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Saline pisciculture effluent as an alternative for irrigation of *Croton blanchetianus* (Euphorbiaceae)[1]

Efluente salino de piscicultura como alternativa para irrigação de *Croton blanchetianus* (Euphorbiaceae)

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HIGHLIGHTS:

Croton blanchetianus *tolerates 3.5 dS m⁻¹ of pisculture saline effluent from fish farming, the maximum level evaluated in this study.*
Up to 3.5 dS m⁻¹ of saline effluent from fish farming can be reused for irrigation of C. blanchetianus *in arid and semiarid regions.*
C. blanchetianus *osmoregulates in the presence of 2.5 and 3.5 dS m⁻¹ of saline effluent from fish farming.*

ABSTRACT: The use of saline effluents from fish farming in agricultural activities has been increasing, especially in semiarid regions. This study was to evaluate the use of saline effluents from fish farming for the irrigation of *Croton blanchetianus* and to analyze the physiological and biochemical responses. After the cuttings were planted, irrigation with the effluent began. Treatments included four salinity levels (control, 1.5, 2.5, 3.5 dS m^{-1}). The control treatment was the supply water (0.56 dS m^{-1}). The evaluated variables were growth, relative water content, dry biomass, and levels of proteins, proline, hydrogen peroxide, malondialdehyde, and photosynthetic pigments. Physiological and biochemical adjustments ensured the maintenance of relative water content and osmotic adjustment under saline conditions without increasing lipid peroxidation. The salinity levels did not affect *C. blanchetianus*, indicating the lack of saline stress. The fish farm effluents are rich in organic matter, from food, and excrement, which was beneficial for the development of *C. blanchetianus*. The reuse of saline effluents from fish farming for the irrigation of *C. blanchetianus* can be a viable environmental alternative, avoiding direct disposal into the environment.

Key words: water reuse, black quince, sustainable development

RESUMO: Efluentes salinos da piscicultura em atividades agrícolas vem crescendo, sobretudo nas regiões semiáridas. Neste sentido, objetivou-se avaliar o efluente salino de piscicultura na irrigação de *Croton blanchetianus* verificando as respostas fisiológicas e bioquímicas. Após o estabelecimento das plantas oriundas de estacas, iniciou-se a irrigação com o efluente, diferenciando-se os tratamentos por quatro níveis de salinidade (controle, 1,5, 2,5 e 3,5 dS m^{-1}). O tratamento controle foi representado pela água de abastecimento (0,56 dS m^{-1}). As variáveis avaliadas foram: crescimento, teor relativo de água, biomassa seca, níveis de proteínas, prolina, peróxido de hidrogênio, malondialdeído e os pigmentos fotossintéticos. Os ajustes fisiológicos e bioquímicos garantiram a manutenção do teor relativo de água e ajuste osmótico em meio à condição salina, sem incremento da peroxidação lipídica. Os níveis de salinidade não afetaram *C. blanchetianus*, indicando que não houve estresse salino, pois, os efluentes da piscicultura são ricos em matéria orgânica, proveniente da alimentação e excrementos, beneficiado o desenvolvimento da espécie. O reuso do efluente salino de piscicultura na irrigação de *C. blanchetianus* pode ser uma alternativa ambiental viável, evitando-se o descarte diretamente no ambiente.

Palavras-chave: reuso de água, marmeleiro, desenvolvimento sustentável

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Introduction

Irregular rains during the year, high evapotranspiration, and scarcity of water in perennial rivers are factors that determine the water deficit in semiarid regions, which is detrimental to the quality of life of the local population (Sá et al., 2018).

Viable alternatives are needed for agricultural production. One alternative is water reuse. Effluents from fish farming can be used to irrigate plant species. One study described the satisfactory development of Nile tilapia (*Oreochromis niloticus*) in desalinized water resulting from a reverse osmosis system (Souza et al., 2022). The findings indicated the advantage of reverse osmosis in providing a valuable use for fish farm effluent. However, the disposal of this effluent has raised concern, given the presence of saline water and high levels of phosphate and nitrates generated by animal excreta and feed waste. Therefore, it is important to assess whether this effluent can be used to cultivate plant species. If so, this sustainable practice could provide economic and social benefits to rural producers.

