

USE OF RUTIN AS A SALT STRESS MITIGATOR FOR YELLOW BELL PEPPER IN A PROTECTED ENVIRONMENT

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Abstract: Bell pepper is one of the main vegetables produced in the world and are among the most commercialized and consumed in Brazil due to their ease of cultivation in small areas and their high economic value. Given the need for vegetable producers to use lower-quality water for irrigation, it is necessary to study inputs that can mitigate the harmful effects of salinity on plants, such as the biostimulant rutin. A study was therefore carried out to assess whether the application of the biostimulant rutin mitigates the harmful effects of salinity when growing yellow peppers in a protected environment. The experimental design used was randomized blocks in subdivided plots with four replications. The plots consisted of four levels of electrical conductivity of the irrigation water (0.3, 1.5, 3.0, and 4.5 dS m⁻¹), and the subplots were four doses of the biostimulant rutin (0.0, 6.0, 12.0, and 18.0 g L⁻¹) applied via foliar. Growth variables such as plant height and stem diameter, production, fruit quality, leaf water potential, gas exchange, brix level, fruit weight, and shoot dry weight were assessed. The data was submitted to analysis of variance and Tukey test at 5%. The doses of the biostimulant rutin did not mitigate the effects of the salts on the plants, and further tests with higher doses of the biostimulant are needed to verify its efficiency. The salts caused a reduction in plant growth, stem diameter, and the dry weight of the aerial part of the yellow bell pepper plants.

Keywords: Capsicum annum, Biostimulant, Salinity, Irrigation, Greenhouse.

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Introduction

Bell pepper (*Capsicum annum* L.) is one of the main vegetables produced in the world. It is among the most commercialized and consumed in Brazil due to its ease of cultivation in small areas and its high economic value. It is estimated that the crop occupies an area of 13,000 hectares in Brazil, producing around 350,000 tons per year (Silva *et al.*, 2022).

Bell pepper can be grown in the open or in protected cultivation. However, in the latter, productivity is higher as there is greater control over environmental conditions, such as rain and wind, as well as facilitating pest and disease control. However, despite the benefits, protected cultivation requires a high implementation cost, thus restricting its use to highly profitable crops, such as the production of colored hybrid bell pepper. According to Rinaldi *et al.* (2008), the bell pepper plant is one of the crops best adapted to planting in a protected environment,

offering advantages such as production in different seasons, especially during the rainy season.

In Brazil, bell pepper production has been growing. However, its production in regions such as the northeastern semi-arid region is quite challenging, mainly due to the limited supply of quality water, which leads most producers to use the water that is available on the property: sometimes it is brackish or saline (Cavalcante *et al.*, 2019).

When plants are irrigated with water containing high concentrations of salts, which is prevalent in the northeastern semi-arid region, they suffer from the accumulation of salts, as it influences the availability of water for cultivated plants, reducing the absorption of water by the roots, consequently resulting in water deficit, a reduction in leaf area and stomatal conductance, thus reducing photosynthesis and plant growth (Sousa *et al.*, 2022), 2022) and causes ionic imbalance (Fang *et al.*, 2021), such effects leading to reduced productivity.

It should be noted that bell pepper is moderately sensitive to soil salinity, tolerating up to 1.5 dS m^{-1} in saline soils without yield losses. Salt stress affects the physiological functions of plants by decreasing the osmotic potential of the soil solution due to its effect on water retention, making it less and less available to the plant (Sousa *et al.*, 2019).

Given the need for many farmers to use lower-quality water for irrigation, researchers must study methods and products that can mitigate the harmful effects of salt on plants, such as the biostimulant rutin, which has been widely used in the pharmaceutical industry for its vasoprotective properties (Muhammad *et al.*, 2015) and for prophylactic purposes (Mishima *et al.*, 2005; Maruyama *et al.*, 2009).

