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NITROGEN AND BIOFERTILIZATION EFFECTS ON BIOCHEMICAL, QUALITATIVE, AND PHYSIOLOGICAL TRAITS OF SORGHUM (*SORGHUM BICOLOR* L.)

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SUMMARY

A field experiment done in the spring of 2022 on *Sorghum bicolor* L. cultivars with different nitrogen and biofertilizer fertilizer regimes proceeded at the Ibn-Al-Bitar Vocational Preparatory School, District Al-Hussainiya, Holy Governorate of Kerbala. Sowing in a randomized complete block design (RCBD) with a factorial experiment and three replications took place in the third week of March 2022, and harvesting in the third week of July 2022. The trial included two factors; the first had three sorghum cultivars (V1 = Al-Khair, V2 = Rabeh, and V3 = Bohuth 70). The second factor was combinations of nitrogen and biofertilizers, i.e., F0 = the complete dose of nitrogen fertilizer (320 kg N ha^{-1}); F1 = 3/4 nitrogen fertilizer + *Azotobacter*; F2 = 1/2 nitrogen fertilizer + *Azotobacter*; F3 = 1/4 nitrogen fertilizer + *Azotobacter*, and F4 = *Azotobacter* with no nitrogen fertilizer. The results showed the superiority of cultivar Bohuth 70 for the traits yield per plant (116.2 g $plant^{-1}$) and nitrogen, phosphorus, and protein percentages in grains (2.212%, 0.3212%, and 13.82%, respectively). Cultivar Al-Khair excelled in total chlorophyll, ash, and potassium percentages in grains (1.057 mg g^{-1}, 2.165%, and 2.085%, respectively). As for the interaction, the combined treatments V3F0 excelled in the traits of yield per plant (134.4 g $plant^{-1}$), nitrogen (2.610%), and protein percentages (16.31%), while the interaction of V1F3 performed better for the variables ash and potassium percentages (2.600% and 2.160%, respectively). However, the reaction of V3F1 surpassed phosphorus and potassium percentages (0.3615% and 2.160%). The research happened to know the extent to reducing the quantities of recommended chemical nitrogen fertilizers and their effect on the qualitative.

Keywords: Nitrogen + *Azotobacter* biofertilizers, sorghum cultivars, genotype by fertilizer interaction, NPK content, protein

Key findings: Biofertilizer *Azotobacter* positively affected almost all the traits except the carbohydrates. The sorghum cultivars varied significantly for the study traits. It is also possible to reduce the use of chemical fertilizers by replacing them with biological fertilizers.

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INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is one of the most valuable multipurpose crops used as human food in developing countries. More than 750 million people worldwide depend directly on food from this crop. With its high protein content, sorghum grains also make the prime component in the concentrated diet of poultry, which reaches 12%. It serves the form of green fodder and silage as animal feed (Wilson, 2011).

Despite being an essential crop, its cultivated area in Iraq is around 34,000 ha, while total productivity is about 42.31 t, with a production rate of 1.18 t ha^{-1} during 2022 (Agricultural Research Department, 2023). Globally, the sorghum cultivated area is 4***2.36*** million ha, with total productivity at 61.82 million t and a production rate of 1.33 t ha^{-1} in 2022 (USDA, 2023).

Cereal crops better respond to nitrogen fertilization, as they enhance vegetative growth and improve the nutritional value of fodder by increasing its protein content and grain yield (AL-Gazhal, 2021). After each grazing or after mowing, a recommended addition of a light dose of nitrogen will stimulate branching and regrowth (Al-Rifi, 2016). Currently, researchers pay more attention to managing nitrogen fertilization due to the global consequences of greenhouse gas emissions, as excessive use of nitrogen fertilizers exacerbates nitrogen oxide, eventually reducing crop yield and delaying the harvest (Snyder *et al*., 2007; Reddy *et al*., 2014; Rifka *et al*., 2020).

