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RESEARCH ARTICLE

Comparative efficacy of phosphate solubilizing bacteria and synthetic phosphate fertilizers on the growth of wheat

Aamir Khan[1], Mohammad Safdar Baloch[1], Naimat Ullah[2]*, Sheikh Zain Ul Abidin[2], Muhammad Zeeshan Bhatti[3], Raees Khan[4], Asghar Ali Khan[1], Hammad Ismail[5], Asma Saeed[6]* & Hadia Gul[6]

[1]Department of Agronomy, Faculty of Agriculture, Gomal University, Dera Ismail Khan, 29050, Pakistan
[2]Institute of Biological Sciences (Botany Program), Gomal University, Dera Ismail Khan, 29050, Pakistan
[3]Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, 46000, Pakistan
[4]Department of Plant Sciences, Quaid-i-Azam University Islamabad, 45320, Pakistan
[5]Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat 59700, Pakistan
[6]Institute of Biological Sciences, Gomal University, Dera Ismail Khan, 29050, Pakistan

*Email: naimat@sabanciuniv.edu, asmasaeed78@gmail.com

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Abstract

Wheat is recognized as one of the most important dietary elements due to its high nutritious content and thus, has become greatest food option all over the world. Phosphorus (P) being major plant food nutrient plays a vital role multiple functions of plant growth and development. The current study was carried out to compare the performance of phosphate solubilizing bacteria (PSB) as bio-fertilizer with commercially available phosphate fertilizers on wheat crop. The trial was designed in randomized complete block (RCB) replicated thrice. Six different sources of phosphate fertilizers (Di-ammonium phosphate as DAP, Nitrophos as NP, Single super phosphate as SSP, Restore as PSB, Marathon as PSB, Nitrogen (N2) fixing bacteria as PSB) followed by control were evaluated for agronomic, physiological and quality attributes of wheat. The results showed that most of the qualitative traits were significantly influenced by different treatments. However, application of N_2 fixing bacteria was more significant in all treatments. Highest total viable count of colony-forming units (14.63×10^6 at 3-WAS & 17.70×10^6 after harvest CFU g^{-1}), maximum tillers' count (337 m^{-2}), grains' count (45.57 $spike^{-1}$), grain yield (2714.3 kg ha^{-1}), LAI (0.67 & 1.16 at 56 & 112 DAS), CGR (13.59 g day^{-1} m^{-2}), photosynthesis rate (26.13 µ mol m^{-2} sec^{-1}) and flag leaf sugar content (0.24%) were recorded on account of using N_2-fixing bacteria applied as PSB. Moreover, NPK content in shoot, grain as well as uptake of NPK by grain were also received as highest in the same treatment. Based on research findings, it is concluded that application of N2-fixing bacteria as PSB (7.5 kg ha^{-1}) might be increasing wheat production in Dera Ismail Khan and other areas of similar environment in Pakistan.

Keywords

Biological processes, phosphate fertilizers, phosphate solubilizing, microorganism, synthetic, wheat

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops, accounting for 1.6% of GDP and 8.9% of agriculture values in Pakistan. Wheat crop cover area of 8.7 million ha in the country with a total production of 25.2 million metric tons (1). Many biotic and abiotic stresses have an impact on wheat yield. Among these, the use of nutrients particularly phosphorus

(P) which is a structural component of the cell membrane, chloroplast, mitochondria and nucleic acids (DNA and RNA) is critical (2, 3). Phosphorus nutrition is associated with the formation of energy compounds (like AMP, ADP and ATP), root development, tillers, flower and seed formation, resistance to plant diseases, and strengthening of cereals straw to reduce lodging. It generate energy and performs various metabolic reactions, it promotes maturation and creates resistance to environmental stress, and thus increase yield (4). Plant dry matter contains 0.1 to 0.4% phosphorus on average, while the average phosphorus content present on soil surface is 0.05%. In 1895, Nobbe and Hiltner made a breakthrough by commercializing "Nitragin" as a biofertilizer in their laboratory and then azotobacter and blue-green algae were discovered for accelerating plant growth and development (5, 6).

Phosphorus deficiency effects approximately 43% of soils globally, and approximately 90% of soils in Pakistan have moderate to severe phosphorus deficit. Although some soils have total phosphorus reserves, the plant available P fraction is approximately one-tenth of total P as documented (7). Phosphorus deficiency is the most common limiting growth factor in crops after nitrogen (8, 9). Its deficiency decreases dry matter accumulation, carbohydrate metabolism, soluble protein content, and cell division (10). Plants absorb a portion of P fertilizers, while soil pH and $CaCO_3$ activity convert the remaining P to fixed/insoluble forms. Pakistani soils are calcareous and alkaline in nature, with high base saturation, low organic matter and a phosphorus deficit, which is the major soil fertility issue (11). The mobility of P in plants and soil is low as compared to other macronutrients including nitrogen and potassium (12). Plants obtain phosphorus from the soil in the form of phosphate ions. It exists in the soil as H_2PO_4 (orthophosphate) and reacts with other nutrients including iron and aluminium oxides to form insoluble compounds that plants cannot use (13, 14). Similarly, P fixation has been identified as a limiting factor in the tropics and a major constraint for crop production (15).

