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Research Article

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Impact of Humic Acid on the Morphological Components and Growth Parameters of Wheat (*Triticum Aestivum* L.) Under Dry Climate of Uthal

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Abstract

Humic acid is natural biological organic, which has a high effect on plant growth and quality. However, the mechanisms of the promoting effect of humic acid on the morphological components, growth and yield parameters were rarely reported. In this study, the effects of soil application of humic acid on the morphological components and growth parameters of wheat under dry climate were explored. Randomized complete block design (RCBD) with five treatments of humic acid and replicated three time, T_1=control, T_2= 4.5kgha^{-1}, T_3= 6 kgha^{-1}, T_4= 9.5 kgha^{-1}, T_5= 12 kgha^{-1} and T_6= 14.5 kgha^{-1}. Findings of this study indicated that with increasing application of humic acid growth and yield also increases plant height (21.97%), spike weight (157%), leaf area (34.12%), grain spike^{-1} (93%), grain yield (49.36%) and biological yield (80.34), though pH of soil also increased. While; results of NO_3-N and K were also significantly different under different level of humic acid and mean maximum were recorded in T_6 (25% and 48%) in comparison of T_1, T_2, T_3, T_4 and T_5. Conclusively; this study revealed that application of humic acid at the rate of 14.5 kg ha^{-1} could improve the growth and yield parameters of wheat and soil fertility.

Keywords: Humic acid, Soil Properties, morphology, wheat, yield.

Introduction

Wheat is the third major crop after corn and rice, grain of wheat are rich in carbohydrate, minerals, vitamins, proteins than other crops and globally 651 million of hector of wheat is cultivated poole *et al*., 2021. Likewise; the vital wheat gluten were ranges from 30 to 35% and play a key role in wheat-baked food product Li *et al*., 2022, Schopf *et al*., 2021. Pakistan agricultural sector not only provides food for the entire population, but also provides raw materials for the industrial sector and a large amount of raw materials for exports. The backbone of the economy, accounting for 19.5% of GDP, providing 42.3% of employment and labor, and providing raw materials for various value-added industries (Usman, 2016, Muhammad et al., 2014). Though; the adoption of Modern technologies, improved seeding methods, chemical fertilizers, pesticides, herbicides, certified seeds and agricultural machinery have not been properly used, promoted and widely adopted by farmers. So for the improvement of biological and grain yield it's necessary to consider the physiological characteristics of the crop. Consequently; for the improvement of crop and management practice start from the bottom with the facilitation of farmers through research scholars to conduct the seminar, workshop on the different aspect of agriculture, soil health, nutrients management in general and particular on wheat as major crop Malik *et al.*, 2015. Soil metal pollution may have some harmful effects on individual physical condition. However, humic acid (HA) components like humic acid and fulvic acid can help plants grow via increasing nutrient absorption and activating biomass production. The goal of this study was to investigate the impact of HA on the photosynthetic pigment and malondialdehyde (MDA) contents of wheat Akcin *et al*., 2019. HA contains a variety of macro and micro nutrients that chelated in certain way. Two separated groups allowing to the polarity and size of dissimilar compounds. Smaller polar component is called fulvic acid, and the larger non-polar component is called humic acid and is the final product of microbial degradation of plant and animal manure, and is an important part of fertile soil De-Melo *et al.*, 2016. Humic acid contains a significant quantity of carbon and a little measure of hydrogen and nitrogen. Marine humic acid has a larger and nitrogen concentration than continental

Turgay *et al*., 2011; Li *et al*., 2019. HA is a remarkable naturally occurring substance that has a more impact on plant growth. With application of humic acid 9% N total, 30% available P and 16% available K could be increases and this directs a significant boost in soil organic carbon and available potassium. As soil fertility level improved that has significant effect on the biological and grain yield of wheat Mahmoud *et al*., 2018, meanwhile, treatment of intensely boosted soil urease, inverses, and phosphatase activities at the maturity. Additionally, the quantity of bacteria declined but the number of fungi grew after humic acid treatment Anwar *et al*., 2021. Ascomycetes on the other than are less abundant in fungi and the majority of the genera that have been found are plant pathogens finally humic acid improves the yields of crop production peanuts through improving physical properties of soil Arslan *et al*., 2021. Enzyme activity and microbial diversity, and differences in plant height, leaf number, total chlorophyll, biological yield, grain yield, and grain total nitrogen are all important Hegab *et al*., 2020. The Chemical properties of humus are quite complex, and apart from some of the many functional groups, they have not been described to any degree. Humic acid contains trace elements, macro elements and trace elements, which can be chelated in a certain way Turgay *et al*., 2011. Humic acids are separated into two groups allowing to the polarity and size of dissimilar compounds. The smaller polar component is called fulvic acid, and the larger non-polar component is called humic acid is the final product of microbial degradation of plant and animal manure, and is an important part of fertile soil Li *et al*., 2019. Generally our soils are calcareous in nature and contain more amount of calcium ions compared to other ions with >7.0 pH. And with rapid cultivation and impediment to boosting productivity is the scarcity of plant nutrients. The application of chemical fertilizers such as urea, DAP correspondingly taking account the problem of soil fertility and low organic matter content Iqbal *et al*., 2021.

Materials and Methods

Description of the Study: Research study was conducted to estimate the impact of humic acid on the Morphological Components and Growth Parameters of Wheat (*Triticum Aestivum* L.) Under Dry Climate of Uthal

Field Area: A plot 3m X 4m in size for present research study was prepared in the experimental field of Lasbela University of Agriculture, Water and Marine Sciences (LUAWMS) Uthal, during Rabi, 2020, with 25.8270 0N and 6636236 0E is the Latitude and longitude.

Climate of Uthal: LUAWMS situated in Uthal, district Lasbela, and intended as a coastal zone of Balochistan, which 125km away from Karachi. Summer is very hot (40-50 0c) with cool winter (25-260c), with limited rain fall 169 mm annually. Lasbela is famous for its unique geographical structure of mountainous regions, and most beautiful beaches such as Gadani, Sonmiani, Kund Malir, Damb etc. summer is very hot with cool winter and annually 169 mm an average of rain fall of the study area. Wheat, Cotton, Tomato, Okra and coaster oil commonly cultivated crop in district Lasbela.

