



Gypsum and bio-fertilizers altered grain yield, forage, and nutrient elements of sorghum (*Sorghum bicolor* L.) in a saline soil

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Abstract

Objective: Soil salinity significantly affects the growth and crop productivity. Application of chemical amendments, such as (GP) ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), has been shown to improve saline-sodic soils, thereby promoting better plant growth and development. This study aimed to evaluate the effects of three levels of GP and different types of bio-fertilizers on grain yield, yield components, forage quality, and concentration of nutrient elements on sorghum in a saline soil.

Methods: This study was laid out as a split plot design based on the randomized complete block with three replications on sorghum at a saline soil ($\text{EC} = 5.9 \text{ dS/m}$). Three levels of GP, including 0 (control), 10, and 20 t/ha, were arranged in the main plots, and five types of bio-fertilizers, including Biosulfur, Phosphosist, Nitroxin, Phosphate bio-fertilizer (Barvar 2), and the control (without application of bio-fertilizer), were arranged in subplots.

Results: The results showed that the application of GP increased grain yield and yield components. The highest grain yield, number of seeds per plant, and 1000-seed weight were obtained with Phosphosist + 20 t/ha of GP. These increases were 58.8%, 37.3%, and 34.4%, respectively, as compared to the control treatment (no bio-fertilizer and no GP). Additionally, GP improved forage quality by increasing the neutral detergent fiber (NDF) in some cases. The highest NDF was obtained at the 20 t/ha GP. The interaction between GP and bio-fertilizers significantly affected the concentration of N, P, Fe, and Cu in the leaves of sorghum; however, the concentration of Ca, K, Mn, and Zn was not affected by this interaction. Nutrient concentration generally increased until the highest application of GP in the soil. The highest concentration of N, Fe, and Cu was obtained for the biosulfur at the rate of 20t/ha GP and of P at the Phosphosist + 20t/ha GP.

Conclusion: The combined application of Gp and phosphosist (as a bio-fertilizer) had the most pronounced positive effect on both grain yield and forage quality in sorghum at a saline soil.

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Introduction

Sorghum (*Sorghum bicolor* L.) ranks fifth among cereal crops in terms of economic impact. Research has demonstrated that sorghum is drought-tolerant and exhibits high water-use efficiency when subjected to drought conditions (Singh and Singh 1995). In addition to drought, soil salinity is a significant environmental limiting factor that adversely affects plant growth and development. Salinity impacts soil biodiversity, microbial activity, and biochemical cycles, disrupting soil respiration and the decomposition of organic residues (Singh 2016; Rajabi Dehnavi *et al.* 2020). The effects of salinity on crop productivity are particularly pronounced in arid and semi-arid regions. Salinity impacts plants by inducing osmotic stress, reducing water uptake, causing ion imbalances, and leading to toxicity (Munns and Tester 2008). Hashemzadeh *et al.* (2024) have stated ionic imbalance due to the excessive accumulation of sodium ions, which reduces the absorption of other mineral nutrients.

Among crop plants, sorghum exhibits moderate salt tolerance (salinity threshold for sorghum is 5-6 dS/m), capable of withstanding salinity levels of 6-8 dS/m. It can maintain photosynthetic activity and dry matter production under stressful conditions, including drought, salinity, and high temperatures (Rooney 2003; Reddy 2019). As a C4 plant, sorghum effectively minimizes resource losses from photorespiration, allowing it to grow rapidly. This rapid growth is associated with significant sucrose translocation from the leaves to the stem (Kanbar *et al.* 2021). Under saline conditions, sorghum demonstrates the ability to exclude Na⁺ and restrict its transport from the roots to the leaves. It also compartmentalizes Na⁺ into cell vacuoles and selectively uptakes and translocates K⁺ and Ca²⁺ over Na⁺ (Shakeri *et al.* 2020).

Gypsum (GP) is one of the most widely used amendments for reclaiming saline soils due to its availability and cost-effectiveness (Ahmad and Salim 2001). Research on the application of gypsum in saline-sodic soils indicates that higher rates of GP application lead to the removal of greater amounts of Na⁺ from soil columns, resulting in a significant reduction in both the sodium adsorption ratio (SAR) and electrical conductivity (EC) of the soil (Hamza and Anderson 2002). Additionally, GP serves as a source of essential plant nutrients, including sulfur and calcium. The application of GP can prevent soil dispersion by maintaining a high Ca²⁺: Na⁺ ratio, which enhances the

flocculation of clay particles and improves the physical stability of soils (Yaduvanshi *et al.* 2008; Naveed *et al.* 2021).

The application of nutrient elements can enhance plant growth. Among various fertilizers, bio-fertilizers play a crucial role in maintaining soil fertility by enriching it with a diverse range of macro- and micro-nutrients through processes such as nitrogen fixation, phosphate solubilization, and potassium mineralization (Sinha *et al.* 2010; Bahrami *et al.* 2016). When bio-fertilizers are applied as soil inoculants or seed treatments, they multiply and contribute to nutrient cycling, ultimately benefiting crop productivity (Singh *et al.* 2011). Azarpour *et al.* (2012) reported that the application of bio-fertilizers, such as Nitroxin, significantly enhanced grain yield and growth characteristics in soybean cultivars. Similarly, Moghimi *et al.* (2012) demonstrated that Nitroxin biological fertilizer positively influenced the grain yield of safflower by facilitating nitrogen fixation and stimulating the production of growth hormones. Ratti *et al.* (2001) investigated various strains of phosphate-solubilizing bacteria and found that their application significantly increased the yield of lemongrass, with notable improvements in plant height and biomass, compared to the control group. Sorghum, being a nutrient-exhaustive crop, requires appropriate fertilizer application to enhance both productivity and the quality of fodder. Verlinden *et al.* (2010) reported that the use of bio-fertilizers positively affected the vegetative growth of grassland plants. Bio-fertilizers not only impact plant growth and biochemical indicators but also promote the synthesis of organic compounds that help protect plants from abiotic stress (Sumbul *et al.* 2020).

