Research Article

Soil Humic Acid Supplementation and Nitrogen Management Positively Regulate the Growth and Yield of Wheat

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Abstract | Humic acid (HA) and nitrogen (N) promotes greater physiological activity in plants. A field trial was conducted to study the effects of HA and N treatments on wheat growth and yield. A randomized complete block design with a split-plot arrangement was applied having 4 levels of HA as main plot and 3 levels of N as sub-plot, with three replications. HA levels (0, 5, 10, 15 kg ha^{-1}) were applied at sowing, while N levels (90, 120, 150 kg ha^{-1}) were applied using three split application methods. The control plots received no HA or N application. The results showed that the plots treated with 10 kg HA ha^{-1} had the highest plant height, grain yield, and harvest index, however, leaf area tiller^{-1}, thousand-grain weight, and biological yield were found higher using 15 kg HA ha^{-1}, but they were statistically at par with 10 kg HA ha^{-1}. Similarly, all these parameters including days to anthesis and physiological maturity, tillers m^{-2}, leaf area index, and yield and yield contributing parameters were observed higher applying 150 kg N ha^{-1} in 3-splits in which tillers m^{-2}, productive tillers m^{-2}, and leaf area tiller^{-1} were statistically at par using N in 2-splits. Conclusively, the application of 10 kg HA ha^{-1} and 150 kg N ha^{-1} in 3-splits were found to be more effective in enhancing wheat production in a profitable manner.

Received | July 08, 2025; **Accepted** | August 03, 2025; **Published** | November 24, 2025
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Citation | Khattak, W.A. and S. Anwar. 2025. Soil humic acid supplementation and nitrogen management positively regulate the growth and yield of wheat. *Journal of Plant Health, Ecology and Evolution,* 01(1): 50-62.
DOI | https://dx.doi.org/10.17582/journal.jphee/2025/01.1.50.62
Keywords | Humic acid, Harvest index, Nitrogen, Growth, Yield, Wheat

Introduction

In agricultural production, various organic materials such as compost, green manures, kitchen wastes, humic acids (HA), etc., are used as soil amendments or soil conditioners (Antonious, 2016). Researchers worldwide try to select sustainable organic sources for soil improvement to increase crop yields (Tahat *et al.*, 2020; Hou *et al.*, 2020). Some researchers recommend humic substances, as the best soil amendments (Kulikova *et al.*, 2021; Farid *et al.*, 2021). Humic acid has proven its significant effectiveness in increasing total crop yield, improving crop quality, resistance to stress, reducing soil pollution, and improving soil physicochemical properties. HA supplementation in small amounts benefits both the soil and the plants. It reduces salinity and drought and improves seed germination, growth, and development (Zhang *et *al*., 2017). HA application to soil also increases the microbial population and improves soil organic matter (Li *et al*. 2019). The HA and trace elements in HA form a complex compound or chelate which dissolves easily and can be readily absorbed by plants, which is helpful for the root system and leaf surface to absorb trace elements (Ampong *et al*., 2022). Humic substances are also believed to contain small amounts of nitrogen (N) in a very stable form that serves as a slow-release N fertilizer. The application of HA alone or in combination with inorganic compound fertilizers was found to be the most economical method for achieving optimum wheat production under rainfed conditions (Khan *et al*., 2015). The HA application significantly affected the initial growth stages of wheat cultivars (Mutlu and Tas, 2022). Moreover, foliar application of HA has limited growth, yield, and quality-enhancing effect on wheat crops compared to split soil application of nitrogenous fertilizers (Zhang *et al*., 2017).

N is one of the limiting essential plant nutrients. Its leaching causes its unavailability to plants. Applying N fertilizer in the form of splits to wheat crops increases its production and quality. Split application of inorganic N-fertilizers not only increases N availability to wheat plants but also increase grain weight and quality more than sole applications (Belete *et al*., 2018). Higher wheat yields can be obtained when N is applied in three equal splits at a rate of 120 kg ha^{-1} rather than in its sole application (Khan *et al*., 2022). A split dose (50 kg N ha^{-1}) of fertilizer application at the sowing and vegetative stage or the vegetative and booting stages significantly increases the number of productive tillers per unit area (Islam and Nahar, 2014). N is essential to chlorophyll, proteins, hormones, nucleotides, enzymes, and vitamins. Application of nitrogen fertilizers improves chlorophyll content, protein level, and protoplasm formation, influencing cell size, leaf area, and photosynthetic activity. Wheat yield can be managed by applying N-fertilizers in full and split applications (Khan *et al*., 2015). N fertilizers and organic fertilizers show a positive interaction (Kong *et al*., 2022).

Wheat (*Triticum aestivum* L.), from the Poaceae family, is a long-day, annual, self-pollinated plant grown in winter. Wheat is a fundamental source of carbohydrate containing gluten, increasing its demand for baking products. Besides, wheat straw is also used as feed, and it is estimated that 5-10% of the wheat grain is consumed as poultry and livestock feed (Bagheri *et al*., 2021). Wheat productivity depends on a range of factors, including soil, water, climate, socioeconomic factors, farm management, and agronomic factors such as quality, quantity, and timing of input use (seed, fertilizers, weedicides, labor, etc.). To increase its production, balanced fertilizers and agronomic measures are required to meet the needs of the present growing population. Considering the importance of HA as a soil amendment, and N fertilizer rate and its application method, the present experiment was conducted to investigate the suitable level of HA and N application rate with appropriate N application method and their mutual interaction for higher growth and yield components of spring wheat grown in arid and semi-arid regions of the world.