Azotobacter are aerobic, free-living soil microbes vital in nature's nitrogen cycle, binding atmospheric nitrogen, which is inaccessible to plants, and releasing it in the form of ammonium ions into the soil (nitrogen fixation) (Gandora *et al*., 1998; Martyniuk and Martyniuk, 2003). Aside from being a model organism for studying diazotrophs, humans use *Azotobacter* to produce biofertilizers, food additives, and biopolymers. *Azotobacter* species are Gram-negative bacteria found in neutral and alkaline soils, water, and some related plants (Tejera *et al*., 2005).

Therefore, the researchers resorted to using biofertilizers for natural agriculture instead of mineral fertilizers. The microorganisms used as fertilizers will increase productivity to obtain higher quality and maintain an environmentally safe and clean environment (Zaki and Abdel-Halim, 2007). The pertinent study aimed to determine how much nitrogen fertilizer reduction can result from inoculating grain with nitrogen-fixing bacteria, reflecting on the sorghum cultivars' qualitative, biochemical, and physiological traits. *Azotobacter* fixes nitrogen, lowers soil pH, and improves plant growth, as it increases plant resistance to stress.

MATERIALS AND METHODS

The presented study came about during the spring of 2022 in the fields of Ibn Al-Bitar Vocational Preparatory School in Al-Husseiniyah District Holy Kerbala, with planting date on 18 March 2022. The experiment included two factors; the first factor has three sorghum cultivars (V1 = Al-Khair, V2 = Rabeh, and V3 = Bohuth-70). The second factor was combinations of nitrogen and biofertilizers, i.e., F0 = the recommended dose of nitrogen fertilizer (320 kg N ha^{-1}), F1 = 3/4 nitrogen fertilizer + *Azotobacter*, F2 = 1/2 nitrogen fertilizer + *Azotobacter*, F3 = 1/4 nitrogen fertilizer + *Azotobacter*, and F4 = *Azotobacter* with no nitrogen fertilizer. The trials were in RCBD with factorial arrangement and three replications.

Azotobacter bacteria contaminated the three sorghum cultivar seeds sown in subplots. Nitrogen addition as urea fertilizer was in two batches, as per transactions. Phosphate and potassium fertilizers augmenting were at the rate of 87.2 and 66.4 kg PK ha^{-1}. All the field operations proceeded according to the recommended technology package for the crop approved by the Ministry of Agriculture. Harvest followed on 20 July 2022.

Data recorded

The data gathering ensued on the following characteristics:

Chlorophyll content (mg g^{-1})

In the green leaves, the total chlorophyll content estimation was according to McKinney (1941). The acquired 200 mg of green leaves, cut by sterilized scissors, underwent grinding in a ceramic mortar with 6 ml of acetone at a concentration of 80%. After the substance's color became free from the green dye, filtrate separation ran by centrifuging for 10 min at 1600 rpm. The resulting extract, placed in dark tubes so the tint would not oxidize in the light, had acetone added to complete the volume. Similarly, preparing a sample (Blank) contains all the materials used in the experiment except for the plant sample, with the optical density of the filter measured through a spectrophotometer at wavelengths 645 and 663 nm, where chlorophyll estimation in plant leaves based on mg g^{-1} fresh plant tissue used the following equation***:***

$$Total\ Chlorophyll = \frac{(22.2 \times D645 + 8.02 \times D633) \times V}{W \times 1000}$$

Where,
V = final volume of filtrate after completion of the centrifugal separation process
D = optical density reading of extracted chlorophyll
W = fresh weight (g)

The unit of measure for chlorophyll is mg g^{-1} fresh tissue.

Nitrogen percentage

Dried and ground samples taken from each experimental unit had an amount of 0.2 g, then digested with the nitrogen content estimation using the Micro Kjeldahl device in the laboratories of the Department of Soil, University of Diyala, Iraq (George *et al*., 2013).

Phosphorus percentage

Calculating the percentage of phosphorus in the grains of the sorghum cultivars ran in the laboratories of the College of Agriculture, University of Kerbala, Kerbala, Iraq, employing a spectrophotometer through the use of ammonium molybdate and ammonium fluoride, at a wavelength of 410 nanometers (Al-Sahhaf, 1989).

