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Combined effects of salinity and *Trichoderma harzianum* on cucumber (*Cucumis sativus* L.) growth

Renato V. da S. Filho[1], Rosa. H. de Almeida[1] and Antônio H. C. do Nascimento[1]

1 Federal Rural University of Pernambuco, Serra Talhada - PE, Brazil

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Abstract

In the Northeast of Brazil, cucumber production is affected due to low rainfall and irrigation waters containing high salt levels, which causes nutritional imbalance due to changes in the absorption, transport, assimilation and distribution of nutrients in the plant. Biological techniques aiming to increase the tolerance of plants to salinity, have been gaining importance, as the use of biostimulants, because it can increase the growth and the vegetal development stimulating the cell division, being able to increase the absorption of water and nutrients by the plants because of its ability to stimulate root growth and thereby attenuating the deleterious effect of salinity on this crop. However, there is little scientific dissemination in this area justifying the development of the present research. Thus, this study aimed to analyze the combined effects of salinity and the *Trichorderma harzianum* fungus on cucumber growth. The experiment was carried out in a greenhouse of UFRPE / UAST, Serra Talhada / PE, in a randomized block design with five levels of salinity (Treatment; T0: 0.02; T1: 1.2; T2: 1.4; T3: 2.0 ; T4: 2.4 dS m^{-1}), three below and two above the threshold salinity of the 2.2 dS m^{-1} culture, with the application of a commercial T-based product based on *T. harzianum* base 15 and 30 days after the start of irrigation. Leaf number, leaf area, main stem length, number of flowers, number, diameter and length of the fruits, as well as root characteristics such as volume, surface area, length, dry and moist root mass were evaluated. The data allow us to infer that there is influence of salinity on cucumber crop. Biometric responses were directly influenced by the interaction of salinity and fungus, so that with the fungus-based product, the cucumber responded positively to salinity.

Key-words: *Cucumis sativus*, Biostimulant, Saline Water

Introduction

Cucumber is of great economic and social importance within the vegetable agribusiness in Brazil and has an annual production that exceeds 200,000 tons. In the domestic market, the commercialization of cucumber has grown in importance among vegetables, due to its appreciation as a salad component (Silva et al., 2014). In addition to the economic and food importance, cucumber cultivation has social prominence, generating many direct and indirect jobs, from cultivation to commercialization (Carvalho et al., 2013).

Cucurbitaceae production in Brazil is mainly found in semiarid regions, which present a great abundance of saline soils, due to the nature of the source material, the water deficit caused by the low rainfall and the high evaporation rate. Therefore the problem is aggravated on intensively cultivated land with the use of irrigation, in the poles of irrigated agriculture (Silva et al., 2011). According to Cavalcante et al. (2006) In this region, the water supply of the soil, which occurs by scarce and irregular rainfall and / or supplemental irrigation, coupled with the strong evaporative demand, imposes the use of water resources of restrictive quality to agricultural production. Thus, water quality often compromises the productive capacity of plants that are less tolerant to salt, especially when the soil does not have physical conditions for salt leaching and sufficient aeration for root expansion.

✉ Rosa. H. de Almeida
E-mail: honoratorh@gmail.com

According to Dias and Blanco (2010) the high saline concentration near the root zone is a stress factor for plants, reducing osmotic potential, retaining water and favoring the action of ions on the protoplasm. Under these conditions, the water is osmotically retained in saline solution, thus reducing the availability of water and reducing the availability of nutrients, leading to deficiency in cultivated plants.

Irrigation is one of the main technologies capable of bringing satisfactory results to the development, yield and quality of agricultural products. However, in addition to the amount of water available to plants, another key factor is related to water quality, especially the concentration of dissolved salts. (Oliveira et al., 2014). Thus, in the semiarid cucumber cultivation problems occur due to salinity in irrigation water, being necessary to identify tolerant cultivars to this condition. (Albuquerque et al., 2016) associated with technologies capable of attenuating the harmful effects of salt, as it is a culture considered moderately sensitive to salinity, presenting a salinity threshold of 2.2 dS m^{-1} (Ayers and Westcot, 1999), which is routinely exceeded by the salinity levels usually found in available irrigation waters, affecting the growth and yield of this crop (Medeiros et al., 2009; Santana et al., 2010). According to Albuquerque et al. (2014) the cucumber can be irrigated with saline water with electrical conductivity of up to 1.8 dSm^{-1} during its initial growth phase. He warns, however, that there may be a difference in response as a function of cultivars and with advancing plant age. About this subject, Santana et al. (2010) have found a negative effect of salinity of 0.1 dS m^{-1} in irrigation water on the redneck cucumber crop, where there is the highest commercial yield, below the tolerated level for the crop, thus demonstrating the high sensitivity of the crop to salinity.

