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RESPONSE OF INTEGRATED USE OF HUMIC ACID AND CHEMICAL FERTILIZER ON GROWTH AND YIELD OF RICE CROP (*ORYZA SATIVA* L.) IN CALCAREOUS SOIL

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Abstract

Poor soil organic carbon and low fertility are interrelated problems that can significantly negatively impact agricultural productivity and ecosystem health. Organic carbon is crucial to soil fertility as it supports soil structure, water retention, and nutrient availability. That's why scientists suggest using organic fertilizers to overcome this issue. Humic acid (HA) is one of such organic amendment that plays a critical role in soil health and fertility. Although a lot of work has been done so far on HA, the novelty of current study was the utilization of K and GA3-enriched humic acid. For experimental purposes, 3 levels of fertilizers (i.e., control, 50% and 100% recommended NPK) were applied with and without HA in rice. There were 3 replicates following the randomized complete block design (RCBD). Results showed that the 50% RDF application rate resulted in a 140.7% increase in plant height, while a 100% RDF application rate led to a 184.2% increase. Specifically, a 50% RDF application rate resulted in a 8.8% increase in spike length, while a 100% RDF application rate led to a 23.4% increase. The use of 50% RDF with humic acid resulted in a significant increase of 41.4%, while the application of 100% RDF with humic acid led to a 40.1% increase compared to the control treatment in 1000 grains weight. In conclusion, the combined use of humic acid and 50% RDF could prove a better strategy to obtain optimum yield in rice crop in calcareous soil than 100% RDF. However, 100% RDF can maximize plant height and spike length. More investigations are recommended at the field level to introduce GA3 and K-enriched HA as effective amendments to improve rice growth under limited fertilizer supply in poor, fertilized calcareous soils.

Key words: Humic acid, Gibberellic acid, rice, Chemical fertilization, Yield, Mineral nutrients.

Introduction

Rice is known as one of Asia's, South America's, Africa's, and Australia's most significant food crops (Yoshida, 1981). Rice is the second largest food crop and is also an exportable item. The crop production declined by 3.3 percent to 7.202 million tons. Low yields have been linked to deteriorating farmers' inability and soil fertility to apply the needed amounts of mineral fertilizers. Nitrogen (N) is the most yield-limiting and critical nutrient in rice cultivation worldwide (Lin *et al*., 2006). It plays a role in various plant activities because its fertilization significantly improve panicle length, dry matter, panicle number per square meter, which are interrelated with yield grain (Bansal *et al*., 1993). Plant requires N and K in relatively greater amount than other nutrients. Deficiency of macronutrients like N and K, resulted in restricted growth of plants, premature flowering, and chlorosis (Lincoln & Edvardo, 2006). It is necessary to supply plant nutrients according to its requirements to achieve the desired yield and fulfill plant nutrient demands. Various organic and inorganic sources of N are being used now a days, among which potassium humate is gaining attention now a days.

The organic matter (OM) content of the soil plays a major role in maintaining or restoring soil fertility. The combined use of inorganic and organic fertilizers has the potential to provide long-term production under intense cropping while sustaining an acceptable nutrient turnover in the plant-soil system. Potassium humate is an organic fertilizer enriched with mineral nutrients and studies also showed that it increased the efficiency of N's efficiency by promoting plant growth (Aman & Rab, 2013). The exact role of potassium humate although the exact mechanism for promoting plant growth is unknown, researchers have proposed several theories, including increased cell membrane permeability, micronutrient availability and transport, nutrient uptake, seed germination, respiration, root elongation and viability stimulation and photosynthesis (Mishra & Srivastava, 1988).

Humic acid (HA) has been used as a soil conditioner and fertilizer in agriculture on a small scale. Humic compounds have been shown to have important effects on plant development and soil structure. Potassium humate in the right quantities can help plants and roots develop faster (Ahmad *et al*., 2013). Many commercialized potassium (K) humate-based products have been created and are extensively sold in light of the potential of HA in agriculture. Most HA products come in the form of potassium (K) humate, a cheap soluble salt (Fong *et al*., 2007). The potassium salt of potassium humate is potassium humate. It is primarily utilized as a soil conditioner and is produced extensively by alkaline extraction of brown coal (lignite) Leonardite. The extraction is carried out in water with the application of potassium hydroxide (KOH), sequestering agents, and hydrotropic surfactants. Heat is used to improve the solubility of potassium humate, allowing for the extraction of additional potassium humate. It is used as a fertilizer addition in agriculture to improve the effectiveness of fertilizers, particularly phosphorus and nitrogen-based fertilizers. Other potassium (K) humate salts are produced, the most common of which is sodium humate, utilized in animal health supplements. It can also be used as an ingredient in aquaculture and oil drilling mud. There are few reports on the use of potassium humate in grain crops. As a result, the impact of HA in combination with chemical fertilizers on rice yield and growth was examined. This study aims to see how the combined application of chemical and HA fertilizer affects rice crop yield and development in calcareous soil.

Material and Methods

Experimental site: To check the response of HA when applied with chemical fertilizers field experiment was conducted at Govt. Reclamation Research Station Chak No.112/15-L, Mian Channu District Khanewal during two successive years (2021-22) in Kharif season. Each plot size was made 25 × 30 ft for cultivation of rice.

GA3 enriched humic acid preparation: Commercial scale humic acid was purchased from local industrial area. The humic acid had 10% humic acid and 3% potassium. After that 10g tablet of 10% GA3 was mixed in 1000ml of humic acid liquid. Final product was used for experimental purpose. The charateritics of humic acid and the experimental site soil were determined before the application of treatment (Table 1).

Treatment plan: The following treatments were used; Control, HA (6 L ha^{-1}) (Saha *et al*., 2013), T2=Recommended dose of fertilizer (RDF), T3=50% RDF + HA and T4= 100% RDF + HA. The field experiment was laid out in a randomized complete block design (RCBD) with three replications each treatment.

