

Article

# Application of Single Superphosphate with Humic Acid Improves the Growth, Yield and Phosphorus Uptake of Wheat (*Triticum aestivum* L.) in Calcareous Soil



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Abstract: In calcareous soil, the significant portion of applied phosphorus (P) fertilizers is adsorbed on the calcite surface and becomes unavailable to plants. Addition of organic amendments with chemical fertilizers can be helpful in releasing the absorbed nutrients from these surfaces. To check out this problem, a field experiment was conducted for two years to determine the effect of P fertilizers and humic acid (HA) in enhancing P availability in soil and their ultimate effect on growth, yield and P uptake of wheat in calcareous soils. The experiment was comprised of five levels of P (0, 45, 67.5, 90 and 112.5 kg  $P_2O_5$  ha<sup>-1</sup>) as a single superphosphate (SSP) and 2 levels of locally produced humic acid (with and without HA) arranged in a two factorial randomized complete block design (RCBD) with three replications. Wheat plant height, spike length, number of grains per spike, 1000-grain weight, grain, straw and biological yield were significantly improved by the addition of HA with SSP. Very often, the performance of 67.5 kg  $P_2O_5$  ha<sup>-1</sup> with HA were either similar or better than 90 or even 112.5 kg  $P_2O_5$  ha<sup>-1</sup> applied without HA. Post-harvest soil organic matter, AB-DTPA extractable and water-soluble P, plant P concentration and its uptake were also significantly improved by the addition of HA with SSP compared to sole SSP application. It was evident that P efficiency could be increased with HA addition and it has the potential to improve crop yield and plants P uptake in calcareous soils.

Keywords: calcareous soil; humic acid; phosphorus uptake; single superphosphate; wheat

#### 1. Introduction

Soil fertility and crop productivity are closely related to three main components of soil ecosystems: the bio-available soil nutrients, soil microbiota and organic matter content [1–5]. Phosphorus is 2nd most yield limiting nutrient after nitrogen in agricultural production across the world [6,7]. Phosphorus (P) plays many key functions in plant life especially in the storage and transfer of energy, photosynthesis, respiration, cell division, and enlargement. Plants take P in  $H_2PO_4^-$  or  $HPO_4^{-2}$  forms from the soil solution. Application of phosphatic fertilizers in a balanced amount and at the correct time with good application techniques and management methods has good impacts on crop yield. However, responses to fertilization can be species and variety-dependent, which greatly influences nutrient accumulation and utilization in the plant [8,9]. Organic Fertilizer addition to soil increases risk of xenobiotic contamination [10–13].

Phosphorus deficiency is often a yield-limiting factor in agricultural soils, particularly in those having high carbonate contents, which reduces phosphorus solubility. In these conditions, achieving a target crop productivity generally demands the use of higher fertilizer rates as a way to account for that increased inefficiency. Besides being expensive [14], use of supra-optimum rates of chemical fertilizers in recent years has been frequently pointed as the reason behind in reduction in organic substances found within the soil [15]. Moreover, excessive usage of chemical fertilizers in agriculture has caused environmental issues like biological processes, physical destruction of the soil and nutritional imbalances [16]. In addition, research shows that increased corn and bean overall yields and quality can be obtained by using organic and chemical fertilizers simultaneously, which in turn aids to reducing the use of chemical fertilizers and improving soil health and overall sustainability [17].

Several studies reported that plant growth and development are greatly related with the movement of specific organic fractions present in both the soil solutions (known as dissolved organic matter) and soil matrix (soil organic matter). These fractions have been defined as humic substances and include humic acids, fulvic acids and humin [1,18]. The advantageous activities of these humic substances in relation to crop production has been attributed to two main effects [19,20]: the indirect effect on soil properties and fertility, related to the ability of these substances to form complexes or chelates with soil metals [14], which impacts soil structure, texture and nutrients availability [19]. While the direct regulating growth effect on plant hormones such as auxin, ethylene, nitric oxide, cytokinins, abscisic acid and reactive oxygen species [20].

Humic acid (HA) is an active ingredient of humus that can play an essential role in improved soil health and plant growth. Physically, it provides good soil structure and enhances the water holding capacity of the soil; biologically it enhances the growth of beneficial soil organisms, while chemically it acts as an adsorption and retention complex for inorganic plant nutrients [21]. Humic acid cannot be only found in soils, but also in peats, rivers, oceans and lignitic coals and can result from the biological decomposition and of organic matter. Humic substances can change the unavailable elements into available forms and can rupture Fe or Al bonded P in acidic soils and Ca in calcareous soils, rendering more soil P to be available for plant uptake. Humic acid can make complexes with Na, K, Mn, Zn, Ca, Fe, Cu and with a variety of other elements to overcome a particular element shortage in the soil. Thus, under certain conditions, the use of HA and its concomitant stimulating effect on various crops has received considerable attention [22]. Chemical composition of HA varies depending on the source and edaphoclimatic conditions where it was formed, but average HA composition contains 51–57% organic C, 4–6% N and 0.2 to 1% P that can be used both for plant nutrition and for improving soil physicochemical and biological parameters [21]. Additionally, HS was found to have a marked effect on the emergence of lateral roots, and the hyper induction of sites for lateral root emergence [23]. Research shows that the effect of Humic substances (HS) on plant growth depends on the source, concentration and molecular weight of humic fractions [24,25]. That is why the present study was conducted to evaluate the role of locally produced HA in enhancing P availability and wheat growth in calcareous soil amended with different P levels as SSP.