Layout of Field: Randomized complete block design (RCBD) replicated three times and a total of 18 plots was prepared before cultivation

Treatments: Six different application of humic acid were applied such as T_1=control, T_2=4.5 $kgha^{-1}$, T_3=6 $kgha^{-1}$, T_4=9.5 $kgha^{-1}$, T_5=12 $kgha^{-1}$, T_6=14.5 $kgha^{-1}$, though the recommended dose of N and P application were also applied at 70 and 9$kgha^{-1}$ respectively.

Growth and yield Parameters: Before harvesting at maturity of crop five healthy plants were collected from each plot of both replication and treatment to estimate the effect of humic acid application on plant height (cm), leaf area (cm), $Grainspike^{-1}$, Fresh & dry root Biomass (g), Grain Yield ($kgha^{-1}$) & Biological Yield ($Kgha^{-1}$)

Soil sampling process and Analysis: thirty six soil samples both before and after harvesting of crop at 0-20cm of soil depth were collected. Sample were packed in well label of polyethylene bags and transport to lab for further analysis. Manually all the plant material and stones were collected and sieved in 2mm sieve and soil extract was prepared (soil and water extraction ratio of 1:2.5) for analysis of soil EC (dSm^{-1}) and pH (by using a digital EC and pH meter), soil organic matter (%) by wet oxidation of (walkely and black, 1934), available P by spectrophotometer and available K data were recorded by using flame photometer followed by (Jackson and Marion, 2005, Estefan et al., 2013)

Statistical Analysis: The collected data was subjected to one-way analysis of variance (ANOVA) suggested by Gomes and Gomes, 1984. Difference among treatments mean will be calculated by Least Significant Difference (LSD) test 5% probability level were used with the help of SPSS Version 20 (IBM.2016)

Result and Discussions

Effect of Humic Acid on soil physico-chemical properties of soil: present study was conducted in dry and hot climate of Uthal, district Lasbela Figure 1 presented the analysis result of both soil pH and EC (dsm^{-1}) before sowing of seed Table 1 and after harvesting of the crop Figure 1.

Table: 1. Physico-chemical Properties of soil before sowing of seed

Treatments	EC (DSm^{-1})	pH	OM (%)	P ($mgkg^{-1}$)	N (%)	K ($mgkg^{-1}$)
T_1	0.54±0.031a	7.84±0.045a	0.64±0.04a	3.69±0.15a	0.038±0.008a	83.67±4.16a
T_2	0.54±0.025a	7.82±0.015a	0.66±0.057a	3.75±0.06b	0.038±0.002a	86.00±6.24b
T_3	0.56±0.028a	7.86±0.043a	0.64±0.037a	3.70±0.16b	0.036±0.008a	83.00±13.11a
T_4	0.53±0.023b	7.87±0.024a	0.58±0.065b	3.59±0.01ab	0.040±0.006b	86.33±10.21b
T_5	0.56±0.013a	7.86±0.049a	0.64±0.045a	3.66±0.15a	0.040±0.002b	84.67±6.027a
T_6	0.56±0.021a	7.83±0.032a	0.60±0.03b	3.67±0.13a	0.038±0.008a	82.00±4.35a
CV	1.49	0.23	5.27	3.18	16.71	4.74
LSD	0.0147	0.0332	0.0703	0.2252	7.865	8.8878
P<value	0.0000	0.0000	0.0004	0.0002	0.0000	0.0002

Note: The mean value of different treatments level of Soil EC (dSm^{-1}), pH, OM (%), p ($mgkg^{-1}$), N (%) and K ($mgkg^{-1}$) in triplicate data with standard error is within the brackets. Different lowercase letters (a, b, c) indicate significant differences at $p< 0.05$ within a column

