

## Exogenous application of biostimulant in zucchini (*Cucurbita pepo* L.) subjected to salt stress<sup>1</sup>

Aplicação exógena de bioestimulante em abobrinha submetida ao estresse salino

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**ABSTRACT** - The physiological potential of seeds is one of the main factors that should be considered at the planting of a crop. This study aimed to evaluate the exogenous application of biostimulant in zucchini, cv. Caserta Italiana, under salt stress conditions. For this, the experiment was divided into two parts. The first one was carried out in the laboratory, using a completely randomized experimental design, in a 5 x 3 factorial scheme (biostimulant doses and seed soaking times), with four replicates of 25 seeds. The second one was carried out in a greenhouse, in a completely randomized experimental design, in a 2 x 6 factorial scheme with four replicates, corresponding to two levels of irrigation water salinity (0.5 and 5.0 dS m<sup>-1</sup>) and six forms of biostimulant application in seeds and leaves. Treatment of zucchini seeds, cv. Caserta Italiana, with biostimulant (Stimulate<sup>®</sup>) at the dose of 10 mL L<sup>-1</sup>, for 8 hours, proved to be viable and resulted in vigorous seedlings when irrigated with 5.0 dS m<sup>-1</sup> saline water.

**Key words:** Cucurbitaceae. Bioregulator. Seed treatment. Salinity.

**RESUMO** - O potencial fisiológico das sementes é um dos principais fatores que devem ser considerados no momento da implantação de uma cultura. Com isso, objetivou-se avaliar a aplicação exógena de bioestimulante em abobrinha, cv. Caserta Italiana, em condições de estresse salino. Para isso, o experimento foi dividido em duas partes, a primeira foi realizada em laboratório, utilizando o delineamento experimental inteiramente casualizado, em esquema fatorial 5 x 3 (dosagens de bioestimulante e tempos de embebição das sementes), com quatro repetições de 25 sementes. A segunda parte, foi realizado em casa de vegetação, em delineamento experimental inteiramente casualizado, no esquema fatorial 2 x 6, com quatro repetições, sendo dois níveis de salinidade da água de irrigação (0,5 e 5,0 dS m<sup>-1</sup>) e seis formas de aplicação do bioestimulante via sementes e folhas. O tratamento de sementes de abobrinha, cv. Caserta Italiana, com bioestimulante (Stimulate<sup>®</sup>) na dosagem de 10 mL L<sup>-1</sup>, durante 8 horas, mostrou-se viável e resultou em mudas vigorosas quando irrigadas com água salina de 5,0 dS m<sup>-1</sup>.

**Palavras-chave:** Cucurbitaceae. Biorregulador. Tratamento de sementes. Salinidade.

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## INTRODUÇÃO

Italian zucchini (*Cucurbita pepo* L.), belonging to the Cucurbitaceae family, is also known as summer squash and is native to Central America, specifically Mexico and southern United States (FILGUEIRA, 2012). This species is among the ten vegetables with the highest economic value and high national production, mainly in south-central Brazil (AZAMBUJA *et al.*, 2015).

Using seeds of high physiological and sanitary potential becomes indispensable because these attributes normally provide fast and uniform germination under field conditions, adequate stand, high yield and quality of the harvested product (PÊGO; NUNES; MASSAD, 2011). In the stage of seedling production, attention should be paid to the quality of water used in irrigation, since most cultivated plants are more affected by salinity in the initial stage of development (ARAÚJO *et al.*, 2016). Therefore, the seedling production stage ends up being one of the most affected by the effects of salinity, so it is necessary to implement management techniques that enable the use of lower quality water in this phase of production (LOPES *et al.*, 2017).

Given the imminent need to use lower quality water for irrigation, research has been conducted with the objective of obtaining adequate management that enables the use of this resource without negatively affecting crop development and yield (OLIVEIRA *et al.*, 2015). Therefore, studies aimed at evaluating crop tolerance to salinity, especially in the initial development, have been conducted with some species of the Cucurbitaceae family, such as squashes and pumpkins (OLIVEIRA *et al.*, 2014), watermelon (SILVA *et al.*, 2014), cucumber (ALBUQUERQUE *et al.*, 2016), melon (ARAÚJO *et al.*, 2016) and gherkin (SOUZA NETA *et al.*, 2018). For these species, the authors found a significant reduction in the initial growth of seedlings in response to salt stress.