### 2. Materials and Methods

## 2.1. Experimental Procedure

A field study was conducted over two years, to investigate the effect of different levels of single superphosphate applied alone and in combination of HA on growth, yield and phosphorus uptake by wheat crop at the Research Farm of the University of Agriculture, Peshawar-Pakistan. The experimental farm is located at 34.01° N latitude, 71.35° E longitude at an altitude of 350 m above sea level in Peshawar valley. The soil in the experimental site was a silt loam, alkaline calcareous, low in organic matter and P contents (Table 1) and was also highly responsive to P application [26]. Treatments included the application of P at 0, 45, 67.5, 90 and 112.5 kg  $P_2O_5$  ha<sup>-1</sup> applied as Single Superphosphate (SSP)without and with the HA addition (i.e., 0 and 5 kg HA ha<sup>-1</sup>). Humic acid was extracted from brown coal collected from Hyderabad, Pakistan in the laboratory of Soil and Environmental\_Sciences, The University of Agriculture Peshawar, Pakistan by following the procedure of Hai and Mir [27]. The experiment was arranged as a two factorial [5 (P levels) and 2 (HA levels)] randomized complete block design (RCBD) with 3 replications. The required P fertilizer along with half recommended levels of N (120 kg ha<sup>-1</sup>) and a full dose of K as 60 kg  $K_2O$  ha<sup>-1</sup> were applied as urea and sulphate of potash respectively, before planting. The remaining half rate of N was applied with the first irrigation. Wheat (*Triticum aestivum* L.) variety "Atta Habib" having a seed rate of 100 kg ha<sup>-1</sup> was sown through handrill with 30 cm row spacing in a plot size of  $4 \text{ m} \times 5 \text{ m}$ . All the agronomic practices and manual weed control were followed as per the standard advised procedure for all treatments uniformly. Plants were harvested at physiological maturity. The field was irrigated as per crop requirements.

Properties	Concentration						
Soil							
Sand (%)	25.00						
Silt (%)	65.23						
Clay (%)	7.00						
Textural class	Silt loam						
pH (1:5)	7.9						
EC $_{(1:5)}$ dS m <sup>-1</sup>	0.20						
Organic matter content (%)	0.72						
AB DTPA extractable P (mg kg <sup>-1</sup> )	4.0						
AB DTPA extractable K (mg kg <sup><math>-1</math></sup> )	110						
Lime contents (%)	14.04						
Humic acid							
Organic C (%)	50.4-60.3						
Total N (%)	3.0–5.5						
AB DTPA Extractable P (mg kg $^{-1}$ )	50.0-52.5						
$Zn (mg kg^{-1})$	05.5-07.3						
$Mn (mg kg^{-1})$	12.2–15.5						
pH (1:5)	5.5–6.0						

Table 1. Characteristics of composite soil of the area and humic acid used in the experiment.

Data on plant height was determined by taking the height of five randomly selected plants from soil surface to the tip of each plant at physiological maturity in each treatment plot and then averaged. To determine the spike length, five spikes were randomly selected in each plot and their length was measured from base of rachis to the tip of uppermost spikelet. After that the spikes were threshed individually to determine grains per spike and their mean were taken as a grains spike<sup>-1</sup>. Thousand grains weight was recorded by counting and weighing thousand grains randomly taken from each treatment plot. Grain yield was recorded after threshing of plants taken from central four rows in each treatment and then converted into kg ha<sup>-1</sup> by using the following formula:

Grain yield (kgha<sup>-1</sup>) = 
$$\frac{\text{Grain yield in kg obtained from harvested rows } \times 10000 \text{ m}^2}{(\text{Row lenght} \times \text{Row spacing in meter} \times \text{No. of rows})}$$

Biological yield (BY) was measured by weighing the entire harvested crop (un-threshed crop i.e., both grain and straw) in each treatment. The BY was then converted in to kg ha<sup>-1</sup> with the following formula:

Biological yield (kgha<sup>-1</sup>) 
$$\frac{\text{Biological yield in kg obtained from harvested rows × 10000 m2}}{(\text{Row lenght × Row spacing in meter × No. of rows)}}$$
 (1)

Straw yield was calculated by subtracting grain yield from biological yield.

### 2.2. Samples Collection and Physicochemical Analysis

Soil samples were taken from (0–30 cm) of the soil used in the field experiment and were prepared for some physical and chemical analysis. Soil properties changes with land use [28–30]. The whole plant samples (shoots + grains) were randomly taken from each treatment plot after harvesting and were dried at 70 °C until constant mass weight. Then, dried samples were grounded to pass a 1-mm screen, as suggested by Weidhuner et al. [30–32] and samples were thoroughly mixed and stored for analysis. Soil EC and pH were quantified in 1:5 soil water suspensions by the procedure of Rhoades [33] and Thomas [34], respectively. Organic matter (O.M.) contents in soil were determined by dichromate oxidation as described by Nelson and Sommers [35], AB-DTPA extractable and water-soluble phosphorus concentration was measured by the standard procedures of Soltanpour and Schwab [36]. The soil was also analyzed for lime content by adopting the procedure of Loeppert and Suarez [37] while soil texture was measured by the protocol of Jones et al. [39]. Plant P concentration and its uptake by wheat were determined by the protocol of Jones et al. [39]. Characterization of soil and HA is provided in Table 1.

### 2.3. Statistical Analysis

Data were subjected to two way ANOVA analysis for the significance of treatment effects and means were separated using the least significant difference (LSD) test with significance set at  $p \le 0.05$  using Staistix 2000 statistical package [40].

## 3. Results

## 3.1. Plant Height (cm)

Application of different levels of P and HA produced significantly ( $p \le 0.05$ ) taller wheat plants than the plots where SSP without HA was applied. When averaged across the P levels, HA produced 84 cm taller plants as compared to 82 cm without HA. When average across the HA levels, the application of P levels at 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> produced maximum taller plants of height 88 cm and the decrease in plant height at a higher level of P indicates that 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> is the optimum dose in the given soil conditions. The interactive effect of HA and P levels was also significant. Maximum plant height 89 cm was observed where 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> with 5 kg HA ha<sup>-1</sup> was applied, whereas a minimum of 77 cm was observed in control.

