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Use of biostimulants in millet as strategies for tolerance to salinity of irrigation water

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ABSTRACT. Millet is grass with high forage potential in semi-arid regions, both for its versatility of use and nutritional quality. The objective of this study was to evaluate the influence of a biostimulant and the plant extract (*Cyperus rotundus*) on the growth forage, and grain production in millet (cultivar IPA BULK 1BF) submitted to salt stress conditions. The research was carried out at the Serra Talhada Academic Unit, Federal Rural University of Pernambuco, in the Semiarid region of the Northeast of Brazil, from February to April 2017. The experiment was installed in randomized blocks, in a 3x4 factorial scheme, composed of a biostimulant (ACADIAN®), nutsedge extract, and the control, in four salinity levels of the irrigation water, electrical conductivities of 0, 1, 2 and 4 dS m^{-1}, with four repetitions. Biometric analysis of all plants was carried out weekly to monitor crop growth. At 77 days after emergence, measures of net CO_2 assimilation and transpiration rates were obtained. The harvest occurred with the maturation of the grains (ED9), being analyzed the dry mass of the different morphological components of the plant. The biostimulant at the level of 2 dS m^{-1} promoted an increase of 66% in the leaf area of millet compared to the control. With 4 dS m^{-1}, the nutsedge extract provided an increase of 253% in the leaf area compared to the control. These expressive results obtained with the use of these compounds reflected in a production of dry leaf blade mass. The IPA BULK 1 BF millet cultivar has tolerance to the salinity levels studied in this research. The nutsedge extract and the biostimulant are alternatives capable of stimulating the growth and the production of forage of millet under the presence of salts in the irrigation water, however, these compounds have no influence on grain production.

Keywords: *Cyperus rotundus*; *Pennisetum glaucum*; saline stress.

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Introduction

Irrigation is an agricultural practice adopted to complement the availability of water from rainfall, being responsible for ensuring agricultural production, especially in semi-arid regions. However, inadequate irrigation management, associated with the characteristics of the source soil material, can promote or accelerate soil degradation through the process of salinization. This problem becomes more noticeable in agricultural areas, where cultivation is more intense and the use of irrigation is necessary (Silva, Alves, Nascimento, Silva, & Medeiros, 2011).

The natural pasture of the Caatinga Biome represents one of the main forage resources used for livestock activity in the Brazilian Semiarid. However, the water deficit provoked by the restricted and irregular rainfall indexes and the high evaporation potential promote seasonality in the production of phytomass in native fields, with marked restriction of plant growth and, consequently, scarcity of natural green forage.

In this scenario, to soften the impacts on the production of food for animals, strategies have been used to produce bulky food using more productive, resistant forage grasses adapted to the edaphoclimatic conditions of the region. Among the forage species commonly used in the semiarid region, millet (*Pennisetum glaucum*) stands out for the production of high quality grains and forage (Pinho et al., 2013; Silva et al., 2015; Almeida et al., 2018; Ullah, Ahmad, Khaliq, & Akhtar, 2017). On the other hand, their production may be limited, since various stages of millet development are negatively affected by the salinity of the irrigation water (Hussain, Ashraf, & Ashraf, 2017).

As the low production of roughages represents a limiting factor for animal production in semi-arid environments, and the excess of soluble salts in the soil solution severely compromises the quality of water for irrigation, it is necessary to provide ways to increase efficiency in food production. One of the proposals is through the use of technologies related to increasing the tolerance of plants, to abiotic stresses, and the most promising one is the use of biostimulants. Their use in seed treatment or seedling development can stimulate root growth, contributing to the accelerated recovery of plants when subjected to unfavorable conditions (Dourado-Neto, Dario, Barbieri, & Martin, 2014; Silva, Cato, & Costa, 2010).

Current literature points out that the nutsedge extract (*Cyperus rotundus*) is efficient in the regulation of substances of plant metabolism in several cultures, due to the high concentration of the phytohormone indolebutyric acid, an auxin that functions as a regulator of many aspects of growth and development of the plants, even being able to promote rooting in a similar way to the use of synthetic auxins (Cavalcante, Lopes, Pereira, Paiva, & Abrantes, 2016; Souza, Pereira, Martins, Coelho, & Pereira Junior, 2012). However, most of the studies are restricted to the early stages of seedling development, and there is no research on the use of this extract in grasses subjected to salt stress conditions in a semiarid environment. Thus, considering the composition of this natural extract, and in an attempt to reduce the use of synthetic hormones, it is essential to study the effects on plants under adverse conditions and during advanced stages of development, serving as a subsidy for more economical exploration.

In view of the above, the objective with this work was to evaluate the influence of a commercial biostimulant and the nutsedge extract on the growth and production of forage and of grains in millet plants submitted to salt stress conditions.

Material and methods

The study was conducted in the experimental area of the Study Group on Forage (GEFOR), Serra Talhada Academic Unit, Federal Rural University of Pernambuco (UAST / UFRPE), located in Serra Talhada, Northeastern Region, Brazil (7°56'15"S, 38°18 '45"W, at an elevation of 429 m). According to the Köppen classification, the climate fits into the BSwh' type, with an average annual precipitation of 632 mm per year and average air temperatures above 25 °C (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013; Leite, Lucena, Sá Junior, & Cruz, 2017).

