



# Quality and Yield of Rice Grain: Effects of Humic Acid and Bean Cake Fertilizers Under Water-Saving Conditions

Zheng Ennan\*, Yin hao Zhu\* and Tianyu Xu\*†

\*School of Hydraulic and Electric Power, Heilongjiang University, Harbin, 150080, China

†Corresponding author: Tianyu Xu: 1044459115@qq.com

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## ABSTRACT

Rice quality and yield traits response to fertilizers under varying field conditions were obtained in our previous study. A better understanding of the intrinsic mechanism of fertilization in regulating rice quality and yield supports field operations and recommendations. This study investigated the potential role of humic acid and bean cake fertilizers as opposed to traditional nitrogen fertilizer (urea) in regulating rice quality and yield traits under water-saving conditions by identifying the quality and yield indicators. Results demonstrated that the application of humic acid and bean cake fertilizers affected the quality and yield compared with the traditional nitrogen fertilizer (urea), which alleviated the deterioration of rice quality and yield caused by excessive fertilizer amounts. The conclusions are that the addition of humic acid and bean cake fertilizers can improve grain quality.

## INTRODUCTION

Rice feeds 50 percent of the global population, and demand is projected to increase by 28 percent by 2050 (Bahuguna 2017). In addition, the demand for high-quality rice is increasing due to rising economic development (Tilman et al. 2011, Foley et al. 2011, Coast et al. 2015), especially in China, the country with the largest population in the world. Rice quality and yield traits are usually affected by many factors such as fertilizers, irrigation methods, planting densities, radiation use efficiency, and climatic conditions (Zhang et al. 2019, Zheng et al. 2018a, Lobell et al. 2012, Shi et al. 2016, Zhou & Sun 2019, Zhang 2019, Lan et al. 2019, Wang et al. 2019). However, fertilizer and water main factors affecting crop quality and yield traits, which have increased gradually because of the expansion of rice-planting areas in recent years (Zhou et al. 2006, Zhang et al. 2017). This has resulted in an abrupt increase in the agricultural water demand as well as an imbalance between supply and demand. Well-irrigated rice accounts for most of the paddy planting area and more than 70% of the regional agricultural water consumption (Fang et al. 2010, Wei et al. 2018). The intensity of fertilizer application in China increased by 36.45% from 2000 to 2015 (Xiao 2018), especially nitrogen fertilizer. Excessive fertilizer and water management not only waste agricultural water but also causes pollution of agricultural surface sources, which damages the ecological environment, and even reduces rice quality and yield traits (Zhang et al. 2017).

As a result, studying the impact of a novel type of fertilizer on rice yield and quality attributes under water-saving settings in Northeast China is critical. In China, new types of ecologically friendly organic fertilizers such as humic acid and bean cake fertilizers are widely employed in agricultural production. However, compare to traditional fertilizer (urea), the associated response relationships of rice quality and yield traits are unknown. In this study, we focused on these traits, under water-saving conditions, to investigate the effects of humic acid and bean cake fertilizers, with an overall goal of saving water and improving the quality and yield traits of rice.

## MATERIALS AND METHODS

### Experimental Site

The experiment was performed in 2018 at the National Key Irrigation Experimental Station located in Heping Town, Qing'an County, Suihua, Heilongjiang, China. The experimental site is located at 45°63'N and 125°44'E at an elevation of 450 m above sea level. This region consists of plain topography and has a semi-arid cold temperate continental monsoon climate, i.e., a typical cold region with a black glebe distribution area. The average annual temperature is 2.5°C, the average annual precipitation is 550 mm, and the average annual surface evaporation is 750 mm. The growth period of crops is 156-171d, and there is a frost-free period of approximately 128 days yr<sup>-1</sup>. The soil at the study site is