Salinity compromises the physiological and biochemical functions of plants, disturbing their water and nutritional relationships (Silva et al., 2019) and reducing leaf growth and biomass (Vieira et al., 2016). Therefore, it is essential to search for plant species that can tolerate and/or metabolize the effluent salts.

Salinity does not affect the essential oil content of some species. *Croton blanchetianus* Baill, a shrub endemic to northeastern Brazil, is a pioneer species adapted to the semiarid region (Silva et al., 2020). A source of essential oils is medically important, given their oils' antimicrobial and anti-inflammatory properties (Firmino et al., 2019; Oliveira et al., 2019).

However, the influence of saline stress on *C. blanchetianus* is unknown. Therefore, the aim of this study was to evaluate the physiological and biochemical responses of *C. blanchetianus* irrigated with fish farming effluents.

Materials and Methods

The study was conducted under greenhouse conditions at the Departamento of Ciências Biológicas, Universidade do Estado do Rio Grande do Norte (UERN), located in the municipality of Mossoró, Rio Grande do Norte in northeastern Brazil. According to the Köppen classification, the climate of the region is BSwh (i.e., dry and very hot). There are two seasons: a dry season from June to January and a rainy season from February to May. The mean annual temperature is 27 °C, the mean annual rainfall is 673 mm, the relative air humidity is 68%, and there are 241.7 hours of daylight per month (Carmo Filho & Oliveira, 1989).

C. blanchetianus seedlings were obtained from the mother plants. Polyethylene pots with a capacity of 8 L contained a substrate consisting of soil and organic polyfertile® compost in a 2:1 ratio. Soil was collected from an area with a population of *C. blanchetianus* on the UERN campus. The composition of the saline effluent from fish farming, whose physicochemical characteristics (Rice et al., 2012) are summarized in Table 1, was obtained from the Rural Federal University of Semiarid (UFERSA) Pisciculture Station, whose electrical conductivity of the fish farm wastewater (ECw) was 13.05 dS m^{-1}. To determine the other treatments (1.5; 2.5; 3.5 dS m^{-1}), the effluent dilutions were performed using an Instrutherm® micro-processed portable conductivity meter (model CD-860) to measure the ECw (Souza et al., 2020).

The experimental design was carried out in randomized blocks, consisting of four treatments related to the levels of EC of the wastewater of fish farming (ECw: 0.56; 1.5; 2.5; 3.5 dS m^{-1}) and six repetitions (blocks), totaling 24 experimental plots.

Pot capacity (PC) was determined using the capillary method. For the calculation, three pots of known weight containing dry substrates, were weighed and then placed in a larger container containing water with a known volume, which ascended through capillary action to saturate the substrate. Upon reaching complete saturation (100%), the PC was determined with a final value of 1 L of water.

Four cuttings were planted per pot to ensure rooting. Water was supplied daily in the morning to maintain soil moisture. At the end of the acclimation period, the 30-day experimental phase began. The same amount of effluent was used for the irrigation of the cuttings.

After the 30-day experiment, physiological and biochemical variables were evaluated. These included branch height, number of leaves (NL), branch diameter (BD), leaf area (LA), and relative water content (RWC). The plants were collected, washed in running water, and divided into leaves, branches, and roots. Each plant portion was placed on Kraft paper and dried in a forced-air circulation oven at 70 °C for 72 hours until a constant weight was obtained. Subsequently, the dehydrated plant material was weighed on a precision analytical scale to determine the leaf dry biomass (LDB), branch dry biomass (BDB), and root dry biomass (RDB). Before being dried in an oven, the roots of each plant were analyzed for root volume (RV), which was evaluated by the displacement of the water volume in a graduated test tube containing a known volume of water.