The assay was installed in randomized blocks, in a 3x4 factorial scheme, composed of a commercial biostimulant (ACADIAN®), nutsedge extract and the control, in four salinity levels of irrigation water, resulting from different electrical conductivities (CEa) (0, 1, 2 and 4 dS m^{-1}), with four repetitions. According to the classification of water for irrigation according to the risk of salinity (Sales, Lopes, Meireles, Chaves, & Andrade, 2014), the levels of CEa (0, 1, 2 and 4 dS m^{-1}) used in the present study are classified, respectively, as C1 (low risk), C2 (medium risk), C3 (high risk) and C4 (very high risk). To obtain the saline levels: 1, 2 and 4 dS m^{-1}, sodium chloride salts (NaCl), corresponding to 0.58, 1.16 and 2.33 g L^{-1}, were diluted in distilled water, respectively. In the treatment with 0 dS m^{-1}, distilled water was used for irrigation.

The experiment was conducted in February, March and April 2017, corresponding to a cycle of evaluation of millet. In this period the accumulated precipitation corresponded to 112.8, 100.7 and 45.8 mm, respectively, thus making a cumulative total of 259.3 mm.

The soil used is classified as inceptisol. A sample composed of soil was collected for purposes of fertility analysis, where the following characterization was obtained: pH (water) = 7.10; P (Mehlich I extractor) = 380 mg dm^{-3}; K^+ = 0.88; Ca^{2+} = 1.20; Mg^{2+} = 0.10; Na^+ = 0.11; Al^{3+} = 0.0; H^+ = 1.00; SB = 2.29; CTC = 3.29 ($cmol_c$ dm^{-3}), m = 0%, V = 69.60% and organic matter = 1.24%. About 10 kg of this soil was stored in plastic pots with a volume of 14.41 dm^3 and distributed in an open area, according to the experimental design.

Three seeds were placed per pot of the millet cultivar IPA BULK 1BF, at a depth of about 2.0 cm. Thinning was carried out seven days after emergence (DAE), keeping one plant per pot. Irrigation was performed based on the reference evapotranspiration (ETo), being determined through the Penman-Monteith equation, parameterized according to FAO Bulletin 56 (Abreu, Silva, Teodoro, Holanda, & Sampaio Neto, 2013). For that, meteorological data were collected from an automated platform, belonging to the National Institute of Meteorology (INMET), located about 300 m from the assay site.

The ACADIAN® biostimulant, a commercial product derived from the seaweed *Ascophyllum nodosum*, was diluted in distilled water, in the dosage recommended by the manufacturer, corresponding to 2.0 mL L^{-1}. Then, this solution was placed in a glass beaker with a volume of 1.0 L, where the seeds were immersed and the solution stirred for one hour, aiming to standardize the distribution of the product over the seeds.

To obtain the nutsedge extract, fresh tubers were collected, washed with running water and mild soap, and subsequently placed to dry on sheets of paper towels. Fifty grams of tubers were weighed and crushed in a blender with 1.0 L of distilled water. After processing, the material was sieved and diluted in distilled water at a concentration of 75%. After obtaining the extract, it was kept in a refrigerator (± 6°C) until use. The millet seeds were immersed in the solution of the nutsedge extract for 24 hours and then were sown directly in the pots. For the treatments that represented the controls, there was no immersion of the seeds in the nutsedge extract and in the biostimulant, being sown directly in the pots.

Irrigation for saline water treatments started at 15 DAE. This period was considered aiming at the full establishment of the seedlings. Applications of biostimulant and nutsedge extract solutions were performed every two weeks. For the biostimulant, the application was carried out via leaf in the dosage of 2.0 mL L^{-1}, following the manufacturer's recommendations. For the nutsedge extract, the solution was applied by watering in a volume of 500 mL.

Biometric evaluations were carried out weekly on the plants, aiming to monitor the influence of treatments on the growth of the crop, through the following parameters: number of tillers (counting all the tillers of the plant), leaf blade length (L, determined along the central vein, considering the point insertion of the ligula with the leaf blade up to its apex) and leaf blade width (W, measured with a measuring tape in the median region) in the older fully expanded leaf, number of live leaves (counted only the leaves that had more than 70% color green) and killed per plant (comprised all leaves with less than 70% green color). The leaf area (LA) was estimated from the regression model $LA = 0.879LW^{0.971}$ (Leite, Lucena, Cruz, Sá Júnior, & Simões, 2019).

In addition to these variables, at 77 DAE, measurements of net CO_2 assimilation and transpiration rates were performed, with a portable photosynthesis system (IRGA - Infrared Gas Analyzer) in the median region of the third fully expanded leaf.

The plant was harvested when the grains were ripe (ED9), and their morphological components were analyzed for the following variables: dry mass of: panicle, 100 grains, leaf blade, stem and root. The leaf blade dry mass was divided into living material (LM) and dead (DM), considering the state of the leaf at the cutting time. From the sum of the dry mass of the panicle, 100 grains, leaf blade and stalk, the total aerial dry mass production (TAP) was determined, and later with the sum of the TAP and the roots dry mass, biomass production was obtained for each treatment. To determine the dry mass, the different morphological components of the plant were dried in an oven with forced air circulation, with a temperature of 65°± 5ºC, until reaching a constant mass (Almeida et al., 2018).

The results were subjected to the test of normality, homogeneity, and subsequently to analysis of variance and regression. The level of 5% probability for rejection of the null hypothesis was adopted, using software R - Project version 2.13.1.

Results and discussion

There was a significant interaction ($p < 0.05$) between the salinity levels and the treatments studied (control, nutsedge extract and biostimulant) only for the variables number of tillers (NT), number of live leaves (NLL) and number of dead leaves (NDL). For leaf area (LA), leaf dry mass, root and biomass only isolated effects of treatments on salinity levels were observed. There was no significant interaction or isolated effect of the factors ($p > 0.05$) for the dry mass of the panicle, 100 grains, stem, total aerial production, net CO_2 assimilation rate and transpiration.