To address or mitigate this problem, many techniques are used to improve salinity tolerance (Abraha and Yohannes, 2013). Among these, one that is gaining importance is the use of biostimulant and, depending on its composition, concentration and proportion of the substances, the bioregulator can increase growth and plant development by stimulating cell division and may also increase the absorption of water and nutrients by plants (Vieira and Castro, 2004) by its ability to stimulate the development of the root system of plants, whose production of substances produce roots with larger volume, change the pH near the rhizosphere, making the plants better nourish and less susceptible to water stress.

One of the most accepted approaches to reduce biotic and abiotic effects on plants is the use of plant growth promoting microorganisms (Saghafi et al., 2018) and among the microorganisms used for this purpose are fungi of the genus *Trichoderma*, capable of colonizing root surfaces and causing substantial changes in plant metabolism, promoting the stimulation of plant defense mechanisms, increased nutrient availability and stress tolerance as stated by Ousley et al. (1994) and Weeden et al. (2008), have being among the most studied and marketed fungi as biofertilizers and soil inoculants, with a growing number of new products registered regularly worldwide (EMBRAPA, 2012). This is because *Trichoderma* spp., Known mainly as biocontrol fungi found in desert, forest and agricultural soils, can colonize plant roots and produce some metabolites by stimulating plant growth (Khoshmanzar et al., 2019).

Studying the use of *Trichoderma* corn seed conditioning, Yesilyurt et al (2018) observed that the treatment improved root and shoot growth system of plants submitted to 50 mM and 100 mM NaCl, suggesting that the fungus has capacity of osmoregulation in situations of high saline levels. Rawat et al. (2012) studying salinity-treated *Trichoderma* strains observed positive responses to salinity attenuation, verifying reduction of detrimental effects of salinity on growth, photosynthetic and biochemical parameters in rice. (Rawat et al., 2012). According to this study, *Trichoderma* pretreated plants responded to salinity stress by modulating physiological and biochemical parameters that lead to restoration of cellular homeostasis, toxin detoxification and growth recovery.

Studies by Harman (2006) also found that incorporating *Trichoderma* enhances deep root growth system, which helps in acquiring more water and absorbing nutrients. According to Weeden et al. (2008) by colonizing the rhizosphere of the root system the fungus produces hormones that promote substantial changes in metabolism in addition to altering the pH of the rhizosphere. This enables plants to form a larger root volume and thus a particular strain of the fungus can increase the number of roots at depth, up to one meter below the soil surface, which allows for greater nutrient absorption. *Trichoderma*

can be applied to seeds, substrate, planting furrows or organic matter that will be incorporated before transplanting Lucon (2009) cited by Machado et al. (2012), however, in Brazil, the applicability of the fungus as a growth promoter through the induction of resistance to salt stress is still little known, leading to the need for such research.

Based on this, this study aims to evaluate the combined effects of salinity and the fungus *Trichorderma harzianum* on cucumber growth and development.

Material and methods

The experiment was carried out in the experimental area of UFRPE / UAST, in the municipality of Serra Talhada / PE, whose geographic coordinates in the SIRGAS 2000 system are 7 ° 57'10 ”south latitude and 38 ° 17'43” west longitude, in the Sertão Pernambucano Mesoregion, Pajeú Microregion, at an elevation of 429 meters. According to KOOPPEN (1948), the climate is very hot and semi-arid BSw'h 'type, with the coldest month temperature exceeding 18 ° C and summer-autumn rainfall. The rainy season begins in November, ending in April. The average annual precipitation is 639 mm and the annual average temperature around 25.2 ºC (LAMEPE/ITEP, 2017).