#### 3.2. Spike Length (cm)

Spike length as influenced by the different levels of P and HA is given in Table 2. When averaged across HA, different levels of SSP significantly increased the spike length as compared to control. On average the maximum spike length (10.77 cm) was recorded in the treatments applied with 112.5 kg  $P_2O_5$  ha<sup>-1</sup> which was statistically higher than the spike length obtained at 67.5 and 45 kg  $P_2O_5$  ha<sup>-1</sup> but not significant from the plots treated with 90 kg  $P_2O_5$  ha<sup>-1</sup>. Application of HA also increased the spike length of wheat plants significantly, but the interactive effect of P and HA was non-significant with a range from 9.75 cm in control to 10.89 cm observed in treatments P at 90 kg  $P_2O_5$  ha<sup>-1</sup> with 5 kg HA ha<sup>-1</sup>.

### 3.3. Grains Spike<sup>-1</sup>

Data showed that both different doses of SSP and HA had a significant effect on the number of grains spike<sup>-1</sup> (Table 2). The number of grains spike<sup>-1</sup> significantly increased with each level of SSP over control. On average maximum, 58 grains spike<sup>-1</sup> were observed in plots treated with 112.5 kg  $P_2O_5$  ha<sup>-1</sup> SSP that was statistically similar to those obtained at 90 kg  $P_2O_5$  ha<sup>-1</sup> SSP, whereas a minimum of 51 grain spike<sup>-1</sup> was recorded in control. Application of HA also showed significant results with maximum grains spike<sup>-1</sup> (56) in the plots receiving 5 kg HA ha<sup>-1</sup> dose which was statistically higher than the plots treated without HA, while the interactive effect was non-significant with a maximum number of 60 grains spike<sup>-1</sup> obtained from the plots treated with 90 kg  $P_2O_5$  ha<sup>-1</sup> with the application of HA.

### 3.4. 1000-Grain Weight

Table 2 shows the 1000-grain weight as influenced by the application of different levels of SSP with and without HA. The data revealed the increase in grain weight with increasing levels of P fertilizer and HA. The non-significant interaction revealed that HA increased the grain size irrespective of P levels. Similarly, when averaged across the HA, the application of 112.5  $P_2O_5$  ha<sup>-1</sup> produced heavier grains amounting 46.05 g per 1000 seeds which was 25.64% heavier than control. When averaged across the P levels, application of HA produced heavier grains of 42.61 g which were statistically higher than the grain weight of the plots which receive no HA.

# 3.5. Grain Yield (kg $ha^{-1}$ )

The addition of HA and P levels significantly increased the grain yield over control. Application of HA showed an additional advantage over the sole application of SSP by increasing the grain yield from 3% in control to 16% at 90 kg  $P_2O_5$  ha<sup>-1</sup> suggesting an increase in P use efficiency with the application of HA with each increment in P level (Table 2). When averaged across HA, grain yield of 2947 kg ha<sup>-1</sup>was recorded in the plots treated with 90 kg  $P_2O_5$  ha<sup>-1</sup> which was statistically higher than the grain yield obtained at 112.5, 67.5 and 45 kg  $P_2O_5$  ha<sup>-1</sup> indicating that the 90 kg  $P_2O_5$  ha<sup>-1</sup> could be the optimum level in the prevailing soil and climatic conditions for the wheat crop. The HA also showed significant results with grain yield of 2540 kg ha<sup>-1</sup> over no HA application with grain yield of 2338 kg ha<sup>-1</sup>.