The tillering capacity in grasses is considered one of the most important characteristics for establishing the productivity of these plants (Sangoi et al., 2011), since the production of dry matter is directly related to the number and size of tillers. In this sense, the results promoted by the stimulants in the millet in the absence and moderate concentration of salts in the irrigation water stand out. The use of nutsedge extract and biostimulant in millet promoted greater tillering in the absence of salinity and at the level of 1 dS m^{-1}, while at the level of 2 dS m^{-1} there was only a significant effect of the nutsedge extract (Table 1).

The high concentration of salts at the level of 4 dS m^{-1} inhibited the effects of extracts on the tillering of millet, that is, there was no difference in relation to the control. These results are related to the negative impacts of excess salts on the availability of nutrients in the soil. The high concentrations of salts in the soil solution promote a nutritional imbalance in the plants, since the excess of Na^{+2} causes disturbances in the absorption of nutrients, such as potassium and nitrogen, in the plant tissues (Oliveira et al., 2011). According to Dias and Blanco (2010), the presence of Na salts tends to reduce the rate of mineralization of N, since with the increase of Na in the soil, there is a reduction in the mineralization of organic. Barbieri et al. (2014) and Oliveira et al. (2013) found that some of the beneficial effects of plant extracts can be inhibited in plants grown under salt stress.

Table 1. Number of tillers of millet as a function of salinity and treatments.

Salinity levels (dS m^{-1})	Treatments (Mean±standard deviation)			*p-value*
	Control	Nutsedge extract	Biostimulant	
	Number of tillers			
0	3.25±0.33 Bc	4.47±0.34 Bb	6.09±0.37 Aa	<0.0001
1	2.91±0.38 Bb	4.63±0.39 Ba	5.19±0.28 ABa	<0.0001
2	3.47±0.40 Bb	5.96±0.42 Aa	4.44±0.41 Bb	<0.0001
4	4.91±0.36 Aa	4.94±0.31 Aa	5.66±0.34 Aa	0.262
p-value	<0.0001	0.016	0.009	

Means followed by equal letters, uppercase in the columns and lowercase in the rows, do not differ statistically according to the Tukey test ($p < 0.05$).

Despite the negative effects of salinity on the availability of nutrients in the soil, some forage grasses, such as the millet, developed mechanisms of tolerance to this stress with the dilution of excess salts in the plant through the formation of new tillers. The high tillering, as observed in the control plants with the highest level of salinity in the irrigation water (Table 1), is considered an important mechanism of salt tolerance, since this increase in NT allows a better dilution of salts in the plant (García & Medina, 2003; Souto Filho, Laime, Fernandes, Suassuna, & Silva, 2014).

The availability of nutrients favors the greater emission of tillers in grasses, since nutrition plays a fundamental role in the formation of axillary buds and initiation of tillers (Sangoi et al., 2011). In this sense, one can correlate the better performance of the nutsedge extract in relation to the other treatments at the level of 2 dS m^{-1} to the greater development of the root system in these plants made possible by phytohormone (indolbutyric acid), found in the nutsedge root extract. Thus, it is worth noting that a greater development of the root system of millet contributes to a better use of water and nutrients present in the soil solution.

The NT in millet ranged from 2.91 tillers, in the condition of 1 dS m^{-1} with the control to 6.09 tillers in the treatment of 0 dS m^{-1} with the biostimulant (Table 1). These values are in agreement with the averages for the NT of millet found by other authors (Almeida et al., 2020; Costa, Domukoski, Ecco, & Duarte Júnior, 2014). Almeida et al. (2020) in a study with the same climatic conditions and the same cultivar as the present research, under water and saline stress, found that irrigation of 100% of crop evapotranspiration and a salinity level of 4 dS m^{-1} provided average of 5.23 tillers per plant at 60 DAE. These same authors noted the increase in NT at the level of 4 dS m^{-1} up to 40 DAE, after this period there was a considerable reduction in the NT of millet with this level of salinity.

Regarding the superior performance of the biostimulant in the optimum condition of the irrigation water (0 dS m^{-1}), the highest NT can be attributed to the composition of this commercial product. In this condition (Figure 1), the biostimulant promoted increases in the NT in the order of 94% and 44% compared to the control and nutsedge extract, respectively. The evaluated biostimulant has algae extract in its composition, associated with plant nutrients and regulators. These algae have, in their constitution, important phytohormones (auxins, gibberellins and cytokinins), capable of promoting cell division and elongation (Arrais et al., 2016).

For the control, nutsedge extract and the biostimulant, the best adjustments were obtained by quadratic regression equations ($p < 0.05$). It was found that for the NT there was no specific pattern of response of the plants to the addition of salts in the irrigation water (Figure 1). These results are related to the nature of the compounds applied and their respective impacts on plant responses when subjected to salinity conditions.

For the control, the maintenance of the NT was observed with the increase of 1 and 2 dS m^{-1} of the electrical conductivity of the irrigation water, however, with the increase of the salinity of the water in the highest level (4 dS m^{-1}), it was verified increase in NT (Table 1). Comparing the values obtained in plants irrigated with water without adding salts to those grown in higher salinity (4 dS m^{-1}), a total increase of 54% in the NT was verified. With the nutsedge extract, increases in NP were observed up to the salinity of 2 dS m^{-1} (5.57 tillers), from that level the impacts of salinity stand out over the beneficial effects of the extract and interrupt the appearance of new tillers. With the biostimulant, results similar to the control were observed, at the level of

2 dS m^{-1} there was a reduction of 36% in the NT, however, the increase in salinity from that level promoted the resumption of tiller emergence. These results demonstrate the mechanism of millet tolerance to salinity through the dilution of salts in the plant with the emission of new tillers regardless of the evaluated treatments (García & Medina, 2003; Souto Filho et al., 2014). It is noteworthy that the level of 4 dS m^{-1} is considered to be of very high risk for plants (Sales et al., 2014).