Rutin used as a biostimulant in agriculture, is extracted from the fava d'anta plant (*dimorphandra mollis*), a species found widely in the northeastern hinterland and the Brazilian Cerrado. It is a bioflavonoid that has various functions in agriculture, such as protecting plants against ultraviolet rays and insects, fungi, viruses, and bacteria. It also has an antioxidant effect, controls the action of plant hormones, inhibits enzymes involved in cellular redox systems, and is an allelopathic agent (Simões *et al.*, 2000).

Given the above, this study aimed to evaluate the responses to the application of the biostimulant rutin associated with irrigation water salinity in the yellow bell pepper crop in a protected environment.

Material and Methods

The research was conducted in the experimental area of the Escola de Floricultura do Ceará – Tecflores, located in the city of São Benedito-CE ($04^{\circ} 02' 55'' \text{ S}$; $40^{\circ} 51' 54'' \text{ W}$; 901 m), belonging to the Instituto Agropolos do Ceará.

The experimental design used was a randomized block design in subdivided plots with four replications. The plots consisted of four levels of electrical conductivity of the irrigation water (0.3, 1.5, 3.0, and 4.5 dS m^{-1}) and the sub-plots consisted of four doses of the biostimulant rutin (0.0, 6.0, 12.0, and 18.0 g L^{-1}) applied via foliar. For each subplot, there were three useful plants in pots, totaling 12 plants per plot, making a total of 48 plants per block with the yellow bell pepper hybrid Rialto 1 MX – Nunhems.

The seedlings were transplanted into pots with a volumetric capacity of 8 liters, which were placed on plastic sheeting so as not to salinize the soil in the experimental area. There was one plant per pot, with a spacing of 0.33 m between plants and 1.5 m between plant rows, with a projected stand of 20,202 plants per hectare. The pots were filled with the local soil and the aim was to leave them with similar weights, to fill them as evenly as possible. It should be noted that three days before the seedlings were transplanted into the pots, they were irrigated until the humidity reached field capacity, to allow the pots to drain initially.

The bamboo stakes were planted every 2.0 m between the pots, in line with the row of plants, with a zip tie to support the plants. The first tie was installed 30 cm above the pots, at the height of the first fork in the bell pepper plant.

Fertilizer management was based on the soil analysis carried out before the experiment and the recommendation for the crop, according to Fertilizer Recommendations for the State of

Pernambuco. For the macronutrients, the following doses were used in the foundation: $\text{N} = 30 \text{ kg ha}^{-1}$, $\text{P} = 120 \text{ kg ha}^{-1}$, and $\text{K} = 40 \text{ kg ha}^{-1}$, and in the top dressing, $\text{N} = 120 \text{ kg ha}^{-1}$ and $\text{K} = 120 \text{ kg ha}^{-1}$. The fertilizers were urea, super simple, and potassium chloride as NPK sources.

From transplanting to harvesting, the bell pepper crop tended to provide the best conditions for plant growth and minimize the occurrence of management, phytosanitary, and or physiological problems. Weed control in the pots was carried out throughout the crop cycle using manual weeding.

After transplanting, the volume of water applied daily per pot was quantified using micro-lysimeters. To do this, four drainage micro-lysimeters were used, one in each block, which were adapted from the pots used in the experiment so that the excess drained was quantified daily.

After quantifying the average volume drained per pot (V_d) and the volume placed per pot (V_c), the volume used per plant (V_u) was calculated, in liters per day, considering it equivalent to the evapotranspiration of the crop in the pot, according to equation 01:

$$V_u = V_c - V_d \quad (01)$$

The volume replenished (V_r) each day was always 20% higher than the consumption forecast so the excess was drained (equation 02).

$$V_r = 1,2 \times V_u \quad (02)$$

The salt solutions used for irrigation were prepared from sodium chloride salt (NaCl), following the methodology of Rhoades *et al.* (2000), in which the electrical conductivity (EC) of the desired water is obtained after the relationship between ECw and its concentration ($\text{mmol}_e \text{ L}^{-1} = \text{EC} \times 10$).

