Studying the effect of humic acid foliar spray and NPK fertilizer application on bread wheat (*Triticum aestivum* L.) productivity and quality

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Abstract

A field experiment was conducted at two distinct locations, Grderasha (GR) and Grdlanka (GL), to evaluate the response of bread wheat (Triticum aestivum L.) to foliar application of humic acid and broadcast NPK fertilization. The experiment was laid out in a factorial design comprising five levels of humic acid (HA0, HA1, HA2, HA3, and HA4) applied as foliar sprays and three levels of NPK fertilizer (F0, F1, and F2) applied through broadcasting, with all treatment combinations assessed at both locations.

Regarding the effect of humic acid foliar application, results revealed that increasing humic acid concentration led to progressive improvements in yield components, particularly at the GL location. Seed number per spike was notably higher at GL, ranging from 518 to 647 seeds, compared to 391 to 480 seeds at GR, with significant treatment responses observed only at GL, while GR exhibited no statistical differentiation among humic acid levels for this trait. Thousand-seed weight showed numerical increases of approximately 37% at GR (from 13.67 to 18.67 g) and 27% at GL (from 17.33 to 22.00 g), although these increases did not reach statistical significance. In terms of grain quality, humic acid application resulted in modest improvements, with higher concentrations associated with slightly elevated protein, gluten, and ash content, though these changes were generally not statistically significant, indicating that humic acid exerts a stronger influence on yield parameters than on grain biochemical composition.

Concerning the effect of broadcast NPK fertilization, the results indicated that NPK treatments produced significant but comparatively smaller effects on yield and quality characteristics than humic acid. Fat content responded significantly to NPK treatments at both locations, ranging from 1.41 to 1.63% at GR and from 1.39 to 1.54% at GL, while fiber content increased by approximately 7% and 9% at GR and GL, respectively. Other nutritional parameters showed limited differentiation among NPK levels, with most sharing similar statistical groupings across F0, F1, and F2, suggesting that the inherent soil fertility at both sites partially met the crop nutrient demand, thereby reducing the additional benefit of higher NPK rates on grain composition.

With respect to the interaction effects between the studied factors, significant location by treatment interactions were detected across several measured characteristics. The GL location consistently produced superior protein content (16.62 to 17.14%), gluten content (24.94 to 25.71%), and ash content (1.67 to 1.96%), whereas the GR location exhibited higher carbohydrate content (66.32 to 68.73%) and energy values (2392 to 2470 kcal/100g). The most favorable outcomes for grain quality optimization were achieved under the highest humic acid and NPK combinations, specifically HA3 and HA4 combined with F1 and F2, which consistently outperformed lower treatment levels at both locations. The GL location demonstrated greater overall responsiveness to the applied treatments compared to GR, highlighting the critical role of site-specific environmental conditions, including differences in soil properties, moisture availability, and microclimatic factors,

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in determining the magnitude of treatment effects. These findings collectively confirm that an integrated nutrient management approach combining foliar humic acid with broadcast NPK fertilization can enhance both wheat productivity and grain quality, provided that application strategies are tailored to the specific environmental characteristics of the production site.

Keywords: Humic acid, NPK fertilizer, Bread wheat, grain quality, Grdarasha, Grdlanka.

Introduction

Bread wheat (*Triticum aestivum* L.) represents a fundamental component of global food security, supplying approximately 20% of daily caloric intake and protein requirements for the human population [1]. The escalating demand for wheat, driven by population growth and dietary shifts, requires substantial increases in production efficiency, simultaneously maintaining or improving grain quality attributes [2]. Contemporary wheat production systems face multifaceted challenges including soil nutrient depletion, climate variability, and the imperative to reduce environmental impacts associated with intensive agricultural practices **[3]**.

Conventional fertilization strategies, particularly broadcast application of NPK fertilizers, remain the predominant method for nutrient delivery in wheat cultivation systems. However, this approach frequently demonstrates limited efficiency, with substantial proportions of applied nutrients being lost through volatilization, surface runoff, and leaching into groundwater systems [4]. Research indicates that nitrogen use efficiency in wheat production rarely exceeds 40% under conventional management practices, representing both economic losses for producers and environmental concerns related to greenhouse gas emissions and water quality degradation [5]. The persistent application of synthetic fertilizers without integrated organic amendments has been implicated in declining soil organic matter content, reduced microbial diversity, and deteriorating soil health indicators (Kopittke et al., 2019) [6].

Humic substances have emerged as promising amendments for sustainable intensification of crop production systems. Humic acid, characterized by complex molecular structures containing aromatic rings and various functional groups, demonstrates multiple beneficial effects on plant physiology and soil properties **[7]**. Foliar application of humic acid has been shown to enhance nutrient assimilation, stimulate enzymatic activities, modulate hormonal balance, and improve stress tolerance mechanisms in cereal crops **[8]**. Recent investigations have documented that humic acid can increase chlorophyll content, enhance photosynthetic rate, and promote root architectural modifications that facilitate improved resource acquisition. The integration of humic acid with mineral fertilizers presents opportunities for developing more resilient and productive cropping systems while addressing sustainability concerns **[9]**.

Wheat performance in response to nutritional management varies substantially across different agro-ecological environments due to variations in soil characteristics, climatic conditions, and genotype-environment interactions **[10]**. Soil properties including texture, pH, organic matter content, and native fertility levels significantly influence nutrient availability and plant uptake efficiency **[11]**. Multi-location experimentation provides essential information regarding the consistency of treatment effects across diverse environmental conditions and enables the identification of location-specific management recommendations. Understanding how combined applications of organic and inorganic nutrient sources perform across contrasting environments is critical for developing adaptable and robust agronomic strategies [12].

Quality attributes of wheat grain, including protein concentration, gluten characteristics, and micronutrient density, are increasingly recognized as important objectives alongside yield maximization. Nutritional interventions can substantially influence grain quality parameters through their effects on nitrogen metabolism, carbon allocation patterns, and the accumulation of bioactive compounds **[13]**. This study was conducted to investigate the effects of different humic acid foliar spray concentrations and varying NPK fertilizer application rates, both individually and in combination, on the productivity and grain nutritional quality of bread wheat (*Triticum aestivum* L.). Specifically, the study aimed to evaluate how each factor independently influences wheat growth, yield components, and grain quality attributes, as well as to examine their interaction effects, with the ultimate goal of identifying an optimum integrated nutrient management combination of humic acid foliar spray and NPK fertilizer that simultaneously maximizes grain yield and enhances the nutritional quality of bread wheat under the prevailing production conditions.

Materials and Methods

Study Site

Two locations in Erbil governorate were selected to do this researching study. First location was in Grdarasha Field (GR), College of Agricultural Engineering Sciences, Salahaddin University Erbil (36˚ N - 44˚ E), and the second location was in Grdlanka (GL), (35°50'0"N - 44°5'0"E).

Experimental Design

The experiment was carried out in two locations, the first location at Grdarasha Field in College of Agricultural Engineering Sciences, Salahaddin University-Erbil. The second location was in Grdlanka village, Erbil that was during winter season at 2024 in the middle of April and early of May 2025. Factorial Randomized Complete Block Design (RCBD), in which three replications each of humic acid (Cosmocel company, H-850 product, Mexico) with 3 levels (HA0, HA1 and HA2); (0, 10 and 15 kg ha^{-1}) was practiced. While, the second factor was NPK fertilizer (12-12-17-2MgO,Iran) added to the plants by the three different levels (F0, F1 and F2); (0, 150 and 200 kg ha^{-1}) in two times, in the first time halve dose with sowing date, and the second dose was added in the tillering stage, in this stage humic acid was also applied using foliar application at concentration of (0, 2 and 4 g L^{-1} water), which was symbolled as (HA0, HA3 and HA4), respectively. Plot area was (2 m^2), the space between plants was 10 cm, also the same space was between row to row, while 1.0 m was the distance between plots and also the same distance was between replications.

