**Research Article** 

# Impacts on growth, water relations and nutritional composition of basil plants submitted to irrigation with saline and wastewater



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#### Abstract

The effect of the application of reclaimed water and saline water on physiological parameters in *Ocimum basilicum* L. plants were studied to evaluate their adaptability to irrigation with saline water, as well as to evaluate the applicability of wastewater as a source for irrigation. Plants of *O. basilicum* grown under greenhouse conditions were submitted to different irrigation treatments: control (deionized water  $3.10^{-6}$  dS m<sup>-1</sup>), deionized water supplemented with 15 mM NaCl ( $1.70 \text{ dS m}^{-1}$ ), deionized water supplemented with 30 mM NaCl ( $2.46 \text{ dS m}^{-1}$ ) and ozonized reclaimed water ( $0.709 \text{ dS m}^{-1}$ ). The results show that reclaimed water did not promote reduction in water potential of leaf. Likewise, growth-related parameters were not affected by the application of this treatment. The analysis of nutrients composition in the vegetal tissues verified a marked effect of the Na<sup>+</sup> exclusion in the root tissues. It did not happen occurred in the roots with the same proportion as in the foliar tissues. Considering the results, the ozonized reclaimed water ( $0.709 \text{ dS m}^{-1}$ ) can be an alternative for irrigation of *O. basilicum* L., if it is properly managed.

Keywords Hydric relations · Plant nutrition · Growth · Wastewater · Salinity

## 1 Introduction

The efficient use of water is a subject of great importance for social, economic and environmental needs. In regions where scarcity and poor distribution of rainfall are limiting factors for water use, wastewater is an effective alternative to irrigation. In the agricultural sector the use of reclaimed water in the irrigation of plants has demonstrated economic, agronomic and environmental importance [1–3]. However, its use is still limited in Brazil.

Reclaimed water is known as low quality and depending on its source may contain several types of salts that can cause salinity problems when not managed properly in irrigation [4]. On the other hand, reclaimed water can be an important nutritional source for plants due to the considerable presence of essential nutrients for their development [5]. However, the effect of the use of this resource for irrigation will depend on the treatment applied to water, its origin and the tolerance to salts of the species to be cultivated [6]. The effects of irrigation with saline water vary according to the different tolerance groups, classified as glycophytes or halophytes; between different species of the same family and genus and between cultivars; moreover it depends on the intensity and duration of the stress, that is, the levels of salts in the irrigation water and the time of exposure [7].Water salinity used for irrigation can rapidly affect plants [4, 8, 9], especially when the concentration of dissolved salts in the soil solution increases, which can

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reduce the osmotic and water potential, decreasing the availability of water and nutrients [10] which may lead the plants to conditions of water stress or physiological dry [11].

 $Na^+$  in high concentrations in the soil can interfere directly with the root absorption of other nutrients such as  $K^+$  and  $Ca^+$ . Although there are several channels of entry through the roots plasma membrane,  $Na^+$  can pass through specific channels of the entrance of other nutrients [5] promoting a nutritional imbalance.

Basil belongs to the *Lamiaceae* family, originating from Southwest Asia and Central Africa [12]. The Ocimum genus comprises around 3,200 species [13] and is considered one of the largest genera in the Lamiaceae family, which are distributed throughout Tropical Asia, Africa, Central America, South America and Europe [14]. There are a large number of species of the Ocimum genus that are known to have several medicinal properties: *Ocimum tenuiflorum L., Ocimum gratissimum L., Ocimum americanum L., O. basilicum* L. among others [15]. Among the various species of the basil genus, *O. basilicum* L. is considered the most important due to the production of aromatic essential oil.

This study aims to know the nutritional, osmotic and / or saline effects derived from reclaimed water on the response to the physiological behavior of *O. basilicum* L. Furthermore, it aimed to know the benefits of reclaimed water as irrigation source.

## 2 Materials and methods

The study was performed at São Paulo State University, in the Department of Chemistry and Biochemistry-IB/UNESP, Botucatu-SP, from January 3 to April 4, 2017 in an agricultural greenhouse with an area of 59.4 m<sup>2</sup>, height of 3 m, at 22°52′55 "S and 48°26′22"W and approximately 840 m of altitude. Botucatu, located in the State of São Paulo-Brazil, presents hot (mesothermic) and humid temperate climate, with an average annual temperature of 20.5 °C [16]. Basil seeds (cv. Genovese), were sown on honeycomb trays and cultivated until the emergence of the first pair of true leaves. After this period, seedlings were transplanted to 5-L pots (a seedling per pot) with medium textured (Led) Dark Red Latosol, according to the classification made by Carvalho [17]. With the result of the soil analysis, the need for dolomitic limestone for soil correction was calculated according to the fertilization recommendation [18]. During germination, the substrate was kept saturated through daily irrigations with deionized water until the treatments application. The drainage of the vessels was handled in a natural way. Reclaimed water (final treated sewage effluent) used in the experiment came from the sewage treatment plant in Botucatu, SP-Brazil, operated by SABESP (*Basic Sanitation Company of São Paulo*). For its use as an irrigation treatment in basil plants, it was submitted to three processes: decantation, sand filtration and ozone treatment. After this process, physical–chemical (Table 1) and microbiological analyzes of reclaimed water were performed, attesting that all the values were below the maximum limits allowed for the use of this type of water.

Irrigation of the different water qualities was performed by drip system. Plants were irrigated with deionized water until the treatments application, which started at the flowering stage of the crop.