albic paddy soil, with a mean bulk density of  $1.01 \text{ g.cm}^{-3}$  and a porosity of 61.8 %. The basic physicochemical properties of the soil are as follows: the mass ratio of organic matter is  $41.8 \text{ g.kg}^{-1}$ , pH value is 6.45, the total nitrogen mass ratio is  $15.06 \text{ g.kg}^{-1}$ , the total phosphorus mass ratio is  $15.23 \text{ g.kg}^{-1}$ , total potassium mass ratio is  $20.11 \text{ g.kg}^{-1}$ , the mass ratio of alkaline hydrolysis nitrogen is  $198.29 \text{ mg.kg}^{-1}$ , the available phosphorus mass ratio is  $36.22 \text{ mg.kg}^{-1}$  and the exchangeable potassium mass ratio is  $112.06 \text{ mg.kg}^{-1}$  (Zheng et al. 2018b).

### Experimental Design

In this experiment, three fertilization treatments were applied: traditional nitrogen fertilizer which was CK treatment (urea + potassium fertilizer + phosphorus fertilizer), humic acid fertilizer (humic acid + potassium fertilizer + phosphorus fertilizer) and bean cake fertilizer (bean cake + potassium fertilizer + phosphorus fertilizer). Urea, humic acid, and bean cake fertilizers were applied according to the proportion of base fertilizer: tillering fertilizer: heading fertilizer (5:3:2). Phosphorus fertilizer was applied once as a basal application. Potassium fertilizer was applied twice: once as basal fertilizer and at the 8.5 leaf age (panicle primordium differentiation stage), at a 1:1 ratio.

The humic acid organic fertilizer was produced by Yunnan Kunming Grey Environmental Protection Engineering Co., Ltd, China. The organic matter  $\geq 61.4\%$ , the total nutrients (nitrogen, phosphorus, and potassium)  $\geq 18.23\%$ , of which  $\text{N} \geq 3.63\%$ ,  $\text{P}_2\text{O}_5 \geq 2.03\%$ ,  $\text{K}_2\text{O} \geq 12.57\%$ . The moisture  $\leq 2.51\%$ , the pH value was 5.7, the worm egg mortality rate  $\geq 95\%$ , and the amount of fecal colibacillosis  $\leq 3\%$ . The fertilizer contained numerous elements necessary for plants. The contents of harmful elements including arsenic, mercury, lead, cadmium, and chromium were  $\leq 2.8\%$ ,  $0.01\%$ ,  $7.6\%$ ,  $0.1\%$ , and  $4.7\%$ , respectively; these were much lower than the test standard.

The bean cake fertilizer was produced by Hebei New Century Zhou Tian Biotechnology Co., Ltd, China. The organic matter  $\geq 40\%$ , the total nutrients (nitrogen, phosphorus, and potassium)  $\geq 8\%$ , the number of active bacteria  $\geq 0.02$  billion/g, the biochemical yellow humic acid  $\geq 6\%$ , and the humic acid  $\geq 10\%$ .

### Water and Cultural Management

The plants were maintained under water-saving conditions, that was, at the re-greening stage, a water layer (0~30 mm) was maintained, but the soil was allowed to dry during the yellow ripeness stage; the water layer was not applied after the irrigation period. The upper limit of irrigation was taken as the saturated water content. At the early and middle tillering stages, jointing stage, heading stage, and milk-ripe

stage, the lower limit of irrigation was 85% of the saturated water content. The soil moisture content was measured using a moisture content analyzer (TPIME-PICO64/32) every day (once at 07:00 and 18:00). When the soil moisture content was lower than or close to the lower limit of irrigation, it was necessary to irrigate to the upper limit.

The local broadly planted rice cultivar Suijing No.18 (Japonica rice) was used for the pot experiment. The plants were grown in plastic pots (40 cm in height, the upper diameter of the pot was 32 cm, the lower diameter of the pot was 28 cm) filled with 30 kg of soil. In the middle of April, rice was sown in the greenhouse; at the seedling stage, irrigation was needed every day. In 2018, on May 21, rice was transplanted from the greenhouse to the pots. Rice was harvested on September 20. The experiment was a randomized complete block design, with at least three replicates for each treatment.