Phosphorus (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	Humic Acid (kg ha <sup>-1</sup> )	Plant Height (cm)	Spike Length (cm)	Grains Spike <sup>-1</sup>	1000-Grain Weight (g)	Grain Yield (kg ha <sup>-1</sup> )	Straw Yield (kg ha <sup>-1</sup> )	Biological Yield (kg ha <sup>-1</sup> )	Harvest Index (%)	Plant P Concentration (g kg <sup>-1</sup> )	P Uptake (kg ha <sup>-1</sup> )
0		$78 \pm 0.13$ e	9.91 ± 0.10 c	51 ± 0.73 d	36.65 ± 0.23 e	1835 ± 33 e	2658 ± 28 d	4493 ± 214 e	$41 \pm 0.60 \text{ ab}$	$2.5 \pm 0.04 \text{ d}$	$11.54 \pm 0.33$ e
45		81 ± 0.03 d	$10.20 \pm 0.18$ b	$53 \pm 0.50$ c	39.95 ± 0.32 d	2237 ± 64 d	3480 ± 109 c	5717 ± 242 d	39 ± 1.21 b	$2.9 \pm 0.10 \text{ c}$	$17.02 \pm 0.67 \mathrm{d}$
67.5		83 ± 0.06 c	$10.34\pm0.01~\mathrm{b}$	$56 \pm 0.17 \text{ b}$	$42.00 \pm 0.45 \text{ c}$	2500 ± 36 c	$3864 \pm 23$ b	6364 ± 229 c	$39 \pm 0.23$ b	$3.1 \pm 0.06$ bc	$19.91 \pm 0.44 \text{ c}$
90		$88 \pm 0.26$ a	$10.67 \pm 0.09$ a	$58 \pm 0.44$ a	$44.87\pm0.67\mathrm{b}$	2947 ± 18 a	3927 ± 97 b	$6874 \pm 369 \text{ b}$	$43 \pm 0.71 \text{ a}$	$3.1 \pm 0.07$ b	$21.41 \pm 0.71 \text{ b}$
112.5		$87 \pm 0.03$ b	$10.77 \pm 0.07$ a	$58 \pm 0.33$ a	$46.05 \pm 0.20$ a	2678 ± 13 b	4549 ± 53 a	7227 ± 267 a	$36 \pm 0.32 c$	$3.3 \pm 0.09 \text{ a}$	$24.30 \pm 0.84$ a
LSD (p :	≤ 0.05)	0.63	0.28	1.33	0.81	95.597	180.96	154.39	2.033	0.1421	1.157
	0	$82\pm0.07~b$	$10.27\pm0.08~\mathrm{b}$	$54 \pm 0.41$ b	$41.20\pm0.40b$	$2338\pm37b$	$3609 \pm 46$	$5947 \pm 1053 \mathrm{b}$	$39 \pm 0.68$	$2.9 \pm 0.04$ b	$17.71 \pm 0.34$ b
	5	$84\pm0.05~\mathrm{a}$	$10.48 \pm 0.12$ a	$56 \pm 0.18$ a	$42.61 \pm 0.29$ a	$2540\pm25~\mathrm{a}$	$3782 \pm 81$	6322 ± 1106 a	$40\pm0.63$	$3.1 \pm 0.07 \text{ a}$	$19.97 \pm 0.62$ a
LSD (p :	≤ 0.05)	0.40	0.18	0.84	0.51	60.461	ns	97.644	ns	0.0898	0.733
0	0	$77 \pm 0.23$ h	$9.75 \pm 0.05$	$51 \pm 0.88$	$35.73 \pm 0.26$	$1807 \pm 43~\mathrm{f}$	$2534\pm90$	4341 ± 65 h	$42 \pm 1.37$	$2.5\pm0.03$	$11.11\pm0.24$
	5	78 ± 0.23 g	$10.06\pm0.17$	$52 \pm 0.58$	$37.57\pm0.23$	$1862 \pm 23 \text{ f}$	$2782 \pm 54$	4644 ± 77 g	$40\pm0.18$	$2.5\pm0.05$	$11.98\pm0.45$
45	0	$80 \pm 0.23$ f	$10.04\pm0.27$	$52 \pm 1.00$	$39.40 \pm 0.40$	2168 ± 57 e	$3377 \pm 114$	$5545 \pm 80 \text{ f}$	$39 \pm 1.35$	$2.9 \pm 0.03$	$16.19 \pm 0.15$
	5	$81 \pm 0.24$ e	$10.36 \pm 0.23$	$54 \pm 1.02$	$40.50\pm0.40$	2306 ± 70 d	$3582 \pm 122$	$5888 \pm 141 \text{ e}$	$39 \pm 1.11$	$3.0 \pm 0.17$	$17.86 \pm 1.20$
67.5	0	$81 \pm 0.23$ e	$10.31\pm0.10$	$55 \pm 0.33$	$41.40\pm0.67$	2439 ± 74 cd	$3763 \pm 84$	$6202 \pm 20 d$	$39 \pm 1.25$	$2.9\pm0.04$	$18.66 \pm 0.20$
	5	85 ± 0.35 d	$10.37 \pm 0.10$	$58 \pm 0.33$	$42.60 \pm 0.36$	2561 ± 26 c	$3965 \pm 125$	6526 ± 121 c	$39 \pm 0.84$	$3.2 \pm 0.09$	$21.16 \pm 0.72$
90	0	$86 \pm 0.44$ c	$10.44 \pm 0.11$	$56 \pm 0.58$	$43.83 \pm 0.49$	2731 ± 35 b	$3882 \pm 35$	6613 ± 61 c	$41 \pm 0.29$	$2.9 \pm 0.05$	$19.49 \pm 0.53$
	5	89 ± 0.15 a	$10.89\pm0.10$	$60 \pm 0.33$	$45.90 \pm 0.86$	3163 ± 69 a	$3972 \pm 174$	7135 ± 109 b	$44 \pm 1.63$	$3.3 \pm 0.09$	$23.33 \pm 0.89$
112.5	0	85 ± 0.31 d	$10.83 \pm 0.06$	$58 \pm 0.02$	$45.63 \pm 0.33$	2547 ± 30 c	$4490 \pm 22$	7037 ± 38 b	$36 \pm 0.28$	$3.2 \pm 0.13$	$23.10 \pm 1.03$
	5	$88\pm0.37~\mathrm{b}$	$10.71\pm0.14$	$58\pm0.67$	$46.47\pm0.45$	$2808\pm29~b$	$4607\pm90$	$7415 \pm 62 a$	$38\pm0.71$	$3.4\pm0.06$	$25.50\pm0.72$
LSD (p :	≤ 0.05)	0.89	ns	ns	ns	135	ns	218	ns	ns	ns

Table 2. Effect of phosphorus and humic acid on the growth, yield and P nutrition of wheat under agro climatic conditions of Peshawar, Pakistan.

Means with same letters are not significantly different from each other. The  $\pm$  values represent stander error of the mean (n = 3) while, ns stands for non-significant difference at  $p \le 0.05$ .

### 3.6. Straw Yield (kg $ha^{-1}$ )

Wheat straw yield as influenced by different levels of SSP with and without HA is presented in Table 2. With an increase in P level, the straw yield significantly increased over control except for 67.5 and 90 kg  $P_2O_5$  ha<sup>-1</sup> which were statistically similar. The maximum straw yield of 4549 kg ha<sup>-1</sup> was recorded from the treatments applied with 112.5 kg  $P_2O_5$  ha<sup>-1</sup> which was 71% higher than control. This increase signifies the function of P in crop growth and productivity in the tested soil and climatic conditions, however, the role of HA was found non-significant. The interactive effect of P levels and HA was also non-significant with maximum straw yield of 4607 kg ha<sup>-1</sup> was observed in the plots treated with 112.5 kg  $P_2O_5$  ha<sup>-1</sup> and HA, while minimum (2534 kg ha<sup>-1</sup>) was in case of control.