Up to 20 days after transplanting (DAT), all the plants were irrigated with water of 0.3 dS m^{-1} (deep tube well). After 20 DAT, treatments with salinity levels were started until 120 DAT.

The four doses of the biostimulant rutin were applied via foliar spraying. Two applications of rutin were made during the Rialto bell pepper crop cycle, with the same concentrations, applying approximately 31.25 mL per plant. The applications took place in the vegetative and pre-flowering phases at 30 and 60 DAT, respectively, in the morning.

For the growth analyses, stem diameter (mm) and plant height (cm) were assessed at 30, 60, and 90 DAT. The diameter of the stem was measured about 1.0 cm above the neck of the plant, using a digital caliper with a precision of 0.05 mm, and the height of the plant was measured with a ruler comprising the gap between the neck of the plant and the apical zone.

After the end of the experimental cycle, at 110 DAT, the dry weight of the aerial part (PSPA) of the yellow bell pepper plants was measured. The three useful plants per experimental unit were used to measure PSPA. The collected plants were placed in paper bags, taken to the laboratory, and dried in a drying oven at 65°C until they reached a constant mass.

The production variables analyzed were: transverse diameter (DT) and longitudinal length (LL) of the fruit, number of

fruits per plant (NF), average fruit weight (PF), yield, and soluble solids content (SS).

The diameter of the fruit was measured with a digital caliper, in mm; the measurements were taken in the middle of the fruit and the number of fruits was determined by direct counting at the time of harvest. The weight of the fruit was determined using a digital scale accurate to 0.001 g. The soluble solids content was quantified using a digital refractometer (MA871 Refractometer). For this assessment, a small fraction of the fruit was macerated and homogenized and then read on the device.

Leaf water potential was determined at 65 DAT using a Schoalander-type pressure pump (model DC CONSOLE, ALLEMAR). To do this, one of the leaf's central leaflets was removed and a segment was immediately placed in the pressure pump chamber, with part of it exposed outside the chamber. Subsequently, the pressure was applied until the water column in the xylem vessels began to move again, as seen by the exudation of water, at which point the pressure was read, which corresponded to the pressure potential, which is known as the leaf's water potential. The readings were taken between 5 and 7 a.m. and the water potential was expressed in MPa.

The rate of net photosynthesis – A, transpiration – E, stomatal conductance – gs, and internal CO₂ concentration – Ci, were determined using a portable infrared gas analyzer – IRGA

(model LCI, ADC, BioScientific, England). The measurements took place on the upper middle leaves of the plant with no morphological defects, between 09:00 and 11:00 in the morning, using an artificial radiation source of 1500 ($\mu\text{mol m}^{-2} \text{s}^{-1}$), with ambient temperature, humidity, and CO₂.

The data for each variable observed was subjected to the normality test (Shapiro-Wilk), and analysis of variance (ANOVA), and when a significant difference was observed between the treatments ($p < 0.05$), it was subjected to regression analysis.

In the regression analysis, the equations that best fit the data were chosen based on the significance of the regression coefficients at $p < 0.05$ (*) and $p \geq 0.01$ (**) and the highest coefficient of determination (R^2). Data analysis was carried out using the SISVAR statistical analysis program (Ferreira, 2019).

Results and Discussion

The variables plant height (HL), stem diameter (SD), shoot dry weight (ASW), and number of fruits (NF) were significant about water salinity alone; there was no statistical difference between the doses of the biostimulant rutin. Similarly, the interaction between salinity levels and doses of the biostimulant rutin also did not lead to significant changes in any of the variables studied (Table 1).

Table 1. Summary of the analysis of variance for the variables of plant height (AL), stem diameter (DC), shoot dry weight (PSPA), and number of fruits per plant (NF) of yellow bell pepper subjected to different levels of electrical conductivity of water (ECw) and doses of the biostimulant rutin in a protected environment.