Fertilizer application: Recommended NPK i.e., 69, 41 and 32 kg/acer were applied at the time of transplantation. Phosphorus and potassium full doses were applied at the time of transplantation. However, nitrogen was added in 3 splits (basal dose, tillering and panicle initiation stage).

Irrigation: Total 16 irrigation of 4 inch per acer were applied according to the requirement of crop.

Harvesting and data collection: The crop was harvested at fully matured stage, and agronomic data i.e., number of productive tillers per m^{-2}, plant height (cm), 1000-grain weight (g), the straw yield and paddy (t ha^{-1}) were taken as per standard procedure.

Electrolyte leakage: To determine the electrolyte leakage (EL) in plants, a fresh plant sample weighing 0.2 g was taken and cut into small pieces. These pieces were then submerged in 10 ml of distilled water and left to incubate at a temperature of 25°C for 24 hours. The first electrical conductivity measurement (EC1) was taken using a pre-calibrated EC meter. To obtain the second electrical conductivity measurement (EC2), the test tubes containing the plant samples were heated in a water bath at a temperature of 120°C for 20 minutes (Bičárová *et al*., 2023). The final value of EL was calculated using the equation as follows;

$$\text{EL (\%)} = \text{EC1/EC2 x 100}$$

Chlorophyll contents: Small pieces of fresh leaves were taken, and 0.5 g leaf samples were immersed in 10 ml of acetone for a period of 24 hours. The chlorophyll extract was then measured, and the color intensity was determined using a spectrophotometer at wavelengths of 645 nm and 663 nm (Arnon, 1949). From intensity values, chlorophyll contents were determined by following formula:

$$\text{Chlorophyll a} \left(\frac{\text{mg}}{\text{g}}\right) = \frac{(12.7 \times \text{A663}) - (2.69 \times \text{A645}) \times \text{V}}{1000 \times \text{W}}$$

$$\text{Chlorophyll b} \left(\frac{\text{mg}}{\text{g}}\right) = \frac{(22.9 \times \text{A645}) - (4.68 \times \text{A645}) \times \text{V}}{1000 \times \text{W}}$$

$$\text{Total Chlorophyll} \left(\frac{\text{mg}}{\text{g}}\right) = 20.2(\text{OD } 645) + 8.02(\text{OD } 663) \times \text{V}/1000\ (\text{W})$$

where, OD = Optical density (wavelength), V = Final volume made, W = Fresh leaf made (g)

Gas exchange attributes: An Infrared gas analyzer (CI-340 Photosynthesis system, CID, Inc. USA) was used to determine the photosynthetic rate and stomatal conductance of rice leaves. The measurements were taken on a sunny day between 10:30 and 11:30 AM when the intensity of light was saturating.

Plant digestion: The plant shoot, leaves, and grains samples were separately oven dried and ground into a homogeneous powder. A di-acid mixture was prepared using a ratio of 10:1:4 of HNO_3, H_2SO_4, and $HClO_4$. The plant material was mixed with concentrated H_2SO_4 and left overnight. Before heating, a 10 ml acid mixture was added to the suspension. The mixture was heated until the solution cleared, and white fumes emerged. The mixture was then cooled and stored for further nutrient analysis (Miller, 1998).

Phosphorus determination: A 10 ml solution of the prepared plant sample was transferred to a flask, and 10 ml of ammonium heptamolybdate and vanadate reagent were added to it. The solution was then diluted with distilled water. A series of standards with concentrations of 0.5, 1, 1.5, 2, and 2.5 ppm were prepared in test tubes and 10 ml of ammonium vanadate reagent was added to each tube. After a 30-minute break, the standards were analyzed using a spectrophotometer at a wavelength of 410 nm (Rashid, 1986).

$$\text{P (\%)} = \text{ppm P (from clibration graph)} \times \frac{\text{Vol}_1}{\text{Wt}} \times \frac{100}{\text{Vol}_2} \times \frac{1}{10000}$$

Table 1. Characteristics of soil properties and humic acid used in experiment.

Soil properties	Year 2019-20	Year 2020-21	References
pH	8.17	8.34	(Page *et al.*, 1982)
EC (dS/m)	1.23	2.12	(Rhoades, 2018)
OM (%)	0.68	0.58	(Nelson & Sommers, 2018)
N (%)	0.02	0.02	(Bremner, 1996)
P (mg/kg)	3.87	4.56	(Kuo, 1996)
K (mg/kg)	75	88	(Pratt, 2016)
Texture	Sandy loam	Sandy loam	(Gee & Bauder, 1986)

Potassium determination: The prepared plant sample was used to know the potassium content by using flame photometer (Pratt, 2016).

Nitrogen determination: To prepare the plant sample for analysis, 1 g of plant material was placed in a digestion tube, and 1 g of catalyst mixture was added to it. The tube was left to sit overnight. The next day, 10 ml of H_2SO_4 was added, and the digestion tube was heated using a block digester until a colorless liquid was obtained. The sample was then distilled using a distillation unit, and the distillate was collected in 20 ml of 4% boric acid. Three to four drops of indicator were added, and the color of the solution changed to purple. After distillation, the solution's color changed to golden yellow. The golden solution was then titrated against 0.1 N sulfuric acid to a purplish end-point (Ryan *et al*., 2001).

$$\mathrm{N}\ (\%) = \frac{14.1 \times \text{Vol. of sulphuric acid used in sample} - \text{Vol. of sulphuric acid used iin blank} \times \text{N of acid}}{\text{wt of sample} \times 10}$$

Statistical analysis

The data for all parameters was statistically analyzed by following standard statistical procedure (Steel *et al*., 1997) using "OriginPro2021" software (Anon., 2021). The average of the results was compared using a randomized complete block (RCB) model, and the importance of the data assembled from the experiment was determined using the analysis of variance technique (ANOVA). The LSD test was used to determine whether the treatment means differed substantially.