Irrigation management was based on the curve of water retention, aiming to maintain soil water content in field capacity. The method of *tensiometry* was used to monitor water content in the soil. The readings were performed from 8 to 10 a.m. before the application of the irrigation blade and the values were converted to soil water potential (-Ψm) in KPa unit, following the model described by *Van Genuchten* [19]. The control of the climatic conditions of the greenhouse was monitored via datalogger to record temperature and relative humidity. The maximum temperature ranged from 25 to 41.4 °C; the minimum temperature varied from 9.8 to 19.9 °C and the relative humidity varied from 46 to 67.5%. The parameters were evaluated

 
 Table 1
 Analysis of nutrients from reuse water collected in the final effluent of SABESP (Basic Sanitation Company of São Paulo, Brazil) and after the process of treatment via ozonation

Water quality parameters	Wastewater	Ozonized waste water	Units
Aspect	yellowish	yellowish	-
Total alcalinity	96.1	76.5	mg $C_a CO_3 L^{-1}$
Color	20	15	-
Calcium	24,9	19.2	mg $L^{-1}$
Chlorid	92,6	90.4	mg $L^{-1}$
Eletric conductivity (EC)	507	432	μS cm
Total organic carbon	37.8	30.4	mg $L^{-1}$
Total hardness	76.0	68.0	mg $C_a CO_3 L^{-1}$
Calcium hardness	62.0	48.0	mg $C_a CO_3 L^{-1}$
Magnesium hardness	11.8	16.8	mg $C_a CO_3 L^{-1}$
Total iron	0.12	0.19	mg $L^{-1}$
Fluorid	0.79	0.77	mg $L^{-1}$
Total phosphor	2.68	3.64	mg $L^{-1}$
Magnesium	2.9	4.1	mg $L^{-1}$
Nitrate	33.32	36.81	mg $L^{-1}$
Nitrite	0.04	4.20	mg $L^{-1}$
Total nitrogen	12.3	9.5	mg $L^{-1}$
pН	7.26	6.65	_
Sulfate	73.4	62.9	mg $L^{-1}$
Total dissolved solids	450	448	mg $L^{-1}$
Turbidity	2.04	2.25	NTU

at 1, 7, 14, 21, 28 and 35 days after flowering (DAF), during the experiment.

The trial was installed in a completely randomized design, consisting of 4 treatments with 3 repetitions each. The experiment was considered as bifactorial with 3 replications, having as main factor the 4 irrigation treatments that differ in the quality of the applied water and the secondary factor composed by the number of days after flowering, which was variable according to each parameter evaluated. It was composed of 4 groups with different water qualities: (T1—Deionized Water (Control), EC=3.10<sup>-6</sup> dS m<sup>-1</sup>; T2—deionized water supplemented with 15 mM NaCl, EC = 1.70 dS m<sup>-1</sup>; T3—ozonized reclaimed water, EC = 0.709 dS m<sup>-1</sup>; T4—deionized water supplemented with 30 mM NaCl, EC = 2.46 dS m<sup>-1</sup>) with 3 replicates each.

Leaf water potential ( $\Psi_{wl}$ ) was determined using the pressure chamber (SAPS II—System Analysis of Plant Stress, mod. 3115) following the technique described by Scholander [20]. The determinations were performed in full expanded leafs, at the isohydric pre-dawn time, taking 6 plants per treatment with 2 plants per repetition. The soil was irrigated to field capacity 12 h before  $\Psi$ wl readings.

The relative water content (RWC) was determined [21] from the following expression: RWC = [(FM-DM) / (DM-TM)] \* 100 where: FM, DM and TM represent, respectively, fresh mass, dry mass and turgid mass of leaf discs with diameter of 1cm<sup>2</sup>, cut from the middle third of 3 plants per treatment, with 1 per replicate. In the determination of dry mass, the leaf discs were dried in an oven at 50 °C until constant weight. For the analysis of growth and biomass parameters, 6 plants per treatment were evaluated, with 2 plants per replicate. In each analyzed plant, the height and stem diameter were measured and the total number of leaves per plant was counted. Afterwards, all leaves of each plant were measured by leaf area integrator LiCOR model LI-3000.

To determinate fresh matter mass (FMM) and dry matter mass (DMM) of the plant material expressed in g plant<sup>-1</sup>, the following parameters were analyzed: Fresh leaf mass (FLM); Fresh stem mass (FSM); Leaf dry mass (LDM); Dry stem mass (DSM) and Root dry mass (RDM). For this determination, the material was packed in paper bags and after weighing the fresh matter, they were kept in an oven with air circulation at 30  $\pm$ 5 °C until constant weight for later measurement of the DMM.

With data obtained in the parameter of growth and plant biomass, it was possible to determine the following growth rates [22]: SLA, (Specific Leaf Area) which indicates the relationship between leaf area (LA) and leaf dry mass (LDM), calculated by the expression: SLA = LA/W (leaf area/LDM), expressed in cm<sup>2</sup> leaves total g<sup>-1</sup> total DM.

LWR (*Leaf Weight Ratio*) that informs the fraction of the total biomass applied in the leaves. It can be calculated

according to the expression: LWR = LDM/TDM (Dry leaf mass/Total dry mass), expressed in g DM  $_{leaves}$  g<sup>-1</sup> total DM.

LAR (*Leaf Area Ratio* is the relation of leaf surface) which reflects on the size of the photosynthetic surface relative to the respiratory mass according to the expression: LAR = LA/ total DM (Leaf area/Total dry mass), expressed in  $m^2_{leaves} g^{-1}$  MS plants.

The analysis of leaves and roots nutrients of *O. basilicum* L was performed following routine methodology [23]. For nutritional analysis of the leaves and roots of *O. basilicum*, 3 plants were collected per treatment, 1 by repetition. After separation into leaves and roots, the plant material was dried in an oven at 30 °C until constant mass. The tissues were ground and analyzed at the FCA/UNESP Plant Mineral Nutrition Lab, to determine macro and micronutrients.

F-test was used with the aid of ASSISTAT statistical program 7.6 [24] to evaluate the effects of the different water qualities on the studied variables.