Materials and Methods

Experimental layout

Field trials was conducted at the Agronomy Research Farm of the University of Agriculture, Peshawar, a relatively semi-arid to arid region of Pakistan. The selection of site was done prior on the basis of previous exhaustive crops such as maize (*Zea mays*) and sesame (*Sesamum indicum*). The experiment was laid out in a randomized complete block design with a split-plot arrangement having three replications. The treatments were designed as Factor (A): HA levels (main plot) having four levels, i.e., HA_0 (0 kg ha^{-1}), HA_5 (5 kg ha^{-1}), HA_{10} (10 kg ha^{-1}), and HA_{15} (15 kg ha^{-1}); Factor (B): N levels (sub-plot) having three levels, i.e., N_{90} (90 kg ha^{-1}), N_{120} (120 kg ha^{-1}), N_{150} (150 kg ha^{-1}); and Factor (C): Nitrogen Application methods (sub-plot) i.e., M_1 {Full dose (at sowing)}, M_2 {two splits (the half at sowing + the half at tillering)}, M_3 {three splits (1/3 at sowing + 1/3 at tillering + 1/3 at anthesis)}. HA levels were applied all at sowing, and a control plot with no N was maintained in the subplot treatment. The subplot size was 4.5 m^2 with a row-to-row distance of 25 cm having six rows. Urea was applied as a source of N. The treatments applied are presented in Table 1. The composite soil samples (n=5) of both sides contained 54.63g kg^{-1} organic matter, 20.12 g kg^{-1} potassium, 6.10 mg kg^{-1} ammonium N, and 35.14 mg kg^{-1} nitrate N. The soil of the study sites was loam to sandy loam. Both HA and N levels were designed as per the soil chemical properties and crop requirements in both study sites. After the application of the above-mentioned study treatments, agronomic practices such as weeding, hoeing, irrigation, etc. were thoroughly practiced when needed.

Table 1: *Distribution of the application of treatments to the experimental field of wheat plants.*

Nitrogen levels ($kg\ ha^{-1}$)	Sowing	Till-ering	An-thesis	Humic acid levels ($kg\ ha^{-1}$)
0	-	-	-	(0, 5, 10, 15) Soil application, all at sowing
90-Full dose (M_1)	90	-	-	
1/2 dose (M_2)	45	45	-	
1/3 dose (M_3)	30	30	30	
120-Full dose (M_1)	120	-	-	
1/2 dose (M_2)	60	60	-	
1/3 dose (M_3)	40	40	40	
150-Full dose (M_1)	150	-	-	
1/2 dose (M_2)	75	75	-	
1/3 dose (M_3)	50	50	50	

Morphophysiological indices

Data concerning days to emergence was recorded by counting the number of days from sowing to date when 80% of the seedlings emerged. Emergence m^{-2} data was recorded by counting the number of plants that emerged in one-meter row length at three randomly selected rows in each subplot and then converted to emergence m^{-2} using the following formula:

$$\text{Emergence } m^{-2} = \frac{\text{No. of seedling counted}}{\text{Row} - \text{row distance (m) x Row length (m) x No. of rows}} \text{x1}$$

Data on days to anthesis was recorded by counting days from the date of sowing till the date of completion of 80% spikes in each subplot. Days to physiological maturity were recorded by counting the days from the sowing date to when 50% of plants became mature in each subplot. Ten plants were randomly selected for the height of each subplot at physiological maturity and then averaged. In each subplot, three rows of one-meter length were selected randomly to count tillers m^{-2}. Counted tillers were converted to tillers m^{-2} accordingly.

The data on the number of productive tillers m^{-2} was recorded by counting the productive tiller in a one-meter-long row at three randomly selected places in each subplot and was converted into m^{-2} area by using the following formula:

$$\text{Productive tillers } m^{-2} = \frac{\text{Total productive tillers counted}}{\text{Row} - \text{row distance (m) x Row length (m) x No. of rows}} \text{x1}$$

After recording the number of leaves $tiller^{-1}$, the leaf area of all leaves was measured, and leaf area $tiller^{-1}$ was calculated:

$$\textit{Leaf area tiller}^{-1}\ (cm^2) = \textit{Leaf area of all leaves/ Number of tillers}$$

The leaf area index at the anthesis stage in each subplot was calculated by substituting the values in the given formula:

$$\textit{Leaf area index} = (\textit{Leaf area tiller}^{-1}) \times (\textit{Number of tillers } m^{-2})$$

Biological and grain yield, and harvest index

Grains of ten randomly selected spikes from each subplot were counted and then averaged for the data regarding grain $spike^{-1}$. From each subplot's grain lot, a thousand grains were taken and weighed to record thousand-grain weight (g). The biological yield was recorded by harvesting four central rows in each subplot, sun-dried, weighed, and converted into $kg\ ha^{-1}$ by using the following formula:

$$\text{Biological yield} (kg\ ha^{-1}) = \frac{\text{Biological yield in four central rows}}{\text{Row} - \text{row distance (m) x Row length (m) x No. of rows}} \text{x10000}$$

The four harvested central rows were dried, threshed, cleaned, and weighed for grain yield. The total grain yield was converted into $kg\ ha^{-1}$ using the following formula:

$$\text{Grain yield} (kg\ ha^{-1}) = \frac{\text{Grain yield in four central rows}}{\text{Row} - \text{row distance (m) x Row length (m) x No. of rows}} \text{x10000}$$

The harvest index was calculated by using the following formula:

$$\textit{Harvest index (\%)} = \textit{Grain yield/ Biological yield} \times 100$$

Statistical analysis

The data was statistically analyzed using the procedure appropriate for randomized complete block (RCB) design with a split-plot arrangement. Means were compared using the least significant difference (LSD) test at a 5% probability level when F-values were significant. Both site's experimental data were analyzed separately for all the studied parameters, however, the data was insignificant between the sites. Therefore, data were averaged and analyzed using a t-test. The correlation analysis was performed using the Pearson Correlation Method, and the heatmap illustration was drawn using TBtools (https://github.

com/CJ-Chen/TBtools) to represent the strength of the relationship of the mean data of all the studied parameters was generated using Circos (http://mkweb.bcgsc.ca/tableviewer/). The data analyzed is represented in the Results section.

Results

Days to emergence and emergence m^{-2}

Data regarding days to emergence and emergence m^{-2} is shown in Table 2. Analysis of variance showed that humic acid levels (HA), nitrogen levels (N), nitrogen application methods (M), and their possible interactions i.e., NxM, HAxN, HAxM, and HAxNxM were found not significant. However, higher emergence of wheat seedlings was noticed in N_{120} using the M_3 method with no HA application (Table 3).

