











## Production of zucchini seedlings in different substrates under salt stress<sup>1</sup>

### Produção de mudas de abobrinha sob estresse salino em diferentes substratos

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#### HIGHLIGHTS:

*Irrigation with brackish water (2.5 dS m<sup>-1</sup>) affects the emergence and development of zucchini seedlings.*

*The use of alternative substrate with castor bean cake, biochar and carbonized rice husk mitigates the deleterious effects of salts.*

*Alternative substrates benefit the emergence and development of zucchini seedlings.*

**ABSTRACT:** Salt stress affects the production of zucchini (*Cucurbita pepo* L.) seedlings. However, the use of alternative substrates can mitigate the deleterious effects caused by using brackish water in irrigation. The aim in this study was to evaluate the emergence and production of zucchini seedlings irrigated with brackish water in different substrates. The experiment was conducted at the Unidade de Produção de Mudas Auroras, belonging to the Universidade da Integração Internacional da Lusofonia Afro-Brasileira, Redenção, CE, Brazil. The experimental design was completely randomized, in a 4 × 2 factorial scheme, referring to four substrate compositions (SB1 = soil + sand + castor bean cake - 7:2:1; SB2 = soil + sand + biochar - 7:2:1; SB3 = soil + sand + carbonized rice husk - 7:2:1; and SB4 = soil) and two levels of electrical conductivity of the irrigation water (0.3 and 2.5 dS m<sup>-1</sup>), with four replicates, with 25 seeds per replicate. Salt stress reduced the emergence percentage, seedling height, root length, shoot, root, and total dry mass and increased the stem diameter of zucchini seedlings. The substrates formulated with castor bean cake, biochar and carbonized rice husk increased the emergence percentage, seedling height, stem diameter, and shoot dry mass of zucchini seedlings. The substrates with castor bean cake and biochar mitigated the effects of salt stress on the emergence speed index and Dickson quality index.

**Key words:** *Cucurbita pepo* L., salinity, emergence

**RESUMO:** O estresse salino afeta a produção de mudas de abobrinha (*Cucurbita pepo* L.). Porém, a utilização de substratos alternativos pode atenuar os efeitos deletérios ocasionados pelo uso de água salobra na irrigação. O objetivo deste estudo foi avaliar a emergência e a produção de mudas de abobrinha irrigada com água salobra em diferentes substratos. O experimento foi conduzido na Unidade de Produção de Mudas Auroras, pertencente à Universidade da Integração Internacional da Lusofonia Afro-Brasileira, Redenção, CE, Brasil. O delineamento experimental foi o inteiramente casualizado, em esquema fatorial 4 × 2, referente a quatro composições de substratos (SB1 = solo + areia + torta de mamona - 7:2:1; SB2 = solo + areia + biochar - 7:2:1; SB3 = solo + areia + casca de arroz carbonizado - 7:2:1; e SB4 = solo) e dois níveis de condutividade elétrica da água de irrigação (0.3 e 2.5 dS m<sup>-1</sup>), com quatro repetições de 25 sementes cada. O estresse salino reduziu a porcentagem de emergência, altura de plântulas, comprimento da raiz, massa seca da parte aérea, raiz, total e aumentou o diâmetro do caule das plântulas de abobrinha. Os substratos formulados com torta de mamona, biochar e casca de arroz carbonizada aumentaram a porcentagem de emergência, altura de plântulas, diâmetro do caule e a massa seca da parte aérea das plântulas de abobrinha. Os substratos com torta de mamona e biochar mitigaram os efeitos do estresse salino para o índice de velocidade de emergência e para o índice de qualidade de Dickson.

**Palavras-chave:** *Cucurbita pepo* L., salinidade, emergência

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## INTRODUCTION

Zucchini (*Cucurbita pepo* L. - Cucurbitaceae) is one of the main vegetables produced and consumed in Brazil. It is grown in tropical and even semi-arid regions, and is cultivated by small producers, with great potential for commercialization (Sousa et al., 2023; Semedo et al., 2024).

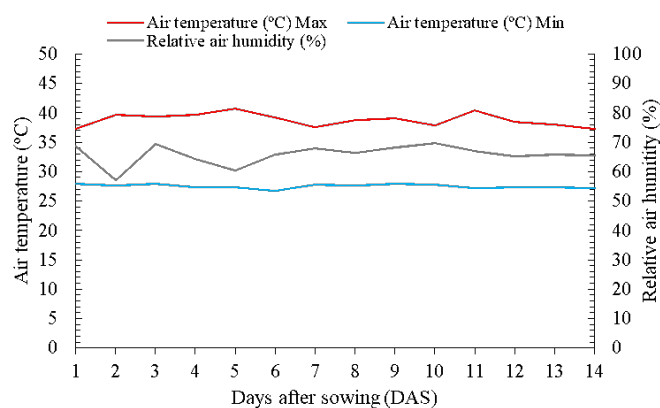
Seedling production is one of the most important stages for the success of the species, requiring the use of high-quality inputs for its cultivation, bearing in mind that in order to obtain a seedling with a high standard of quality, the choice of substrates to be used is extremely important, as well as their volume and proportion (Lessa et al., 2022; Monteiro Neto et al., 2024).

Various attributes and properties of the substrate can influence the growth and vegetative development of seedlings, such as structure, pH, aeration, water retention capacity and the degree of contamination by pathogens, as well as the availability of nutrients, oxygen, temperature and light and organic matter content (Sousa et al., 2023). In research carried out by Santos et al. (2023), it was found that carbonized rice husk and biochar were very efficient as alternative substrates in the production of zucchini seedlings.

