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# Quantitative and qualitative responses of *Catharanthus roseus* to salinity and biofertilizer

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#### ABSTRACT

The development of agriculture in the northeastern semi-arid region depends, at least in part, on the correct exploitation and efficient use of natural resources. The objective of this study was to evaluate the effects of the use of saline water on morphophysiological responses and sensory analysis of 'Boa noite' plants (*Catharanthus roseus*) in soil fertilized with bovine biofertilizer. A completely randomized design was used in the split plot arrangement, the plots being formed by the five irrigation water salinity levels (0.5, 2.5, 5.0, 7.5 and 10 dS m<sup>-1</sup>), and the subplots by two frequencies of application of the 150 mL pot<sup>-1</sup> of liquid bovine biofertilizer (divided into one and five times), with five replicates. The analysed variables were: leaf gas exchange relative chlorophyll index, biometry, dry matter (leaf, stems, flowers and roots) and sensory analysis (general appearance and buyers' preference). Quantitative (growth and physiological) and qualitative (sensory analysis) responses show that *Catharanthus roseus* plants can be produced using saline water at the level of 2.5 dS m<sup>-1</sup>, and the plants of this treatment were preferred by the judges in sensory analysis. Biofertilizer application frequency did not attenuate the effects of salinity. Therefore, the liquid bovine biofertilizer can be applied all at once, reducing costs.

**Palavras-chave:** plantas ornamentais tolerância à salinidade análise sensorial

## Respostas quantitativas e qualitativas de *Catharanthus roseus* à salinidade e biofertilizante

#### RESUMO

O desenvolvimento da agricultura no semiárido nordestino depende, sobretudo, da exploração correta e do uso eficiente dos recursos naturais. Objetivou-se avaliar os efeitos do uso de águas salinas, em solo adubado com biofertilizante bovino, sobre as respostas morfofisiológicas e na qualidade (análise sensorial) de plantas de Boa noite (Catharanthus roseus). Usou-se o delineamento inteiramente casualizado com tratamentos arranjados em parcelas subdivididas, sendo as parcelas formadas por cinco níveis de salinidade da água de irrigação (0,5; 2,5; 5,0; 7,5 e 10 dS m<sup>-1</sup>) e as subparcelas por duas frequências de aplicação da dose de 150 mL de biofertilizante bovino líquido por vaso (dividida em uma e cinco vezes), com cinco repetições. As variáveis analisadas foram: trocas gasosas foliares índice relativo de clorofila, biometria, matéria seca de folhas, hastes, flores e raízes e análise sensorial (aparência geral e preferência dos compradores). Com as análises quantitativas (crescimento e fisiológicas) e qualitativas (análise sensorial), notou-se que plantas de Catharanthus roseus de cor branca podem ser produzidas utilizando-se água salina em nível de 2,5 dS m<sup>-1</sup>, sendo as plantas deste tratamento as preferidas dos julgadores na análise sensorial. A frequência de aplicação do biofertilizante não atenuou os efeitos da salinidade. Desta forma, pode-se aplicar o biofertilizante bovino líquido de uma única vez, diminuindo custos.



#### **INTRODUCTION**

Rapid growth of urban populations and intense competition for good-quality water between agriculture, industry and recreational and domestic users have led to increased interest in the use of alternative water sources in various sectors. For application in the sector of irrigated agriculture, some of these water sources stand out, such as saline and brackish waters, common in semi-arid regions (Lacerda et al., 2011), agricultural drainage water (Oliveira et al., 2012), rejects of desalinators and wastewaters (Porto et al., 2001; Medeiros et al., 2008).

Among the sectors that depend on irrigation, at least in one part of the year, garden and landscaping areas could primarily be supplied with lower-quality waters. Hence, it becomes necessary to sort and identify ornamental plants tolerant or moderately tolerant to water salinity, which can allow the expansion in the use of saline water for garden irrigation and seedling production (Niu et al., 2012; Wu et al., 2016; García-Caparrós et al., 2016). In Brazil, however, ornamental plants are irrigated with good-quality water and information on their tolerance to salinity, and particularly 'Boa noite' (*Catharanthus roseus*), is limited and sporadic.