Soil Analysis

Chemical and physical properties of the both study sites

The GR sample is a clay loam soil (34.47% clay, 49.24% silt, 16% sand) with moderate cation exchange capacity (22.39 cmolc/kg) and bulk density (1.03 g/cm^3). It exhibits severely deficient macronutrient levels with very low nitrogen (72 ppm), phosphorus (5.12 ppm), and potassium (15.2 ppm), alongside low organic matter content (1.73%) and low moisture content (1.58%). The soil is alkaline (pH 8.04) and moderately calcareous (15.17% $CaCO_3$), which may restrict micronutrient availability, particularly iron and zinc. However, it shows no salinity concerns (EC 0.19 dS/m). This soil requires comprehensive fertilization with NPK and organic amendments to improve fertility and productivity for agricultural use.

The GL sample is a loam soil (26% clay, 29% silt, 46% sand) with moderate cation exchange capacity (20.15 cmolc/kg) and ideal bulk density (1.0 g/cm³) for root penetration. The soil exhibits very low nitrogen (58 ppm), very low phosphorus (2.58 ppm), but adequate potassium (1.36 ppm), with critically low organic matter (0.98%) and moisture content (1.62%). It is alkaline (pH 8.03) and calcareous (20% $CaCO_3$), which limits nutrient availability. A significant constraint is moderate salinity (EC 1.30 dS/m), which may affect salt-sensitive crops and reduce yields. Management priorities include nitrogen and phosphorus fertilization, organic matter incorporation, and salinity mitigation through improved drainage or leaching, while potassium supplementation is not immediately necessary.

Table 1. Soil analysis of both locations (GR and GL)

GL		**GR**	
58	Nitrogen (ppm)	72	Nitrogen (ppm)
2.58	Phosphorous (ppm)	5.12	Phosphorous ppm
13.6	Potassium (ppm)	15.2	Potassium (ppm)
20	CaCO3	15.17	$CaCO_3$
0.98	Organic matter	1.73	Organic matter
8.03	pH	8.04	pH
1	Bulk density (g/cm³)	1.03	Bulk density (g/cm³)
1.3	EC (dS/m)	0.19	EC (dS/m)
20.15	CEC (cmolc/kg -)	22.39	CEC (cmolc/kg -)
45.55	Snad	16.28	Snad
29	Silt	49.24	Silt
25.45	Clay	34.47	Clay
1.62	Moisture content	1.58	Moisture content

Sampling Method and Data Collection

The plants were manually harvested six months after planting. From each treatment plot, 7 plants were selected randomly which was inside rows. Overall, there were 315 plants on all plots. Then, yield and yield component parameters (Manual method) were taken from selected plants. Additionally, it was also used to determine chemical components (NIR in house method) in the grain.

Statistical Analysis

Yield and yield component parameters, and also seed components were statistically analyzed according to the technique of analysis of variance (ANOVA) for randomized complete block design, (RCBD) using IBM SPSS Statistics program (27.0) the mean comparison was fulfilled according to Duncan multiple range test at the level of significant 0.05.

Results and Discussion

The results revealed that humic acid foliar spray had a significant and consistent effect

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on all measured productivity parameters of bread wheat, exhibiting a clear dose-dependent response across both growing conditions. Progressive increases in humic acid concentration from HA0 to HA4 resulted in statistically significant improvements in total weight, seed weight, and bran weight. The highest performance was recorded under the HA3 treatment, which achieved total weight, seed weight, and bran weight values of 1104.44, 1335.56, and 253.78 g under GR conditions, and 435.56, 897.78, and 901.11 g under GL conditions, respectively (Table 2). These findings suggest that humic acid, when applied at optimum concentration, enhances the plant's ability to absorb and utilize nutrients more efficiently, thereby promoting greater biomass accumulation, grain filling, and ultimately higher grain and bran yields. Statistical groupings further confirmed that HA3 and HA4 were significantly superior to lower concentration treatments, while intermediate treatments showed overlapping performance, indicating that a threshold concentration is required to achieve meaningful productivity gains. Furthermore, the superiority of foliar application treatments (HA3 and HA4) over broadcasting practices (HA1 and HA2) as shown in Table 2, underscores the importance of application method in determining the effectiveness of humic acid, as foliar delivery ensures more direct and efficient uptake by the plant.

NPK fertilizer treatments also produced significant effects on bread wheat productivity parameters, though the magnitude of improvement was comparatively more modest than that observed with humic acid treatments. With respect to total weight, the F3 fertilizer level produced the highest value of 1032.00 g under GR conditions, indicating that adequate NPK supply supports enhanced grain development and overall biomass production.

Similarly, bran weight responded positively to NPK application, with the F3 level recording the highest bran weight of 434.20 g, though this peak performance was observed under GL conditions rather than GR. This variation between growing conditions suggests that the response of bran weight to NPK fertilization is sensitive to the prevailing environmental and moisture conditions. The relatively smaller differences observed between NPK treatment levels compared to humic acid treatments imply that, under the tested conditions, NPK fertilization alone may not be sufficient to drive substantial productivity gains, and that its effectiveness is likely enhanced when combined with humic acid foliar supplementation.

Growing conditions had a substantial influence on all measured productivity parameters. GL conditions consistently outperformed GR conditions across all treatments and parameters, with the most pronounced differences observed in seed weight, particularly at higher humic acid concentrations. This pattern suggests that favorable growing conditions, likely associated with enhanced water availability and better soil moisture retention under GL, create a more conducive environment for nutrient uptake and utilization. Notably, the beneficial effects of humic acid supplementation were more strongly expressed under GL conditions, implying that optimal growing conditions amplify the positive influence of humic acid on wheat productivity. This interaction between growing environment and humic acid application highlights the importance of considering site-specific conditions when designing integrated nutrient management strategies for bread wheat production.

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Table 2. Single effects of humic acid and NPK fertilizers on yield parameters of wheat (*Triticum aestivum* L.)

Weight of bran (g)		Seed weight (g)		Total weight (g)		Treatments
GL	GR	GL	GR	GL	GR	
Humic acid*						
794.22a	751.67c	356.89c	90.56c	1171.11b	896.11c	**HA0**
827.22a	828.56b	368.00bc	115.33bc	1199.44b	928.89c	**HA1**
857.78a	838.33ab	376.00bc	138.89c	1238.00ab	1001.67b	**HA2**
867.33a	844.00ab	410.67ab	166.11b	1271.67ab	1072.78a	**HA3**
901.11a	897.78a	435.56a	253.78a	1335.56a	1104.44a	**HA4**
NPK fertilizer						
831.00a	821.53a	357.73b	145.87a	1202.33a	979.67b	**F0**
841.47a	821.80a	376.33b	154.47a	1263.47a	990.67ab	**F1**
876.13a	852.87a	434.20a	158.47a	1263.67a	1032.00a	**F2**

*HA: Humic acid, HA0, HA1, HA2 (0, 2, and 4 g. L^{-1} water), as foliar, HA3 and HA4 (10 and 15 kg. ha^{-1}), as broadcasting, F: NPK fertilizer, F0, F1, and F2 (0, 150, and 200 kg. ha^{-1}), GR= Grdarasha, GL= Grdlanka

Single effects of humic acid and NPK fertilizers on chemical components of wheat

Humic acid foliar spray treatments exhibited modest but progressive effects on the nutritional composition of bread wheat grain. Although most measured nutritional parameters did not show statistically significant differences across humic acid concentration levels, as indicated by shared letter groupings in the statistical analysis, consistent numerical trends of gradual improvement were observed with increasing humic acid concentration. The HA4 treatment recorded the highest values for protein content (16.70% under GR and 17.04% under GL conditions), fat content (1.55% under GR and 1.52% under GL conditions), and ash content (1.69% under GR and 1.91% under GL conditions) (Table 3). Similarly, energy value reached 2433.22 kcal under GR and 2396.42 kcal under GL conditions, while gluten content attained its highest values of 24.55% under GR and 25.57% under GL conditions under the same treatment (Table 4). Protein and gluten content are particularly critical quality indicators for bread wheat, as they directly determine dough strength, loaf volume, and overall bread-making quality. The non-significant but progressive increases observed in these parameters with rising humic acid concentration suggest that humic acid may contribute to marginal improvements in grain nutritional composition, possibly through enhanced nitrogen uptake and assimilation facilitated by the chelating properties of humic substances. Nevertheless, when compared to the control treatment, only modest compositional changes were recorded, indicating that under the tested conditions, humic acid foliar spray primarily exerts its influence on wheat productivity parameters such as yield and biomass accumulation rather than substantially altering grain nutritional composition.