Together with the use of substrates, irrigation is seen as one of the factors that directly influence the emergence process and the final quality of the seedlings. However, due to the scarcity of good-quality water, farmers in the northeastern semi-arid region have been forced to use brackish water in agricultural production systems (Sousa et al., 2022; Lessa et al., 2023a; Semedo et al., 2024), which can result in the salinization of substrates, restricting the absorption of water and mineral nutrients by seedlings (Sousa et al., 2023). In view of the above, the aim in this study was to evaluate the emergence and production of zucchini seedlings irrigated with brackish water in different substrates.

## MATERIAL AND METHODS

The experiment was conducted in an arch-type greenhouse, covered with 150-micron agricultural diffusive plastic, with a galvanized steel structure and dimensions of 8 × 15 m, during October 2022 at the Unidade de Produção de Mudas Auroras (UPMA), belonging to the Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Auroras campus, Redenção, Ceará (4° 13' 86.33" S; 38° 43' 39" W, with altitude of 92 m), Brazil. The climate of the region is of the BSh' type, characterized as tropical rainy, with very high temperatures and predominant rainfall in the summer and autumn seasons (Alvares et al., 2013). Air temperature and



**Figure 1.** Mean values of maximum (Max) and minimum (Min) temperatures and relative air humidity observed during the experimental period

relative air humidity data were monitored by a Data logger (HOBO® U12-012 Temp/RH/Light/Ext) (Figure 1).

The experimental design used was completely randomized, in a 4 × 2 factorial scheme, referring to four substrate compositions (SB1 = soil + sand + castor bean cake - 7:2:1; SB2 = soil + sand + biochar - 7:2:1; SB3 = soil + sand + carbonized rice husk - 7:2:1; and SB4 = soil) and two levels of electrical conductivity of the irrigation water (0.3 and 2.5 dS m<sup>-1</sup>), based on a study conducted by Sousa et al. (2022), with four replicates, with 25 seeds per replicate.

The soil used to prepare the substrates was classified as Argissolo vermelho-amarelo (Ultisol - Soil Survey Staff, 2022). The samples of the substrates used in the experiment were sent to the Soil and Water Laboratory of the Soil Science Department of the Universidade Federal do Ceará (UFC) for analysis of the chemical attributes (Table 1), according to the methodology described by Teixeira et al. (2017).

The zucchini seeds were sown in polyethylene trays with 200 cells of 40 cm<sup>3</sup>, at a depth of 2 cm. The cultivar used was Caserta hybrid (Topseed®), bush zucchini, also known as Italian zucchini, which has bushy growth characteristics, measuring up to 90 cm in height, with flower and fruit formation on the stem.

The highest salinity water (2.5 dS m<sup>-1</sup>) was prepared using NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O and MgCl<sub>2</sub>·6H<sub>2</sub>O salts in an equivalent ratio of 7:2:1, following the relationship between EC<sub>w</sub> and the respective molar concentration (mmol<sub>c</sub> L<sup>-1</sup> = EC × 10) (Rhoades et al., 2000). The lowest salinity water (0.3 dS m<sup>-1</sup>) used for irrigation came from the local unit supply. The EC<sub>w</sub> was monitored periodically with a benchtop water tester (AZ® 806505 pH/Cond./TDS/Sal).

Irrigation was carried out manually daily, adhering to the method outlined by Marouelli & Braga (2016). Water

**Table 1.** Chemical characteristics of the substrates (SB1 = soil + sand + castor bean cake - 7:2:1; SB2 = soil + sand + biochar - 7:2:1; SB3 = soil + sand + carbonized rice husk - 7:2:1; SB4 = soil)

| Sub | OM<br>(g kg <sup>-1</sup> ) | N<br>(mg kg <sup>-1</sup> ) | P<br>(mg kg <sup>-1</sup> ) | Ca <sup>2+</sup> | K <sup>+</sup> | Mg <sup>2+</sup> | Na <sup>+</sup> | H <sup>+</sup> + Al <sup>3+</sup> | Al   | SB    | CEC   | V<br>(%) | ECse<br>(dS m <sup>-1</sup> ) | pH<br>H <sub>2</sub> O |
|-----|-----------------------------|-----------------------------|-----------------------------|------------------|----------------|------------------|-----------------|-----------------------------------|------|-------|-------|----------|-------------------------------|------------------------|
| SB1 | 22.03                       | 1.37                        | 83                          | 6.10             | 1.72           | 2.60             | 0.30            | 0.17                              | 0.00 | 10.72 | 10.89 | 98       | 2.14                          | 6.8                    |
| SB2 | 8.69                        | 0.51                        | 85                          | 2.50             | 0.51           | 1.60             | 0.18            | 0.66                              | 0.05 | 4.79  | 5.45  | 88       | 0.78                          | 7.1                    |
| SB3 | 10.55                       | 0.65                        | 78                          | 4.50             | 0.56           | 0.60             | 0.17            | 0.99                              | 0.05 | 5.83  | 6.82  | 85       | 0.76                          | 7.2                    |
| SB4 | 4.03                        | 0.24                        | 2                           | 2.50             | 0.06           | 0.30             | 0.57            | 0.33                              | 0.00 | 3.43  | 3.76  | 91       | 0.37                          | 7.6                    |

Sub - Substrates; OM - Organic matter; SB - Sum of bases (Ca<sup>2+</sup> Mg<sup>2+</sup> + Na<sup>+</sup> + K<sup>+</sup>); CEC - Cation exchange capacity - [(Ca<sup>2+</sup> Mg<sup>2+</sup> + Na<sup>+</sup> + K<sup>+</sup> + (H<sup>+</sup> + Al<sup>3+</sup>))]; V - Base saturation - (Ca<sup>2+</sup> Mg<sup>2+</sup> + Na<sup>+</sup> + K<sup>+</sup> / CEC) × 100; ECse - Electrical conductivity of the substrate saturation extract

was applied until it drained from the bottom of the trays, maintaining a consistent leaching depth of 8% (every three days).