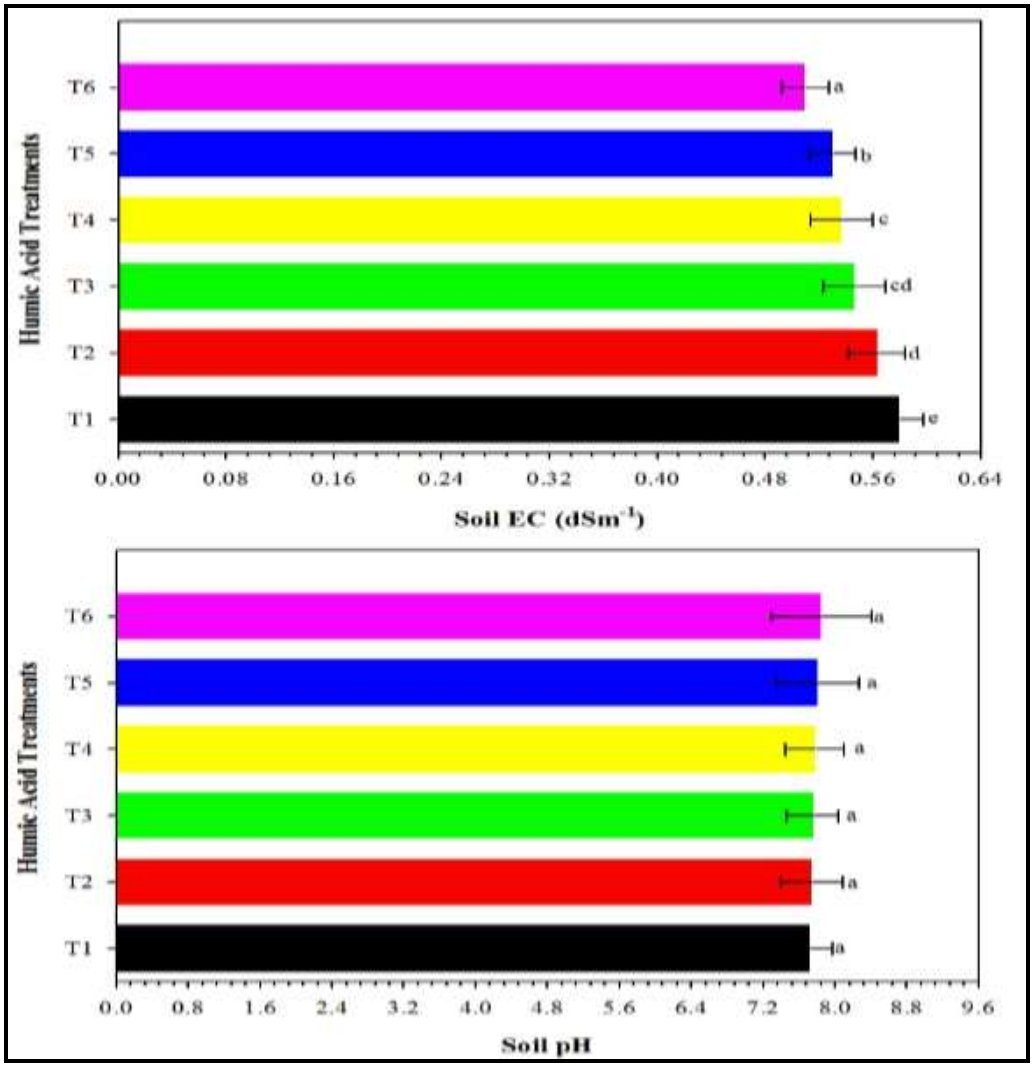

Figure1. Effect of application of humic acid on soil EC (dSm^{-1}) and pH after harvesting of crop of wheat crop. Vertical bar indicates the standard errors of triplicates and different alphabetic letters the significant difference ($p\leq0.05$).

Analysis result showed the non-significant different at both soil layers before sowing of seed (Table 1), whereas the some minor significant different among the EC and HA application were recorded after the harvesting of crop Figure 1. Though, mean maximum soil EC was recorded in T_6 followed $T_5>T_4>T_3$ and $>T_2$ (Figure 1) respectively. Increasing of soil EC (dsm^{-1}) in T_6 and T_5 may be due to increasing application of HA. The study finding were also in line with the findings of Muindi, 2019; Khan *et al*., 2018 that with increasing level of humic acid EC (dSm^{-1}) also increases.

Both before and after harvesting of crop the analysis results of both soil organic matter and

Nitrogen are presented (Table 1 and Figure 2). Result revealed that the application of HA could improve the organic matter in the soil and meanwhile improved soil properties particularly formation soil aggregation Kalhoro *et al.,* 2019, uptake of nutrients, germination and growth of plant. Statically the data are non-significant ($p<0.05$). Mean maximum and minimum were recorded in T_6 and T_1 respectively (0.82 and 0.57%), followed by T_2, T_3, T_4, and T_5 (0.58, 0.60, 0.76 and 0.79%) Figure 2. Thus finding reveals that addition of HA with in the rotation of crop can increase soil nutrient content and improve soil properties particularly soil OM Li *et al*., 2019. Furthermore; in case of the analysis of N-No^{-}_3, both before and after harvesting of the crop are non-significant ($p<0.05$), whereas; the significant differences among the application of HA were also recorded Figure 2. Mean minimum of N-NO^{-}_3 were recorded in T_1 (0.03-0.04mg kg^{-1}) and the maximum were recorded in T_6 & T_5 (0.077 & 0.075mgkg^{-1}) Figure 2. The study findings are in line with the findings of Marzadori, *et al*., 2000 study on the interaction of HA with urease activities. A few enzymes also have been discovered in HA, including urease being one of the most common and important for crop cultivation. Organic material boosts microbial population and several soil enzymatic activities such as urease, alkaline phosphatase, and glycosidase Maji *et al*., 2017