The application of growth regulators can promote greater growth of the root system, enabling rapid recovery of the plant after a period of water stress, besides providing greater tolerance to insects, pests, diseases and nematodes. Growth regulators also promote quick and uniform establishment of seedlings, which may lead to greater uptake of nutrients and yield (DANTAS *et al.*, 2012).

Some researchers have found that the beneficial effect of biostimulants can be inhibited in plants grown under water stress (ÁVILA *et al.*, 2010) or salt stress (OLIVEIRA *et al.*, 2013). On the other hand, Souza Neta *et al.* (2018), found a significant increase in the average fruit weight and production of gherkin, also belonging to the Cucurbitaceae family, when using the biostimulant Stimulate® under salt stress.

In view of the above, the objective of this study was to evaluate the exogenous application of biostimulant in zucchini, cv. Caserta Italiana, under salt stress conditions.

## MATERIAL AND METHODS

### Experiment I

The first experiment was conducted at the Seed Analysis Laboratory of the Department of Agronomic and Forestry Sciences (DCAF) of the Federal Rural University of the Semi-Arid Region (UFERSA), using seeds of zucchini, cultivar Caserta Italiana, purchased at the local market.

The seeds were pre-soaked in solutions of 0, 5, 10, 15 and 20 mL L<sup>-1</sup> of biostimulant (Stimulate®) for 6, 8 and 10 hours. The liquid formulation of this compound consists basically of 0.005% of indole butyric acid (auxin), 0.009% of kinetin (cytokinin) and 0.005% of gibberellic acid (gibberellin) plus some inert ingredients.

According to each treatment, the seeds were fully immersed in the biostimulant solutions at ambient temperature of 27 °C and then placed on paper towels to drain excess solution. Sowing was carried out in trays (5 x 12 x 16 cm), containing sterile sand moistened up to 50% of its field capacity. The trays were arranged in a laboratory environment at 27 °C during the tests. Irrigation was performed daily using a sprayer with distilled water.

The seeds were evaluated by means of the germination test conducted with four replicates of 25 seeds per treatment, with counts performed at four and eight days after sowing (BRASIL, 2009). The values were expressed as a percentage based on the number of normal seedlings for each treatment, in relation to the number of seeds tested.

Germination speed index was determined together with the germination test, by calculating the number of seeds germinated daily, from the fourth to the seventh day after sowing, using the formula proposed by Maguire (1962):  $GSI = G1/N1 + G2/N2 + \dots + Gn/Nn'$  where GSI - germination speed index; G1 - number of seedlings germinated in the first count; N1 - number of days for the first count; G2 - number of seedlings germinated in the second count; N2 - number of days for the second count; Gn - number of seedlings germinated in the last count; Nn - number of days for the last count.

At the end of the germination test, seedling length was measured with a graduated ruler (cm) considering the distance from the root meristem to the apex of the primary leaves. Then, the measured seedlings were placed in a paper bag and dried in an oven with forced air circulation

at 65 °C until reaching constant weight. After this period, they were weighed on an analytical scale (0.01 g), and the results were expressed in g seedling<sup>-1</sup>.

The experimental design used was completely randomized, in a 5 x 3 factorial scheme (biostimulant concentrations x soaking periods), totaling 15 treatments in four replicates of 25 seeds. The results were subjected to analysis of variance and, in case of significance ( $p < 0.05$ ), the data referring to soaking periods were compared by Tukey test ( $p < 0.05$ ). Data of biostimulant concentrations were subjected to polynomial regression analysis. Statistical analysis was performed using the program SISVAR 5.6 (FERREIRA, 2011).