### Sampling and Measurements

**Milling and appearance quality traits:** rice grain samples harvested at physiological maturity were air-dried at room temperature before shelling and milling. The brown rice rate and milled rice rate were determined by the processing machinery SY88-TH & SY88-TRF (Wu xi Shang long Grain Equipment Co., Ltd., China), according to the national standard for rice quality evaluation GB/T17891/1999, the People's Republic of China. The grain size characteristics (length and width) of the brown rice were measured using digital vernier calipers (CD-15CP; Mitutoyo Corp., Kawasaki, Japan). Chalk characteristics of the brown rice were observed by using a rice grain appearance quality scanning machine (SC-E, Wanshen Technology Company, Hangzhou, China) according to the China National Standard GB/T 17897-1999 (She et al. 2019).

**Nutritional quality traits:** the protein and amylose contents were measured using a Near-infrared Grain Analyzer (VECTOR 22/N, Foss, BRUKER company, Germany).

**Cooking quality traits:** the cooking quality was measured by a Rice Taste Meter (STA1A, SATAKE company, Japan).

**K<sup>+</sup> and Na<sup>+</sup> contents:** Whole grain meal (100 mg) was incubated in 5 mL 95% (V/V) sulphuric acid for complete digestion using 30% H<sub>2</sub>O<sub>2</sub> as a catalyst. The digestion solution was transferred into a 50 mL volumetric flask and diluted: ion contents were determined using a TAS986 atomic absorption spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China) (Xia et al. 2016).

**Yield:** rice yield was determined per pot, and the harvested paddy grain yield was adjusted to 14.5% grain moisture content. The number of spikes per pot, number of kernels per spike, 1000-kernel mass, and the grain-filling rate were measured by harvesting in each pot. The yield was calculated

ed by multiplying the number of spikes per pot, number of kernels per spike, 1000-kernel mass, and grain-filling rate.

### Statistical Analysis

For statistical analysis, data processing was completed using Microsoft Excel 2010 followed by analysis with SPSS 19 software. The statistical results are reported as the mean value and were confirmed with an LSD (least significant difference) test.

## RESULTS

### Yield and Yield Components

The bean cake and humic acid treatments did not affect the number of spikes per pot, the number of kernels per spike, or the 1000-kernel mass when compared to the urea treatment (Table 1). The grain yield, on the other hand, was increased. There were substantial changes in grain yield between the bean cake, humic acid, and urea treatments. When bean cake and humic acid fertilizers were used, grain yield increased by 5.2 percent and 4.8 percent, respectively. Since these four component parameters impacted the yield (Shao et al. 2019). Although the fertilizers had no effects on the component factors of yield, the yield that was multiplied by them was significantly different compared to urea.

Table 1: Yield and yield component factors.

Treatments	Spikes per pot	Kernel per spike	1000-Kernel mass [g]	Grain filled rate	Grain yield [g.pot <sup>-1</sup> ]
Urea	73.33a	38.22a	48.23a	95.23%a	128.72b
Bean cake	75.00a	39.12a	49.06a	94.15%a	135.52a
Humic acid	74.33a	38.64a	49.25a	95.37%a	134.90a

Note: Different letters within columns significant difference at  $P < 0.05$  according to the LSD test.

Table 2: Milling parameters.

Treatments	Kernel weight [g]	Brown rice weight [g]	Milled rice weight [g]	Brown rice rate [%]	Milled rice rate [%]	Intact Milled rice rate [%]	Broken kernel rate [%]
Urea	202.30a	146.50b	110.30b	72.42b	54.52b	25.11c	53.95a
Bean cake	199.00a	159.10a	110.74b	79.95a	55.65b	47.13b	15.30b
Humic acid	197.80a	160.40a	129.15a	81.09a	65.29a	59.68a	8.60c

Note: Different letter within columns significant difference at  $P < 0.05$  according to the LSD test.