### 3.7. Biological Yield (kg $ha^{-1}$ )

Both the P levels, HA and their interaction significantly ( $p \le 0.05$ ) increased the biological yield of wheat (Table 2). The interaction of P levels with HA exhibited that biological yield increases with increasing application of P but HA application further intensify such improvement. On average, each increment of P produced higher biological yield than the preceding lower dose indicating the role of P in increasing the crop growth in the given soil and climatic conditions. The maximum biological yield (7227 kg ha<sup>-1</sup>) was recorded in the plots treated with 112.5 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> that was statistically higher than the plots applied with 90, 67.5 and 45 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Table 3 and Figure 1A). The data regarding biological yield also revealed that the increase over control with 90 kg P<sub>2</sub>O<sub>5</sub> kg ha<sup>-1</sup> and HA was 64% which was close to 62% increase observed with 112.5 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> applied without HA suggesting that P application dose could be reduced with HA (Figure 1B). Similarly, when averaged across the P levels, the HA application produced 6322 kg biological yield ha<sup>-1</sup> on dry weight basis which was significantly higher than the plots which does not receive HA advocating the increasing role of P with HA. A remarkable percent increase of 6.2%, 5.2%, 7.9% and 5.4% in biological yield was observed with the addition of HA and P levels over the sole application of P levels.

P Levels (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	Humic Acid Levels (kg ha <sup>-1</sup> )	OM (%)	Extractable P (mg kg <sup>-1</sup> )	WSP (mg kg <sup>-1</sup> )	Extractable K (mg kg <sup>-1</sup> )
0		$0.83 \pm 0.02 \text{ c}$	5.05 ± 0. 06 e	0.139 ± 0.01 e	86 ± 0.58 e
45		$1.00 \pm 0.01 \text{ b}$	$5.93 \pm 0.07 \text{ d}$	$0.155 \pm 0.02 \text{ d}$	97 ± 0.88 d
67.5		$1.04 \pm 0.05 \text{ b}$	$6.88 \pm 0.15 \text{ c}$	$0.185 \pm 0.01 \text{ c}$	$107 \pm 1.01 \text{ c}$
90		$1.13 \pm 0.02$ a	$7.78 \pm 0.12 \text{ b}$	$0.206 \pm 0.01 \text{ b}$	$121 \pm 0.51 \text{ b}$
112.5		$1.16 \pm 0.04$ a	$8.68 \pm 0.10$ a	$0.232 \pm 0.01$ a	$134 \pm 0.84$ a
LSD	$(p \le 0.05)$	0.063	0.121	0.016	2.461
	0	$0.95 \pm 0.02 \text{ b}$	$6.12 \pm 0.12 \text{ b}$	0.165 ± 0.01 b	$102 \pm 0.19$ b
	5	$1.11 \pm 0.03$ a	$7.61 \pm 0.08$ a	$0.201 \pm 0.02$ a	$116 \pm 1.01 \text{ a}$
LSD ( $p \le 0.05$ )		0.040	0.076	0.015	1.556
0	0	0.83 ± 0.03 f	$4.20 \pm 0.06$ j	$0.135 \pm 0.01 \text{ f}$	81 ± 1.45 h
	5	$0.82 \pm 0.02 \; f$	$5.90 \pm 0.06$ h	$0.144 \pm 0.01 \text{ ef}$	91 ± 1.47 g
45	0	$0.89 \pm 0.04 \text{ ef}$	$5.07 \pm 0.09$ i	$0.144 \pm 0.01 \text{ ef}$	$90 \pm 0.88$ g
	5	$1.11 \pm 0.03 \text{ cd}$	$6.80 \pm 0.07 \; \text{f}$	$0.165 \pm 0.02 \text{ de}$	$103 \pm 0.91 \text{ e}$
67.5	0	$0.93 \pm 0.06 \text{ e}$	$6.05 \pm 0.21 \text{ g}$	$0.171 \pm 0.01 \text{ d}$	$98 \pm 1.01 \text{ f}$
	5	$1.15 \pm 0.04$ bc	7.67 ± 0.09 d	$0.198 \pm 0.01 \text{ c}$	116 ± 1.73 c
90	0	$1.04 \pm 0.01 \text{ d}$	$7.10 \pm 0.15 \text{ e}$	$0.180 \pm 0.02 \text{ cd}$	112 ± 1.45 d
	5	$1.22 \pm 0.04$ ab	$8.47\pm0.12~\mathrm{b}$	$0.233 \pm 0.01 \text{ b}$	$130 \pm 0.89 \text{ b}$
112.5	0	$1.08 \pm 0.03$ cd	8.13 ± 0.12 c	$0.198 \pm 0.01 \text{ c}$	$127 \pm 1.20$ b
	5	$1.24 \pm 0.04$ a	$9.23 \pm 0.09$ a	$0.266 \pm 0.03$ a	$141\pm0.58~\mathrm{a}$
LSD ( $p \le 0.05$ )		0.0886	0.171	0.222	3.480

**Table 3.** Effect of different levels of phosphorus and humic acid on post-harvest soil properties of wheat under field conditions of Peshawar, Pakistan.

The mean while tanders Means with same letters are not significantly different from each other. OM and WSP stand for organic matter and water soluble P respectively. The  $\pm$  values represent stander error of the mean (n = 3).



**Figure 1.** Comparative increase in biological yield of wheat after the application of SSP with and without HA; observed values (**A**) and increase over control (**B**).