#### **3** Results and discussion

The application of saline treatments (15 and 30 Mm NaCl) significantly reduced leaf water potential (Ψwl) of basil plants (Fig. 1), from the 14th day after flowering ( $p \le 0.01$ ) and this effect became more accentuated as the days progressed, differing from the control and reclaimed water treatments. On the contrary, the treatment with reclaimed water, which did not differ from the control during the whole experiment, indicated that the salts did not promote restriction in water absorption and did not reduce the water potential in plants of O. basilicum L. Depending on the salt concentrations in water, these salts may accumulate around the roots. This accumulation can result in decrease of water potential of this region, increasing the difficulty of plants in absorbing water by the roots [25]. Plants can lose water inside the roots instead of absorbing it, because its osmotic potential is higher than soil's, which is the major cause of the reduction in plant growth [8].

Although there was a significant reduction of water potential in plants in both saline treatments (15 and 30 mM NaCl), it is not possible to characterize these values as indicative of water stress. Generally, water potentials with values from -6.2 kPa are considered slightly stressful for basil [26]. In the present study, the lowest value we found for this variable was -3.41 kPa in the treatment with 30 mM NaCl (Fig. 1) that can be considered above the potentials found in plants under water stress, indicating that plants of all treatments remained well hydrated.

In contrast to the water potential, the application of the different qualities of water did not promote alteration in the relative water content (RWC) that remained stable during the 1st, 14th and 28th DAF (Fig. 2). The results Fig. 1 Water potential KPa ( $\Psi_w$  leaf) in plants of *O. basilicum* L. irrigated with different qualities of water. Means followed by the same letter represents the comparison between treatments by the Tukey's test at 1% probability



**Fig. 2** Relative water content (RWC) in leaf discs of *O. basilicum* L. irrigated with different water qualities

evidenced in this work show that the irrigation of plants with reclaimed water did not bring any harm to the availability of water in the foliar tissues with a similar result to the control treatment. Similarly, saline treatments were not able to significantly alter this variable (Fig. 2). Similar results [27] were found for cowpea seedlings exposed to concentrations of 0, 25, 50, 75 and 100 mM NaCl in which there was no change in RWC.

Decreased RWC in plant tissues is generally related to the difficulty of cell expansion, development of tissues, besides affecting several metabolic processes. Some authors [28, 29] reports that when the plants are submitted to water stress, the growth processes are rapidly affected. In this study, this condition is not observed since the growth parameters in *O. basilicum* L. crop as: plant height, stem diameter, leaf area and number of leaves were not significantly altered by the application of the different qualities of water (Table 2).

Likewise, Tarchoune et al. [30] evaluating the effect of salinity on plants of *O. basilicum* L. cv. Genovese, observed that the concentrations of 50 mM NaCl applied in hydroponic solution did not promote significant changes in growth parameters (leaf, stem and root, dry weight, leaf area, shoot height and root length) remaining constant in comparison with the control. According to Prisco and Gomes, (2010) [31], changes in plant growth subjected to

Table 2	Growth of O. basilicum
L. irriga	ted with different water
qualitie	S

Parameters	Treatments	Days after flowering							
		1	7	21	28	35			
Height (cm)	Control	50.00	50.50	70.50	76.33	71.66			
	15 mM NaCl	49.66	50.83	70.16	77.16	79.16			
	Reclaimed water	49.00	51.16	66.66	76.00	76.50			
	30 mM NaCl	48.33	50.66	73.16	77.50	76.33			
		ns	ns	ns	ns	ns			
Stem diameter (mm)	Control	5.00	5.66	6.50	6.50	6.50			
	15 mM NaCl	5.00	6.16	6.50	6.58	6.16			
	Reclaimed water	5.00	5.50	6.33	6.83	6.33			
	30 mM NaCl	5.00	5.33	6.00	6.66	6.66			
		ns	ns	ns	ns	ns			
Leaf area	Control	0.05	0.11	0.12	0.15	0.14			
(m <sup>2</sup> of leaf plant <sup>-1</sup> )	15 mM NaCl	0.04	0.11	0.14	0.12	0.12			
	Reclaimed water	0.05	0.10	0.14	0.13	0.13			
	30 mM NaCl	0.05	0.08	0.11	0.12	0.11			
		ns	ns	ns	ns	ns			
Number of leaves	Control	107	140	215	231	209			
	15 mM NaCl	108	153	209	229	240			
	Reclaimed water	107	140	180	242	253			
	30 mM NaCl	100	128	208	233	229			
		ns	ns	ns	ns	ns			

Tukey's test:  $p \le 0.05$ ;  $p \le 0.01$ ; *ns* not significant

salinity will depend on the stress characteristics, such as the concentration and ionic composition of the solution in soil, root or shoot part exposed to stress and how stress is applied. The same authors affirm that the stress characteristics can result in tolerance or susceptibility of the plant, depending on the capacity of response or adaptation to the applied stress.

The fresh leaf mass (FLM) and fresh stem mass (FSM) did not change due to the application of different water qualities used in the irrigation. However, when leaf dry mass (LDM) production was evaluated, it was observed that the treatment with higher salt concentration promoted the reduction of this variable (Table 3). This decrease in growth can be understood as a physiological mechanism to minimize the loss of water by transpiration [32]. The highest total dry mass accumulation (TDM) was observed in the treatment with 15 mM NaCl and reclaimed water. When the partitioning of these assimilates is considered in different plant structures, the highest accumulation of leaf dry mass (LDM), shoot dry mas (ShDM) and root dry mass (RDM) was observed in the treatment with 15 mM NaCl. This fact is an indication that in the lower concentration of NaCl, plants may have invested more in RDM, which is numerically (but not statistically) higher in the treatment 15 mM NaCl and may justify the higher production of TDM in this treatment (Table 3). According to Acosta-Motos et al. [7] an adequate root system can guarantee the uptake of water and nutrients and improve the resistance of plants to saline conditions. It was verified that plants irrigated with reclaimed water showed higher DSM at 35 DAF, showing a probable acceleration of stem biomass accumulation in the last days (Table 3). The fact of TDM being higher due to the irrigation with reclaimed water with results superior to the control (Table 3), reflects a good response of the plants to this treatment. Bione et al. [33] states that the crop of *O. basilicum* L. can be classified as tolerant to irrigation water salinity levels up to 1.70 dS m<sup>-1</sup> and to values between 3.80 and 6.08 dS m<sup>-1</sup> classified as moderately sensitive, following the criteria presented by Fageria et al. (2010) [34].

