect of Different Combinations of B-RP and DAP on Wheat Nutritional Quality

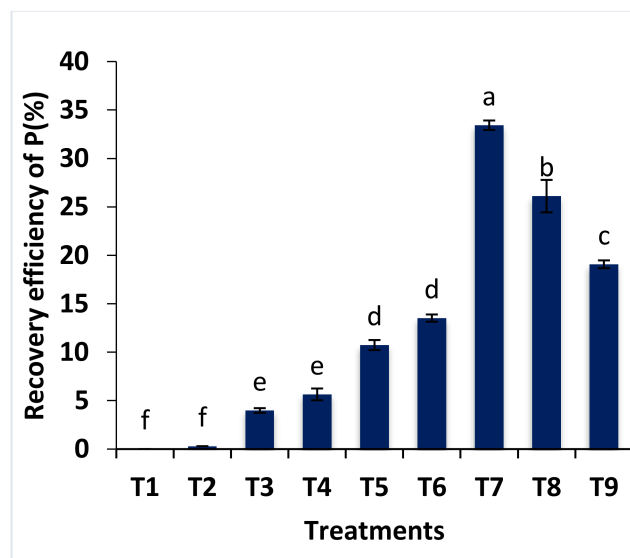
Different combinations of P sources (B-RP + DAP at 50:50 and 25:75 ratios) were found either superior or equivalent to the sole application of DAP. Dry matter in T<sub>7</sub> treatment (B-RP + DAP at 50:50 ratio) increased up to 28.8% over that of sole DAP application as shown in Table 3. Sole application of B-RP and its all combinations with DAP were statistically at par with the sole application of DAP. For crude protein estimation, the two leading combinations i.e., B-RP+DAP at 50:50 and 25:75 ratios (T<sub>7</sub> and T<sub>8</sub>), produced 28.8 and 17.6% increase, respectively, over the sole DAP application (Table 3). Even B-RP + DAP at 75:25 ratio and B-RP at 100% P were at par with that of DAP (at 100% P). However, DAP at 100% P, was superior to all the treatments including control (00% P), RP, AM and PM (each applied with 100% recommended P).

Fats/lipids contents were found minimum in control (0.55%) and sole RP (0.63%) treatments. Maximum (1.49%) fats were estimated in combination of B-RP+DAP at 50:50 ratio (T<sub>7</sub>) which elucidated 19.2% increase over that of DAP applied at the rate of 100% P. The other two combinations (B-RP+DAP at 25:75 and 75:25 ratios) were found to be statistically equivalent with sole DAP. It could be noted that the treatments of B-RP in different combinations with DAP performed better than the treatment with the sole application of B-RP at 100% P. Maximum (1.29%) crude fiber was determined by applying B-RP + DAP at 50:50 ratio, followed by B-RP + DAP at 25:75 ratio of P and DAP at 100% P (with 1.11 and 1.02% crude fiber, respectively). These two proficient combinations of B-RP + DAP at 50:50 and 25:75 ratios (T<sub>7</sub> and T<sub>8</sub>) were observed with 26.5 (significant) and 8.8% (non-significant) increases, respectively, over DAP applied alone. Again, minimum crude fiber was estimated in control and sole RP treatments (0.44 and 0.48%). Ash content

is also an important estimation for the presence of vital minerals necessary to maintain the minerals balance and normal metabolic activities. It was estimated maximum (1.28%) in B-RP + DAP at 50:50 ratio. Although this treatment was at par with B-RP + DAP at 25:75 ratio of P and DAP at 100% P (with 1.17 and 1.15% mineral matter, respectively) yet it produced 11.3% increase (statistically non-significant) over DAP (100% P).



(A)



(B)

**Figure 4.** Effect of different combinations of B-RP and DAP on total P uptake (A) and recovery efficiency of P (B) of wheat. Bars sharing similar letters don not differ significantly at  $P < 0.05$ ; The standard error (SE) represents variation of population mean from sample mean ( $n = 3$ ); T1: Control (0% P), T2: RP (100% P), T3: AM (100% P), T4: PM (100% P), T5: B-RP (100% P), T6: B-RP (75% P) + DAP (25% P), T7: B-RP (50% P) + DAP (50% P), T8: B-RP (25% P) + DAP (75% P), T9: DAP (100% P). RP: Rock phosphate, AM: Animal manure, PM: Poultry manure, B-RP: Bio-activated rock phosphate, DAP: Diammonium phosphate.

