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Enhancing wheat photosynthetic efficiency and productivity via co-application of humic acid and phosphate solubilizing bacteria under varying phosphorus levels

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Abstract: Phosphorus (P) deficiency severely constrains global wheat production, hindering photosynthetic efficiency and ultimately reducing crop yields. This is primarily due to phosphorus fixation in the soil, which diminishes the bioavailability of this crucial nutrient, thereby impairing vital functions like ATP synthesis and photosynthesis. To address this challenge, a two-year field experiment was conducted during the 2021 and 2022 *rabi* seasons at G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand (India). The study employed a factorial randomized block design with 12 treatment combinations, involving three phosphorus levels (control, 50%, and 100%, corresponding to 0, 30, and 60 kg of P_2O_5/ha) and four phosphate solubilizers (control, humic acid (HA) @ 10 kg/ha, phosphate solubilizing bacteria (PSB) biofertilizer @ 10 g/kg seed, and a combination of HA @ 10 kg/ha and PSB biofertilizer @ 10 g/kg seed). Results showed that P fertilizer application at 50% and 100% of the recommended dose significantly enhanced Soil Plant Analysis Development (SPAD) and Normalized Difference Vegetation Index (NDVI) values, particularly at 60 and 90 days after sowing (DAS). The combined application of HA and PSB yielded the highest gains in both SPAD and NDVI values, indicating improved P availability and uptake. Specifically, SPAD values increased by 8.6-12.7% and NDVI values by 2.9-8% with the combined use of HA and PSB over control. These positive findings highlight the potential of integrating phosphorus management with natural solubilizers to significantly boost photosynthetic efficiency and overall performance in wheat. This approach offers a promising, practical solution for farmers facing nutrient limitations.
Keywords: *Humic acid, NDVI values, Phosphate solubilizing bacteria, SPAD values, Wheat.*

1. Introduction

Phosphorus (P) is one of the three primary macronutrients required by plants, along with nitrogen (N) and potassium (K). It is involved in several vital physiological processes in plants, including photosynthesis, energy transfer (through ATP), and the synthesis of nucleic acids [1]. Phosphorus is essential for early root formation and development, which, in turn, influences overall plant growth and yields. Despite the widespread application of P fertilizers in many intensive cropping systems, only a small fraction i.e. ~10-25% of the applied phosphorus is effectively utilized in the year of application [2]. The remaining phosphorus either accumulates in the soil or runs off into water bodies, leading to eutrophication - a condition characterized by excessive algae growth and subsequent depletion of oxygen in aquatic ecosystems [3]. Furthermore, global phosphorus supplies are under threat due to the depletion of high-quality rock phosphate reserves. It is estimated that high-quality reserves may be exhausted within this century, forcing the agriculture industry to rely on lower-grade rock phosphate, which would be more costly to process [4]. In light of these environmental and resource-related challenges, optimizing fertilizer usage and improving phosphorus use efficiency (PUE) are crucial. One

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promising approach to enhancing PUE is the use of phosphate solubilizers, such as HA and PSB, which can reduce the need for P fertilizer without jeopardizing crop yields [5, 6].

Humic products, including humic acid, fulvic acid, and humin, play a crucial role in making P more available to plants by transforming insoluble P compounds into forms that plants can readily absorb. Specifically, in acidic soils, they break down P bonded with iron (Fe) and aluminum (Al), while in calcareous soils, they disrupt calcium (Ca)-bonded P [7]. Humic acid, containing 51-57% organic carbon, 4-6% nitrogen, and 0.2-1% phosphorus, improves soil properties and nutrient supply. When applied along with phosphatic fertilizers, humic acid further boosts P mobility and availability by facilitating ligand exchange and solubilization, and by releasing hydrogen ions (H^+) in the rhizosphere. This acidification of the soil enhances phosphate uptake by plants. This combined action of humic acid significantly improves both soil health and crop yields [8]. Microorganisms are equally essential to the soil's phosphorus cycle, moving P between various soil reserves and making it biologically available to plants. Phosphate Solubilizing Microorganisms (PSM), a group that includes both bacteria and fungi, converts both inorganic and organic soil P into plant available forms through processes like solubilization and mineralization. Prominent among these are phosphate solubilizing bacteria (PSB), particularly species from *Pseudomonas* and *Bacillus* genera. These bacteria secrete organic acids such as formic, fumaric, gluconic, acetic, 2-ketogluconic and fumaric etc. which effectively dissolve insoluble phosphates. This acidification process increases the amount of soluble P, thereby improving plant uptake and contributing to sustainable soil fertility and crop productivity [9]. Field investigations have consistently demonstrated that integrating humic acid (HA) and phosphate solubilizing bacteria (PSB) with conventional phosphorus (P) fertilizers can lead to a significant reduction in P fertilizer application, potentially by up to 50% [6, 10, 11]. Recognizing the critical role of HA and PSB in enhancing P utilization efficiency, the current research aimed to optimize chlorophyll content (SPAD) and NDVI in wheat while concurrently minimizing the use of synthetic phosphorus fertilizers through the strategic application of these phosphate solubilizers.