There is insufficient evidence regarding the effects of gypsum and bio-fertilizer applications on sorghum grown in saline soils. Therefore, this study aimed to examine the impacts of gypsum and different types of bio-fertilizers on grain yield, yield components, forage quality traits, and the concentration of nutrient elements in the leaves of sorghum cultivated in saline soil.

Materials and Methods

A field experiment was conducted in the north Khorasan province, Jajarm, Iran (latitude of 56° 25 'N and longitude of 36° 57' E with an elevation of 122 m) in 2022. The soil was sandy loam in texture, having a pH of 7.7, an EC of 5.9 ds/m, 0.265% of organic carbon, 0.019% of N, 7.3, and 270 ppm of available P and K, respectively.

Table 1. Chemical analysis of the gypsum used in this study.

Mn	Cu	Zn	Fe	K	P	N	CaSO ₄ .2H ₂ O	EC	pH
mg.kg ⁻¹							%	dS/m	
12	2.3	14	471	750	2.5	0.1	96.3	1.3	7.3

The experiment was laid out as a split plot design, based on the randomized complete block design with three replications. Three levels of GP, 0 (control), 10, and 20 t/ha, were arranged in main plots and five types of bio-fertilizers, including Biosulfur, Phosphosist, Nitroxin, Phosphate bio-fertilizer (Barvar 2), and no bio-fertilizer (control), in subplots.

Biosulfur contains a collection of the most effective sulfur-oxidizing microorganisms (*Thiobacillus*). These microorganisms can oxidize sulfur and lead to sulfate. Then, it is easily absorbed by the plants. Nitroxin contains *Azotobacter* and *Azospirillum* bacteria, and it is most effective in nitrogen fixing and phosphate solubilizing. Phosphosist contains phosphobacter and provides phosphate for plants. Nitroxin and phosphosist are soluble and were used at 4 and 5 liters per hectare, respectively. They were used at the 4-leaf stage with irrigation water. Phosphate bio-fertilizer (Barvar 2) contains phosphate-solubilizing bacteria (*Pantua aglomerans* and *Pseudomonas putida*). It can produce organic acids and phosphatase enzymes around the root and cause phosphate ion release. In this experiment, phosphate bio-fertilizer (Barvar 2) and biosulfur were combined with seeds before sowing.

The chemical analysis of GP is shown in Table 1. GP was mixed with soil before sowing. Seeds of sorghum (Sepideh cultivar) were sown on 5 July 2022, at the experimental plots of 3 × 4 m in dimensions. The Sepideh cultivar is a medium-sized, single-stemmed, and short-stemmed genotype, with an average plant height of 135 cm, high yielding, and suitable for temperate, warm, hot, and dry regions. The rows were 45 cm apart in each plot with an intra-row distance of 6 cm. Weeds were removed by hand, and the plots were irrigated (EC= 2.1dS/m) as required throughout the growing season.

To measure biomass and grain yield, harvesting was done after the grains reached physiological maturity, when a black layer was formed at the base of each grain. Harvesting took place from a one square meter area in each plot. To measure the yield components, including the number of seeds per panicle, 1000 seed weight, and plant height, five plants were randomly harvested from each plot at maturity. Additionally, during the flowering stage, samples were collected from the terminal leaves of the plants to measure mineral elements' concentration.

The contents of P, N, and K in the leaves were determined by using the Kjeldahl method, spectrophotometer, and Jenway PFP7 flame photometer, respectively. Additionally, the concentrations of Mn, Fe, Cu, and Zn in the leaves were measured by using atomic absorption (Shimadzu AA6200 model).

At the flowering stage, among the forage quality characteristics, we measured the soluble carbohydrates in leaves according to the Schlegel (1956), neutral detergent fiber (NDF), and dry matter digestibility (DMD) based on the methods described by Goering and Van Soest (1970).

Statistical analyses

Data were analyzed by constructing the analysis of variance table. Then, the differences between individual means were determined using the LSD test. Data were analyzed with SAS software (version 9.2).

Results

Grain yield and yield components

Analysis of data showed that the interaction between GP and kinds of bio-fertilizers had a significant effect on grain yield, biomass, number of seeds per plant, and 1000-seed weight (Table 2). Figures 1 to 4 showed that the highest amount of grain yield, number of seeds per plant, and 1000-seed weight were obtained for the Phosphosist bio-fertilizer together with the application of 20 t/ha gypsum in the soil. The highest biomass (12659.6 kg/ha) was observed for Nitroxin with the application of 20 t/ha of GP in the soil. In all of the traits related to grain yield and yield components, the lowest amount was observed when no bio-fertilizers and GP were used. The amount of increase compared to the control for grain yield, biomass, number of seeds per plant, and 1000-seed weight was 58.8%, 51.2%, 37.3%, and 34.4%, respectively (Figures 1-4).

