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Use of fish-farming wastewater in lettuce cultivation

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A B S T R A C T

Lettuce stands out as the main leafy vegetable consumed in Brazil, with divergence regarding adaptability to different environmental conditions, which include salinity. The objective of the work was to evaluate the response of lettuce cultivars to the use of saline wastewater from fish farming. The experiment was conducted in a completely randomized design with four replicates, in a factorial 7 x 4, corresponding to seven cultivars (Regiane, Vera, Isabela, Elisa, Amelia, Lavinia and Angelina) and four salt concentrations (1.2, 2.2, 3.2 and 4 2 dS m^{-1}). The evaluated characteristics were: number of leaves, leaf area, crown diameter and shoot fresh and dry matter. Diluted wastewater from fish farming can be used in the preparation of solution for fertigation of lettuce. The cultivars Regiane and Elisa had higher production of leaves and leaf area, while Amélia and Angelina were the most productive in terms of biomass. The cultivars Vera, Isabela, Amélia and Angelina were more tolerant to the salinity of the fertigation solution.

Palavras-chave:
Lactuca sativa
recursos hídricos
salinidade
sustentabilidade

Uso de água residuária da piscicultura no cultivo de alface

R E S U M O

A alface se destaca como a principal hortaliça folhosa consumida no Brasil apresentando divergência para adaptabilidade a diferentes condições ambientais, entre as quais a salinidade. O trabalho teve, como objetivo, avaliar as respostas de cultivares de alface ao uso de água salina residuária da atividade da piscicultura. O experimento foi desenvolvido sob delineamento inteiramente casualizado, com quatro repetições, em esquema fatorial 7 x 4 sendo sete cultivares (Regiane, Vera, Isabela, Elisa, Amélia, Lavínia e Angelina) e quatro níveis de salinidade (1,2; 2,2; 3,2 e 4,2 dS m^{-1}). As características avaliadas foram: número de folhas, área foliar, diâmetro da coroa, massa fresca e seca da parte aérea. Verificou-se que a água residuária da piscicultura diluída pode ser utilizada na irrigação da alface cultivada em vasos. As cultivares Regiane e Elisa apresentaram maior emissão de folhas e área foliar e as cultivares Amélia e Angelina foram as mais produtivas em termos de biomassa. As cultivares Vera, Isabela, Amélia e Angelina se mostraram mais tolerantes à salinidade da solução de fertirrigação.

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Introduction

Lettuce (*Lactuca sativa* L.) is a leafy vegetable widely consumed in the world, especially fresh, in the form of salads. It has vitamins and mineral salts and, due to its low content of calories and easy digestion, it is recommended for fiber-rich diets (Sala & Costa, 2012). It is a species cultivated in all regions of Brazil due to its wide genetic variability, which facilitates the acquisition of these materials by farmers (Sousa et al., 2007).

In northeastern Brazil, especially in the semiarid region, the problem related to water scarcity affects many families of small rural farmers, negatively reflecting in the production of food.

Recently, the use of wastewater has been evaluated in the production of vegetables, such as lettuce (Dias et al., 2011; Sarmento et al., 2014) and rocket (Santos et al., 2012), besides fish-farming wastewater in rocket (Souza Neta et al., 2013) and lettuce (Baumgartner et al., 2007). As to the utilization of fish-farming wastewater, the last three authors obtained satisfactory results when using it in the preparation of nutrient solutions for rocket, basil and marjoram in hydroponic system; for these species, diluted wastewater constituted a viable alternative source of water and nutrients to plants without yield loss.

However, most studies were developed using Nutrient Film Technique (NFT) hydroponic system or in cultivation with inert substrate (Santos et al., 2012; Souza Neta et al., 2013) and there are only a few studies on the use of this type of wastewater for lettuce cultivated in soil (Baumgartner et al., 2007). In this context, aiming at the sustainable use of waters and given the socioeconomic importance of lettuce, this study aimed to evaluate the use of diluted fish-farming wastewater in the cultivation of lettuce cultivars.