Chemical fertilizers are commonly used to compensate for soil nutrient deficiencies and increase crop productivity. The rising prices of synthetic fertilizers and their detrimental effects on soil fertility, tilth, and the health of human beings are the major problems (16, 17). The chemical fertilizers application is economical and less laborious but it is deleterious to ecosystem. Moreover, continued use of synthetic fertilizers reduces soil microbial activity and organic matter (18, 19). Therefore, it is emphasized that an alternative source, which overcomes phosphorus deficiency and reliance on costly synthetic fertilizers. Under these conditions, the use of plant root zone bacteria in conjunction with a small amount of inorganic fertilizers appears to be viable alternative to mitigate the practice of chemical fertilization and subsequent improvement of plant and soil health (20). Previously, it was proposed that using PSB and organic phosphate together would increase soil Olsen-P, bacterial population and soil organic matter. It will reduce the use of chemical fertilizers without compromising plant growth. Similarly, research findings showed that commercial bio-inoculants (Bacillus and Pseudomonas species of P-solubilizing bacteria) significantly increased seed P content, tiller formation and wheat yield when used in single and dual combination (21-23). The use of PSB appears to be a profitable and sustainable approach in P solubilisation (24).

Soil microorganisms (e.g. P solubilizing bacteria-PSB) increase plant nutrient accumulation. They perform many biological activities including the conversion of insoluble soil phosphorus (25). The bio-inoculation of PSB is important in maintaining soil nutrient status and structure as well as providing new avenues for improved crop production (26). PSB has great potential as a bio-fertilizer and it is cost-efficient, environmentally friendly, and increases crop productivity (27). P-solubilizing microorganisms such as bacteria, actinomycetes and fungi provide phosphorus to plants as well as plant hormones, such as gibberellin and auxin as well as vitamins which are used to supplement chemical fertilizers in order to maintain soil health and maximize crop production (19, 28). These bacteria either directly promote plant growth by solubilizing phosphorus, zinc and potassium or indirectly by releasing a siderophore, hydrogen cyanide and ammonia (29, 30). PSB not only solubilizes synthetic phosphate but nitrogen-fixing bacteria may also solubilize phosphorus (31).

The plant growth, development, and enhancement of soil fertility could be enhanced in different locations by the application of PSB along with rock phosphate (29). Since several environmental factors influence PSB performance in soil, it is suggested that factors influencing the survival mechanism and function of these P-inoculants be identified in order to understand the efficacy and behaviour of these microbes in different regions (32). Understanding the dynamics of bacterial functioning and interference in soils is required in order to develop specific inoculant formulations for improved phosphorus utilization in different soils and plants. The availability of P in the plant roots with soil nutritional status, soil microenvironment, and plant species (33).

Therefore, the current trial was initiated to investigate the impact of P and N solubilizing bacteria as well as various sources of synthetic P fertilizers on wheat quality, yield and agronomic attributes. Given the forgoing, it was felt necessary to initiate a research study with the goals of assessing the efficacy of PSB in wheat crop, investigating the impact of chemical P fertilizers on wheat yield, and comparing the effects of PSB and synthetic fertilizers on nutrients in shoots, seed and their uptake in wheat.

Materials and Methods

Study area

The research was carried out in the research area, Department of Agronomy, Gomal University, Dera Ismail Khan, Pakistan using a randomized complete block design (RBCD) with 3 replications. Each replication was divided into 7 units, resulting in 21 plots measuring 5 × 1.2 square meters each.

Variety and Treatment

Wheat variety "Israr Shaheed" was used @ 150 kg ha^{-1} by a man-driven hand drill. Nitrogen (N) and potash (K) were applied uniformly @ 150-90 kg ha^{-1}, whereas various sources of phosphorus (P) were applied to fulfil 120 kg ha^{-1} phosphorus in the form of Di-ammonium phosphate (DAP), Nitrophos (NP) and Single super phosphate (SSP). Phosphorus solubilizing bacteria (PSB) were applied as Restore, Marathon and N_2-fixing bacteria. Just before sowing, half dose of nitrogen along with full doses of phosphorus (in respective P-sources) and potash were applied. The remaining half of the N was applied when the 1st irrigation was given to crop.

The following treatments were studied:

T1 – Control (no phosphorus)

T2 – DAP (120 kg P ha^{-1})

T3 – NP (120 kg P ha^{-1})

T4 – SSP (120 kg P ha^{-1})

T5 – Restore (2.5 kg ha^{-1} as PSB)

T6 – Marathon (62.5 kg ha^{-1} as PSB)

T7 – Nitrogen (N2) fixing bacteria (7.5 kg ha^{-1} as PSB)

Data analysis

The following data were collected with the procedures given below:

Days to germination

Total number of days taken to start germination in each sub-plot was counted from sowing.

Bacterial count/total viable count

Total counting of available bacteria in 1 g soil was performed twice (3 weeks after sowing [WAS] and at crop harvest). One-g soil was mixed in 9 mL sterile DI water and placed on shaker for 15 minutes (250 rpm), serial dilution of the same amount up to 10^{-6} was done thereafter. Micropipette was used to take 0.1 mL of this solution and spread on surface of plant count agar media. These plates were retained in an incubator (25 °C ± 5 °C) for three days, after that the bacteria colonies were counted using colony counter.

Number of tillers

Total quantity of off-shoots appeared in 1 m^2 area per sub-plot was recorded at maturity.

Spike length, spikelet and grains

Average length, number of spikelet and grains of ten spikes in each experimental unit was measured and recorded.

1000-grain weight

A sample of 1000 grains was collected per sub-plot and weighed separately.