#### 3.8. Harvest Index (%)

Application of different P levels significantly affect the harvest index of wheat crop, where the HA addition and interaction of P levels and HA non-significantly affect the harvest index of wheat plants (Table 2). When averaged across the HA levels, maximum harvest index of 43% was observed in the plots that received 90 kg  $P_2O_5$  ha<sup>-1</sup>, whereas lowest harvest index of 36% was noted in plots that receive 112.5 kg  $P_2O_5$  ha<sup>-1</sup> in the form of SSP.

# 3.9. Plant Phosphorus Concentration $(g kg^{-1})$

Both P levels and HA showed significant effects and increased plant phosphorous concentration (Table 2). The application of 112.5 kg  $P_2O_5$  ha<sup>-1</sup> enhanced the plant shoots [P] in leaves with a value of 3.3 g kg<sup>-1</sup> which was statistically higher than other treatments. On average maximum plant [P] of 3.1 g kg<sup>-1</sup> was observed in the plots applied with 5 kg HA ha<sup>-1</sup> which was statistically higher than 2.9 g kg<sup>-1</sup> observed in plots that receive no HA. The interactive effect of P levels with HA was non-significant with maximum P concentration of 3.4 g kg<sup>-1</sup> in plots where 112.5 kg  $P_2O_5$  ha<sup>-1</sup> was used with HA, while the minimum of 2.5 g kg<sup>-1</sup> was noted in control plots.

# 3.10. Phosphorous Uptake by the Plant (kg $ha^{-1}$ )

Regarding the P-uptake of wheat plants, the addition of different levels of SSP with HA showed superior results over sole SSP application (Table 2). When averaged across the HA, maximum P uptake of 24.30 kg ha<sup>-1</sup> was noted in plots that were treated with 112.5 kg  $P_2O_5$  ha<sup>-1</sup>, followed by 21.41 kg ha<sup>-1</sup> in plots received 90 kg  $P_2O_5$  ha<sup>-1</sup>, whereas, the minimum was noted in control plots. When averaged across P levels, the maximum P uptake of 19.97 kg ha<sup>-1</sup> which was 13% higher than uptake in plots treated without HA (Figure 2A). Regarding different P levels, addition of HA increases the P uptake from 10.3 to 21.2% as compared to plots where P levels were applied without HA. The interaction of P levels and HA was non-significant with the maximum uptake of 25.50 kg ha<sup>-1</sup> observed with 112.5 kg  $P_2O_5$  ha<sup>-1</sup> applied with HA. The total uptake of P at 90 kg  $P_2O_5$  ha<sup>-1</sup> applied with HA (23.33 kg ha<sup>-1</sup>) was similar to uptake of 23.10 kg ha<sup>-1</sup> at 112.5 kg  $P_2O_5$  ha<sup>-1</sup> without HA suggesting the increase in P use efficiency and reduction in P requirements for optimum crop growth and yield. Similarly, the percent increase over control with HA-SSP at 67.5 kg ha<sup>-1</sup> was 90.5% that was more than the percent increase over control at 90 kg SSP alone (75.4%) confirming the fact that P use efficiency increased with HA application (Figure 2B).



**Figure 2.** Comparative increases in plant P uptake after the application of SSP with and without HA; observed values (**A**) and Increase over control (**B**).

#### 3.11. Soil Organic Matter

Soil organic matter (SOM) content was significantly affected by P levels, HA and their interaction. Treatments receiving SSP and HA had significantly ( $p \le 0.05$ ) higher organic matter than sole SSP levels at each increment of P levels from 45 to 112.50 kg  $P_2O_5$  ha<sup>-1</sup> (Table 3). When averaged across P levels, the HA had 1.11% organic matter which was significantly higher than 0.95% calculated for plots received no HA. Similarly, when averaged across the HA, the maximum soil organic matter contents of 1.16% was observed in plots treated with higher doses of SSP of 112.5 kg  $P_2O_5$  ha<sup>-1</sup> that was statistically identical to 1.13% observed in the plots treated with 90 kg  $P_2O_5$  ha<sup>-1</sup> but higher than other treatments and control. The interactive effect of HA and SSP was significant with the highest organic matter contents of 1.24% in plots treated with 112.5 kg  $P_2O_5$  ha<sup>-1</sup> and HA, and the lowest of 0.82% was noted in control. It was evident from the interaction (HA\*P) that SOM increased with the application of HA regardless of P rate except in control and plots treated with 45 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> where SOM content was similar for with and without HA treated plots. The organic matter content observed for 112.50 kg  $P_2O_5$  ha<sup>-1</sup> without HA was at par to 67.5 kg  $P_2O_5$  ha<sup>-1</sup> with HA which were significantly lower than plots amended with 90 kg  $P_2O_5$  ha<sup>-1</sup> with HA. Similarly, OM observed at 45 kg  $P_2O_5$  ha<sup>-1</sup> with HA was statistically similar to that observed for 67.5 kg  $P_2O_5$  ha<sup>-1</sup> without HA. These finding suggested that, HA application can preserved soil organic matter content when applied with SSP.

### 3.12. AB-DTPA Extractable Phosphorus

Results showed that treatments receiving HA + SSP had significantly ( $p \le 0.05$ ) more soil AB-DTPA extractable P than SSP alone at each increment of P levels from 45 to 112.50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Table 3). When averaged across the P levels, HA-SSP produced 7.61 mg P kg<sup>-1</sup> which was significantly higher than 6.12 mg P kg<sup>-1</sup> observed in no HA plots. Addition the SSP with HA increased the post-harvest soil AB-DTPA P over alone SSP levels with a mean value of 24.3%. Similarly, when averaged across the fertilizer HA, the AB-DTPA extractable P increased with each increment of P. The mean maximum AB-DTPA ext. P of 8.68 mg kg<sup>-1</sup> was recorded in treatments applied with 112.5 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> that was statistically higher than the phosphorous content of other treatments. The minimum AB-DTPA ext. P with a value of 5.05 mg kg<sup>-1</sup> was observed in control. The interactive effect of SSP and HA was also significant showing increases with an increase in P levels. The maximum AB-DTPA ext. P of 9.23 mg kg<sup>-1</sup> was recorded in plots receiving 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as HA + SSP while the minimum of (4.20 mg kg<sup>-1</sup>) was recorded in control.