Results

In terms of the effect of humic acid on plant height, the data revealed that HA resulted in a higher plant height than the control treatment, with an increase of 93.7% for the 50% RDF and 63.1% for the 100% RDF application rates. These results suggest that HA can promote plant growth and development, consistent with previous studies showing the positive effects of humic acid on plant growth. Regarding the effect of fertilizer application rates on plant height, the results demonstrated that the use of fertilizer led to a significant increase in plant height compared to the control treatment. The 50% RDF application rate resulted in a 140.7% increase in plant height, while a 100% RDF application rate led to a 184.2% increase. When considering the combined effects of both factors, the results showed that the highest plant height was achieved by applying both HA and fertilizer at 100% RDF, resulting in a 315.1% increase compared to the control treatment (Fig. 1A).

Regarding the effect of humic acid on spike length, the results indicated that the use of HA resulted in a slightly higher spike length compared to the control treatment, with an increase of 0.9% for the 50% RDF and 16.3% for the 100% RDF application rates. The effect of fertilizer application rates on spike length demonstrated that fertilizer led to a significant increase in spike length compared to the control treatment. Specifically, a 50% RDF application rate resulted in a 8.8% increase in spike length, while a 100% RDF application rate led to a 23.4% increase. When considering the combined effects of both factors, the results showed that the highest spike length was achieved with the application of both HA and fertilizer at 100% RDF, resulting in a 29.0% increase compared to the control treatment (Fig. 1B).

The results indicated that the use of humic acid led to a moderate increase in the number of spikes, with a rise of 31.3% observed for both 50% and 100% RDF application rates when compared to the control treatment. Additionally, the results showed that the application of fertilizer led to a significant increase in the number of spikes, with a 57.7% and 178.7% rise noted for 50% and 100% RDF application rates, respectively, when compared to the control treatment. Moreover, when examining the combined effects of humic acid and fertilizer, the results showed that the application of both factors at 50% RDF resulted in the highest number of spikes, with a 299.4% increase observed when compared to the control treatment (Fig. 1C).

The results indicated that the use of humic acid had a moderate effect on the number of tillers, with a 27.2% increase observed for the 50% RDF application rate compared to the control treatment. Similarly, a significant increase of 44.4% was observed for the 100% RDF application rate compared to the control treatment. Furthermore, the results revealed that the application of fertilizer had a significant effect on the number of tillers, with a 50% and 100% RDF application rate resulting in a 87.5% and 45.8% increase, respectively, compared to the control treatment. Additionally, when examining the combined effects of humic acid and fertilizer, the results showed that the highest number of tillers was achieved with the application of humic acid and fertilizer at 50% RDF, resulting in a 58.3% increase compared to the control treatment (Fig. 1D).