2. Material and Methods

The study was conducted during the *rabi* seasons of 2021 and 2022 at the N. E. Borlaug Crop Research Centre, located at G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand (India). The soil of the experiment site was sandy loam in texture with a neutral pH of 7.1, containing 0.77% organic carbon, 167.3 kg/ha of available nitrogen, 15.6 kg/ha of available phosphorus, and 166.1 kg/ha of available potassium. The experiment comprised 12 treatment combinations, including 3 phosphorus levels (control, 50%, and 100%, corresponding to 0, 30, and 60 kg of P_2O_5/ha) and 4 phosphate solubilizers (control, humic acid (HA) @ 10 kg/ha, phosphate solubilizing bacteria (PSB) biofertilizer @ 10 g/kg seed, and a combination of HA @ 10 kg/ha and PSB biofertilizer @ 10 g/kg seed). This was arranged in a factorial randomized block design (FRBD) with three replications. The recommended dose of fertilizer was 120:60:40 kg $N:P_2O_5:K_2O$/ha. One-third of the nitrogen and the full amount of potassium, along with phosphorus as per the treatment requirements, were applied as a basal dose before sowing, followed by the soil application of humic acid (10 kg/ha). The remaining two-thirds of the nitrogen was top-dressed in two equal splits at 30 and 60 days after sowing (DAS). HD 2967 wheat seeds, both those treated with the NE10 strain of PSB and untreated, were manually sown at a rate of 100 kg/ha in furrows spaced 22.5 cm apart and immediately covered to improve seed-soil contact. All recommended agricultural practices were adopted throughout the experiment, with the exception of specific treatments.

2.1. Soil Plant Analysis Development (SPAD)

The SPAD values, which indicate the chlorophyll content of leaves, were recorded at 30, 60, and 90 days after sowing (DAS) using the OPTI-SCIENCES' CCM-200 Chlorophyll Content Meter. This device operates by measuring the difference in light transmittance through a leaf at two specific wavelengths: red light at around 650 nm, and near-infrared light at around 940 nm. Chlorophyll in the

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leaf absorbs the red light, while the near-infrared light passes through with minimal absorption. Based on the difference in absorption between the red and near-infrared light, the SPAD meter calculates the chlorophyll content.

2.2. Normalized Difference Vegetation Index (NDVI)

The NDVI values were recorded at 30, 60, 90, and 120 days after sowing (DAS) using a Trimble Green Seeker. This sensor collects data by emitting pulses of red and near-infrared (NIR) light towards the target, such as a plant or crop, and measures the amount of light reflected back from the plant's surface. As the sensor moves across the crop, it continuously samples the area as long as the trigger is engaged. The reflected light data is processed to calculate an NDVI value, which is then displayed on the sensor screen. The NDVI values range from 0.00 to 0.99, indicating plant health; higher values suggest better crop condition and vigor.

$$\text{NDVI} = \frac{NIR - Red}{NIR + Red}$$

3. Results

3.1. Effect on SPAD Values

The study demonstrated that the application of varying phosphorus levels and phosphate solubilizers significantly influenced the SPAD values of wheat, particularly at 60 and 90 days after sowing (DAS). Notably, there were no significant effects observed at 30 DAS. The application of 50% phosphorus resulted in substantial increases in SPAD values when compared to the control in 2021, with enhancements of 11.95% at 60 DAS and 8.54% at 90 DAS. In the following year, 2022, this trend continued and the SPAD values for the same treatment further increases to 13.33% at 60 DAS and to 13.51% at 90 DAS. Furthermore, the application of 100% phosphorus led to even greater improvements in SPAD values compared to the control, with increases of 15.21% at 60 DAS and 13.10% at 90 DAS in 2021, and 24% at 60 DAS and 15.67% at 90 DAS in 2022.

The application of humic acid led to percentage increases in SPAD values of 7.93% at 60 DAS and 8.78% at 90 DAS, and 9.16% at 60 DAS and 7.32% at 90 DAS compared to the control for the year 2021 and 2022, respectively. Similarly, phosphate solubilizing bacteria also contributed to improved SPAD values, yielding increases of 7.40% at 60 DAS and 7.08% at 90 DAS in 2021, and 7.12% and 7.06%, respectively, in 2022. The combined use of humic acid and phosphate solubilizing bacteria showed the greatest improvements, with SPAD values increasing by 9.78% at 60 DAS and 11.33% at 90 DAS in 2021, and by 12.72% at 60 DAS and 11.25% at 90 DAS in 2022 (Table 1).