Forage quality traits

NDF and DMD are two factors that determine forage quality characteristics in forage crops. In this study, analysis of data showed that the interaction of GP with bio-fertilizers was significant for NDF and DMD in sorghum plants (Table 2). As shown in Figure 5, increasing GP from 0 to 20 t/ha increased the amount of NDF in biosulfur, when no bio-fertilizer was used. The highest amount of NDF (60.1%) was obtained at the GP of 20 t/ha without the use of bio-fertilizer, which had an increase of about 36.4%, compared to the related control.

As seen in Figure 6, in the absence of bio-fertilizers, the application of GP reduced the DMD. However, the application of bio-fertilizers increased the amount of DMD, and by increasing the content of GP, the DMD increased. Amongst bio-fertilizers, the Phosphosist and Phosphate bio-fertilizers had the highest effect. And the highest amount of DMD was obtained at the Phosphosist +

Table 2. Analysis of variance for the effects of gypsum and bio-fertilizer on grain yield, yield components, mineral elements, and forage quality of sorghum.

SOV	df	Mean squares							
		DMD	NDF	SC	N	P	K	Ca	Mg
Block	2	18.7*	34.3 ^{ns}	48.02**	0.210 ^{ns}	0.0005 ^{ns}	0.15 ^{ns}	0.520**	0.036 ^{ns}
Gypsum (GP)	2	0.51 ^{ns}	101.9**	22.8**	1.610**	0.0270**	2.04**	1.550**	0.339**
Error a	4	2.39	17.7	1.47	0.033	0.0004	0.37	0.125	0.024
Bio-fertilizer (B)	4	266.3**	60.6**	8.45**	2.350**	0.0048**	0.32 ^{ns}	0.134 ^{ns}	0.058 ^{ns}
GP × B	8	50.7**	84.9**	1.41 ^{ns}	0.380**	0.0020*	0.45 ^{ns}	0.099 ^{ns}	0.091*
Error b	24	2.83	8.53	1.72	0.081	0.0007	0.23	0.076	0.038
CV (%)		2.54	6.04	8.51	8.96	10.97	17.97	18.77	23.92

Table 2 continued

SOV	df	Mean squares							
		Mn	Zn	Cu	Fe	NSPP	TSW	GY	Bio
Block	2	1084.3**	3.94 ^{ns}	7.88*	12961**	219 ^{ns}	0.90 ^{ns}	8279 ^{ns}	4996005**
Gypsum (GP)	2	1700.3**	429.01**	18.45**	67766**	192358**	38.1**	1032121**	73828860**
Error a	4	102.8	13.76	4.43	7712	8132	1.63	31093	565606
Bio-fertilizer (B)	4	326.1**	63.49*	63.25**	17215**	23292**	8.71**	158357**	9170624**
GP × B	8	78.1 ^{ns}	10.71 ^{ns}	11.21**	5681*	5073*	6.46**	56980**	2001782*
Error b	24	34.4	15.60	1.61	2241	1972	1.48	11172	831380
CV (%)		14.04	12.57	6.05	14.77	4.67	5.62	7.71	10.46

ns, *, **: Not significant and significant at 0.05 and 0.01 probability levels, respectively; DMD: Dry matter digestibility, NDF: Neutral detergent fiber, SC: Soluble carbohydrates, NSPP: Number of seeds per plant, TSW; 1000 seed weight, GY: grain yield, Bio: Biomass.

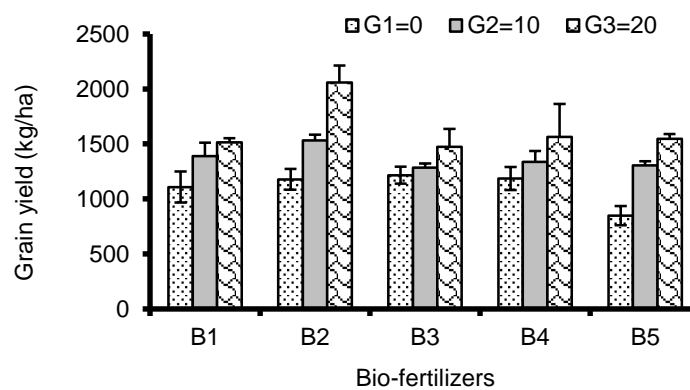
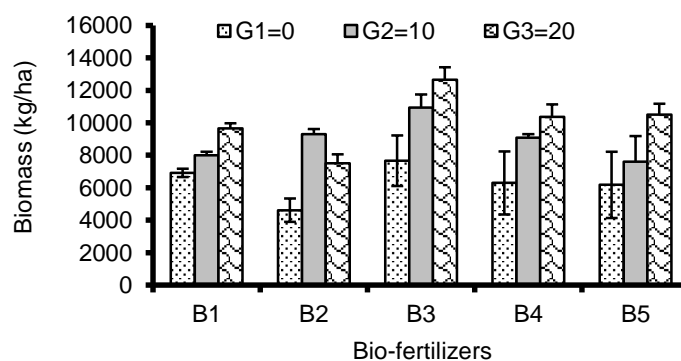
20 t/ha GP treatment, which had an increase of about 30.3% compared to the no biofertilizer + 20 t/ha of GP, which had the lowest DMD value (Figure 6).

GP and bio-fertilizers had a significant effect on the accumulation of soluble carbohydrates in the leaves of sorghum plants, but the interaction between these two factors was not significant (Table 2). As shown in Table 3, carbohydrate accumulation in leaves decreased with increasing the GP level. By increasing GP from 0 to 20 t/ha, the amount of carbohydrates decreased by about 14.3%. With the application of bio-fertilizers, changes in the soluble carbohydrates were also observed. The highest amount of carbohydrates was obtained by the Phosphate bio-fertilizer (Barvar 2), the control without bio-fertilizer, and Phosphosist, respectively (Table 3).