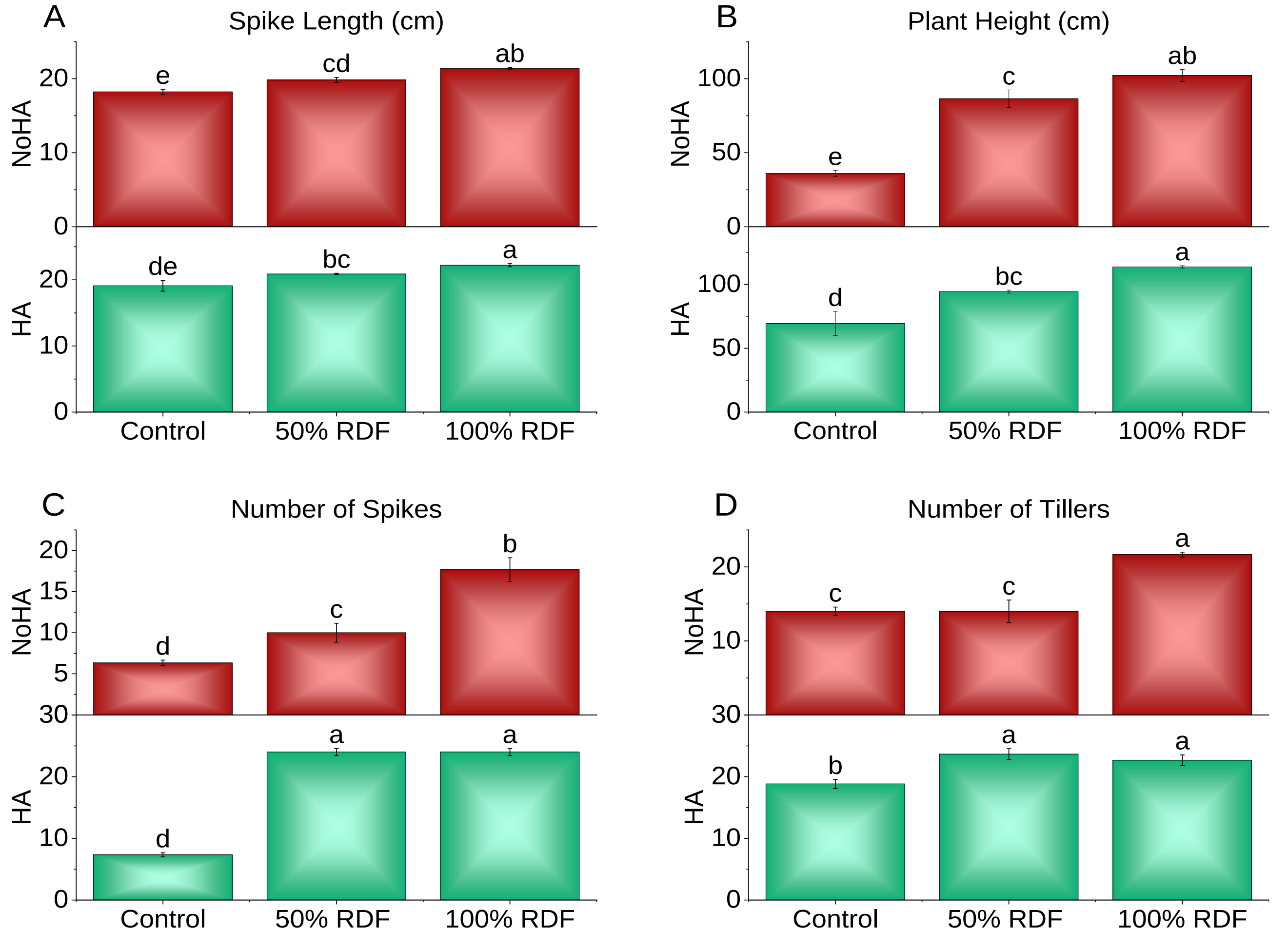

Fig. 1. The impact of different levels of recommended fertilizer application rate, with and without the addition of recommended humic acid application, on the growth parameters of rice, including spike length (A), plant height (B), number of spikes (C), and number of tillers (D). The bars presented represents the average of three replicates, with standard error (SE). Statistical analysis using Fisher LSD revealed significant differences ($p \leq 0.05$) between treatments, as indicated using different letterings on the bars. Recommended dose of fertilizer = RDF; Humic acid = HA.

NoHA Control treatment resulted in an average 1000 grains weight of 17.56 g, while the NoHA 50% RDF treatment showed a slightly higher weight of 18.69g. However, the NoHA 100% RDF treatment had the highest 1000 grains weight of 21.67 g, indicating a positive effect of the increased fertilizer application rate on the weight of the grains. For the HA treatments, the control group had an average weight of 21.19 g, which was surpassed by both the HA 50% RDF and HA 100% RDF treatments. The 50% RDF HA treatment had a 1000 grains weight of 24.82 g, while the HA 100% RDF treatment had a slightly lower weight of 24.53 g. The use of 50% and 100% RDF application rates led to a moderate increase in 1000 grains weight compared to the control treatment, with an increase of approximately 6.6% and 23.7%, respectively. However, when humic acid was applied along with fertilizer, the effect on 1000 grains weight was more pronounced. The use of 50% RDF with humic acid resulted in a significant increase of 41.4%, while the application of 100% RDF with humic acid led to a 40.1% increase compared to the control treatment (Fig. 2A).

Based on results, the highest mean yield of rice per plant was obtained from the treatment with HA applied at 100% RDF, with a mean yield of 42.42 grams per plant. This was followed by the treatment with HA applied at 50% RDF, with a mean yield of 40.22 grams per plant. The treatment with NoHA at 100% RDF also had a relatively high mean yield of 33.99 grams per plant. The mean yield of the NoHA treatment at 50% RDF was 29.38 grams per plant, which was lower than the mean yields of the HA treatments at both 50% and 100% RDF. The lowest mean yield was obtained from the NoHA treatment at control, with a mean yield of 17.63 grams per plant. Compared to the control treatment, which had a mean yield of 17.63 grams per plant, the HA treatments at both 50% and 100% RDF resulted in a significant increase in yield. The HA treatment at 50% RDF showed a 128.3% increase in yield compared to the control, while the HA treatment at 100% RDF showed a 141.9% increase in yield compared to the control. Similarly, the NoHA treatment at 100% RDF showed a 93.9% increase in yield compared to the control (Fig. 2B).

According to results, the HA treatment at 50% RDF had the highest average photosynthetic rate of 26.33 µmol $CO_2/m^2/s$, followed by the NoHA treatment at 100% RDF with an average rate of 22.52 µmol $CO_2/m^2/s$. The HA treatment at control also had a relatively high mean rate of 17.84 µmol $CO_2/m^2/s$. Conversely, the mean rates of the NoHA treatment at control, 50% RDF, and HA treatment at 100% RDF were 9.35 µmol $CO_2/m^2/s$, 12.88 µmol $CO_2/m^2/s$, and 24.3 µmol $CO_2/m^2/s$, respectively. Compared to the control treatment, which had an average photosynthetic rate of 9.35 µmol

$CO_2/m^2/s$, all the treatments significantly increased photosynthetic rate. The HA treatment at 50% RDF exhibited the highest increase in rate with a 181.1% rise compared to the control. The NoHA treatment at 100% RDF showed a 140.3% increase in rate compared to the control, while the HA treatment at control showed a 90.2% increase in rate compared to the control. Additionally, the HA treatment at 100% RDF and NoHA treatment at 50% RDF also showed significant increases in photosynthetic rate compared to the control, with increases of 159.6% and 37.7%, respectively (Fig. 2C).

The NoHA treatment had a baseline electrolyte leakage value of 26.9%, while the NoHA treatment at 50% RDF resulted in a 2.49% decrease in leakage compared to the control. However, the NoHA treatment at 100% RDF resulted in a 9.17% decrease in leakage compared to the control. On the other hand, the HA treatment at control resulted in a 16.08% decrease in leakage compared to the control. However, the HA treatment at 50% RDF resulted in a 28.86% decrease in leakage compared to the control, which was the largest decrease among all treatments. The HA treatment at 100% RDF resulted in a 36.07% decrease in leakage compared to the control (Fig. 2D).

The NoHA treatment had a baseline straw N content value of 0.88333, while the NoHA treatment at 50% RDF resulted in a 36.92% increase in straw N content compared to the control. The NoHA treatment at 100% RDF showed an even greater increase in straw N content, with a 51.1% increase compared to the control. On the other hand, the HA treatment at control resulted in a 24.13% increase in straw N content compared to the control. The HA treatment at 50% RDF showed a further increase in straw N content, with a 49.46% increase compared to the control, which was the largest increase among all treatments. The HA treatment at 100% RDF resulted in a similar straw N content value as the NoHA treatment at the same level, showing a 50.55% increase compared to the control (Fig. 3A).

The baseline grain N content value for the NoHA treatment was 0.44. Compared to the control, the NoHA treatment at 50% RDF showed a significant increase of 54.15% in grain N content, while the NoHA treatment at 100% RDF showed a higher increase of 65.91%. Meanwhile, the HA treatment at control resulted in a significant 37.06% increase in grain N content compared to the control. The largest increase was observed in the HA treatment at 50% RDF, with a significant 74.24% increase in grain N content compared to the control. The HA treatment at 100% RDF resulted in a grain N content value similar to that of the NoHA treatment at the same level, showing a 75% increase compared to the control (Fig. 3B).