The influence of NPK fertilizer application on grain nutritional quality parameters was similarly limited, though marginal differences between treatment levels were observed. The F2 treatment produced the highest values for most nutritional parameters, particularly protein content (16.70% under GR

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conditions), fat content (1.50% under GR conditions), and energy value (2429.49 kcal under GR conditions). However, statistical analysis revealed minimal differentiation between fertilizer levels, with most nutritional parameters sharing identical letter groupings across the F0, F1, and F2 treatments, as shown in Tables 3 and 4. This limited response of grain nutritional composition to NPK fertilizer levels may be attributed to the fact that grain protein and other compositional traits are largely determined during the grain filling stage and are more sensitive to the timing and form of nitrogen supply rather than the overall NPK application rate. These findings suggest that while NPK fertilization plays an important role in supporting wheat productivity, its capacity to significantly modify grain nutritional quality under the tested conditions was constrained, and that higher or more targeted fertilizer management strategies may be required to produce meaningful improvements in grain compositional attributes.

Unlike the substantial differences observed between GR and GL growing conditions in yield-related parameters, the influence of growing conditions on grain nutritional composition was comparatively minimal. Nutritional parameters remained relatively stable across both growing environments, with only slight and inconsistent variations recorded. GL conditions showed marginally elevated protein content across most treatments, which may be associated with better water availability supporting more efficient nitrogen uptake and translocation to the grain. Conversely, GR conditions demonstrated marginally higher energy values, though these differences were not consistently significant across treatments. The relative stability of nutritional composition across growing conditions suggests that grain quality attributes in bread wheat are more strongly governed by genetic factors and specific nutrient management practices than by the broader growing environment, at least under the range of conditions examined in this study.

Table 3. Single effects of humic acid and NPK fertilizers on protein, fat, ash and fiber of wheat (*Triticum aestivum* L.)

Fiber		**Ash**		**Fat**		**Protein**		**Treatments**
GL	**GR**	**GL**	**GR**	**GL**	**GR**	**GL**	**GR**	
								Humic acid*
2.82a	2.83a	1.71a	1.63b	1.45b	1.44b	16.77a	15.89a	**HA0**
2.85a	2.84a	1.72a	1.66ab	1.46ab	1.44b	16.85a	15.90a	**HA1**
2.87a	2.84a	1.72a	1.67ab	1.50ab	1.47ab	16.89a	16.22a	**HA2**
2.89a	2.86a	1.76a	1.68ab	1.50ab	1.48ab	17.00a	16.52a	**HA3**
2.89a	2.88a	1.91a	1.69a	1.52a	1.55a	17.04a	16.70a	**HA4**
								NPK fertilizer
2.83a	2.84a	1.75a	1.64b	1.47a	1.46a	16.89a	15.93b	**F0**
2.88a	2.85a	1.76a	1.67ab	1.49a	1.47a	16.91a	16.11ab	**F1**
2.88a	2.86a	1.78a	1.68a	1.49a	1.50a	16.92a	16.70a	**F2**

*HA: Humic acid, HA0, HA1, HA2 (0, 2, and 4 g. L^{-1} water), as foliar, HA3 and HA4 (10 and 15 kg. ha^{-1}), as broadcasting, F: NPK fertilizer, F0, F1, and F2 (0, 150, and 200 kg. ha^{-1}), GR= Grdarasha, GL= Grdlanka

Table 4. Single effects of humic acid and NPK fertilizers on moisture, carbohydrate, energy and gluten of wheat (*Triticum aestivum* L.)

Gluten		Energy		Carbohydrate		Moisture		Treatments
GL	GR	GL	GR	GL	GR	GL	GR	
								Humic acid*
25.15a	23.51a	2387.10a	2413.94a	66.15a	66.96a	10.60b	10.58a	**HA0**
25.28a	23.85a	2388.31a	2417.42a	66.17a	67.10a	10.71ab	10.64a	**HA1**
25.33a	24.33a	2391.07a	2419.46a	66.26a	67.15a	10.71ab	10.72a	**HA2**
25.50a	24.45a	2393.25a	2425.63a	66.33a	67.36a	10.72ab	10.70a	**HA3**
25.57a	24.55a	2396.42a	2433.22a	66.44a	67.60a	10.81a	10.77a	**HA4**
								NPK fertilizer
25.34a	23.70a	2386.39a	2414.52a	66.13a	66.99a	10.66b	10.65a	**F0**
25.36a	24.16a	2392.92a	2421.79a	66.32a	67.22a	10.66b	10.66a	**F1**
25.38a	24.54a	2394.38a	2429.49a	66.36a	67.48a	10.81a	10.76a	**F2**

*HA: Humic acid, HA0, HA1, HA2 (0, 2, and 4 g. L^{-1} water), as foliar, HA3 and HA4 (10 and 15 kg. ha^{-1}), as broadcasting, F: NPK fertilizer, F0, F1, and F2 (0, 150, and 200 kg. ha^{-1}), GR= Grdarasha, GL= Grdlanka

Interaction effects of humic acid and NPK on seed number spike^{-1}

The combined effects of humic acid and NPK fertilizer on wheat seed number showed different patterns at the two locations. At GR, seed number ranged from (391 to 480 seeds spike^{-1}) (23% increase), but these differences weren't statistically significant. This suggests that environmental factors at this site may have prevented the treatments from showing their full potential.

In contrast, Grdlanka produced much higher seed numbers (518-647 seeds spike^{-1}), about (32-35%) more than GR. More importantly, the statistical analysis revealed clear differences here: the lowest treatment (HA0F0) performed significantly worse than the treatments (HA4F1 and HA1F1). This indicates that GL growing conditions allowed the fertilizer treatments to express their benefits more clearly. These results highlight how location matters when applying humic acid and NPK fertilizers, with Grdlanka proving more responsive to these inputs (Figure 1). The differential response across locations reflects the critical role of environmental and edaphic factors in determining treatment efficacy. Meta-analytical evidence shows humic acid efficacy varies substantially with environmental conditions, with optimal effects in regions having annual precipitation exceeding 300 mm, mean temperatures above 10°C, and moderate soil pH, while effects are attenuated in alkaline soils and under high nitrogen or low organic carbon conditions **[14]** . The non-significant response at GR despite a 23% numerical increase suggests site-specific limitations constrained treatment benefits. The significantly higher seed production at Grdlanka and clear treatment differentiation indicate more favorable conditions for nutrient utilization. Combined humic acid and NPK application can increase spike-related parameters by 8.8% to 23.4%, while humic-based products enhance plant growth through direct effects on nutrient uptake and indirect effects on soil physicochemical properties **[15]**. Research on barley shows humic acid combined with phosphorus fertilizer can increase grain yield by 49-65% **[16]**, and field experiments demonstrate humic acid urea increased winter wheat yield by 5.14-6.24%**[17]**, confirming that integrated organic-mineral fertilization enhances productivity.

The contrasting responses underscore the importance of site-specific management strategies. Meta-analysis found humic acid increased crop yield by 12% on average, but effects were highly variable depending on climatic and soil conditions **[14]**. The superior performance at Grdlanka suggests this location possessed optimal soil characteristics and environmental conditions that facilitated enhanced nutrient uptake, emphasizing the necessity for location-specific optimization of fertilizer management practices in wheat production systems.

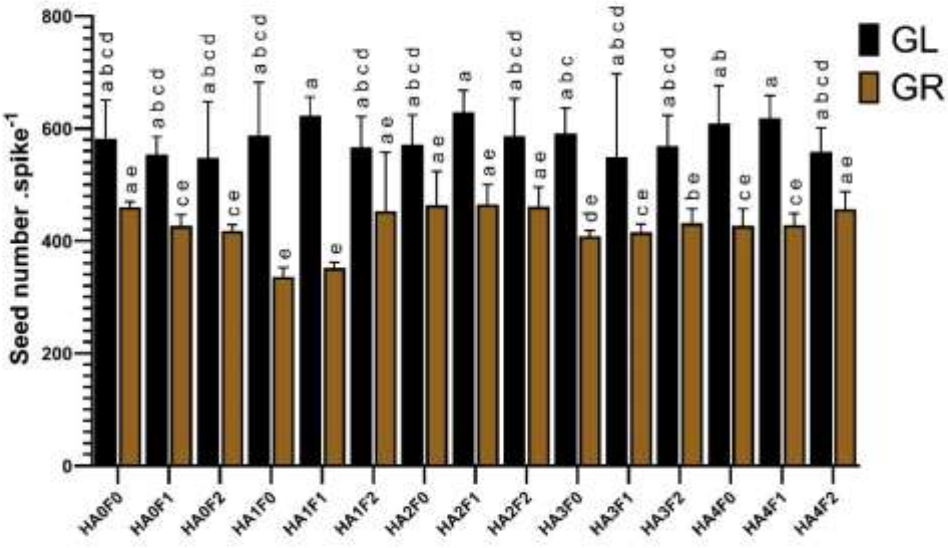

Figure 1. Interaction effects of humic acid and NPK on seed number spike^{-1} at both locations.