Tarchoune et al. [30] evaluating the effect of salinity on physiological aspects of *O. basilicum* L. cv. Genovese, observed that the concentration of 50 mM NaCl applied in hydroponic solution did not promote significant changes in the production of dry mass of the plants.

Based on the observed data for leaf area and fresh/dry matter of the different plant organs, an allocation study by growth rates was performed. These rates allowed to evaluate biomass fractions invested in different parts of the plant, which may reflect development strategies and establishment of tolerance to the physical environmental factors (saline water and reclaimed water).

SLA is calculated by dividing the total leaf area of a plant by the dry mass of its leaves. The values found in

#### Table 3 Plant biomass of O. basilicum L. irrigated with different qualities of water

Parameters	Treatments	Days afte	Days after flowering					
		1	7	21	28	35		
Leaf fresh mass	Control	15.20	33.16	51.48	46.87	38.33		
(LFM, g plant <sup>-1</sup> )	15 mM NaCl	16.38	32.26	44.52	41.12	44.36		
	Reclaimed water	15.89	31.56	56.13	41.38	43.63		
	30 mM NaCl	15.02	26,10	42,17	37,35	36,65		
		ns	ns	ns	ns	ns		
Stem fresh mass	Control	7.82	22.54	35.41	45.60	47.79 b		
(SFM, g plant <sup>-1</sup> )	15 mM NaCl	8.08	24.70	39.34	42.62	53.76 ab		
	Reclaimed water	8.00	20.80	39.08	51.91	59.68 a		
	30 mM NaCl	8.17	18.63	39.26	46.45	49.03 b		
		ns	ns	ns	ns	*		
Leaf dry mass (LDM, g $plant^{-1}$ )	Control	2.86	5.72	7.00	8.25	7.16 a		
	15 mM NaCl	3.16	5.49	7.19	6.95	8.78 a		
	Reclaimed water	2.74	5.47	6.62	6.68	7.17 a		
	30 mM NaCl	2.86	4.44	6.38	6.47	6.70 b		
		ns	ns	ns	ns	*		
Leaf, stem and root dry mass (TDM g plant <sup>-1</sup> )	Control	5.14	12.19	16.81	21.64	24.08 b		
	15 mM NaCl	5.92	11.74	17.45	20.79	27.51 a		
	<b>Reclaimed</b> water	5.30	11.86	17.02	21.28	26.53 a		
	30 mM NaCl	5.39	10.24	16.96	19.25	23.93 b		
		ns	ns	ns	ns	*		
Stem dry mass	Control	1.09	3.50	6.23	8.94	12.05 b		
$(SDM, g plant^{-1})$	15 mM NaCl	1.18	3.67	7.03	8.83	13.70 ab		
	Reclimed water	1.10	3.58	6.87	9.76	14.58 a		
	30 mM NaCl	1.15	3.19	7.78	8.93	12.47 ab		
		ns	ns	ns	ns	*		
Root dry mass	Control	1.18	2.96	3.58	4.43	4.86		
$(RDM, g plant^{-1})$	15 mM NaCl	1.57	2.57	3.22	5.00	5.02		
	Reclaimed water	1.46	2.80	3.51	4.83	4.78		
	30 mM NaCl	1.37	2.60	2.79	3.84	4.74		
		ns	ns	ns	ns	ns		
Shoot dry mass (leaf and stem) (ShDM, g plant <sup>-1</sup> )	Control	3.96	9.22	13.23	17.20	19.22 b		
	15 mM NaCl	4.35	9.16	14.22	15.79	22.48 a		
	Reclaimed water	3.84	9.06	13.50	16.45	21.75 ab		
	30 mM NaCl	4.01	7.63	14.16	15.40	19.18 b		
		ns	ns	ns	ns	*		

Tukey test: \* $p \le 0.05$ ; \*\* $p \le 0.01$ ; ns not significant

this relation indicate the relationship between the area and the leaf mass. In this experiment, no significant differences between the different qualities of water were identified (Table 4).

Although it is not confirmed by statistical analysis, saline treatments apparently showed a decrease in SLA in the mean of treatments (Table 4). Considering only as a trend, this effect confirms a reaction that can be observed in many short-cycle vegetables under stress. In this case, the decrease in leaf area seems to be a strategy

to resist the osmotic and ionic effect of the salts and to maintain high water potential.

Likewise, for the size of the photosynthetic surface in relation to the respiratory mass of the plants, obtained by the ratio between the Leaf Area and total dry mass (LAR), the lowest results were obtained for saline treatments (15 and 30 mM NaCl) (Table 4).

According to Benincasa (2003) [35], the size of the photosynthetic surface in relation to the respiratory mass represents the useful leaf area for photosynthesis, with the Table 4Growth rates in leavesof O. basilicum L. irrigated withdifferent water qualities

Parameters	Treatments	Days after flowering							
		1	7	21	28	35			
SLA	Control	212.97	203.38	175.07	185.50	201.53			
(cm <sup>2</sup> leaves g <sup>-1</sup> DM leaf)	15 mM NaCl	189.35	202.32	199.99	179.02	139.95			
	Reclaimed water	214.82	199.16	214.21	202.83	182.27			
	30 mM NaCl	201.96	201.02	171.77	198.02	165.95			
		ns	ns	ns	ns	ns			
LAR	Control	0.0117	0.0096	0.0071	0.0071	0.0058			
(m <sup>2</sup> leaves g <sup>-1</sup> DM plant)	15 mM NaCl	0.0101	0.0095	0.0082	0.0060	0.0045			
	Reclaimed water	0.0112	0.0092	0.0083	0.0063	0.0049			
	30 mM NaCl	0.0108	0.0087	0.0064	0.0065	0.0046			
		ns	ns	ns	ns	ns			
LWR	Control	0.55	0.47	0.41	0.38	0.29			
(g DM leaves g <sup>-1</sup> total DM)	15 mM NaCl	0.53	0.46	0.41	0.33	0.31			
	Reclaimed water	0.52	0.46	0.38	0.31	0.26			
	30 mM NaCl	0.53	0.43	0.37	0.33	0.27			
		ns	ns	ns	ns	ns			

Tukey's test: \* $p \le 0.05$ ; \*\* $p \le 0.01$ ; *ns* not significant

ratio between the leaf area, responsible for the interception of light energy and absorbed  $CO_2$  and total dry mass. It would be plausible the occurrence of LAR decrease in plants grown under salt stress as a mechanism to reduce water loss.