Days to anthesis and physiological maturity

Statistical analysis of the data revealed that days to anthesis and physiological maturity were significantly affected by HA, N, M, N x M, and HA x N.

Table 2: *ANOVA of days to emergence (DE), emergence rate (E), days to anthesis (DA), days to physiological maturity (DPM), and plant height (PH). Here, HA: Humic Acid, N: Nitrogen Levels, and M: Nitrogen application methods.*

		DE		E (m^{-2})		DA		DPM		PH (cm)	
SOV	**df**	**F-ratio**	**P**	**F-ratio**	**P**	**F-ratio**	**P**	**F-ratio**	**P**	**F-ratio**	**P**
Replication	2	3.08	0.12	4.79	0.057	4.01	0.078	3.75	0.088	1.81	0.242
HA	3	0.46	0.72	0.47	0.716	190.79	0.000	183.48	0.000	4.08	0.067
Error I	6								0.000		
Treatment	9	1.19	0.316	2.17	0.034	95.96	0.000	123.51	0.000	14.74	0.000
Control vs. rest	1	0.41	0.523	5.58	0.021	297.27	0.000	374.47	0.000	48.79	0.000
N	2	0.6	0.552	2.99	0.057	170.04	0.000	229.76	0.000	3.86	0.025
M	2	0.6	0.552	1.69	0.192	108.09	0.000	131.95	0.000	32.74	0.000
N x M	4	1.97	0.108	1.15	0.342	2.54	0.047	3.42	0.013	2.67	0.039
HA x T	27	1.49	0.094	0.67	0.879	2.08	0.007	2.03	0.009	1.07	0.394
HA x N	6	1.59	0.162	0.77	0.595	4.71	0.000	3.39	0.005	1.87	0.097
HA x M	6	0.9	0.496	0.2	0.974	1.75	0.122	2.19	0.053	0.55	0.772
HA x N x M	12	1.48	0.153	0.81	0.643	0.37	0.971	0.7	0.744	1.05	0.412
HA x Control vs. rest	3	2.48	0.068	0.83	0.48	4.33	0.007	4.33	0.007	0.61	0.609
Error II	72										
Total	119										

Table 3: *Mean data of emergence rate (E/m^{-2}), days to anthesis (DA), days to physiological maturity (DPM), plant height (PH), number of tillers (NT), productive tillers (PT), leaf area tiller^{-1} (LAT), leaf area index (LAI), grains spike^{-1} (GS), thousand-grain weight (TGW), biological yield (BY), grain yield (GY), and harvest index (HI).*

		E/m^{-2}	**DA**	**DPM**	**PH (cm)**	**NT (m^{-2})**	**PT (m^{-2})**	**LAT**	**LAI**	**GS**	**TGW**	**BY**	**GY**	**HI**
Humic acid (kg ha^{-1})	HA_0	92	124 d	162 d	87.2 c	288	273	105.70 c	3.05	46	42.30 b	8397 b	2911 c	34.61 c
	HA_5	90	125 c	164 c	92.4 b	304	290	109.85 bc	3.35	46.48	44.30 a	8773 b	3228 b	36.67 b
	HA_{10}	90	128 b	166 b	96.8 a	304	290	111.32 ab	3.39	46.22	44.85 a	9338 a	3544 a	38.05 a
	HA_{15}	90	129 a	167 a	94.4 ab	298	283	114.43 a	3.41	47.67	45.04 a	9362 a	3332 b	35.53 bc
Nitrogen application method	M_1	91	125 c	164 c	91.0 c	288 b	274 b	107.78 b	3.11 b	44.83 c	43.22 b	8519 b	2959 c	34.81 b
	M_2	90	127 b	165 b	92.5 b	307 a	292 a	111.97 a	3.45 a	46.33 b	43.86 b	9103 a	3326 b	36.39 a
	M_3	91	128 a	166 a	94.6 a	300 a	286 a	111.22 a	3.35 a	48.61 a	45.28 a	9280 a	3477 a	37.44 a
Nitrogen (kg ha^{-1})	90	89	125 c	163 c	90.7 c	284 b	269 b	100.32 c	2.85 c	44.44 c	42.22 c	8580 c	2975 c	34.70 b
	120	92	127 b	165 b	93.1 b	303 a	288 a	111.57 b	3.38 b	46.97 b	43.25 b	8940 b	3289 b	36.73 a
	150	91	128 a	167 a	94.3 a	309 a	294 a	119.08 a	3.68 a	48.36 a	46.89 a	9382 a	3498 a	37.21 a

However, HAxM and HAxNxM were found not significant. Control vs. rest and its interaction with HA was found significant for both the studied parameters (Table 2). With increasing HA levels, days to anthesis increased significantly (Table 3). More days to anthesis (4%) were observed in the plots using HA_{15} compared to HA_0. Days to anthesis were also increased with an increasing rate of nitrogen from 90 to 150 kg ha^{-1} i.e., 125 days to 128 days. Similarly, the application of nitrogen in splits significantly affected days to anthesis. Nitrogen applied in splits showed a linear increase for days to anthesis, recorded 3% more for rest treatments over control (no nitrogen). Interactive response of N and M (NxM) showed more days to anthesis (129) in N_{150} applied as M_3. For HAxN, more days to anthesis (132) were recorded in HA_{15} applied with N_{150} using the M_3 method. Similarly, days to physiological maturity were observed more (159) in the plots treated with HA_{15} compared to HA_0. Days to physiological maturity also increased linearly with an increasing rate of nitrogen from 90 to 150 kg ha^{-1} (155 to 159). More (158) days to physiological maturity were recorded in the M_3 application method compared to the M_1 (156) application method. Rest plots had more (157) days to physiological maturity than control (153) plots. In interaction, NxM showed more days to physiological maturity (160) in plots with N_{150} applied as M_3 and HAxN showed more days to physiological maturity (162) using HA_{15} with N_{150}.