Interaction effects of humic acid and NPK on thousand-seed weight (g)

Seed weight at GR increased from 13.67 g to 18.67 g (37% gain) as treatment levels increased, though statistically these differences weren't significant. This suggests that factors other than nutrition-controlled grain filling at this location. Grdlanka produced heavier seeds overall (17.33-22.00 g), roughly 18-27% heavier than GR. However, like GR, no statistical differences appeared between treatments. While, both locations showed numerical trends favoring higher inputs, the lack of statistical significance suggests that seed weight may be controlled more by genetics and environmental conditions during grain filling than by the nutritional treatments tested. The consistently heavier seeds at Grdlanka point to better growing conditions (Figure 2). Meta-analysis across China demonstrated that humic acid application increased 1000-grain weight by only 4.3% on average (Bao-chong et al., 2024) **[18]** , while field experiments in calcareous soils showed humic acid with phosphorus fertilizer increased 1000-grain weight by 25.64%, **[19]** indicating highly variable responses. Grain weight is predominantly regulated by complex molecular networks involving transcriptional and post-translational regulators, with starch synthesis-related genes exerting important effects **[20]**, and grain size is a complex quantitative trait controlled by multiple genes and determined through several developmental stages **[21]**.

The consistently heavier seeds at Grdlanka despite non-significant treatment effects indicate superior environmental conditions for grain filling. Most research demonstrates wheat grains are not limited by assimilate supply during post-anthesis, except under extreme source restriction **[22]**, suggesting the non-significant fertilizer effects may reflect genetic constraints rather than nutritional

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limitations. Phenotypic characteristics of wheat are influenced by both genetics and environment, with environmental factors such as temperature, precipitation, soil water content, and organic matter differentially affecting grain weight (Zhang et al., 2024) **[23]**. The numerical trends favoring higher inputs at both sites, though statistically non-significant, align with evidence that humic-based products improve plant growth through both direct effects on nutrient uptake and indirect effects on soil physicochemical properties **[15]**, effects that may require longer-term application or different environmental conditions to manifest statistically significant differences in grain weight.

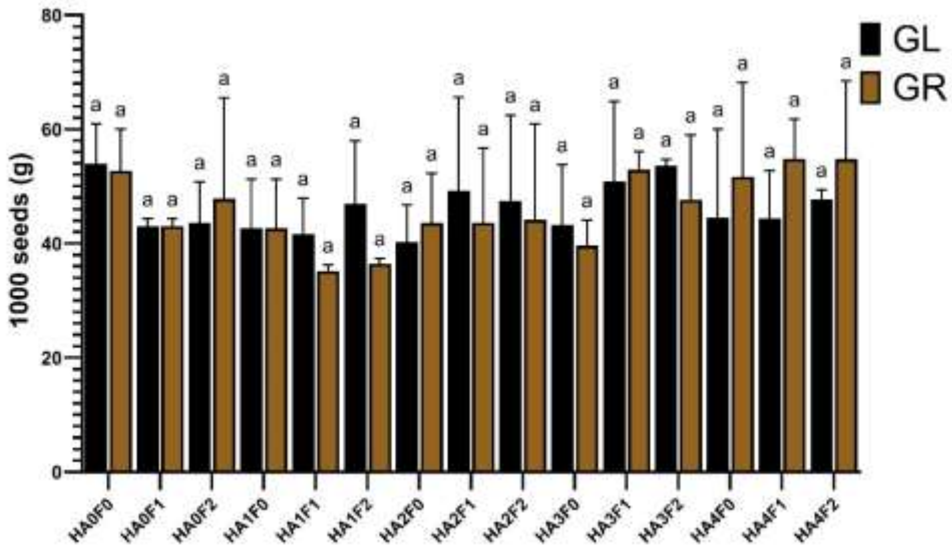

Figure 2. Interaction effects of humic acid and NPK on thousand-seed weight (g) at both locations.

Interaction effects of humic acid and NPK on grain chemical components

Protein levels ranged from (15.10-16.89 %) at GR and (16.62-17.14 %) at Grdlanka, with no statistical differences among treatments at either site. Grdlanka consistently produced about 10% more protein. This superior protein content makes Grdlanka wheat better suited for bread making.

The data shows that GL consistently maintains protein levels around 17% across all interactions (HA0F0 through HA4F2), with values marked as 'a' indicating no significant differences within this group. In contrast, GR exhibits lower protein percentages, ranging from approximately 14-17%, with greater variability among interactions as indicated by different letter designations (a, b, c, ac, bc).

Statistical analysis reveals that GL protein content remains relatively stable regardless of the interaction treatment applied. However, GR shows significant variations, with some interactions (HA0F0, HA4F2) displaying notably lower protein percentages (marked 'c' or 'bc') compared to most other treatments. The letters above bars indicate significant differences based on post-hoc analysis (different letters denote $p<0.05$) (Figure 3).

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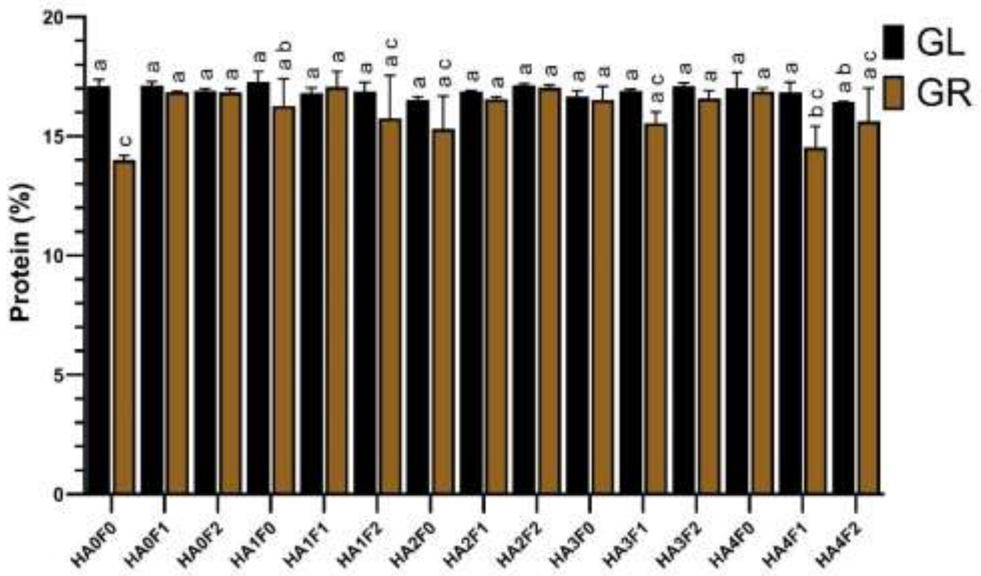

Figure 3. Interaction effects of humic acid and NPK on protein content (%) at both locations.

Fat content responded significantly to treatments at both locations. At GR (1.41-1.63%), the lowest treatments were statistically inferior while the highest treatment (H4F2) was superior. Grdlanka showed similar patterns (1.39-1.54%), confirming that higher humic acid and NPK levels effectively increase grain fat content.

GR showed no treatment differences in ash content (1.61-1.72%). However, Grdlanka displayed significant variation (1.67-1.96%), with higher treatments producing more minerals. This location-specific response suggests GL conditions better support mineral uptake and movement into the grain.

Both locations showed significant fiber increases with higher treatments. At GR fiber rose from 2.75% to 2.94% (7% increase), while Grdlanka showed 2.76-3.01% (9% increase). The clear statistical differences at both sites confirm that humic acid and NPK fertilization effectively boosts grain fiber content.

