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Irrigation with saline water in soybean (*Glycine max* (L.) Merr.) in a soil with bovine biofertilizer

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Key words:
organic fertilizer
saline stress
photosynthesis

ABSTRACT
Salinity is a complex phenomenon that affects the metabolic processes of the plant, changing the physiological and biochemical parameters. In this context, the objective of this study was to evaluate the effect of irrigation water salinity on growth, biomass and gas exchanges in soybean crop in soil with and without biofertilizer. The experiment was carried out in a greenhouse at the experimental area of the Agrometeorological Station of the UFC, Fortaleza, CE, Brazil, from May to June 2016. The treatments were distributed in randomized blocks in a 5 × 2 factorial scheme, corresponding to the levels of irrigation water salinity: 0.8; 1.6; 2.4; 3.2 and 4.0 dS m^{-1}, in soil without and with bovine biofertilizer, in five replicates. The following variables were evaluated: growth (plant height, number of leaves, stem diameter, and leaf area), biomass (leaf, root and total dry matter) and gas exchanges (photosynthesis, stomatal conductance and transpiration). Irrigation water salinity reduced photosynthesis, stomatal conductance and transpiration, but with less intensity in the plants that received bovine biofertilizer. The aerobically fermented bovine biofertilizer attenuates saline stress on the initial growth and biomass of soybean plants.

Palavras-chave:
adubo orgânico
estresse salino
fotossíntese

Irrigação com água salina na soja (*Glycine max* L.) em solo com biofertilizante bovino

RESUMO
A salinidade é um fenômeno complexo que afeta os processos metabólicos da planta, alterando o crescimento e os índices fisiológicos. Objetivou-se nesse trabalho avaliar o efeito da salinidade da água de irrigação no crescimento, na biomassa e nas trocas gasosas da cultura da soja em solo na presença e ausência de biofertilizante. O experimento foi conduzido em casa de vegetação na área experimental da UFC, Fortaleza, CE, no período de maio a junho de 2016. Os tratamentos foram distribuídos em delineamento inteiramente casualizado, em esquema fatorial 5 × 2, referente aos níveis de salinidade da água: 0,8; 1,6; 2,4; 3,2 e 4,0 dS m^{-1}, no solo sem e com biofertilizante bovino, com cinco repetições. Foram avaliadas as seguintes variáveis: crescimento (altura da planta, número de folha, diâmetro do caule e área foliar), biomassa (massa seca da parte aérea, raiz e total) e trocas gasosas (fotossíntese, condutância estomática e transpiração). A salinidade da água de irrigação reduziu a fotossíntese, a condutância estomática e a transpiração, porém com menor intensidade as plantas que receberam biofertilizante bovino. O biofertilizante bovino de fermentação aeróbia atenua o estresse salino no crescimento inicial e na biomassa das plantas de soja.

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Introduction

Soybean (*Glycine max* L.) is one of the most important crops in Brazil and its yield is 3,038 kg ha^{-1} in the Midwest region, which concentrates most of the area planted with this oilseed crop (CONAB, 2015). According to Ayres & Westcot (1999), the soybean crop is considered as moderately tolerant to salinity and resistant to irrigation using salinity water with electrical conductivity of up to 3.3 dS m^{-1}, without reduction in yield.

In many irrigated areas worldwide, good-quality water supply may not be sufficient to maintain irrigated agriculture, but soil drainage techniques have been successfully adopted in crops irrigated with this type of water source (Lima et al., 2016).

The use of saline waters for irrigation must be carefully performed because, when used to irrigate sensitive plants, these waters can cause significant reduction of growth. Munns (2005) claims that inhibition of growth in plants under salinity occurs because of two reasons: the first one is related to the osmotic effect caused by salinity, which reduces water absorption, and the second one is due to the specific effect or the excess of the ions, which enter the transpiration flow and eventually cause damages to the leaves, reducing growth or negatively influencing the absorption of essential elements.

Saline stress is a complex phenomenon for plants, involving physiological alterations and affecting photosynthesis, which can be inhibited by the accumulation of Na^+ and/or Cl^- ions in the chloroplasts (Taiz & Zeiger, 2013). Confirming this information, Prazeres et al. (2015) evaluated the gas exchanges of cowpea plants and observed that saline stress reduced their photosynthetic rate.

