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ORIGINAL ARTICLE

Bacillus sp., fertilization forms, and salt stress on soybean production[1]

Bacillus sp., formas de adubação e estresse salino na produção da soja

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HIGHLIGHTS:
Bovine biofertilizer promotes promising results for soybean production.
Organomineral fertilization mitigates the deleterious effects of excess salts on soybean plants.
Inoculation alone does not mitigate the negative effects of excess salts on soybean.

ABSTRACT: The use of *Bacillus* sp. mitigates salt stress and increases the productive yield in soybean plants. In this context, the objective of the present study was to evaluate the production of soybean grown under different forms of fertilization and salt stress, inoculated with *Bacillus* sp. The experiment was performed in the experimental area of the University of International Integration of Afro-Brazilian Lusophony (UNILAB), Redenção, Ceará, Brazil. A completely randomized design was used in a 4 x 2 x 2 factorial scheme, with five replications, corresponding to four forms of fertilization (F1 = 100% of the NPK recommendation; F2 = 50% of the NPK recommendation; F3 = 100% bovine biofertilizer; F4 = organomineral fertilization - 50% NPK + 50% bovine biofertilizer), two electrical conductivities of the irrigation water (ECw - 0.3 and 4.0 dS m^{-1}), with and without inoculation of *Bacillus* sp. The forms of fertilization organic with 100% of the recommendation through bovine biofertilizer, organomineral fertilization - 50% mineral and 50% organic with bovine biofertilizer, and 50% of the NPK recommendation promote greater productive performance of the soybean crop irrigated with water of lower salinity. Organomineral fertilization - 50% mineral and 50% organic with bovine biofertilizer was more efficient for soybean production, in the absence or presence of *Bacillus* sp. Salt stress negatively affected the production components of soybean crop under all forms of fertilization.

Key words: *Glycine max* (L.) Merr., salinity, plant nutrition

RESUMO: O uso de *Bacillus* sp. atenua o estresse salino e aumenta o rendimento produtivo em plantas de soja. Neste contexto, o objetivo do presente estudo foi avaliar a produção da soja cultivada sob diferentes formas de adubação e estresse salino, inoculada com *Bacillus* sp. O experimento foi realizado na área experimental da Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Redenção, Ceará. Utilizou-se um delineamento inteiramente casualizado em esquema fatorial 4 x 2 x 2, com cinco repetições, correspondente a quatro formas de adubação (F1 = 100% da recomendação de NPK; F2 = 50% da recomendação de NPK; F3 = 100% de biofertilizante bovino; F4 = organomineral - 50% de NPK + 50% de biofertilizante bovino), duas condutividades elétricas da água de irrigação (CEa - 0,3 e 4,0 dS m^{-1}), com e sem inoculação de *Bacillus* sp. As formas de adubação orgânica com 100% da recomendação através de biofertilizante bovino, adubação organomineral - 50% mineral e 50% orgânica com biofertilizante bovino e 50% da recomendação de NPK, proporcionam maior desempenho produtivo da cultura soja irrigada com água de menor salinidade. A adubação organomineral - 50% mineral e 50% orgânica com biofertilizante bovino, foi mais eficiente para produção da cultura da soja, na ausência ou presença de *Bacillus* sp. O estresse salino afetou negativamente os componentes de produção da cultura da soja em todas as formas de adubação.

Palavras-chave: *Glycine max* (L.) Merr., salinidade, nutrição de plantas

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Introduction

Soybean (*Glycine max* L.) is one of the most economically important crops in Brazil, making the country stand out on the world stage as one of the leaders in the production and export of the grain (Marques et al., 2022), mainly due to global demand for food and animal feed (Cunha et al., 2022), reaching an average yield of 3.508 kg ha^{-1} in 2023 (CONAB, 2022). Soybean yield can decrease mainly due to climatic variables, including reduced water availability and the uneven distribution of rainfall in different regions of the country (Barbosa et al., 2020; Oliveira et al., 2020). This effect can be more severe in the Brazilian semi-arid region, due to excess salts in the soil or in the water used for irrigation.

Salinity negatively affects plants through a reduction in osmotic potential, resulting in disturbances in the absorption of water and nutrients and physiological functions, in addition to exerting negative effects through the accumulation of specific toxic ions and, consequently, decreasing yield (Taiz et al., 2017; Silva et al., 2022).