Grain yield

Harvested material of each sub-plot was threshed separately and weighed for grain yield.

Chlorophyll content

SPAD meter readings were recorded twice (56 and 112 days of sowing) for chlorophyll content.

Leaf area index and duration

These were measured after collecting data at 56 & 112 days after sowing.

Crop growth and net assimilation rate

Data on these attributes were measured after 56 and 112 days of sowing with the following formulas:

$$\text{CGR} = \frac{(w_2 - w_1)}{(t_2 - t_1)}$$

$$\text{NAR} = \frac{(w_2 - w_1) \times (LnLA_2 - LnLA_1)}{(t_2 - t_1) \times (LA_2 - LA_1)}$$

Photosynthesis rate

Data on rate of photosynthesis was recorded at 112 days after sowing using digital photosynthesis meter device.

Flag leaf sugar content

Prior to heading of wheat, sample leaves were collected at 112 DAS for sugar analysis. TSS of flag leaf was measured as per following equation.

$$Y = 0.8111\,x - 0.37285$$

(where "Y" is % sugar content "x" is leaf juice brix)

Nutrient status in shoot/seed

Status of nutrients in shoot/seed was measured by multiplying the nutrient percentage and grain yield (kg ha^{-1}), divided by 100.

Statistical analysis

The results so obtained were analysed statistically using analysis of variance techniques (34) and comparison of individual treatment values was tested through $LSD_{0.05}$. Computer software "Statistic ver. 8.1" was used for analysis.

Results and Discussion

Seed germination

The number of days to germination was non significantly affected by various treatments. The results elucidated that among treatments, N2 fixing bacteria took comparatively fewer days to germinate (11.33 days), followed by Restore (12.00 days) and Marathon (12.33 days). The difference among treatments non-significant statistically (Table 1). Control (without P application) took numerically greater number of days (13.67) to germinate. The use of bio priming (seed inoculation with bacteria) might have accelerated the germination process in N2 fixing bacteria treatment more than all other protocols used in this study. Previously, days to germination were not considerably influenced by phosphorous fertilizers (24). However, our findings on the wheat crop are consistence with already reported studies (35, 36). Significant increase in germination percentage was also recorded by the application of phosphorus solubilizing microbes (37).

Table 1. Effect of P solubilizing bacteria (biofertilizers) and synthetic P fertilizers on days to germination, number of bacteria and tillers of wheat.

Treatments	Days to germination	Total viable count of colony-forming units (CFU) g^{-1} soil		Number of tillers (m^{-2})
		3 weeks after sowing (10^6)	After crop harvest (10^8)	
T1 – Control (no phosphorus)	13.67 ^{ns}	12.23 ^{b}	10.60 ^{b}	211.33 ^{e}
T2 – DAP (120 kg P ha^{-1})	13.67 ^{ns}	12.43 ^{b}	14.10 ^{ab}	285.67 ^{c}
T3 – NP (120 kg P ha^{-1})	12.67 ^{ns}	13.00 ^{ab}	15.00 ^{ab}	317.00 ^{ab}
T4 – SSP (120 kg P ha^{-1})	13.33 ^{ns}	12.37 ^{b}	13.57 ^{ab}	245.00 ^{d}
T5 – Restore (2.5 kg ha^{-1} as PSB)	12.00 ^{ns}	13.17 ^{ab}	17.10 ^{a}	312.33 ^{ab}
T6 – Marathon (62.5 kg ha^{-1} as PSB)	12.33 ^{ns}	13.87 ^{ab}	15.97 ^{ab}	300.67 ^{bc}
T7 – N2 fixing (7.5 kg ha^{-1} as PSB)	11.33 ^{ns}	14.63 ^{a}	17.70 ^{a}	337.00 ^{a}
$LSD_{0.05}$	–	1.79	5.46	26.47

Mean sharing different letter are statistically different at 5% level of probability (*n=5*); ns: non-significant, LSD: Least Significant Difference, Lettering in each parameter is assigned in descending order i.e. alphabet "a" corresponds to maximum value.

Total viable count after 3 weeks of sowing and wheat harvest

The data about total viable count indicated a higher number of bacteria (14.63 x 10^6) at 3 weeks after sowing in the N2 fixing bacteria treatment, which was followed by Marathon (13.87 x 10^6), Restore (13.17 x 10^6) and Nitrophos (13.00 x 10^6). In the control group, the number of bacteria was lowest (12.43 x 10^6) (Table 1). A similar trend was found after one week of crop harvest, wherein N2 fixing bacteria possessed the maximum number of bacteria (17.70 x $10^{8)}$, followed by Restore (17.10 x 10^8), Marathon (15.97 x 10^8), Nitrophos (15.00 x 10^8), DAP (14.10 x 10^8) and SSP (13.57 x 10^8), respectively. The lowest number of bacteria (10.60 x 10^8) was noted in the control. Previous, findings showed that P solubilizing bacteria are present in soil which release and solubilize synthetic phosphate into plant available form, which they can utilize directly. Nitrogen-fixing bacteria may also solubilize phosphorus (31). Such types of P-solubilizing microorganisms or plant growth-promoting microbes are basically a group of soil microorganisms that mineralize organic phosphorus or solubilize insoluble phosphate and release phosphorus and thus enhance growth under normal and stressful environments (28). These bacteria promote plant growth directly by solubilizing phosphorus, zinc and potassium or indirectly by releasing a siderophore, hydrogen cyanide and ammonia (30). Biofertilizers contain plant essential nutrients, hormones such as gibberellin, cytokinins and auxins, antibiotics and vitamins (19). Bacterial inoculants are capable of promoting plant growth, improve nutrient availability and uptake of plants (38). The phosphate-solubilizing bacteria promote the enhancement of bacteria numbers for fixing capabilities in the soil (39). However, similar results for P solubilizing bacteria were reported (1).