Recent studies point to the utilization of organic fertilizers as management strategy to reduce the impacts of salinity. Among these techniques there is the use of bovine biofertilizer, which has attenuated the deleterious effects of irrigation water salinity on some crops, as reported by Silva et al. (2011) in cowpea, Gomes et al. (2015) in sunflower and Sousa et al. (2016) in radish.

Bovine biofertilizer attenuates saline stress on the initial growth and gas exchanges of the soybean crop. In this context, this study aimed to evaluate the effect of irrigation water salinity on the growth, biomass and gas exchanges of the soybean crop in soil with bovine biofertilizer.

Material and Methods

The study was conducted in protected environment at the experimental area of the Meteorological Station of the Federal University of Ceará (UFC), situated in the municipality of Fortaleza, Ceará, Brazil (3° 45' S; 38° 33' W; 19 m), from May to June 2016. According to Köppen (1923), the local climate is Aw', with annual rainfall of 1,564 mm, mean temperature of 27.0 °C and mean relative air humidity of 78%, according to the data of the station located at 50 m away from the experimental area.

The substrate used in the experiment was composed of sand, fine sand and bovine manure in proportion of 3:3:2, respectively. Table 1 shows some chemical attributes and density of the substrate before applying the treatments, determined according to Silva (1999).

Table 1. Physical and chemical characteristics of the substrate Fortaleza-CE, Brazil 2016

Variables	Unit	Values
Ds	g cm^{-3}	1.49
pH	-	6.9
OM	g kg^{-1}	5.48
Ca	$cmol_c$ kg^{-1}	1
Mg	$cmol_c$ kg^{-1}	1.8
Na	$cmol_c$ kg^{-1}	0.31
K	$cmol_c$ kg^{-1}	2.09
H + Al	$cmol_c$ kg^{-1}	0.99
SB	cmolc kg^{-1}	5.2
CEC	$cmol_c$ kg^{-1}	6.19
V	%	84
ECse	dS m^{-1}	2.25
ESP	%	5

Ds - Soil bulk density; SB - Sum of exchangeable bases; CEC - Cation exchange capacity; V - Base saturation; ESP - Exchangeable sodium percentage

Seeds of soybean (transgenic cultivar M9144) were germinated in polyethylene plastic bags containing 6 kg of soil. At 10 days after sowing (DAS), thinning was performed to leave only the most vigorous plant per pot.

The experimental design was completely randomized in a 5 x 2 factorial scheme, with five replicates. Treatments consisted of five levels of irrigation water electrical conductivity (ECw: 0.8; 1.6; 2.4; 3.2 and 4.0 dS m^{-1}), applied in pots with soil with and without bovine biofertilizer.

Saline waters were prepared using the salts NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$, at proportion of 7:2:1, following the relationship between ECw and their concentrations ($mmol_c$ L^{-1} = EC x 10) (Rhoades et al., 2000).

Irrigation with saline waters began after thinning (10 days after emergence) on a daily frequency, through the pot weighing method (Puértolas et al., 2017), by supplying the evapotranspired water volume every 24 h to maintain the substrate with moisture corresponding to 90% field capacity, in order to avoid the risks of leaching of the biofertilizers of each treatment (Lima Neto et al., 2015).

Bovine biofertilizer was prepared from a mixture of equal parts of fresh bovine manure and water under aerobic fermentation, for 30 days, in a plastic container. The biofertilizer was applied all at once, in a volume equivalent to 10% of the substrate volume (600 mL $plant^{-1}$). Contents of nutrients present in the chemical composition of the bovine biofertilizer (Table 2) were determined according to the methodology described by Silva (1999).

At 40 days after sowing (DAS), plants were harvested and the following variables were evaluated: number of fully open leaves per plant (NL), by direct counting the leaves; plant height (PH), measured using a tape measure graduated in centimeters; stem diameter (SD), measured at the base of the stem at approximately 2 cm height from soil surface with a digital caliper; leaf area (LA), measured using an image digitizer (Scanner) attached to a microcomputer and analyzing the image through the program Sigmascan® to calculate the area.

Table 2. Chemical characteristics of the aerobically fermented bovine biofertilizer

	N	P	K	Ca	Mg	Na	EC	pH
	g L^{-1}						dS m^{-1}	
Biofertilizer	1.07	0.58	0.97	1.3	0.4	5.4	3.8	7.0

To determine leaf and root dry biomass (40 DAS), the collected plants were identified, placed in paper bags and dried in an oven at 60 °C until constant value of dry matter.