## Experiment II

The experimental design used was completely randomized, with treatments arranged in a 2 x 6 factorial scheme. The first factor corresponded to two salinity levels of the water used for irrigation (0.5 and 5.0 dS m<sup>-1</sup>), in which the water with lowest salinity came from the water supply network, while the highest salinity was obtained by diluting sodium chloride (NaCl - A. R.) in water (S1), considering the relationship between the electrical conductivity of water (EC<sub>w</sub>) and the concentration of salts ( $10 \cdot \text{meq L}^{-1} = 1 \text{ dS m}^{-1}$  of EC<sub>w</sub>), as suggested by Rhoades *et al.* (1992), which is valid for EC<sub>w</sub> ranging from 0.1 to 5.0 dS m<sup>-1</sup>. The second factor corresponded to six forms of biostimulant application (B1 - absence; B2 - seeds; B3 - seeds + leaves at 5 mL L<sup>-1</sup>; B4 - seeds + leaves at 10 mL L<sup>-1</sup>; B5 - leaves at 5 mL L<sup>-1</sup> and B6 - leaves at 10 mL L<sup>-1</sup>), as follows: B1 - absence of biostimulant (soaking for 8 hours with distilled water), B2 - application by seed treatment (soaking for 8 hours at a dose of 10 mL L<sup>-1</sup>); B3 and B4 - applications by seed treatment (soaking for 8 hours at doses of 5 and 10 mL L<sup>-1</sup>) plus foliar application (doses of 5 and 10 mL L<sup>-1</sup>, respectively) at 8 days after sowing; and B5 and B6 - foliar applications (doses of 5 and 10 mL L<sup>-1</sup>, respectively), also applied at 8 days after sowing.

Emergence test was carried out in a 21-m-long, 7.0-m-wide arched greenhouse at the Department of Agronomic and Forestry Sciences (DCAF), covered by a 0.10-mm-thick, transparent low-density polyethylene film, protected from the action of ultraviolet rays. Its front and sides consist of anti-aphid screens and the 0.30-m-high wall is made of reinforced concrete. Seeds of the cultivar Caserta Italiana were sown in expanded polystyrene trays with capacity for 180 pyramid-shaped cells, by placing one seed per cell. Substrate consisted of coconut powder (Golden Mix - Granulado®), composed of 100% coconut fiber with fine texture and no basal fertilization.

After emergence, four days after sowing, treatment with salt stress began with daily applications of nutrient

solution via fertigation through the floating-type seedling irrigation system. This apparatus was installed on a wooden bench (5.0 x 1.0 m) and supported by 1.0-m-high trestles. The bench was divided into three parts of 1.6 x 0.8 m, using pieces of wood (rafters). Each part was covered with plastic tarpaulin, forming a micro-pool with capacity to hold four trays (OLIVEIRA *et al.*, 2014).

The nutrient solution followed the recommendation of Adams (1994), with the following nutrient concentrations: 8, 2, 4, 2, 1 and 1 mol L<sup>-1</sup> of N, P, K, Ca, Mg and S, respectively, and 35, 19, 21, 4, 0.9 and 0.7 μmol L<sup>-1</sup> of Fe, Mn, B, Zn, Cu, and Mo, respectively.

The seedlings were collected at 15 days after sowing (DAS), and 10 seedlings per experimental unit were analyzed for the following characteristics: seedling height (H) - measured with millimeter ruler (cm), from the collar region to the apical bud; stem diameter (SD) - measured at the base of the collar, using a digital caliper (Digimess®) (0.01 mm); number of leaves (NL) - obtained by simple counting of leaves longer than 3 cm; main root length (MRL) - measured with a millimeter ruler (cm) from the collar to the tip of the longest root; leaf area (LA) - obtained by the leaf disc method (SOUZA NETA *et al.*, 2018); and specific leaf area (SLA) - obtained by dividing the value of leaf area by leaf dry mass.

To obtain shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM), the seedlings were separated into shoots and roots, placed in paper bags and dried in an oven with forced air circulation at 65 °C until reaching constant weight. After being dehydrated, the samples were weighed on an analytical scale (0.01 g) to determine SDM and RDM, while TDM was obtained by the sum of these two (SDM and RDM).