Recent studies have described the beneficial effects of mineral or organic fertilizers on plants grown in saline environments (Rodrigues et al., 2022). In the study conducted by Silva et al. (2022), it was possible to verify that fertilization with 100% mineral fertilizer and 100% plant ash mitigated salt stress and increased foliar N and Ca content in peanut crop. However, the participation of inoculation with bacteria in soybean plants also reveals a mitigating effect on salt stress (Costa-Gutierrez et al., 2020). Confirming this information, El-Esawi et al. (2018) observed that inoculation with *Bacillus firmus* in soybean plants promoted a mitigating effect on salt stress, showing a positive effect on growth, gas exchange, and nutrient absorption. In view of the above, the objective of the present study was to evaluate the production of soybean grown under different forms of fertilization and salt stress, inoculated with *Bacillus* sp.

Material and Methods

The experiment was carried out from January to April 2023, in the experimental area of the Auroras Seedling Production Unit - UPMA, belonging to the Universidade da Integração Internacional da Lusofonia Afro-Brasileira - UNILAB, Redenção, Ceará. The region's climate is classified as Aw', characterized as tropical rainy, very hot, with rainfall predominating in the summer and autumn seasons (Alvarez et al., 2023). The meteorological data obtained during the period of the experiment are shown in Figure 1.

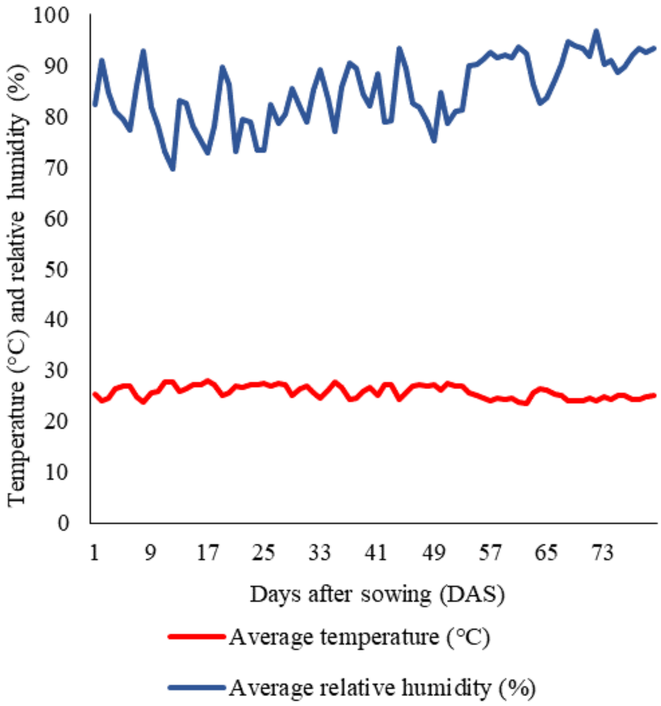


Figure 1. Average values for air temperature (°C) and relative humidity (%) during the experimental period (January 10th to April 1st, 2023)

The experimental design used was completely randomized, in a 4 x 2 x 2 factorial scheme, referring to four forms of fertilization (F1 = fertilization with 100% of the NPK recommendation; F2 = fertilization with 50% of the NPK recommendation; F3 = organic fertilization with 100% of the recommendation through bovine biofertilizer; F4 = organomineral fertilization - 50% mineral and 50% organic with bovine biofertilizer); two levels of salinity (electrical conductivity) of the water used for irrigation (A1 = 0.3 dS m^{-1} and A2 = 4.0 dS m^{-1}) in the presence and absence of inoculant with *Bacillus* sp.

Seeds of the soybean 'Brasmax Olimpo 80I82 RSF IPRO' were sown in polyethylene pots with capacity of 9 dm^3. Ten days after sowing (DAS), the seedlings were thinned out, keeping only two plants per pot. The substrate used was a mixture of sand, arisco (light-textured sandy material normally used in constructions in Northeast Brazil), and cattle manure in a ratio of 7:2:1 v/v (Table 1), respectively. It was then sent to the Soil and Water Laboratory of the Department of Soil Sciences/ Federal University of Ceará for the determination of chemical attributes, according to the methodology described by Teixeira et al. (2017).