Plant height

Data concerning plant height is given in Table 3. Statistical analysis of the data revealed that the plant height of wheat was significantly affected by HA, N, and M, however, their possible interactions except NxM were recorded as not significant. Control vs. rest was found significant also, while its interaction with HA was not significant (Table 2). Higher plant heights (96.8 cm) and lower (87.2 cm) were recorded in HA_0 and HA_{10}, respectively, which were statistically similar (94.4 cm) to HA_{15}. Nitrogen applied at the rate of 150 kg ha^{-1} showed greater values (94.3 cm), while lower values (90.7 cm) were recorded in 90 kg N ha^{-1} treated plots. Plant height was higher (94.6 cm) using nitrogen in three splits (M_3), while lower (91 cm) at full dose (M_1). Control plots showed lower (86.6 cm) plant height in comparison to the rest (92.7 cm). Interaction of N and M showed higher (97.2 cm) plant height for N_{150} applied as M_3, while lower (90 cm) was recorded in N_{90} using the M_1 method. The interaction HAxNxM was found insignificant but a higher (102.2 cm) value of plant height was noticed treating HA_{10} combinedly with N_{150} using the M_3 method.

Number of tillers m^{-2}

Data regarding the number of tillers m^{-2} are given in Table 3. A perusal of the data showed that N, M, and HAxN significantly affected the number of tillers m^{-2}, while no significant results were found for HA, NxM, HAxM, and HAxNxM. Similarly, Control vs. rest was found significant, but its interaction with HA was insignificant (Table 4). Plots treated with 120 and 150 kg N ha^{-1} had a higher (303 and 309) number of tillers m^{-2}, while 90 kg N ha^{-1} treatments had a lower (288) number of tillers m^{-2}. Nitrogen application as M_2 and M_3 resulted in a higher (307 and 300) number of tillers m^{-2} which was statistically similar when applying N as M_1 (253). The interaction between HA and N also affected the number of tillers m^{-2}. In plots that received 10 kg HA ha^{-1} with 120 kg N ha^{-1}, more (326) tillers m^{-2} were recorded. The number of tillers m^{-2} was recorded more (298) for rest plots and less (236) for control plots. However, the three-way interaction, i.e., HAxNxM, was not significant, but a greater value (350) was observed in plots without HA and 150 kg N ha^{-1} applied in two splits.

Number of productive tillers m^{-2}

Data on the number of productive tillers m^{-2} are reported in Table 4. Data analysis indicated that N and M significantly affected the number of productive tillers m^{-2}, while HA was insignificant. Similarly, all possible interactions except HAxN were recorded as not significant. Control vs. rest was significant but found non-significant for its interaction with HA (Table 4). Nitrogen application resulted in a linear increase in productive tillers m^{-2}. Nitrogen applied at the rate of 120 kg ha^{-1} and 150 kg ha^{-1} showed insignificant results (288 and 294), which were higher than those plots treated with 90 kg N ha^{-1} (269). Similarly, nitrogen applied in two and three splits showed higher (292 and 286) productive tillers m^{-2} which were statistically at par with each other, but less (274) productive tillers m^{-2} were recorded for its application using the M_1 method. Rest plots produced more (284) productive tillers m^{-2} than control (222). Interaction HAxN also significantly affected productive tillers m^{-2}. Plots treated with HA_{10} and N_{120} produced more (313) productive tillers m^{-2}, respectively.

Table 4: *ANOVA of the number of tillers (NT), productive tillers (PT), leaf area tiller^{-1} (LAT), and leaf area index (LAI). Here, HA: Humic Acid, N: Nitrogen Levels, and M: Nitrogen application methods.*

	df	NT (m^{-2})		PT (m^{-2})		LAT		LAI	
		F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Replication	2	1.49	0.298	1.16	0.376	0.33	0.732	0.93	0.444
HA	3	0.92	0.484	0.67	0.601	0.78	0.546	0.11	0.954
Error I	6								
Treatment	9	11.71	0.000	11.96	0.000	7.41	0.000	10.29	0.000
Control vs. rest	1	71.39	0.000	72.59	0.000	36.57	0.000	57.73	0.000
N	2	10.4	0.000	10.81	0.000	5.17	0.008	8.87	0.000
M	2	5.32	0.007	4.87	0.01	4.39	0.016	7.54	0.001
N x M	4	0.65	0.632	0.93	0.452	2.76	0.034	0.51	0.728
HA x T	27	1.81	0.025	1.84	0.021	1.7	0.039	1.04	0.435
HA x N	6	4.13	0.001	3.99	0.002	0.69	0.655	1.35	0.246
HA x M	6	0.59	0.736	0.78	0.589	3.72	0.003	0.99	0.438
HA x N x M	12	1.57	0.119	1.64	0.099	1.59	0.115	1.08	0.39
HA x Control vs. rest	3	0.52	0.672	0.48	0.698	0.11	0.954	0.34	0.799
Error II	72								
Total	119								

Leaf area tiller^{-1}

Statistical analysis of the leaf area tiller^{-1} data revealed significant results for HA, N, M, and NxM. Humic acid interactions with N, M, and Control vs. rest were insignificant, however, Control vs. rest alone was found significant (Table 4). Expanded (114.43) leaf area tiller^{-1} was recorded using HA at the rate of 15 kg ha^{-1}, while lesser (105.70) leaf area tiller^{-1} was recorded at HA_0. Leaf area tiller^{-1} increased with the increase in N levels from 90 to 150 kg ha^{-1}. Leaf area tiller^{-1} was recorded higher (119.08) in N_{150}, while nitrogen applied at 90 kg ha^{-1} showed lesser (100.32) leaf area tiller^{-1}. The application of nitrogen as M_2 and M_3 showed greater leaf area tiller^{-1} (111.97 and 111.22) than less (107.78) in the M_1. Leaf area tiller^{-1} was recorded more (110.32) for rest plots than control (80.24). Interaction NxM responded higher (121.05) at 150 kg N ha^{-1} in three splits, while it was lower (98.17) at 90 kg N ha^{-1} in three splits.