#### 3.13. Water-Solublephosphorus

Analysis of variance (ANOVA) revealed that P levels, humic acid and their interaction significantly affected soil water soluble P. Water-soluble phosphorus significantly ( $p \le 0.05$ ) increased with HA and P levels (Table 3). HA treated plots, when averaged across P levels, maintained higher water-soluble P of  $(0.201 \text{ mg kg}^{-1})$  than plots receiving no HA which had 0.165 mg kg<sup>-1</sup>water-soluble P, on an average basis. The percent increase in water-soluble phosphorous with HA+SSP levels over respective sole P levels ranged from 6.7% in control to 34.3% in treatments receiving 112.5 kg  $P_2O_5$  ha<sup>-1</sup> suggesting higher release from HA+SSP than commercially available SSP. The interactive effect of HA and SSP was significant with maximum water-soluble P of (0.266 mg kg<sup>-1</sup>) observed in 112.5 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as HA-SSP while a minimum of  $(0.135 \text{ mg kg}^{-1})$  was recorded in control. The significant interaction of HA\*P demonstrated that, WSP increased with increasing P level, however, this increase was more in plot treated with 5 ton HA ha<sup>-1</sup> compared to control HA at respective P levels. Furthermore, with respect to WSP the response of 45 kg  $P_2O_5$  ha<sup>-1</sup> with HA was significantly better than 67.5 kg  $P_2O_5$  ha<sup>-1</sup> without HA and the performance of 67.5 kg  $P_2O_5$  ha<sup>-1</sup> with HA was at par to 90 kg  $P_2O_5$  ha<sup>-1</sup> without HA. It was also evident that 90 and 112.5 kg  $P_2O_5$  ha<sup>-1</sup> with HA performed similar which was significantly superior than 112.5 kg  $P_2O_5$  ha<sup>-1</sup> without HA. Thus, it can be deduced that, HA enhances P availability in soil amended with P as chemical fertilizers.

#### 4. Discussion

Application of P levels with humic acid (HA) produced significantly taller wheat plants than sole P application at each increment of P levels from 45 to 112.50 kg  $P_2O_5$  ha<sup>-1</sup>, whereas the interactive effect of P and HA was also significant. The increase over control with 67.5 kg  $P_2O_5$  kg ha<sup>-1</sup> and HA was 10.4% which was close to 11.7% increases observed with the application of 90 kg  $P_2O_5$  ha<sup>-1</sup> alone indicating that P application dose could be reduced with HA. Such differential increases revealed that P use efficiency was increased with HA over sole application of P as commercial SSP fertilizer and as such could reduce the farmer input cost without compromise on yield and crop productivity. These results revealed that the linear increase in plant height could be achieved with P increased levels and HA applications. Ahmad et al. [26] also suggested that plant height can be increased with a higher P rate application. Khattak and Dost [41] also reported the increase in plant height with HA which has suggested that the application of HA with different fertilizers may cause beneficial effects on plant growth and nutrients uptake. This could be associated with the capability of HA to improve the biochemical environment of soil by promoting soil enzymatic activities, microbial activities and population, cation exchange capacity and water retention of soil that ultimately enhance the plant growth and nutrients uptake. These results were also in accordance with Tahir et al. [42] who reported that the application of HA significantly improved the plant height. The application HA with SSP improved the quality of wheat produced as indicated by grain size and weight. Combine application of SSP and HA produced higher 1000-grain weight than SSP alone at each P level, while their interaction remained non-significant at  $p \le 0.05$ . These results advocated the role of P in increasing the size and quality of seed in the present study. These results were similar to the study of Kaleem et al. [9] who reported that maximum phosphorus dose enhanced the number of grains spike<sup>-1</sup>, tillers number, thousand-grain weights and grain yield due to the highest accumulation of photosynthates in the plants and increased grain ripening which resulted in heavier grains. The results were also in close consistency with the findings of Ibrahim et al. [43] who stated that wheat 1000 grains weight could be increased significantly with the combined application of chemical and organic fertilizers. Wheat spike length and grains per spike showed almost similar trend and an increase was observed with increasing P and HA levels. Addition of HA with all P levels showed increased spike length except 112.5 kg  $P_2O_5$  ha<sup>-1</sup> which showed a decrease of 1.11%. This negative increase may be associated with the imbalance in plant nutrients caused by increased concentration of phosphorus. It is an established fact that higher doses of one nutrient can have detrimental effect on the absorption of others and as such reduce the crop performances. The results also depicted that balance ratio of P fertilizers is essential to