SV	Mean Square				
	DF	AL	DC	PSPA	NF
Repetition	3	39.68 ^{ns}	1.42 ^{ns}	233.26 ^{ns}	4.93 ^{ns}
Salinity	3	668.77 ^{**}	16.18 ^{**}	2049.31 ^{**}	49.82 ^{**}
Error 1	9	17.21 ^{ns}	2.63 ^{ns}	129.85 ^{ns}	3.38 ^{ns}
Rutin	3	47.85 ^{ns}	1.34 ^{ns}	17.48 ^{ns}	0.38 ^{ns}
S x R	9	31.34 ^{ns}	0.69 ^{ns}	146.04 ^{ns}	1.28 ^{ns}
Error 2	36	20.04 ^{ns}	0.91 ^{ns}	107.77 ^{ns}	1.17 ^{ns}
CV 1 (%)		7.4	11.7	19.26	30.85
CV2 (%)		7.99	6.89	17.55	18.19

SV- Source of variation; DF- Degree of freedom; S x R- Interaction between salinity and rutin; CV- Coefficient of variation; ** and * - significant by the F test at 5 and 1%, respectively; ^{ns} - not significant by the F test at 5%.

In this study, no positive results were found for the biostimulant rutin, so it appears that rutin did not mitigate the effect of the different levels of salinity on the variables AL, DC, PSPA, and PF. Recent research has found results that corroborate this work, such as that by Alfosea-Simón *et al.* (2020) on tomato cultivation.

Figure 1A shows that when the plants received water with ECs of 0.3, 1.5, 3.0, and 4.5 dS m⁻¹, the bell pepper plants reached ALs of 61.86, 60.42, 54.06, and 47.77 cm, with a linear decrease as a result of the increase in the electrical conductivity of the irrigation water.

It should be noted that there was a significant effect of the salinity levels applied on the height of the bell pepper plants, showing that bell pepper plants are sensitive to salinity in irrigation water from 1.5 dS m⁻¹ onwards, which was reduced by 22% at 4.5 dS m⁻¹ compared to the control, whose EC was 0.3 dS m⁻¹ (Figure 1A).

A similar effect was found by Barros *et al.* (2021), studying the tolerance of bell pepper cultivars under salinity in germination and initial growth, who observed that as the EC of the water increased, there was a linear decrease in the length of the seedlings, reaching a reduction of 0.39 cm. Reges *et al.* (2017) also found in their results that when salinity increased, there was a decline in the development of the bell pepper crop.