One day after sowing, the number of emerged seedlings began to be counted, using the appearance of fully expanded cotyledonary leaves as a criterion until stabilization (14 days after sowing - DAS). During this period, the following variables were assessed: emergence percentage (EP), obtained by the ratio between the number of normal seedlings emerged and the number of seeds sown; emergence speed index (ESI), following the methodology recommended by Maguire (1962) through daily counts of emerged seedlings; and mean time of emergence (MTE), through daily counts of seeds, according to the methodology proposed by Labouriau (1983), with the result expressed in days.

At 14 DAS, the following growth variables were analyzed: seedling height (SH) using a ruler graduated in cm, measuring from the base to the apex of the seedling; stem diameter (SD) using a digital caliper graduated in mm, measuring close to the substrate; and root length (RL) also using a ruler graduated in cm.

To obtain the biomass, the seedlings were placed in paper bags, identified and dried in an oven at 65 °C for 72 hours until they reached a constant mass. Shoot dry mass (SDM) and root dry mass (RDM) were then assessed using an analytical balance and total dry mass was obtained by summing their values ( $TDM = SDM + RDM$ ), in g per seedling. Based on the data obtained in the previous evaluations, the quality of the seedlings was determined using the Dickson quality index (DQI) (Dickson et al., 1960), according to Eq. 1:

$$DQI = \frac{TDM}{\frac{SH}{SD} + \frac{SDM}{RDM}} \quad (1)$$

where:

DQI - Dickson quality index;  
TDM - total dry mass (g per seedling);  
SH - seedling height (cm);  
SD - stem diameter (mm);  
SDM - shoot dry mass (g per seedling); and,  
RDM - root dry mass (g per seedling).

The results were initially analyzed to determine the homogeneity of variance and subjected to the Kolmogorov-Smirnov test ( $p \leq 0.05$ ) to assess the normality of the data. After checking for normality, the data were subjected to analysis of variance and the means were compared by Tukey's test ( $p \leq 0.05$ ), using Assistat software, version 7.7 Beta (Silva & Azevedo, 2016).

## RESULTS AND DISCUSSION

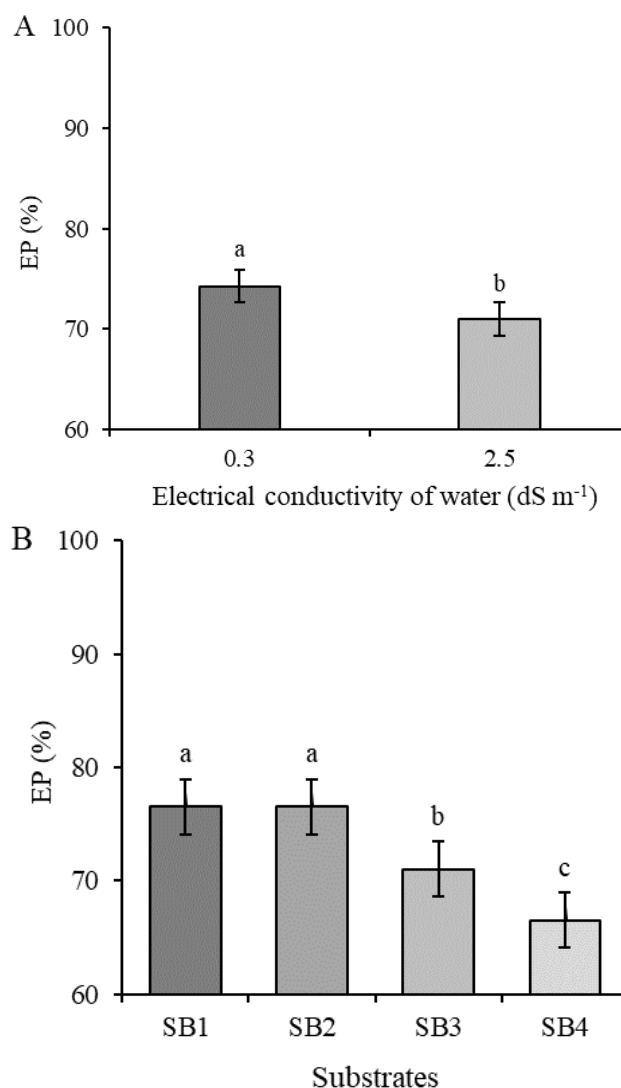
The analysis of variance showed that there was a significant interaction between the different substrates (SB) and the electrical conductivity of the irrigation water (ECw) for the variables emergence speed index ( $p \leq 0.01$ ) and mean time of emergence ( $p \leq 0.05$ ), while emergence percentage was individually affected by both factors ( $p \leq 0.01$ ) (Table 2).

**Table 2.** Summary of the analysis of variance for emergence percentage (EP), emergence speed index (ESI), and mean time of emergence (MTE) of zucchini seedlings subjected to two electrical conductivities of irrigation water (ECw) and different substrates (SB)

| Sources of variation | DF | Mean squares       |        |         |
|----------------------|----|--------------------|--------|---------|
|                      |    | EP                 | ESI    | MTE     |
| ECw                  | 1  | 105.62**           | 1.55** | 11.64** |
| SB                   | 3  | 233.95**           | 1.55** | 4.25**  |
| ECw × SB             | 3  | 3.95 <sup>ns</sup> | 0.25** | 0.59*   |
| Residual             | 32 | 4.06               | 0.03   | 0.13    |
| Total                | 39 | -                  | -      | -       |
| CV%                  | -  | 2.78               | 7.03   | 5.65    |

DF - Degrees of freedom; \* - Significant by the F test at  $p \leq 0.05$ ; \*\* - Significant by the F test at  $p \leq 0.01$ ; ns - Not significant; CV - Coefficient of variation

The emergence percentage of zucchini seedlings was relatively low under electrical conductivity of the irrigation water of 2.5 dS m<sup>-1</sup>, obtaining a value of 71%, and being inferior to the water of low salinity (ECw = 0.3 dS m<sup>-1</sup>), at which an emergence percentage of 74.25% was obtained (Figure 2A).