Sowing of the Aodai cucumber variety was carried out directly in pots with a capacity of 20 liters, containing sieved soil which is classified as Cambisol, humus and sand (ratio 5: 3: 2). Three seeds were placed in the sowing per pot, leaving only the most vigorous at the time of thinning when it presented the second true leaf. The plants presenting the third true leaf were vertically tutored by means of a string. The fungus was applied twice: 15 and 30 days after the beginning of irrigation. The application of the product (ECOTRICH wp), which contains 1x1010 CFU / g of the commercial product, recommending to use 0.15 kg ha^{-1}, The volume of solution to be used was in accordance with the total volume of water applied in the day's irrigation, besides the aid of the manual of the Biological Institute of São Paulo, where it presents greater diversity of cultures to be worked with the fungus.

The fertilization was carried out based on the Pernambuco State Corrective and Fertilizer Recommendation Bulletin (IPA, 2008), aiming to meet the need per hectare for cucumber cultivation, using as a source of NPK the commercial formulation 10-10- 10 (3.72 g per pot), available in local trade, considering the pot area of 0.062 m2.

Table 1 below shows the chemical characterization of the soil after the analysis, allowing a diagnosis enabling future decision making.

Table 1. Chemical characterization of the substrate used in the experiment.

pH	K	Na	Al	Ca	Mg	H+Al	SB	CTC
	$cmol_c\ dm^{-3}$							
7.1	2.30	0.30	0.00	8.60	5.60	0.00	16.80	16.80

Fe	Cu	Zn	Mn	P	V	C	m	M.O.
$mg\ dm^{-3}$					(%)			
303.0	0.6	14.4	44.00	520.00	100	1.93	0.00	3.33

The experiment was carried out in a randomized block design with five levels of salinity (Treatment/T0: 0,02; T1: 1,2; T2: 1,4; T3: 2,0 ; T4: 2,4 dS m^{-1}), three below and two above the threshold salinity of the crop 2.2 dS m^{-1} (Ayers and Westcot, 1999), with application of a commercial product based on *Trichorderma harzianum*, only in treatments T1, T2, T3, T4 totaling twenty experimental units. The conductivity of the irrigation water was obtained by diluting the salt with a minimum content of 99.0% sodium chloride (NaCl) PA in water from the Supply Company, using the methodology proposed by Gheyi et al. (2016), according to Equation 1.

SDT = CEa x 640 (01),

If: 0,1 < CEa < 5,0 dS m^{-1}. Where: SDT = Total dissolved salts (mg L^{-1}); CEa = Electrical conductivity of water (dS m^{-1}).

Irrigation management was performed daily, calculated based on crop evapotranspiration (ETc), which, according to Equation 2, determined by Allen et al. (1998), The reference evapotranspiration (ETo) obtained by the Class A Tank (close to the experiment) and the culture coefficient (Kc) equal to 1.20 (phase I - Initial at 19 DAE) were used; 1.30 (phase II - development at 39 DAE); 1.15 (phase III - Final at 50 DAE), totaling a total applied final water levels of 8.16 mm per experimental unit.

ETc = ETo x Kc (02),

In which: Etc - Culture Evapotranspiration (mm day^{-1}); Eto - Reference Evapotranspiration (mm day^{-1}); Kc – Culture Coefficient (dimensionless).

For the estimation of the ETo we chose the class A tank method (TCA) –

Equation 3:ET_O = ECA x Kp (03),

In which: ET_O - Reference Evapotranspiration by Class A Tank (mm d^{-1}); ECA - Tank Evaporation Class A (mm d^{-1}); Kp – Tank Coefficient (dimensionless).

Os Daily Kp values were determined by Allen and Pruitt (1991) methodology presented in Equation (4).

"Kp "="0,108 - 0,000331U "+" 0,0422Ln"(F) +"
0,1434Ln"(H)" -0,000631"["Ln"(F)]^2 "Ln"(H)

where: U - wind speed at 2,0 m from the ground in km d-1; F - distance from the border surrounding the tank, considering 10.0 m of grass, in the present work; H - is the relative average humidity (%).

Relative air humidity and wind speed data are collected daily by the automated agrometeorological station located at UFRPE / UAST through the INMET website (Figure 7), taking care to convert the wind speed data from 10 meters above ground to 2 meters through equation 5.

$$Uz = U_{10}\frac{4,27}{\ln\left[(67,8xALT)-5,42\right]} \quad (05)$$

where: Uz - Wind speed at 2m, in km d^{-1}; U10 - Wind speed at 10m, in km d^{-1}; ALT - Site Altitude, in m.