Proline content was determined as previously described (Bates et al., 1973). Malondialdehyde (MDA) content in the plant tissue was measured, and the results were expressed in nmol of MDA g^{-1} of fresh matter (Heat & Packer, 1968). Hydrogen peroxide (H_2O_2) was measured and the results were expressed in μmol of H_2O_2 g^{-1} of fresh matter (Alexieva et al., 2001). The extraction and quantification of total soluble

Table 1. Physical-chemical characteristics of saline effluents from fish farming (EP) and water supply (AS) used in the experiment

	pH Water	EC (dS m^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	CO_3^{2-}	HCO_3^-	SAR (mmol L^{-1})$^{0.5}$	Hardness (mg L^{-1})	Cations	Anions
			(mmol$_c$ L^{-1})									(mmol$_c$ L^{-1})	
EP	7.30	13.05	2.0	44.91	15.10	25.70	52.80	0.00	3.80	9.9	2040	87.7	56.6
AS	7.0	0.56	0.25	4.44	1.00	0.90	2.4	0.00	3.40	4.6	95	6.60	6.50

EC - Electrical conductivity; SAR - Sodium adsorption ratio protein content were performed using bovine serum albumin as the standard (Bradford, 1976). Photosynthetic pigments (chlorophyll and carotenoid content) were measured as previously described (Lichtenthaler, 1987).

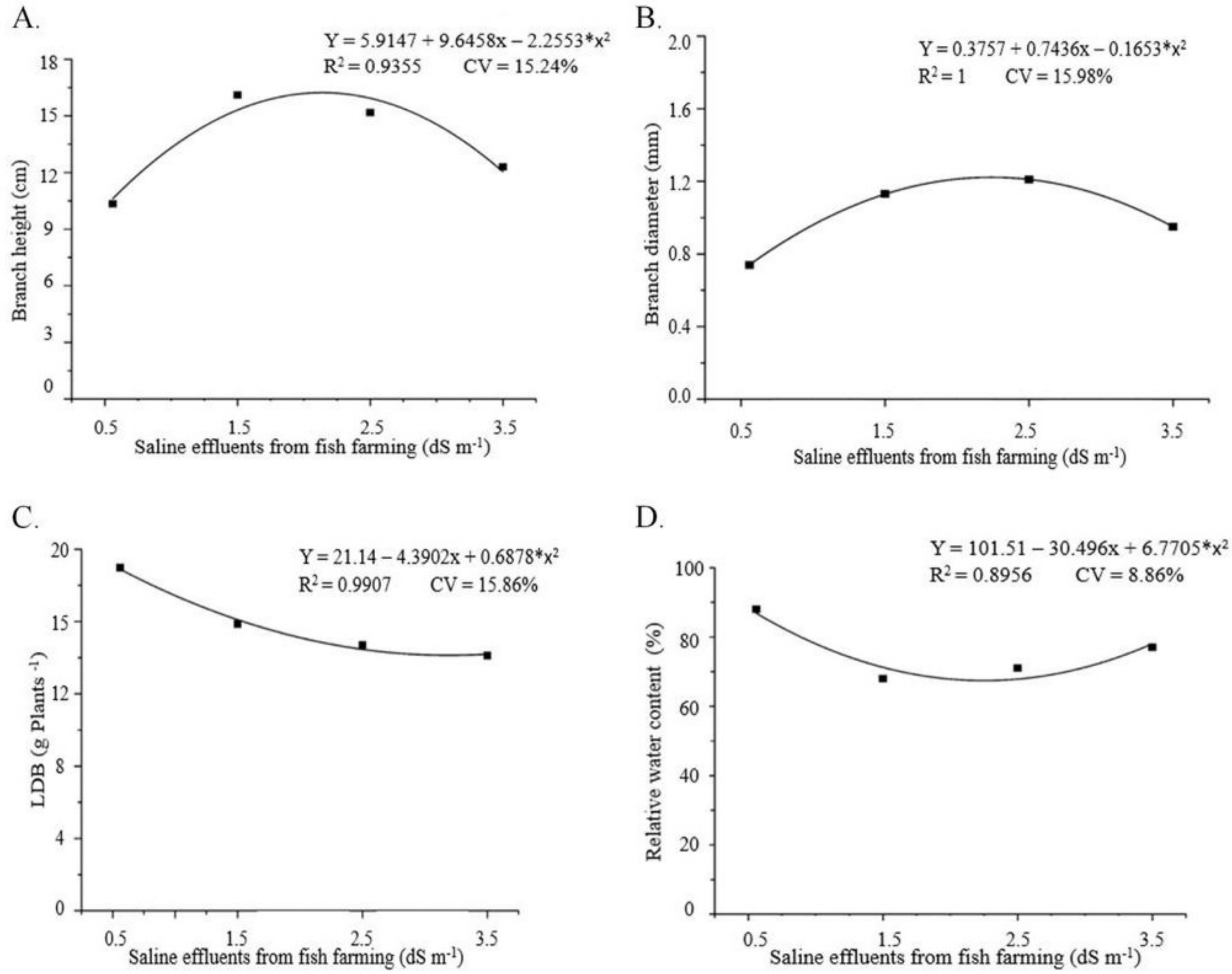
Data were subjected to analysis of variance (ANOVA) using the SISVAR statistical program. Regression analyses were performed at a significance level of 0.05.