Means followed by different letters indicate significant differences by Tukey's test ( $p \leq 0.05$ ). Vertical bars represent the standard error of the mean ( $n = 4$ ). SB1 - Soil + sand + castor bean cake - 7:2:1; SB2 - Soil + sand + biochar - 7:2:1; SB3 - Soil + sand + carbonized rice husk - 7:2:1; and SB4 - Soil

**Figure 2.** Emergence percentage of zucchini seedlings as a function of the electrical conductivity of the irrigation water (A) and different substrates (B)

The high salt content in the substrate, especially due to the excess of  $\text{Na}^+$  and  $\text{Cl}^-$  ions, causes a decrease in the water potential of the medium due to the increase in osmotic potential, hindering the seed soaking mechanism, negatively affecting the processes of cell division and elongation and interfering with seedling emergence (Liu et al., 2023).

When assessing the production of zucchini seedlings under salt stress, Santos et al. (2023) also found a negative effect of salinity on the percentage of seedling emergence, leading to a reduction in EP as the EC<sub>w</sub> increased. Similarly, Sousa et al. (2023) also found a reduction in the emergence percentage of watermelon seedlings with the increase in salts in the irrigation water.

Regarding the influence of the substrates, the average values show that castor bean cake (SB1) and biochar (SB2) were superior to the other substrates for the emergence percentage of zucchini seedlings, both leading to 76.5% EP (Figure 2B). This result may be related to their ability to adsorb nutrients on their surface and release them into the substrate, particularly  $\text{Ca}^{2+}$ ,  $\text{K}^+$  and  $\text{Mg}^{2+}$ . These are essential for enzyme activation and biochemical processes in emergence. Calcium stabilizes cell membranes, potassium regulates turgidity and water transport, and magnesium supports energy needs during early development, which favors the physiological process of emergence and seedling establishment (Qin et al., 2020; Santos et al., 2023; Sousa et al., 2023).

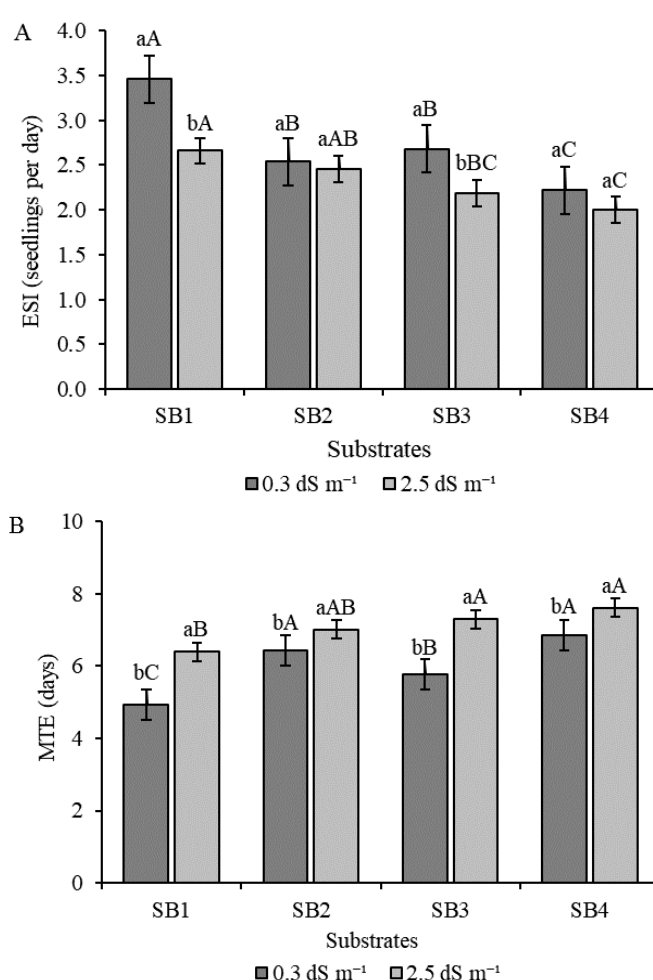
In agreement with the result found, Chrysargyris et al. (2020) observed a higher emergence percentage of *Lactuca sativa* L. seedlings with the use of biochar as a substrate.

The highest averages for ESI (Figure 3A) were obtained with the castor bean cake (SB1) regardless of the electrical conductivity ( $0.3 \text{ dS m}^{-1} = 3.46$  and  $2.5 \text{ dS m}^{-1} = 2.66$  seedlings per day) in the treatments with the low salinity water, while in the other substrates there was no significant difference between the EC<sub>w</sub> levels. The lowest average ESI was obtained in the treatment with soil (SB4) regardless of the electrical conductivity ( $0.3 \text{ dS m}^{-1} = 2.22$  and  $2.5 \text{ dS m}^{-1} = 2.00$  seedlings per day).

This result indicates that the addition of organic sources in the formulation of substrates in satisfactory quantities, in addition to favoring the emergence and proper development of seedlings, can also act to mitigate salt stress (Sousa et al., 2023). Thus, the lower amount of organic matter in SB4, combined with the effect of salt stress, reduced the absorption of water and nutrients by the seed, delaying the emergence physiological process.