Material and Methods

The experiment was carried out in a greenhouse from April to May 2014 at the Department of Environmental and Technological Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró, RN, Brazil (5° 11' 31" S; 37° 20' 40" W; 18 m).

The greenhouse used in the experiment (6.40 x 22.5 m) is covered by a 0.10-mm-thick, transparent, low-density polyethylene cover, treated against the action of ultraviolet rays, and has an arched frame. Side and front walls consisted of anti-aphid screen and a 0.30-m-high base wall made of reinforced concrete. During the experiment, minimum and maximum temperatures inside the greenhouse were 15 and 35 °C and the relative air humidity, 60%.

Sowing was performed on polyethylene trays with capacity for 128 cells, filled with coconut fiber, and three seeds were placed in each cell; thinning was performed later, leaving only one seedling in each cell. After thinning, the seedlings were fertigated for 15 days through a capillary system using the nutrient solution recommended by Furlani et al. (1999), diluted to 50%.

The seedlings were transplanted 25 days after sowing to plastic pots with capacity for 12 L containing 10 L of soil classified as eutrophic Red Yellow Argisol (EMBRAPA, 2006) with the following chemical characteristics: pH = 6.5; O.M. = 10.2; N = 0.6 (g kg^{-1}); P = 10.7; K = 176.7; Na = 35.4 (mg dm^{-3}); Ca = 2.9; Mg = 1.44; H = 1.2 cmol$_c$ dm^{-3} (EMBRAPA, 1997).

The experimental design was completely randomized, in a 7 x 4 factorial scheme with four replicates, represented by a pot containing one plant. The factors corresponded to seven lettuce cultivars (Regiane, Vera, Isabela, Elisa, Amélia, Lavínia and Angelina) and four salinity levels of the fertigation solution (S_1 = 1.2; S_2 = 2.2; S_3 = 3.2 and S_4 = 4.2 dS m^{-1}). The saline levels corresponded to the salinity of the waters after the addition of fertilizers (mixture). Saline waters were obtained by mixing freshwater from the water supply system of the UFERSA campus (S_1) with wastewater from the Fish Farming Sector (FFW) of the UFERSA.

Samples of the waters used in the preparation of nutrient solutions were collected for physicochemical analysis (Table 1). According to the classification of Richards (1993), the FFW is classified as C4S4, corresponding to water with high electrical conductivity (EC) and sodium adsorption rate (SAR), which may cause serious environmental problems if deposited directly in water sources.

The volume of water from each source to obtain the saline levels was determined through the equation recommended by Lacerda et al. (2010):

$$EC_{wf} = \frac{\left(EC_{w1} \cdot V_{w1}\right)}{\left(V_{w1+w2}\right)} + \frac{\left(EC_{w2} \cdot V_{w2}\right)}{\left(V_{w1+w2}\right)}$$

where:

EC_{wf} - final electrical conductivity of the mixture;

EC_{wl} - electrical conductivity of the water of lower salinity;

EC_{w2} - electrical conductivity of the water of higher salinity, dS m^{-1};

V_{wl} - volume of the water of lower salinity;

V_{w2} - volume of the water of higher salinity;

V_{w1+w2} - final volume of the mixture;

V_{w1}/V_{w1+w2} - represents the proportion of the water of lower salinity (P_{w1}); and,

V_{w2}/V_{w1+w2} - represents the proportion of the water of higher salinity (P_{w2}).

Daily fertigations were performed based on the nutrient concentration recommended for lettuce in hydroponic cultivation, applying the amount of water sufficient to increase soil moisture to the maximum water holding capacity. Irrigation stopped when drainage started.