Table 1.
Effect of phosphorus levels and phosphate solubilizers on SPAD values.

Treatments	**SPAD values**					
	30 DAS		**60 DAS**		**90 DAS**	
	2021	**2022**	**2021**	**2022**	**2021**	**2022**
Phosphorus levels						
Control	31.9	32.7	36.8	37.5	35.1	37.0
50% phosphorus	34.0	35.2	41.2	42.5	38.1	42.0
100% phosphorus	34.0	37.8	42.4	46.5	39.7	42.8
SEm±	1.0	1.2	0.7	0.4	0.6	0.8
CD (p = 0.05)	NS	NS	2.0	1.4	1.7	2.2
Phosphate solubilizers						
Control	31.4	32.8	37.8	39.3	35.3	38.2
HA	33.9	36.2	40.8	42.9	38.4	41.0
PSB	33.9	35.6	40.6	42.1	37.8	40.9
HA + PSB	34.1	36.6	41.5	44.3	39.3	42.5
SEm±	1.2	1.4	0.8	0.5	0.7	0.9
CD (p = 0.05)	NS	NS	2.3	1.6	2.0	2.6

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3.2. Effect on NDVI values

The results of this study illustrate the influence of different phosphorus levels and phosphate solubilizers on NDVI values recorded at 30, 60, 90, and 120 days after sowing (DAS) over the course of two years, 2021 and 2022. At 30 DAS, NDVI values showed minimal variation among treatments, suggesting that varying phosphorus levels and phosphate solubilizers did not significantly affect early-stage NDVI values. At 60 DAS, noticeable differences emerged as NDVI values increased with phosphorus application. Specifically, a 50% phosphorus application resulted in an enhancement of 3.75%, while a 100% phosphorus application yielded a 3.70% increase from 2021 to 2022. At 90 DAS, the 50% phosphorus application demonstrated a 3.89% rise, with the 100% phosphorus application showing a 2.5% improvement. By 120 DAS, the trend continued, with the 50% phosphorus application leading to a 2.98% rise, and the 100% phosphorus treatment resulting in a notable 4.28% improvement in NDVI values from 2021 to 2022 (Table 2).

Further, the application of 50% phosphorus resulted in increases in NDVI values compared to the control in 2021, with enhancements of 5.26% at 60 DAS, 6.94% at 90 DAS, and 3.07% at 120 DAS. In 2022, the NDVI values for the same treatment showed similar positive trends, with increases of 5.06% at 60 DAS, 5.26% at 90 DAS, and 2.89% at 120 DAS. The application of 100% phosphorus yielded even more pronounced improvements. In 2021, NDVI values increased by 6.57% at 60 DAS, 11.11% at 90 DAS, and 7.69% at 120 DAS compared to the control. In 2022, the increases for 100% phosphorus treatment were observed as 6.32% at 60 DAS, 7.31% at 90 DAS, and 8.95% at 120 DAS, indicating consistent benefits from higher phosphorus application.

On the other hand, the application of humic acid resulted in percentage increases of NDVI values of 6.66% at 60 DAS, 2.70% at 90 DAS, and 3.03% at 120 DAS compared to the control in 2021. In 2022, this treatment similarly yielded increases of 2.5% at 60 DAS, 3.89% at 90 DAS, and 2.94% at 120 DAS. Applying phosphate solubilizing bacteria also improved NDVI values, with increases of 5.33% at 60 DAS, 2.70% at 90 DAS, and 1.51% at 120 DAS in 2021, while in 2022, the enhancements were 2.5%, 2.59%, and 1.47%, respectively. The combined application of humic acid and phosphate solubilizing bacteria exhibited the most substantial improvements, with percentage gains of 8% at 60 DAS, 6.75% at 90 DAS, and 4.54% at 120 DAS relative to the control in 2021. Similar improvements were noted in 2022, with increases of 5%, 6.49%, and 7.35% at these growth stages.

Table 2.
Effect of phosphorus levels and phosphate solubilizers on NDVI values.