The relative chlorophyll index (SPAD unit) was evaluated indirectly by the chlorophyll concentration, using a chlorophyll meter (ClorofiLOG®, CFL 1030 model), operated according to the manufacturer's instructions. The values obtained were expressed in Relative Chlorophyll Index (RCI).

The results were subjected to analysis of variance and, in case of significance ( $p < 0.05$ ), the data were compared by Tukey test ( $p < 0.05$ ). Statistical analysis was performed using the program SISVAR 5.6 (FERREIRA, 2011).

## RESULT AND DISCUSSION

### Experiment I

The interaction between soaking time and biostimulant concentrations (T x C) was significant for dry

mass ( $p < 0.05$ ). There were significant effects of soaking time on germination ( $p < 0.05$ ) and dry mass ( $p < 0.01$ ). Biostimulant doses significantly influenced germination ( $p < 0.05$ ). Germination speed index (GSI), seed coat release (SCR) and seedling length (SL) were not influenced ( $p > 0.05$ ) by the factors studied (Table 1).

Seeds soaked with biostimulant for 6 h obtained higher germination percentage, and there was no difference between treatments soaked for 8 and 10 h (Table 1). For GSI, the highest values occurred in seeds soaked for 10 h, followed by the treatments of 6 and 8 h of soaking (Table 1). Studies conducted by Silva *et al.* (2014), with Stimulate® in watermelon found no significant response for germination and seedling length, but there was a significant response in the germination speed index. This divergence can be attributed, in addition to the species used, to the form of application of the biostimulant, since the authors applied the product directly to the seed.

Germination was quadratically affected by biostimulant concentrations, with the highest germination (96.4%) obtained with the dose of 11.8 mL L<sup>-1</sup>, promoting an increase of around 7.5% compared to the germination obtained in the absence of biostimulant (89.7%) (Figure 1A).

The positive effect of biostimulant application in the seed on germination occurs due to the physiological functions of the Stimulate® components. The gibberellins present in Stimulate® can stimulate the synthesis of

enzymes that digest the reserves stored in the endosperm, forming simple sugars, amino acids and nucleic acids (TAIZ *et al.*, 2017). Also according to these authors, these compounds are absorbed and transported to the embryo growth regions, stimulating cell elongation and causing the radicle to rupture the seed coat, accelerating germination and promoting greater uniformity. In addition to gibberellins, cytokinins and auxins participate in several processes of physiological development, including seed germination and breakage of dormancy of buds (BEWLEY; BLACK, 1994).

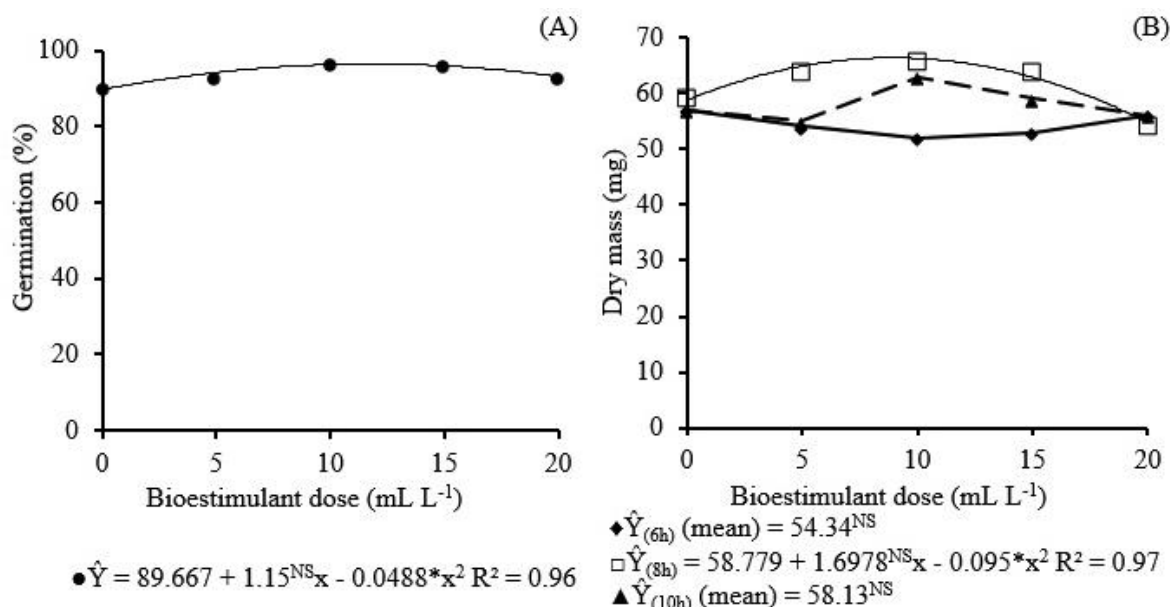
The dry mass of seedlings at the soaking times of 6 and 10 hours did not have significant fit for the biostimulant doses, obtaining means of 54.34 and 58.13 mg seedling<sup>-1</sup>, respectively. In seeds soaked for 8 h, the response was quadratic, obtaining 66.36 mg seedling<sup>-1</sup> at the concentration of 9 mL L<sup>-1</sup>, which corresponded to a 12.9% increase compared to the dry mass obtained in the absence of biostimulant (58.78 mg seedling<sup>-1</sup>) (Figure 1B).