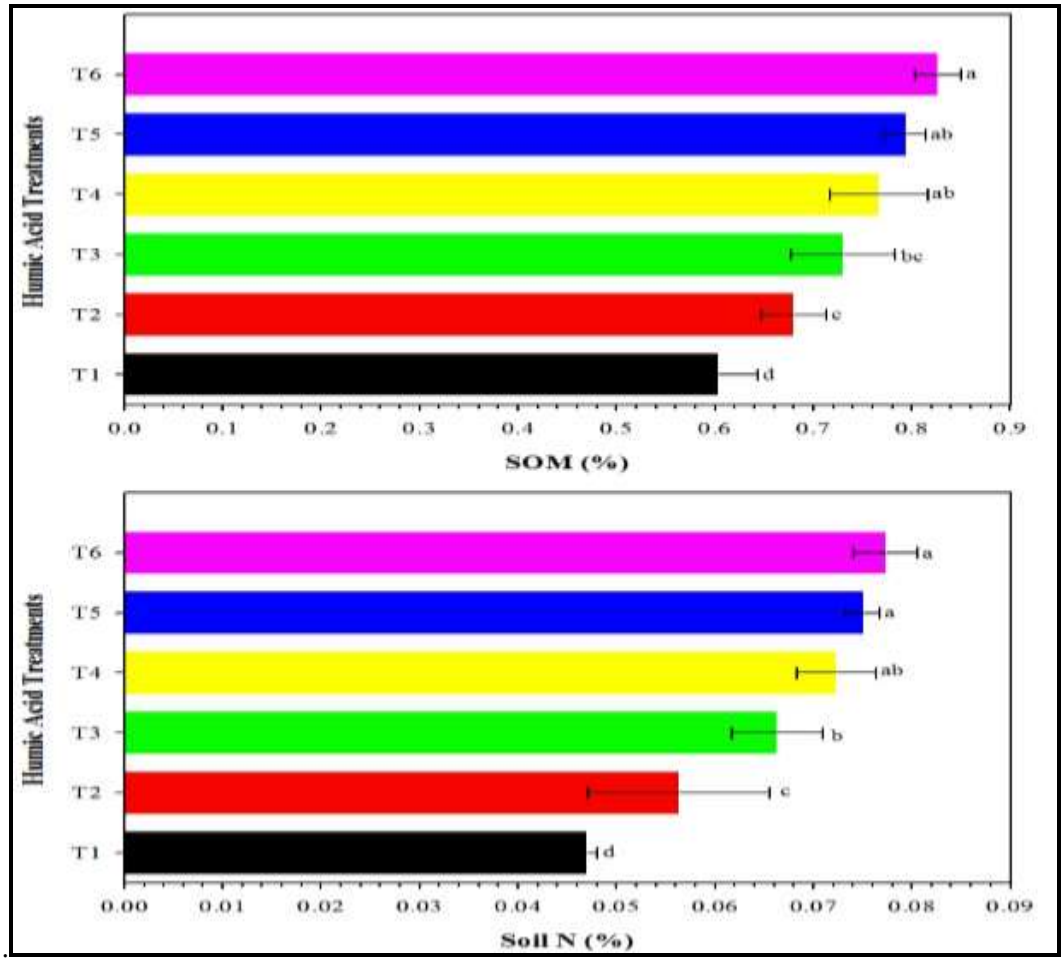

Figure 2. Effect of application of humic acid on soil organic matter (%) and Nitrogen (%) of soil samples after harvesting of wheat crop. Vertical bar indicates the standard errors of triplicates and different alphabetic letters the significant difference ($p\leq0.05$)

The analysis results of available phosphorous and potassium (mgkg^{-1}) of both before and after harvesting of crop are detailed in Figure 3. Statically data was non-significant ($p<0.05$) Furthermore; analysis results showed that available P in plant was improved after the application of humic acid (Figure 3). In comparison of p application of fertilizer and humic acid T_6 contain maximum amount of available P (4.06mgkg^{-1}) compared to other treatments Figure 3. Whereas; in-comparison of fertilizer and humic acid application, the improved available p was recorded with in humic acid treatments Figure 3. Overall the order was recorded as $T_1>T_2>T_3>T_4>T_5$ and $>T_6$ (Figure 3). Findings are in line with Khattak *et al*., 2013 conducted research on alkaline phosphate activity in saline soil incubated for two weeks with combine application of HA. Furthermore; analysis results of Potassium (K) both before sowing of seed and after harvesting of crop are presented in Figure 3. Statically data were non -significant ($p<0.05$). Minimum mean were recorded in control T_1 (83.0 mg kg^{-1}) compared to other treatments (T_2, T_3, T_4, T_5 and T_6) Figure 3. Though;

the significant difference was also recorded under different application of humic acid and K application Figure 3. Generally the order were recorded as $T_6>T_5>T_4>T_3>T_2$ and $>T_1$ (118.68, 112.33, 112.33, 105.83, 87.67 and 83.0mgkg^{-1}) respectively Figure 3. Additionally; enriched N&P nutrients with combine application of HA could improve chlorophyll concentration and significant impact on shoot growths Sible *et al*., 2021.

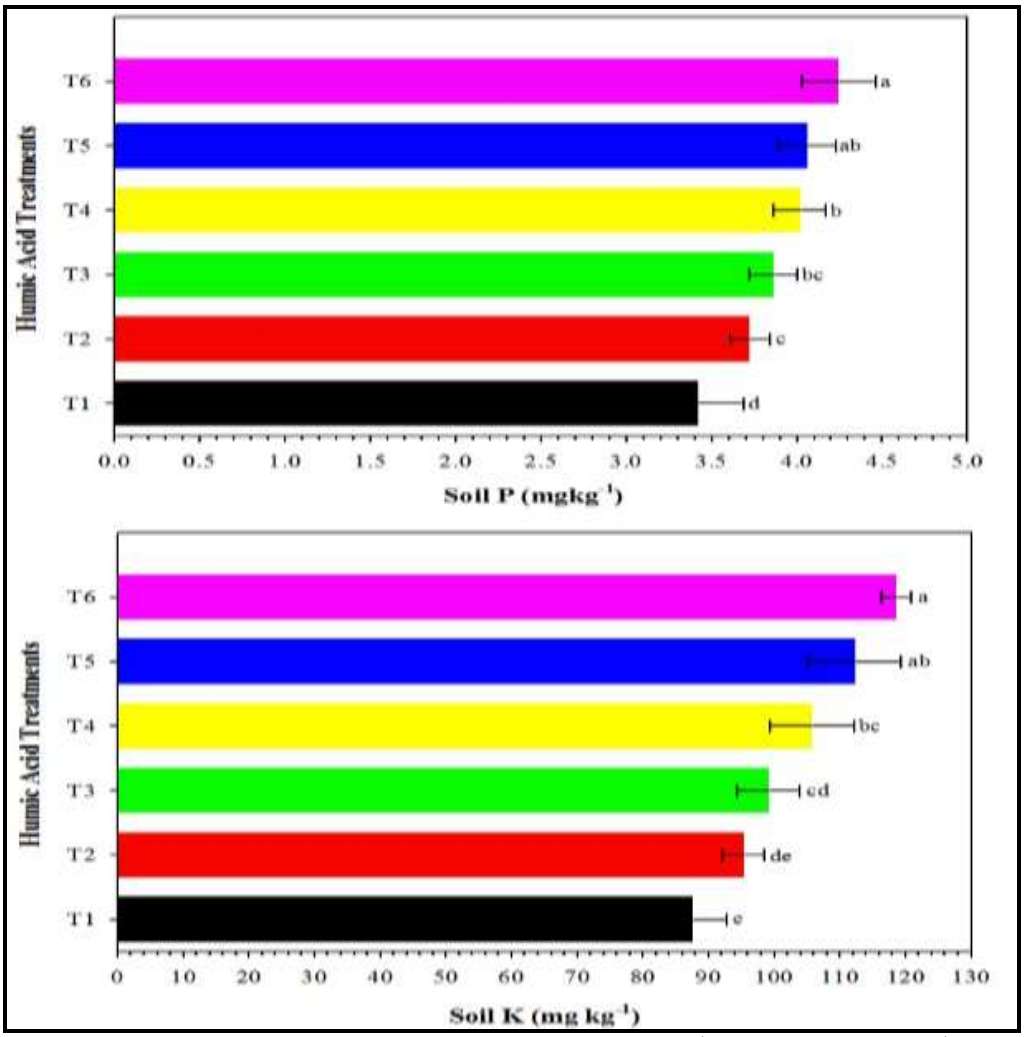

Figure 3. Effect of application of humic acid on available Phosphorous (mgkg^{-1}) and Potassium (mgkg^{-1}) of soil samples after harvesting of wheat crop. Vertical bar indicates the standard errors of triplicates and different alphabetic letters the significant difference ($p\leq0.05$)