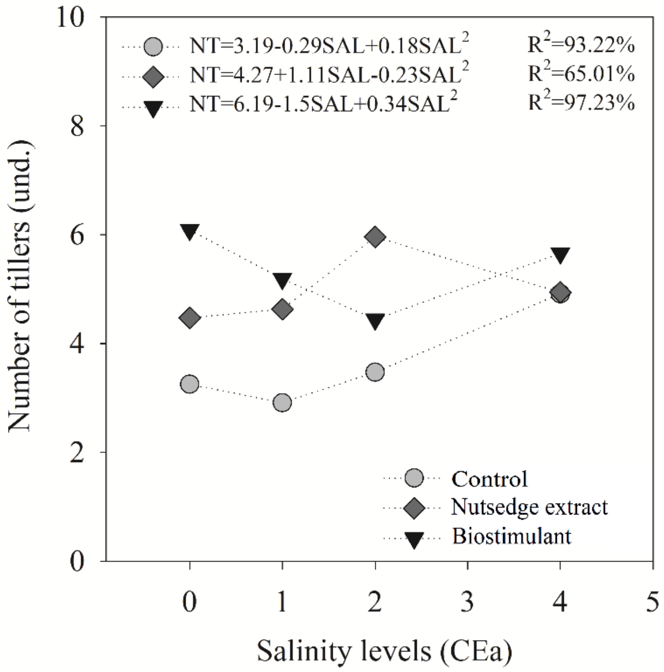

Figure 1. Effect of plant extracts on the number of tillers (NT) of millet grown under different levels of salinity (SAL, electrical conductivities, CEa in dS m^{-1}), at 56 days after emergence.

The nutsedge extract, due to its influence on root growth, was more tolerant to the effects of salinity. Under this extract, the addition of salts up to 2 dS m^{-1} was sufficient to activate the salinity tolerance mechanism by diluting the salts in the plant with the increase in the number of tillers. However, with the increase up to the level of 4 dS m^{-1}, due to the possible greater absorption of salts by the roots, there was a reduction in the number of tillers. The quadratic behavior that suggests a greater sensitivity to the effects of salts up to the level of 2 dS m^{-1} observed with the biostimulant may be related to the mode of action of the product, since it was applied via the leaves and, consequently, concentrates its effects on the aerial part of plants. In this case, the effects provided by the biostimulant play significant actions only in the absence of salinity condition, as it possibly does not bring the benefits of a greater use of the soil by the roots compared to nutsedge extract.

The results observed for the NLL are similar to those seen with the NT (Table 2). Significant increases ($p < 0.05$) were found in NLL through the beneficial effects of the biostimulant in the absence of salinity. At the level of 1 dS m^{-1}, the nutsedge extract and the biostimulant presented means higher than those verified with the control, while at the level of 2 dS m^{-1} only the nutsedge extract was superior to the control. As with tillering, the excess of NaCl salts at the level of 4 dS m^{-1} inhibited the beneficial effects of extracts on NLL. One of the main effects of salinity on plants is the limitation of growth due to increased osmotic pressure in the soil, resulting in a reduction in water absorption by plants, even under apparently moist soil (physiological drought) (Dias & Blanco, 2010).

Under stress conditions, plants develop tolerance mechanisms, resulting from biochemical processes and/or morphological changes. Among the morphological changes related to plant tolerance, the reduction of NLL stands out, in order to reduce the leaf area and, consequently, the transpirant surface (Oliveira, Medeiros, Cunha, Souza, & Lima, 2016). As observed for the NLL, there was no specific pattern of response of the plants to the addition of salts in the irrigation water. In fact, it was found that with the control the highest mean for the NLL was obtained with maximum salinity, while with the nutsedge extract and the biostimulant there was no significant difference between the absence of salts and the highest level (4 dS m^{-1}).

Table 2. Number of live leaves and number of dead leaves of millet as a function of salinity and treatments.

Salinity levels (dS m^{-1})	Treatments (Mean±standard deviation)			*p-value*
	Control	Nutsedge extract	Biostimulant	
Number of live leaves				
0	22.25±2.45 ABb	23.09±2.46 Ab	37.03±2.45 Aa	<0.0001
1	15.09±2.46 Bb	25.75±2.45 Aa	30.78±2.46 ABa	<0.0001
2	19.78±2.46 ABb	32.00±2.46 Aa	22.97±2.45 Bb	<0.0001
4	26.00±2.45 Aa	26.81±2.46 Aa	27.78±2.46 Ba	0.853
p-value	0.002	0.05	0.011	
Number of dead leaves				
0	5.47±1.11 Bb	10.88±1.12 Aa	13.22±1.11 Aa	<0.0001
1	8.13±1.12 ABa	8.03±1.11 Aa	9.72±1.11 ABa	0.364
2	9.16±1.11 ABa	10.22±1.11 Aa	9.00±1.12 Ba	0.603
4	9.81±1.11 Aa	9.31±1.12 Aa	12.00±1.11 ABa	0.198
p-value	0.003	0.2921	0.024	

Means followed by equal letters, uppercase in the columns and lowercase in the rows, do not differ statistically according to the Tukey test ($p < 0.05$).