Number of tillers

Biofertilizers and synthetic P fertilizers momentously affected the number of offshoots per unit area in wheat. The offshoots data exhibited a higher tillers' count (337.00 m^{-2}) in the treatment where N2 fixing bacteria was used. It was, however, statistically similar to Nitrophos (317.00 m^{-2}), Restore (312.33 m^{-2}) and followed by Marathon (300.67 m^{-2}). Lower tillers production (211.33 m^{-2}) was noted in control plots (Table 1). The impact of commercial bio-inoculants such as bacillus and pseudomonas species of P-solubilizing bacteria, which increased seed P content, tiller formation and grain yield when used solely, and in combination (24). The increase in tillers production was possibly due to efficient and balanced availability of nutrients to crop as plants develop better roots with the balanced availability of nutrients (40, 41). It was also revealed that nitrogen fixing and PSB increased offshoot (tillers) production (42).

Spike length

Biofertilizers and synthetic P fertilizers notably increased spike measurement. All treatments except the control showed increased spike length. The longest spikes (10.08 cm) were noted in N2 fixing bacteria treatment, which was statistically at par with Nitrophos (9.88 cm), Restore (9.80 cm), Marathon (9.69 cm), DAP (9.37 cm) and SSP (9.48 cm) treatments respectively. The control treatment had a 9.05 cm spike length. The maximum spike length was recorded in N2 fixing bacteria (150:00:90 NPK kg ha^{-1}) + (7.5 kg phosphate solubilizing bacteria) treatment while the minimum was noted in control (Table 2). It was probably due to the optimum supply of nutrients from PSB, which improved spike length and other growth characteristics. The integrated use of phosphatic fertilizers along with nitrogen-fixing bacteria enhanced the growth characteristics of wheat crops (43).

Number of grains spike^{-1}

The significant differences in grain numbers per spike in various treatments N2 fixing bacteria treatments produced a greater number of seeds (45.57 $spike^{-1}$), which was statistically similar to Nitrophos (41.33) and followed by Restore (40.13), Marathon (40.13), DAP (40.00) and SSP (38.27). Minimum grains (32.17 $spike^{-1}$) were noted in control plots (Table 2). The increment in grains production might be due to a higher number of kernels ($spike^{-1}$) which possessed more grains. Reports are on the application of 75 kg P_2O_5 ha^{-1} and recorded the highest seeds per spike than all other treatments (22). Phosphatic biofertilizers (having bacteria) help in increasing the accessibility of fixed phosphates for plant growth by solubilization, which shows that PSB inoculants can make soil indigenous P available for plant growth and uptake (44). An increase of 6.1% of grains per spike with inoculation treatments was observed (45). It was also noted similar results. However, the highest number of seeds per spike in phosphate solubilizing bacteria treatment (23).

Spikelets (spike^{-1})

Biofertilizers and phosphatic fertilizers significantly improved the number of spikelets producted. Among treatments, N2 fixing bacteria had higher spikelets (17.13 spike^{-1}) production, which was however statistically similar to Nitrophos (17.10), Restore (16.83), Marathon (16.67), DAP (16.43) and SSP (16.03) respectively. While the untreated control had the lowest spikelets initiation (14.87 spike^{-1}) (Table 2). Hence, biofertilization increases spikelets production because it improves nitrogen nourishment, and hence plant produces fertile spikelets and flowers. The nitrogen-fixing and phosphate-solubilization abilities of bacteria promote root growth and increase yield (46). An increasing trend in spikelet production with an increment in P level shows the usefulness of phosphorus in seed formation and grain filling (43, 47). Moreover, the inoculation of microbes increased the plant growth and yield-related traits (48). The higher number of fertile tillers of wheat might be attributed to the accessibility of P during the establishment of seedlings (49). As P is directly involved in grain formation and development, its availability throughout the growth period improves the weight and number of grains (50).

Table 2. Effect of P solubilizing bacteria (biofertilizers) and synthetic P fertilizers on spike length, number of grains (spike^{-1}) and spikelet (spike^{-1}) of wheat.

Treatments	Spike length (cm)	Number of grains (spike^{-1})	Spikelet (spike^{-1})
T1 – Control (no phosphorus)	9.05 ^{b}	32.17 ^{c}	14.87 ^{b}
T2 – DAP (120 kg P ha^{-1})	9.37 ^{ab}	40.00 ^{b}	16.43 ^{ab}
T3 – NP (120 kg P ha^{-1})	9.88 ^{ab}	41.33 ^{ab}	17.10 ^{a}
T4 – SSP (120 kg P ha^{-1})	9.48 ^{ab}	38.27 ^{b}	16.03 ^{ab}
T5 – Restore (2.5 kg ha^{-1} as PSB)	9.80 ^{ab}	40.13 ^{b}	16.83 ^{a}
T6 – Marathon (62.5 kg ha^{-1} as PSB)	9.69 ^{ab}	40.13 ^{b}	16.67 ^{a}
T7 – N2 fixing (7.5 kg ha^{-1} as PSB)	10.08 ^{a}	45.57 ^{a}	17.13 ^{a}
$LSD_{0.05}$	0.97	5.38	1.73

Mean sharing different letter are statistically different at 5% level of probability (*n*=5); ns: non-significant. LSD: Least Significant Difference, Lettering in each parameter is assigned in descending order i.e. alphabet “a” corresponds to maximum value.