Moisture content varied minimally (10.39-10.88% at GR, 10.59-10.83% at Grdlanka) with inconsistent patterns, suggesting post-harvest handling influences this trait more than pre-harvest nutrition. Carbohydrate content showed significant treatment responses at both locations, with Grderasha producing higher overall values (66.32-68.73%) compared to Grdlanka (65.79-66.80%). Energy values followed similar patterns, ranging from 2392-2470 kcal/100g at GR and 2376-2409 kcal/100g at Grdlanka. The higher energy at GR reflects its greater carbohydrate content.

The two locations produced different quality profiles: Grdlanka excelled in protein, gluten, and minerals, while GR had higher carbohydrates and energy content. Location strongly influenced how treatments performed, with significant interactions observed for most quality parameters. The highest humic acid and NPK combinations (H3-H4 with F1-F2) consistently improved grain quality, especially for fat, fiber, and carbohydrates. These findings demonstrate that integrated humic acid and NPK management enhances wheat quality, but location-specific conditions play a crucial role in determining both the size and significance of these improvements.

Both GL and GR exhibit relatively consistent fat content ranging from approximately 1.4-1.7% across most interactions, with minimal variation between the two groups. The

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majority of treatments show no significant differences, as indicated by shared letter designations (predominantly 'bc' and 'c').

A notable exception occurs at interaction HA1F2, where GL demonstrates a significantly elevated fat percentage (~1.7%, marked 'a') compared to other treatments. Conversely, interactions HA0F0 and HA3F1 show slightly higher values (marked 'ab') for both groups relative to the baseline treatments.

Statistical analysis reveals limited significant variation within each genotype across treatments, suggesting that fat accumulation in wheat grain remains relatively stable and is less responsive to the applied interaction conditions compared to protein content. The overlapping letter designations between GL and GR indicate no substantial genotypic differences in fat percentage, contrasting with the more pronounced differentiation observed in protein accumulation patterns (Figure 4).

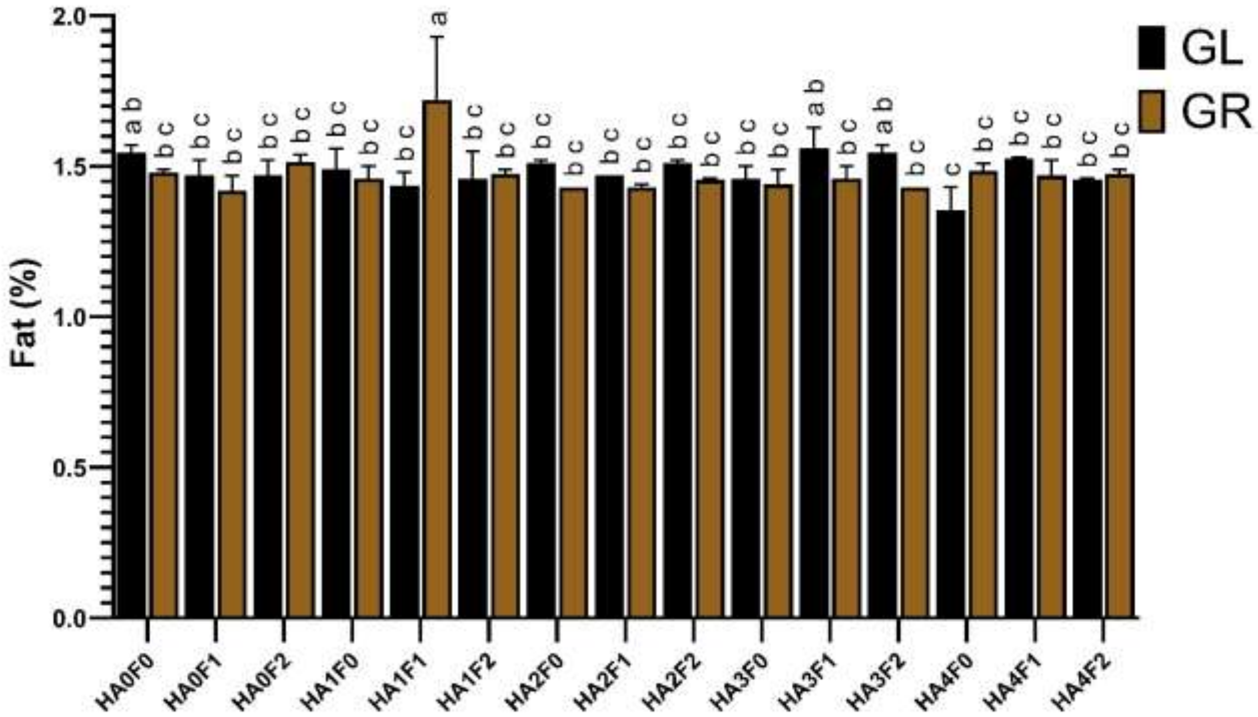

Figure 4. Interaction effects of humic acid and NPK on fat (%) at both locations.

Ash content, representing the mineral fraction of wheat grain, ranges from approximately 1.6-2.1% across all interactions. GL exhibits slightly higher ash percentages in certain treatments, particularly at HA1F0 and HA1F2 (both marked 'a' and 'ab', respectively), reaching peak values of ~2.0-2.2%. These treatments show significantly elevated ash content compared to most other interactions. GR demonstrates more uniform ash accumulation across treatments, with values predominantly marked 'c', indicating lower and more consistent mineral content (approximately 1.6-1.8%). A few interactions (HA0F0, HA1F0, HA2F2) show intermediate values (marked 'bc') in GR. Statistical analysis reveals that GL generally accumulates higher ash content than GR, particularly in specific interaction conditions (HA1F0, HA1F2), suggesting genotypic differences in mineral uptake or retention capacity. The majority of treatments, however, maintain relatively stable ash percentages around 1.6-1.8%, with letter designations 'bc' and 'c' predominating (Figure 5).

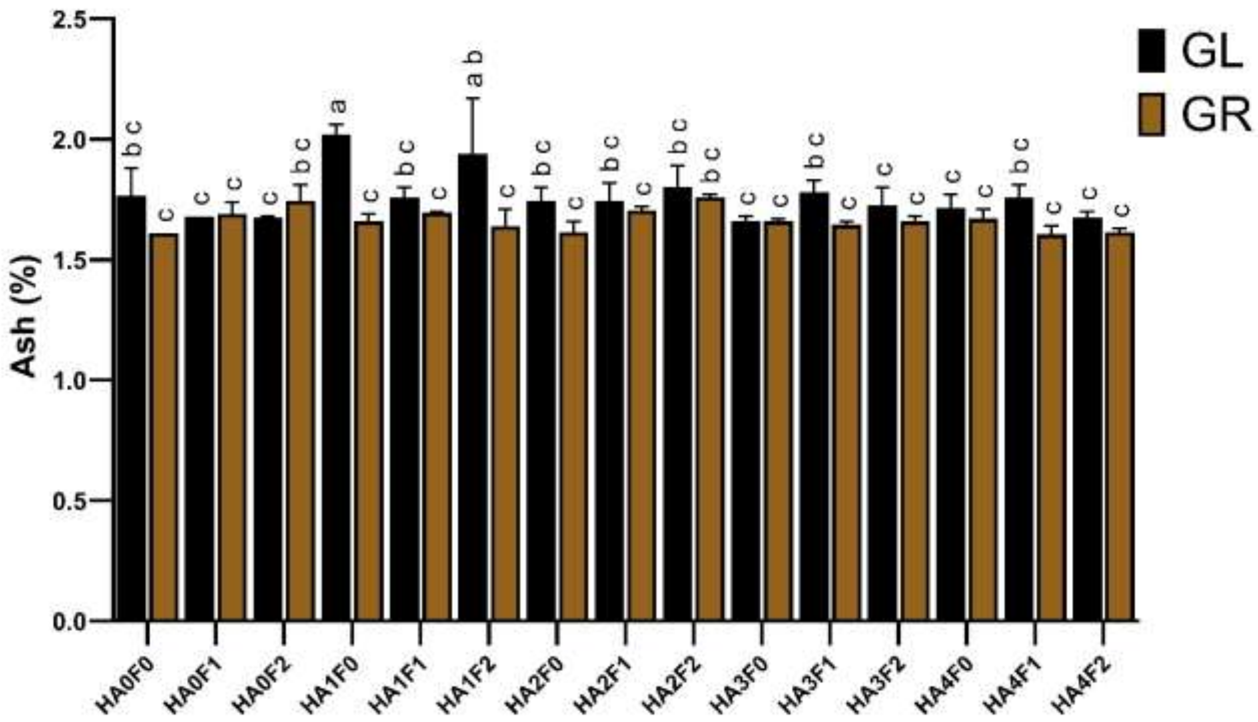

Figure 5. Interaction effects of humic acid and NPK on ash (%) at both locations.