Table 3. Mean comparison of soluble carbohydrate and ion content in sorghum forage as affected by Gypsum and bio-fertilizers.

Factors	Soluble carbohydrates (μmol Glucose/g FW)	Ion content			
		K (mg/g DW)	Ca (mg/g DW)	Mn (mg/kg DW)	Zn (mg/kg DW)
Gypsum (t/ha):					
0	16.7	2.35	1.43	52.83	25.8
10	15.1	2.61	1.16	41.04	32.14
20	14.3	3.07	1.81	31.58	36.5
LSD5%	0.97	0.37	0.22	10.28	2.93
Bio-fertilizers:					
Biosulfur	13.8	2.82	1.28	51.14	35.3
Phosphosist	16.0	2.73	1.42	42.86	29.1
Nitroxin	15.1	2.82	1.56	37.42	32.07
Phosphate bio-fertilizer (Barvar 2)	16.16	2.63	1.49	41.96	32.1
Control	16.01	2.36	1.56	35.69	28.9
LSD5%	1.28	- ⁺	- ⁺	5.71	3.84

⁺: F for bio-fertilizers was not significant in the analysis of variance table.

**Figure 1.** Interaction of gypsum and bio-fertilizers for the grain yield in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control; LSD5% = 178.13.**Figure 2.** Interaction of gypsum and bio-fertilizers for the biomass in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 1536.61.

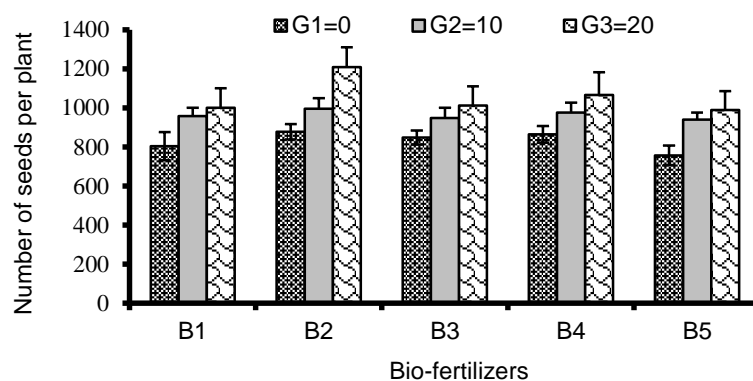


Figure 3. Interaction of gypsum and bio-fertilizers for the number of seeds per plant in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 74.84.

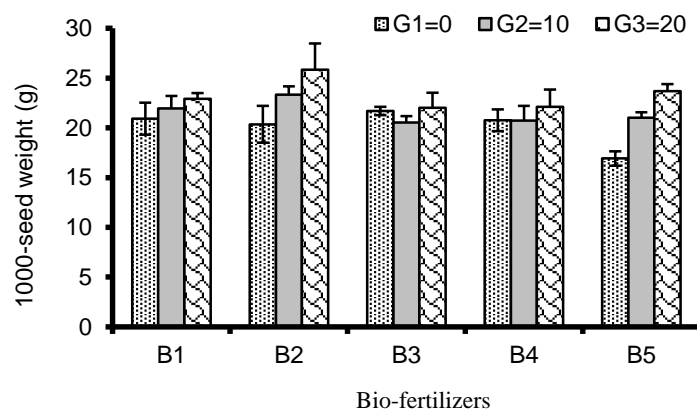


Figure 4. Interaction of gypsum and bio-fertilizers for the 1000-seed weight in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 2.05.

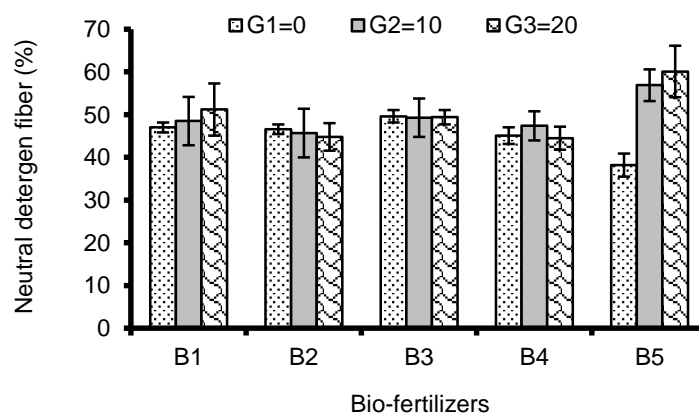


Figure 5. Interaction of gypsum and bio-fertilizers for the neutral detergent fiber in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 4.92.

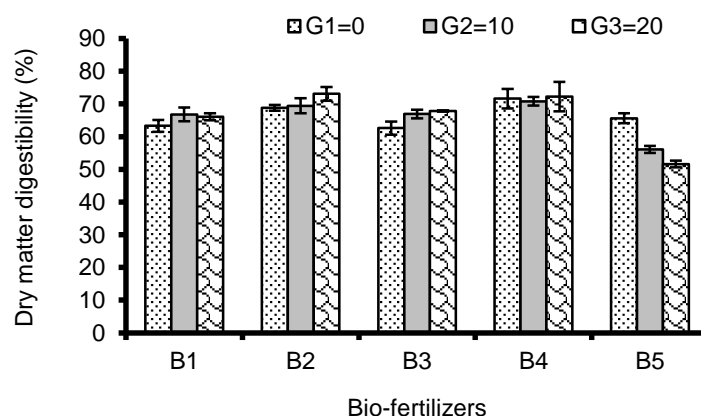


Figure 6. Interaction of gypsum and bio-fertilizers for the dry matter digestibility in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 2.84.