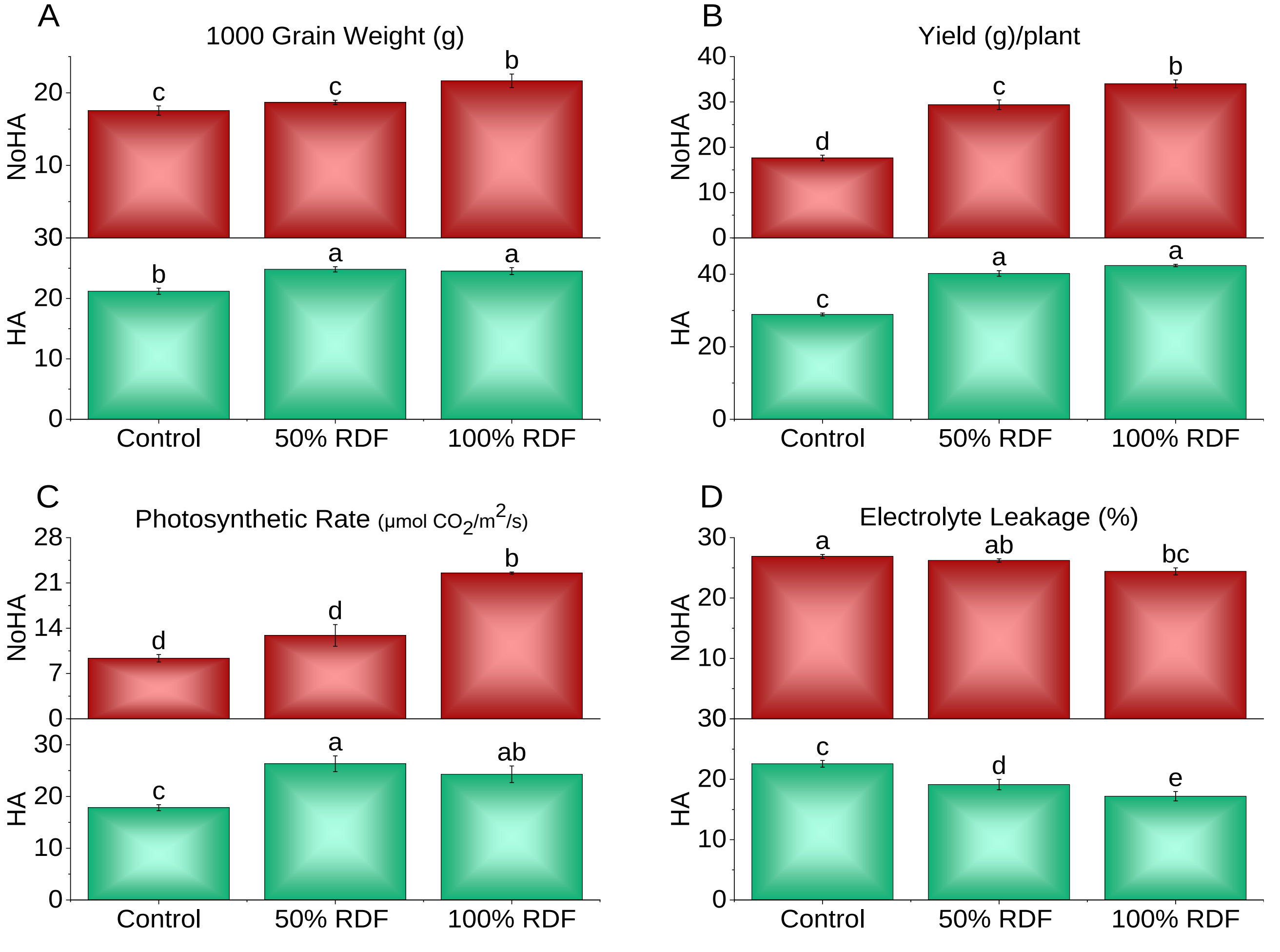

Fig. 2. The impact of different levels of recommended fertilizer application rate, with and without the addition of recommended humic acid application, on the growth parameters of rice, including 1000 grains weight (A), yield (B), photosynthetic rate (C), and electrolyte leakage (D). The bars presented represents the average of three replicates, with standard error (SE). Statistical analysis using Fisher LSD revealed significant differences ($p \leq 0.05$) between treatments, as indicated by the use of different letterings on the bars. Recommended dose of fertilizer = RDF; Humic acid = HA.