In this same period (40 DAS), during the vegetative growth state, the following parameters were measured in fully expanded leaves: photosynthesis (A), transpiration (E) and stomatal conductance (gs), using an infrared gas analyzer (LCi System, ADC, Hoddesdon, UK), in open system, with air flow of 300 mL min^{-1}. Measurements were taken always between 10 and 11 a.m., using an artificial radiation source (approximately 1,200 μmol m^{-2} s^{-1}).

The results were subjected to analysis of variance and regression, and the means were compared by Tukey test with $p < 0.05$, using the program Assistat 7.7 Beta (Silva & Azevedo, 2016). In the regression analysis, the equations which fitted best to the data were selected based on the significance of regression coefficients at 0.01 and 0.05 significance levels by F test, and on the highest coefficient of determination (R^2).

Results and Discussion

As shown in Table 3, the interaction between salinity and bovine biofertilizer influenced the number of leaves, plant height, leaf area, stem diameter, shoot dry matter, root dry matter, transpiration, stomatal conductance and photosynthesis at 0.01 significance level.

Saline stress significantly reduced the number of leaves of soybean, but with lower intensity in the treatments with bovine biofertilizer (Figure 1A).

The small attenuating effect of the biofertilizer on the number of leaves may be related to its high salt content and low availability of essential nutrients (N, P and K) (Table 1) during the studied period. In other words, the presence of sodium chloride in the soil solution shows an antagonism of Na^+ with P and K, and of Cl^- with N in the plants.

Similar results in which bovine fertilizer attenuated saline stress on the number of leaves have been reported by Silva et al. (2011) and Sousa et al. (2016) during the initial growth of cowpea and radish under saline water irrigation.

As soil salinity increased there was a reduction in plant height in the soil with and without bovine biofertilizer (Figure 1B). The positive effect of the organic input in the saline environment for this variable can be related to the greater contribution of essential elements to plants (N, P and K), as reported by Silva et al. (2011) for cowpea and Regis et al. (2017) in bell pepper crop.

Studying the cowpea crop under greenhouse conditions, Silva et al. (2011) obtained similar results to those of the present study, in which high contents of salts in the irrigation water affected plant height. For the effect of the biofertilizer in soil under saline water irrigation, the data behavior is similar to that reported by Gomes et al. (2015) in sunflower plants with bovine biofertilizer.

Leaf area (Figure 1C) were also reduced by the increase in saline stress. Cavalcante et al. (2011) noted similarity for jatropha leaf area, using increasing levels of irrigation water salinity. Regarding the organic input used in this study, the result corroborates the data found by Gomes et al. (2015), who evaluated the leaf area of sunflower irrigated with saline water and observed lower reduction in the leaf area of plants in the treatments with bovine biofertilizer.

According to the regression analysis, stem diameter data fitted to a linear model as a function of irrigation water salinity (Figure 1D). Reduction in SD leads to delayed growth, since it is influenced by the reduction of available water in the soil, causing the plant to require more energy to absorb water and develop (Souto et al., 2013).

In agreement with these results, Santos et al. (2013) worked with castor bean irrigated with saline water and also found a reduction in stem diameter.

Regarding the deleterious effect of salts in the soil with bovine biofertilizer on the jatropha crop, Cavalcante et al. (2011) observed lower reduction in stem diameter in treatments that received organic fertilizer. Likewise, Sousa et al. (2014) also reported higher SD in cowpea under saline stress in soil with crab biofertilizer.

The linear model fitted best to SDM, but the treatments without bovine biofertilizer were superior up to ECw of 2.4 dS m^{-1}, and the biofertilizer attenuated salinity only at ECw levels of 3.2 and 4 dS m^{-1} (Figure 1E). These results can be related to the metabolic cost of energy associated with the acclimation to salinity (Oliveira et al., 2014).

Sousa et al. (2014), working with cowpea, observed that SDM values decreased with the increment in irrigation water salinity. The same trend was found by Cavalcante et al. (2011), who evaluated irrigation water salinity on jatropha plants in a greenhouse and concluded that SDM decreased as saline stress increased.