Plant responses to salinity have been observed especially in terms of growth, yield and physiological variables (Munns & Tester, 2008; Rahnama et al., 2010). In the case of ornamental plants, it is also fundamental to evaluate the effects on plant quality particularly relative to the visual aspect, based on analysis by the future consumers, because the beauty in the acquisition of an ornamental plant is not always associated with its size (Cassaniti et al., 2013).

In this context, this study aimed to evaluate the impacts of using saline waters, in soil fertilized with bovine biofertilizer, on growth, physiological responses and quality (sensory analysis) of 'Boa noite' plants (*Catharanthus roseus*).

#### MATERIAL AND METHODS

The experiment was carried out in the experimental area of the Meteorological Station of the Federal University of Ceará, in Fortaleza, CE, Brazil (3° 45' S; 38° 33' W; 19 m). During the experiment, temperature and relative air humidity data inside the greenhouse were monitored by a data logger of the Onset Computer Corporation (model HOBO<sup>°</sup> U12-012 Temp/RH/ Light/Ext). Mean air temperature varied from 28 to 31 °C, while relative humidity oscillated between 59.0 and 68.5%.

The experiment was installed in a completely randomized design, with treatments arranged in split-plot scheme, in which plots were formed by five levels of irrigation water salinity and the subplots by two frequencies of application of the 150 mL dose of liquid bovine biofertilizer per pot (divided into one and five times), with five replicates, in a total of 50 experimental units (one plant per experimental plot).

The experiment used seedlings of 'Boa noite' (*Catharanthus roseus*), white color, with 45 days of age, which were transplanted to 7 L pots filled with substrate made by a mixture of arisco (fine sand-sized particles used in constructions) + earthworm humus, at proportion of 2:1. After transplanting,

plants were irrigated with low-salinity water  $(0.5 \text{ dS m}^{-1})$  for 15 days, to recover from the stress suffered in the transplantation and start producing new roots and leaves. After this period, plants were irrigated with saline water according to the respective treatments, until 75 days after transplantation.

Irrigation was performed using waters with different saline concentrations, according to the treatments: S1 - 0.5; S2 - 2.5; S3 - 5.0; S4 - 7.5 and S5 - 10.0 dS m<sup>-1</sup>. The solutions were prepared using NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O and MgCl<sub>2</sub>.6H<sub>2</sub>O salts at proportion equivalent to 7:2:1 (mmol<sub>c</sub> L<sup>-1</sup> = EC x 10), according to Rhoades et al. (2000). The applied water volume was estimated in order to cause drainage, so that water drained through the bottom of the pots, with a leaching fraction of 0.15. Water was locally applied to avoid direct contact with the leaves.

The liquid bovine biofertilizer used in the experiment was prepared using fresh manure, anaerobically obtained, according to Mesquita et al. (2012), and applied at dose of 150 mL per pot in a single application in the B1 treatment and split into 5 times in the B5 treatment. Synthetic mineral nutrients were not applied, only the liquid bovine biofertilizer. In the B5 treatment, the biofertilizer was always applied one day before the readings of leaf gas exchanges.

At 30, 45 and 60 days after transplanting (DAT), stomatal conductance (gs, in mol m<sup>-2</sup> s<sup>-1</sup>), transpiration rate (E, in mmol m<sup>-2</sup> s<sup>-1</sup>) and photosynthetic rate (A, in µmol m<sup>-2</sup> s<sup>-1</sup>) were measured in fully expanded leaves using an IRGA device (model LI6400XT, Licor, USA). Readings were taken with attached light source with intensity of 1500 µmol m<sup>-2</sup> s<sup>-1</sup> and under natural conditions of air temperature and CO<sub>2</sub> concentration, between 8 and 11 h. In the same leaves, the relative chlorophyll index was determined using a portable SPAD meter (Minolta).