The biofertilizer used in the experiment was prepared from a mixture of fresh cattle manure and water in equal proportions (1:1, v/v). The mixture was stored in a 100-L container and underwent an aerobic fermentation process for 30 days. Table 2 shows the results of the chemical analysis of the biofertilizer according to the methodology described by Teixeira et al. (2017).

Inoculation was carried out via seed at the time of sowing, with the commercial product BiomaPhos® as recommended

Table 1. Chemical attributes of the substrate used before the treatments were applied

Chemical attributes of the substrate									
OM	N	P	Mg	K	Ca	Na	pH	ECse	ESP
			(g kg^{-1})				water	(dS m^{-1})	(%)
0.8	0.21	0.068	0.03	0.28	0.07	0.11	6.5	0.37	3.4

OM - organic matter; ECse - electrical conductivity of the soil saturation extract; pH_{water} -1:2.5 soil-water ratio; P - Mehlich extractor; ESP - exchangeable sodium percentage

Table 2. Chemical characterization of the biofertilizer used before applying the treatments

Chemical characteristics of biofertilizer								
N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
($g L^{-1}$)					($mg L^{-1}$)			
0.82	1.4	1.0	2.5	0.75	142	1.92	68	14.72

by the manufacturer. The product contains a mixture of the bacterial strains BRM 119 (*Bacillus megaterium*) and BRM 2084 (*B. subtilis*). The volume recommended by the manufacturer was used, which corresponds to 100 mL of the product for every 60,000 soybean seeds.

Mineral fertilization was carried out following the recommendations of EMBRAPA (2013), corresponding to 20 kg ha^{-1} of N, 80 kg ha^{-1} of P_2O_5, and 60 kg ha^{-1} of K_2O. For pot fertilization purposes, a stand of 10,000 plants ha^{-1} was considered, with the plants fertilized with 100% mineral fertilizer (treatment F1) receiving 2.0 g of N, 8.0 g of P_2O_5, and 6.0 g of K_2O, through the sources urea, single superphosphate, and potassium chloride, respectively. Treatment F2 (50% of the recommended dose) used 1.0 g of N, 4.0 g of P_2O_5, and 3.0 g of K_2O. Treatment F4 (organomineral) used 50% in mineral form, in the quantities of 1.0 g $plant^{-1}$ of N, 4.0 g of P_2O_5 $plant^{-1}$ and 3.0 g $plant^{-1}$ of K_2O, while the other half (50%) was in organic form with biofertilizer.

To prepare the water with electrical conductivity of 4.0 dS m^{-1}, the soluble salts NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ were used in an equivalent ratio of 7:2:1 between Na, Ca, and Mg, respectively, following the relationship between ECw and salt concentration according to the methodology of Rhoades et al. (2000).

Irrigation with water of higher electrical conductivity began at 10 days after sowing (DAS) and continued until harvest. Irrigation was carried out on a daily basis and calculated according to the drainage lysimeter principle (Bernardo et al., 2019), keeping the soil close to field capacity, according to Eq. 1:

$$VI = \frac{Vp - Vd}{1 - LF} \quad (1)$$

where:

VI - volume of water to be applied in the irrigation (mL);
Vp - volume of water applied in the previous irrigation (mL);
Vd - volume of water drained (mL); and,
LF - leaching fraction of 0.15.

At 80 DAS, the following production components were assessed: number of pods per plant (NPP); pod mass (PM, g) - determined by weighing the pods from each experimental plot; pod length (PL, cm) - measured using a ruler graduated in centimeters; pod diameter (PD, mm) - measured using a digital caliper; number of grains per pod (NGP) - obtained by counting the grains; and production (PROD, g pot^{-1}) - measured on a precision analytical balance.

To assess the normality of the data, the variables were subjected to the Kolmogorov-Smirnov test ($p \leq 0.05$). The data were then subjected to analysis of variance, and the Tukey's test for comparing means ($p \leq 0.05$) was carried out using the ASSISTAT 7.7 BETA program (Silva & Azevedo, 2016).

Results and Discussion

The analysis of variance (Table 3) showed a significant interaction between forms of fertilization and salinity for pod length (PL), and a significant effect for forms of fertilization and salinity (individually) for pod diameter (PD). The variable pod mass (PM) showed a significant response to the interactions between forms of fertilization and salinity and inoculation versus salinity. The number of grains per pod (NGP) was significantly influenced by the interaction between forms of fertilization and salinity, as were the variables number of pods per plant (NPP) and production (PROD), which were also influenced by the interaction between forms of fertilization and inoculation.