Leaf area index

Data concerning the leaf area index are presented in Table 3. Data analysis showed significant results for N and M. HA and all possible interactions between HA, N, and M were found to be non-significant except HAxN. Control vs. rest was also found significant (Table 4). As the amount of N increased, the leaf area index increased linearly, resulting in significant differences between the other levels (Table 3). Nitrogen applied at the rate of 150 kg ha^{-1} increased the leaf area index (3.68), while nitrogen applied at 90 kg ha^{-1} showed lower results (2.85). Nitrogen applied as M_2 and M_3 showed a higher leaf area index (3.45 and 3.35), while M_1 showed a lower leaf area index (3.11). The leaf area index was recorded more for rest (3.302) than for control plots (1.891). HA applied at the rate of 5 kg ha^{-1} and N at 150 kg ha^{-1} showed a higher leaf area index (3.93), respectively.

Grains spike^{-1}

Table 3 presented the data on grains spike^{-1} and the analysis of the data is presented in Table 5. The perusal of data showed that grains spike^{-1} was significantly affected by N, M, and HAxN, while not significantly affected by HA, NxM, HAxM, HAxNxM, and HA x Control vs. rest. Similarly, Control vs. rest was found to be significant. Increasing nitrogen from 90 to 150 kg ha^{-1} increased grains spike^{-1}, respectively. A greater number (48.36) of grains spike^{-1} was recorded in N_{150} compared to N_{90}, which produced fewer (44.44) grains spike^{-1}. Grains spike^{-1} was also significantly affected by nitrogen application methods. Nitrogen applied using the M_1 method resulted in lower (44.83) grains spike^{-1}, while higher results (48.61) were observed in plots applied N as M_3. Control plots showed lower (33) grains spike^{-1} than rest plots (46.59). Humic acid interacting with N-affected grains spike^{-1} significantly also. Higher values (49.56) were recorded for humic acid application at the rate of 15 kg ha^{-1} in combination with 150 kg N ha^{-1}.

Table 5: *ANOVA of grains spike^{-1} (GS), thousand-grain weight (TGW), biological yield (BY), grain yield (GY), and harvest index (HI). Here, HA: humic acid, N: nitrogen levels, and M: nitrogen application methods.*

		GS		TGW		BY		GY		HI	
SOV	**df**	**F-ratio**	**P**	**F-ratio**	**P**	**F-ratio**	**P**	**F-ratio**	**P**	**F-ratio**	**P**
Replication	2	2.12	0.201	0.95	0.437	5.05	0.052	1.91	0.229	1.23	0.358
HA	3	0.12	0.945	1.63	0.279	17.08	0.002	13.93	0.004	2.84	0.128
Error I	6										
Treatment	9	34.58	0.000	28.23	0.000	20.91	0.000	34.84	0.000	26.05	0.000
Control vs. rest	1	279.69	0.000	135.53	0.000	95.78	0.000	180.86	0.000	157.4	0.000
N	2	6.01	0.004	40.27	0.000	16.85	0.000	19.06	0.000	6.91	0.002
M	2	4.54	0.014	10.75	0.000	21.66	0.000	35.57	0.000	19.38	0.000
N x M	4	2.62	0.042	4.13	0.005	3.85	0.007	5.87	0.000	6.11	0.000
HA x T	27	1.43	0.115	2.07	0.008	2.1	0.007	1.55	0.074	1.22	0.253
HA x N	6	2.19	0.054	4.3	0.001	4.15	0.001	2.02	0.074	2.41	0.035
HA x M	6	2.03	0.072	1.55	0.174	0.96	0.461	0.45	0.845	0.38	0.891
HA x N x M	12	1.1	0.37	1.5	0.143	1.75	0.075	1.82	0.061	1.05	0.412
HA x Control vs. rest	3	0.05	0.985	0.88	0.454	1.68	0.179	1.69	0.177	1.15	0.335
Error II	72										
Total	119										

Thousand grains weight

The mean data regarding thousand-grain weight (g) is given in Table 3. Data analysis showed that thousand-grain weight was significantly affected by HA, N, and M, but their interactions were not significant. Control vs. rest comparison was significant for the thousand-grain weight (Table 5). HA applied at the rate of 15 kg ha^{-1} showed higher results (45.04); however, these results are statistically similar to that of HA_5 and HA_{10}. Nitrogen applied at different levels also significantly affected thousand-grain weight. Nitrogen applied at the rate of 150 kg ha^{-1} showed more (46.89) thousand-grain weight followed by N_{120} (43.25), while N_{90} showed lower values (42.22). Nitrogen application in full and split doses also affected thousand-grain weight, and significant differences were found among different methods. Nitrogen application in both M_2 and M_3 resulted in higher (43.86 and 45.28) thousand-grain weights and was statistically similar to its application as a full dose, M_1 (43.22). Compared to the rest, control plots were observed less for thousand-grain weight, i.e., 37.58 and 44.12.

Biological yield

Mean data regarding biological yield is reported in Table 3. Analysis of the data shows that the biological yield of wheat was significantly affected by HA, N, M, and NxM. At the same time, HA interaction with N, M, and Control vs. rest was found non-significant. Similarly, the comparison of Control vs. rest was also found significant for biological yield (Table 5). The biological yield was linearly increased with the increase in humic acid levels from 0 to 15 kg ha^{-1}. Biological yield (9338 and 9362) was noticed to be higher in 10 and 15 kg HA ha^{-1}, respectively, which were statistically similar for HA_5 and HA_0. Application of nitrogen at the rate of 150 kg ha^{-1} showed a higher (9382) biological yield, while a lower value (8580) was recorded for 90 kg N ha^{-1} treated plots. Nitrogen application in two and three splits is statistically similar to each other (9103 and 9280) and was found to be higher than the full dose (8519). The biological yield was recorded more for rest (8967) than for control (7323). Looking at the interactive response, NxM showed more biological yield (9683) for N_{150} applied as M_2, and HAxNxM showed maximum biological yield (10473) for HA_{10} combined with N_{150} applied in two splits; however, the three-way interaction was found insignificant.