Exogenous application of Stimulate® promotes the balance of the concentration of hormones existing in the seeds, leading to more vigorous seedlings, because bio-activators are complex organic growth-modifying substances, capable of acting on the DNA transcription in the plant, gene expression, membrane proteins, metabolic enzymes and mineral nutrition (TAIZ *et al.*, 2017). Positive effect of seed treatment with biostimulant on biomass accumulation has been observed in seedlings of several vegetables, such as

**Table 1** - Summary of analysis of variance and test of means for germination (GER), germination speed index (GSI), seedling length (SL), dry mass (DM) and seed coat release (SCR) of zucchini seedlings, cv. Caserta Italiana, as a function of seed treatment with different doses of biostimulant and soaking times

SV	DF	Mean squares				
		GER	GSI	SL	DM	SCR
Time (T)	2	88.55*	6.82**	1.39 <sup>NS</sup>	212.54**	60.72 <sup>NS</sup>
Concentrations (C)	4	89.01*	1.79 <sup>NS</sup>	0.36 <sup>NS</sup>	28.87 <sup>NS</sup>	64.64 <sup>NS</sup>
T x C	8	27.14 <sup>NS</sup>	1.30 <sup>NS</sup>	1.06 <sup>NS</sup>	47.81*	21.47 <sup>NS</sup>
Error	45	25.75	1.15	0.70	18.27	54.52
CV (%)		5.41	12.35	5.25	7.40	9.06
Tukey test ( $p < 0.05$ )						
		GER (%)	GSI	SL (cm)	DM (mg)	SCR (%)
Time (h)	6	95.93 a	8.79 ab	16.06 a	54.34 b	81.80 a
	8	92.00 b	8.04 b	16.12 a	61.51 a	79.65 a
	10	93.60 ab	9.18 a	15.64 a	58.13 ab	83.10 a

\* and \*\* - Significant at 5 and 1% probability levels, respectively; ns - not significant. Means followed by the same letter in the column do not differ by Tukey test at 5% probability level

**Figure 1** - Germination (A) and dry mass (B) of zucchini seeds, cv. Caserta Italiana, as a function of soaking with different concentrations of biostimulant

\*and NS = significant at 5% probability level ( $p < 0.05$ ) and not significant, respectively

lettuce (ALBUQUERQUE *et al.*, 2009; SOARES *et al.*, 2012), sweet potato (RÓS; NARITA; ARAÚJO, 2015) and gherkin (OLIVEIRA *et al.*, 2017).

## Experiment II

The interaction between salinity and biostimulant (S x B) was significant for the relative chlorophyll index (RCI), leaf area (LA), shoot dry mass (SDM) and total dry mass (TDM), at 1% probability level, and for specific leaf area (SLA) and root dry mass (RDM) at 5% probability level. Seedling height (H) and stem diameter (SD) were significantly influenced ( $p < 0.01$ ) by the treatment with biostimulant. Main root length (MRL) and number of leaves (NL) were not influenced by the treatments studied (Table 2).