In this sense, the absence of deleterious effects of salinity on NLL is related to the species' intrinsic tolerance to the levels of salts evaluated in this study. In addition to millet tolerance, it is noteworthy that during the conduct of the experiment there was an accumulation of 259.3 mm of rainfall, which may have caused a dilution of salts and a consequent reduction in their concentration in the soil.

Plants treated with nutsedge extract or biostimulant were superior to the control in three salinity levels for NLL. These results are explained by the composition of these extracts. According to Khan, Hiltz, Critchley, and Prithiviraj (2011), biostimulants from the extract of *A. nodosum*, even if in small quantities, can have positive effects on plant growth, as they present several hormones in their composition, in addition to other compounds with activity similar to that of plant hormones. Regarding the superior response of the nutsedge extract, the results are related to the presence and consequent beneficial effect of indolebutyric acid, which corresponds to a phytohormone responsible for the formation and growth of the roots (Cavalcante et al., 2016).

Regarding millet NDL (Table 2), there was a significant effect of the extracts only at the level of 0 dS m^{-1}, with the highest number of dead leaves found in plants treated with nutsedge extract and biostimulant. This should not be considered a negative factor for the use of these plant extracts, since this morphogenic variable has an equilibrium relationship with the NLL, the higher the NLL, the greater the senescence and death of leaves in the plant.

For the NLL and NDL significant responses of the addition of salts were observed only with the control plants and the biostimulant, and, for all cases, the adjustments were obtained by quadratic regression equations (Figures 2), except for the treated NDL with nutsedge extract, whose best fit was observed through a cubic model. Through the regression analysis it was noted that the superiority of the plants treated with the nutsedge extract in the conductivity of 2 dS m^{-1} remains with the increase of up to 1 dS m^{-1} in the electrical conductivity of the irrigation water. The plants of this treatment under salinity equivalent to 3 dS m^{-1} are superior to the control in 22% and to the biostimulant in 27%. From that level there is no difference between the control plants and those treated with stimulants. It is also worth noting the significant increase in NLL provided by the biostimulant in the absence of salinity. In this condition, this treatment promoted increases in the NDL in the order of 72% and 80% in comparison to the nutsedge extract and the control, respectively.

Regarding the NDL, it is possible to state that despite the tolerance mechanisms developed by millet to stress due to salinity, the excess of salts in the soil caused higher leaf mortality. Comparing the values obtained in the control plants irrigated with water without adding salts to those grown in higher salinity (4 dS m^{-1}), an increase of 78% in NDL was verified. With the plants treated with nutsedge extract and biostimulant there was no significant impact of salinity on NDL. These results are directly related to the toxic effects of excess ions (Na^+ and Cl^-) absorbed by plants. The damage caused by chloride toxicity results in the burning of the apex of the leaves, which, in advanced stages, reaches the edges and promotes premature senescence. While the typical symptoms of Na toxicity appear in the form of a burn or necrosis, along the edges and progressively progress to the center of the leaves (Dias & Blanco, 2010).

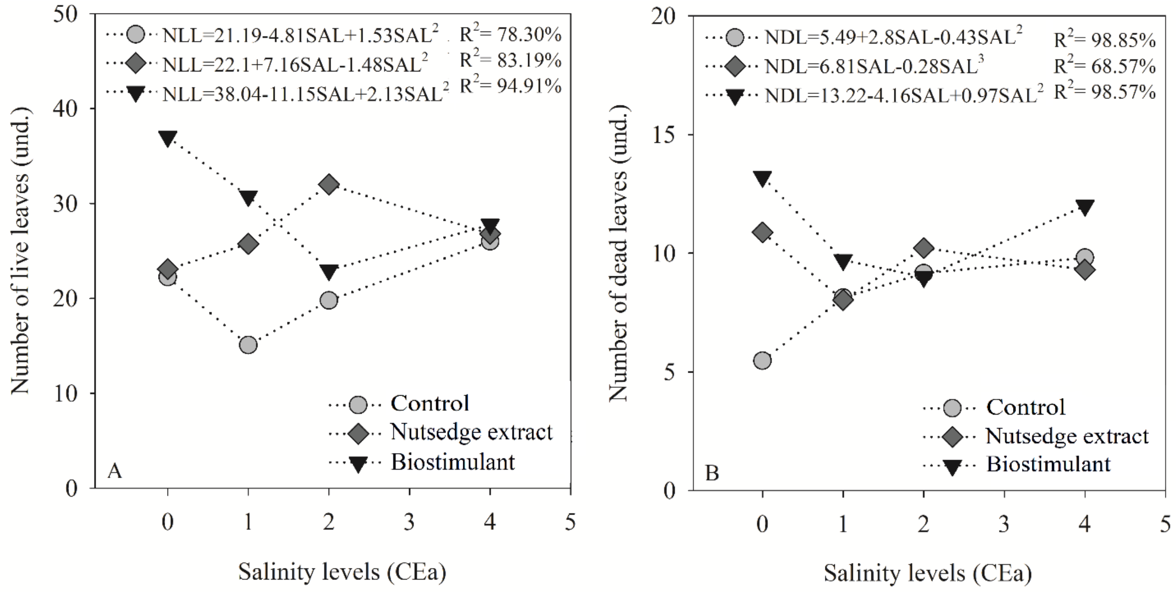

Figure 2. Plant extracts effect on the number of live leaves (NLL) and number of dead leaves (NDL) of millet grown under different levels of salinity (SAL, electrical conductivities, CEa in dS m^{-1}), at 56 days after emergence.