Ion content

Macronutrients in the leaves: The interaction between bio-fertilizers and GP was significant for the concentration of N, P, and Mg in the leaves. However, for the K and Ca content, only the effect of GP was significant. The effect of bio-fertilizer and its interaction with GP were not significant (Table 2).

By increasing GP from 0 to 20 t/ha, the concentration of N and P increased in all bio-fertilizer treatments, except for the Phosphate bio-fertilizer (Barvar 2), which showed a decreasing trend in N. The application of bio-fertilizers had different effects on N and P. As seen in Figure 7, the highest N concentration in the leaves was obtained with the Nitroxin bio-fertilizer + 20 t/ha GP (4.32%) and Biosulfur + 20 t/ha GP (4.21%), respectively. For the P, as shown in Figure 8, the highest amount was obtained with the Phosphosist + 20 t/ha of GP (0.34 mg/g DW). The increase rate for P and N, compared to the control (without bio-fertilizer and GP) was 50% (Figure 8).

In contrast to Ca and K, the interaction between GP and bio-fertilizers was significant for the Mg concentration in the sorghum leaves (Table 2). As shown in Figure 9, by increasing the GP from 0 to 20 t/ha, the concentration of this element increased in the presence of the bio-fertilizers. The bio-fertilizers had different effects, and Biosulfur and Nitroxin had the highest effect when 20 t/ha GP was applied.

As shown in Table 2, GP had a significant effect on the concentration of K and Ca in the leaves of sorghum. However, bio-fertilizer and its interaction with GP were not significant for these two elements. By increasing the GP level to 20 t/ha, the concentration of these two elements in the leaves increased, and the highest content was obtained when GP was applied at the rate of 20 t/ha. These increases were 20.9 and 23.4%, respectively, compared to the control.

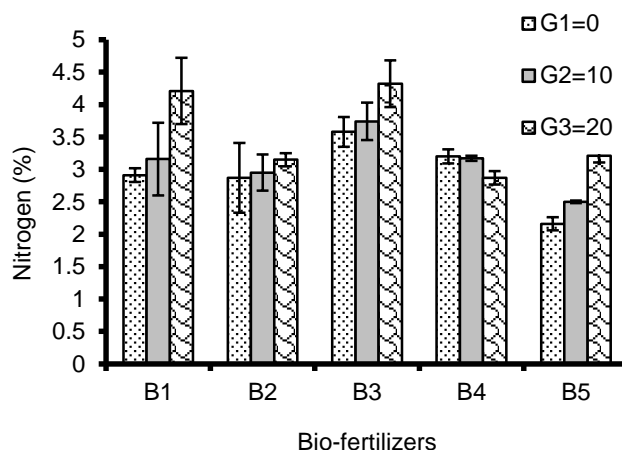


Figure 7. Interaction of gypsum and bio-fertilizers for the nitrogen content in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 0.48.

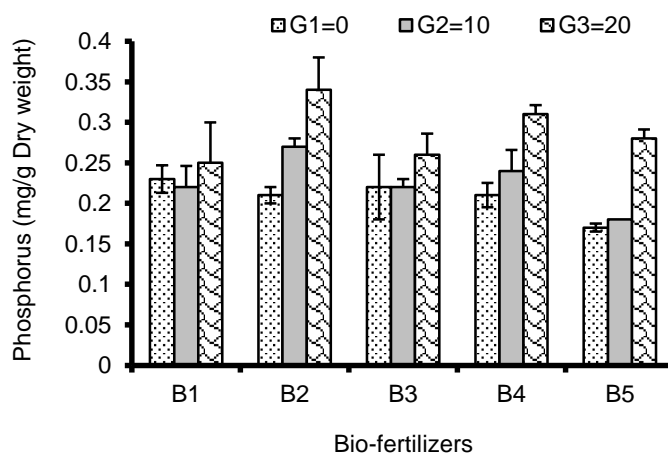


Figure 8. Interaction of gypsum and bio-fertilizers for the phosphorus content in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 0.04.

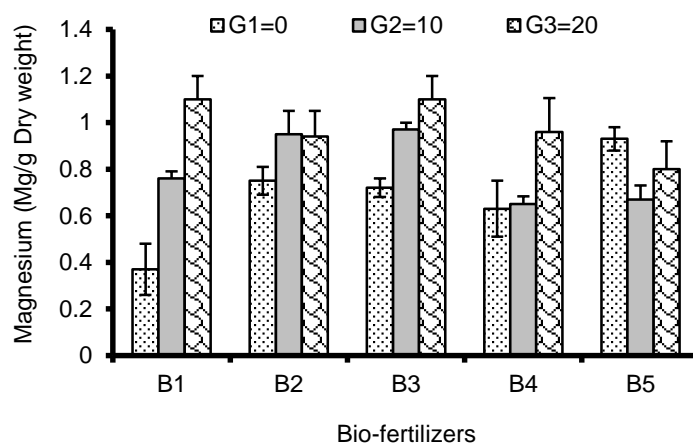


Figure 9. Interaction of gypsum and bio-fertilizers for the magnesium content in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 0.33.