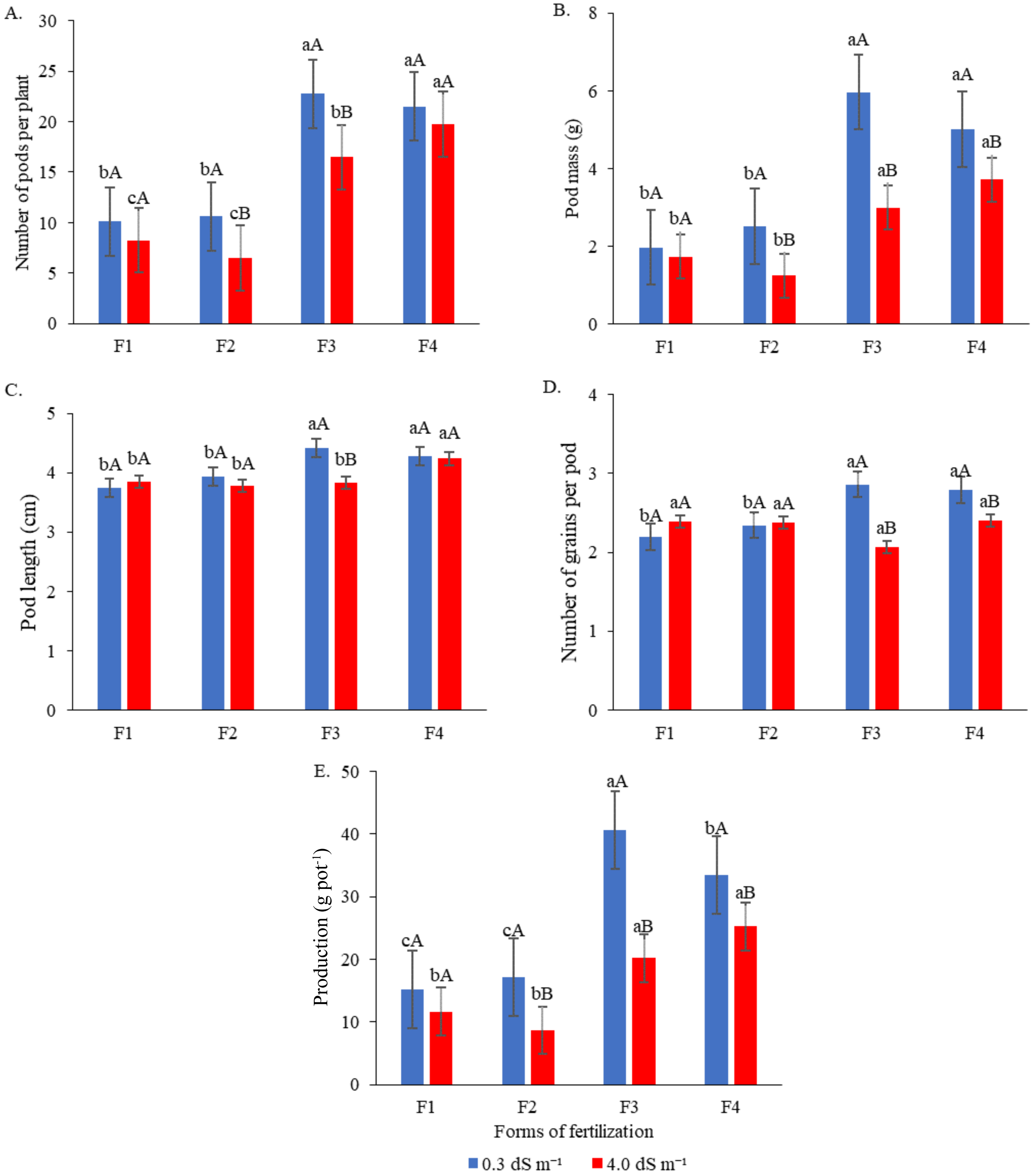
Fertilization methods F2 and F3 associated with the use of lower-salinity water promoted the highest values for the number of pods per plant (Figure 2A). Due to the accumulation of soluble salts from the irrigation water, there was a reduction in water absorption by the plant caused by reductions in the osmotic potential of the substrate, damaging plant development (Taiz et al., 2017).