Contrary to this result, Santos et al. (2023), when studying the influence of substrates (carbonized rice husk and biochar) on the production of zucchini seedlings under salt stress, found higher values for the emergence speed index when seedlings were irrigated with an EC<sub>w</sub> of  $2.5 \text{ dS m}^{-1}$ . Silva Junior et al. (2020) also found a higher emergence speed index for watermelon seedlings growing with biochar as a substrate under EC<sub>w</sub> of 0.8 and  $2.5 \text{ dS m}^{-1}$ .

The highest values for mean time of emergence were obtained with the water with the highest electrical conductivity in all the substrates, showing a significant difference compared to the water with the lowest salinity (Figure 3B).



The same lowercase letters comparing the mean values of electrical conductivity of the water in each substrate and the same uppercase letters comparing the mean values of the different substrates at the same level of electrical conductivity of the water do not differ statistically from each other by Tukey test at  $p \leq 0.05$ . Vertical bars represent the standard error of the mean ( $n = 4$ ). SB1 - Soil + sand + castor bean cake - 7:2:1; SB2 - Soil + sand + biochar - 7:2:1; SB3 - Soil + sand + carbonized rice husk - 7:2:1; and SB4 - Soil + sand + soil - 7:2:1.

**Figure 3.** Emergence speed index (A) and mean time of emergence (B) of zucchini seedlings as a function of the electrical conductivity of the irrigation water and different substrates

This effect is the result of the reduction in water absorption by the seeds due to the higher concentration of  $\text{Na}^+$  and  $\text{Cl}^-$  ions from the irrigation water in the substrates, causing a delay in the start of the metabolic processes that initiate germination and seedling emergence due to osmotic stress, affecting the seed soaking process and delaying the emergence process and time (Sousa et al., 2023). This effect was mainly enhanced under SB1, where the initial salinity of the substrate saturation extract was  $2.14 \text{ dS m}^{-1}$  (Table 1).

Similar to the results found in this study, Ó et al. (2020) also observed an increase in the mean time of emergence of mini watermelon seedlings when irrigated with higher salinity water (0.33, 1.5, 3.5, and  $5.5 \text{ dS m}^{-1}$ ). In contrast, Santos et al. (2023) found a longer mean time of emergence in zucchini seedlings when irrigated with lower salinity water in a substrate composed of biochar.

The variables seedling height ( $p \leq 0.01$ ), stem diameter ( $p \leq 0.01$ ), root length ( $p \leq 0.05$ ), and shoot dry mass ( $p \leq 0.05$ ) showed significant individual effects of the different substrates (SB) and electrical conductivity of the irrigation water (EC<sub>w</sub>)



**Table 3.** Summary of the analysis of variance for seedling height (SH), stem diameter (SD), root length (RL), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM) and Dickson quality index (DQI) of zucchini seedlings subjected to two electrical conductivities of the irrigation water (ECw) and different substrates (SB)

| Source of variation | DF | Mean square        |                    |                    |                     |                    |                    |                      |
|---------------------|----|--------------------|--------------------|--------------------|---------------------|--------------------|--------------------|----------------------|
|                     |    | SH                 | SD                 | RL                 | SDM                 | RDM                | TDM                | DQI                  |
| ECw                 | 1  | 258.36**           | 1.09**             | 20.05**            | 0.064**             | 0.26**             | 0.59**             | 0.0002 <sup>ns</sup> |
| SB                  | 3  | 27.37**            | 0.41**             | 2.78*              | 0.006*              | 0.01 <sup>ns</sup> | 0.02 <sup>ns</sup> | 0.0006 <sup>ns</sup> |
| ECw × SB            | 3  | 3.10 <sup>ns</sup> | 0.10 <sup>ns</sup> | 0.94 <sup>ns</sup> | 0.001 <sup>ns</sup> | 0.02 <sup>ns</sup> | 0.03 <sup>ns</sup> | 0.0029*              |
| Residual            | 32 | 1.13               | 0.08               | 0.67               | 0.001               | 0.01               | 0.01               | 0.0010               |
| Total               | 32 | -                  | -                  | -                  | -                   | -                  | -                  | -                    |
| CV (%)              |    | 8.58               | 11.00              | 12.63              | 9.01                | 25.90              | 13.18              | 19.88                |

DF - Degrees of freedom; \* - Significant by the F test at  $p \leq 0.05$ ; \*\* - Significant by the F test at  $p \leq 0.01$ ; ns - Not significant; CV - Coefficient of variation

( $p \leq 0.01$ ) (Table 3). Root dry mass and total dry mass were significantly affected only by ECw ( $p \leq 0.01$ ). Only the Dickson quality index was significantly affected by the interaction between SB and ECw ( $p \leq 0.05$ ).

The height of the zucchini seedlings was lower under the water with the higher electrical conductivity, with a reduction of approximately 33.95% compared to the water of low salinity (Figure 4A). This result can be attributed to the excess of saline ions in the substrate, causing changes in the osmotic potential of the medium, reducing the availability of water and mineral nutrients and subsequently causing ionic toxicity, which inhibits cell elongation and division, and consequently a reduction in seedling growth (Munns & Tester, 2008; Lessa et al., 2022).

Similar results regarding the negative effect of salt stress caused by excess salts in irrigation water on the initial growth of seedlings were reported by Freitas et al. (2024). These authors found reductions in the height of *Celosia argentea* L. seedlings with an increase in the electrical conductivity of the irrigation water from 0.5 to 4.5 dS m<sup>-1</sup> in a substrate containing organic compost, a 51.26% reduction in seedling height.