Results and Discussion

The results of the development of *C. blanchetianus* plants revealed an effect of effluent only for the height (Figure 1A) and BD (Figure 1B). In general, a moderate increase in the salinity of the fish farming effluent did not affect plant growth. The greatest heights and diameter of branches were observed in plants irrigated with fish farming effluent with a salinity of 1.5 and 2.5 dS m^{-1}. For these levels of conductivity, the respective heights were 16.1 and 15.2 cm, and the respective diameters of the branches were 1.13 and 1.21 mm. These values were significantly higher than those observed with the other treatments.

Under some environmental conditions, moderate salinity levels can cause plants to self-stress, a condition of mild stress. Simultaneously, conditions are stimulating for some plant species. Self-stress is a favorable and positive factor, as it increases the physiological activities of the plant, favoring cell metabolism, plant growth, and development (Lichtenthaler, 2004). In general, branch growth is associated with self-stress caused by an increase in ECw. Because fish farming effluents contain organic matter and nutrients, this waste can contribute to plant nutrition, as the effluents are rich in nitrogen and phosphorus added to the water through the food provided to the fish, along with animal excreta (Nascimento et al., 2016). It is likely that the effluent used in this experiment had these nutritional characteristics. Similar results were reported by Rosa et al. (2018) in papaya plants (*Carica papaya*) irrigated with fishpond waste, which showed increased growth and better plant performance.

ANOVA revealed no significant effect of the salinity of the irrigation water for the variables number of leaves (NL) and LA (both p > 0.05). The number and size of *C. blanchetianus* leaves were not affected by moderate salt concentrations, indicating that other physiological mechanisms were activated. Maintenance of NL and LA can be understood as a plant adaptation strategy, demonstrating that the species maintains



* - Significant at 0.05 of probability; CV - Coefficient of variation

Figure 1. Regression analysis for branch height (A), branch diameter (B), leaf dry biomass (C), and relative water content (D) of *Croton blanchetianus* plants irrigated with saline effluents from fish farming

leaf production even under conditions of abiotic stress (Araújo Junior et al., 2018). This behavior is important because it is directly related to other physiological activities, such as the rate of transpiration, light interception, and CO_2 assimilation, which contribute to photosynthesis (Taiz & Zeiger, 2013). Therefore, in the present study the maintenance of NL and LA contributed to better investment in the height and diameter of the species.