The water levels were applied with the aid of buckets and 500 mL graduated beaker. To evaluate the effects of fungal interaction and salinity on cucumber culture, biometric data were collected at the end of the experiment. Regarding biometrics, the number of leaves, leaf area, length of the main stem, number of flowers, number, diameter and length of the fruits, besides the volume, surface area, length, dry and moist mass of the roots were verified. For the number of leaves it was determined that the leaves were fully expanded, with a minimum length of two centimeters and with at least 50% of their area with green color, as shown Grimes and Carter (1969).

Data were collected weekly for leaf number, stem diameter and main stem length, extending to the fruiting phase, where five readings were taken, the first one before the fungus application (*Trichoderma harzianum*). Leaf area, volume, surface area and root length were obtained at the end of the experiment.

The diameter of the vest was obtained with the aid of a caliper (in mm) two centimeters above the ground. The length of the main stem comprised the distance from the base to the apical meristem, obtained with a string, which was later related to a graduated ruler. The leaf area was determined using the Lafore program, where at the end of the experiment all leaves of each experimental unit were removed, scanned and converted to JPG format and submitted to the mentioned program. Root data were obtained using Safira software, with a similar procedure to the one that has been done with the leaves.

To obtain the dry biomass, the roots were separated, washed and packed in paper bags and dried in an oven with forced air circulation, kept at 80ºC for a period of 24 hours (Nakagawa, 1999). After this period, each repetition had the mass evaluated on a scale with accuracy of 0.001g.

The determination of the electrical conductivity of the soil saturation extract (ECse) was taken from substrate samples (mixture) at the beginning and end of the experiment. In the laboratory these samples went through a procedure, basically consisting of mixing air-dried fine earth (ADFE) with distilled water to obtain the “saturated paste”. This slurry was then placed on a Büchner funnel with filter paper, kitassate and vacuum pumps for suction application and the extract collected. The determination of ECse was performed soon after obtaining the extract by means of a TDS, thus performing the transformation of the values obtained in PPM to dSm^{-1}, whose results are presented in Table 2.

Table 2. Electrical conductivity values of substrate saturation extracts used in the experiment.

Levels applied (dSm^{-1})	Initial condition (substrate)	0.02	1.2	1.4	2.0	2.4
ECse (dSm^{-1})	0.36	0.67	1.89	2.24	2.77	3.38

Quantitative data were subjected to regression analysis seeking to adjust equations with plausible meanings to the treatments used. In the regression analysis, the best fit equations were chosen based on the significance of the regression coefficients at 1% (**) and 5% (*) probability by the F test and the

highest determination coefficient (R2). For this, the software Excel and Sisvar 5.6 were used.

Results and discussion

In Figure 1 we can observe the mean tests for root volume and number of leaves, after performing the analysis of variance at 5% probability by the F test, thus finding that there was a significant difference in the variables studied. There was an increase in root volume with increasing salinity of irrigation water (Figure 1A). Regarding the number of fruits, the treatment with higher EC in the irrigation water presented a lower fruit yield (Figure 1B).

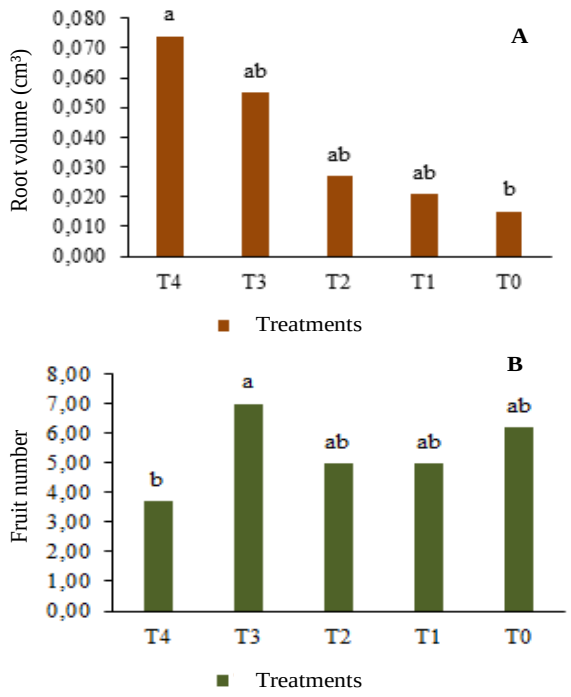

Fig. 1. Mean root volume (A) and number of cucumber fruits (B) obtained by the use of fungo *T. harzianum* fungus in association with salinity. Serra Talhada-PE. * Different lowercase letters in columns represent different averages at 0.05 probability by Tukey test.