As for the influence of the substrates (Figure 4B), the highest mean values for seedling height were obtained with the substrates containing biochar (SB2) and carbonized rice husk (SB3), differing significantly from the other substrates and promoting an increase of 23.48 and 26.61% in seedling height compared to the substrates with castor bean cake (SB1) and no added organic material (SB4), respectively. This result can be explained by the fertility of the substrates, as the substrate with the lowest values (SB4) has a low level of nitrogen in its composition, compared to the higher ones, mainly SB1 and SB3 (Table 1), since this macronutrient acts directly on cell elongation and expansion. Santos et al. (2023) also found positive results from biochar as a substrate for zucchini seedling height, while Salé et al. (2021) found positive effects of carbonized rice husk on the height of *Ocimum basilicum* L. seedlings.

The stem diameter of the zucchini seedlings increased by 11.96% when they were irrigated with an electrical conductivity of 2.5 dS m<sup>-1</sup>, obtaining a value of 2.76 mm. On the other hand, in seedlings irrigated with lower salinity water, the stem diameter was 2.43 mm, which is less than that observed under salt stress (Figure 4C). This mechanism may be based on a change in biomass allocation, as under stress conditions plants favor structural and reproductive parts to ensure support and transport of water and nutrients, seeking homeostasis (Oliveira et al., 2022).

Corroborating this study, Oliveira et al. (2022) found an increase of 11.22% in the stem diameter of tomato seedlings with an increase in the electrical conductivity of the irrigation water from 0.2 to 3.0 dS m<sup>-1</sup>. Similarly, Santos et al. (2023) also found an increase in the stem diameter of zucchini seedlings as the salinity of the irrigation water increased from 0.8 to 2.5 dS m<sup>-1</sup>.

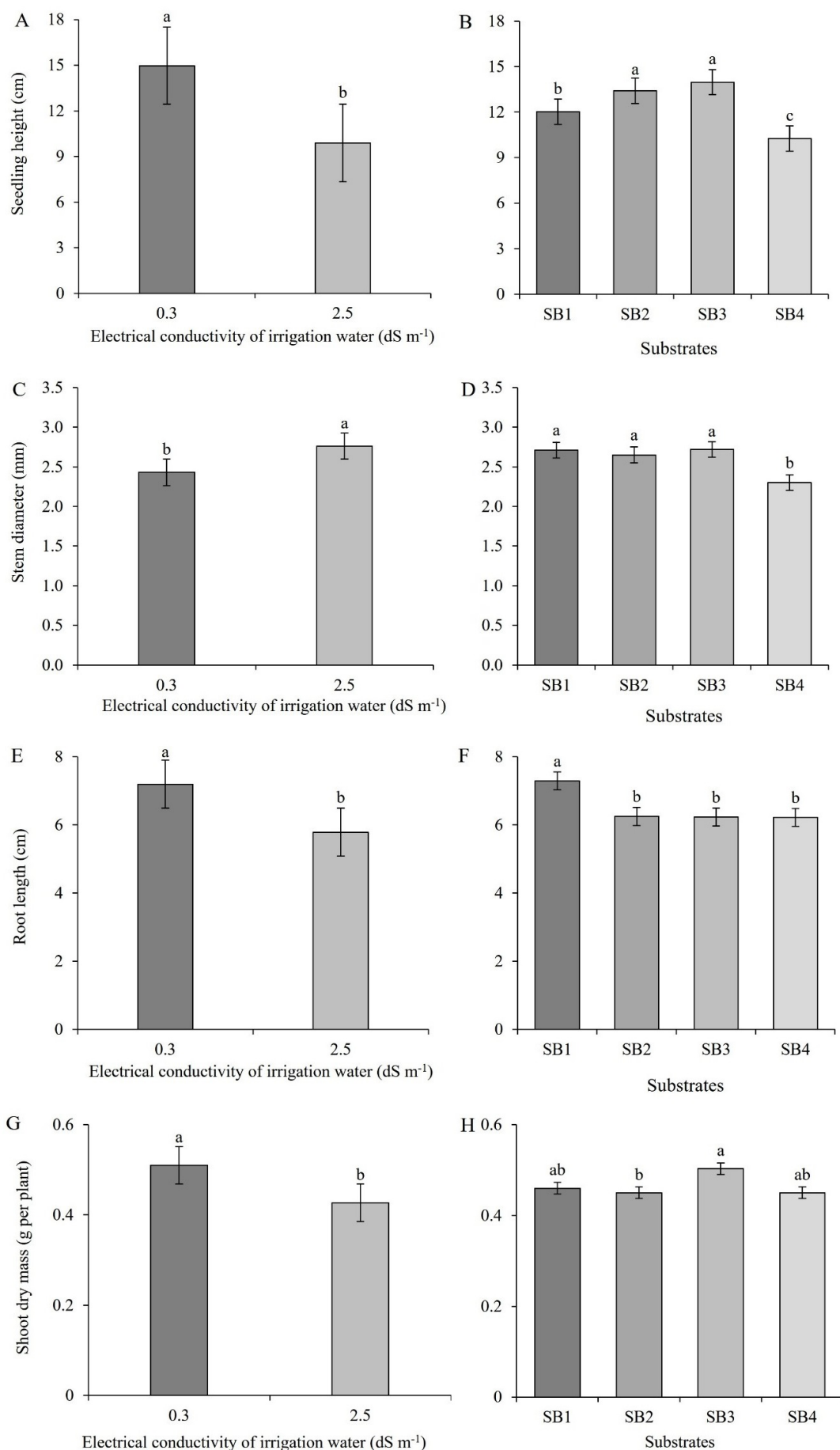
Seedlings grown in the substrates containing castor bean cake (SB1), biochar (SB2), and carbonized rice husk (SB3) had a larger stem diameter, with increases of 15.13, 13.21, and 15.44%, respectively, compared to the soil (SB4) (Figure 4D). The better performance of substrates containing organic sources indicates the ability of these materials to provide a high supply of nutrients, positively influencing better absorption efficiency and consequently greater diameter. Contrary to this result, Silva Junior et al. (2020) and Lessa et al. (2023b) found no influence of substrates based on biochar and carbonized rice husk on the stem diameter of passion fruit and watermelon seedlings, respectively.