**Table 3.** Effect of different combinations of B-RP and DAP on nutritional quality parameters of wheat.

Treatments	Dry Matter (%)	Crude Protein (%)	Fats/Lipids (%)	Fibre (%)	Mineral Matter (%)
T <sub>1</sub> : Control (0% P)	73.6 ± 1.6 d	5.10 ± 0.18 f	0.55 ± 0.04 g	0.44 ± 0.03 g	0.36 ± 0.05 g
T <sub>2</sub> : RP (100% P)	73.9 ± 1.8 d	5.56 ± 0.22 f	0.63 ± 0.07 fg	0.48 ± 0.04 fg	0.44 ± 0.03 fg
T <sub>3</sub> : AM (100% P)	75.5 ± 2.2 cd	7.38 ± 0.41 e	0.77 ± 0.03 efg	0.55 ± 0.05 fg	0.58 ± 0.07ef
T <sub>4</sub> : PM (100% P)	77.2 ± 0.8 bcd	7.83 ± 0.53 e	0.84 ± 0.05 ef	0.62 ± 0.06 ef	0.65 ± 0.07 de
T <sub>5</sub> : B-RP (100% P)	82.2 ± 2.3 abc	9.44 ± 0.32 d	0.95 ± 0.09 de	0.73 ± 0.05 de	0.81 ± 0.07 cd
T <sub>6</sub> : B-RP (75% P) + DAP (25% P)	82.5 ± 2.4 ab	11.33 ± 0.40 bc	1.07 ± 0.11 cd	0.85 ± 0.06 cd	0.96 ± 0.13 bc
T <sub>7</sub> : B-RP (50% P) + DAP (50% P)	85.8 ± 2.6 a	13.42 ± 0.36 a	1.49 ± 0.05 a	1.29 ± 0.07 a	1.28 ± 0.05 a
T <sub>8</sub> : B-RP (25% P) + DAP (75% P)	84.0 ± 3.3 a	12.25 ± 0.72 ab	1.30 ± 0.14 ab	1.11 ± 0.08 b	1.17 ± 0.09 a
T <sub>9</sub> : DAP (100% P)	82.7 ± 3.2 ab	10.42 ± 0.55 cd	1.25 ± 0.07 bc	1.02 ± 0.09 bc	1.15 ± 0.03 ab
HSD	6.742	1.261	0.224	0.174	0.201

All values are average of three replicates. Means sharing similar letters in a column do not differ significantly from one another ( $p \geq 0.05$ ); The standard error (SE) represents variation of population mean from sample mean ( $n = 3$ ); RP: Rock phosphate, AM: Animal manure, PM: Poultry manure, B-RP: Bio-activated rock phosphate, DAP: Diammonium phosphate. T<sub>1</sub>: Control (0% P), T<sub>2</sub>: RP (100% P), T<sub>3</sub>: AM (100% P), T<sub>4</sub>: PM (100% P), T<sub>5</sub>: B-RP (100% P), T<sub>6</sub>: B-RP (75% P) + DAP (25% P), T<sub>7</sub>: B-RP (50% P) + DAP (50% P), T<sub>8</sub>: B-RP (25% P) + DAP (75% P), T<sub>9</sub>: DAP (100% P).

So, it could be concluded that the combination of B-RP + DAP with equal share of P resulted in 3.8, 28.8, 19.2, 26.5 and 11.3% increases in dry matter, crude protein, fats, fiber and mineral matter, respectively, over the results estimated in the treatment of DAP with 100% P.