Grain yield

Statistical analysis of the data revealed that grain yield was significantly affected by HA, N, M, and NxM, while HAxN, HAxM, HAxNxM, and HAxControl vs. rest were found not significant. The comparison of Control vs. rest was found significant (Table 5). Humic acid application increased grain yield, and significant differences were found among its various levels. Higher grain yield (3544) was recorded in the plots treated with 10 kg HA ha^{-1}, while lower (2911) was observed for no HA application. Grain yield was also increased with an increasing rate of nitrogen from 90 to 150 kg ha^{-1} (2975 to 3498). Nitrogen application in splits significantly affected grain yield. Nitrogen applied as M_3 produced more grain yield (3477) than M_1 (2959 kg ha^{-1}). Grain yield was recorded more for the rest (3254) than control plots (2103). Looking at the interactive response of NxM, more grain yield (3778) was recorded for N_{150} applied in three splits, while less (2846) grain yield was recorded at N_{90} in full dose. Three-way interaction, i.e., HAxNxM was found to be not significant; however, it produced a higher grain yield.

Harvest index

Mean data regarding the harvest index are shown in Table 3. Analysis of variance revealed that HA, N, M, NxM, and HAxN were significant, but, HAxM and HAxNxM were insignificant. The comparison, Control vs. rest, was also significant, but its interaction with HA was not significant (Table 5). The harvest index was significantly affected by humic acid levels. A higher (38.05) harvest index was recorded in plots treated with HA_{10} as compared to other levels. Nitrogen levels also increased the harvest index linearly. Nitrogen applied at the rate of 150 kg ha^{-1} showed a higher (37.21) harvest index which is statistically similar to 120 kg N ha^{-1} (36.73). Nitrogen applied in full and splits also significantly affected the harvest index. Nitrogen application as M_2 and M_3 is statistically similar (36.93 and 37.44), followed by M_1 (34.81). The harvest index was recorded more for rest (36.21) than for control plots (28.71). In an interaction between N and M, more harvest index (39.60) was recorded for N_{150} applied in the M_3 method. In the case of HA and N interaction, more harvest index (40.59) was recorded in the plots treated with HA_{10} in combination with N_{120}.

Correlation analysis

Correlation analysis was performed to assess the strength of the relationships of all the studied morpho-physiological and yield indices with HA, N, and M treatments (Figure 1). Preferably, the focus was on assessing the relationships between all yield and yield-contributing parameters (productive tillers m^{-2}, no. of grain spike^{-1}, thousand-grain weight, and grain yield) in connection with HA, N, and M treatments. The positive relationship or strong strength was

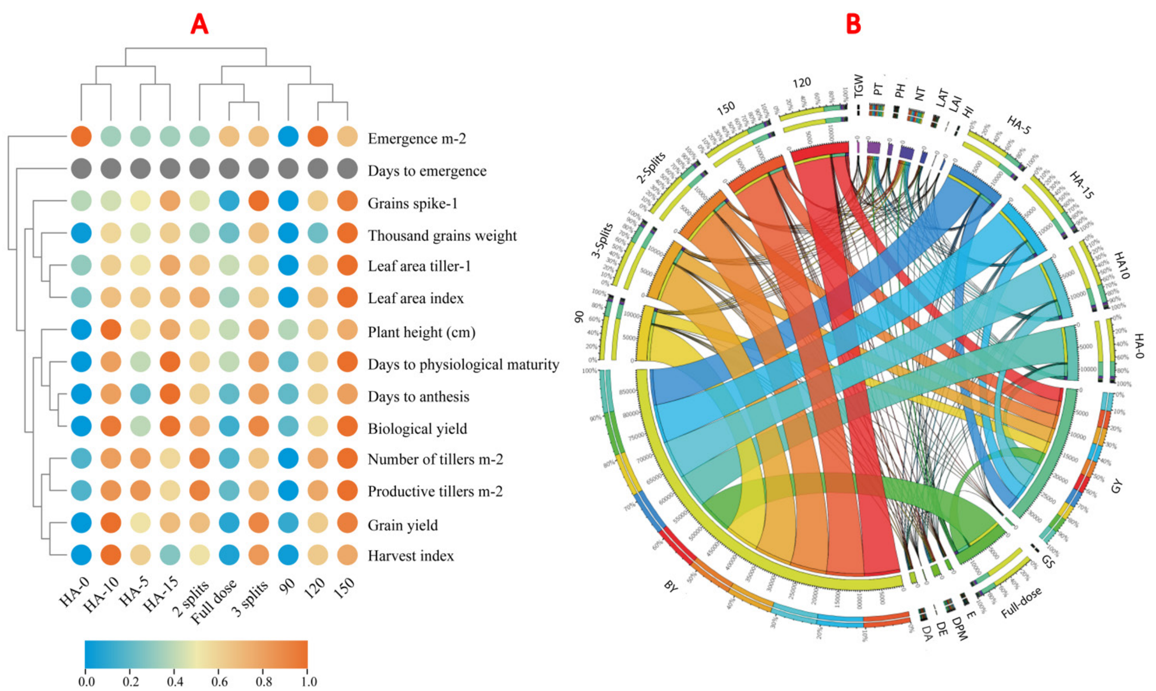

Figure 1: *Correlation (A) and Circos visualization (B) among morphophysiological indices and yield-related parameters of wheat. HA-0, HA-5, HA-10, HA-15: (Humic Acid, HA); 90, 120, 150: (Nitrogen levels, N); full dose, 2 splits, 3 splits (Nitrogen application methods, M). DE: Days to emergence, DA: Days to anthesis; E: Emergence m^{-2}; DPM: Days to physiological maturity; PH: plant height; TGW: thousand-grain weight; LAT: leaf area tiller^{-1}; LAI: leaf area index; NT: number of tillers m−2; PT: productive tillers m^{-2}; GS: grain spike^{-1}; BY: biological yield; GY: grain yield; HI: harvest index.*

mentioned using red, while the weak one was mentioned using blue. It is obvious from the figure that the mentioned growth and yield components were strongly affected using HA at the rate of 10 or 15 kg ha^{-1} as compared to no HA. Similarly, applying N at the rate of 150 kg ha^{-1} using the M_3 method showed a positive relationship and boosted all yield and its components for wheat. Figure 1B is a Circos plot drawn to visualize the strength of the relationship between any two studied parameters. Similarly, it was drawn to clarify the exact strength of every treatment impacting a specific parameter, in our case, wheat's growth and yield components. The color and fatness of a line elaborate the exact strength and relationship between any two study parameters and similarly, the specific treatment applied to it.