Table 3: Appearance parameters.

Treatments	Chalk rate [%]	Tarnishing kernel	Crack kernel	Kernel length [mm]	Kernel width [mm]	Length/Width	Chalk area [%]	Chalkiness[%]
Urea	8.85a	0.00a	11.15a	4.75a	2.50a	1.90a	7.7a	4.8a
Bean cake	1.40b	0.10a	1.70b	4.50a	2.80a	1.61b	1.2b	1.1b
Humic acid	0.25c	0.05a	0.60c	4.70a	2.70a	1.74b	0.4c	0.1c

Note: Different letters within columns significant difference at  $P < 0.05$  according to the LSD test.

### Milling and Appearance Quality Traits

The effects of fertilizer application on rice milling and appearance quality traits are shown in Table 2 and Table 3 respectively. The results indicated that the application of humic acid fertilizer increased grain milled rice weight, milled rice rate, brown rice weight, and brown rice rate (Table 2), while there was no significant change in the grain length and width, the ratio of the grain shape was affected compared to the urea treatment (Table 3). The milling suitability of the grain mostly increased in response to the humic acid, as evidenced by the increased intact milled rice percentage. A higher intact milled rice value suggests fewer broken kernels are produced during the milling process (Wang & Frei 2011).

Compared with urea, the bean cake and humic acid treatments significantly affected rice chalky rate, chalky area, and chalkiness. The application of bean cake and humic acid under water-saving conditions reduced the chalky rate, chalky area, and chalkiness compared to the urea treatment. The lowest values of the chalk traits were obtained in the humic acid treatment. As the most important indicator of rice appearance quality, chalkiness is mainly caused by the inconsistent development of starch granules and protein bodies, resulting in loosely arranged voids and white opaque parts (She et al. 2019). In our study, application of the bean cake and humic

acid fertilizers may not be the cause of the inconsistent development of starch granules and protein bodies, and therefore, may not cause the incidence of chalkiness compared to urea. The decrease of appearance traits was mainly attributed to the occurrence of chalkiness. However, the responses of chalkiness to the application of fertilizers were not consistent across years (Zhao et al. 2017, Wang & Wu 2019), suggesting the complexity of quality formation induced by fertilizers.

### Nutritional Quality and Ion Contents

The bean cake and humic acid fertilizers did not affect the  $K^+$  and  $Na^+$  contents in the grains, compared to urea (Fig.1-a). The  $K^+$  contents in the urea, bean cake, and humic acid fertilizer treatments were 5.9 mg.g<sup>-1</sup>, 6.2 mg/g, 6.3 mg/g, respectively. The corresponding  $Na^+$  contents were 1.2 mg.g<sup>-1</sup>, 1.3 mg.g<sup>-1</sup>, 1.4 mg.g<sup>-1</sup>, respectively. Fertilization did not cause an obvious imbalance between  $K^+$  and  $Na^+$  in the grain, i.e., the  $K^+/Na^+$  ratio was similar among all the treatments. Bean cake and humic acid increased the  $K^+$  and  $Na^+$  contents; however, the effects of different fertilizers on the accumulation of  $K^+$  and  $Na^+$  contents in the grains

have not been sufficiently documented in the literature. In the present study, the difference in the contents among the different treatments was not significant.