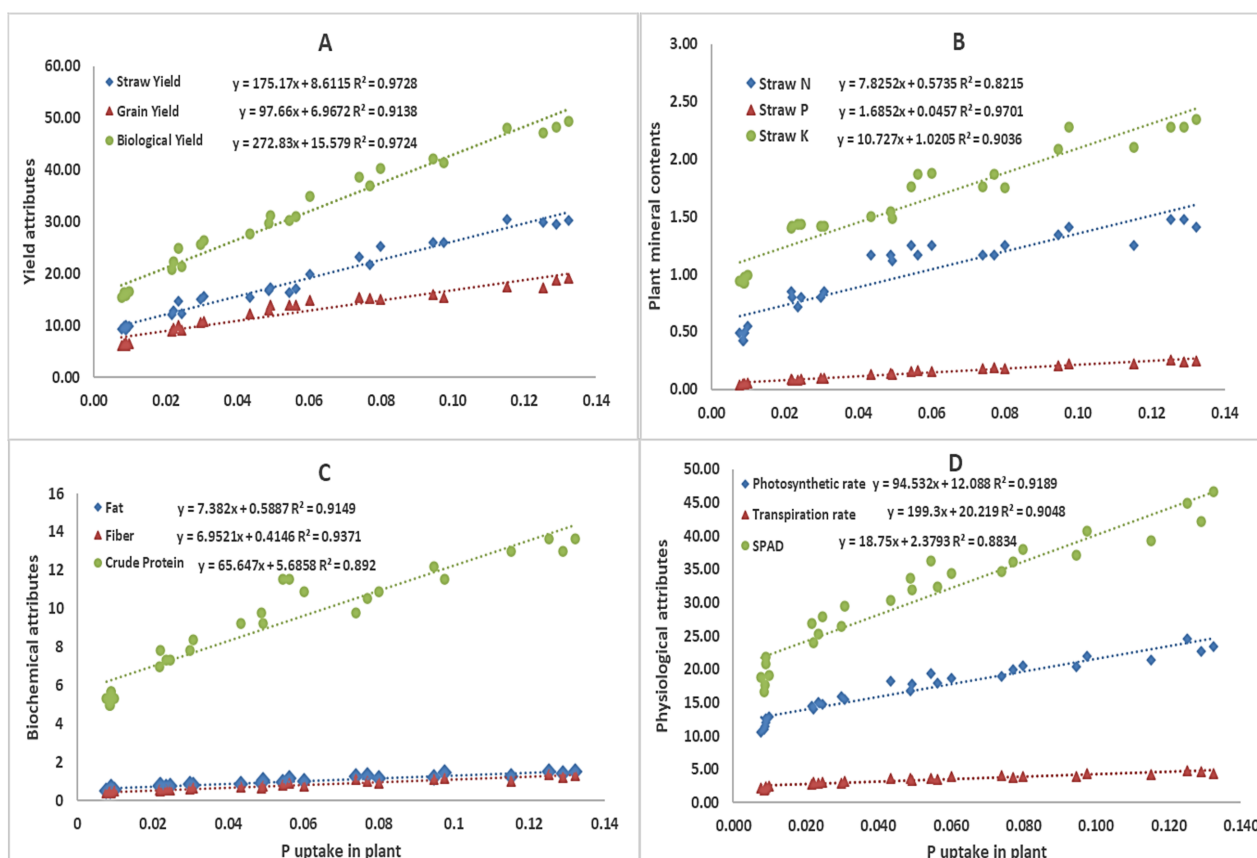
### 3.5. Correlation and Regression Analysis

Significant positive correlations were observed among plant yield and nutrients (straw yield, grain yield, straw N, straw P, straw K, P uptake), physiological (chlorophyll contents, photosynthetic rate, transpiration rate) and biochemical parameters (crude protein, fat contents, fiber contents, dry matter) along with P sources in soil and plant tissues (Table 4). Similarly, significant regression lines were analyzed when different growth, nutrients, physiological and biochemical parameters were with plant P uptake (Figure 5).

**Table 4.** Pearson correlation among the plant growth, physiological, biochemical nutritional parameters of wheat plant.

Variables	DM	SY	SN	SP	SK	Fat	Fib	CP	GY	PU	PR	TR
SY	0.920											
SN	0.988	0.919										
SP	0.966	0.988	0.964									
SK	0.938	0.963	0.963	0.978								
Fat	0.955	0.984	0.957	0.994	0.974							
Fib	0.941	0.991	0.932	0.993	0.961	0.994						
CP	0.975	0.943	0.986	0.979	0.986	0.971	0.958					
GY	0.986	0.959	0.990	0.988	0.977	0.986	0.971	0.989				
PU	0.928	0.992	0.920	0.989	0.959	0.982	0.994	0.950	0.960			
PR	0.974	0.970	0.981	0.992	0.980	0.993	0.980	0.987	0.994	0.971		
TR	0.980	0.958	0.990	0.985	0.976	0.984	0.966	0.986	0.995	0.958	0.998	
SPAD	0.971	0.966	0.977	0.988	0.977	0.990	0.977	0.984	0.991	0.970	0.998	0.995

The abbreviations are as: straw yield (SY), grain yield (GY), straw N (SN), straw P (SP), straw K (SK), crude protein (CP), fat contents (Fat), fiber contents (Fib), dry matter (DM), P uptake (PU), chlorophyll contents (SPAD), photosynthetic rate (PR), transpiration rate (TR).