Potassium percentage

Potassium content estimation in grains used a flame photometer, according to Page *et al*. (1982)***.***

Grain protein percentage%)

The protein percentage calculation followed the equation (A.O.A.C., 1975)***:***

$$Protein\ percentage\ (\%) = Nitrogen\ percentage\ (\%) * 6.25$$

Ash percentage

The ash percentage estimates in grains transpired in the laboratories of the Department of Chemistry, College of Science, University of Baghdad, Iraq. A 2 g sample taken from each treatment underwent drying at a temperature of 60 °C for 30 min, then burned in an oven at a temperature of 550 °C for 10 h (A.O.A.C., 1975***)***:

$$Ash\ percentage = \frac{(crucible\ wt. + sample\ wt.\ after\ combustion) - (Crucible\ wt.)}{Sample\ wt.} \times 100$$

Carbohydrates percentage

The percentage of carbohydrates estimation employed the method of Herbert *et al.* (1971), taking 1 g of dry ground grains and adding 50 ml of boiled distilled water to it, then placed in a water bath for half an hour at a temperature of 80 °C. Then filtering the sample, the filtrate was added to 50 ml of distilled water, mixing 1 ml of the sample filtrate well with 1 ml of phenol reagent 5%. Adding 5 ml of concentrated sulfuric acid followed, with 10 ml of distilled water added for diluting purposes. Color intensity measurement used a spectrophotometer at a wavelength of 488 nm.

Grain yield per plant

The grain yield per plant calculation extracted the average weight of five heads.

Statistical analysis

All the data analysis as per RCBD design with split-plot arrangement used the Genstat program (Oehlent, 2010). Engaging the least significant difference (LSD) test also aided the comparison and separation of mean.

RESULTS AND DISCUSSION

Total chlorophyll

Results revealed significant differences among the cultivars, combinations of nitrogen and biofertilizers, and their interactions for total chlorophyll (Table 1). The F0 treatment gave the highest mean of total chlorophyll content (1.065 mg g^{-1}); however, it did not differ significantly from the F1, F2, and F3 treatments. Although, the treatment F4 provided the lowest mean for total chlorophyll (0.905 mg g^{-1}). These results might be due to the role of nitrogen in the composition of porphyrin, which works to build chlorophyll leading to an increase in the chlorophyll concentration in the leaves. The cultivar Al-Khair achieved the highest mean value for the total chlorophyll characteristic (1.054 mg g^{-1}), whereas genotype Bohuth-70 showed the lowest mean for this trait (0.923 mg g^{-1}).

The interaction indicated the superiority of the V1F3 treatment (1.185 mg g^{-1}), yet, it did not differ significantly from the others, i.e., V1F0, V1F2, V1F4, V2F0, V2F1, V2F2, V2F3, V2F4, V3F0, V3F1, V3F2, and V3F3. The interchange V3F4 showed the lowest estimate of the total chlorophyll content (0.801 mg g^{-1}). These results were consistent with past findings in various crop plants (Ali *et al*., 2014). The differences in the genotypes and their interactions with nitrogen and biofertilizers might be due to their genetic potential and response to various fertilizers and varied doses (Jassim, 2018).

Nitrogen percentage

Significant differences occurred among the various nitrogen and biological fertilization treatments for grain nitrogen percentage (Table 2). The F0 treatment excelled, having the highest nitrogen percentage (2.352%); however, it showed no significant differences from the other doses, F1 and F2. Meanwhile, the F4 treatment gave the lowest nitrogen percentage (1.784%). The reason may be due to an increasing nitrogen availability as a result of adding in the form of a fertilizer. A possibility may also refer to the compensation and reduction of chemical fertilizers as a result of the fixation of atmospheric nitrogen by *Azotobacter* bacteria, which reflected positively on the absorption of nitrogen by the roots and its accumulation in the plant, with results in line with the findings of Kumar *et al*. (2017).

Table 1. Effect of nitrogen and biofertilizers on total chlorophyll in the green leaves of three sorghum cultivars (mg g^{-1}).