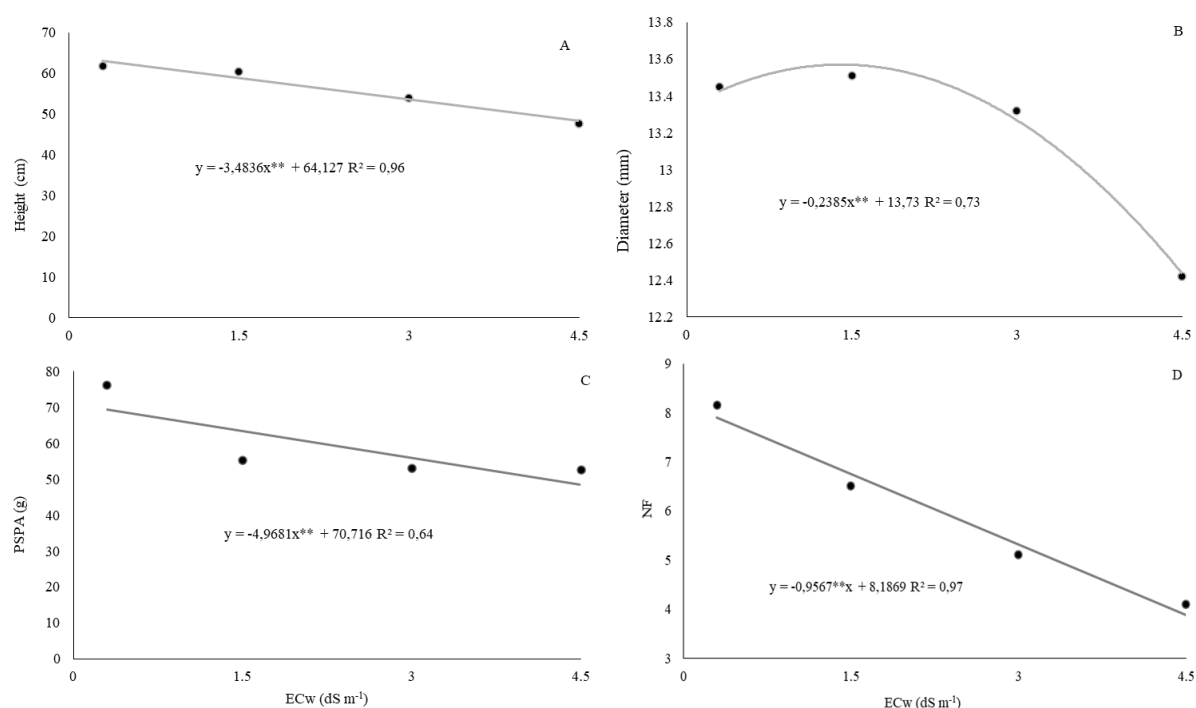


Figure 1. Averages of the following variables: plant height – AL (A), stem diameter – DC (B), dry weight of the aerial part – PSPA (C), and number of fruits – NF (D) of yellow bell pepper as a function of the levels of electrical conductivity of the water used for irrigation.

Quadratic behavior was observed for the DC of yellow bell pepper plants under different ECw (Figure 1B), with a maximum value of 13.5 mm in the plants that received irrigation with ECw of 1.5 dS m⁻¹, whose reduction was more pronounced from the electrical conductivity of 3.0 dS m⁻¹. Therefore, it was noted that as the salinity of the irrigation water increased, this variable suffered, with a reduction of (16.3 %) about the lowest level of salinity in the irrigation water (0.3 dS m⁻¹).

Similar results were found by Lemos Neto *et al.* (2012) with the bell pepper crop, where the diameter of the stem was reduced as the salinity of the irrigation water increased, with lower diameter values from 3.5 dS.m⁻¹. Cavalcanti *et al.* (2005), studying the tolerance of castor bean (*Ricinus communis* L.) to salinity, found that stem diameter had a significant effect on salinity, linearly decreasing, with a decline of 0.2 mm (1.45%) for each unit increase in ECw. Dias *et al.* (2020) reported that when cotton plants were exposed to irrigation with saline water, there was also a reduction in stem diameter.

It can be seen in Figure 1C that the PSPA value was 76.05 g with the 0.3 dS m⁻¹ irrigation water; on the other hand, with the use of 1.5 dS m⁻¹ water there was a significant decrease in PSPA with an average of 55.16 g; with the 3.0 dS m⁻¹ water an average of 52.91 g was found and with the 4.5 dS m⁻¹ water a value of 52.54 g was observed. PSPA decreased by approximately 20.89 g when comparing the first level of salinity (76.05 g; 0.3 dS m⁻¹) with the second level (55.16 g; 1.5 dS m⁻¹). Divergent results were found by Karaki *et al.* (2023), working with lettuce, who made foliar applications of a biostimulant with amino acids to the plants and achieved positive effects on plant height, dry and fresh weights, and leaf chlorophyll content.

For the NF, it was observed that when the plants were irrigated with water with an ECw of 0.3 dS m⁻¹, there was an average of 8.15 fruits per plant; with irrigation water with an EC of 1.5 dS m⁻¹, there was a decrease to 6.51 fruits and 5.1 fruits with water of 3.0 dS m⁻¹. And 4.09 fruits per plant for water with a salinity of 4.5 dS m⁻¹, there was a reduction in NF as the salinity of the irrigation water increased (Figure 1D). The results of Silva *et al.* (2020) corroborate the results of the present study, where the authors also reported a reduction in the number of chili fruits with the increase in salts in the irrigation water.