The fraction of total biomass allocated in the leaves (LWR- Leaf Weight Ratio) in *O. basilicum* L. (Table 4) shows that the application of the treatment with higher salinity irrigation (30 mM NaCl) gradually reduced leaf biomass at a faster rate than the other treatments. Regarding the treatment of irrigation with reclaimed water, there was also a decrease of LWR, but only from 21 DAF on, similar to the treatment with 15 mM NaCl and Control. As the commercial interest of this crop is based mainly on the use of the leaves for the production of essential oil, production of drugs and cosmetics, the application of reclaimed water, although numerically lower than the means of the other treatments, did not affect the LWR (Table 4).

Acosta-Motos (2014) [36, 37] showed that reclaimed water used in irrigation with 8 dS  $m^{-1}$  electrical.

conductivity (EC) was able to promote SLA reduction and increase of LWR in ornamental plants of *Eugenia myrtifolia* and *Myrtus communis*. However, changes in these variables are observed with higher salinity levels than those applied in the present study. Thus, the evaluation of growth rates was not able to identify occurrences of changes promoted by the increase of salinity in this experiment.

The increase in the concentration of salts in the soil solution around the roots induces an increase in the flow of ions towards the epidermis cells, leading to elevation of the ionic concentrations in the apoplast near the plasma membrane [38]. The existence of membranespecific transporters promotes the entry of ions into the cytosol. Regarding K<sup>+</sup> and Na<sup>+</sup>, which share the same carrier, the binding of the salts to the membrane carrier is facilitated by increasing the ion concentration in the soil solution. However, the disproportionate increase in the concentration of ions favors the transport of the more concentrated salt.

In this study, the concentration of K<sup>+</sup> ion in the soil solution was higher in the saline treatments at the end of the crop cycle, indicating a lower absorption of this ion by the plants in these treatments (Table 5). This information becomes more consistent when K<sup>+</sup> concentration is numerically lower in the root tissues of the same treatments (Table 5). Similarly, Na<sup>+</sup> increased significantly ( $p \le 0.01$ ) in the soil solution in saline treatments, which may have contributed to the limitation of K<sup>+</sup> uptake in these treatments.

Among the factors studied to characterize the effects of saline stress, as well as the ability of plants to tolerate salinity, the nutritional state of the plants should be considered [11]. The elevation of Na<sup>+</sup> concentration in the soil can directly interfere with the root absorption of other nutrients, such as K<sup>+</sup> and Ca<sup>+</sup>. According to Tester and Davenport [39] as they share the same membrane co-transporter, K<sup>+</sup> and Na<sup>+</sup> are competing elements during the process of root absorption. Thus, the adequate supply of K<sup>+</sup> is fundamental to minimize the absorption of Na<sup>+</sup>. In addition, the ability to maintain high levels of K<sup>+</sup> and Ca<sup>+</sup> and low levels of Na<sup>+</sup> in plant tissues is a key mechanism for greater tolerance to salinity [11]. Table 5Mineral composition,pH and electrical conductivityof soil solution duringcultivation of *O. basilicum* L. atthe end of the vegetative stage

Treatments	Р	К	Ca	Mg	Na	рН	EC
	$\mu g L^{-1}$						$mS m^{-1}$
Control	0.63 ab	7.66 a	27.66 b	14.66 a	113 c	7.55 a	0.23 c
15 mM NaCl	0.67 a	14.00 a	142.33 a	75.00 a	30.600 b	6.95 a	2.77 b
Reclaimed water	0.54 b	9.33 a	86.33 ab	54.66 a	4656c	7.52 a	0.91 c
30 mM NaCl	0.54 b	12.66 a	135.33 a	73.00 a	72.933 a	6.79 a	4.72 a
CV%	6.54	26.71	39.67	51.55	20.74	5.15	28.14

Means followed by the same letter represents the comparison between treatments by Tukey's test at 5% probability

Table 6 Composition of leaves nutrients of *O. basilicum* L. irrigated with different water qualities (T1—Control, T2—15 mM NaCl, T3—reclaimed water and T4—30 mM NaCl)