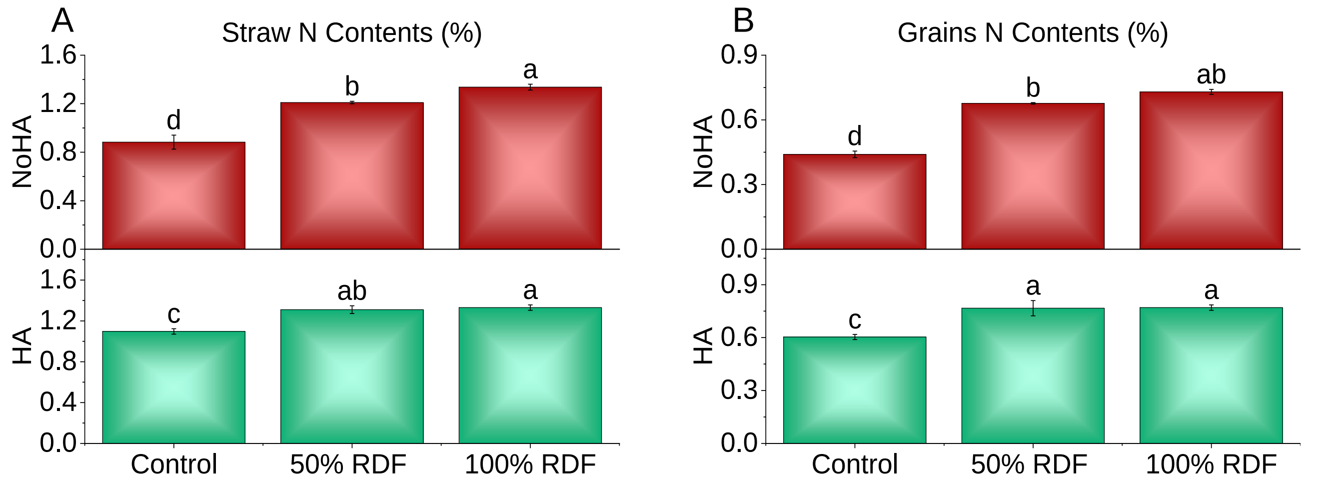

Fig. 3. The impact of different levels of recommended fertilizer application rate, with and without the addition of recommended humic acid application, on the growth parameters of rice, including straw N contents (A) and grains N contents (B). The bars presented represents the average of three replicates, with standard error (SE). Statistical analysis using Fisher LSD revealed significant differences ($p \leq 0.05$) between treatments, as indicated by the use of different letterings on the bars. Recommended dose of fertilizer = RDF; Humic acid = HA.

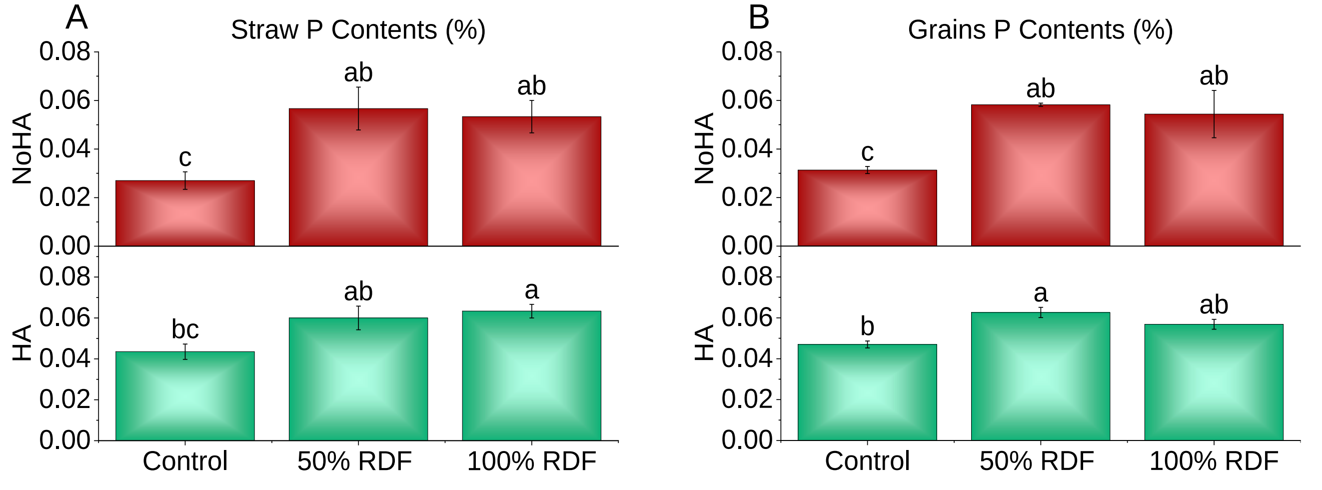

Fig. 4. The impact of different levels of recommended fertilizer application rate, with and without the addition of recommended humic acid application, on the growth parameters of rice, including straw P contents (A) and grains P contents (B). The bars presented represents the average of three replicates, with standard error (SE). Statistical analysis using Fisher LSD revealed significant differences ($p \leq 0.05$) between treatments, as indicated by the use of different letterings on the bars. Recommended dose of fertilizer = RDF; Humic acid = HA.

Compared to the control, the NoHA treatment at 50% RDF showed a significant increase of 109% in straw P content, with a value of 0.057. The NoHA treatment at 100% RDF also showed an increase in straw P content, with a value of 0.053. The HA treatment at control resulted in a straw P content value of 0.044, which was a 58.97% increase compared to the control. The HA treatment at 50% RDF also showed a significant increase of 120% in straw P content compared to the control, with a value of 0.06. The HA treatment at 100% RDF showed a slightly higher value of 0.06333, indicating a further increase in straw P content compared to the other treatments (Fig. 4A).

For grains P content, the NoHA treatment at control had a value of 0.03133, while the NoHA treatment at 50% RDF resulted in a 85.96% increase in grain P content compared to the control. The NoHA treatment at 100% RDF showed a similar value as the NoHA treatment at 50% RDF, with a 74.28% increase compared to the control. The HA treatment at control resulted in a 50.12% increase in grain P content compared to the control. The HA treatment at 50% RDF showed a further increase in grain P content, with a 99.94% increase compared to the control, which was the largest increase among all treatments. The HA treatment at 100% RDF resulted in a value similar to the NoHA treatment at the same level, showing a 81.2% increase compared to the control (Fig. 4B).

The results for straw K content showed that all three levels of RDF led to a significant increase in K content compared to the control for both NoHA and HA treatments. The NoHA treatment showed a 79.87% increase in K content at 50% RDF and a 75.15% increase at 100% RDF compared to the control. Similarly, the HA treatment showed a 28.45% increase at control, a 28.57% increase at 50% RDF, and a 36.44% increase at 100% RDF compared to the control. These findings suggest that the use of RDF in conjunction with NoHA or HA can significantly increase straw K content (Fig. 5A).