Fiber content demonstrates remarkable stability across all interactions, ranging narrowly from approximately 2.7-3.0% for both genotypes. All treatments are marked with the letter 'a', indicating no statistically significant differences among any of the interaction conditions or between the two groups ($p>0.05$). Both GL and GR exhibit nearly identical fiber accumulation patterns, with values consistently hovering around 2.8-2.9% regardless of treatment application. The minimal error bars further confirm the low variability within each treatment group, suggesting that fiber content is highly conserved and minimally influenced by the experimental conditions tested.

This uniformity contrasts sharply with the variability observed in protein, fat, and ash content, indicating that fiber percentage in wheat grain is a stable biochemical parameter that remains largely unaffected by genotypic differences or environmental/treatment interactions. Such consistency suggests strong genetic regulation of fiber biosynthesis and accumulation, making it a reliable grain quality characteristic (Figure 6).

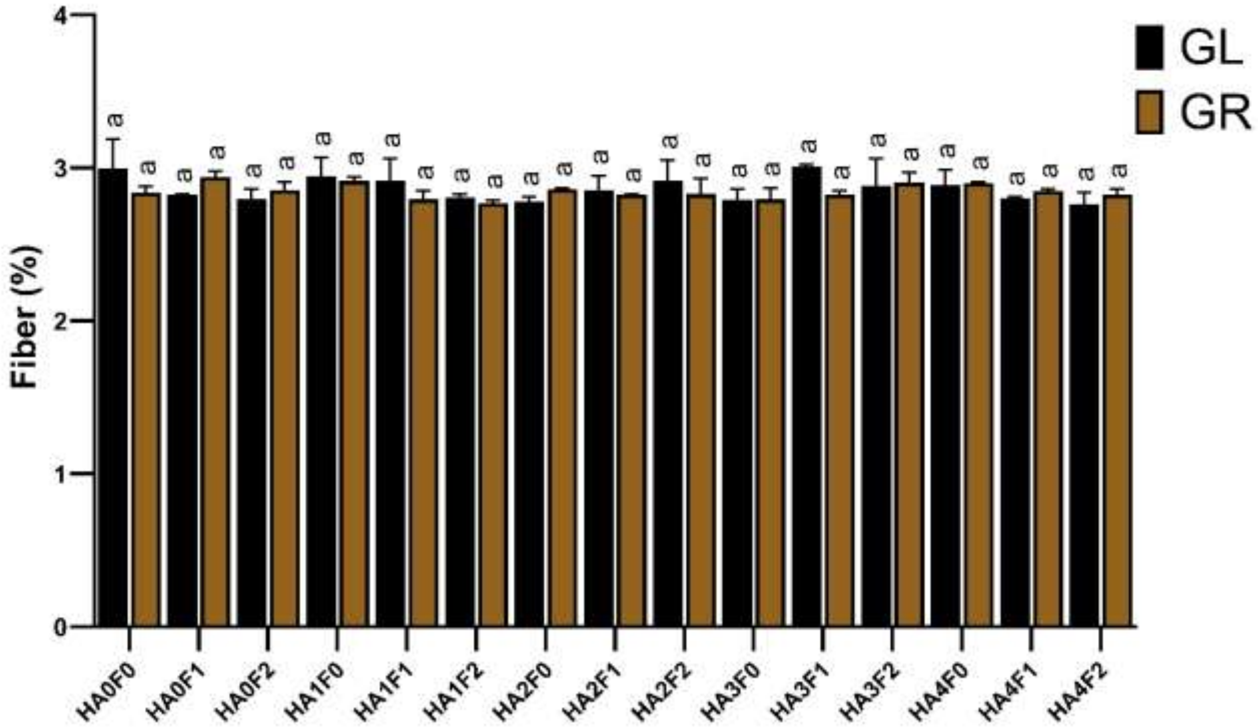

Figure 6. Interaction effects of humic acid and NPK on fiber (%) at both locations.

Moisture content exhibits relatively consistent values across all interactions, ranging from approximately 10.5-11.0% for both genotypes. Statistical analysis reveals minimal significant variation, with all treatments marked predominantly as 'a', 'ab', or 'b', indicating limited differentiation among interaction conditions (p>0.05). GL displays slightly lower moisture percentages in several treatments (marked 'a'), clustering around 10.5-10.8%, while GR tends toward marginally higher values (marked 'ab' or 'b'), typically ranging from 10.8-11.0%. However, these differences are relatively small and the overlapping letter designations suggest no substantial genotypic divergence in moisture retention capacity. The narrow range of variation and consistent error bars indicate that grain moisture content remains relatively stable across the experimental conditions tested. This stability is expected, as moisture percentage is largely determined by post-harvest handling and storage conditions rather than pre-harvest treatments or genotypic factors (Figure 7).

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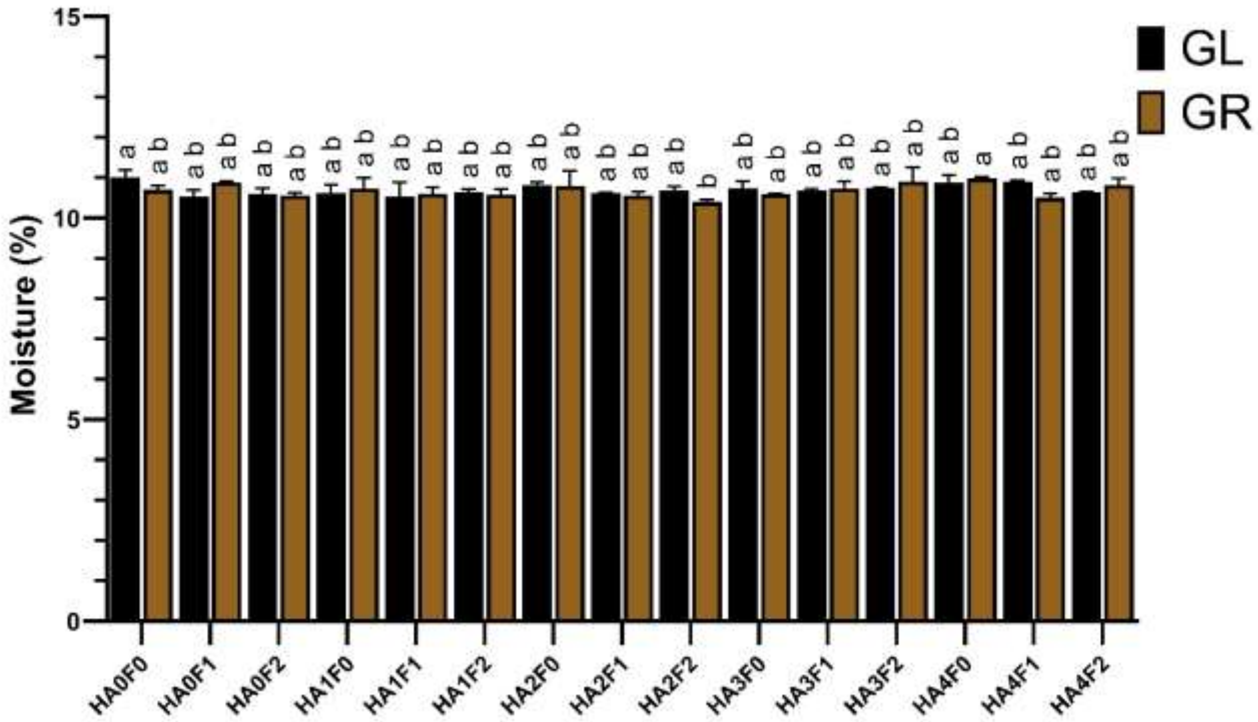

Figure 7. Interaction effects of humic acid and NPK on moisture (%) at both locations.