The bean cake and humic acid fertilizers influenced the protein and amylose contents in the grains (Fig.1-b). The protein contents in the bean cake and humic acid treatments were 1.19 and 1.18 times that in the urea treatment, respectively. The amylose contents in the grain decreased in response to bean cake and humic acid treatments. Specifically, the amylose contents in the bean cake and humic acid treatments were 17.41% and 7.31% lower, respectively, than that in the urea treatment. Rice, one of the most important staple crops, provides essential minerals and nutrients for humans such as protein and amylose (Xia et al. 2016). The starch in rice grain accounts for about 90% of the brown rice weight. The process of rice grain filling is the process of starch accumulation. Starch synthesizes and accumulates from the inner to outer of rice endosperm, granule-bound starch synthase is mainly responsible for the biosynthesis of amylose, compared to urea, the bean cake, and humic acid with low expression of granule-bound starch synthase, leading to the

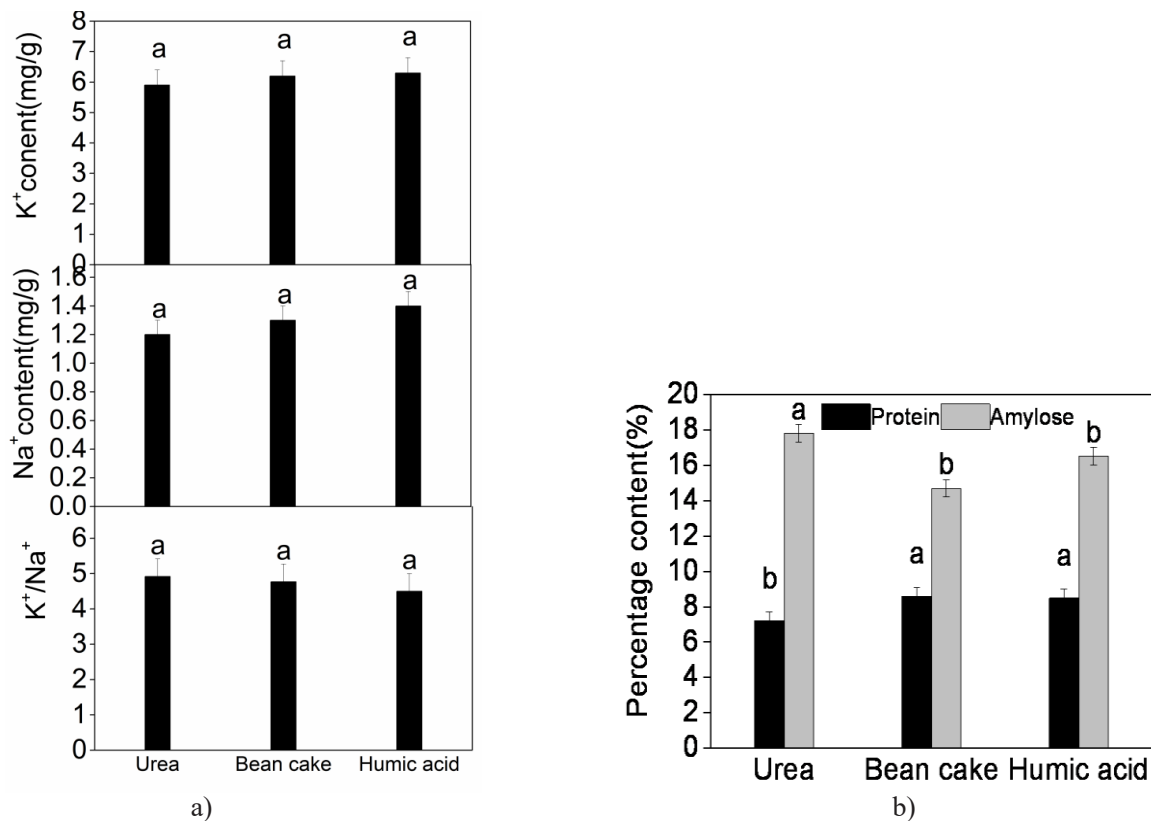


Fig. 1: The  $K^+$  and  $Na^+$ , protein, and amylose content in the grain (Note: Different letters indicate significant differences at  $P < 0.05$  according to the LSD test).

lower amylose content in the grain (Zhao et al. 2004). Protein contents in rice grain which are essential for human beings are affected by transamination in plants, the aminotransferase is mainly responsible for the biosynthesis of protein (Wang et al. 2015), application the bean cake and humic acid in the soil increased the content of aminotransferase at the heading stage, facilitating the formation of proteins. Therefore, the increase in the content of protein in response to bean cake and humic acid fertilizers in the present study indicated an improvement in the nutritional value of rice grains.