Thousand-grain weight

The substantial effect of both biofertilizers and phosphatic fertilizers was observed on wheat grain weight. A maximum weight (37.57 g) of 1000-grain was obtained in the N2 fixing bacteria and Nitrophos (35.26 g) treatments. These were followed by Restore (34.39 g), Marathon (32.00 g), SSP (31.25 g) and DAP (30.35 g) treatments. A minimum grain weight of 29.08 g was noted in control (Table 3). The P-solubilizing bacteria increase both soil and synthetic P nutrition, which may improve wheat metabolic activities and produce healthier grain (51). The probable reason may also be due to the high utilization of PSB, optimum solar light utilization and higher starch production resulting in increased grain size and higher grain weight (52). Studies reported that phosphorus and organic matter alone and in combination with each other increased the grain weight more than other studied treatments. PSB enhances the grain weight of wheat as compared to the control treatment. These findings are also consistent with previously reported grain weight of wheat (53).

Grain yield

The seed yield of the crop is the resultant of its yield contributing attributes. The maximum grain yield in the N2 fixing bacteria treatment was 2714.3 kg ha^{-1}, which was comparable to Nitrophos (2601.7 kg ha^{-1}) and Restore (2592.3 kg ha^{-1}). These were followed by Marathon (2433.7 kg ha^{-1}), DAP (2225.7 kg ha^{-1}), and SSP (2144.3 kg ha^{-1}),

Table 3. Effect of P solubilizing bacteria (biofertilizers) and synthetic P fertilizers on 1000-gràin weight, and grain yield of wheat.

Treatments	1000-gràin weight (g)	Grain yield (kg ha^{-1})
T1 – Control (no phosphorus)	29.08 ^{d}	1507.7 ^{d}
T2 – DAP (120 kg P ha^{-1})	30.35 ^{cd}	2225.7 ^{bc}
T3 – NP (120 kg P ha^{-1})	35.26 ^{ab}	2601.7 ^{ab}
T4 – SSP (120 kg P ha^{-1})	31.25 ^{bcd}	2144.3 ^{c}
T5 – Restore (2.5 kg ha^{-1} as PSB)	34.39 ^{abc}	2592.3 ^{ab}
T6 – Marathon (62.5 kg ha^{-1} as PSB)	32.00 ^{bcd}	2433.7 ^{abc}
T7 – N2 fixing (7.5 kg ha^{-1} as PSB)	37.57 ^{a}	2714.3 ^{a}
$LSD_{0.05}$	4.82	390.31

Mean sharing different letter are statistically different at 5% level of probability (*n*=5); ns: non-significant. LSD: Least Significant Difference, Lettering in each parameter is assigned in descending order i.e. alphabet “a” corresponds to maximum value.

while control produced minimum grain yield of1507.7 kg ha^{-1} (Table 3). An increase in grain yield (30-40%) where P fertilizer was inoculated with bacterial strains, either single or dual, and 20% in dual inoculation without P fertilizer when compared to control (54). Similarly, P solubilizing bacteria inoculation @ 25 mL kg^{-1} of seed could produce a cost-effective and reasonable yield (22). Plants showed better growth with P application and resulted in improved agronomic traits, which lead toward improved grain yield (55). Studies showed that highest grain yield was achieved by organic phosphorous fertilization (42). The higher yield by using TSP may be attributed to enhance crop growth and net assimilation rate which boosted grain yield substantially (56).

Chlorophyll contents (56 & 112 days after sowing)

Overall, chlorophyll content was not influence by biofertilizers and synthetic P fertilizers at 56 days after sowing (DAS). A maximum value of 40.73 µg cm^{-2} was noted in N2 fixing bacteria treatment while a minimum of 39.03 µg cm^{-2} was obtained in control (Table 4). Biofertilizers and synthetic phosphatic fertilizers substantially influenced chlorophyll contents at 112 DAS. The trend of producing chlorophyll content was same at 56 DAS. Among various treatments, N2 fixing bacteria gave maximum SPAD value of 51.78 µg cm^{-2}, which was as par statistically with Nitrophos (51.72 µg cm^{-2}), Restore (50.02 µg cm^{-2}), Marathon (49.75 µg cm^{-2}), DAP (49.14 µg cm^{-2}) and SSP (48.49 µg cm^{-2}) than the untreated control (48.33 µg cm^{-2}) (Table 4). The impact of organic phosphate alone and in integration with PSB on wheat was studied, and it was said that application of both PSB and organic phosphate substantially improved the yield of wheat compared to control. They further reported that half a dose of nitrogen and phosphorus fertilizer improved growth of wheat while a full dose of NP fertilizer and application of PSB and organic phosphate enhanced crop growth rate, straw yield and chlorophyll contents of wheat (57). Other studies also revealed that organic and inorganic phosphorous enriched the total chlorophyll content and leaf proteins (58).