Element	DAF	AF Treatments			CV% Element	DAF	Treatments				CV%		
(g kg <sup>-1</sup> )		T1	T2	Т3	T4		$(mg kg^{-1})$		T1	T2	T3	T4	
N	14	20 b	22 ab	25 a	23 ab		В	14	33 a	38 a	47 a	41 a	
	35	15 a	14 a	16 a	13 a			35	44 a	43 a	42 a	38 a	
	Mean	17 a	18 a	20 a	18 a	10.4		Mean	39 a	40 a	45 a	40 a	16.53
Ρ	14	1.8 a	1.7 a	1.9 a	1.9 a		Cu	14	5.0 a	5.0 a	5.0 a	5.0 a	
	35	1.3 a	1.4 a	1.2 a	1.2 a			35	4.6 a	4.0 a	5.0 a	4.6 a	
	Mean	1.5 a	1.5 a	1.6 a	1.5 a	9.37		Mean	4.8 a	4.5 a	5.0 a	4.8 a	12.05
К	14	13 a	13 a	15 a	14 a		Fe	14	138 a	130 a	153 a	142 a	
	35	10 a	11 a	10 a	11 a			35	145 a	142 a	143 a	150 a	
	Mean	12 a	12 a	13 a	13 a	19.61		Mean	142 a	136 a	148 a	146 a	17.61
Ca	14	15 a	19 a	19 a	19 a		Mn	14	30 a	45 a	35 a	48 a	
	35	21 a	21 a	21 a	21 a			35	45 b	72 a	62 ab	67 ab	
	Mean	18 a	20 a	20 a	20 a	14.77		Mean	38 b	58 a	49 ab	57 a	21.78
Mg	14	7 a	8 a	9 a	10 a		Zn	14	35 a	41 a	39 a	46 a	
	35	8 a	10 a	9 a	9 a			35	33 b	46 ab	43 ab	49 a	
	Mean	7 a	9 a	9 a	10 a	17.86		Mean	34 b	44 ab	41 ab	48 a	16.14
S	14	2.3 ab	1.9 b	2.5 ab	2.5 a		Na	14	53 a	70 a	60 a	76 a	
	35	2.2 a	1.5 b	1.6 b	1.6 b			35	100 a	136 a	103 a	136 a	
	Mean	2.3 a	1.7 b	2.0 ab	2.1 ab	11.08		Mean	76 a	103 a	81 a	106 a	51.03

Means followed by the same letter do not differ statistically by the Tukey's test at 5% probability

Changes in leaf nutrient concentrations were observed in sulfur (S), manganese (Mn) and zinc (Zn) (Table 6). In the treatment with 15 mM NaCl (T2), a reduction of leaf S concentration was observed in comparison to the control, which is not different from the other treatments (Table 6). On the contrary, the element Mn presented greater accumulation in the leaves of the saline treatments plants. Zn presented an increase in the treatment with the highest level of salinity (30 mM NaCl) and presented a greater leaf accumulation than the control. Although there were no significant differences for sodium (Na<sup>+</sup>), the treatments that received NaCl present numerically higher concentrations of this element than the control and reclaimed water. It is important to note that the accumulation of Na<sup>+</sup> for control and reclaimed water is similar (Table 6). The evaluation of leaf nutrients indicated a tendency to increase the concentration of Na<sup>+</sup> in *O. basilicum* L. between the 14th and 35th DAF. It was observed that the increase of Na<sup>+</sup> coincides with the tendency of K<sup>+</sup> reduction in the same period. This fact may be indicative of the occurrence of K<sup>+</sup> and Na<sup>+</sup> antagonism, with Na<sup>+</sup> elevation probably favoring its absorption in detriment to K<sup>+</sup> absorption. When the different water qualities are compared, greater Na<sup>+</sup> leaf accumulation was observed in saline treatments, although these results are not statistically significant (Table 6).

When we consider root tissues, the treatments T2 (15 mM NaCl) and T3 (reclaimed water) also presented a N<sup>+</sup> increase being statistically superior to the control (T1) and 30 mM NaCl (T4). The accumulation of P was higher in the

treatments with NaCl and reclaimed water. However, only the least concentrated NaCl treatment differed from the control. Zn showed significantly higher accumulation in T2 (15 mM NaCl) compared to T1 (Control) and T3 (Reclaimed water) (Table 7).

The results showed a significantly higher Na<sup>+</sup> accumulation in the saline treatments compared to T1 (Control). Reclaimed water (T3) did not differ from the control (T1), indicating that this treatment did not promote accumulation of salts in the roots of O. basilicum L.

Considering that there was no significant accumulation of Na<sup>+</sup> in the leaves of O. basilicum L., even in the treatments where the accumulation of this element was significantly higher in the roots, it must be considered that these plants avoid raising this element to the leaves. Some authors [32, 39] report that plant tolerance to salinity can occur through distinct mechanisms: individual cell resistance, usually by Na<sup>+</sup> compartmentalization in cell vacuoles and damage repair mechanisms. These mechanisms can affect all cells by supplying the plant with resistance to salinity. Control of the movement of Na<sup>+</sup> between different plant tissues, preventing excess salts to reach the leaves, or the ability to exclude radicular Na<sup>+</sup>. In this study, the Na<sup>+</sup> absorbed by the roots does not reach the totality of the leaves, and this mechanism can contribute to avoid salinity damages and give a certain degree of tolerance to salinity.

Analysis of K<sup>+</sup>/Na <sup>+</sup> ratio in the root tissues showed that saline treatments and reclaimed water promoted a significant reduction of this ratio in comparison with the control on all days after flowering (Table 8).

In plants that received the treatment of 15 mM NaCl, the reduction of this ratio is associated with an increase in Na<sup>+</sup> content in the tissues, since the concentration of K<sup>+</sup> did not reduced significantly. When the plants were irrigated with 15 Mm NaCl, a decrease of K<sup>+</sup>/Na<sup>+</sup> was observed, which was promoted by the significant  $(p \le 0.05)$  drop in K<sup>+</sup> content in the root tissues, evidencing the antagonistic effect between these two elements (Table 8).

**Table 8** Root ratio of  $K^+/Na^+$  in plants of O. basilicum L. irrigated with different water qualities

Treatments	Days after flowe	ring
	14	35
Control	2.58 a	1.89 a
15 mM NaCl	0.66 b	0.25 b
Reclaimed water	0.97 b	0.55 b
30 mM NaCl	0.41 b	0.31 b