### Cooking Quality Traits

Significant impacts of the different fertilizers were observed in terms of rice cooking quality traits of the test cultivar, except for the completeness and taste (Table 4). The bean cake and humic acid fertilizers significantly increased the aroma, burnish, and mouthfeel compared to the urea treatment. The greatest effect was for burnishing, of which the highest value was obtained in the bean cake treatment; it was significantly ( $P < 0.05$ ) higher than that in the urea and humic acid fertilizers treatments. Overall, the bean cake and humic acid fertilizers increased the cooking quality traits. Chalkiness, protein, and amylose are the most important factors affecting the cooking quality traits; the cooking quality traits were negatively correlated with chalkiness and amylose and positively correlated with protein (Liu et al. 2019). In the present study, chalkiness and amylose were lower in the bean cake and humic acid treatments, and the protein was higher than that in the urea treatment (Table 3 and Fig. 1-b). Therefore, the comprehensive scores for the bean cake and humic acid treatments were better compared to the urea treatment.

### Correlation Among the Quality Traits

The cooking quality traits were positively and significantly correlated with the protein contents in the grains; however, they were negatively correlated with the amylose (Table 5). An opposite situation was found for the appearance quality traits of chalk. A significant negative correlation between the brown rice rate and chalk was observed in the present study. However, the milled rice rate was not correlated with the appearance, nutritional content, or cooking quality traits except that a significantly positive relationship was found for the intact milled rice rate ( $R=0.83$ ).

Table 4: Cooking parameters.

Treatments	Aroma	Burnish	Completeness	Taste	Mouthfeel	Comprehensive scores
Urea	7.10b	7.05c	7.26a	7.81a	7.08b	76.76b
Bean cake	7.48a	7.77a	7.12a	8.08a	7.55a	80.03a
Humic acid	7.46a	7.44b	7.33a	7.93a	7.32a	79.43a

Note: Different letters within columns significant difference at  $P < 0.05$  according to the LSD test.

### DISCUSSION

The quality and yield are affected by many factors such as climate conditions and management methods, especially the water and fertilizer. As an important source of grain in China, the rice should be fertilized 3-5 times in a growth cycle. However, in China, the nitrogen use efficiency is only about 30%. Most of the nitrogen fertilizer is lost in the environment through leakage, runoff, and volatilization, causing serious environmental pollution to soil, water, and atmosphere and influencing the growth of the crop. To implement the work of "zero growth of chemical fertilizer", the application of humic acid and bean cake fertilizers were used as a breakthrough to reduce the amount of chemical fertilizer used in rice and investigated its effects on the quality and yield. Yao et al. (2020) found that the rate of brown rice, milled rice, and head rice was significantly increased by applying slow and controlled release fertilizer, the chalkiness of conventional fertilization was significantly higher than those of other fertilization treatments and blank treatments. Combined with an organic fertilizer such as peanut bran, it can not only ensure rice yield, but also improve the crude protein of rice grain, and its rich nutrient elements such as amino acids are significantly improved (Li et al. 2019). Combined application of biochar and nitrogen fertilizer could significantly affect the processing quality of rice, such as milled rice rate, chalky grain rate, and grain length, and nutritional quality such as gel consistency, amylose, and protein content, but had no significant effect on brown rice rate and chalkiness, the optimal biochar ( $5 \text{ t} \cdot \text{hm}^{-2}$ ) combined with nitrogen fertilizer (80%) had the best effect on soil microbial carbon and nitrogen, rice yield and quality (Shi et al. 2020). Jiang et al. (2019) thought that When the total amount of nitrogen decreased by 10%, the nutritional quality was improved. In our study, we found that application the of humic acid and bean cake could be improved the quality and yield of ac compared to the area. The grain yield increased by 5.2% and 4.8% when the respective bean cake and humic acid fertilizers were applied. Because the application of humic acid and bean cake changed the soil thermal nutrients, the supply of soil organic nutrients was sufficient and can change the soil bulk density, increase the gas exchange of roots, which is conducive to the growth of rice. The results indicated that the application of humic acid fertilizer increased grain milled rice weight, milled rice rate,

Table 5: Correlation among the quality traits.