**Figure 5.** Relationship among plant P uptake and different yield (A), nutritional (B), biochemical (C) and (D) physiological attributes of wheat ( $n = 3$ ).

#### 4. Discussion

It is vital to improve P availability and efficiency for better yield and improved quality of wheat grain [7,50]. The solubility of RP can be improved through its composting, by using phosphate solubilizing microbes along with organic manures [51–53]. P-solubilizing bacteria (PSB) make P more available for plant uptake [54] through; i) excretion of organic acids [54,55], ii) mineralization of soil organic compounds [56], iii) conversion of tri-calcium phosphate to soluble P [35,57–60], iv) mineralization by the enzymes mainly by phosphatases and phytases enzymes [61] and phytases [62], v) and production some mineral acids such as hydrochloric acid (HCl), nitric acid (HNO<sub>3</sub>) and sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) [63,64] and anionic compound i.e., carboxylic anions [33]. Urea and molasses cause higher rate of decomposition of organic wastes [65–68] through provision of a readily available C-source and narrowing down the C:N ratio [69,70].

So, composting of RP along with organic manures bio-augmented with PSB and bio-stimulated with molasses and urea has great potential to bring insoluble soil phosphates in the RP into soluble forms through the secretion of organic and mineral acids [26,71,72]. Therefore, in the present pot experiment, application of B-RP prepared through composting of RP, AM and PM with the help of PSB supplied with molasses and urea was explored to evaluate its effectiveness for improving quality, quantity and PUE of wheat.

The results of this pot study manifested that the treatment combination of B-RP and DAP in equal proportion fetched encouraging and marvelous results in improving not only growth and yield physiological and chemical parameters of wheat but also enhances its nutritional quality and PUE as well. The combined application of B-RP and DAP in 25:75% ratio of recommended P, respectively, followed it. The leading combination efficiently improved yield and yield contributing factors like plant height, number of fertile



tillers, 100 grain weight and shoot biomass by 19.4, 48.4, 18.6 and 25.2%, respectively, over sole DAP (100% P). Similarly, plant physiological parameters i.e., chlorophyll contents, photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs) were also enhanced by treatments of B-RP in different combinations with DAP. Photosynthetic pigments (chlorophyll) are responsible for absorbing the light energy of definite wavelengths necessary for photosynthesis [73]. Loss of water in the form of transpiration is coupled to the process of photosynthesis and stomatal conductance [74,75]. Tiny pores in the plant leaves in the form of stomata permitted gas exchange at cost of water loss [74,76]. The opening and closing activity of stomata is regulated by special guard cells [3,74–76]. This might be explained as this integrated approach resulted in better soil environment and its physico-chemical properties [77]. These manipulated conditions might have improved organic matter content, soil porosity, water and nutrient holding capacities which assured continuous and uninterrupted nutrient supply to the wheat crop to meet its nutritional requirements resulting in improved wheat yield [78,79]. Comparatively inferior results by sole application of DAP (100% P) might have resulted because of the inability of DAP to supply continuous nutrients, especially P. As P from soluble DAP was vulnerable to its fixation to the soil particles, so at crucial stages it might not supply adequate amount of P due to P-immobilization either through adsorption and/or chemical precipitation which might have reduced the wheat yield [80,81]. Moreover, there are different mechanisms through which PSB in B-RP result not only improved P-solubilization but their secretions also reduce P-fixation. These bacteria excrete the organic acids which enhance the solubility of the rock phosphate or inorganic phosphate complexes [55]. The insoluble forms of P like tri-calcium phosphate may be converted to soluble P by PSB [57] through principal means of secretion of low molecular weight organic acids by these microorganisms [58,59]. For, organic-P, the mineralization process transforms organic-P into soluble-P [60] which is assisted by the enzymes mainly by phosphatases and phytases enzymes and phytases [61,62]. Further, PSB produce some mineral acids such as hydrochloric acid (HCl), nitric acid (HNO<sub>3</sub>) and sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) [63,64]. These acids also accelerate the process of dissolution of insoluble tri-calcium phosphate (TCP) and enhance P-availability to plants. These PSB also produce anionic compound i.e., carboxylic anions increasing available phosphorus by anionic exchange which reduces P-fixation/ unavailability [33].