Regarding the biomass produced by the species during the experimental phase, ANOVA showed that the salinity of the irrigation water significantly affected ($p \leq 0.05$) the production of LDB (Figure 1C). The decrease in LDB production by *C. blanchetianus* can be explained by the osmotic effect generated by the salts around the roots or soil solution, and the accumulation of potentially toxic ions (Na^+ and Cl^-) in the leaf tissues, which affect the development of some plant species (Coelho et al., 2018). In response to these adverse conditions, plants can divert part of their energy to osmoregulation, especially to the synthesis of molecules, such as proline (Lacerda et al., 2015). This was verified in this study; in plants treated with higher salinity concentrations, the level of proline was higher (Figure 2A). A decrease in biomass at the expense of osmotic adjustment has also been observed in *Lippia gracilis* (Ragagnin et al., 2014), and *Hyptis suaveoles* (Arruda et al., 2018; Souza et al., 2020). In addition to its effect on osmotic adjustment, proline is an osmoprotector of membranes that protects plants against dehydration.

Another mechanism that can be used by plants is greater control over the opening and closing of the stomata. This control avoids excessive water loss through transpiration. However, stomatal closure influences the diffusion of CO_2 into cells, which consequently reduces the rate of photosynthesis and results in lower biomass production (Silva et al., 2015). Therefore, the reduction in LDB observed in this study is part of an adaptive mechanism that seeks to find conditions to maintain vital activities, even if it is reduced. Salinity acts on the turgor pressure of cells owing to the decrease in tissue water content, resulting in a decline in cell wall expansion and less biomass production (Souza et al., 2017a). There are no reports on the effect of salinity on *C. blanchetianus*; however, similar results were reported by Oliveira et al. (2019), who studied an effluent consisting of sodium chloride, calcium chloride dihydrate, and magnesium chloride hexahydrate under greenhouse conditions. At higher saline concentrations (9.9 dS m^1), the authors verified lower biomass production by *Lippia gracilis* leaves. Similar to *C. blanchetianus*, *L. gracilis* is adapted to semiarid regions and, under saline conditions, maintains its metabolism at the expense of productivity. This physiological behavior has been observed in a study that evaluated the effects of salinity on plant species (Araújo et al., 2016).

For the BDB, RDB, and RV of *C. blanchetianus* plants, no significant differences were observed among the different treatments. Regarding the root system, salinity may or may not result in changes in root morphology and physiology, causing interruption in cell homeostasis, ionic imbalance in the soil-plant interphase, and plant toxicity due to the decrease in water potential (Nascimento et al., 2017). This study revealed no changes in the biomass or RV of *C. blanchetianus*.

The fact that there was no significant effect ($p > 0.05$) of salinity on RDB and RV may indicate that, even under saline conditions, the plants were able to produce a root system comparable to that of plants in the control group. The production of root biomass may indicate that the studied species can tolerate saline conditions. Such behavior was not described in studies that have evaluated the influence of salinity on plant species. Similar results were obtained by Souza et al. (2019), who found that salinity did not interfere with the development of bamboo roots (*Bambusa vulgaris*).

We observed a significant effect of salinity on the relative water content (RWC) ($p \leq 0.05$). On average, plants in the control group showed the highest value (88%) (Figure 1D). RWC is a fundamental index that quantifies the water retention capacity of plant leaf tissues.