Fertilizer treatments	Sorghum cultivars			Means (F)
	V1	V2	V3	
F0	1.026	1.113	1.056	1.065
F1	0.858	1.109	1.087	1.018
F2	1.253	0.943	0.934	1.043
F3	1.185	1.023	0.754	0.991
F4	0.947	0.967	0.801	0.905
Means (V)	1.054	1.031	0.923	
$LSD_{0.05}$	Treatments	Cultivars	Interactions	
	0.1461	0.1131	0.2530	

Table 2. Effect of nitrogen and biofertilizers on the nitrogen percentage in grains of three sorghum cultivars.

Fertilizer treatments	Sorghum cultivars			Means (F)
	V1	V2	V3	
F0	2.310	2.137	2.610	2.352
F1	1.833	2.270	2.433	2.179
F2	2.153	2.250	2.330	2.244
F3	1.817	1.723	1.867	1.802
F4	1.743	1.790	1.820	1.784
Means (V)	1.971	2.034	2.212	
$LSD_{0.05}$	Treatments	Cultivars	Interactions	
	0.2072	0.1605	0.3589	

Table 3. Effect of nitrogen and biofertilizers on the phosphorus percentage in grains of three sorghum cultivars.

Fertilizer treatments	Sorghum cultivars			Means (F)
	V1	V2	V3	
F0	0.2693	0.3000	0.3584	0.3174
F1	0.2680	0.2901	0.3615	0.3065
F2	0.2717	0.2742	0.3172	0.2877
F3	0.2495	0.2705	0.2791	0.2644
F4	0.2670	0.2348	0.2896	0.2638
Means (V)	0.2700	0.2739	0.3212	
$LSD_{0.05}$	Treatments	Cultivars	Interactions	
	0.03576	0.0277	0.06194	

Results also revealed that cultivar Bohuth-70 outperformed the rest of the cultivars by showing the highest nitrogen percentage (2.212%), whereas cultivar Al-Khair provided the lowest nitrogen percentage (1.971%). It might be due to the genetic difference among the cultivars and their ability to absorb more nitrogen by roots. These findings may also refer to the superiority of the sorghum cultivar Bohuth-70 in some important growth traits, such as plant height, number of leaves, 1000-grain weight, and grain yield, with study results aligning with the findings of Tandel *et al*. (2020). On interactions between the cultivars and fertilizer combinations, the combination of V3F0 signified the highest nitrogen percentage (2.610%). However, such interaction did not differ significantly from others, viz., V3F1, V3F2, V2F1, and V1F0. The interaction V2F3 came with the lowest nitrogen percentage (1.723%).

Phosphorus percentage

For phosphorus percentage in grains, notable differences among the sorghum cultivars appeared, along with combinations of nitrogen and biofertilizers and their interactions (Table 3). The sorghum cultivars differed substantially for phosphorus percentage, with Cultivar Bohuth-70 recorded with the highest rate of phosphorus (0.3212%) and Al-Khair with the lowest (0.2700%). An explanation might be due to the genetic variations among the sorghum cultivars, which affect the process of absorption of various elements, and reflected in the phosphorus absorption (Tandel *et al*., 2020).

The F0 treatment shone with the highest phosphorus percentage (0.3174%), yet, no significant difference occurred from F1 and F2 treatments. Meanwhile, treatment F4 has the lowest phosphorus percentage

Table 4. Effect of nitrogen and biofertilizers on the potassium percentage in the grains of three sorghum cultivars.

Fertilizer treatments	Sorghum cultivars			Means (F)
	V1	V2	V3	
F0	2.080	1.780	2.040	1.967
F1	2.020	1.770	2.160	1.983
F2	2.160	1.740	1.933	1.944
F3	2.067	1.680	1.853	1.867
F4	2.100	1.520	1.763	1.794
Means (V)	2.085	1.698	1.950	
$LSD_{0.05}$	Treatments	Cultivars	Interactions	
	0.0979	0.0758	0.1695	

(0.2638%). Given the positive effect of the nitrogen elements and its increased concentration in plants, the vegetative and root systems enhanced and thus reflected positively on the absorption of phosphorus (Ali *et al*., 2014). In various interactions of sorghum cultivars and fertilizers, the combination of V3F1 provided the highest phosphorus percentage (0.3615%). Still, the promising interaction did not differ meaningfully from the others, i.e., V3F0, V3F2, and V2F0. However, the interchange of V2F4 revealed the lowest phosphorus percentage (0.2348%).