Effect on leaf area index

The effect of biofertilizer and synthetic P fertilizers on leaf area index (LAI) after (56 DAS) was non-significant. However, maximum LAI (0.67) was noted in N2 fixing bacteria and a minimum (0.47) in control. LAI was substantially influenced by both biofertilizers and inorganic P fertilizers at 112 DAS. N2 fixing bacteria excelled all other treatments by producing highest LAI (1.16), which was statistically on par with earlier reports (1.15, 1.14), followed by Restore (1.13), DAP (1.12) and SSP (1.11). The lowest LAI (1.10) was recorded in untreated control (Table 4). These results suggest that bacteria have dissolved P from insoluble P, other studies also indicated increased yields and growth of plant parts due to use of PSB (40, 58).

Leaf area duration

The time in which plant possesses leaves is known as leaf area duration (LAD). The biofertilizers and P fertilizers non-significantly affected LAD at 56 days after sowing. However, maximum LAD (5.33) was recorded in N2 fixing bacteria while minimum in control (3.73) (Table 4). LAD was substantially influenced by both biofertilizers and inorganic P fertilizers at 112 DAS. N2 fixing bacteria again surpassed all other treatments having LAD value of 18.56 though it was statistically at par with Nitrophos (18.24) and Marathon (18.24). These treatments were followed by Restore, DAP and SSP with LAD values of 18.13, 17.92 and 17.71 respectively. The minimum LAD (17.55) was noted in control (Table 4). As a result, higher LAD in the current study could be attributed to better P accumulation in plants by N2 fixing bacteria. It might be due to maximum P availability from organic phosphate sources, which improves root establishments (59). It was also concluded that leaf area duration increases by bio fertilization of phosphate (60).

Crop growth rate

GR shows the rate of dry matter accumulation at a specific time. It is usually high at later stages of crop growth (i.e. flowering) while low at early stages. The data indicated that biofertilizers and chemical P fertilizers significantly affected crop growth rate. Among various treatments, the

Table 4. Effect of P solubilizing bacteria, (biofertilizers) and synthetic P fertilizers on chlorophyll contents, leaf area index and leaf area duration of wheat.

Treatments	Chlorophyll contents (µg cm^{-2})		Leaf area index		Leaf area duration	
	56 DAS	112 DAS	56 DAS	112 DAS	56 DAS	112 DAS
T1 – Control (no phosphorus)	39.03 ^{ns}	48.33 ^{b}	0.47 ^{ns}	1.10 ^{d}	3.73 ^{ns}	17.55 ^{d}
T2 – DAP (120 kg P ha^{-1})	40.86 ^{ns}	49.14 ^{ab}	0.63 ^{ns}	1.12 ^{bcd}	5.07 ^{ns}	17.92 ^{bcd}
T3 – NP (120 kg P ha^{-1})	41.14 ^{ns}	51.72 ^{a}	0.60 ^{ns}	1.15 ^{a}	4.80 ^{ns}	18.24 ^{ab}
T4 – SSP (120 kg P ha^{-1})	39.44 ^{ns}	48.49 ^{ab}	0.60 ^{ns}	1.11 ^{cd}	4.80 ^{ns}	17.71 ^{cd}
T5 – Restore (2.5 kg ha^{-1} as PSB)	39.21 ^{ns}	50.02 ^{ab}	0.53 ^{ns}	1.13 ^{abc}	4.27 ^{ns}	18.13 ^{abc}
T6 – Marathon (62.5 kg ha^{-1} as PSB)	39.38 ^{ns}	49.75 ^{ab}	0.57 ^{ns}	1.14 ^{ab}	4.53 ^{ns}	18.24 ^{ab}
T7 – N2 fixing (7.5 kg ha^{-1} as PSB)	40.73 ^{ns}	51.78 ^{a}	0.67	1.16 ^{a}	5.33	18.56 ^{a}
$LSD_{0.05}$	–	3.33	–	0.03	–	0.50

Mean sharing different letter are statistically different at 5% level of probability (*n=5*); ns: non-significant. LSD: Least Significant Difference, Lettering in each parameter is assigned in descending order i.e. alphabet “a” corresponds to maximum value.

highest CGR (13.59 g m^{-2} day^{-1}) was noted in N2 fixing bacteria, which was statistically on par with Nitrophos (13.27 g m^{-2} day^{-1}), Marathon (12.81 g m^{-2} day^{-1}), Restore (12.79 g m^{-2} day^{-1}), DAP (12.27 g m^{-2} day^{-1}), and SSP (12.13 g m^{-2} day^{-1}) treatments. In control CGR was minimum of 10.71 g m^{-2} day^{-1} (Table 5). Plants with nitrogen and PSB improve N and P nutrition, which results in higher crop growth rate. Therefore, as a major function of P fertilization is the enhancement of vegetation and plant growth, the above results are clearly indicating the significant response of crop growth rate by P fertilization. It was also possibly due to the proper availability of organic P from PSB that increased the growth rate of the wheat crop. Regarding P availability in soil, release of phosphorous by PSB is an important aspect. Bacterial biomasses take up P from soil and prevent it from fixation, which gives rise to plant growth rate. They positively affect soil fertility by storage of nutrients, mineralization and decomposition. They also solubilize precipitated P and enhance P accessibility (44).

Net assimilation, rate

Table 5. Effect of P solubilizing bacteria (biofertilizers) and synthetic P fertilizers on crop growth rate and net assimilation rate, photosynthesis rate, flag leaf sugar content of wheat.