Micronutrients: Interaction between bio-fertilizers and application of GP had a significant effect on the concentration of micronutrients such as Fe and Cu in the leaves of sorghum (Table 2). Figures 10 and 11 showed that the concentration of Fe increased with the application of GP, even in the absence of bio-fertilizers. However, in the case of Cu, the application of GP was only increased in the presence of Biosulfur, Nitroxin, and the control. Amongst bio-fertilizers, the highest concentration of Fe (474.4 mg/kg DW) and copper (28.5 mg/kg DW) was obtained for the Biosulfur + 20 t/ha GP. The increase for Fe and Cu was 53.8% and 43.5%, compared to the lowest amount.

About Mn and Zn, only the main effects of GP and bio-fertilizers were significant, but their interaction was not significant (Table 2). Application of GP up to 20 t/ha caused an increase in the concentration of these two elements in the leaves. These increases over the control for Mn and Zn were 30.6 and 29.3%, respectively (Table 3). Bio-fertilizers had different effects on the concentration of Mn and Zn in the sorghum leaves. In the case of Mn, the highest concentration was obtained with Nitroxin, and the highest amount of zinc was in Biosulfur (Table 3).

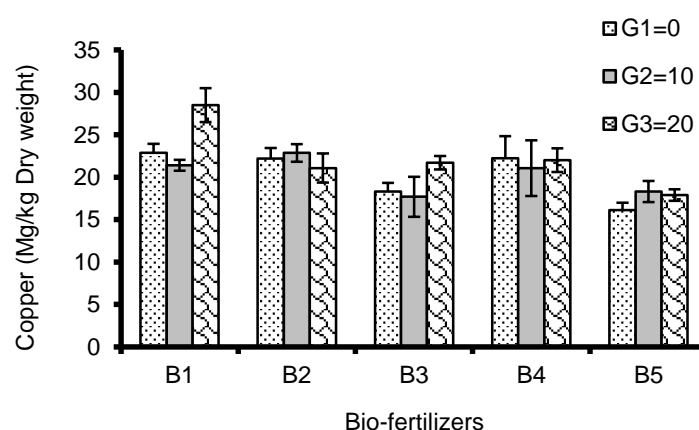


Figure 10. Interaction of gypsum and bio-fertilizers for the copper content in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 2.14.

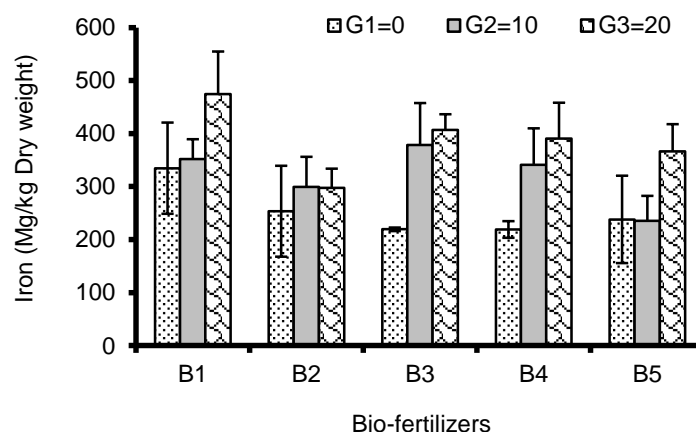


Figure 11. Interaction of gypsum and bio-fertilizers for the iron content in sorghum; B1 = Biosulfur, B2 = Phosphosist, B3 = Nitroxin, B4 = Phosphate bio-fertilizer (Barvar 2), and B5 = Control, LSD5% = 79.78.

Discussion

GP is the most widely used amendment for reclaiming saline-sodic soils due to its general availability, low cost, and abundant supply of calcium ions (Ca^{2+}) (Murtaza *et al.* 2009). Salinity negatively affects crop growth through ion toxicity, osmotic stress, and nutrient imbalances (Gong *et al.* 2018). GP enhances the ability of food crops to maintain favorable K^+/Na^+ and $(\text{Ca}^{2+}/\text{Na}^+)$ ratios, lowers soil pH, and provides essential sulfur (S) nutrition in saline conditions (Ahmed *et al.*, 2016). Research in saline-sodic soils has demonstrated that GP application effectively removes Na^+ from the soil columns, leading to significant reductions in soil EC and sodium adsorption ratio (SAR) (Hamza and Anderson 2003; Shahzad *et al.* 2019). In this study, applying GP at a rate of up to 20 t/ha in the saline soil improved the grain yield and yield components of sorghum (Figures 1-4), likely due to the enhanced nutrient absorption. Hosseini-Boldaji *et al.* (2020) indicated that under salinity conditions, stomata conductance in alfalfa decreased. This reduced the rate of photosynthesis and consequently reduced yield. We also found that the application of 20 t/ha GP significantly increased the concentrations of macro and microelements measured in the leaves of sorghum (Figures 7-11).

Bio-fertilizers are natural products containing living microorganisms sourced from the roots or cultivated soil, which do not adversely affect the soil health. In addition to their roles in atmospheric N fixation and P solubilization, bio-fertilizers also stimulate plant growth (Kushwaha *et al.* 2018). These microorganisms solubilize Zn and P, fix N, and make other macro- and micronutrients available, promoting plant growth under abiotic stress conditions (Singh *et al.* 2022). In this study, we found that bio-fertilizers enhanced growth and grain yield in sorghum, particularly when combined with GP in the soil. As illustrated in Figures 1 to 4, the highest biomass was recorded for the Nitroxin + 20 t/ha of GP, while Phosphosist + 20 t/ha of GP had the highest impact on yield and yield components. Esmailpour *et al.* (2013) reported that the application of the bio-fertilizer *Azotobacter* increased wheat grain yield to 3.360 kg/ha, compared to the control yield of 2.839 kg/ha. Similarly, Ghaderi-Daneshmand *et al.* (2012) found that the use of bio-fertilizers (Nitroxin and Biophospor) resulted in significant increases in the number of grains per spike and grain weight, compared to the control, ultimately enhancing the final yield.