obtain higher yield of wheat against the common farmer's practice in the area who do not bother to keep in mind the balance of different fertilizers at the time of sowing [9]. These improvements revealed that application of P with HA improved the storage of photosynthates in the plants. This accumulation of photosynthates in plants enhances the enzymatic, microbial and catalytic activities in plants and thus produces higher grains spike $^{-1}$ , grain yield, straw yield and biomass as well [9]. Results regarding the grain yield showed that the percent increases of 41.7% with HA and 67.5 kg  $P_2O_5$  ha<sup>-1</sup> is closely resembled to 40.9 and 51% obtained with SSP alone at 90 and 112.5 kg  $P_2O_5$  ha<sup>-1</sup>, respectively, revealing a reduction in P requirements and increase in P fertilizer use efficiency with the addition of HA. A similar effect was noted by Khattak and Dost [41] who stated that the combined use of fertilizers and HA could increase the yield of different crops and reduce the crop fertilizer requirements without compromise on yields and quality. The substantial increase in wheat yield over control with SSP and HA suggested its potential use as an effective P fertilizer. The increase in grain yields with P levels is a well-established fact in P deficient soils. The HA would have increased the P availability by making its soluble complexes and stimulating plant growth [24] as well as by providing a good physicochemical and biological environment of the soils to the plants [21]. Proliferation of rhizobacteria also played an imperative role in better uptake of phosphorus when applied in the presence of organic carbon [44–47]. Improvement in straw and biological yield revealed that a significant increase in straw and biological yield could be obtained by the application of phosphorus and HA [27]. The increase in growth parameter and yield may be related with the stimulating effect of P and HA on plant growth by the assimilation of major and minor elements, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis that ultimately enhances the biomass or biological production [48,49]. The results were also in consistence with Sarir et al. [50] and Sharif et al. [51] who reviewed that HA application can improve biological yield up to a prominent level. Data regarding the P concentration in plant shoots suggested that the application of SSP with HA increased the plant P concentration as 3.5, 10.4, 6.9 and 6.3% with 45, 67.5, 90 and 112.5 kg  $P_2O_5$ , respectively, over the same levels of SSP alone indicating that HA increased the P use efficiency and uptake of plants. The results of the study were similar to the study of Khattak and Dost [41] who reported that HA increased the plant growth and nutrients uptake capability through the improvement of soil enzymatic system and microbial activities and population ultimately making the soil conditions favourable for plant uptake. The results of the study can also be supported by the results of Majumdar et al. [52] who stated that the application of rock phosphate mixed with different organic manures could increase P concentration in plants significantly. The higher P concentration in plant leaves with the application of HA along with SSP was in line with the study of Cooper et al. [53] and Atiyeh et al. [54]. They stated that the addition of HA in the soils enhanced the root growth as well as the proliferation, branching, and initiation of root hairs and thus able the roots towards more nutrient capturing and increase nutrient concentrations in the plants. A lot of studies showed an increase in root length, root number and root branching with the application of HA. However, the increase in root growth is generally more noticeable than shoot growth [55]. Pettit [56] also reported a prominent increase of root initiation and increased root growth with the application humic and fulvic acids to the soil ultimately increasing the nutrient concentrations in plant tissues. The uptake of P indicated that the observed increases in wheat growth and yield with SSP levels and HA in the present study. The increase in P uptake with increasing P levels is an accepted fact [57] and the additional advantage with HA is in consistence with the findings of Erdal et al. [55] who stated a prominent accumulation of nutrients in the plant with the application of organic materials along with mixing of inorganic fertilizers. These results can also be supported by Sharif et al. [51] who reported that P and N uptake could be increased with the addition of P fertilizers (SSP) along with organic materials. The results regarded the soil organic matter contents indicated that both SSP and HA could improve the soil organic matter content. The improvement in the organic matter could be attributed to higher biomass and bumper root growth and as such more leftover fraction as also indicated by close resemblance between plant biomass and soil organic matter. These results are in line with the findings of Sharif et al. [51] who reported that the use of inorganic fertilizers along with organic

fertilizers (humic acid and FYM) increased soil organic matter content. Similar findings were reported by Tamayo et al. [58] and Han et al. [59], who stated an increase in soil organic carbon with the use of chemical fertilizer along with the organic. The data regarding the AB-DTPA extractable P revealed that the increase over control with 67.5 kg  $P_2O_5$  kg ha<sup>-1</sup> and HA was 83% which was more than 70% increases observed with 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> applied alone suggesting that P application dose could be reduced with the HA. The results of the study are similar to the findings of [60] who reported that the application of HA to acidic and alkaline soil decreases the P complex formation and dissolves the insoluble and unavailable P thus enhance the availability of phosphorus to the plants. Similar results were reported by Sharif et al. [51] and Majumdar et al. [52] who stated that phosphorus concentration could be increased with the application of rock phosphate (RP) along with the mixing of different organic materials. An increase in water-soluble P is related to the accessibility of phosphorus added to the soil as well as with HA which decreases the P fixation and provides more water-soluble P for plants. These results were in uniformity with the findings of several researchers [26,61–65]. They reported that the supplementation of P from various P sources including rock phosphate and HA application increased the soil solution P whereas the high pH and lime contents in calcareous soils reduced it by making its insoluble complexes. Like other organic materials, the HA make soluble complexes with P and increase its concentration in the soil solution. However, plant and microbial exudates neutralized the rhizosphere by producing  $(H^+)$  as a result of cation uptake can increase the availability of P in the soil.

#### 5. Conclusions

Application of P as SSP fertilizer and humic acid (HA) significantly improved wheat growth, yields, P uptake and post-harvest soil AB-DTPA extractable and water-soluble P contents. The interactive effect of P and HA were found significant for plant height, grain and biological yield and soil AB-DTPA extractable P and K. The significant superiority of SSP and HA over sole SSP at almost every application rate suggested improvement in P fertilizer use efficiency with HA. The grain yield obtained at 67.5 kg  $P_2O_5$  ha<sup>-1</sup> with HA was statistically comparable to 112.5 kg  $P_2O_5$  ha<sup>-1</sup> applied as commercial SSP suggested that the input expenditures of fertilizer may be reduced up to 50% by combine application of chemical fertilizers with HA. Similarly, HA with SSP maintained higher AB-DTPA extractable, water-soluble P, soil organic matter contents, plant P concentrations and P uptake over commercial SSP in the soil, which further signifies the importance of HA in enhancing P availability.

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