Opposite results were found by Gomes et al. (2015), who applied saline water in sunflower in soil with bovine biofertilizer and obtained higher SDM in the treatments with

Table 3. Summary of analysis of variance for number of leaves (NL), plant height (PH), leaf area (LA), stem diameter (SD), shoot dry matter (SDM), root dry matter (RDM), transpiration (E), stomatal conductance (gs) and photosynthesis (A) of soybean (*Glycine max (L.)*) as a function of irrigation water salinity levels in soil with and without bovine biofertilizer

SV	DF	Mean square								
		NL	PH	LA	SD	SDM	RDM	E	gs	A
Treatment	9	836.25**	207.13**	229654.36**	2.81**	15.37**	0.21**	16.8**	0.04**	52.07**
Salinity (S)	4	646.98**	229.47**	191713.8**	2.30**	15.06**	0.3**	14.01**	0.03*	69.44**
Biofertilizer (B)	1	706.88**	95.22^{ns}	145999.86*	1.13^{ns}	5.42^{ns}	0.14*	1.38ns	0	0.86ns
B x S	4	1057.88**	212.77**	288508.55**	3.74**	18.17**	0.15**	23.44**	0.05**	47.50**
Residual	40	83.99	34.72	32101.7	0.3	1.46	0.03	2.62	0.01	9.36
Total	49									
Overall mean	-	35.04	31.98	453.89	4.01	3.81	0.64	8.99	0.38	22.03
CV (%)	-	26.15	18.43	29.47	13.82	31.71	27.94	17.99	27.7	13.89

SV – Source of variation; DF – Degrees of freedom; *Significant at 0.05 probability level by Tukey test; **Significant at 0.01 probability level by Tukey test; ^{ns}Not significant; CV - Coefficient of variation