There was no significant effect of salinity on the leaf area (Figure 3) and on all yield components (Figure 4). These results show that due to the photosynthetic system, type C_4, millet has a high efficiency in the use of water, as well as being able to withstand the levels of salinity evaluated in this study. However, isolated effects were observed between treatments (control, nutsedge extract and biostimulant) on the salinity levels for the leaf area (LF), leaf dry mass (live and dead material), root and biomass.

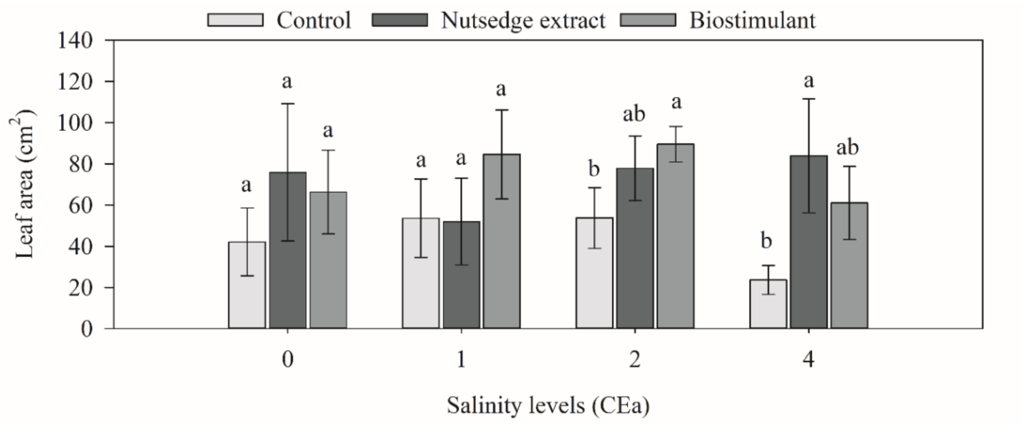

Figure 3. Effect of plant extracts on the leaf area of millet cultivated under different levels of salinity (electrical conductivities, CEa in dS m^{-1}), at 56 days after emergence.

The biostimulant use promoted significant increases in the leaf area of millet with a salinity of 2 dS m^{-1} compared to control plants. At the level of 4 ds m^{-1} only significant effects of the nutsedge extract were observed in relation to the control (Figure 3). These results reflected in higher leaf blade dry mass production (Figure 4) with these treatments at the respective levels discussed, which confirms the hypothesis that the benefits promoted by these stimulants can serve as a strategy to increase forage plant biomass production under the presence of salts in the irrigation water.

The biostimulant promoted an increase of 66% in the leaf area of millet at the level of 2 dS m^{-1} compared to the control. For the condition of greater salinity evaluated in this study, it was observed increments provided by the nutsedge extract in comparison to the control in the order of 253%. As previously discussed, the results verified with the use of nutsedge extract and biostimulant are directly related to the composition of these compounds (Cavalcante et al., 2016; Khan et al., 2011).

These expressive results obtained with the nutsedge extract use in the leaf area of the millet reflected in greater production of leaf blade dry mass in living material (Figure 4A). The use of nutsedge extract and biostimulant s reduced the toxic effects of salts in the leaves, with reduction of the leaf blade in dead material (Figure 4B).