Carbohydrate content, representing the predominant macronutrient component in wheat grain, exhibits high values ranging from approximately 70-73% across all interactions. Both genotypes demonstrate remarkably similar carbohydrate accumulation patterns, with values consistently clustering around 72-73% for most treatments. Statistical analysis reveals minimal significant variation among treatments, with letter designations showing overlapping categories ('a', 'ad', 'abc', 'abcd', 'cd'). A few interactions display slightly elevated carbohydrate percentages (marked 'a'), including HA2F2 and HA3F0, while treatments HA4F0 and HA4F1 show marginally lower values (marked 'cd' and 'ad') around 71-72%.

The consistency in carbohydrate content across both genotypes and most treatments is noteworthy, as carbohydrates constitute the major fraction of grain dry matter. The inverse relationship between carbohydrates and other grain components (proteins, fats, ash, fibers, moisture) is reflected in the data, where variations in protein content (observed in earlier figures) are balanced by corresponding but minimal adjustments in carbohydrate percentage (Figure 8).

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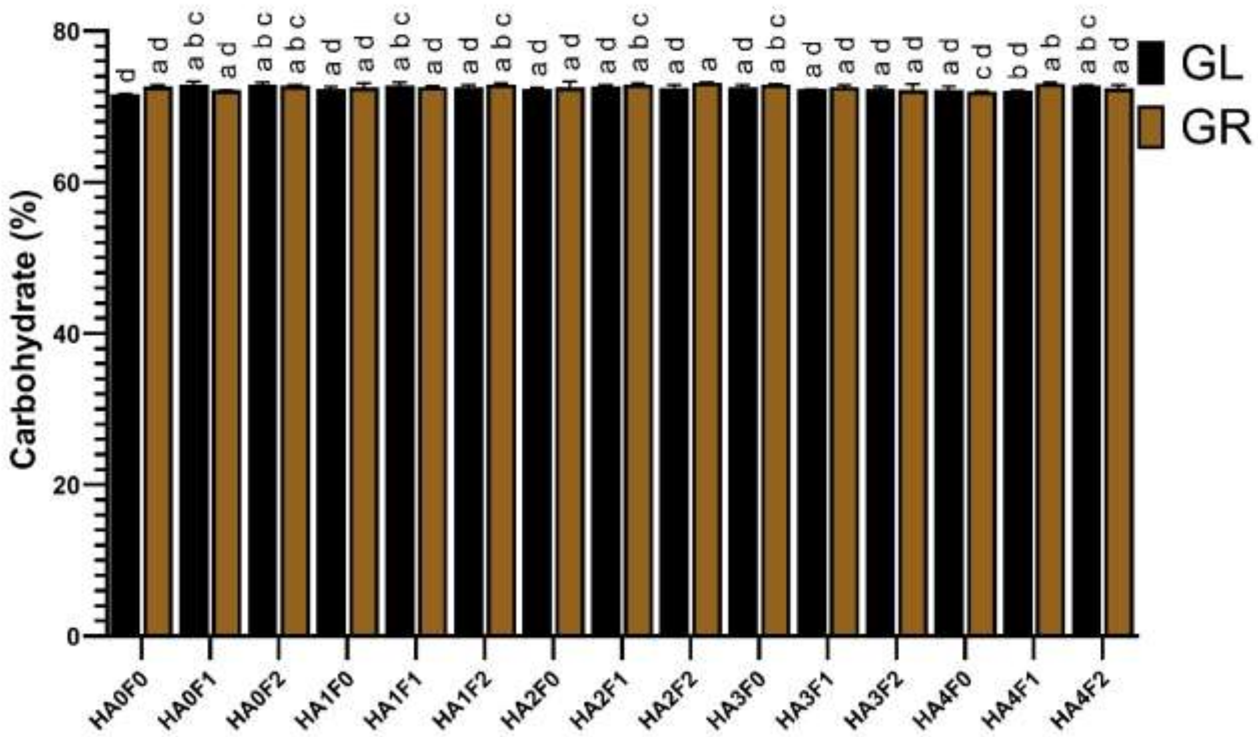

Figure 8. Interaction effects of humic acid and NPK on carbohydrate (%) at both locations.

Energy values remain remarkably consistent across all interactions, ranging from approximately 2400-2500% (likely representing kcal/100g or similar energy units) for both genotypes. Statistical analysis reveals minimal significant variation, with letter designations predominantly showing 'cd', 'bd', 'abc', and related overlapping categories, indicating no substantial differences among most treatments ($p>0.05$). Both GL and GR exhibit nearly identical energy content patterns, with values clustering tightly around 2450% across the majority of interactions. Treatments HA0F0 and HA4F1 display marginally higher values (marked 'a' or 'ab'), while most others maintain consistent energy levels (marked 'cd' or 'bd'). The minimal error bars and narrow range of variation confirm the stability of this parameter. The consistency in energy content directly reflects the stable carbohydrate percentages observed previously, as carbohydrates represent the primary energy-yielding component in wheat grain. The slight variations in energy content align with minor fluctuations in macronutrient composition (particularly proteins and fats), though these effects are minimal given the predominance of carbohydrates (Figure 9).

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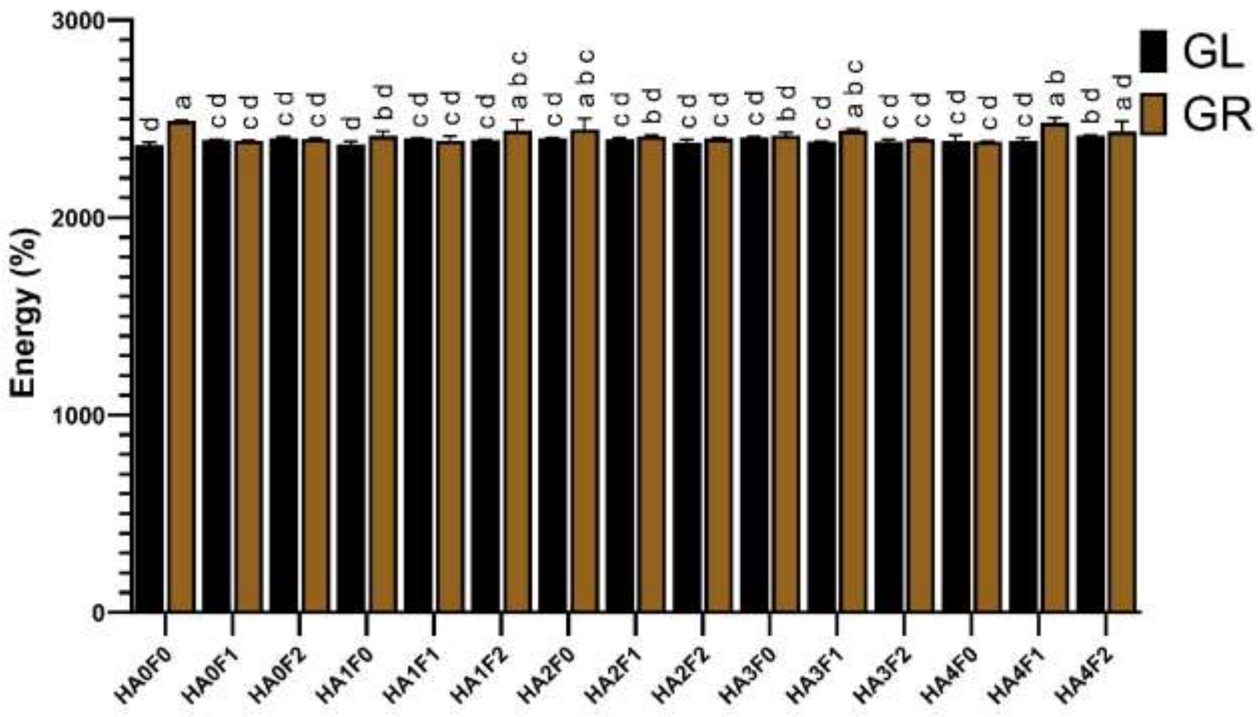

Figure 9. Interaction effects of humic acid and NPK on energy (%) at both locations.