Fruit weight (PF), transverse diameter (DT), and longitudinal length (LL) were significant ($p < 0.01$) for the ECw levels used; these variables were influenced by the salinity of the irrigation water. On the other hand, the soluble solids (SS) content was not influenced by the salinity of the irrigation water (Table 2).

The variables analyzed showed no significant difference between the application of rutin and the salinity-rutin interaction. This confirms that there was no effect of the doses of rutin, showing that there was no attenuation of the effect of salinity on yellow bell pepper plants by the doses of rutin. However, the literature states that plant biostimulants are highly beneficial for plants, as they confer greater tolerance to stress and increase productivity. This is closely linked to their ability to influence metabolic, cellular, and physiological processes in plants (Omoarelojie *et al.*, 2021).

Table 2. Summary of the analysis of variance for the post-harvest variables: soluble solids (SS), fruit weight (PF), longitudinal length (LL), and transverse diameter (DT) of yellow bell pepper fruit subjected to different levels of electrical conductivity of the irrigation water (ECw) and doses of the biostimulant rutin in a protected environment.

SV	Mean Square				
	DF	SS	PF	LL	DT
Repetition	3	1.53 ^{ns}	1276.95 ^{ns}	27.61 ^{ns}	266.61 ^{ns}
Salinity	3	0.74 ^{ns}	2709.92 ^{**}	349.09 ^{**}	309.02 ^{**}
Error 1	9	0.51	372.76	15.05	29.36
Rutin	3	0.29 ^{ns}	280.53 ^{ns}	33.01 ^{ns}	18.48 ^{ns}
S x R	9	0.14 ^{ns}	163.84 ^{ns}	13.13 ^{ns}	32.18 ^{ns}
Error 2	36	0.20	176.02	27.86	42.19
CV1 (%)			21.74	5.65	7.77
CV2 (%)			14.94	7.69	9.32

SV- Source of variation; DF- Degree of freedom; S x R- Interaction between rutin and salinity; CV- Coefficient of variation; * and ** - significant by F test at 5 and 1%, respectively; ^{ns} - not significant by F test at 5%.

Figure 2A shows that the longitudinal length and transverse diameter (Figure 2B) showed a linear decrease in both variables as the salinity of the irrigation water increased, following the same behavior for fruit weight (Figure 2C).

Oliveira *et al.* (2023). working with cucumber using four levels of electrical conductivity of the nutrient solution (ECns) 2.1; 3.6; 5.1 and 6.6 dS m⁻¹ concluded that the electrical conductivity of the nutrient solution negatively affected the length and diameter of cucumber fruit with reductions of 3.78% in the average fruit length and 3.76% in the average fruit diameter.