At 65 DAT, plants were collected, separated into leaves (leaf blades), stems (branches and petioles), flowers and roots, stored in paper bags, properly identified and, later dried in an oven at 60 °C, weighed to determine dry matter production. Plant height was measured using a ruler, number of branches was manually counted and leaf area was measured during plant collection (Area meter, LI-3100, Licor, USA).

Plants of all treatments, after reaching the commercial point, were selected and subjected to sensory analysis, according to Ureña et al. (1999), based on hedonic scale, with nine numerical points whose limits were one (extremely disliked) and nine (extremely liked), and the affective method (preference test), with the question: "In your opinion as a consumer, which of the plants would you buy?". Such analysis was made by 40 judges randomly selected among students, staff and professors of the Federal University of Ceará. The experimental design was completely randomized, with 40 replicates, represent by the untrained judges.

For the sensory evaluation test, the positions of the samples were randomly selected and randomized, identified as: A, B, C, D, E, F, G, H, I and J, in the form used in the sensory analysis. The evaluation tests of attributes and preference, based on data of identification, consisted of a sample with greater number of male untrained judges (27 male judges and 15 female judges), with average age of 18 to 35 years and predominance of university students. Statistical analyses were performed using the software Assistat, developed by Silva & Azevedo (2016). Growth and physiological variables data were subjected to analysis of variance to verify the isolated and interactive effects. Effects of salinity levels were tested by regression, using the F test to verify the significance (p < 0.05) and selecting the model with best fit, with highest coefficient of determination ( $\mathbb{R}^2$ ). The results of the sensory analysis were evaluated according to Ureña et al. (1999).

Data of growth, dry biomass production and the mean values of net  $CO_2$  assimilation (A), stomatal conductance (gs), transpiration rate (E) and relative chlorophyll index (RCI) were used to calculate the percent reductions in the different saline treatments compared with the control, and the salinity tolerance indices were established according to the methodology described by Fageria (1985): tolerant (reductions from 0 to 20%), moderately tolerant (20.1 to 40%), moderately sensitive (40.1 to 60%) and sensitive (reduction superior to 60%).

#### **RESULTS AND DISCUSSION**

For shoot biomass production, only water salinity had significant effect (p < 0.05), and there was no influence of biofertilizer application frequency or the interaction between factors. According to Figure 1, water salinity had a decreasing linear effect on leaf biomass (Figure 1A) and stem biomass (Figure 1B), but a quadratic effect on flower biomass production (Figure 1C). In the latter case, maximum flower production was estimated at salinity level of 3.9 dS m<sup>-1</sup>.

It is also important to point out that the reduction in leaf and stem biomass production for that salinity level was below 20%, indicating that plants did not suffer major reduction in vegetative growth and partially compensated it, increasing flower production. Similar results were obtained by Alvarez & Sánchez-Blanco (2015), who found that irrigation water salinity of 4.0 dS m<sup>-1</sup> caused reduction of 39% in the biomass production of *Callistemon laevis* plants, but did not affect flowering.

Since there was no effect of biofertilizer application frequency or interaction between factors (p > 0.05) on growth data, all tolerance indices were presented based on the mean values relative to the factor salinity (Table 1). For the thirteen indices used, plants were tolerant to water salinity of 2.5 dS m<sup>-1</sup>, with mean reduction of 6.2%. Maximum reduction on the order of 14.5% was observed in leaf dry matter and minimum of 0% in flower dry matter and mean photosynthetic rate. For salinity of 5.0 dS m<sup>-1</sup>, plants were predominantly classified as moderately tolerant, with mean reduction of 27.8%. For higher salinity levels, the classification of moderately sensitive prevailed, with reductions from 40 to 60%.