The NoHA treatment had a baseline grain K content value of 0.59333, while the NoHA treatment at 50% RDF resulted in a 56.59% increase in grain K content compared to the control. The NoHA treatment at 100% RDF showed a slight decrease in grain K content, with a 29.22% decrease compared to the control. On the other hand, the HA treatment at control resulted in a 27.78% increase in grain K content compared to the control. The HA treatment at 50% RDF showed a further increase in grain K content, with a 55.60% increase compared to the control. The HA treatment at 100% RDF resulted in a slight increase in grain K content, showing a 63.26% increase compared to the control (Fig. 5B).

Principal Component Analysis (PCA) was performed on a set of 18 attributes related to the growth and yield of wheat plants. The results of PCA are presented in the table above, which includes the Eigenvalue, Percentage of Variance, Cumulative Percentage, and scores for the first two principal components (PC1 and PC2). PC1 explained 67.5% of the total variation and was positively loaded with Plant Height, 1000 Grain Weight, Yield (g)/plant, Photosynthetic Rate, Electrolyte Leakage, Straw N Contents, Grains N Contents, Straw P Contents, Grains P Contents, Straw K Contents, Grains K Contents, and pH. This suggests that these attributes have a strong correlation with each other and can be summarized by PC1. PC2 explained 10.1% of the total variation and was positively loaded with Spike Length, Number of Spikes, Number of Tillers, and EC (dS/m), and negatively loaded with Plant Height, 1000 Grain Weight, and Yield (g)/plant (Fig 6). This indicates that these attributes have a weaker correlation with each other and can be summarized by PC2. Furthermore, the PCA results suggest that attributes such as OM, N, P (mg/kg), and K (mg/kg) have low Eigenvalues and do not contribute much to the total variation. Overall, the PCA results can be used to identify the most important attributes for the growth and yield of wheat plants and to reduce the dimensionality of the data for further analysis (Table 2).

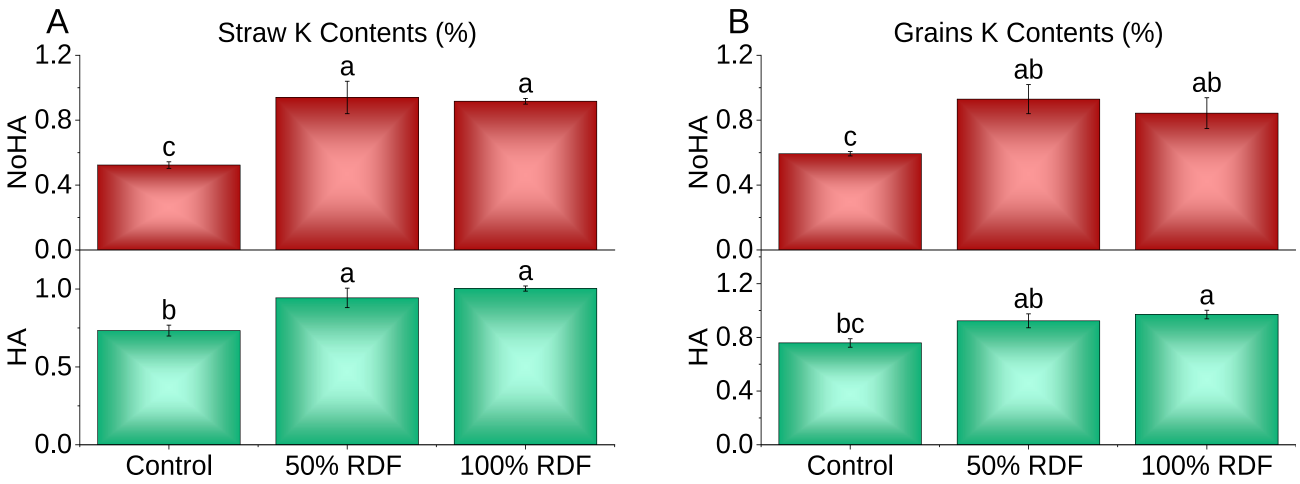

Fig. 5. The impact of different levels of recommended fertilizer application rate, with and without the addition of recommended humic acid application, on the growth parameters of rice, including straw K contents (A) and grains K contents (B). The bars presented represents the average of three replicates, with standard error (SE). Statistical analysis using Fisher LSD revealed significant differences ($p \leq 0.05$) between treatments, as indicated by the use of different letterings on the bars. Recommended dose of fertilizer = RDF; Humic acid = HA.