The negative effect of salt stress on the variable number of pods per plant can be attributed to the reduction in the absorption of nutrients such as nitrogen and potassium, hindering quantity and quality. In this context, Tareq et al.

Table 3. Summary of the analysis of variance for the number of pods per plant (NPP), pod mass (PM), pod length (PL), pod diameter (PD), number of grains per pod (NGP), and production (PROD) in soybean plants under forms of fertilization, electrical conductivity of irrigation water in the presence and absence of inoculation with *Bacillus* sp.

SV	DF	Mean squares					
		NPP	PM	PL	PD	NGP	PROD
Forms of fertilization (FF)	3	848.3854**	43.4528**	0.9501**	1.5577**	0.3504*	1857.7291**
Electrical conductivity of water (ECw)	1	245.0000**	41.7164**	0.5417**	0.6747*	1.1400**	2051.1205*
Inoculation (I)	1	6.3281ns	0.3886ns	0.0016ns	0.0003ns	0.0007ns	1.9903ns
FF x ECw	3	22.7604*	6.4173**	0.4332**	0.2454ns	0.9977**	258.3059**
FF x I	3	21.4843*	2.2954ns	0.0793ns	0.4005ns	0.2845ns	135.9800**
ECw x I	1	1.9531ns	0.0005*	0.1917ns	0.0004ns	0.0250ns	1.2741ns
FF x ECw x I	3	10.6510ns	0.6676ns	0.0195ns	0.2604ns	0.1561ns	63.2030ns
Residual	64						
Total	79						
CV (%)		16.25	30.41	5.87	4.40	14.61	24.6

SV - Source of variation, DF - degree of freedom, CV (%) - coefficient of variation; *, **, ns - Significant at 0.05 and 0.01 probability level and not significant by F-test, respectively



Uppercase letters compare the average values of ECw in each form of fertilization and lowercase letters compare the average values of the different forms of fertilization at the same level of ECw. Means with same letters do not differ statistically from each other by Tukey's test ($p \leq 0.05$); vertical bars represent standard error (n=5)

Figure 2. Number of pods per plant (A), pod mass (B), pod length (C), number of grains per pod (D), and production (E) of soybean plants subjected to different forms of fertilization (F1= 100% NPK; F2= 50% NPK; F3= 100% biofertilizer; F4= organomineral - 50% NPK + 50% bovine biofertilizer) and irrigated with brackish water (0.3 and 4.0 dS m^{-1})

(2022), when evaluating soybean crop under salt stress, also recorded a similar trend, where excess salts in the seawater affected the quality and the number of pods per plant. Regarding chemical fertilization with NPK, Sousa et al. (2023), when studying peanut crop fertilized with NPK, obtained results similar to those found in this study. For the purpose of organic fertilizer, which offers several benefits to plants, although with a slower release of mineral elements, Goes et al. (2021) detected a positive effect on pod number in peanut plants irrigated with brackish water.

With regard to pod mass (Figure 2B), a similar response was observed for fertilizer forms F2, F3, and F4, which led to statistically higher average values with lower-salinity water. Biofertilizers and organic fertilizers have different types of microorganisms in their composition, which can increase the availability of nutrients to plants through biological processes, which may explain the positive effect compared to other forms of fertilization (Zainuddin et al., 2022). Corroborating the results obtained, Sousa et al. (2023), when assessing the agronomic performance of peanut crop under salt stress and different forms of fertilization, found that bovine biofertilizer promoted greater pod mass compared to mineral fertilization with NPK in plants irrigated with water of electrical conductivity of 5.0 dS m^{-1}.

The treatment with 100% organic fertilization through bovine biofertilizer (F3) showed higher average values for pod length with lower-salinity water than with higher-salinity water (Figure 2C). This result may be related to the effect of the humic substances and enzymes present in the organic input, attenuating the salt stress and promoting greater availability of nutrients and consequently greater pod length (Alves et al., 2019).

Studies conducted by Oliveira et al. (2015) in cowpea crop irrigated with brackish water and under application of biostimulant, resulted in reductions in the average length of pods from the salinity level of 1.25 dS m^{-1}. The opposite trend was reported by Sousa et al. (2023) when investigating the use of mineral fertilization with NPK and organic fertilization with bovine biofertilizer on peanut irrigated with brackish water.