The water with the lower salinity promoted the zucchini seedlings with the greatest root length (7.2 cm) and was statistically superior to the water with the higher electrical conductivity, which led to a value of 5.79 cm, a 17.11% reduction in root length (Figure 4E). In glycophyte species, the osmotic and ionic stress caused by salinity leads to excessive production of reactive oxygen species, resulting in oxidative damage to cell organelles and membranes, impairing the formation of root tissues and compromising root development (Hasanuzzaman et al., 2021).

A similar result regarding the negative effect of salt stress on seedling root length was reported by Lessa et al. (2022). These same authors found a reduction in the root length of yellow passion fruit seedlings with an increase in the electrical conductivity of the irrigation water from 0.3 to 3.0 dS m<sup>-1</sup>.

As for the influence of the substrates (Figure 4F), it was found that the greatest root length of zucchini seedlings was obtained in the substrate containing castor bean cake (SB1), differing significantly from the other substrates and promoting an increase of approximately 14% in root length. This result can be attributed to the high contents of organic matter and nitrogen, which provide nutritional input, influencing greater root growth (Santos et al., 2023). When studying the application of humic substances to tomato seedlings, Qin & Leskovar (2020) found a greater root length compared to the control treatment.

The shoot dry mass of the zucchini seedlings was lower under the water with the higher electrical conductivity, with



Means followed by different lowercase letters indicate significant differences by the Tukey test at  $p \leq 0.05$ . Vertical bars represent the standard error of the mean ( $n = 4$ ). SB1 - Soil + sand + castor bean cake - 7:2:1; SB2 - Soil + sand + biochar - 7:2:1; SB3 - Soil + sand + carbonized rice husk - 7:2:1; and SB4 - Soil

**Figure 4.** Seedling height, stem diameter, root length and shoot dry mass of zucchini seedlings as a function of the electrical conductivity of the irrigation water (A, C, E, and G) and different substrates (B, D, F, and H)

a reduction of 16.27% compared to the water with of low salinity (Figure 4G). High concentrations of salts in substrates cause cytotoxic effects on seedlings, leading to denaturation of proteins and destabilization of cell membranes, affecting the dynamics of nutrient absorption and reducing the growth and biomass of the aerial part (Oliveira et al., 2022; Freitas et al., 2024). Similarly, Tarchoun et al. (2022) also found a reduction in the shoot biomass of *Cucurbita maxima* Duchesne seedlings in response to an increase in the salt concentration of the irrigation water.

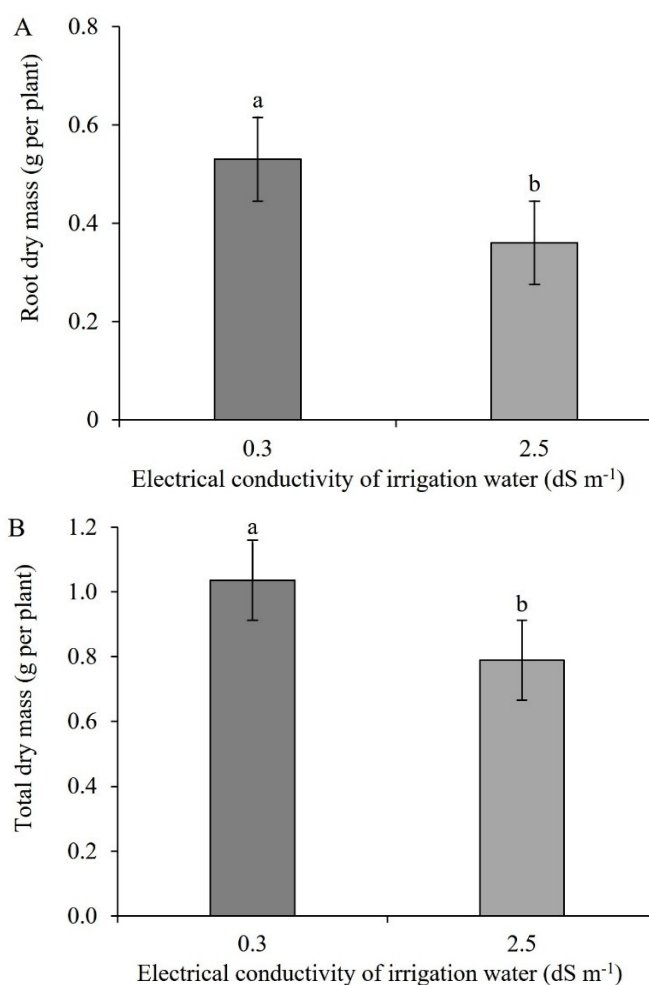
The substrates SB1, SB3 and SB4 were statistically superior to SB2, promoting greater shoot dry mass of zucchini seedlings (Figure 4H). This effect may be related to the chemical composition of the substrates (Table 1), which possibly increased the availability of nutrients (NPK) to the seedlings (Salé et al., 2021). Significant results of carbonized rice husk in the composition of substrates for seedling production were also reported by Watthier et al. (2017). These researchers found greater growth in the shoot dry mass of lettuce seedlings when they associated carbonized rice husk with organic tung compost.