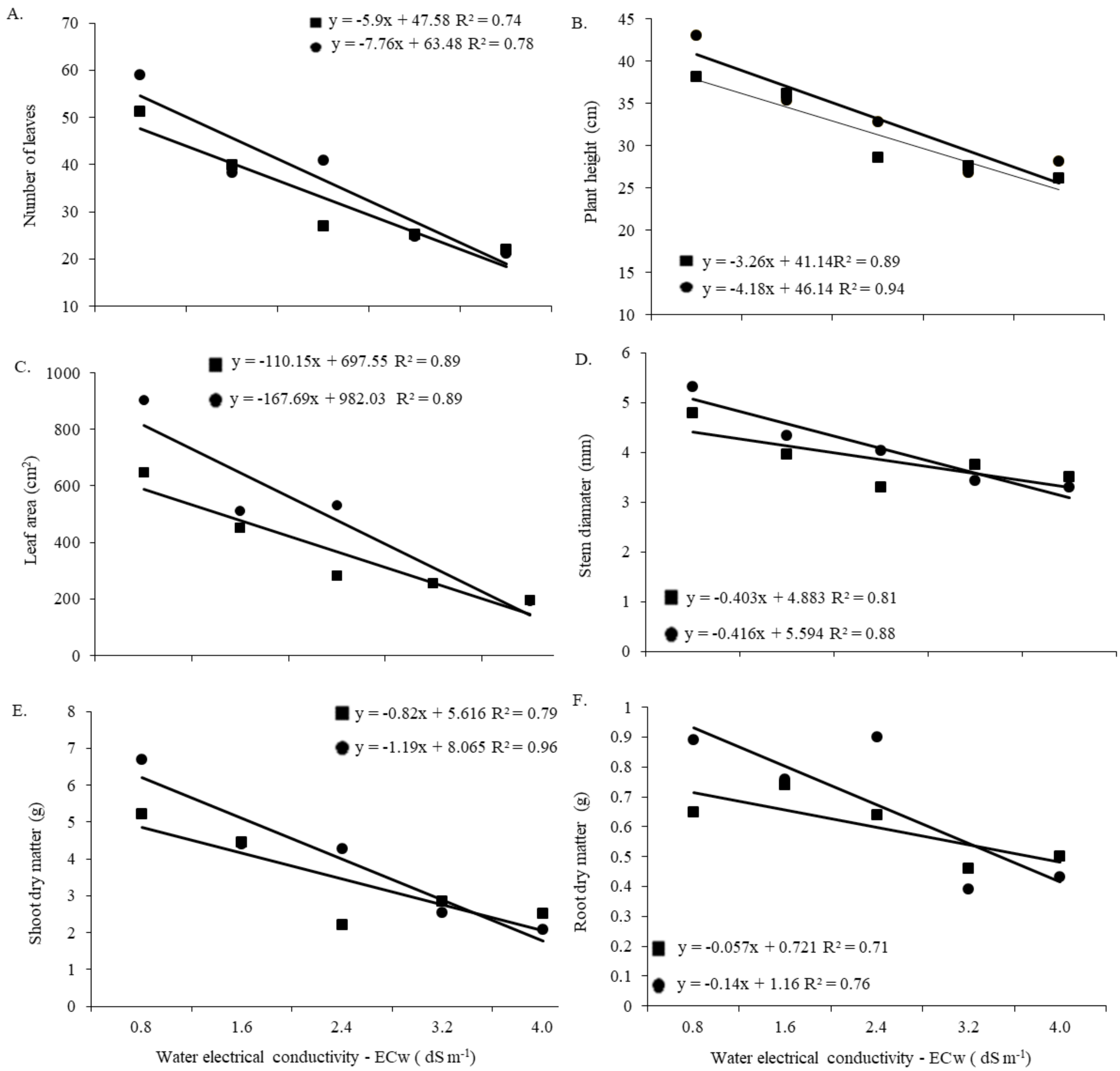

Figure 1. Number of leaves – NL (A), plant height – PH (B), leaf area – LA (C), stem diameter – SD (D), shoot dry matter – SDM (E) and root dry matter – RDM (F) of soybean plants irrigated with saline water in the soil with (■) and without (●) bovine biofertilizer

the organic input. Silva et al. (2011), testing the effect of saline stress on cowpea, found increase in SDM when plants were fertilized with the bovine biofertilizer.

RDM was inhibited by the increase in water salinity, but with lower intensity in the presence of the bovine biofertilizer (Figure 1F). This effect is consistent with the information provided by Larcher (2006), who explained that the plant under stress spends more energy to absorb water from the soil and performs the biochemical adjustments necessary for survival. Sousa et al. (2012b) also reported reduction in the RDM of corn plants under saline stress.

Medeiros et al. (2011), studying the effect of saline stress on cherry tomato in soil with and without bovine biofertilizer, concluded that RDM decreased but with lower intensity when the organic fertilizer was used. Such attenuating effect of the biofertilizer in soil irrigated with saline water was also observed by Campos et al. (2009) in the castor bean crop and by Gomes et al. (2015) in the cowpea crop.

Increasing irrigation water salinity linearly affected transpiration in soybean plants in the absence of bovine biofertilizer (Figure 2A).

Such behavior can be related to the tolerance of the crop, limiting the flux of salts to the shoots due to the lower transpiration rate (Prazeres et al., 2015) or to the greater availability of essential nutrients (Table 1) in this organic fertilizer. Reduction in transpiration rate is associated with the fact that plants under saline stress close their stomata to not absorb harmful salts (Na^+ and Cl^-) and, because of that, there is a reduction in CO_2 absorption (Prazeres et al., 2015).

Working in a greenhouse, Gomes et al. (2015) studied the sunflower crop and observed that transpiration was affected by the increase in irrigation water salinity, but plants were less