Effect of Humic Acid on plant growth: Figure 4 presents the analysis results of plant height (cm) and leaf area (cm^2). Analysis results showed that a significant difference ($p<0.05$) among the treatments in both parameters. Mean maximum of plant height and leaf area were recorded in T_6 followed by T_5 Figure 4. Whereas the minimum was recorded in T_1 followed by T_2 (43.98cm) Figure 4. Overall the ascending order trend in both parameters was recorded as T_6, T_5, T_4, T_3, T_2 and T_1 respectively. Nasiroleslami *et al*., 2021 conducted a two years field study on combine application of urea and folair application of humic acid in Iran and reported that the different doses of humic acid significantly effect on spike/plant, root biomass, and biological yield of wheat.

In-comparison of both grainspike^{-1} and spike weight a significant different ($p<0.05$) among the treatments were recorded Figure 5. Mean maximum were recorded in T_6 and T_5 compared to T_4, T_3, T_2 and T_1. And whole ascending order was recorded as T_1, T_2, T_3, T4, T_5 and T_6 respectively. The finding of this study is in line with Anwar *et al*. 2021. 2014 conducted the experiments on humic acid fertilizers application, and concluded that applications of humic acid could improve the fertility and productivity of the crop. Furthermore, the indirect beneficial effect of humic acid on chlorophyll concentration could explain the higher number of grainspod^{-1}. Increased chlorophyll concentration stimulates photosynthetic activity, directing more photo-assimilates to a greater number of sinks Nordi *et al*., 2002. Additionally; Khan *et al*., 2018, Mohammed *et al*., 2019 reported that the combine application of HA with NPK fertilizations could improve the plant growth parameters. HA-rich zeolite rates did not have an effect on the agronomic performance of canola, but

urea and zeolite alone significantly increased agronomic parameters Bybordi and Ebrahimian, 2013.

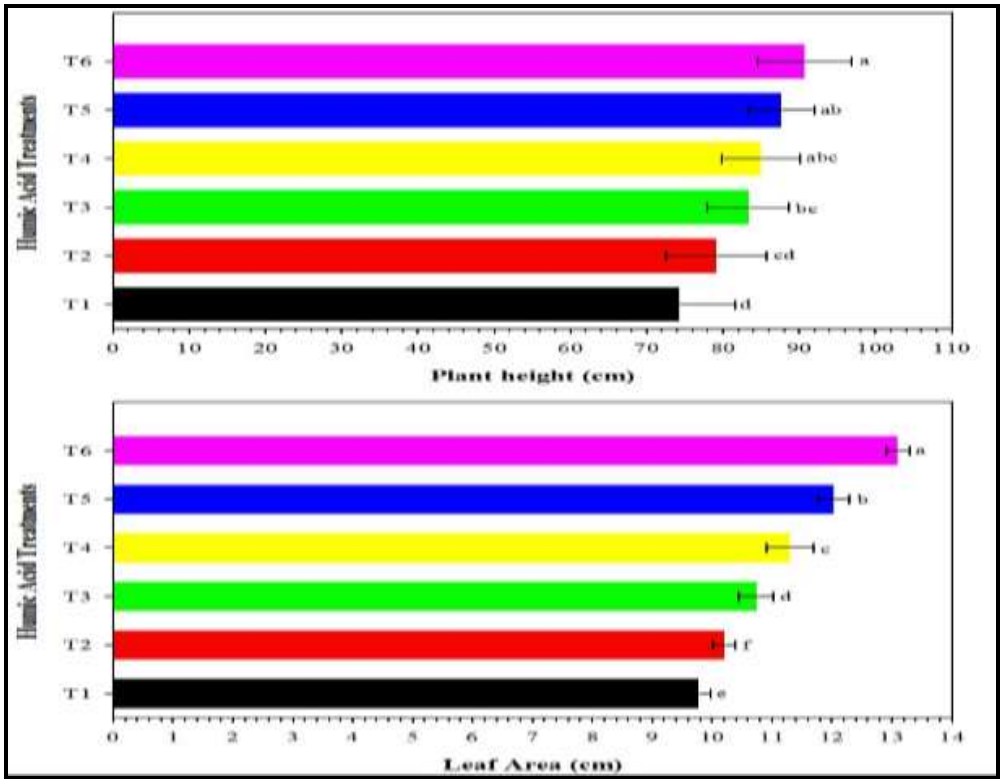

Figure 4: The mean of triplicates of plant height (cm) and leaf area (cm) under different applications of humic acid, vertical bar indicates the standard errors of triplicates and different alphabetic letters the significant difference ($p \leq 0.05$)