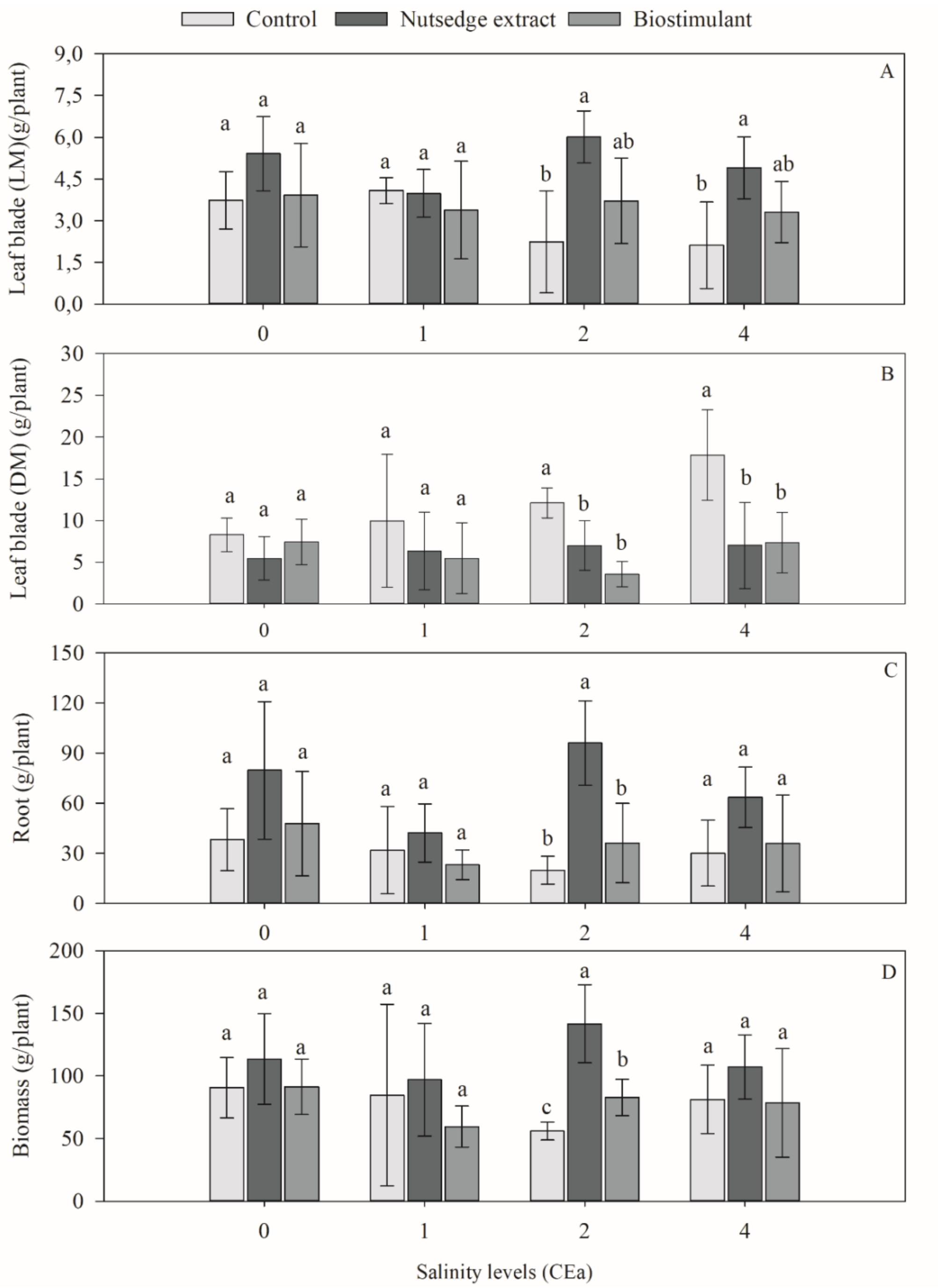

Figure 4. Effect of plant extracts on the leaf blade dry mass production in live material - LM (A) and dead - DM (B), root (C) and biomass - aerial part + root (D) of millet cultivated under different salinity levels (electrical conductivities, CEa in dS m^{-1}), at 84 days after emergence. Means followed by equal letters for each level of salinity, do not differ statistically according to the Tukey test ($p < 0.05$).

With the highest concentrations of NaCl (2 and 4 dS m^{-1}), it was found in plants treated with the tiririca extract averages of 6.01 and 4.9 g $plant^{-1}$ for the production of dry leaf blade mass, considering the living matter (LM) of this constituent (Figure 4A), these values being more than the double of the production found with the control. However, the averages of leaf blade production with nutsedge extract did not differ from the yields of 3.71 and 3.31 g $plant^{-1}$ obtained with the biostimulant at the highest salinity levels.

The stimulants significantly reduced the losses in quantity and quality of the dry matter production of the leaf blade, since with the application of these treatments it was possible to keep the leaves alive in the plants for a longer period of time and, consequently, the maintenance of the nutritional value. The use of nutsedge extract and biostimulant in the salinity of 2 dS m^{-1} reduced the losses due to mortality in leaf dry matter by 72% and 236%, respectively, compared to the control plants. With the electrical conductivity of 4 dS m^{-1}, the reductions were 153% and 142% for the nutsedge extract and biostimulant, respectively (Figure 4B). These results indicate the benefits of using the nutsedge extract and the biostimulant, aiming at a higher forage yield of the millet culture.

The accumulation of sodium and chloride in plants promotes damage to the cytoplasm, mainly in the border and at the apex of the leaves and, as the problem intensifies, the necrosis advances to the center of the leaves (Dias & Blanco, 2010). These changes caused by the exposure of plants to salt stress are associated with the reduction of photosynthetic pigments. According to Chutipaijit, Cha-Um, and Sompornpailin (2011), the decrease in chlorophyll content under salt stress is a phenomenon frequently reported. In several studies, the amount of chlorophyll was used as an indicator of the cellular metabolic state, due to the possible relationship with the degradation of the chloroplast membrane, due to the toxic effects of the accumulation of salts in the leaves. This reduction in chlorophyll content induces the excitation of chlorophyll molecules that still remain unchanged, favoring the formation of free radicals capable of promoting lipid peroxidation, thus affecting more chlorophyll molecules and other membranes in the photosynthetic process, which results in photooxidation and subsequent death of the leaves (Oliveira et al., 2018).

Therefore, chlorophyll is the main responsible for the green color of the plants and approximately 50 to 70% of the nitrogen in the leaves is associated with these photosynthetic pigments (Taiz, Zeiger, Moller, & Murphy, 2017). Thus, considering that nitrogen is the essential constituent of proteins and that the supply of the nutritional needs of ruminants depends mainly on the energy and protein content of the diet (Viana et al., 2012), it is suggested that the results obtained with the control plants for the production of dry matter from the dead fraction of the leaf blade can safely represent a reduction in the nutritional value of these plants.

Significant effects of the nutsedge extract were observed in the production of dry matter from the roots and biomass with only the salinity of 2 dS m^{-1} (Figure 4C and D). In this condition, the nutsedge extract promoted greater growth of the millet roots, with 381% and 165% of increases in the root dry mass, compared to the control plants and those treated with the biostimulant, respectively. The considerable increases in the roots dry mass provided by the use of the nutsedge extract in the CEa of 2 dS m^{-1} favored the greater production of millet biomass, being 152% and 70% higher in relation to control and the biostimulant. Still under this condition, differences were verified between the production of biomass with the biostimulant and the control, where there was an increase of 48% in the biomass of the plants due to the application of the biostimulant.

Despite the absence of significant effects between the averages of the treatments with the conductivity of 4 dS m^{-1} for the production of dry mass of roots and biomass, it is worth mentioning that with this level of salinity the nutsedge extract provided a higher average for mass production root drought, equivalent to 111% and 77% of the control and biostimulant plants, while in the production of biomass these increases were 32% and 36%. This treatment also showed superiority in comparison to the control in the dry matter production of the leaf blade (LM) at the levels of 2 and 4 dS m^{-1}, which shows the relationship of greater root growth in the production of dry leaf mass.