Various ecological, cultural, and physical factors, including the management of fertilizers and bio-fertilizers, significantly influence the nutritional and chemical composition of plants, as well as their anatomical and morphological structures (Salunkhe and Kadan 1998; Fasusi *et al.* 2021). As illustrated in Figures 7 to 11, this experiment demonstrated that the application of bio-fertilizers and GP increased the concentrations of macro and microelements in the leaves of sorghum plants. However, different types of bio-fertilizers exhibited varying effects on these elements.

Conclusion

The application of bio-fertilizers positively influenced crop yield. Findings of this experiment clearly demonstrated that the combined application of bio-fertilizers with 20 kg/ha of GP enhanced growth, grain yield, ion content, and forage quality in sorghum plants grown in the saline soil. The results indicated that Phosphosist had the highest impact on the forage quality, grain yield, and yield components in sorghum. Among the bio-fertilizers tested, Biosulfur (B₁) showed the greatest effect on most of the macro and microelements, including N, Fe, and Cu in the leaf tissues, particularly when paired with 20 t/ha of GP in the saline soil.

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Conflict of Interest

The authors declare no conflict of interest with any individual or organization.

References

- Ahmad M, Salim M. 2001. Agricultural use of gypsum in Pakistan: background and recommendations. National Workshop. Int J Agric Biol. 3: 339-340.
- Ahmed K, Qadir G, Jami AR, Saqib AI, Nawaz MQ, Kamal MA, Haq E. 2016. Strategies for soil amelioration using sulphur in salt affected soils. Agron Res Moldavia. 49: 5–16. <https://doi.org/10.1515/cerce-2016-0021>
- Azarpour E, Moradi M, Bozorgi HR. 2012. Effects of vermicompost application and seed inoculation with biological nitrogen fertilizer under different plant densities in soybean [*Glycine max* (L.) cultivar, Williams]. Afr J Agric Res. 7(10): 1534-1541. <https://doi.org/10.5897/AJAR11.1767>
- Bahrami M, Heidari M, Ghorbani H. 2016. Variation in antioxidant enzyme activities, growth and some physiological parameters of bitter melon under salinity and chromium stress. J Environ Biol. 37(4): 529-535.

- Esmailpour A, Hassanzadehdelouei M, Madani A. 2013. Impact of livestock manure, nitrogen and bio-fertilizer (Azotobacter) on yield and yield components of wheat (*Triticum aestivum* L.). *Agron Res Moldavia*. 46(2): 5-15. <https://doi.org/10.2478/v10298-012-0079-5>
- Fasusi OA, Cruz C, Babalola OO. 2021. Agricultural sustainability: microbial bio-fertilizers in rhizosphere management. *Agriculture*. 11: 163. <https://doi.org/10.3390/agriculture11020163>
- Ghaderi-Daneshmand N, Bakhshandeh A, Rostami MR. 2012. Bio-fertilizer affects yield and yield components of wheat. *Int J Agric Res Rev*. 2(6): 699-704.
- Goering HK, Van Soest PJ. 1970. Forage fiber analysis: apparatus, reagents, procedures and some applications. USDA-ARS Agricultural Handbook 379, Washington DC, USA.
- Gong DH, Wang GZ, Si WT, Zhou Y, Liu Z, Jia J. 2018. Effects of salt stress on photosynthetic pigments and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase in *Kalidium foliatum*. *Russ J Plant Physiol*. 65: 98–103. <https://doi.org/10.1134/S1021443718010144>
- Hamza MA, Anderson WK. 2003. Responses of soil properties and grain yields to deep ripping and gypsum application in a compacted loamy sand soil contrasted with a sandy clay loam soil in Western Australia. *Aust J Agric Res*. 54: 273-282. <https://doi.org/10.1071/AR02102>
- Hashemzadeh M, Maleki M, Rahimi M. 2024. The impact of silicon dioxide on bread wheat seedling under saline stress. *J Plant Physiol Breed*. 14(1): 77-88. <https://doi.org/10.22034/jppb.2023.58596.1321>
- Hosseini-Boldaji SA, Babakhani B, Hassan-Sajedi R, Houshani M. 2020. Antioxidant properties of two alfalfa ecotypes in response to sodium chloride salinity stress. *J Plant Physiol Breed*. 10(2): 45-58. <https://doi.org/10.22034/jppb.2020.13192>
- Kanbar A, Mirzai M, Abuslima E, Flubacher N, Eghbalian R, Garbev K, Bergfeldt B, Ullrich A, Leibold H, Eiche E, *et al.* 2021. Starve to sustain—an ancient Syrian landrace of sorghum as tool for phosphorous bio-economy? *Int J Mol Sci*. 22(17): 9312. <https://doi.org/10.3390/ijms22179312>
- Kushwaha M, Singh M, Pandey AK, Kar S. 2018. Role of nitrogen, phosphorus and bio-fertilizer in improving performance of fodder sorghum-a review. *J Hill Agric*. 9(1): 22-29. <https://doi.org/10.5958/2230-7338.2018.00004.6>
- Moghimi F, Yousefirad M, Karimi M. 2012. Effects of nitroxin biological fertilizer and EDTA on nitrogen concentration, yield and yield components of safflower (Mexican cultivar). *Ann Biol Res*. 3(12): 5724-5728.
- Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Ann Rev Plant Biol*. 59: 651-681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>