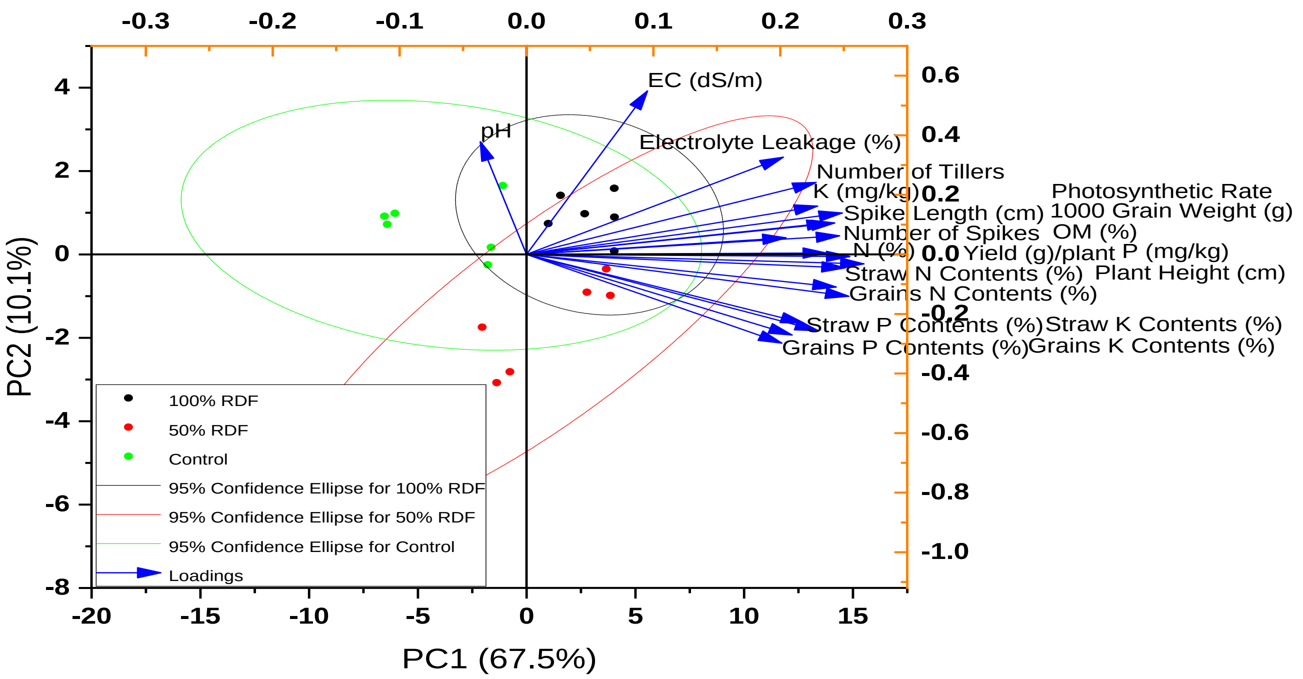

Fig. 6. Principal component analysis for studied attributes.

Table 2. Eigenvalues of studied PCA attributes.

Principal component number	Eigenvalue	Percentage of variance (%)	Cumulative (%)	PC1 (67.5%)	PC2 (10.1%)
Plant height (cm)	13.49981	67.49904	67.49904	0.251	-0.04543
Spike length (cm)	2.01922	10.09611	77.59515	0.23932	0.10653
Number of spikes	1.54456	7.72279	85.31795	0.24657	0.06204
Number of tillers	0.80934	4.04669	89.36464	0.22816	0.2412
1000 Grain weight (g)	0.61189	3.05944	92.42408	0.24276	0.10564
Yield (g)/plant	0.37793	1.88966	94.31374	0.26588	-0.03178
Photosynthetic RATE	0.33206	1.66031	95.97404	0.24879	0.13897
Electrolyte leakage (%)	0.24799	1.23994	97.21399	0.2023	0.32626
Straw N contents (%)	0.14388	0.71941	97.9334	0.24399	-0.10961
Grains N contents (%)	0.12756	0.6378	98.5712	0.25393	-0.14054
Straw P contents (%)	0.08038	0.40189	98.97309	0.21869	-0.23087
Grains P contents (%)	0.0643	0.32152	99.2946	0.20119	-0.298
Straw K contents (%)	0.05338	0.26691	99.56151	0.22991	-0.25656
Grains K contents (%)	0.04179	0.20895	99.77046	0.20921	-0.27093
pH	0.03508	0.1754	99.94586	-0.03639	0.37828
EC (dS/m)	0.00741	0.03705	99.98291	0.0953	0.54857
OM (%)	0.00342	0.01709	100	0.20439	0.05553
N (%)	1.76025E-30	8.80123E-30	100	0.23627	0.00456
P (mg/kg)				0.25471	-0.0072
K (mg/kg)				0.22934	0.16237

Discussion

When GA3 and humic acid are mixed together and applied to rice plants, they can work together to enhance plant growth and development in multiple ways. One of the primary benefits of this combination is an increase in chlorophyll content, which is essential for photosynthesis and plant growth (Anwar *et al*., 2020, Ali *et al*., 2022). Humic acid contains a variety of functional groups, including carboxylic acids, phenols, and quinones, which can interact with GA3 through hydrogen bonding and other non-covalent interactions (Souza *et al*., 2022). These interactions can enhance the uptake and translocation of GA3 within the plant (Mahmood *et al*., 2019), increasing cell division and elongation (Abd El-Naby *et al*., 2019). As a result, the plant can produce more chlorophyll, which is essential for photosynthesis and plant growth (Yang *et al*., 2023). In addition to improving chlorophyll content, the combination of GA3 and humic acid can also decrease electrolyte leakage in rice plants (Hizal *et al*., 2023, Ritonga *et al*., 2023). Electrolyte leakage is a measure of membrane damage in plant cells, which can occur due to various stresses such as drought, salinity, and extreme temperatures. When plant cells are damaged, electrolytes such as potassium and sodium leak out, leading to decreased plant growth and yield (Bičárová *et al*., 2023). Studies have shown that the combined use of GA3 and humic acid can reduce electrolyte leakage in rice plants, indicating that the plant cells are less damaged and more resistant to stress (Hizal *et al*., 2023, Ritonga *et al*., 2023). The chelating properties of humic acid may play a role in this effect, as they can help to bind minerals and other nutrients in the soil, making them more available to the plant (Nikoosefat *et al*., 2023). Humic acid can increase soil organic matter content by acting as a soil conditioner (Chen *et al*., 2023). It can help to break down organic matter in the soil, releasing and increasing nutrients uptake essential for plant growth (Rahi *et al*., 2021). Overall, the combination of GA3 and humic acid can benefit rice plants, including improved growth, yield, chlorophyll content, and stress tolerance. By enhancing the uptake and translocation of GA3 and improving nutrient availability through the chelating properties of humic acid, this combination can help to maximize the potential of rice plants and increase crop productivity.

Conclusion

Applying humic acid and chemical fertilizer improved rice crop growth and yield in calcareous soil. The combined effect of HA and 50% RDF increase the plant height, spike length, grain yield, and soil fertility by increasing the efficiency of applied nutrients. No dominant change was noted in 1000 grains weight when 50% and 100% RDF were applied with GA3 and K enriched HA. Gowers are suggested to incorporate 50% RDF along with HA for improvement in rice growth and yield. However, for maximization of growth and yield of rice 100% RDF and HA is recommended. As a future prospectives more investigation s are suggested at field levels on different cereal crops especially consideration cost analysis for declaration of 50% RDF and HA as effectives amendment compared to 100% RDF and HA.

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