Salt stress caused a 32.08% reduction in the root dry mass (0.36 g per seedling) of zucchini seedlings, compared to the control treatment, which obtained a RDM value of 0.53 g per seedling (Figure 5A). This reduction can be attributed to the excess of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in the substrates, damaging the cells of the root system and reducing nutrient absorption. Similarly, Sousa et al. (2023) also observed a reduction in root dry mass in watermelon seedlings as the salinity level of the irrigation water increased. Freitas et al. (2024) also reported a reduction in the root dry mass of *C. argentea* seedlings under salt stress.

The total dry mass of zucchini seedlings was negatively influenced by the water with the higher electrical conductivity, which caused a reduction of 23.82% compared to the water with the lower salinity (Figure 5B). This result shows that the high salt content in the irrigation water led to morphological and anatomical changes in the shoots and roots of the seedlings, due to the greater energy expenditure to absorb water and nutrients, resulting in lower dry mass accumulation by the zucchini seedlings. Similar to this study, Santos et al. (2023) also observed a decrease in total dry mass in zucchini seedlings as the electrical conductivity of the irrigation water increased from 0.8 to 2.5  $\text{dS m}^{-1}$ .

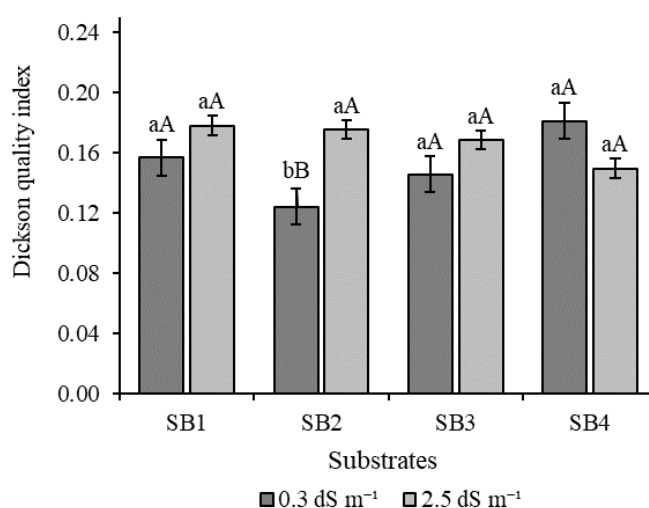
For the Dickson quality index of zucchini seedlings, the substrate composed of biochar (SB2) associated with the water with the higher EC<sub>w</sub> led to the highest average value (0.17), differing significantly from the water with the low salinity. However, there was no statistical difference between the two levels of electrical conductivity of the irrigation water for the other substrates (Figure 6). It should be noted that, at the higher EC<sub>w</sub>, the substrates did not show significant differences in the averages. At the lower EC<sub>w</sub>, the SB2 substrate contributed to the lowest average, while there was no significant difference between the other substrates.

Biochar has a high capacity to adsorb  $\text{Na}^+$ , reducing the absorption of this element by the seedlings and promoting greater availability of mineral nutrients in the substrate (Lessa et al., 2023b; Santos et al., 2023). It is worth noting that



Means followed by different lowercase letters indicate significant differences by the Tukey test at  $p \leq 0.05$ . Vertical bars represent the standard error of the mean ( $n = 4$ )

**Figure 5.** Root dry mass (A) and total dry mass (B) of zucchini seedlings as a function of the electrical conductivity of the irrigation water



The same lowercase letters comparing the mean values of electrical conductivity of the water in each substrate and the same uppercase letters comparing the mean values of the different substrates at the same level of electrical conductivity of the water do not differ statistically from each other by Tukey test at  $p \leq 0.05$ . Vertical bars represent the standard error of the mean ( $n = 4$ ). SB1 - Soil + sand + castor bean cake - 7:2:1; SB2 - Soil + sand + biochar - 7:2:1; SB3 - Soil + sand + carbonized rice husk - 7:2:1; and SB4 - Soil

**Figure 6.** Dickson quality index of zucchini seedlings as a function of the electrical conductivity of the irrigation water and different substrates

the highest values for zucchini seedling stem diameter were obtained with water of higher electrical conductivity (Figure 4C), which also indicates the highest DQI values.

Similar to this result, Lessa et al. (2023b) in yellow passion fruit seedlings, also observed a higher value for DQI with a substrate containing biochar associated with an electrical conductivity of the irrigation water of 3.0 dS m<sup>-1</sup>. Sousa et al. (2023) recorded a decrease in DQI in watermelon seedlings also grown in a substrate containing biochar, when the electrical conductivity of the irrigation water was increased from 0.5 to 4.5 dS m<sup>-1</sup>.

In light of the results, it is possible to identify ways of producing Italian zucchini seedlings under biosaline conditions. The use of alternative substrates appears to be a viable alternative to produce better quality seedlings. The results of this study show that substrates formulated with castor bean cake and biochar partially mitigated the deleterious effects of salts on seedling emergence and growth. This is confirmed by the Dickson quality index, showing quality seedlings even under high salinity. It is therefore crucial to identify alternatives for seedling production under salt stress, in order to seek less drastic reductions and greater financial returns under adverse salinity conditions, as the seedling stage is essential for the good productive development of the crop.

## CONCLUSIONS

1. Salt stress reduced the emergence percentage, seedling height, root length, shoot, root and total dry mass and increased the stem diameter of zucchini seedlings.

2. The substrates formulated with castor bean cake (SB1), biochar (SB2), and carbonized rice husk (SB3) increased the emergence percentage, seedling height, stem diameter and shoot dry mass of zucchini seedlings.

3. The SB1 and SB2 substrates mitigated the effects of salt stress on the emergence speed index and Dickson quality index.

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