Treatments	Crop growth rate (g m^{-2} day^{-1})	Net assimilation, rate (g m^{-2} day^{-1})	Photosynthesis rate (µ mol m^{-2} sec^{-1})	Flag leaf sugar content (%)
T1 – Control (no phosphorus)	10.71 ^{b}	0.18 ^{ns}	12.13 ^{c}	0.07 ^{ns}
T2 – DAP (120 kg P ha^{-1})	12.27 ^{ab}	0.22 ^{ns}	15.27 ^{bc}	0.08 ^{ns}
T3 – NP (120 kg P ha^{-1})	13.27 ^{a}	0.22 ^{ns}	21.93 ^{ab}	0.23 ^{ns}
T4 – SSP (120 kg P ha^{-1})	12.13 ^{ab}	0.19 ^{ns}	13.90 ^{bc}	0.08 ^{ns}
T5 – Restore (2.5 kg ha^{-1} as PSB)	12.79 ^{ab}	0.22 ^{ns}	20.00 ^{abc}	0.21 ^{ns}
T6 – Marathon (62.5 kg ha^{-1} as PSB)	12.81 ^{ab}	0.21 ^{ns}	18.60 ^{abc}	0.18 ^{ns}
T7 – N2 fixing (7.5 kg ha^{-1} as PSB)	13.59 ^{a}	0.25 ^{ns}	26.63 ^{a}	0.24 ^{ns}
$LSD_{0.05}$	2.32	–	9.10	–

Mean sharing different letter are statistically different at 5% level of probability (*n=5*); ns: non-significant. LSD: Least Significant Difference, Lettering in each parameter is assigned in descending order i.e. alphabet "a" corresponds to maximum value.

It shows the net photosynthesis accumulated by the plants. The data related to that showed non-significant differences among treatments for producing net assimilation rate in wheat. However, N2 fixing bacteria gave maximum NAR (0.25) over all other treatments, including control (0.18). This might be due to phosphate sources, which distribute phosphorous evenly throughout the plant growth (Table 5). Several physiological parameters were non-significantly influenced by certain sources of organic and commercial P (61).

Photosynthesis rate

Photosynthesis data indicated that both biofertilizers and inorganic P fertilizers significantly affected the photosynthesis rate. Among treatments, N2 fixing bacteria (26.63 µmol m^{-2} sec^{-1}), and Nitrophos (21.93 µmol m^{-2} sec^{-1}) showed higher photosynthesis rate than Restore (20.00 µ mol m^{-2} sec^{-1}), Marathon (18.60 µmol m^{-2} sec^{-1}), DAP (15.27 µmol m^{-2} sec^{-1}), and SSP (13.9 µ mol m^{-2} sec^{-1}) compare to control (12.13 µmol m^{-2} sec^{-1}). Enhancing microbial activity through P solubilizing inoculants might increase releasing of bound P and contribute to an increased photosynthetic rate (Table 5). These results are in agreement with previous findings, which confirmed these results by indicating similar findings on photosynthesis rate (62). Moreover, scientist also reported that organic sources of phosphate fertilizers increased the photosynthetic rate and crop growth (63).

Flag leaf sugar content (%)

Flag leaf plays an important role in crop nutrition. Its sugar contents also represent crop assimilates percentage (or quantity). The data exhibited non-significant variations in sugar contents among treatments, though maximum (0.24%) was noted in N2 fixing bacteria and minimum (0.07%) in control (Table 5). Bacteria performed best which might be due to rhizobacteria ability to solubilize P, increasing nutrient uptake and hence development of larger root surface area associated with additional root hairs and lateral root development (64, 65). Sugar content in flag leaf of the crop was non-significantly influenced by various organic and commercial sources of P fertilizers (66).

N, P and K concentration in wheat shoot

Wheat biofertilizers and synthetic P fertilizers improved wheat shoot's P and K while nitrogen showed non-significant variations. The N2 fixing bacteria treatment had the highest N, P, and K (0.91, 0.51, and 2.20% respectively), which was statistically comparable to Nitrophos (0.87, 0.49 and 2.06%), Restore (0.84, 0.44 and 1.90%), Marathon (0.77, 0.46 and 1.75%), DAP (0.76, 0.38 and 1.67%) and SSP (0.75, 0.37 and 1.42%) respectively. Minimum values (0.66, 0.36 and 1.32% NPK respectively) were noted in control (Table 6). This might be due to the P sources, which give rise to wheat shoot P and K contents. Microorganism (with P solubilizing potential) increases the availability of soluble P and enhances plant growth by improving biological nitrogen fixation. The higher K and P contents due to use of commercial and organic sources of P fertilizers (67).

N, P and K concentration in wheat grain

Biofertilizers and phosphatic fertilizers significantly improved wheat grain's N, P and K. Maximum N, P and K (2.48, 1.25 and 0.45% respectively) values were obtained in

N2 fixing bacteria. It was statistically comparable to Nitrophos (2.16, 0.87 and 0.43% NPK), Restore (2.12, 0.83 and 0.39%), Marathon (2.08, 0.84 and 0.40%), DAP (2.01, 0.31 and 0.36 %) and SSP (1.84, 0.81 and 0.38% respectively), while control had minimum NPK values (1.69, 0.66 and 0.33% respectively) (Table 6). PSB mobilize soil inorganic P and increase its bioavailability for plant use. This promotes sustainable agriculture, improve soil fertility and hence increases grain quality and promotes the enrichment of NPK content in grains. The use of PSB as microbial inoculants is a new way to improve plant productivity (68). The NPK assimilations in grain are directly proportional to the nitrogen-fixing PSB, which greatly enhances the grain nitrogen, phosphorous and potash contents (69).