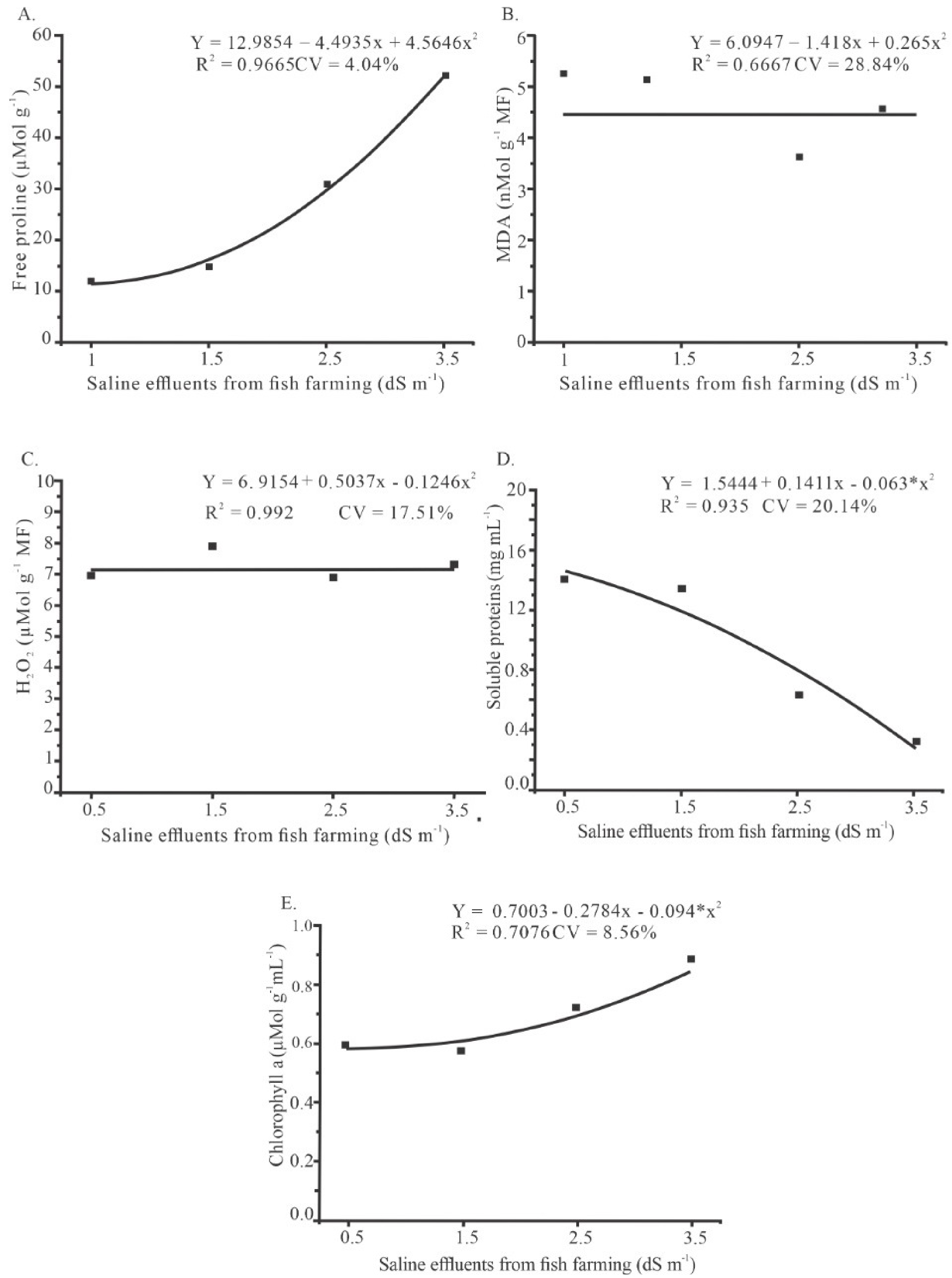
The water deficit induced by the increase in ECw may have directly affected the RWC in the cells, with this characteristic being more evident in plants irrigated with effluents with salinities of 1.5, 2.5, and 3.5 dS m^{-1}, resulting in RWC values of 68, 71, and 76%, respectively (Figure 1D). Depending on the degree and exposure to salinity, a plant may lose water inside the roots, instead of absorbing water. This is due to the potential difference between the cells present in the root and the soil solution through a process known as plasmolysis (Souza et al., 2017b). It is important to highlight the increase in the RWC we observed at the highest ECw (Figure 1D). This was probably due to the increase in proline to the same level as ECw (Figure 2A). When plants are subjected to an excess of salts, osmotic adjustment mechanisms are triggered to maintain cell turgidity. Similar findings were reported by Sarwat et al. (2016) and Souza et al. (2019).