- Murtaza G, Ghafoor A, Owens G, Qadir M, Kahlon UZ. 2009. Environmental and economic benefits of saline-sodic soil reclamation using low-quality water and soil amendments in conjunction with a rice-wheat cropping system. *J Agron Crop Sci.* 195: 124-136. <https://doi.org/10.1111/j.1439-037X.2008.00350.x>
- Naveed M, Aslam MK, Ahmad Z, Abbas T, Al-Huqail AA, Siddiqui MH, Ali HM, Ashraf I, Mustafa A. 2021. Growth responses, physiological alterations and alleviation of salinity stress in sunflower (*Helianthus annuus* L.) amended with gypsum and composted cow dung. *Sustainability.* 13(12): 6792. <https://doi.org/10.3390/su13126792>
- Rajabi Dehnavi A, Zahedi M, Ludwiczak A, Cardenas Perez S, Piernik A. 2020. Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy.* 10(6): 859. <https://doi.org/10.3390/agronomy10060859>
- Ratti N, Kumar S, Verma HN, Gautam SP. 2001. Improvement in bioavailability of tricalcium phosphate to *Cymbopogon martinii* var. Motia by rhizobacteria, AMF and azospirillum inoculation. *Microbiol Res.* 156(2): 145-149. <https://doi.org/10.1078/0944-5013-00095>
- Reddy PS. 2019. Breeding for abiotic stress resistance in sorghum. In: Aruna C, Visarada KBRs, Bhat BV, Tonapi VA (eds.) Breeding sorghum for diverse end uses. Woodhead Publishing Series in Food Science, Technology and Nutrition. Sawston, UK: Woodhead Publishing, pp. 325-340. <https://doi.org/10.1016/B978-0-08-101879-8.00020-6>
- Rooney LW. 2003. Overview: Sorghum and millet food research failures and successes. In: Belton PS, Taylor JPN (eds.). Proceedings of the AFRIPRO Workshop on the Proteins of Sorghum and Millets: Enhancing Nutritional and Functional Properties for Africa. 2–4 April, Pretoria, South Africa, pp. 35-44.
- Salunkhe DR, Kadan SS. 1998. Handbook of Vegetable Science and Technology Production, Composition and Processing. New York: Marcell Dekker Publication. <https://doi.org/10.1201/9781482269871>
- Schlegel HG. 1956. Die verwertung organischer säuren durch chlorella im licht. *Planta.* 47(8): 510-526. <https://doi.org/10.1007/BF01935418>
- Shahzad H, Ullah S, Iqbal M, Bilal HM, Shah GM, Ahmad S, Zakir A, Ditta A, Farooqi MA, Ahmad I. 2019. Salinity types and level-based effects on the growth, physiology and nutrient contents of maize (*Zea mays*). *Ital J Agron.* 14(4): 199-207. <https://doi.org/10.4081/ija.2019.1326>
- Shakeri E, Emam Y, Pessarakli M, Tabatabaei SA. 2020. Biochemical traits associated with growing sorghum genotypes with saline water in the field. *J Plant Nut.* 43(8): 1138-1153. <https://doi.org/10.1080/01904167.2020.1724307>

- Singh K. 2016. Microbial and enzyme activities of saline and sodic soils. *Land Degrad Dev.* 27(3): 706-718. <https://doi.org/10.1002/ldr.2385>
- Singh BR, Singh DP. 1995. Agronomic and physiological responses of sorghum, maize and pearl millet to irrigation. *Field Crops Res.* 42: 57-67. [https://doi.org/10.1016/0378-4290\(95\)00025-L](https://doi.org/10.1016/0378-4290(95)00025-L)
- Singh JS, Pandey VC, Singh DP. 2011. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric Ecosyst Environ.* 140: 339-353. <https://doi.org/10.1016/j.agee.2011.01.017>
- Singh P, Arif Y, Miszczuk E, Bajguz A, Hayat S. 2022. Specific roles of lipoxygenases in development and responses to stress in plants. *Plants.* 11: 979. <https://doi.org/10.3390/plants11070979>
- Sinha RK, Valani D, Chauhan K, Agarwal S. 2010. Embarking on a second green revolution for sustainable agriculture by vermiculture biotechnology using earthworms: reviving the dreams of Sir Charles Darwin. *J Agric Biotech Sustain Dev.* 2(7): 113-128. <https://doi.org/10.5897/JABSD.9000017>
- Sumbul A, Ansari RA, Rizvi R, Mahmood I. 2020. Azotobacter: a potential bio-fertilizer for soil and plant health management. *Saudi J Biol Sci.* 27(12): 3634-3640. <https://doi.org/10.1016/j.sjbs.2020.08.004>
- Verlinden G, Coussens T, De Vlieghe A, Baert G, Haesaert G. 2010. Effect of humic substances on nutrient uptake by herbage and on production and nutritive value of herbage from sown grass pastures. *Grass Forage Sci.* 65(1): 133-144. <https://doi.org/10.1111/j.1365-2494.2009.00726.x>
- Yaduvanshi NPS, Sharma DR. 2008. Tillage and residual organic manures/chemical amendment effects on soil organic matter and yield of wheat under sodic water irrigation. *Soil Tillage Res.* 98(1): 11-16. <https://doi.org/10.1016/j.still.2007.09.010>