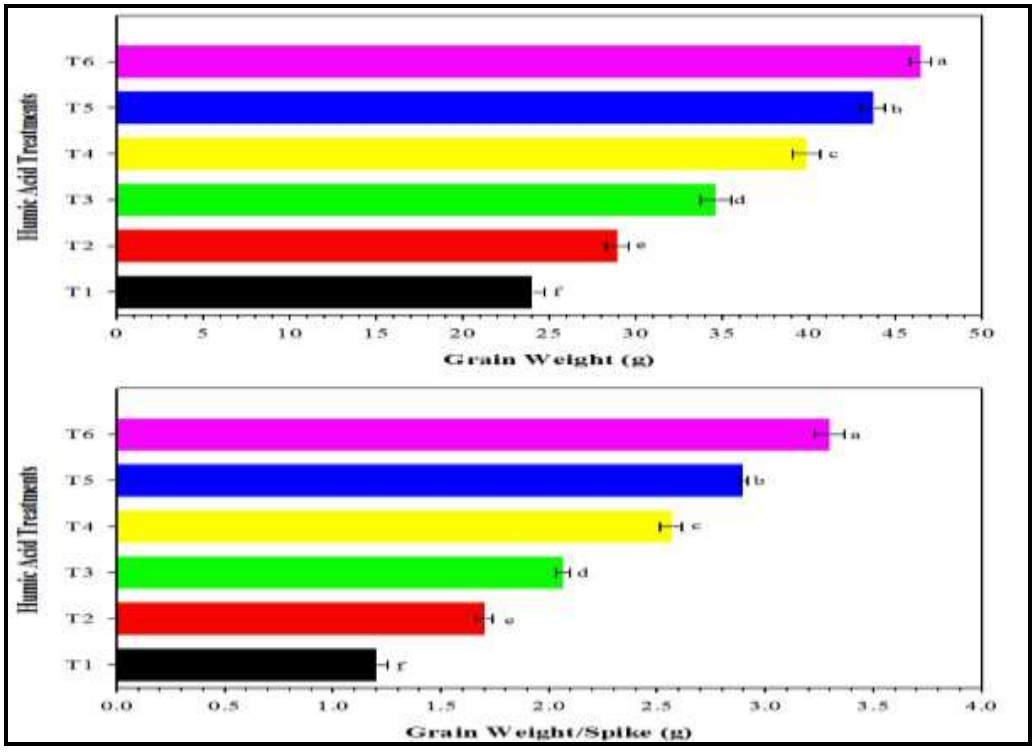

Figure 5: The mean of triplicates of grainspike^{-1} (g) and spike weight (g) under different applications of humic acid, vertical bar indicates the standard errors of triplicates and different alphabetic letters the significant difference ($p \leq 0.05$)

Effect of Humic acid on Biological and Grain Yield (kg ha^{-1}): Figure 6 present the analysis results of both biological yield and grain yield (kgha^{-1}). Statically the result of both analysis (biological yield and grain yield) was highly-significant ($p<0.05$) with in the treatments Figure 6. Mainly in-comparison of T_1, T_2 and T_5, T_6 Figure 6. Moreover grain yield mean maximum was recorded in T_6 (3933.3 kgha^{-1}) with in the maximum dose of fertilizer and humic acid applications Figure 6. Though; in-comparison of T_5 and T_6 a minor and statically non-significant different were recorded Figure 6. Conclusively it's also recorded that with increasing dose of humic acid grain yield also increased the similar findings were also recorded by Khan *et al*., 2021 and suggested that using humic acid since it increases grain yield in a number of crops such as wheat, aerobic rice, peanuts, mustered, Brassica raya, and barley. Furthermore, bigger yield components in the same plots might explain for the increased grain yield in 3 kgha^{-1} treated plots.

Statically the analysis results of biological yield are significant different ($p<0.05$) among the treatments Figure 6. The maximum mean were recorded in T_6 16110g followed by T_5 and T_4 plot^{-1}. Whereas; the lowest were recorded in control T_1 8933 and 15533g plot$^{-1.}$ Findings are consistent with the findings of Arjumend *et al*., 2015, who found that application of humic acid has a significant effect on wheat productivity and yields component such as 1000-grain weight, biological yield, dry matter yield, grain yield, and harvest index. Furthermore; HA150 and HA 200 regimes yielded and seems to have a concentration-dependent positive effect on yield and yield attributes. Lower HA concentrations/rates, such as 50 and 100 mg HAkg^{-1}, were shown to be less effective and yield was considerably lower when compared to higher rates, such as 150 and 200 mg Kg^{-1}.

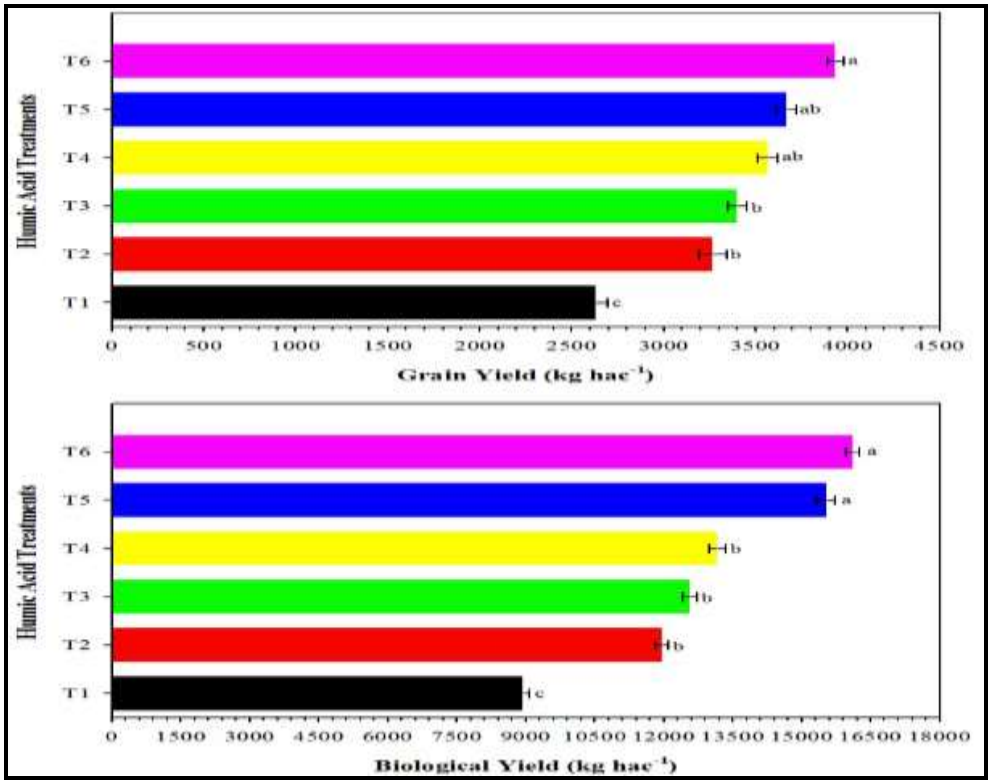

Figure 6. The mean of triplicates of grain yield (kgha^{-1}) and biological yield (kgha^{-1}) with effect of different applications of humic acid, vertical bar indicates the standard errors of triplicates and different alphabetic letters the significant difference ($p\leq0.05$)