Despite the reduced RWC values, linear growth was observed between the treatments with effluent use (Figure 1D) as ECw increased. This may be related to the presence and accumulation of compatible solutes, such as proline, an amino acid with increased synthesis and accumulation under stressful conditions. Osmotic adjustment is a process by which the plant's water potential decreases without a decrease in turgor (Taiz & Zeiger, 2013). When this happens, the plants are able to absorb water through the roots. The proline data indicate that this may have occurred in the plant species used in this experiment.

The levels of proline accumulated in the leaves of *C. blanchetianus* plants are shown in Figure 2A. Regression analysis revealed a significant difference between the levels of salinity in the irrigation water for this variable ($p \leq 0.05$) (Figure 2A).

The greatest increases in free proline levels were observed in plants irrigated with fish farming effluent at salinity levels of 2.5 and 3.5 dS m^{-1}. The increases exceeded those of plants in the control group. This behavior may represent an important mechanism in the turgor maintenance of *C. blanchetianus*, allowing it to endure saline conditions during the development of the species due to the accumulation of this solute in the cytosol (Lima et al., 2016).

Among the organic solutes involved in the osmotic adjustment process, proline is the amino acid that is produced at high concentrations by most species as a strategy to tolerate



** - Significant at 0.01 of probability; * - Significant at 0.05 of probability; CV - Coefficient of variation; Figures 2B (MDA) and 2C (H_2O_2): coffecients were not significant

Figure 2. Regression analysis for free proline (A), accumulation of malondialdehyde (MDA) (B), hydrogen peroxide (H_2O_2) (C), soluble proteins (D), and chlorophyll a contents (E) in *Croton blanchetianus* plants irrigated with saline effluents from fish farming

and survive stress conditions (Lacerda et al., 2015). The proline accumulation observed in this study was essential for *C. blanchetianus* to maintain water potential and intracellular osmotic balance, especially in treatments with higher concentrations.

The presence of this amino acid in the cytosol, in addition to osmoregulation, favors redox balance in stressed cells (Lima et al., 2016). The proline content explains the maintenance of tissue hydration in spite of the increase in ECw (Figure 1D) and the decrease in LDB (Figure 1C), which, due to osmoregulation, diverted metabolic energy to such adjustment, partially compromising the production of biomass. These observations clearly indicate that *C. blanchetianus*, under saline conditions, invests in energy production aimed at the synthesis of proline in an attempt to osmotically adjust its cells.

The content of MDA did not differ statistically between the control treatment and the saline effluent under different conductivities ($p > 0.05$, f value = 0.2514^{ns}) (Figure 2B). This finding indicates a lack of stress on the irrigated plants due to the increase in effluent salinity. MDA is an important biochemical marker of the degree of lipid peroxidation in plant cells subjected to different abiotic stresses (Kumar et al., 2018). The present result concerning MDA indicates that effluent salinity probably did not cause peroxidation of unsaturated fatty acids in the cell membrane. Physiological and biochemical responses are reportedly sufficient to maintain constant MDA levels under moderate stress compared with the control (Morais et al., 2019). The authors also emphasized that the maintenance of MDA content and small growth inhibition suggests the efficiency of plant acclimation for cultivation under moderately saline conditions.

The accumulation of H_2O_2 in the leaves of *C. blanchetianus* did not differ significantly between the saline concentrations and the control ($p > 0.05$, f value = 0.5975^{ns}) (Figure 2C). This indicates that the effluent did not cause oxidative stress in the plants. H_2O_2 is a reactive oxygen species (ROS) produced in plants during normal metabolism. Production of ROS is intensified when plants experience environmental stress. At high concentrations in the cell, H_2O_2 can be converted into more harmful reactive species, such as hydroxyl radicals, which contribute to increased membrane damage (Demidchik, 2015).

Different results were reported by Khan et al. (2014). The authors evaluated the effects of salt stress on *Vigna radiata* L. plants and observed an increase in H_2O_2. Plants have different physiological and biochemical mechanisms for survival, maintenance, and tolerance to stress caused by excess salts (Taiz & Zeiger, 2013). In the case of *C. blanchetianus*, investment in the production of molecules, such as proline, may be related and could justify the results observed for MDA and H_2O_2. In addition to its osmoregulatory function, proline protects the integrity of biomolecules and increases the activity of enzymes responsible for eliminating ROS (Lacerda et al., 2015; Lima et al., 2016).