Discussion

Both days to emergence and emergence m^{-2} were not significantly influenced by any treatment used in this experiment. These results parallel the previous study of Daur (2013), who reported that germination could not be affected using HA in the soil. This might be because HA and inorganic fertilizer action at that stage takes more time to be observed and considered. Moreover, the seed at this stage uses stored food in the endosperm instead of external nutrients. Another possible reason may be that seed germination is a chemical change, for it only needs water instead of fertilizer to start and sprout. Moreover, Szabó *et al.* (2022) reported that nitrogen application also has no effect on the emergence m^{-2}, which is similar to our findings.

Statistical analysis of the data revealed that days to anthesis and physiological maturity were significantly affected by HA, N, M, and NxM, HAxN while not affected by HAxM and HAxMxN interactions. Humic acid levels significantly increased days to anthesis and physiological maturity. The results are similar to the Iqbal *et al.* (2022) study findings, which reported that HA applied at higher doses significantly affected days to anthesis and physiological maturity. This may be due to HA's availability of nutrients to the plant for a longer time. Nitrogen levels also significantly increase days to anthesis and maturity. Heavy N dose prolongs vegetative growth and maturity (Anas *et al.* 2020). Our results are also aligned with Belete *et al.* (2018), who documented that a longer vegetative period results from higher N rates at the anthesis stage. Split dose N application significantly increases days to anthesis and delayed maturity. Anas *et al.* (2020) reported that N-application in splits promotes growth and development, ultimately prolonging vegetative growth, and thus maturity is also delayed.

Data regarding plant height was significantly affected by HA levels. The HA application resulted in higher plants and increased total phenolic contents (Gholami *et al.*, 2018). N levels also showed significant results. An increase in N level from 90 to 150 kg ha^{-1} showed significant differences. Nitrogen applied at the rate of 150 kg ha^{-1} showed statistically higher results, and 90 kg N ha^{-1} treated plots showed lower results for plant height. Similarly, N application methods were also noticed significantly. The results are at par with Szabó *et al.* (2022) findings, who recorded N least effectively applied all at seedbed preparation. An increase in plant height may be attributed to the large biomass production, which increased internodal length and, consequently, taller plants. Islam and Nahar (2014) findings also support our results; they noticed taller plants using a full dose of urea (215 kg ha^{-1}) in three splits compared to the control.

It is obvious from the results that N, M, and HA and their interactions significantly affected the number of tillers m^{-2}. Nitrogen applied at the rate of 120 and 150 kg ha^{-1} showed statistically similar results and was found higher for tiller^{-2} than 90 kg ha^{-1}. This phenomenon may be due to N improvement in WUE, enhancing vigorous growth and development (Iqbal *et al.*, 2022). Nitrogen application methods also significantly improved tillers m^{-2}. The single-dose application showed lower results than two and three-splits. It may be attributed to more nitrogen availability at mean stages. Significant results had also been reported by other researchers (Khan *et al.*, 2022; Kong *et al.*, 2022; Luo *et al.*, 2020). The interaction of HA and N was found significant, which may be due to the improved soil physio-chemical properties, drought resistance, and reduction of N loss from the soil (Rasouli *et al.*, 2022).

Nitrogen and its application methods significantly affected the number of productive tillers m^{-2}. However, HA was found not significant, but its interaction with nitrogen showed significant results. It might be due to HA activity promoting nutrient uptake, increasing NUE, enhancing plant growth, and higher uptake rate of soil nutrients (Anas *et al.*, 2020; Kong *et al.*,

2022) independent of nutrition (Rasouli *et al*., 2022). Kong *et al*. (2022) demonstrated that HA applied with nitrogenous fertilizer showed a higher number of productive tillers. A linear increase was found in N application from 90 to 150 kg ha^{-1} and showed significant differences among each other. Application of N at higher rates increased productive tillers m^{-2} (Szabó *et al*., 2022). These lines are in favor of our findings. Moreover, using N in splits compared to all at once enhances the number of productive tillers m^{-2}, which is favored by the Islam and Nahar (2014) experimental results.

Leaf area tiller^{-1} showed a significant and linear increase in using HA (0 to 15 kg ha^{-1}) and N from 90 to 150 kg ha^{-1}. Plots that received no HA were found less for leaf area tiller^{-1} than other levels, which all were statistically at par with each other. Applying N_{150} was noted higher in leaf area tiller^{-1} than in lower level (90 kg ha^{-1}). Similarly, N applied in splits significantly influenced leaf area tiller^{-1} more than full dose application. The probable cause might be more N availability during growth, resulting in more chlorophyll formation and leaf expansion to the sun. Islam and Nahar (2014) findings were similar and in line with our experimental findings. Anas *et al*. (2020) reported that using a higher rate of N at the panicle emergence stage produced maximum leaf area plant^{-1}.

The leaf area index was not significantly affected by HA levels but was significantly enhanced by N and its application methods. All possible interactions were found not significant except HAxN. Nitrogen linearly increased the leaf area index from 90 to 150 kg ha^{-1}. It might be due to higher absorption and available nitrogen to the tissues that, in response, increases elongation and expansion. Our results are in line with the findings of Azeem *et al*. (2014), who reported that the leaf area index increased with higher N levels. The reverse was true for control plots (no N). These results are in conformity with Liu *et al*. (2018) findings, who recorded maximum LAI in the plots that received N in different splits at later stages of the crop. Higher amounts of N enhance cell division and augmented photosynthate construction (Luo *et al*., 2020).

Grains spike^{-1} was significantly affected by nitrogen, nitrogen application methods, and HAxN but was not significantly affected by humic acid levels. Nitrogen used at 150 kg ha^{-1} showed increased grains spike^{-1} than 90 kg ha^{-1}. Higher yield components were achieved using nitrogen at the rate of 120 kg ha^{-1}. Similarly, NUE was also increased with 120 kg N ha^{-1} (higher rates) applied in three equal splits as 1/3rd basal, top dress, and 1st node stage (Belete *et al*., 2018). Grains spike^{-1} was found to increase with splitting nitrogen. Splitting nitrogen into three equal splits showed higher grains spike^{-1}, statistically different from applying it in the full dose and two splits. Similar results were also reported by Szabó *et al*. (2022). El-Hashash *et al*. (2022) also reported higher grains spike^{-1} for using N fertilizer in M_2 and M_3 compared to M_1 applications. In combination with nitrogen-treated plots, HA was also significant in grains spike^{-1}. Humic acids and other nutrients seemed economical in production in saline calcareous soil (Tahat *et al*., 2020).