	Brown rice rate [%]	Milled rice rate [%]	Intact Milled rice rate [%]	Chalk area [%]	Chalkiness [%]	Protein [%]	Amylose [%]	Aroma	Burnish	Completeness	Taste	mouthfeel	Comprehensive
Brown rice rate (%)	1												
Milled rice rate (%)	0.68	1											
Intact Milled rice rate (%)	0.97**	0.83*	1										
Chalk area (%)	-0.99**	-0.66	-0.96**	1									
Chalkiness (%)	-0.99**	-0.73	-0.68	0.99**	1								
Protein (%)	0.98**	0.53	0.91**	-0.97**	-0.96**	1							
Amylose (%)	-0.74	0.001	-0.55	0.75	0.68	-0.85	1						
Aroma	0.98**	0.53	0.91**	-0.98**	-0.96**	0.99**	-0.85*	1					
Burnish	0.83*	0.14	0.66	-0.84*	-0.78	0.92**	-0.99**	0.91**	1				
Completeness	-0.07	0.69	0.17	0.09	0.0001	-0.25	0.73	-0.24	-0.62	1			
Taste	0.77	0.05	0.60	-0.78	-0.71	0.87*	-0.99**	0.87*	0.99**	-0.70	1		
Mouthfeel	0.80*	0.1	0.63	-0.81*	-0.75	0.90**	-0.99**	0.89*	0.99**	-0.65	0.99**	1	
Comprehensive	0.95**	0.43	0.86*	-0.96**	-0.93**	0.99**	-0.90**	0.99**	0.95**	-0.36	0.92**	0.94**	1

Note: The \* significant difference at  $P < 0.05$  according to the LSD test; the \*\* significant difference at  $P < 0.05$  according to the LSD test

brown rice weight, and brown rice rate as compared to the bean cake and urea, however, the appearance parameters were decreased significantly, and the lowest cooking parameters were obtained in the urea treatment. Because a lot of trace elements were contained in the humic acid, and bean cake also could lots of organic nutrients, when applying the humic acid and bean cake in the soil, It is helpful to improve the quality of rice as compared to the urea treatment. Therefore, compared with inorganic fertilizer, it is more environmental protection, less pollution to the environment, and rice, which is conducive to improving the utilization rate of resources. When improving soil conditions and cultivating fertile soil, the application of humic acid bean cake and other organic fertilizers plays an important role in the growth and development of rice, which could ensure the quality of rice and improve the yield of rice. Therefore, when planting rice, we should strengthen the research on organic fertilizer, and application with inorganic fertilizer reasonably to improve the utilization rate of organic fertilizer, promote the development of the rice planting industry, and maximize economic benefits.

## CONCLUSION

In conclusion, the application of the bean cake and humic acid fertilizers increased the yield compared with traditional urea under water-saving conditions. However, the  $K^+$  and  $Na^+$  contents in the grain and the  $K^+/Na^+$  ratio did not change between treatments. The application of bean cake and humic acid fertilizers enhanced the milled rice quality traits but decreased the appearance quality traits compared with the urea treatment. The best values of the milled quality traits were obtained in response to humic acid treatment, which had the lowest appearance quality traits. Higher protein content was measured in the bean cake and humic acid treatments than in the urea treatment, and the opposite situation was found with amylose; however, there was no significant difference between the bean cake and humic acid treatments.

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