The presence of *Trichoderma sp*. in the rhizosphere or rhizoplane region, colonizing this organ endophytically may have contributed to the release of growth promoting substances. Carvalho Filho et al. (2008) have found significant levels of IAA concentration in *T. harzianum* isolate culture filtrate. Weeden et al. (2008) have found in their studies that a particular strain of fungus demonstrated an increase in root numbers to a depth of one meter below the soil surface. In Figure 1B it can be observed that the irrigation with the use of water 2.0 dS m^{-1} the crop presented higher number of fruits, although it did not differ from the treatments 0.02, 1.2 and 1.4 dS m^{-1} statistically, which did not differ from the treatment of 2.4 dSm^{-1}, despite the fact that it had a smaller number of fruits, this data corroborates the variable number of flowers, a result already expected due to the higher salt accumulation. Ghanem et al. (2009) reported the largest flower abortion is due to reduced transport of soluble carbohydrates from leaves to flowers, reducing pollen viability, yet according to the author the effect of saline stress on flower abortion is more evident when it is applied at the beginning of flowering.

Figure 2 shows that when irrigated with 2.0 dS m^{-1} water, the culture presented higher final leaf area, followed by treatments 0.02, 1.2 and 1.4 dS m^{-1} that did not differ statistically, the 2.4 dS m^{-1} EC treatment presented the lowest number of final leaf area.

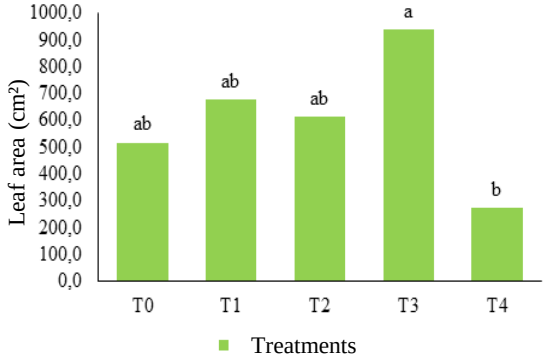

Fig. 2. Leaf area averages obtained by the use of *T. harzianum* fungus in association with salinity.

According to Munns (2002) and Lacerda et al. (2003) Reductions in leaf area size are associated with osmotic, toxic and nutritional effects of salt stress, which interfere with net CO2 assimilation and inhibit leaf expansion and accelerate senescence of mature leaves, thus reducing the area destined the photosynthetic process and the total production of photoassimilates. As an explanation for the highlighting of the 2.0 dS m^{-1} EC submitted crop, it can be suggested that the plants under these conditions had a smaller fall of the larger leaves, resulting in this value, since the leaf area was only determined at the end of the experiment.

In Figure 3 we verify the leaf number variable, thus showing the curve of the behavior of the crop by the different levels of salinity. Leaf growth increased to 1.4 dS m^{-1} level. In the variable is observed a

growth of the number of leaves until the level of 1.4 dS m^{-1}, with a decrease then this reduction in leaf number can be attributed to the higher amount of toxic ions in the plant, such as sodium which triggers a series of physiological, hormonal and nutritional disorders under the plant limiting its growth and development (Epistein and Bloom, 2006; Sá et al., 2013; Taiz and Zaiger et al., 2013).

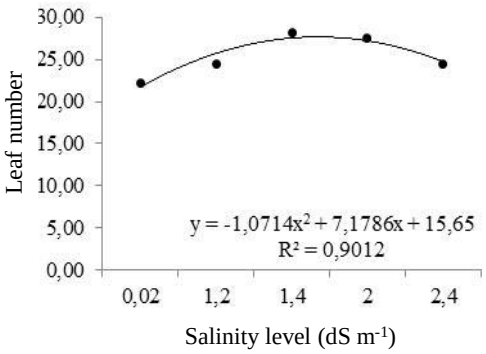

Fig. 3. Regression analysis of cucumber leaf number obtained by the use of *T. harzianum* fungus in association with salinity.