Application of HA level significantly affected thousand grains weight. The application of chemical fertilizer with HA increased its accessibility and increased its cell number and yield (El-Hashash *et al*., 2022). Nitrogen and its application methods enhanced thousand-grain weight. Grain weight was increased with the application of N fertilizer at a higher level than a lower level by Khan *et al*. (2022). Similarly, thousand-grain weights were also increased in the treatments having 160 kg N ha^{-1} (Azeem *et al*., 2014). Yang *et al*. (2022) reported that N applied in three equal splits at the sowing, tillering, and flowering stages significantly increased thousand-grain weight. Similarly, inorganic fertilizers applied with organic fertilizers showed significant effects on yield parameters (Khan *et al*., 2022).

The biological yield was influenced using HA, N, and M but their interactions with each other, except NxM, were not significant. Humic acid had a positive and significant effect on biological yield. Higher levels (10, 15 kg ha^{-1}) of HA were recorded as statistically similar and showed more biological yields. The results coincide with Daur (2013), who concluded that an increase in soil application of HA levels significantly improves wheat biological yields by 20-25%. Application of inorganic N at the rate of 150 kg ha^{-1} showed enhanced biological yields than 90 kg ha^{-1}. Our results are best supported by the findings of Anas *et al*. (2020), who obtained higher biological yields using a high rate of nitrogen. Nitrogen application methods also positively responded to biological yield. This observed fact may be due to higher plant height, density, grains spike^{-1}, and grain weights which were

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enhanced by a higher N dose. Application of N in splits showed higher values than full dose application. Belete *et al.* (2018) reported that various yield components increased with splitting N application at different growth stages. Khan *et al.* (2015) also reported that HA applied with half (30:20 kg ha^{-1}) rate of N appeared economical to obtain higher wheat yields under rainfed conditions.

Grain yield was noticed significantly using various HA levels. This increase in grain yield might be due to more availability of major and minor nutrients and enhanced growth regulators by HA (Rasouli *et al.*, 2022). Our results are also confirmed by Zhang *et al.* (2017), who documented that adding humic acid to the soil significantly increased green herbage, plant height, grain yield, and crude protein. Similar results were reported by Rasouli *et al.* (2022) that applying HA either in sole or combined with nitrogenous fertilizer increased dry matter and grain yield compared to the control. Nitrogen application also significantly affected grain yield, and a linear increase was found in grain yield with increasing N levels. This increase in grain yield can be correlated to more crop growth, leaf area plant^{-1}, spikes m^{-2}, grains ear^{-1}, and heavier grains due to more N availability. Our findings are supported by the research work of Azeem *et al.* (2014), who concluded that N applied at the rate of 160 kg ha^{-1} increased 1000 grains weight, grains cob^{-1}, and grain yield. Yang *et al.* (2022) also reported that a greater number of grains was produced by plots treated with higher N rates. Nitrogen application in splits at different stages was found to be significant. Higher grain yields with increasing nitrogen splits may result from more N availability and fewer N losses due to leaching at mean stages. Luo *et al.* (2020) reported that N was consumed efficiently when applied at the rate of 150 kg ha^{-1} in between tillering and stem elongation stages.

The harvest index significantly increased with increasing levels of HA. The harvest index was higher at 10 kg ha^{-1} than at rest HA levels. This may be due to the enhancement of micronutrients and activation of physiological processes by HA (Abbas *et al.*, 2022) that increased grain yields. Our results also correlate with Liu *et al.* (2018) and Kulikova *et al.* (2021) findings, who reported that seed yield and harvest index were increased with the application of HA compared to the control. Higher levels of nitrogen promote harvest index as compared to low levels. Application of N_{120} and N_{150} showed more harvest index than 90 kg N ha^{-1}, similar to the findings of Iqbal *et al.* (2022), who reported more harvest index in N-treated plots compared to the control. Nitrogen application methods affected the harvest index, and a higher harvest index was noted in a split application than in a full dose. The harvest index was affected significantly by N in three splits at different stages (Ali *et al.*, 2018), whereas; it was not affected by N-application time (Anas *et al.*, 2020).

Conclusions

It is concluded from the results that HA applied at the rate of 10 and 15 kg ha^{-1} showed significantly higher values for yield and yield contributing parameters. Similarly, higher rates of nitrogen (150 kg ha^{-1}) applied in three equal splits (M_3) produced maximum grain yield and contributed more to yield components of wheat. Moreover, HA in combination with nitrogenous fertilizer was easily manageable and farmers friendly in terms of cost-benefit ratio. It is, therefore, recommended that the application of HA at the rate of 10 kg ha^{-1} and N at the rate of 150 kg ha^{-1} in three equal splits is economical and advised to farmers for obtaining higher yield and yield components of wheat in the arid and semi-arid regions of the world.

Acknowledgments

The present work is based on the MS thesis of first author submitted to the department of Agronomy, Faculty of Crop Production Sciences, The University of Agriculture, Peshawar, Pakistan. The authors are grateful to all the faculty members and colleagues for their boundless support during conducting this research work.

Novelty Statement

This study identified the optimal Humic acid (10 kg per ha) and Nitrogen (150 Kg per ha) combination in three equal splits and application timing (three equal) for maximum productivity of wheat, promoting economically sustainable wheat production systems.

Author's Contribution

Both authors are equally contributed in developing this article. The first author (Wajid Ali Khattak) conducted the research work and wrote the original draft of this article. Coauthor (Shazma Anwar) edited the final version of the paper. Both authors are agreed with the final version of this article.

Generative AI and AI-assisted technology statement

No generative AI and AI-assisted Technology has been used in this article.

Conflict of interest

The authors have declared no conflict of interest.

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