N, P and K uptake in wheat grains

Grain nutrient uptake was substantially affected by biofertilizers and synthetic P fertilizers. Maximum N, P, and K uptake (67.28, 33.83 and 12.18 kg ha^{-1}) was observed in the N2 fixing bacteria treatment, which was comparable to Nitrophos (67.28, 33.83 and 12.18 kg ha^{-1}) and Restore (55.52, 21.79 and 10.11 kg ha^{-1}) and was followed by Marathon (50.88, 20.23 and 9.68 kg ha^{-1}), DAP (44.83, 17.70 and 8.12 kg ha^{-1}) and SSP (39.43, 17.28 and 8.12 kg ha^{-1} respectively), while control had minimum uptake (32.16, 12.65 and 6.39 kg N-P-K ha^{-1}) (Table 6). This was due to N fixing ability and P solubilisation of bacteria which made available N, P and K to grain and increased the seeds' capability of up taking N, P and K (70). Inorganic phosphorus decreases the uptake of N, P and K content as there are greater chances of volatilization and less available nutrients tend to decrease grain NPK proportions. Bacillus, Enterobacter and Pseudomonas are very effective for increasing plant growth, the yield of crops and P availability in soil. Therefore, evaluation of PSB through bio fertilization has great importance for using natural reserves of phosphate rocks and increasing bonded P in the soil (71, 72).

Table 6. Effect of P solubilizing bacteria (biofertilizers) and synthetic P fertilizers on N, P and K contents in shoot before heading of wheat and seed N, seed P and seed K of wheat

Treatments	Wheat shoot Percentage			Wheat grain Percentage			Uptake of wheat grain (kg ha^{-1})		
	N	P	K	N	P	K	N	P	K
T1 – Control (no phosphorus)	0.66 ^{ns}	0.36 ^{b}	1.32 ^{b}	1.69 ^{c}	0.66 ^{b}	0.33 ^{b}	32.16 ^{d}	12.65 ^{c}	6.39 ^{c}
T2 – DAP (120 kg P ha^{-1})	0.76 ^{ns}	0.38 ^{ab}	1.67 ^{ab}	2.01 ^{bc}	0.81 ^{b}	0.36 ^{ab}	44.83 ^{bcd}	17.70 ^{bc}	8.12 ^{bc}
T3 – NP (120 kg P ha^{-1})	0.87 ^{ns}	0.49 ^{ab}	2.06 ^{a}	2.16 ^{ab}	0.87 ^{b}	0.43 ^{a}	56.28 ^{ab}	22.69 ^{b}	11.21 ^{ab}
T4 – SSP (120 kg P ha^{-1})	0.75 ^{ns}	0.37 ^{b}	1.42 ^{b}	1.84 ^{bc}	0.81 ^{b}	0.38 ^{ab}	39.43 ^{cd}	17.28 ^{bc}	8.12 ^{bc}
T5 – Restore (2.5 kg ha^{-1} as PSB)	0.84 ^{ns}	0.44 ^{ab}	1.90 ^{ab}	2.12 ^{ab}	0.83 ^{b}	0.39 ^{ab}	55.52 ^{ab}	21.79 ^{b}	10.11 ^{ab}
T6 – Marathon (62.5 kg ha^{-1} as PSB)	0.77 ^{ns}	0.46 ^{ab}	1.75 ^{ab}	2.08 ^{b}	0.84 ^{b}	0.40 ^{ab}	50.88 ^{bc}	20.23 ^{bc}	9.68 ^{abc}
T7 – N2 fixing (7.5 kg ha^{-1} as PSB)	0.91 ^{ns}	0.51 ^{a}	2.20 ^{a}	2.48 ^{a}	1.25 ^{a}	0.45 ^{a}	67.28 ^{a}	33.83 ^{a}	12.18 ^{a}
$LSD_{0.05}$	–	0.15	0.65	0.37	0.29	0.09	15.41	8.94	3.30

Mean sharing different letter are statistically different at 5% level of probability (*n=5*); ns: non-significant. LSD: Least Significant Difference, Lettering in each parameter is assigned in descending order i.e. alphabet "a" corresponds to maximum value.

Conclusion

The current study elucidated that use of PSB had significant impact on growth, quality and yield status of wheat. Bio and synthetic P fertilizers at different concentrations enhanced utilization efficiency, biological N2-fixation, NPK uptake, morphological and yield-related traits in comparison with control. The results indicated that most of the attributes were considerably influenced by various treatments; however, the use of N2 fixing bacteria (used as PSB) excelled in all treatments. Therefore, we conclude that PSB is recommended for obtaining higher wheat yield in district Dera Ismail Khan and other areas of Pakistan. However, the use of nitrophos (NP) as synthetic instant P fertilizer might be a viable option for increased productivity of wheat in non-availability of PSB. Moreover, PSB might also be evaluated in extensive field trials to investigate their potential as biofertilizer.

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Authors contributions

AK and MSB carried out the experimental and investigate; NU, MZB and AS participated in the study design; RK, AAK and HG carried out the data analysis and validation; NU, HI and SZUA, carried out the written and drafted the manuscript; AS and MZB finalized the manuscript All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: All the authors declared that they have no competing interest related to this article.

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