Further, this composite source of B-RP and DAP (50:50 ratio) improved not only chemical constituents (N, P and K) of wheat grain and straw but also yielded grains with better nutritional quality by improving nutrient use efficiencies, especially PUE. The cumulative effect of PSB enriched composted RP applied with DAP was found more effective and efficient approach for improving yield and nutrients uptake of wheat and mung bean by earlier studies of [82,83]. As composted RP is a vital source of organic materials for sustained microbial population of PSB, it not only improves P-solubilization of RP but also enhances solubilization of fixed soil-P. Moreover, application of DAP along with composted RP ensures optimum nutrient uptake through creating suitable and conducive soil environment [84,85]. The enhancement in growth, yield and nutrient contents of wheat in the present study could be attributed to direct effects of bioactivated rock phosphate through increased P supply and indirectly through the enhancement of microbial activity due to addition of organic materials used in bio-activation process. Moreover, as the P sources in the present study were bioaugmented with P-solubilizing microbes, these microbes might have enhanced indigenous and applied P solubilization through diverse mechanisms such as rhizospheric acidification and nutrient solubilization which in turn have improved plant growth and yield contributing factors in the present study. These results are in accordance with previously reported works [7,86].

The combination of B-RP and DAP with equal share of P resulted in 3.8, 28.8, 19.2, 26.5 and 11.3% increases in dry matter, crude protein, fats, fiber, and mineral matter, respectively over the results estimated in the treatment of DAP with 100% P. It could be concluded that this integrated approach might have maintained the nutrients demand on continuous basis throughout the growing season due to the presence of organic and mineral sources

of P. Organic matter assures avoidance of P-fixation and slow release of nutrients while DAP supplies a quick source of P-availability [83,87,88]. In addition to this, there are additive advantages of using consortium or mixed culture of PSB as they increase soil P by producing organic acids, inorganic/mineral acids, chelators and phosphatase enzymes, leading to enhanced P-availability for longer and continuous supply of P and is evidenced by previous studies as well [89–93]. Moreover, the application of organic materials might have improved the physical conditions of soil through enhanced soil aggregation and soil aggregate stability [94], which in turn have improved water holding capacity and nutrient retention in the soil after fertilizer application.

Similarly, using combined organic sources might have fetched similar results not only for continuous nutrient supply but also the sustained solubilization of RP [95]. Biochemical variations of different substrates (Table 1) depicts varying organic carbon, nitrogen and ash contents. These substrates have different biochemical variations among those carbon to nitrogen ratio (C:N) is of vital importance for PSB. The narrower the C:N the more will be mineralized, and the broader the C:N the more will be immobilized. Hence, PM and AM, due to these structural/biochemical variations (C:N ratio is more critical), release available P by accelerating rate of biodegradation/composting. So, the composite organic source (AM + PM) results in more and prolonged P-supply through mineralization of composite source by PSB as main agent during earlier stages is PM (due to narrow C:N ratio) and during later stages it is AM bearing relatively broader C:N ratio [96,97]. Furthermore, in the present study, we found higher nutritional quality parameters of wheat grains under the combined application of P sources. This could be attributed to the presence of ammonium in DAP fertilizer which on hydrolysis might have caused acidification and resultantly enhanced solubility of bound P from Ca-P complexes [7]. The increased grain quality parameters in the present study may also be due to the enhanced supply of micronutrients such as Fe, Zn, Cu and Mn to wheat crop [98,99].

## 5. Conclusions

It was concluded that nutrient uptake of wheat grain can be improved through integrated approach of manipulating B-RP and DAP in combined application. In the present experiment, the combined application of B-RP and DAP, particularly in equal proportions, was found superior to their sole application as well as different treatment combinations in improving plant growth, shoot biomass, total P-uptake, PUE, dry matter, lipid and protein content, and other yield, physiological and nutritional quality parameters of wheat. This eco-friendly approach manages organic wastes on one hand and adds to the better livelihood of the farmers on the other hand through enhanced crop production. More importantly, utilization of national/indigenous RP sources could profitably alleviate the problem of huge amounts spent on importing DAP from foreign countries every year and improving wheat yield both quantity and quality wise. The findings of this experiment encourage to verify these results in field study for better exploration of this technology.

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