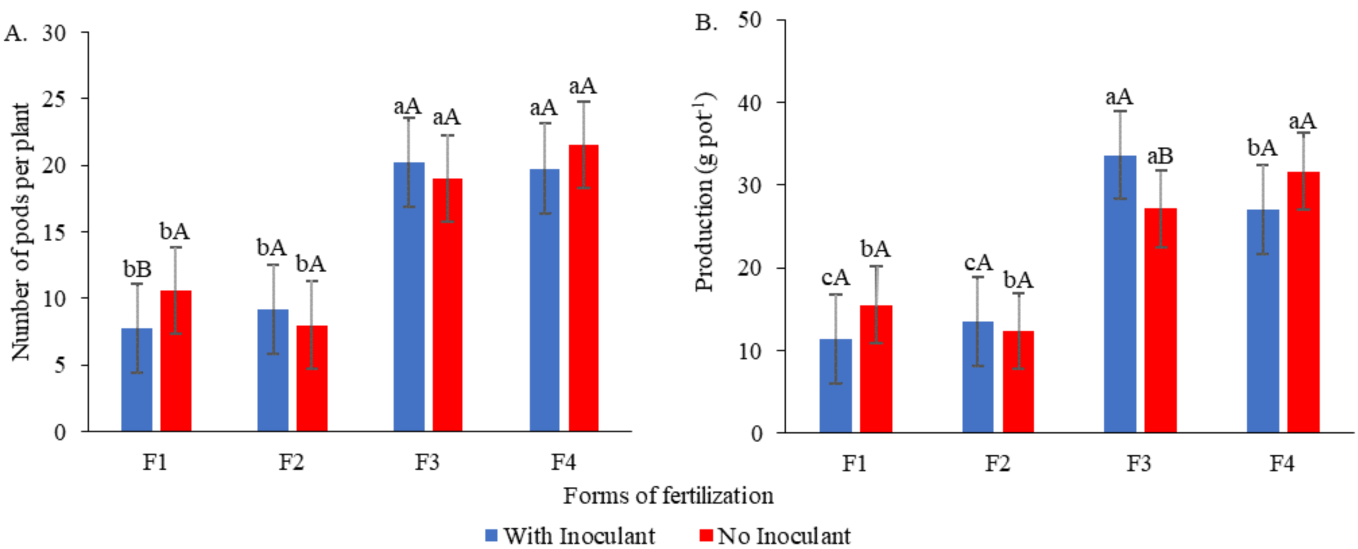
With regard to the number of grains per pod (Figure 2D), it can be seen that the use of water with lower salinity in the F3 and F4 fertilization forms led to statistically higher values than the other forms. Organic and organo-mineral fertilizations promote a greater nutritional balance, which contributes to physiological and biochemical processes, directly and positively affecting production components (Silva et al., 2019).

With the use of higher-salinity water, there was no difference between F1 and F2 for the number of grains per pod (Figure 2D), showing a mitigating effect of these forms of fertilization in a saline environment, but in F3 and F4, excess salts reduced the number of grains per pod. This negative effect of F3 and F4 may be related to the low content and solubility of K present in the organic input, which resulted in the lower grain performance. Tareq et al. (2022), when evaluating water salinity in soybean crop, observed results similar to those found in this study, with reduction in the number of grains per pod in plants irrigated with 5.0 dS m^{-1} water.

Production was affected by the interaction between fertilization and salinity, with plants fertilized with F2, F3, and F4 and irrigated with lower-salinity water showing statistically higher values (Figure 2E). It is noteworthy that salt stress causes a nutritional imbalance in the soil solution, generating antagonism with nutrients such as N, P, and K, which are important in productive performance. In their study, Tareq et al. (2022), recorded a negative effect of salt stress on the productive performance of soybean crop. As for the effect of mineral fertilization, Sousa et al. (2023) found good productive performance of the peanut crop fertilized with NPK and irrigated with brackish water. For these same authors, this result may be related to the supply of nutrients resulting from mineral fertilization, which was able to attenuate the displacement of salts (Na^+ and Cl^-) to the photoassimilates and subsequently to the grains, promoting greater production.