Figure 4 shows the values of root wet and dry mass as a function of irrigation water salinity. The behavior of the wet mass occurred so that the control presented a higher value, occurring a reduction of the mass with increase of the electrical conductivity of the water (Figure 4A). The root dry mass presented the inverse behavior of the dry mass with an increase of the biomass up to 1.4 dS m^{-1} (Figure 4B).

It can be observed in Figure 4 that the curve of the moist root mass (Figure 4A), presented greater value in the treatment, occurring a weight reduction with the increase of the electrical conductivity of the water, this characteristic happens due to the presence of salts in the soil solution causing a decrease of the external water potential (Epstein and Bloom, 2006). Thus, the addition of soluble salts in the soil solution increases the osmotic pressure and can reach a level where the plants will not have sufficient suction force to overcome the osmotic potential and thus the plant will not absorb water. This factor can also be explained by the fact that salinity treatments have larger root diameters than the control, so that thinner roots have greater water absorption efficiency.

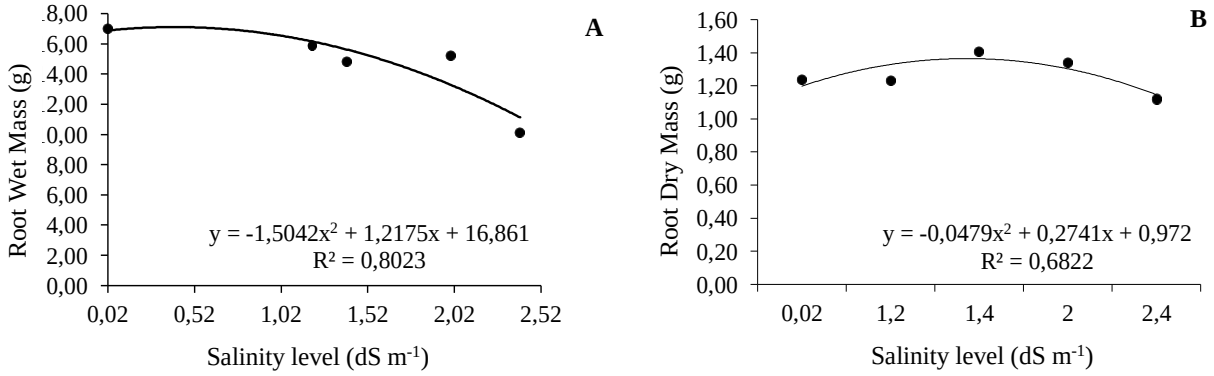

Fig. 4. Regression analysis for cucumber root mass (Figure 4A) and cucumber root dry mass (Figure 4B) obtained by the use of the fungus *T. harzianum* in association with salinity.

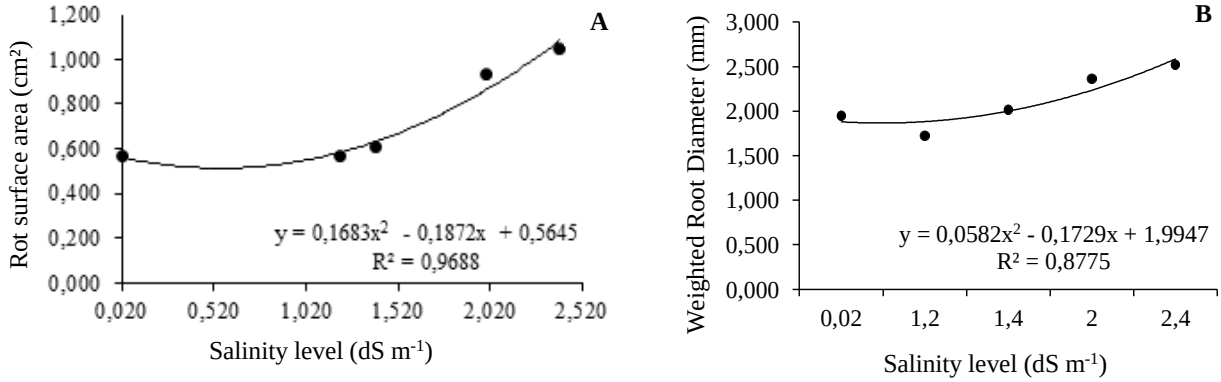

Fig. 5. Regression analysis of cucumber root surface area (cm^2) (A) and weighted root diameter (mm) (B) obtained by the use of the fungus *T. harzianum* in association with salinity. Serra Talhada-PE.