The nutsedge extract has in its composition a high concentration of endogenous auxins, such as indolacetic acid (IAA) and indolebutyric acid (Dias et al., 2012). Auxins are compounds that have the ability to promote cell division and stretching, and are also responsible for root formation and development (Taiz et al., 2017). According to Meneguzzi et al. (2015), the IAA is responsible for the formation of apical buds, lateral buds and the apex of the roots. According to these authors, this phytohormone can promote greater rooting when used in optimal concentrations. Moreira and Gliglio (2012) observed positive results of the nutsedge extract in the length of the corn root. In the wheat crop, these same authors found that the nutsedge extract promoted greater height of the aerial part and greater mass of the seedlings.

At 84 DAE, in analysis independent of the salt level, percentages of dry mass of the panicle in relation to the aerial part were verified, corresponding to 7.4% with the control, 8.92% for the nutsedge extract and 11.87% with the plants treated with the biostimulant. In this sense, according to Avelino et al. (2011) a higher percentage of panicles is fundamental for the production of high quality silage, since this constituent has a high amount of total digestible nutrients, and contributes considerably to a higher production of dry mass of the aerial part.

In glycophyte species, classified as moderately tolerant to salinity, such as millet, tolerance to salt stress is extremely linked to the plant's ability to prevent the accumulation of toxic ions such as Na^+ and Cl^- in the aerial part. On the other hand, sensitive species are unable to prevent this accumulation and to compartmentalize these ions in a specific tissue or leaf blade cell, suffering, as a consequence, their interference in the cellular metabolism, which will cause a reduction in plant growth and development (Boursier & Läuchli, 1989). In this sense, it is evident that the absence of a deleterious effect of salinity for all yield components in millet (Figure 4) is directly associated with the ability of this crop to prevent the accumulation of potentially toxic ions in the aerial part, including in the their reproductive structures.

Hussain et al. (2017), evaluating two strains of millet, one considered tolerant and the other sensitive to salinity, showed that the differential growth between these two strains may be due to differences in the rates of transfer and accumulation of ions in the aerial part of the plants. The tolerant strain showed lower transfer rates of Na^+ and Cl^- under saline stress, due to better control of the absorption of the root system of these ions, as well as greater salt retention in the roots. Also according to these authors, the highest content of Na^+ and Cl^- ions were found in order, in stems and sheaths, roots, young leaves (2nd leaf, top to botton) and old leaves (3rd leaf, top to botton). These results of retention of potentially toxic ions in the stem and sheath were considered as an example of a millet tolerance mechanism to salinity, suggesting that this species exports little Na^+ from the stem to the leaf limbs, thus avoiding the excess of potentially ions toxic in photosynthetic tissues.

Salinity levels and application of treatments to millet seeds and plants had no influence on photosynthesis at 77 DAE. These results are good indicative of the intrinsic tolerance of millet culture to saline stress. However, it is worth mentioning once again that during the conduct of the experiment there was an accumulation of 259.3 mm of rainfall, which may have promoted the leaching of salts and a consequent reduction in their concentration in the soil placed in the pots. Among the harmful factors of salinity related to photosynthetic efficiency, water deficiency is one of the main and primary factors, since the presence of salts interferes with the water potential of the soil, reducing the potential gradient between the soil and the plant. In this sense, salt tolerance is directly related to the plant's ability to use the water available in the soil. Millet, as a C_4 and glycophyte plant, has a better efficiency in the use of water compared to C_3 plants, when subjected to limiting environmental conditions such as, for example, salt stress.

According to Machado (1988), the species that present the C_4 metabolism have the capacity to concentrate CO_2 in the cells of the vascular sheath, which favors the activity of the ribulose 1.5 bisphosphate carboxylase (RubisCO), and, consequently, reduces the occurrence of photorespiration in these plants. Thus, C_4 grasses become more efficient in maintaining photosynthetic activity at normal levels, even when subjected to abiotic stresses.

It was observed that the salinity levels and the application of the treatments had no influence on transpiration, at 77 DAE. The species that have the C_3 and C_4 photosynthetic systems respond in different ways to salt stress, mainly due to the greater efficiency in the use of water by C_4 plants and their general adaptation to locations with adverse development conditions. This better use of water is due to the greater efficiency in capturing and storing carbon from CO_2, which allows C_4 plants to better manage stoma opening, thus contributing to the control of leaf transpiration (Marin & Nassif, 2013).

Under conditions of abiotic stress, such as saline and water stress, plants, in general, decrease stomatal conductance, in order to reduce water loss by the leaves. However, even though this reduction in the rate of water loss may represent an advantage to avoid tissue dehydration, it is worth noting that this mechanism can directly affect the sensitive heat balance on the leaves, and, in addition, due to the reduction perspiration, reduce CO_2 absorption and, consequently, limit photosynthesis (Nascimento, Bastos, Araújo, Freire Filho, & Silva, 2011).

Therefore, as the rate of transpiration in the present study was not influenced by the concentrations of salts in the irrigation water, it is evident that the millet culture did not present physiological indications of saline stress, as well as it was not affected by the limitation of availability of water for plants, by reducing the potential gradient between the soil and the plant.

Conclusion

The IPA BULK 1BF millet cultivar has tolerance to the salinity levels studied in this research.

The nutsedge extract and the biostimulant reduce the losses in quantity and quality of the dry matter production of the leaf blade of millet.

Stimulants are alternatives capable of increasing the growth and forage production of millet under the presence of salts in the irrigation water, however, these compounds have no influence on grain production.

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