Treatments	NDVI values							
	30 DAS		60 DAS		90 DAS		120 DAS	
	2021	2022	2021	2022	2021	2022	2021	2022
Phosphorus levels								
Control	0.67	0.68	0.76	0.79	0.72	0.76	0.65	0.67
50% phosphorus	0.68	0.70	0.80	0.83	0.77	0.80	0.67	0.69
100% phosphorus	0.70	0.72	0.81	0.84	0.80	0.82	0.70	0.73
SEm±	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
CD (p = 0.05)	NS	NS	0.03	0.02	0.02	0.02	0.02	0.03
Phosphate solubilizers								
Control	0.67	0.69	0.75	0.80	0.74	0.77	0.66	0.68
HA	0.69	0.70	0.80	0.82	0.76	0.80	0.68	0.70
PSB	0.68	0.70	0.79	0.82	0.76	0.79	0.67	0.69
HA + PSB	0.70	0.71	0.81	0.84	0.79	0.82	0.69	0.73
SEm±	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
CD (p = 0.05)	NS	NS	0.04	0.03	0.03	0.03	0.02	0.04

4. Discussion

The observed increase in chlorophyll content, as indicated by higher SPAD values, suggests that elevated phosphorus levels significantly enhance photosynthetic activity and overall plant vigor. This increase may correlate with improved leaf development and nutrient uptake, as phosphorus is essential for energy transfer and root development [12]. The results of this study confirm the benefits of both individual and combined applications of humic acid and PSB in increasing SPAD values, suggesting their potential in reducing the need for synthetic phosphorus fertilizers [13]. The sustained increases in chlorophyll content with these treatments can contribute to higher photosynthetic activity, improving crop growth and productivity. Humic acid is known for its ability to improve soil structure, enhance water retention, and promote microbial activity in the rhizosphere, which collectively contribute to increased nutrient availability, including nitrogen, potassium, and phosphorus, essential for chlorophyll synthesis and overall plant growth [11]. In addition to humic acid, phosphate solubilizing bacteria plays a vital role in mobilizing phosphorus in the soil, making it more available for plant uptake through mechanisms such as organic acid secretion and mineral solubilization [14]. The combined application of humic acid and PSB not only exhibited all the aforementioned stimulatory effects but also enhanced phosphorus availability, resulting in optimal performance with respect to SPAD values. This approach could be particularly beneficial in phosphorus-deficient soils or when aiming to reduce the dependency on synthetic phosphorus fertilizers, making it a sustainable and eco-friendly strategy for enhancing crop productivity.