Potassium percentage

Noteworthy variances showed among the sorghum cultivars, combinations of nitrogen and biofertilizers, and their interactions for potassium percentage (Table 4). Among the sorghum genotypes, Al-Khair outperformed the other cultivars by giving the highest rate (2.085%), whereas cultivar Rabeh scored the lowest mean percentage for potassium (1.698%). It might be due to variations in the architectural structure of various cultivars' roots, which influenced the absorption process of potassium elements (Kumar *et al*., 2017).

The F1 treatment topped by showing the highest percentage (1.983%); however, it gave no considerable distinction from the other doses, F0 and F2. The treatment F4 resulted in the lowest rate of the said element (1.794%). It may be because nitrogen is vital in building a dense root system, which reflected positively to increase the root hairs contributing to the absorption of more potassium (Kumar *et al*., 2017). As for the interaction, two combinations, viz., V1F2 and V3F1, provided the highest potassium percentage (2.160%), which did not differ significantly from five other interactions, i.e., V1F0, V1F1, V1F3, V1F4, and V3F0. The interaction V2F4 exhibited the lowest percentage of potassium in the grains (1.520%).

Protein percentage

For protein percentage, remarkable diversities surfaced among the sorghum genotypes, nitrogen and biofertilizer combinations, and their interactions in the grains (Table 5). Treatment F0 indicated the highest protein percentage (14.70%), which did not differ considerably from other treatments, F1 and F2, with treatment F4 showing the lowest mean for the said trait (11.14%). The enhancement in the protein percentage is attributable to the increase in nitrogen content of the plant, which is the cornerstone in forming amino acids that enter into the protein formation, as nitrogen enters the carbon synthesis and stimulates the protease enzyme responsible for protein synthesis. Sorghum cultivar Bohuth-70 excelled all others by providing the highest protein percentage (13.82%), whereas Al-Khair had the lowest protein mean (12.40%). It might be due to the genotypes' genetic differences and variations in nitrogen absorption (Prajapati *et al*., 2017). In interactions, the combination V3F0 bested all other reactions, giving the highest protein percentage (16.31%); however, it did not

Table 5. Effect of nitrogen and biofertilizers on the protein percentage in the grains of three sorghum cultivars.

Fertilizer treatments	Sorghum cultivars			Means (F)
	V1	V2	V3	
F0	14.43	13.35	16.31	14.70
F1	11.45	14.18	15.20	13.61
F2	13.49	14.06	14.56	14.04
F3	11.35	10.77	11.66	11.26
F4	10.89	11.18	11.37	11.14
Means (V)	12.40	12.71	13.82	
$LSD_{0.05}$	Treatments	Cultivars	Interactions	
	1.299	1.271	1.382	

Table 6. Effect of nitrogen and biofertilizers on the ash percentage in the grains of three sorghum cultivars.

Fertilizer treatments	Sorghum cultivars			Means (F)
	V1	V2	V3	
F0	2.250	1.493	2.430	2.058
F1	1.797	1.543	1.487	1.609
F2	2.407	1.560	1.830	1.932
F3	2.600	1.830	1.287	1.906
F4	1.773	2.350	1.630	1.918
Means (V)	2.165	1.755	1.733	
$LSD_{0.05}$	Treatments	Cultivars	Interactions	
	N.S	0.4274	0.9557	

differ significantly from the others, i.e., V3F1, V3F2, V2F1, and V1F0. The combination V2F3 gave the lowest mean for said trait (10.77%).