Based on the results obtained by Cai et al. (2014), who studied five rose genotypes subjected to salinity levels of 1.5, 4.0 and 8.0 dS m<sup>-1</sup>, it was observed that most genotypes tested were moderately tolerant to salinity from 3.0 to 4.0 dS m<sup>-1</sup>. These authors found that, at salinity level of 4.0 dS m<sup>-1</sup>, reductions in shoot biomass production varied from 22 to 40%. At the highest salinity level (8.0 dS m<sup>-1</sup>), shoot dry matter in the five cultivars was reduced by 41 to 69%. In contrast, Niu et al.



Figure 1. Dry matter of leaves - LDM (A), stems - StDM (B) and flowers - FDM (C) of 'Boa noite' plants (*Catharanthus roseus*) at 65 DAT, as a function of levels of irrigation water salinity

Table 1. Percent reduction of biomass production (total, shoots, leaves, flowers, stems and roots), number of branches, plant height, leaf area, stomatal conductance (gs), photosynthetic rate (A), transpiration rate (E) and relative chlorophyll index (RCI), and classification regarding tolerance to salinity of 'Boa noite' plants (*Catharanthus roseus*) irrigated with saline waters

Variables	Percent reduction (%) – dS m <sup>-1</sup>			
	2.5	5.0	7.5	10.0
TDM	7.0 <sup>⊤</sup>	34.5™	49.4 <sup>™S</sup>	55.4 <sup>™S</sup>
ShDM	4.9™	31.3™	47.8 <sup>MS</sup>	54.7 <sup>™S</sup>
LDM	<b>1</b> 4.5 <sup>⊤</sup>	34.9™	51.7 <sup>™s</sup>	54.6 <sup>MS</sup>
FDM	0 <sup>т</sup>	0 <sup>τ</sup>	14.1⊺	34.4™
StDM	-2.8 <sup>⊤</sup>	33.7™	46.3 <sup>™S</sup>	57.1 <sup>™S</sup>
RDM	14.2 <sup>⊤</sup>	45.4 <sup>™S</sup>	54.9 <sup>™S</sup>	57.5 <sup>MS</sup>
NB	12.2 <sup>⊤</sup>	20.4™	38.7™	43.2 <sup>™S</sup>
PH	<b>-</b> 1.3 <sup>⊤</sup>	4.2 <sup>™</sup>	14.9 <sup>⊤</sup>	24.1 <sup>™™</sup>
LA	6.2 <sup>⊤</sup>	16.4⊺	43.3 <sup>™S</sup>	51.7 <sup>™S</sup>
<b>gs</b> <sub>mean</sub>	10.3 <sup>⊤</sup>	39.0™	52.4 <sup>™s</sup>	70.1 <sup>s</sup>
A <sub>mean</sub>	0.0 <sup>T</sup>	22.3™	33.0 <sup>™⊺</sup>	44.5 <sup>™S</sup>
Emean	7.6 <sup>⊤</sup>	30.4™	40.6 <sup>™S</sup>	55.5 <sup>™S</sup>
RCImean	1.5™	21.6 <sup>™™</sup>	23.9 <sup>MT</sup>	25.9 <sup>MT</sup>
Overall mean	6.2 <sup>⊤</sup>	27.8™	41.7 <sup>™S</sup>	49.5 <sup>™S</sup>

<code>T; MT; MS; STOlerant; Moderately tolerant; Moderately sensitive; Sensitive, respectively; TDM = Total dry matter; ShDM = Shoot dry matter; LDM = Leaf dry matter; FDM = Flower dry matter; StDM = Stem dry matter; RDM = Root dry matter; NB = Number of branches; PH = Plant height; LA = Leaf area; gs\_mean = Mean stomatal conductance; A\_mean = Mean photosynthetic rate; E\_mean = Mean photosynthetic rate; RCI\_mean = Mean relative chlorophyll index = Number of the store of th</code>

(2008), working with three rose rootstocks [*Rosa x fortuniana* Lindl., *R. multiflora* Thunb., and *R. odorata* (Andr.) Sweet], observed that shoot dry matter decreased by 33, 49 and 55% in *R.* × *fortuniana*, *R. odorata*, and *R. multiflora*, respectively, when irrigation water salinity increased from 1.6 to 6.0 dS m<sup>-1</sup>.