Treatment of seeds with Stimulate® (B1, B2, B4 and B6) promoted the highest growth of zucchini seedlings, whereas foliar application (B3 and B5) led to the lowest heights, although these did not differ from B1 and B2, indicating that the biostimulant action varies with the form of application (Table 3). In the study conducted by Oliveira *et al.* (2017), with Stimulate® in gherkin seedlings produced in coconut fiber substrate, there was no influence of seed treatment with water or biostimulant on seedling height.

In stem diameter, the applications of biostimulant in seeds + leaves or only in leaves promoted higher values, while the lowest values of diameter occurred

in the absence of biostimulant (B1) (Table 3). Positive effect of biostimulant application on stem diameter was also observed by Silva *et al.* (2014), in watermelon seedlings, a behavior not observed in gherkin seedlings (OLIVEIRA *et al.*, 2017). It is worth mentioning that, in relation to morphology, zucchini has a more voluminous stem than gherkin, which may result in a greater possibility of response to the treatments applied.

The relative chlorophyll index (RCI) was affected by salinity and according to the application of biostimulant. RCI was affected by salinity only when biostimulant was applied in seeds + leaves  $5 \text{ mL L}^{-1}$  (B3) and only in leaves  $5 \text{ mL L}^{-1}$  (B5), when RCI was reduced by salt stress (Table 4).

The reduction in chlorophyll contents in plants grown in saline medium has been attributed to the increase in chlorophyllase, inducing destruction of the chloroplast structure and instability of pigment protein complexes (JAMIL *et al.*, 2007).

The biostimulant did not influence RCI at low salinity, with a mean RCI of 15.74. On the other hand, in seedlings subjected to salt stress, the application of the biostimulant through the leaves, either alone or associated with seed treatment, reduced the values of this variable. There was no significant difference when only seed treatment with water and biostimulant was performed. An increase in RCI was expected with the application of biostimulant through the leaves, because the positive

effect of Stimulate® on the relative chlorophyll index comes from cytokinins. Cytokinins induce the synthesis of proteins and enzymes, maintaining cell vigor and the metabolic processes of absorption and assimilation of nutrients, besides delaying the degradation of proteins and chlorophyll (COLL *et al.*, 2001).

There was no significant difference between the levels of salinity for the variable leaf area in the absence of biostimulant (B1) and when the biostimulant was applied only in seeds (B2); on the other hand, salinity reduced leaf area in the other forms of biostimulant application (Table 4). At both levels of salinity, the use of biostimulant increased leaf area, and for the

non-saline condition the highest values occurred with the application of biostimulant through the leaves at a dose of 10 mL L<sup>-1</sup> (B6), although this treatment did not differ from B3 and B4. With the use of saline water, the application of biostimulant in seeds + leaves at 10 mL L<sup>-1</sup> (B4) led to the highest values, although they did not differ from the values obtained in B3, B5 and B6.

For specific leaf area, the effect of salinity was significant when the biostimulant was applied through the leaves at doses of 5 and 10 mL L<sup>-1</sup>, causing reductions of 15.22 and 18.38%, respectively (Table 4). Specific leaf area expresses the area of leaf blade available to produce one unit of leaf dry mass, so that it represents

**Table 2** - Summary of analysis of variance for height (H), stem diameter (SD), main root length (MRL), number of leaves (NL), leaf area (LA), specific leaf area (SLA), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM) and relative chlorophyll index (RCI) in zucchini seedlings, cv. Caserta Italiana, as a function of salinity and form of application of the biostimulant

SV	DF	Mean squares				
		H	SD	MRL	NL	LA
S	1	0.29 <sup>NS</sup>	0.68 <sup>NS</sup>	0.42 <sup>NS</sup>	0.02 <sup>NS</sup>	3636.74 <sup>**</sup>
B.	5	3.29 <sup>**</sup>	0.47 <sup>**</sup>	1.65 <sup>NS</sup>	0.02 <sup>NS</sup>	1898.46 <sup>**</sup>
S x B	5	1.57 <sup>NS</sup>	0.12 <sup>NS</sup>	0.35 <sup>NS</sup>	0.12 <sup>NS</sup>	1309.04 <sup>**</sup>
Error	36	0.67	0.07	1.13	0.31	447.57
CV (%)	47	10.84	7.71	12.14	12.37	11.03