Effect of Humic Acid Application on Nutrient Content in Plant: The analysis results of both N and P content in plant are present in Figure 7. The results revealed non-significant ($p<0.05$) among the treatment. Whereas; a significant difference among the control were recorded Figure 7. Positive indication the effect of different level of humic acid on availability of N & P content in plant. Overall the descending order among the treatments was recorded as T6<T5<T4<T3<T2 and <T1. sharma et al., 2013 also reported that different doses of both humic acid and N&P fertilizer also improve the availability of P in plant. Zhu et al., 2018, Ampong et al., 2022 and Sible et al., 2021 also conducted research studies on humic acid with combine application of fertilizers and reported that both combine application could improve the chlorophyll concentration and availability of N&P in plant.

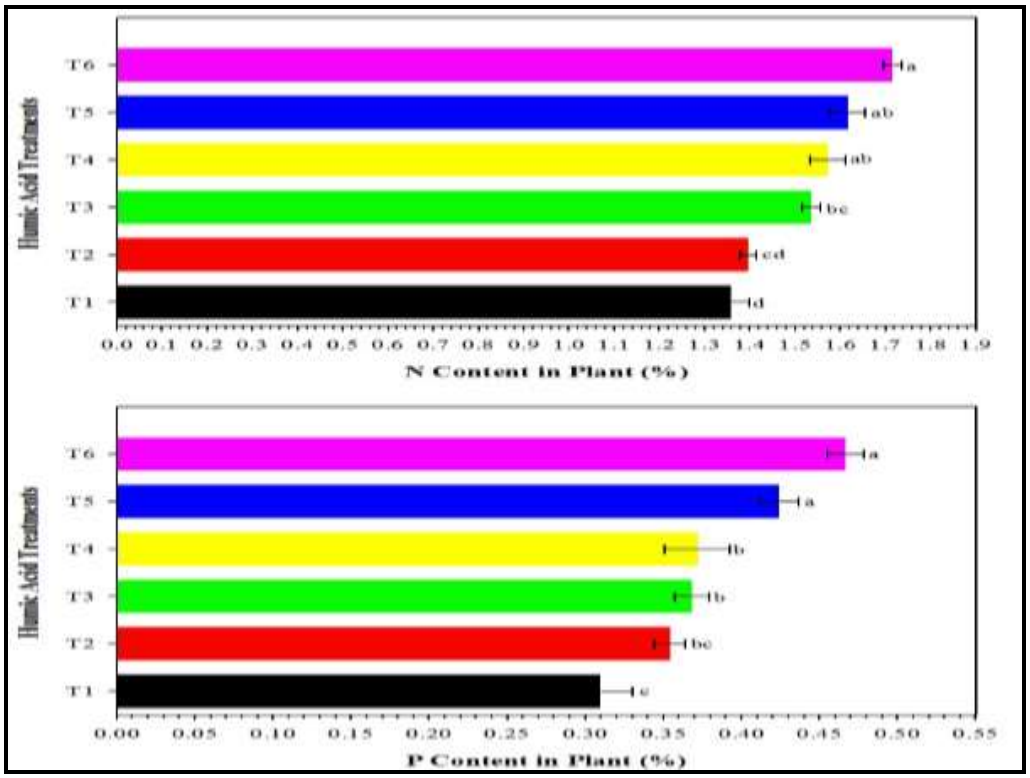

Figure7: The mean of triplicates of Nitrogen content in plant (%) and Phosphorous content in plant (%) with effect of different applications of humic acid, vertical bar indicates the standard errors of triplicates and different alphabetic letters the significant difference ($p \leq 0.05$)

Effect of humic acid on Root biomass: The analysis results of fresh root and dry root biomass are present in Figure 8. Statically both results revealed a significant ($p<0.05$) among the treatments, mean maximum of fresh root was (6.3667g) recorded in T_6 application of humic acid was @ rate of 14.8 Kg ha^{-1} (6.3667g) and T_5 12 Kg ha^{-1} (5.6000g) respectively. Though, minimum mean value (3.2333g) of fresh root was recorded in control Figure 8. On the other side same type of findings was also recorded in dry root biomass mean maximum were recorded in T_6 (3.62g) compared to others Figure 8. Whereas; the lowest both frsh and dry root biomass (3.2333 and 1.75g) was recorded in control Figure 8, Fatima *et al*., 2021 reported that the use of humic acid has the potential to improve plant and root development. In-comparison of all treatments T_1, T_2, T_3, T_4, T_5 and T_6 significant results were recorded in T_6 and T_5 compared to T_1, T_2, T_3, and T_4. The combine application of humic acid and fertilizer increase the root formation which support the plant to deeply uptake of water and nutrients (Ampong *et al*., 2022, Olaetxea *et al*., 2020). Deeply rooted plant are also maintain the water availability of the plant Kalhoro *et al*., 2018. Though, the roots of the plant is not deeply expanded the, the shallow stored water is limited and in results the losses of the crop yield Kalhoro *et al*., 2017. Some previous studies indicated that the combine application of humic acid and N&P fertlizers has been stimulate the growth of roots and shoots with progress in production Olaetxea *et al*., 2020. With improving of growth hormones and enzymes i.e auxin and cytokin chlorophyll concentration, root/shoot growth, photosynthesis rate and grain yield also increase with application of humic acid Bybordi and Ebrahimian, 2013. Humic acid derived from organic waste could significant effect on the agronomic parameters including leaf chlorophyll content, root/shoot weight, thousand grain weight, biological yield and grain yield of wheat. The application of HA derived from organic waste significantly increased agronomic parameters of chrysanthemum including the chlorophyll content, leaf area, and root and shoot dry weight (Fan *et al*., 2014, Arjumend *et al*., 2015). Consequently, during agricultural production in a semi-arid area, choosing wheat cultivars of different resistance in different quantities of nitrogen fertilizer to benefit the root distribution and absorbing ability that can improve wheat photosynthetic response and productivity.