As for the positive effect of the biofertilizer used for fertilization (F3), it may be due to the release of humic substances into the soil, as well as the relative water content, proline content, soluble sugar content and improved efficiency of enzymes in plant leaves, such as catalase, peroxidase, and polyphenol oxidase (Babaei et al., 2017), which justifies the positive effect on yield of organic and organomineral fertilization. Similar results were observed by Sousa et al. (2023) when they evaluated irrigation with brackish water and the use of bovine biofertilizer in peanut cultivation.

Figure 3A shows the results for the number of pods per soybean plant as a function of the forms of fertilization with and without inoculant, where F1 was the only one to show



Columns followed by the same uppercase letter for inoculation and lowercase letter for forms of fertilization do not differ by Tukey's test ($p < 0.05$); vertical bars represent standard error (n = 5)

Figure 3. Number of pods per plant (A) and production (B) of soybean plants subjected to different forms of fertilization (F1= 100% NPK; F2= 50% NPK; F3= 100% biofertilizer; F4= organomineral - 50% NPK + 50% bovine biofertilizer), in the presence and absence of inoculant

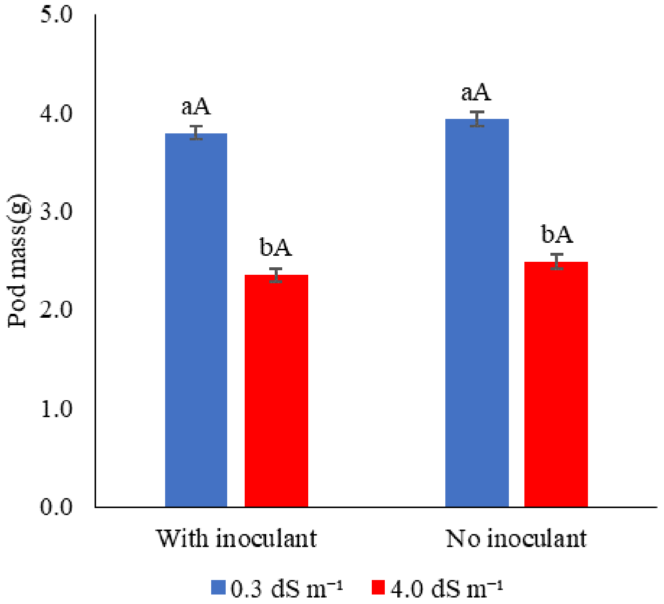
a significant difference, and in the absence of inoculant it performed better.

This result shows that the microorganisms together with NPK fertilization were not effective in increasing the release of these elements during biochemical mineralization, through the excretion of extracellular enzymes (Masrahi et al., 2023). Cordeiro & Echer (2019) observed an increase in the number of pods per inoculated soybean plant under nitrogen fertilization.

For the interaction between fertilization and inoculation for the yield variable (Figure 3B), plants fertilized with bovine biofertilizer (F3) in the presence of inoculant were statistically superior to those under the other forms. The application of microorganisms alone or in combination with chemical fertilizers can positively influence the physiological responses of cultivated plants, generating increases in production (Silva et al., 2023). In the study by Ilangumaran et al. (2021), with co-inoculation with *Bradyrhizobium japonicum* 532C in soybean plants, it was possible to observe similar yield with water of higher and lower salinity. A similar trend to that found in this study was reported by Sousa et al. (2023) when they verified a reduction in the production of peanut crop fertilized with bovine biofertilizer as a biostimulant. There was a decrease in pod mass with and without inoculant in plants irrigated with higher-salinity water (Figure 4).

This study shows that legumes such as soybeans, grown under ideal nutritional conditions, increase the rate of nodulation and consequently the nitrogen provided by biological fixation, showing greater help in the production of organic molecules capable of resisting various stresses, such as salt stress, i.e., their antioxidant system is more efficient (Lima et al., 2021; Milléo et al., 2023). A similar trend was reported by Sousa et al. (2023) when they evaluated bovine biofertilizer as a biostimulant in peanut cultivation under salt stress.