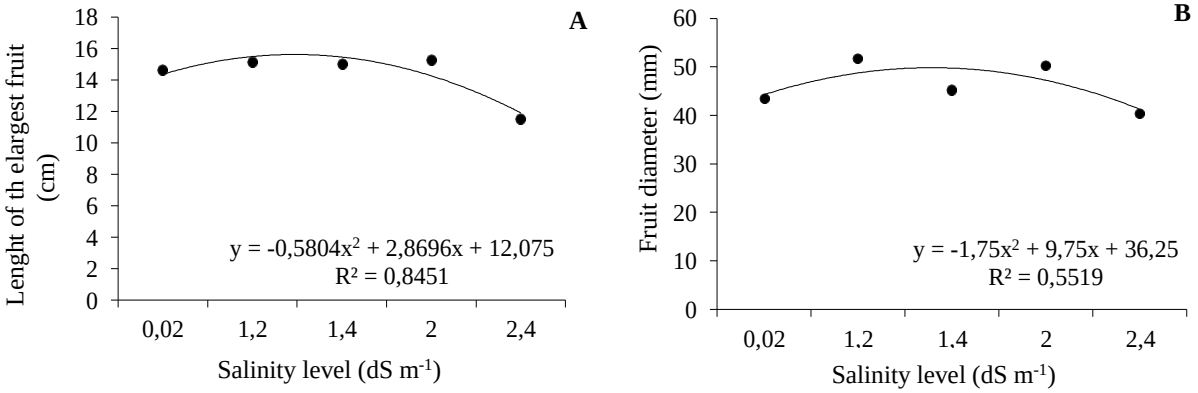

Fig. 6. Regression analysis of the length of the largest fruit (cm) (A) and fruit diameter (mm) (B) of cucumber obtained by the use of the fungus *T. harzianum* in association with salinity

The root dry mass (Figure 4B) presented an inverse behavior to the wet mass, where even with increase of the electric conductivity of the irrigation water there was an increase of the biomass up to 1.4 dS m^{-1} level and from that point on this decrease can be explained by the higher salt accumulation provided by the irrigation water. According to Willadino et al. (2011) the low growth and production of phytomass by glycophyte plants in general, including cucumber, in saline environment responds to nutritional imbalance and toxicity, which result in loss of respiration, root expansion, water absorption and CO_2 fixation. However, this consequence may have been attenuated due to the beneficial effects that occur in plant interactions with *Trichoderma* sp., so that colonizing root surfaces produces enzymes that cause changes in plant metabolism, in order to promote the stimulation of plants, plant defense mechanisms, increased nutrient availability and stress tolerance.

Figure 5 shows the behavior of root surface area curves (Figure 5A) and weighted root diameter (Figure 5B) as a function of different salinity levels. It is observed that the surface area and weighted diameter of the root exhibit a similar behavior, where there is a root growth in these variables with increasing salinity. This growth occurred probably due to the interaction of the plant-microorganism, which directly influenced its further development.

Figure 6 presents the biometric data of fruits at each salinity level of irrigation water. When analyzing the data of length (Figure 6A) and diameter of the largest fruit (Figure 6B), it can be observed that the treatment that was irrigated with water at the 2.4 dS m^{-1} level presented lower value for the two variables with values of 11.5 cm and 40.5 cm respectively. The other treatments presented similar values in relation to these two variables, although the treatment with salinity of 2.4 dS m^{-1} had the lowest values, but the fruit is within the market standards that are 15 cm long and 5 to 6 cm in diameter (EMBRAPA, 2013).

It is noteworthy that cucumber crop is classified as moderately sensitive to salinity (MAAS, 1984). According to Gomes et al. (2005), salinity affects many aspects of plant metabolism such as reductions in perspiration, photosynthesis, translocation, respiration, ionic and / or water imbalance, as well as toxic effects of Na^+ and Cl^- ions.

Conclusions

Biometric variables were directly influenced by the interaction of salinity and fungus, but treatments with *Trichoderma harzianum* product obtained positive responses to salinity.

Because it is a product for other purposes, some control measures should be taken into account, such as appropriate irrigation management, considering the addition of salts to the soil.

Conflict of interest: All authors declare no conflict of interest.

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