Table 1. Analyses of the waters used in the preparation of nutrient solutions

Water source	pH	EC (dS m^{-1})	K^+	Na^+	Ca^{++}	Mg^{++}	Cl^-	CO_3^{-2}	HCO_3^-	SAR (mmol L^{-1})$^{0.5}$
			(mmol$_c$ L^{-1})							
FFW*	8.41	32.9	820.8	227.4	45.3	74.3	73.0	1.0	2.0	29.4
SSW	8.02	0.5	2.0	0.9	2.8	0.4	4.0	0.2	1.8	2.3

* FFW - Fish-farming wastewater; SSW - Water from the supply system of the UFERSA campus

The contents of nutrients present in the FFW were not taken into account in the preparation of the solutions. The composition of the nutrient solution contained the following doses of fertilizers (mg L^{-1}): 750 of calcium nitrate; 500 of potassium nitrate; 150 of monoammonium phosphate; 400 of magnesium sulfate; 0.15 of copper sulfate; 0.50 of zinc sulfate; 1.50 of manganese sulfate; 1.50 of boric acid; 0.15 sodium molybdate and 60 of 6% Fe-EDDHA (Furlani et al., 1999). The pH of the solution was adjusted between 6.0 and 6.5 through the application of 0.1 mol L^{-1} solutions of KOH or HCl.

Harvest was performed 35 days after transplantation and the plants were analyzed for the following characteristics: a) number of leaves - only green leaves and leaves with midrib length greater than 4 cm; b) leaf area - determined through the disc method, using a hole puncher with area of 1.78 cm^2; c) mean crown diameter - determined using a ruler, at the tip of the leaf (two measurements in perpendicular directions); d) shoot fresh and dry matter - at harvest, leaves that did not have commercial standard were discarded; the other ones were weighed on a digital scale with precision of 0.01 g. For dry matter determination, plants were placed in paper bags and dried in a forced-air oven at 65 °C (±1) until constant weight, and then weighed (0.001 g).

The obtained data were subjected to analysis of variance by F test and the means referring to the cultivars were compared by Tukey test at 0.05 probability level. The results obtained as a function of the salinity levels were subjected to regression analysis. The analyses were performed using the program SISVAR (Ferreira, 2008).

Results and Discussion

According to the analysis of variance, there was significant effect of the interaction between cultivars and salinity levels for the number of leaves (NL) and mean crown diameter (MCD) at 0.05 probability level, as well as for leaf area (LA), shoot fresh matter (SFM) and shoot dry matter (SDM) at 0.01 probability level. Regarding the isolated effect of the factors, there was significant difference among cultivars for all analyzed variables ($p < 0.01$), while no significant response of NL was observed for the factor salinity ($p > 0.05$). On the other hand, there was significant response for SD at 0.05 probability level and for LA, MCD, SFM and SDM at 0.01 probability level (Table 2).

The cultivars Regiane and Elisa stood out from the others with the highest values of number of leaves and leaf area, not differing statistically from each other or from the cultivar Lavínia for the variable crown diameter. On the other hand, the cultivars Amélia and Angelina obtained higher values of shoot fresh and dry matter in comparison to the others (Table 3).

In Brazil, it is notorious the tendency of consuming processed and packed lettuce leaves, as in the United States and in Europe (Sala & Costa, 2012). Thus, plants with greater number of commercial leaves are desirable, in order to meet this increasing demand of the market.

Fitted regression equations representing the behavior of the cultivars in response to the increase in the salinity of the fertigation solution for the analyzed variables are shown in Table 4.

For the number of leaves (NL), there was significant response to salinity only by the cultivars Regiane and Elisa; for Regiane, the response was increasing and linear, with increment of 1.2 leaf per plant in response to the increase in salinity, and the highest NL (28.3 leaves) occurred at the salinity level of 4.2 dS m^{-1}. On the other hand, cultivar Elisa showed quadratic response since, initially, there was an increment in NL with the increase in salinity until the level of 2.6 dS m^{-1} (20 leaves), decreasing from this point on (Table 4).

These results corroborate, in part, with those obtained by Dias et al. (2011), who used the lettuce cultivars Verônica and Quatro Estações. These authors also observed varied responses in which the increase in salinity caused reduction and increment in NL for the cultivars Quatro Estações and Verônica, respectively.