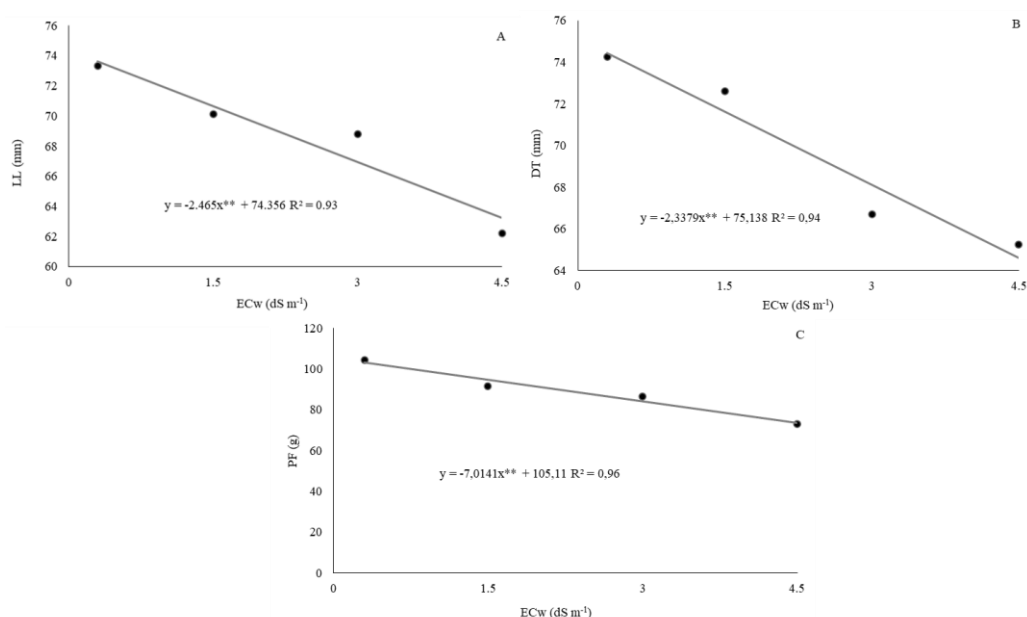


Figure 2. Averages the variables: longitudinal length – LL (A) transverse diameter – DT (B) and fruit weight – PF (C) of yellow bell pepper as a function of the levels of electrical conductivity of the water used for irrigation.

There was a linear decrease in the fruit weight (PF) of the yellow bell pepper plants. It was observed that the highest PF was 104 g when irrigated with good quality water (0.3 dS m⁻¹) and 72.88 g when irrigated with water of higher salinity (4.5 dS m⁻¹) there was a reduction of approximately 30.16 g. Showing that fruit weight was negatively influenced by salinity from 1.5 dS m⁻¹ onwards the PF of yellow peppers decreases with saline stress (Figure 2C).

Physiological variables such as stomatal conductance (gs), transpiration (E), net photosynthesis (A), and leaf water potential ($\square$ w) were not influenced by salinity. Only the internal CO₂ concentration variable was significant for salinity alone (Figure 3).

As for the salinity versus rutin interaction, there was also no significant effect on the physiological variables (Ci, E, gs, A, and $\square$ w). Abiotic-induced stress affects the phytohormonal balance in plants, which can affect stress adaptation mechanisms such as stomatal closure and carbon partitioning (Moyo *et al.* 2021).

Table 3. Summary of the analysis of variance for the physiological variables: internal CO₂ concentration (Ci), transpiration (E), stomatal conductance (gs), net photosynthesis (A), and leaf water potential (Ψ_w) of yellow bell pepper plants subjected to different levels of electrical conductivity of the irrigation water (ECw) and doses of the biostimulant rutin in a protected environment.

SV	DF	Mean Square				
		Ci	E	gs	A	Ψ _w
Repetition	3	0.03 ^{ns}	144.5 ^{ns}	0.61 ^{ns}	0.011 ^{ns}	0.11 ^{ns}
Sanity	3	0.36 [*]	405.08 ^{ns}	0.21 ^{ns}	0.008 ^{ns}	4.81 ^{ns}
Error 1	3	0.03	148.58	0.3	0.013	1.86
Rutin	3	0.11	1393.75 ^{ns}	0.42 ^{ns}	0.03 ^{ns}	1.57 ^{ns}
S x R	9	0.05 ^{ns}	1010.72 ^{ns}	0.49 ^{ns}	0.04 ^{ns}	1.78 ^{ns}
Error 2	12	0.28	880.22	0.77	0.021	1.73
CV 1 (%)		0.02	5.64	15.39	35.04	19.22
CV 2 (%)		0.06	13.74	24.47	44.03	18.57

SV- Source of variation; DF- Degree of freedom; S x R - Interaction between rutin and salinity; CV - Coefficient of variation; ** and * - significant by F test at 5 and 1%. respectively; ^{ns} - not significant by F test at 5%.