Means followed by the same letter represents the comparison between treatments by Tukey's test at 1% probability

reclaimed water and T4—30 mM NaCl)													
Element	DAF	Treatments				CV%	Element	DAF	Treatmen	ts			CV%
(g kg <sup>-1</sup> )		T1	T2	T3	T4		(mg kg <sup>-1</sup> )		T1	T2	T3	T4	
N	14	9.3 b	11.1 a	11.3 a	9,2 b		В	14	63.3 a	66.0 a	71.2 a	74.6 a	
	35	6.9 a	8.0 a	8.4 a	7,9 a			35	80.8 a	78.7 a	77.0 a	104.5 a	
	Mean	8.1 c	9.7 ab	9.8 a	8.5 bc	8.16		Mean	72.0 a	72.3 a	74.1 a	89.6 a	19.99
Р	14	1.0 b	1.4 a	1,0 ab	1.1 ab		Cu	14	13.3 a	13.0 a	13.3 a	14.0 a	
	35	0.9 a	1.0 a	0.9 a	1.0 a			35	13.3 a	11.3 ab	10.6 b	11.6 ab	
	Mean	0.9 b	1.2 a	1,0 ab	1.1 ab	14.56		Mean	13.3 a	12.1 a	12.0 a	12.8 a	7.95
К	14	9.3 a	6.6 ab	6.9 ab	5.9 b		Fe	14	3469.3 a	2295.0 b	2903.3 ab	2857.5 ab	
	35	7.7 a	4.5 b	5.4 ab	3.6 b			35	4001.3 a	3591.6 a	3788.0 a	3638.3 a	
	Mean	8.5 a	5.5 b	6.1 b	4.7 b	21.66		Mean	3735 a	2943 a	3345 a	3247 a	15.07
Ca	14	6.1 a	6.2 a	6.0 a	5.7 a		Mn	14	60 a	58 ab	43 ab	37 b	
	35	8.7 a	8.0 a	8.1 a	8.2 a			35	46 a	38 a	43 a	44 a	
	Mean	7.4 a	7.1 a	7.0 a	6.9 a	9.28		Mean	53 a	48 a	43 a	40 a	20.15
Mg	14	14.4 a	9.5 b	9.4 b	9.1 b		Zn	14	56 b	78 a	64 ab	63 ab	
	35	8.4 a	8.5 a	6.4 a	6.3 a			35	32 b	58 a	33 b	48 ab	
	Mean	11.4 a	9.0 ab	7.9 b	7.7 b	18.18		Mean	44 b	68 a	49 b	55 ab	15.72
S	14	2.0 a	1.9 a	1.9 a	1.9 a		Na	14	3683 b	11.066 ab	8503 ab	14.450 a	
	35	1.8 b	2.0 ab	2.2 a	1.8 b			35	4143 c	18.366 a	9866 bc	16.266 ab	
	Mean	1.9 a	1.9 a	2.0 a	1.8 a	8.69		Mean	3913 c	14.716 ab	9185 bc	14.358 a	33.64
Moons fol	llowed b	v camo lo	ttor do n	ot diffor	statistical		ov's tost at 5	% proba	bility				

Table 7 Nutrient composition of roots of O. basilicum L. irrigated with different water gualities (T1-Control, T2-15 mM NaCl, T3-

Means followed by same letter do not differ statistically by Tukey's test at 5% probability

## **4** Conclusions

The ability of *O. basilicum* L. to prevent leaf accumulation of Na +, even with the significant increase in salinity in the soil solution and in the root system, suggests the existence of a resistance / survival mechanism of plants to saline conditions. It was also concluded that *O. basilicum* L. can be safely cultivated using water with a salinity level equal to or less than 15 mM NaCl and that irrigation with reuse water, with the characteristics similar to that used in this experiment, can be used without any damage to plant development.

### **Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- 1. Pedrero F, Allende A, Gil MI, Alarcón JJ (2012) Soil chemical properties, leaf mineral status and crop production in a lemon tree orchard irrigated with two types of wastewater. Agric Water Manag 109:54–60
- 2. Santos OSN, Paz VS, Gloaguen TV, Teixeira MB, Fadigas FS, Costa JA (2012) Growth and nutritional status of helicônia irrigated with treated wastewater in greenhouse. Rev Bras Eng Agr Amb 16:820–827
- Castro AAS, Damásio AOC, Menezes FS, Souza JA, Santana SF, Mendonça D, Faccioli GG (2016) Análise do impacto do uso de efluentes nas características do solo da cultura do feijão-caupi brs novaera (*Vigna unguiculata* L.walp.). Rev Agroforestalis News 1:41–47
- Bañón S, Miralles J, Ochoa J, Franco A, Sánchez-Blanco MJ (2011) Effects of diluted and undiluted treated wastewater on the growth, physiological aspects and visual quality of potted lantana and polygala plants. Scientia Hort 129:869–876
- Mancuso PCS, Santos HF (2003) A escassez e o reúso de água em âmbito mundial. In: Mancuso PC, Santos HF, Philippi A Jr (eds) reúso de água, 1st edn. Manole, Barueri, p 18
- 6. Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. Trends Plant Sci 6:371–379
- Acosta-Motos JR, Ortuño MF, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco MJ, Hernandez JA (2017) Plant Responses to Salt Stress: Adaptive Mechanisms. Agronomy 7:18
- Schossler TR, Machado DM, Zuffo AM, Andrade FR, Piauilino AC (2012) Salinidade: efeitos na fisiologia e na nutrição mineral de plantas. Enciclopédia Biosfera 8:1563–1578
- 9. Guimarães IP, Oliveira FN, Vieira FER, Torres SB (2013) Efeito da salinidade da água de irrigação na emergência e crescimento inicial de plântulas de mulungu. Braz J Agr Sci 8:137–142
- Alves FAL, Silva SLF, Silveira JAGS, Pereira VLA (2011) Efeito do Ca<sup>2+</sup> externo no conteúdo de Na<sup>+</sup> e K<sup>+</sup> em cajueiros expostos a salinidade. Braz J Agr Sci 6:602–608
- Dias ND, Blanco FF (2010) Efeitos dos sais no solo e na planta. In: Gheyi HR, Dias NS, Lacerda CF (eds) Manejo da salinidade na agricultura: Estudos básicos e aplicados. Instituto Nacional de Ciência e Tecnologia em Salinidade, Fortaleza, pp 129–130