Other studies conducted with ornamental plants confirm the existence of genetic variability in these species (Wu et al., 2016). For Cassaniti et al. (2009) and Sabra et al. (2012), the tolerance of ornamental plants to salts can be associated with the lower absorption and transport of potentially toxic ions (Na and Cl) to leaf tissues, which reduces the toxic effects and burning and death of leaves.

Tolerance to salinity can be evaluated based on indices of growth, burning of leaves and physiological responses (Fageria, 1985; Niu & Cabrera, 2010; Rahnama et al., 2010). However, in the case of ornamental plants, the aesthetic value also assumes important role in the analysis of the final product (Bernstein et al., 1972). Therefore, visual quality, although it may be subjective, is an important parameter to evaluate tolerance to salinity in ornamental plants (Niu & Rodriguez, 2006a, b; Niu et al., 2007).

According to Figure 2A, salinity treatments S1 (0.5 dS m<sup>-1</sup>) and S2 (2.5 dS m<sup>-1</sup>) showed the highest number of grades equal to or higher than 7 for overall plant appearance, and this trend was similar for single application (B1) and split application (B5) of biofertilizer. In contrast, among the judges, the treatments S2B1 and S2B5 showed greater purchase intents (Figure 2B), being the preference of 15 and 17 judges, respectively, while for the treatments S3B1, S5B1 and S5B5, none of the judges expressed purchase intent. Four, six, six, three and one judges expressed purchase intent relative to S1B1, S1B5, S3B5, S4B1 and S4B5, respectively. These results demonstrate that plants irrigated using saline water with EC of 2.5 dS m<sup>-1</sup> (S2) were the preferred ones, and this result was not influenced by the biofertilizer application frequency.

A possible explanation for these results of the sensory analysis (Figure 2) is related to the small reduction in the vegetative biomass and increase in flower production (Figure 1) observed in plants that received water with salinity of





2.5 dS m<sup>-1</sup>, compared with the treatment of lowest salinity. In contrast, low grades and low preference of the judges for the most saline treatments reflect the negative effects of salinity on all variables (Table 1), in both quantitative (lower growth) and qualitative or visual terms (such as reduction in relative chlorophyll index).

According to Cai et al. (2014), flower production, a preponderant factor for ornamental plants, decreases with the increase of salinity, but this response depends on the studied genotype. These authors observed that, from six rose varieties, two did not show reduction in flower production when irrigated using water of up to 4.0 dS m<sup>-1</sup>, a result similar to that of the present study.

Niu et al. (2007), working with ten ornamental species irrigated using saline solutions with EC of 0.8, 3.2 and 5.4 dS m<sup>-1</sup>, observed genetic variability in terms of both quantitative responses (growth) and visual aspects. While *Rudbeckia hirta* L. plants did not survive when irrigated using water with salinity level of 3.2 and 5.4 dS m<sup>-1</sup>, plants of *A. millefolium, G. aristata, L. x hybrida, L. japonica*, and *R. officinalis* exhibited a small reduction in growth and maintenance of aesthetic quality (visual appearance) when irrigated using water with salinity level of up to 5.4 dS m<sup>-1</sup>.

#### Conclusions

1. Quantitative (growth and physiological) and qualitative (sensory) analyses showed that white 'Boa noite' (*Catharanthus roseus*) plants can be produced using saline water of 2.5 dS m<sup>-1</sup>, and plants of this treatment were the preferred ones by the judges in the sensory analysis.

2. Biofertilizer application frequency did not attenuate the effects of salinity. Thus, liquid bovine biofertilizer can be applied all at once, reducing costs.

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