There was a linear increase in the internal CO₂ concentration as the ECw used in irrigation increased (Figure 3). The first phenomenon that occurs in plants under saline stress is stomatal closure caused by a decrease in the osmotic potential of the water in the soil which makes it difficult for the roots to absorb water. Thus, stomatal closure ensures less water loss to the atmosphere, but also becomes a physical barrier to the entry of CO₂ into mesophyll cells, reducing carboxylation rates to the lowest level (Branco *et al.*, 2020).

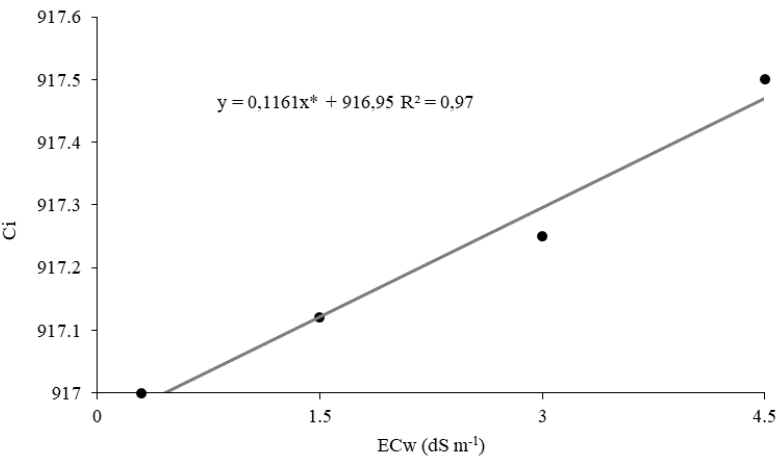


Figure 3. Internal CO₂ concentration of yellow peppers as a function of the electrical conductivity of the irrigation water (ECw).

The results found in the present study corroborate Veloso *et al.* (2021), these authors also observed that the internal CO₂ concentration (Ci) in bell pepper plants increased with saline stress by 6.50% per unit increase in the EC of the irrigation water. Lima *et al.* (2020), studying the effect of salinity on passion fruit plants, also found an increase in the internal CO₂ concentration (Ci) variable.

Tabela 4. Equações de regressão resultantes das interações da salinidade.

	$y = -3.4836x^{**} + 64.127$	PF	$y = -7.0141x^{**} + 105.11$	Ci	$y = 0.1161x^{*} + 916.95$
AL	$R^2 = 0.9648$		$R^2 = 0.9651$		$R^2 = 0.9759$
	CV1=7.4%		CV1=21.74		CV1=0.02%
	CV2=7.99%		CV2=14.94%		CV2=0.06%
DC	$y = -0.2385x^{**} + 13.73$	DL	$y = -2.465x + 74.356$		
	$R^2 = 0.7281$		$R^2 = 0.9258$		
	CV1=11.7 %		CV1=5.65%		
	CV2=6.89 %		CV2=7.69%		

	$y = -4.9681x + 70.716$	DT	$y = -2.3379x^{**} + 75.138$
PSPA	$R^2 = 0.6404$		$R^2 = 0.9402$
	CV1=19.6%		CV1=7.77%
	CV2=17.55%		CV2=9.32%
	$y = -0.9567^{**}x + 8.1869$		
NF	$R^2 = 0.9774$		
	CV1=30.85%		
	CV2=18.19%		

Conclusions

1. The doses of the biostimulant rutin did not mitigate the effects of the salts on the Rialto hybrid yellow bell pepper plants, further studies with higher doses of rutin are needed to verify its efficiency.
2. The salts caused an accentuated unevenness in the bell pepper plants, implying a reduction in the growth variables of the Rialto yellow bell pepper plants.
3. The salts had no negative effects on the gas exchange variables, except for the internal CO₂ concentration inside the protected environment.
4. From the salinity of 1.5 dS m⁻¹ in the irrigation water, the yellow bell pepper plants decrease linearly in terms of growth and post-harvest variables

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