SV	DF	Mean squares				
		SLA	SDM	RDM	TDM	RCI
S.	1	3929.69 <sup>*</sup>	43266.02 <sup>**</sup>	274.56 <sup>*</sup>	50433.85 <sup>**</sup>	13.55 <sup>NS</sup>
B.	5	31999.70 <sup>**</sup>	4159.78 <sup>*</sup>	846.00 <sup>**</sup>	3925.59 <sup>*</sup>	20.34 <sup>**</sup>
S x B	5	1309.04 <sup>*</sup>	4985.36 <sup>**</sup>	200.68 <sup>*</sup>	6148.05 <sup>**</sup>	24.47 <sup>**</sup>
Error	36	447.57	1236.24	42.19	1271.04	4.15
CV (%)	47	11.03	11.87	20.56	10.88	13.40

\* and \*\* - Significant at 5 and 1% probability levels, respectively; ns - not significant; S - salinity; B - biostimulant

**Table 3** - Seedling height and stem diameter in zucchini seedlings, cv. Caserta Italiana, as a function of the form of application of the biostimulant

Biostimulant	B1-Absence	Seedling height	Stem diameter
	B2-Seeds	8.12 ab	3.61 a
	B3-Seeds+leaves 5	7.07 b	3.47 a
	B4-Seeds+leaves 10	8.09 ab	3.66 a
	B5-Leaves 5 mL L <sup>-1</sup>	6.89 b	3.35 ab
	B6-Leaves 10 mL L <sup>-1</sup>	8.31 a	3.58 a
	Means	7.59	3.45

Means followed by the same letter in the columns do not differ by Tukey test at 5% probability level; B3 - seeds + leaves (5 mL L<sup>-1</sup>), B4 - seeds + leaves (10 mL L<sup>-1</sup>)

**Table 4** - Relative chlorophyll index (RCI), leaf area (LA), specific leaf area (SLA), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) in zucchini seedlings, cv. Caserta Italiana, as a function of water salinity and form of application of biostimulant

Biostimulant	Waters			
	Non-saline	Saline	Non-saline	Saline
	Relative chlorophyll index – RCI		Leaf area (cm <sup>2</sup> )	
B1-Absence	15.65 Aa	18.54 Aa	27.90 Ac	30.71 Ab
B2-Seeds	16.33 Aa	18.42 Aab	28.77 Ac	30.80 Ab
B3-Seeds+leaves 5	17.27 Aa	11.61 Bc	69.53 Aab	39.94 Bab
B4-Seeds+leaves 10	14.17 Aa	13.29 Ac	74.17 Aab	49.17 Ba
B5-Leaves 5	16.73 Aa	12.00 Bc	64.05 Ab	43.50 Bab
B6- Leaves10	14.27 Aa	14.18 Abc	80.69 Aa	46.53 Bab
Means	15.74	14.67	57.52	40.11
	Specific leaf area (cm <sup>2</sup> g <sup>-1</sup> LDM)		Shoot dry mass (mg seedling <sup>-1</sup> )	
B1-Absence	104.24 Ab	123.82 Ab	311.10 Aa	291.28 Aab
B2-Seeds	104.56 Ab	108.47 Ab	318.30 Aa	332.55 Aa
B3-Seeds+leaves 5	241.38 Aa	221.94 Aa	323.35 Aa	211.93 Bc
B4-Seeds+leaves 10	243.38 Aa	216.87 Aa	353.80 Aa	263.78 Babc
B5-Leaves 5	250.12 Aa	212.05 Ba	259.93 Aa	244.18 Bbc
B6- Leaves10	261.39 Aa	213.34 Ba	354.88 Aa	253.38 Bbc
Means	200.48	182.75	326.23	266.18
	Root dry mass (mg seedling <sup>-1</sup> )		Total dry mass (mg seedling <sup>-1</sup> )	
B1-Absence	20.00 Ab	19.93 Ab	331.10 Aa	311.20 Aab
B2-Seeds	18.88 Ab	19.63 Ab	337.18 Aa	352.18 Aa
B3-Seeds+leaves 5	42.40 Aa	25.28 Bb	365.75 Aa	237.20 Bb
B4-Seeds+leaves 10	46.30 Aa	45.68 Aa	400.10 Aa	309.45 Bab
B5-Leaves 5	35.42 Aa	33.30 Aab	331.35 Aa	277.48 Bab
B6- Leaves10	40.95 Aa	31.45 Bb	395.83 Aa	284.83 Bab
Means	33.99	29.21	360.22	295.39

Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ by Tukey test at 5% probability level; B3 - seeds + leaves (5 mL L<sup>-1</sup>); B4 - seeds + leaves (10 mL L<sup>-1</sup>)

the thickness of the leaf blade; therefore, the larger the specific leaf area, the smaller this thickness. Thus, it is verified that applications by seed soaking led to lower values compared to treatments with foliar application, both in the non-saline condition and in the high-salinity condition. Salinity resulted in the production of thicker leaves, indicating that the effect of salinity was higher on leaf blade expansion than on biomass accumulation. This behavior was observed by Oliveira *et al.* (2014), when analyzing the development of pumpkin and squash cultivars under salt stress, as well as by other researchers with other cucurbits, such as sponge gourd (MEDEIROS *et al.*, 2014).

Root, shoot and total dry masses showed varied responses to the biostimulant, according to the salinity used (Table 4). For root dry mass, there was significant effect of salinity when the biostimulant was applied through seeds + leaves at 5 mL L<sup>-1</sup> and through leaves at 10 mL L<sup>-1</sup>, causing reductions of 40.38 and 23.20%, respectively. There was no significant effect of the application of biostimulant in seeds on RDM; however, the other forms of applications of the biostimulant caused increase in this variable, not differing from each other. When saline water was used, the use of biostimulant in seeds + leaves at 10 mL L<sup>-1</sup> (B4) and in leaves at 5 mL L<sup>-1</sup> (B5) promoted higher values of root dry mass, although

the latter (B5) did not differ from the others (Table 4). According to Castro and Vieira (2001), biostimulants have the ability to stimulate root development, due to the stimulation of cell division, differentiation and elongation. This behavior favors the absorption of water and nutrients by the roots, an important factor for plants under salt stress.

Shoot and total dry masses were not influenced by salinity in the absence of biostimulant (B1), when only seed application (B2) was performed. However, in the other forms of biostimulant application, these variables were reduced and the lowest losses were observed in B3 and B6 (for both variables). In this form of application, salinity caused reductions in shoot dry mass of 34.46 and 28.60% in B3 and B6, respectively, whereas for TDM salt stress caused reductions of 35.15% in B3 and 37.14% in B6.

Biostimulant application in the seeds (B2) attenuated the effect of salinity on SDM. However, with foliar application (B3, B4, B5 and B6) there were reductions in the SDM and TDM of zucchini seedlings (Table 4). The reductions in SDM and TDM corroborate those verified for LA and SLA and are related to the tolerance mechanism of plants, in reducing leaf area and increasing leaf thickness in order to minimize water losses by transpiration and, consequently, the absorption of salts via irrigation water (SÁ *et al.*, 2013), since there were no effects of salinity on the growth in height, stem diameter and root length, as well as on the production of leaves (Table 2). Similar results were observed by Oliveira *et al.* (2013), with cowpea crop, for which the foliar application of biostimulant also reduced leaf area and shoot dry mass.

## CONCLUSION

Treatment of zucchini seeds, cv. Caserta Italiana, with biostimulant (Stimulate®) at dose of 10 mL L<sup>-1</sup>, for 8 hours, is feasible and results in vigorous seedlings when irrigated with 5.0 dS m<sup>-1</sup> saline water.

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