The reduction in NL under saline stress conditions is one of the alternatives of the plants to maintain water absorption as a consequence of morphological and anatomic alterations, causing reduction in the production of new leaves and/or leaf senescence and reflecting in the reduction of transpiration (Tester & Davenport, 2003). As to LA, there were quadratic responses for all cultivars; however, there was difference in

Table 3. Mean values of number of leaves (NL), leaf area (LA), mean crown diameter (MCD), shoot fresh matter (SFM) and shoot dry matter (SDM) in lettuce cultivars under fertigation with saline solutions

Cultivars	NL	LA (cm^2)	MCD (cm)	SFM (g $plant^{-1}$)	SDM (g $plant^{-1}$)
Regiane	20.62 a	1702.44 ab	34.56 a	62.06 b	4.49 c
Vera	11.37 b	1391.56 c	29.57 c	61.50 b	4.88 c
Isabela	11.50 b	1354.25 cd	26.25 d	50.37 d	3.93 d
Elisa	17.94 a	1809.50 a	34.42 a	62.18 b	4.52 c
Amélia	11.50 b	1537.05 bc	30.12 bc	75.00 a	6.15 a
Lavínia	11.68 b	1156.69 d	32.51 ab	54.37 cd	3.81 d
Angelina	11.25 b	1391.94 c	29.57 c	73.18 a	5.79 a

Means followed by the same letters in the columns do not differ by the Scott-Knott test (0.05)

Table 2. Summary of the analysis of variance for number of leaves (NL), leaf area (LA), mean crown diameter (MCD), shoot fresh matter (SFM) and shoot dry matter (SDM) in lettuce cultivars subjected to different levels of irrigation water salinity

Source of variation	DF	Mean square				
		NL	LA	MCD	SFM	SDM
Cultivars (C)	6	242.80**	119218.20**	142.11**	1307.86**	11.69**
Salinity (S)	3	2.03^{ns}	98597.85**	138.54**	210.60**	5.06**
C x S	18	5.21*	29374.76**	11.55*	380.62**	1.64**
Residual	84	2.89	4496.67	5.96	28.53	0.27
CV (%)		12.42	11.57	11.72	12.53	10.93

CV - Coefficient of variation, ns - Not significant, * - Significant at 0.05 probability level, ** - Significant at 0.01 probability level by F test

Table 4. Regression equations for the number of leaves, leaf area, mean crown diameter, shoot fresh matter and shoot dry matter in lettuce cultivars under fertigation with saline solutions

Cultivars	Regression equations	R^2
	Number of leaves	
Regiane	NL = 1.21x + 23.49	0.872
Vera	NL = 11.35	ns
Isabela	NL = 11.47	ns
Elisa	NL = $-1.71x^2$ + 8.91x + 8.38	0.989
Amélia	NL = 11.47	ns
Lavínia	NL = 11.67	ns
Angelina	NL = 11.25	ns
	Leaf area	
Regiane	LA = $-221.2x^2$ + 1097.1x + 629.2	0.991
Vera	LA = $-87.2x^2$ + 494.4x + 798.7	0.755
Isabela	LA = $-288.9x^2$ + 1514.1x + 217.1	0.873
Elisa	LA = $-128.7x^2$ + 625.1x + 1141.1	0.917
Amélia	LA = $-130.6x^2$ + 514.9x + 1262.1	0.998
Lavínia	LA = $-109.7x^2$ + 498.9x + 746.5	0.979
Angelina	LA = $-168.7x^2$ + 827.9x + 647.5	0.851
	Mean crown diameter	
Regiane	MCD = 2.99x + 42.49	0.866
Vera	MCD = 2.02x + 34.55	0.801
Isabela	MCD = 2.25x + 32.3	0.936
Elisa	MCD = $-1.62x^2$ + 8.18x + 26.20	0.781
Amélia	MCD = 30.07	ns
Lavínia	MCD = -1.92x + 37.68	0.806
Angelina	MCD = 29.32	ns
	Shoot fresh matter	
Regiane	SFM = -5.82x + 75.76	0.893
Vera	SFM = $-4.51x^2$ + 21.72x + 41.34	0.941
Isabela	SFM = $-4.87x^2$ + 23.57x + 28.35	0.997
Elisa	SFM = -3.62x + 71.92	0.899
Amélia	SFM = $-6.12x^2$ + 30.42x + 45.03	0.994
Lavínia	SFM = -6.67x + 72.18	0.809
Angelina	SFM = $-9.82x^2$ + 50.74x + 21.81	0.715
	Shoot dry matter	
Regiane	SDM = $-0.425x^2$ + 2.30x + 1.88	0.895
Vera	SDM = 0.58x + 3.33	0.944
Isabela	SDM = 3.9	ns
Elisa	SDM = 0.36x + 3.53	0.661
Amélia	SDM = $-0.75x^2$ + 3.99x + 1.78	0.903
Lavínia	SDM = 0.41x + 2.72	0.930
Angelina	SDM = $-1.01x^2$ + 5.26x + 0.238	0.944