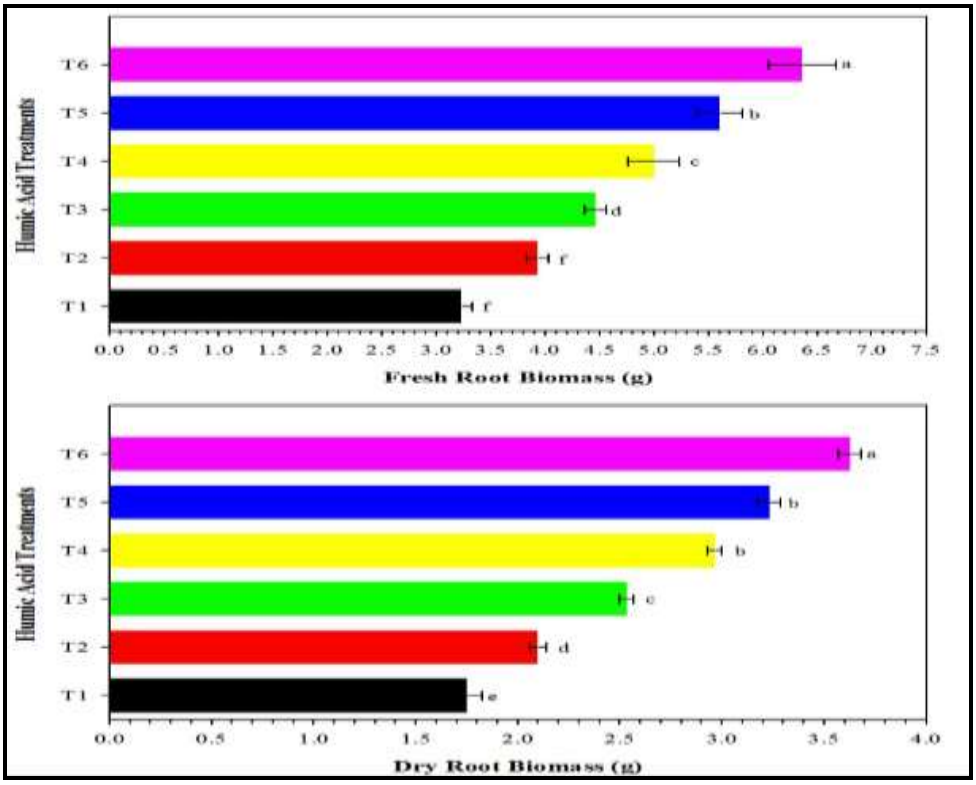

Figure 8: The mean of triplicates of Fresh root biomass (g) and Dry root biomass (g) content of wheat crop with effect of different applications of humic acid, vertical bar indicates the standard errors of triplicates and different alphabetic letters the significant difference ($p \leq 0.05$)

Table 2: Effect of humic acid application on yield parameters of wheat

Humic acid dose Kg ha $^{-1}$	**CV**	**LSD**	**p< value**
Grain /spike (g)	3.00	1.9803	0.0000
Grain yield (Kg ha^{1})	7.88	488.91	0.0026
Plant height (cm)	3.97	7.0033	0.0015
Spike weight (g)	6.81	0.2851	0.0000
Leaf area (cm^2)	2.36	0.4798	0.0000
Biological yield (Kg ha^{-1})	7.80	1851.8	0.0001
Nitrogen in plant	5.59	0.1560	0.0038
Phosphorous in plant	6.77	0.0471	0.0003
Fresh root (g)	4.42	0.3831	0.0000
Dry root (g)	6.41	0.3152	0.0000

Note: The reported values in the table represent the variability including the coefficient of variation (CV), least significant difference (LSD), and p-values of growth and yield parameters of wheat (*Triticum Aestivum*)

Conclusion

On the basis of study observation the study indicated that with increasing application of humic acid along with the recommended dose of NP fertilizations could significantly effect on the soil properties particularly on plant growth parameters such as plant height (cm), leaf area (cm), spike length (cm), 1000 grain weight (g), biological yield (kgha^{-1}) and grain yield (kgha^{-1}). Before and after harvesting of soil physico-chemical properties are also significantly improved with the application of humic acid. On the other side; the increase of soil EC (dSm^{-1}) and pH also increase and possibly could be improve with the application of farm yard manure. Consequently; future study could be conducted on humic acid with combine application of different farm yard manures to evaluate soil aggregate and aggregate stable that play a major role to retain soil moisture, uptake of nutrients and that effect on the productivity of the cultivated crop.

Author's Contributions

Siraj Ahmed-Conducted research study and organized 1st draft, Shahmir Ali Kalhoro- over all supervision, organized research study and edit article, Bilal Ahmed-field work, Kashif Ali Kubar-review article, Mehar un Nisa Narejo-review and edit article, Qamar Sarafraz-statically data analysis, Muneer Ahmed Baloch-field work, Khalid Hameed Mengal-soil sampling and preparation, Shabir Ahmed-field and lab, Sami ullah-edit revision and suggestion, field & lab

Conflict of Interest

All authors certify that no conflict of interest exists, and that they have no financial arrangement with any company whose product figures prominently in the submitted manuscript or with a company making a competing product.

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