- 12. Pereira RCA, Moreira ALM (2011) Manjericão: cultivo e utilização. Embrapa Agroindústria Tropical, Documentos 136:31p
- 13. Blank AF et al (2010) Comportamento fenotípico e genotípico de populações de manjericão. Hortic Bras 28:305–310
- Soboti SN, Poushpangadan P (1982) Studies in the genus Ocimum: cytogenetcs, breeding and production of new strains of economic importance. In: ATAL CK, KAPUR BM (Ed) Cultivation and utilization of aromatic plants. Jammu-Tawi: Regional Laboratory Council of Scientific and Industrial Research, 3: 606p.
- Padalia RC, Verma RS (2011) Comparative volatile oil composition of four Ocimum species from northern India. Nat Prod Res 25:569–575
- 16. Cunha AR, Martins D (2009) Classificação climática para os municípios de Botucatu e São Manuel, SP. Irriga 14:1–11
- Carvalho WA (1983) Levantamento de Solos da Fazenda Lageado-Estação Experimental Presidente Médici. Botucatu-SP, Brazil, UNESP, p 95
- Raij VB, Andrade JC, Cantarella H, Quaggio JA (2012) Análise química para avaliação de fertilidade de solos tropicais. SP, Brazil, Campinas, p 285
- Van Genutchen MTH (1980) A closed-from equation for predicting the hydraulic conductivity of insatured. Soil Sci Soc Am J 41:892–898
- 20. Scholander PF, Hammel HT, Bradstreet ED (1965) Sap pressure in vascular plants. Science 148:339–346
- Barrs HD (1968) Determination of water deficits in plant tissues. In: Kozlowsky TT (ed) Water deficits and plant growth. Academic press, New York, pp 235–268
- 22. Hunt R (1982) Plant Growth Curves. The functional approach to growth analysis. Edward Arnold, London, p 248
- 23. Malavolta E, Vitti GC, Oliveira SA (1997) Avaliação do estado nutricional das plantas princípios e aplicações, 2nd edn. Potafos, Piracicaba, p 319
- 24. Silva FAS, Azevedo CAV (2009) Principal components analysis in the software assistat-statistical attendance. In: World congress on computers in agriculture, 7, Reno-NV-USA: 2009, American Society of Agricultural and Biological Engineers.
- 25. Cavalcante VS, Santos VR, Neto ALS, Santos MAL, Santos CG, Costa LC (2012) Biomassa e extração de nutrientes por plantas de cobertura. Rev Bras Eng Agr Amb 16:521–528
- Simon JE et al (1992) Water stress-induced alterations in essential oil content and composition of sweet basil. J Essent Oil Res 4:71–75
- Maia JM, Silva-Ferreira SL, Voigt EL, Macedo CEC, Pontes LFA, Silveira JAG (2012) Atividade de enzimas antioxidantes e inibição do crescimento radicular de feijão caupi sob diferentes níveis de salinidade. Acta Bot Bras 26:342–349
- Larcher W (1995) Gas exchange in plants. In: Larcher W (ed) Physiological plant ecology, 3rd edn. Springer, Berlin, pp 74–128
- 29. Távora FJAF, Ferreira RG, Hernandez FFF (2001) Growth and water relations in guava plants under NaCl saline stress. Rev Bras Frut 23:441–446
- Tarchoune I, Innocenti ED, Kaddour R, Guidi L, Lachaa M, Navari-Izzo F, Ouerghi Z (2012) Effects of NaCl or Na<sub>2</sub>SO<sub>4</sub> salinity on plant growth, ion content and photosynthetic activity in *Ocimum basilicum* L. Acta Phys Plant 34:607–615
- Prisco JT, Gomes Filho E (2010) Fisiologia e bioquímica do estresse salino em plantas. In: Ghey HR, Dias NS, Lacerda CF (eds) Manejo da salinidade na agricultura Estudos básicos e aplicados. INCSal, Fortaleza, p 472
- 32. Jha D, Shirley N, Tester M, Roy SJ (2010) Variation in salinity tolerance and shoot sodium accumulation in *Arabidopsis* ecotypes linked to differences in the natural expression levels of transporters involved in sodium transport. Plant, Cell Environ 33:793–804

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- Bione MAA, Paz VS, Silva FS, Ribas RF, Soares TM (2014) Crescimento e produção de manjericão em sistema hidropônico NFT sob salinidade. Rev Bras Eng Agr Amb 18:1228–1234
- Fageria NK, Soares Filho WS, Gheyi HR (2010) Melhoramento genético vegetal e seleção de cultivares tolerantes à salinidade. In: Gheyi HR, Dias NS, Lacerda CF (eds) Manejo da salinidade na agricultura: Estudos básicos e aplicados. INCTSal, Fortaleza, pp 205–216
- 35. Benincasa MMP (2003) Análise de crescimento de plantas: noções básicas. Jaboticabal, SP, Brazil, FUNEP, p 42
- Acosta-Motos JR, Álvarez S, Hernández JA, Sánchez-Blanco MJ (2014) Irrigation of *Myrtus communis* plants with reclaimed water: morphological and physiological responses to different levels of salinity. J Hortic Sci Biotechnol 89:487–494
- Acosta-Motos JR, Álvarez S, Barba-Espín G, Hernández JA, Sánchez-Blanco MJ (2014) Salts and nutrients present in regenerated waters induce changes in water relations, antioxidative

metabolism, ion accumulation and restricted ion uptake in *Myrtus communis* L. plants. Plant Physiol Biochem 85:41–50

- Silveira JAG, Silva SLF, Silva EM, Viégas RA (2010) Mecanismos biomoleculares envolvidos com a resistência ao estresse salino em plantas. In: Greyi HR, Dias NS, Lacerda CF (eds) Manejo da salinidade na agricultura: Estudos básicos e aplicados. Instituto Nacional de Ciência e Tecnologia em Salinidade, Fortaleza, CE, Brazil, pp 161–180
- 39. Tester M, Davenport R (2003) Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plants. Ann Bot 91:503–527

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