the maximum values obtained. In general, considering all evaluated cultivars, there was an increase in LA with the increment in salinity until the levels of 2.48, 2.83, 2.62, 2.43, 1.97, 2.27 and 2.45 dS m^{-1}, with maximum LA values of 1,989.5, 1,499.5, 2,200.9, 1,900.1, 1,769.6, 1,313.7 and 1,663.2 cm^2 plant^{-1}, which were equivalent to gains of 22.3, 18.4, 36.0, 11.4, 4.6, 10.6 and 19.0% in comparison to the values obtained at salinity of 1.2 dS m^{-1}, for the cultivars Regiane, Vera, Isabela, Elisa, Amélia, Lavínia and Angelina, respectively. On the other hand, considering the LA values obtained in plants subjected to the extreme salinity levels used in the present study (1.2 and 4.2 dS m^{-1}), there were reductions of 21.8, 5.3, 9.3, 14.0, 50.9, 31.0 and 21.7%, respectively, in the cultivars Regiane, Vera, Isabela, Elisa, Amélia, Lavínia and Angelina (Table 4).

These results are partially different from those obtained by Oliveira et al. (2011) and Dias et al. (2011), who observed linear reduction in LA in response to the increase in salinity; however, it should be pointed out that fish-farming wastewater was used in the present study, which may have contributed to increasing plant tolerance to salinity, due to the presence of nutrients in the water, a fact also observed by Souza Neta et al. (2013) in rocket.

The dimension of the total leaf area of a plant results from the product between the number of leaves and the area of the leaf blade; thus, the effect of salinity on leaf area is related to both the reduction in the production of new leaves and the death and fall of leaves, besides reducing leaf blade expansion (Mahmoud & Mohamed, 2008). However, as observed in the present study, the effect of using saline water from fish farming was higher on leaf blade expansion, since there was little influence on the number of leaves.

Soil salinity reduces the osmotic potential, reflecting in the decrease of water absorption by plants and compromising physiological processes. Hence, plants may show morphophysiological modifications in order to increase their tolerance to salinity, especially reduction in leaf area, due to the decrease in the volume of cells. With reduction in LA and increase in the total concentration of solutes in the leaf, there is the osmotic adjustment of the cells, guaranteeing water absorption by plants (Tester & Davenport, 2003).

Mean crown diameter (MCD) was not affected by salinity in the cultivars Amélia and Angelina. On the other hand, the cultivars Regiane, Vera, Isabela and Lavínia showed a negative linear response to the increase in salinity, and the lowest values were observed at the highest salinity level (4.2 dS m^{-1}). Based on the fitted regression equations, the cultivar Regiane showed the highest reduction in MDC, with loss of 2.99 cm per unit increase in salinity. Considering the highest salinity level, there were total losses of 26.9, 22.2, 26.7 and 19.3%, for the cultivars Regiane, Vera, Isabela and Lavínia, respectively (Table 4).

For the cultivar Elisa, there was a quadratic response and the highest MCD (36.51 cm) was observed at salinity of 2.5 dS m^{-1}, from which there was a reduction in MCD, to 31.9 cm at 4.2 dS m^{-1} (Table 4).