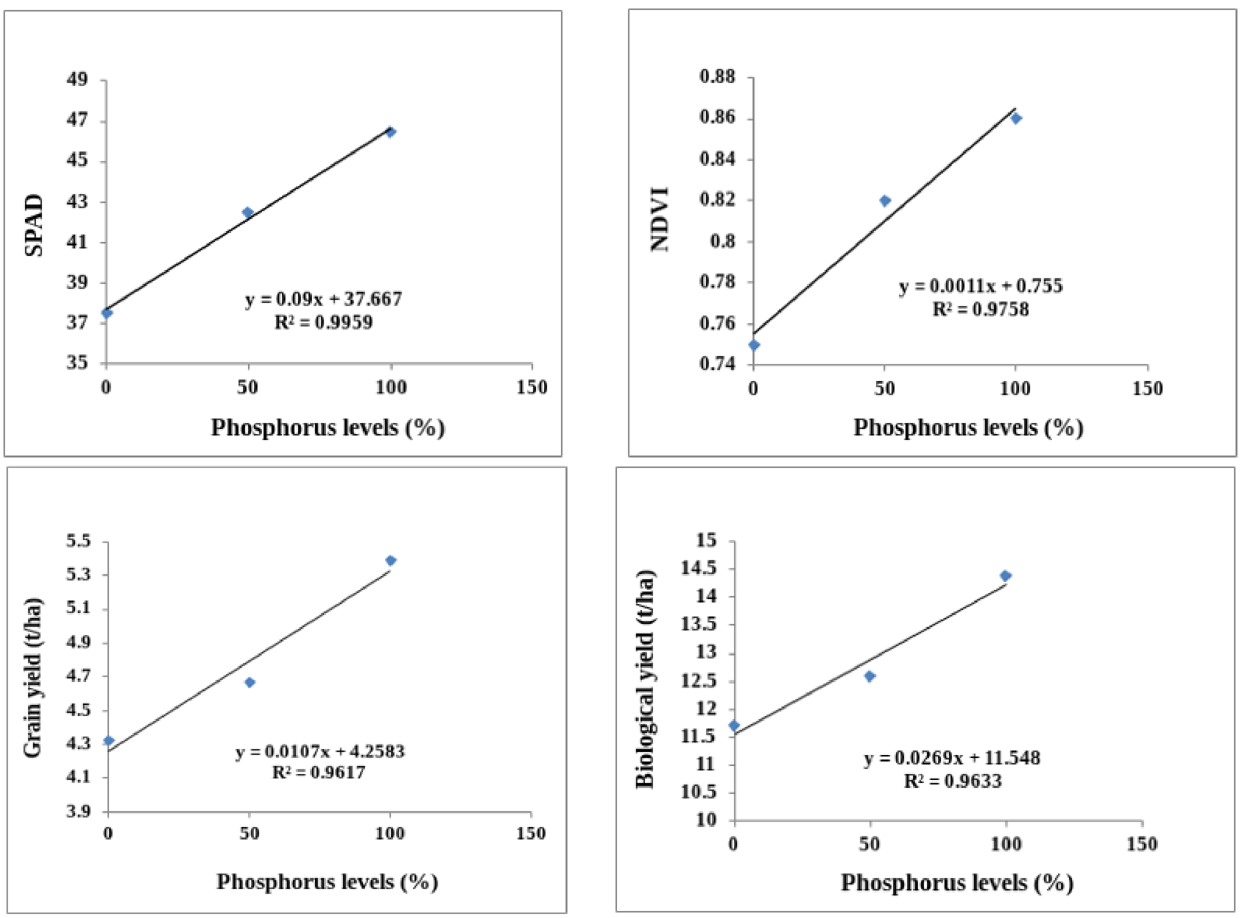

Figure 1.
Pearson correlation matrix of phosphorus levels, SPAD (60 DAS), NDVI (60 DAS) and wheat productivity during 2022 ($p < 0.01$).

Phosphorus is essential for various physiological processes, including photosynthesis and root development. Increased phosphorus application leads to notable improvements in growth parameters and enhances nutrient uptake, which is reflected in elevated NDVI values [6]. Healthier plants with greater chlorophyll content tend to absorb more near-infrared light and reflect less red light, resulting in higher NDVI readings [15]. Further, the elevated NDVI values observed at 60 and 90 DAS may be attributed to the impact of top-dressed nitrogen applied at 30 and 60 DAS. This nitrogen likely interacted with phosphate ions to form nucleotides essential for amino acid synthesis, supporting the growth and development of wheat [16]. Humic acid stimulates root growth and soil microbial activity, which, when coupled with PSB, increases phosphorus, allowing plants to absorb nutrients more effectively. This synergy promotes better chlorophyll production, essential for NDVI measurement, leading to healthier, greener crops [17]. The consistent improvement in NDVI values over both years suggests that the integration of humic acid and PSB can enhance phosphorus availability and uptake, contributing to higher photosynthetic activity and overall crop performance.

The study highlights phosphorus management as a critical factor for enhancing wheat productivity, with strong correlations observed between phosphorus levels and physiological plant characteristics. SPAD demonstrates a very strong positive relationship with phosphorus ($r = 0.995$), while NDVI ($r = 0.975$), grain yield ($r = 0.961$), and biological yield ($r = 0.963$) also show significant associations. These correlations suggest that increased phosphorus availability supports photosynthetic efficiency through higher pigment concentrations, directly improving grain filling processes and overall productivity. The yield of grain increases with the amount 0.9617 R^2 value indicates yield variation is more likely to be the result of applied phosphorus. Without phosphorus, the minimum yield (grain) rests at 4258.3 kg/ha and is estimated to increase by 10.7 kg/ha for every 1% increase in phosphorus (Figure 1). Research by Ali, et al. [6] and da Silva, et al. [17] confirms that enhanced photosynthetic machinery drives these yield gains, while Li, et al. [15] validates NDVI's utility as a non-invasive monitoring tool for nutrient management through its strong link to chlorophyll levels.

5. Conclusions

The Results indicate that the combined application of humic acid (HA) and phosphate solubilizing bacteria (PSB) yielded the highest chlorophyll content (SPAD values) and NDVI values at critical growth stages, particularly at 60 and 90 days after sowing. Specifically, SPAD values increased by 8.6% to 12.7% and NDVI values by 2.9% to 8% with the combined use of humic acid and PSB over control. The addition of HA and PSB significantly enhanced phosphorus availability and uptake, thereby improving chlorophyll content and crop vigor. Overall, the combined use of HA and PSB with 50% phosphorus (30 kg P_2O_5/ha) achieved results comparable to the full phosphorus application (60 kg P_2O_5/ha), demonstrating their potential to cut synthetic phosphorus fertilizer usage by half. This approach not only helps to reduce dependency on finite phosphorus resources but also minimizes the environmental risks associated with excessive phosphorus runoff. By adopting this integrated nutrient management strategy, wheat growers can achieve sustainable crop productivity while contributing to environmental conservation and resource optimization.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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