The soluble protein content in *C. blanchetianus* leaves was significantly affected by irrigation with fish farming effluents ($p \leq 0.05$). Regression analysis results revealed a decrease in soluble protein levels from the 2.5 dS m^{-1} level of ECw (Figure 2D).

Water deficit, induced by an excess of salts in the soil solution, affects nitrogen metabolism in plants, decreasing protein synthesis and accumulation of amino acids, ammonia, and free polyamines (Demidchik, 2015). Calvet et al. (2013) evaluated the growth and accumulation of solutes in cowpea (*Vigna unguiculata* L.) plants irrigated with different levels of salinity. The authors observed a decrease in soluble protein content in the leaves, as there was a significant increase in salinity levels.

The reduction in the concentration of soluble proteins can be attributed to the increase in proteases, which catalyze the degradation of other proteins. When plants are under unfavorable environmental conditions, such as salt stress, amino acid production is reduced. Therefore, the reduction/breakdown of soluble proteins observed in this study probably occurred to make important amino acids available to the species, including proline, which helps in osmoregulation (Lima et al., 2016).

Regarding the concentration of pigments in the leaves of *C. blanchetianus*, an increasing response was observed only for chlorophyll a in relation to ECw (Figure 2E). The increase in the levels of chlorophyll a was more significant in plants irrigated with 2.5 and 3.5 dS m^1, with a statistically significant difference compared to control plants. The increase in chlorophyll content may be a result of chloroplast development (increase in the number of thylakoids) or the increase in the number of thylakoids, suggesting the activation of a protective mechanism for the photosynthetic apparatus (Silva et al., 2016).

No significant effect of salinity on the other analyzed pigments (chlorophyll b, total chlorophyll, and carotenoids) was evident (all $p > 0.05$).

The data on chloroplast and carotenoid pigments indicate that the photochemical apparatus was not damaged by variations in ECw. The *C. blanchetianus* plants, even under saline conditions, managed to produce and maintain the same concentration of pigments as those observed in the control group. This is important because it is directly related to species development and growth. Similar results were observed in Jatropha (*Jatropha curcas* L.) seedlings (Souza et al., 2010), submitted to different levels of salinity, which maintained unchanged chlorophyll concentrations, suggesting that the synthesis of these pigments was not impaired by exposure to salt.

These results can be part of the species integrated process of adaptation to salinity, aiming to maintain photosynthetic activity favorable to its growth during the stress period. In the case of *C. blanchetianus* plants, the results indicate that these plants maintained pigment synthesis and/or managed to control their photodegradation, allowing the photosynthetic apparatus to function adequately up to an electrical conductivity of 3.5 dS m^{-1}.

Thus, in an attempt to modulate itself to saline conditions, *C. blanchetianus* invested in the production of photosynthetic pigments. In addition to acting on the photosynthetic apparatus, these pigments act as non-enzymatic components of the antioxidant system in photoprotection and energy dissipation, promoting a protective response against ROS and their formation, consequently reducing harmful effects on the plant's photosynthetic apparatus. The photoprotective effect of carotenoids facilitates energy dissipation by exposure to high temperatures and light through the proteins present in the thylakoid lumen (Domonkos et al., 2013).

Conclusions

1. Electrical conductivity of 3.5 dS m^{-1} of the saline effluent from fish farming did not promote lipid peroxidation or generate stress in *C. blanchetianus* plants.

2. *C. blanchetianus* plants produced higher concentrations of proline in the presence of fish farm effluent containing salinity levels of 2.5 and 3.5 dS m^{-1}.

3. The collective findings indicate that the use of saline effluents from fish farming is a viable alternative for water reuse in the irrigation of *C. blanchetianus* cultivation.

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