Figure 5A shows that the average pod diameter was higher in plants that were fertilized with bovine biofertilizer (F3). The positive effect observed may be related to the microbiological

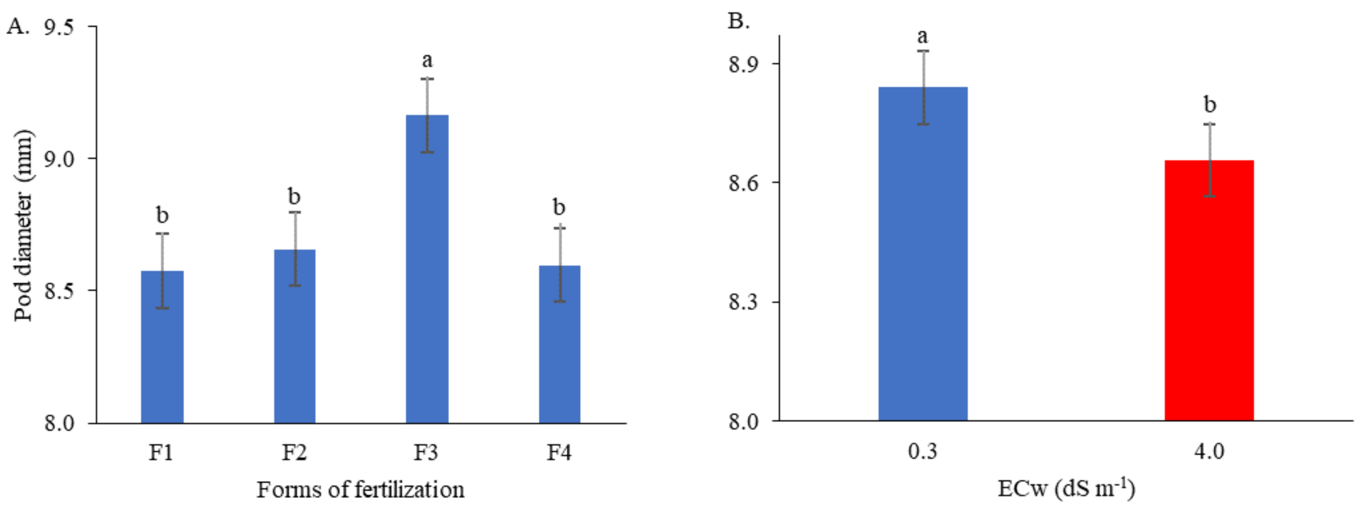


Lowercase letters comparing the mean values of ECw, with and without inoculant and uppercase letters comparing the mean values of inoculation at the same level of ECw do not differ statistically by Tukey's test ($p \leq 0.05$); vertical bars represent standard error (n=5)

Figure 4. Pod mass of soybean in the presence and absence of inoculant and irrigated with brackish water (0.3 and 4.0 dS m^{-1})

action promoted by the input, thus favoring the mineralization and availability of nutrients to the plants (Sousa et al., 2018). Similar results were observed by Souza et al. (2019) when they detected a greater pod diameter of the peanut crop with the use of bovine biofertilizer as a fertilizer source. Magalhães et al. (2017) also found an increase in the pod diameter of the cowpea crop fertilized with chicken manure.

Pod diameter was reduced with salt stress (Figure 5B). Reductions in the substrate's water potential due to the accumulation of salts can induce greater energy expenditure to maintain metabolic activities, which results in damage to plant development, such as smaller fruit diameter (Silva et al., 2019). Similar to the results obtained in this study, Guilherme et al. (2021) observed a reduction in pod diameter values in peanut plants subjected to irrigation with brackish water.



Columns followed by the same letter do not differ by Tukey's test ($p < 0.05$); vertical bars represent standard error (n=5)

Figure 5. Pod diameter of soybean plants subjected to different forms of fertilization (A) (F1= 100% NPK; F2= 50% NPK; F3= 100% biofertilizer; F4= organomineral - 50% NPK + 50% bovine biofertilizer) and irrigated with brackish water (B) (0.3 and 4.0 dS m^{-1})

Conclusions

1. The forms of fertilization organic with 100% of the recommendation through bovine biofertilizer, organomineral fertilization (50% mineral and 50% organic with bovine biofertilizer, and 50% of the NPK recommendation) promote greater productive performance of the soybean crop irrigated with water of lower salinity.

2. Organomineral fertilization (50% mineral and 50% organic with bovine biofertilizer) was more efficient for soybean crop yield, in the absence or presence of *Bacillus* sp.

3. Salt stress negatively affected the production components of soybean crop under all forms of fertilization.

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