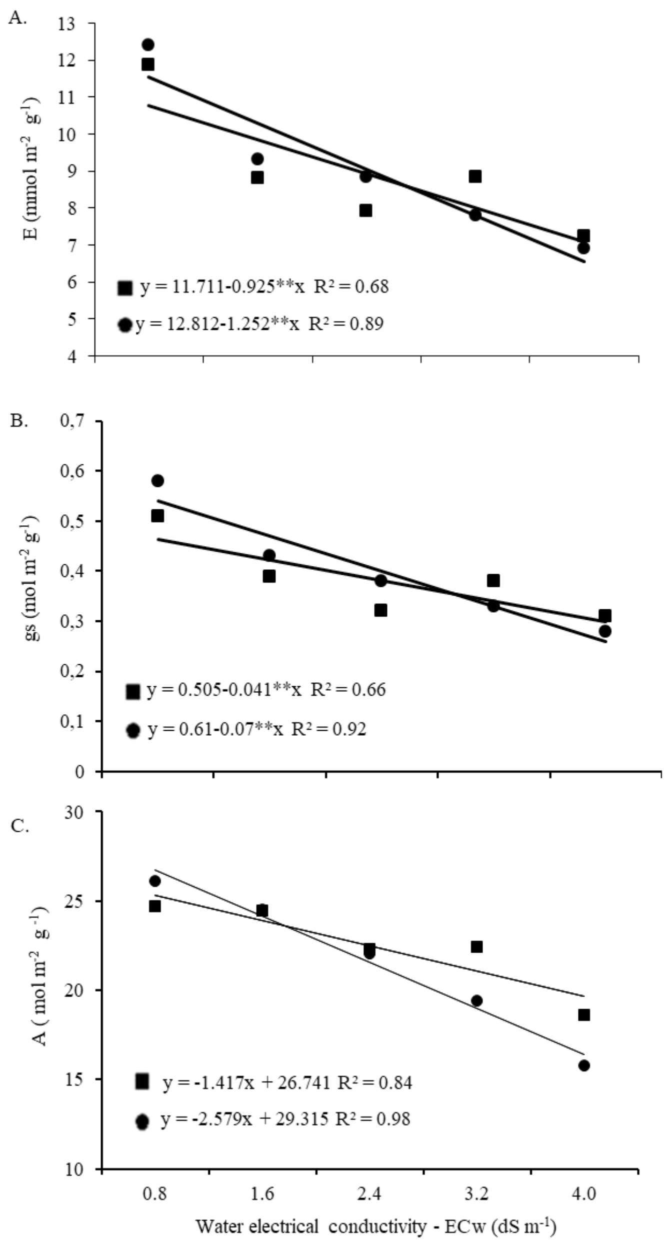

Figure 2. Photosynthesis - E (A), stomatal conductance - gs (B) and transpiration - A (C) in soybean plants irrigated with saline water in soil with (▪) and without (●) bovine biofertilizer

affected when bovine biofertilizer was used. The same effect was found by Silva et al. (2011), applying saline water and bovine biofertilizer in cowpea plants.

Figure 2B reveals that the decreasing linear model was the most adequate for the stomatal conductance of soybean plants as a function of irrigation water electrical conductivity in soil with and without bovine biofertilizer. It is worth highlighting that plants under saline stress close their stomata to avoid excessive loss of water, which is poorly absorbed by the roots at lower osmotic potential in the soil solution (Lima et al., 2011), avoiding harmful effect on stomatal opening for increasing the resistance to CO_2 diffusion (Praxedes et al., 2014).

Prazeres et al. (2015), studying stomatal conductance in cowpea plants subjected to saline stress, also observed reduction in the values of stomatal conductance.

On the other hand, plants that received the organic fertilizer had similar results to those of Gomes et al. (2015), who worked with sunflower plants in a greenhouse, and of Sousa et al. (2014) with cowpea; these authors observed reduction in stomatal conductance with the increment in irrigation water salinity. However, these authors described that, when bovine and crab biofertilizers were applied, plants resisted more to saline stress and consequently showed higher stomatal conductance.

According to Figure 2C, saline stress linearly inhibited photosynthesis but with lower intensity in the presence of the bovine biofertilizer. Silva et al. (2011) claim that plants close their stomata due to the osmotic effect of salinity, reducing transpiration and, as a consequence, there is a decrease in photosynthetic rate.

Prazeres et al. (2015), investigating the effect of saline water irrigation on cowpea plants, observed that there was a reduction in photosynthesis as the saline levels increased. Similarly, Sousa et al. (2012a) studied the effect of saline water irrigation on jatropha plants and found a decrease in photosynthesis as water salinity increased. Nevertheless, these authors claim that the reduction was less expressive than in stomatal conductance.

Works reporting positive effect of the biofertilizer in saline environment corroborate with Sousa et al. (2014) in cowpea plants and Gomes et al. (2015) in sunflower. For these studies, although photosynthesis was inhibited by the increase in irrigation water salinity, the results were always superior in the treatments with biofertilizer.

Conclusions

1. Irrigation water salinity reduced photosynthesis, stomatal conductance and transpiration, but with lower intensity in plants that received bovine biofertilizer.

2. Aerobically fermented bovine biofertilizer attenuates saline stress on the initial growth and biomass of soybean plants.

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