As to shoot fresh matter (SFM), there was effect of salinity, which varied according to the analyzed cultivar. The cultivars Regiane, Elisa and Lavínia were linearly and negatively affected by the saline stress; as salinity increased, there were SFM reductions of 5.84 , 3.63 and 6.67 g plant^{-1} with per unit increase in electrical conductivity, which corresponded to percent per unit and total losses, respectively, of 7.98 and 29.5% for the cultivar Regiane; 5.16 and 19.1% for the cultivar Elisa; and 9.68 and 35.8% for the cultivar Lavínia (Table 4).

These results corroborate those obtained by Dias et al. (2011), who studied the cultivars Verônica and Quatro Estações subjected to salinity levels, in coconut fiber, and obtained decreasing linear responses, with relative losses of approximately 8.05 and 5.91% per unit increase in salinity. Paulus et al. (2010), working with lettuce, also observed reductions that ranged from 6 to 6.5%. Additionally, Santos et al. (2010) used the cultivars Vera and AF-1743 and observed losses of 17.06 and 15.74%, respectively.

For the other cultivars, there were quadratic responses and the highest values of SFM occurred at salinity of 2.42 dS m^{-1} for the cultivars Vera and Isabela, which showed maximum values of 67.5 and 5.8 g plant^{-1}, respectively. For the cultivars Amélia and Angelina, the maximum SFM values occurred at salinity levels of 2.48 and 2.58 dS m^{-1}, respectively, with 82.8 and 87.3 g $plant^{-1}$ (Table 4). Therefore, among the studied cultivars, Lavínia showed higher sensitivity, while Vera, Isabela, Amélia and Angelina stood out for having greater tolerance.

There were also varied responses of the cultivars to the increase in salinity for the accumulation of shoot dry matter (Table 4). There was no significant effect for the cultivar Isabela, which showed mean SDM of 3.9 g $plant^{-1}$. On the other hand, there were quadratic responses of the cultivars Regiane, Amélia and Angelina, and the highest values were obtained at salinity levels of 2.71, 2.66 and 2.63 dS m^{-1}, with 5.0, 7.1 and 7.2 g $plant^{-1}$, respectively. Comparing these values with those obtained at the lowest salinity level, there were increases of 23.9% for the cultivar Regiane, 29.3% for Amélia and 40.9% for Angelina. From these salinity levels on, the cultivars showed reduction in the production of shoot dry matter.

The cultivars Vera, Elisa and Lavínia showed linear reduction in dry matter in response to the increase in salinity, with decreases of 0.58, 0.36 and 0.40 g $plant^{-1}$ with per unit increase in salinity, which resulted in total losses of 65.9, 34.9 and 53.6%, respectively (Table 4).

These results corroborate those found by other researchers, who observed varied responses of different lettuce cultivars subjected to salinity (Magalhães et al., 2010; Paulus et al., 2010; Oliveira et al., 2011).

Such variation is consistent with Flowers & Flowers (2005), who pointed out that the degree of tolerance of crops to salinity is variable among species and even within same species, according to phenological stages, in which the tolerance to salinity is controlled by more than one gene and highly influenced by environmental factors.

The reduction in dry matter accumulation in plants subjected to saline stress occurred because the excess of salts in the soil solution modifies the metabolic activities of the cells in the process of cell elongation, limiting the elasticity of the cell wall, reducing cell elongation and, consequently, plant growth (Taiz & Zeiger, 2009). In this context, Chen & Jiang (2010) claim that the effect of salts causes reduction in the shoots of certain plants, because they do not have an osmotic adjustment as a mechanism of adaptability to the excess of salts in the soil solution.

Conclusions

1. The cultivars Regiane and Elisa stand out for their greater leaf development (number of leaves and leaf area) and the cultivars Amélia and Angelina are the most productive in terms of fresh and dry matter.

2. The cultivars Vera, Isabela, Amélia and Angelina are more tolerant to the salinity of the fertigation solution, with no yield loss until the electrical conductivity of 3.5 dS m^{-1}.

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