Gluten content, a critical quality parameter for wheat bread-making properties, shows distinct patterns between genotypes. GL consistently maintains high gluten levels around 25-26% across all interactions, with values uniformly marked as 'a', indicating no significant differences within this group. In contrast, GR exhibits substantially lower gluten percentages, ranging from approximately 21-25%, with greater variability among treatments as indicated by different letter designations (a, b, c, ac, bc). Statistical analysis reveals significant genotypic differences, with GL demonstrating superior gluten accumulation capacity compared to GR. Within GR, certain interactions (HA0F0, HA2F0, HA4F2) show notably reduced gluten content (~21-22%, marked 'c' or 'bc'), while others approach GL levels. This pattern closely mirrors the protein content distribution observed previously, which is expected given that gluten proteins constitute a major fraction of total grain protein.

The stability of gluten content in GL across all treatments suggests strong genetic regulation and resilience to environmental or nutritional variations. Conversely, GR's responsiveness to interaction treatments indicates that gluten synthesis or accumulation in this genotype may be more susceptible to external factors (Figure 10).

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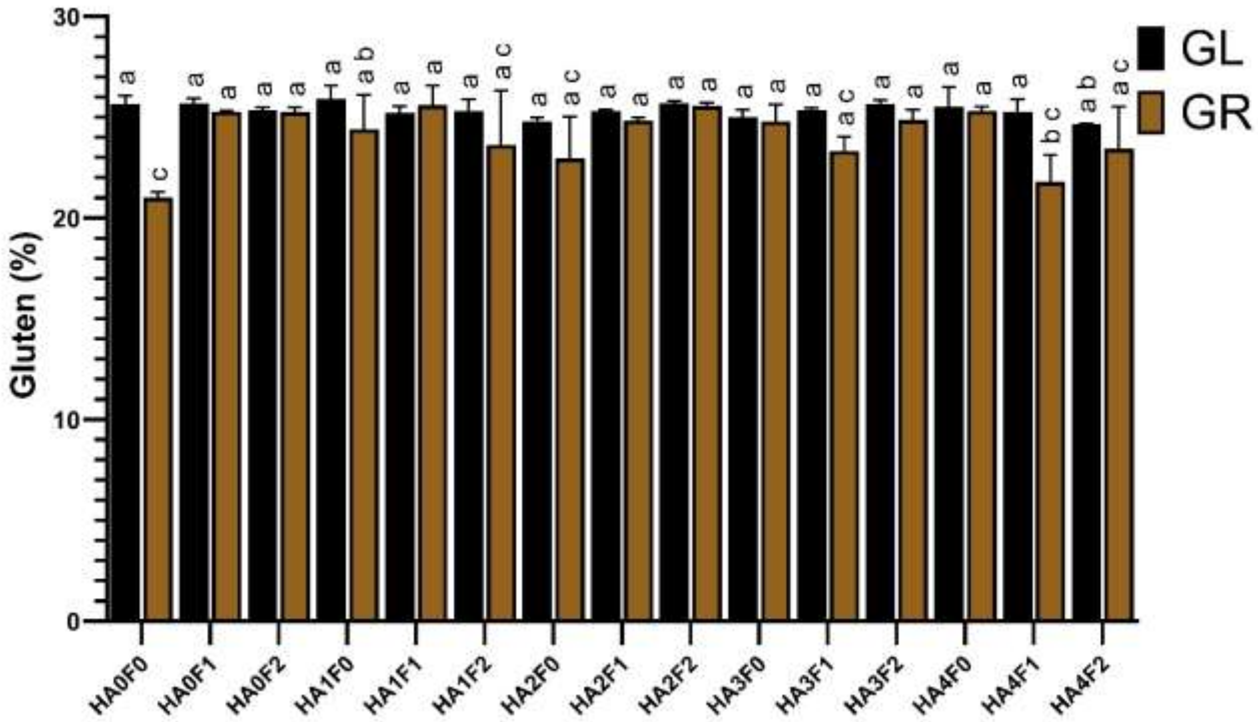

Figure 10. Interaction effects of humic acid and NPK on gluten (%) at both locations.

Protein levels ranged from 15.10-16.89% at GR and 16.62-17.14% at Grdlanka, with no statistical differences among treatments at either site, though Grdlanka consistently produced about 10% more protein. Similarly, gluten content showed no treatment differences, with Grdlanka averaging 12-13% higher values (24.94-25.71% versus 22.09-25.34%). The non-significant treatment responses contrast with findings that mycorrhiza and azotobacter combined with foliar-applied humic acid significantly increased protein content to 12.76% and gluten content to 30.90% (Lamlom et al., 2023) **[24]**, suggesting that biofertilizer integration may be necessary to enhance protein accumulation. Humic acid fertigation and its interaction with Azotobacter improved dry gluten and protein concentration of wheat grain and flour compared to control **[25]**, indicating that application method and biological interactions influence protein responses.

Fat content responded significantly to treatments at both locations. At GR (1.41-1.63%) and Grdlanka (1.39-1.54%), the highest treatment (H4F2) was superior, confirming that higher humic acid and NPK levels effectively increase grain fat content. Ash content showed location-specific responses—GR exhibited no differences (1.61-1.72%), while Grdlanka displayed significant variation (1.67-1.96%), with higher treatments producing more minerals. Fiber content increased significantly at both sites: GR showed 7% increase (2.75-2.94%), while Grdlanka exhibited 9% increase (2.76-3.01%). Humic-based products enhance plant growth through direct effects on nutrient uptake and assimilation, and indirect effects on soil physicochemical properties including enhanced nutrient availability **[15]**, explaining the improved mineral and fiber accumulation with integrated fertilization.

Carbohydrate content showed significant treatment responses, with GR producing higher values (66.32-68.73%) than Grdlanka (65.79-66.80%), corresponding to energy values of 2392-2470 versus 2376-2409 kcal/100g, respectively. The inverse

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relationship between protein and carbohydrate content resulted in distinct quality profiles: Grdlanka excelled in protein, gluten, and minerals, while GR had higher carbohydrates and energy. Humic acid-functionalized fertilizers regulate nutrient release and promote wheat productivity and grain quality **[26]**, supporting the observed quality improvements. The highest humic acid and NPK combinations (H4-H4 with F1-F2) consistently improved grain quality, particularly for fat, fiber, and carbohydrates,

Conclusions

Growing location played a decisive role in determining the productivity response of bread wheat to integrated humic acid and NPK fertilization. The GL (Grdlanka) site consistently outperformed GR, recording 32–35% more seeds per spike and 18–27% heavier seeds, reflecting more favorable growing conditions that supported superior grain development. Seed number per spike exhibited a clear dose-dependent response to integrated treatments at GL, where the highest combinations H4F1 and H4F2 significantly outperformed the control H0F0, suggesting that combined humic acid and NPK supply collectively enhances floret fertility and grain set. In contrast, seed weight remained unresponsive to all treatment combinations at both locations, indicating that individual grain filling is predominantly governed by genetic and environmental factors rather than external nutrient management, and that increasing nutrient supply is more effective in improving grain number than grain size.

Grain nutritional quality parameters responded differentially to integrated treatments and growing conditions. GL conditions produced 10–13% higher protein and gluten content, reflecting its suitability for bread-making quality, while GR conditions favored higher carbohydrate and energy values, indicating a location-driven trade-off between protein and carbohydrate accumulation. Among quality parameters, fat and fiber content showed the greatest

demonstrating that integrated management enhances wheat quality, though location-specific conditions critically determine the magnitude and significance of these improvements. Similarly, gluten content showed no treatment differences, though Grdlanka averaged 12-13% higher values (24.94-25.71% versus 22.09-25.34%). This superior protein and gluten content make Grdlanka wheat better suited for bread making.

sensitivity to integrated treatments, recording significant increases

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of 7–9% at higher treatment combinations, likely due to enhanced nutrient partitioning facilitated by the synergistic interaction between humic acid and NPK fertilization. Ash content exhibited location-specific responses, while protein and gluten remained statistically unaffected by treatments, confirming their stronger dependence on genetic and environmental control. Overall, the highest integrated combinations of H3 and H4 paired with F1 and F2 consistently produced the most favorable grain quality outcomes, representing the most effective strategy for simultaneously enhancing wheat productivity and nutritional quality under the tested conditions.

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