Ash percentage

Significant disparities were observable among the sorghum cultivars and nitrogen and biofertilizer combinations for ash percentage (Table 6). Cultivar Al-Khair outperformed the rest of the cultivars by showing the highest ash percentage (2.165%); however, detecting no notable distinction between it and the cultivar Rabeh might refer to differences in the genetic makeup of the genotypes. Cultivar Bohuth-70 recorded the lowest mean at 1.733%. In previous studies, the cultivar Al-Khair was superior in vegetative growth characteristics, such as leaf area (Telleng *et al*., 2016). For interactions between the cultivars and combinations of nitrogenous and biofertilizers, the combination V1F3 appeared with the highest ash percentage (2.600%), which did not differ significantly from the rest interactions, i.e., V1F0, V1F1, V1F2, V1F4, V2F3, V2F4, V3F0, and V3F2. The V3F3 reaction revealed the lowest mean for ash percentage (1.287%).

Carbohydrates percentage

For carbohydrates percentage, nonsignificant differences occurred among the sorghum genotypes, the combinations of nitrogen and biofertilization, and the interactions between them (Table 7)***.***

Grain yield per plant

Sorghum cultivars, the combinations of nitrogen and biofertilizers, and their interactions revealed sizable dissimilarities with grain yield per plant (Table 8). Treatment F0 excelled by giving the highest grain yield (115.3 g plant^{-1}), which did not differ significantly from F1 and F2, whereas treatment F4 provided the lowest grain yield per plant (95.6 g plant^{-1}). It may be because of the correlation of individual yield components with vegetative growth, which positively

Table 7. Effect of nitrogen and biofertilizers on the carbohydrates percentage in the grains of three sorghum cultivars.

Fertilizer treatments	Sorghum cultivars			Means (F)
	V1	V2	V3	
F0	80.00	74.13	80.25	78.13
F1	84.00	77.42	76.75	79.39
F2	78.93	77.75	79.65	78.78
F3	78.50	78.77	77.10	78.12
F4	84.92	80.97	81.17	82.35
Means (V)	81.27	77.81	78.98	
$LSD_{0.05}$	Treatments	Cultivars	Interactions	
	N.S	N.S	N.S	

Table 8. Effect of nitrogen and biofertilizers on the grain yield per plant of the three sorghum cultivars.

Fertilizer treatments	Sorghum cultivars			Means (F)
	V1	V2	V3	
F0	125.2	86.3	134.4	115.3
F1	115.0	88.0	119.9	107.6
F2	116.6	82.9	120.1	106.5
F3	110.4	84.0	110.7	101.7
F4	109.0	82.0	95.6	95.6
Means (V)	115.3	84.6	116.2	
$LSD_{0.05}$	Treatments	Cultivars	Interactions	
	9.58	7.42	16.59	

increased the individual grain yield. Sorghum cultivar Bohuth-70, recorded with the highest mean for grain yield per plant (116.2 g $plant^{-1}$), did not differ significantly from Al-Khair, with the cultivar Rabeh showing the lowest grain yield per plant (84.6 g $plant^{-1}$). The reason may indicate the genetic nature and the difference between cultivars, which eventually resulted in grain yield differences. Past findings enunciated that sorghum cultivars showed remarkable divergences for 1000-grain weight and grain yield per plant (Al-Khazali, 2013; Najm, 2016). The interaction V3F0 produced the highest average grain yield per plant (134.4 g $plant^{-1}$) but showed no extensive variations from the five others, i.e., V3F1, V3F2, V1F0, V1F1, and V1F2. The interchange V2F4 revealed the lowest grain yield per plant (82.6 g $plant^{-1}$).

CONCLUSIONS

Results revealed that adding the bacterial biofertilizer (*Azotobacter*) affected the studied traits of various sorghum cultivars and the nitrogen fertilizer. Adding 50% of the recommended dose of nitrogen fertilizer with the biofertilizer proved better responsive. However, the percentage of nitrogen, phosphorus, potassium, and protein, and grain yield per plant in grains did not differ significantly with the 100% nitrogen fertilizer addition.

RECOMMENDATIONS

Biofertilizer application, added to mineral fertilizer (50% of the recommended dose), was found superior in response and economical. Biofertilizers can be vital in reducing environmental pollution resulting from extreme chemical fertilizer use.

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