

Photochemical efficiency, biomass and chlorophyll of *Physalis peruviana* under salinity and biostimulant

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ABSTRACT: *Physalis peruviana* L. has great exploitation potential especially for small producers in the Brazilian Northeast. However, production in semiarid regions may be limited by high salt content in soils and water sources used for irrigation. Thus, this study evaluated the application of *Ascophyllum nodosum* seaweed extract as saline stress attenuator in *P. peruviana* L. The randomized block design was used, in an incomplete 5 × 5 factorial scheme, with five of electrical conductivities of the irrigation water (0.50, 1.23, 3.00, 4.77 and 5.50 dS m⁻¹) and five increasing concentrations of kelp extract (0.00, 1.45, 5.00, 8.55 and 10.00 mL L⁻¹), with nine combinations generated by the Central Box matrix, with four replicates of four plants to evaluate the effect on phytomass accumulation, chlorophyll fluorescence and pigment content. Results showed, salinity significantly reduced biomass production and chlorophyll fluorescence and pigment indices. However, when plants were treated with 9.9 mL L⁻¹ seaweed extract they increased biomass production and seedling quality. On the other hand, up to 4.1 mL L⁻¹, the biostimulant increased dark-adapted fluorescence indices which indicate it reduces damages to the photosynthetic apparatus caused by salt-stress and thus improving photosynthetic activity and photoassimilates production. Thus the application of *A. nodosum* seaweed extract attenuates the deleterious effect of salt stress in photosynthesis and thus in biomass production of *P. peruviana* plants.

Key words: *Ascophyllum nodosum* L.; *Physalis peruviana* L.; plant physiology; saline stress

Eficiência fotoquímica, biomassa e clorofila da *Physalis peruviana* sob salinidade e biostimulante

RESUMO: A *Physalis peruviana* L., apresenta grande potencial de exploração, especialmente para pequenos produtores do Nordeste Brasileiro. No entanto, a produção em regiões semiáridas pode ser limitada pelo alto teor de sais contidos no solo e nas fontes de água utilizadas na irrigação. Assim, este estudo avaliou a aplicação do extrato de algas marinhas *Ascophyllum nodosum* como atenuador de estresse salino em *P. peruviana* L. O delineamento de blocos casualizados foi utilizado, em esquema fatorial incompleto 5 × 5, sendo cinco de condutividades elétricas da água de irrigação (0,50, 1,23, 3,00, 4,77 e 5,50 dS m⁻¹) e cinco concentrações crescentes do extrato de alga marinha (0,00, 1,45, 5,00, 8,55 e 10,00 mL L⁻¹), com nove combinações geradas pela matriz Central de Box, com quatro repetições de quatro plantas para avaliar o efeito no acúmulo de fitomassa, fluorescência e teor de pigmentos da clorofila. Os resultados mostraram que a salinidade reduziu significativamente a produção de biomassa e os índices de fluorescência e pigmentos da clorofila. No entanto, quando as plantas foram tratadas com 9,9 mL L⁻¹ de extrato de algas marinhas, elas aumentaram a produção de biomassa e a qualidade das mudas. Por outro lado, até 4,1 mL L⁻¹, o bioestimulante aumentou os índices de fluorescência adaptada ao escuro, o que indica que ele reduz os danos ao aparato fotossintético causados pelo estresse salino, melhorando assim a atividade fotossintética e a produção de fotoassimilados. Assim, a aplicação de extrato de alga marinha *A. nodosum* atenua o efeito deletério do estresse salino na fotossíntese e, conseqüentemente, na produção de biomassa de plantas de *P. peruviana*.

Palavras-chave: *Ascophyllum nodosum* L.; *Physalis peruviana* L.; fisiologia vegetal; estresse salino

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Associate Editor: Maria do Céu Monteiro Cruz

Introduction

Physalis peruviana L., known as camapú and fisális, has gained wide acceptance among farmers and consumers, due to their peculiar organoleptic traits of fruits, and high vitamins A and C content as well as medicinal substances (Fischer et al., 2014). However, *P. peruviana* exploitation in semiarid regions is severely affected by environmental and edaphoclimatic conditions.

Brazilian Northeast show low rainfall and suffers from water scarcity and high salt content in soils and water sources used for irrigation. Such conditions severely affect the development of crops in the region (Almeida et al., 2019). The high salt content in water and soil causes salt stress in plants which may disturb growth and development, by mainly affecting the photosynthetic apparatus (Cavalcante et al., 2019), resulting in biomass production losses.

To mitigate the deleterious effects of salt stress on plants, organic products have been used to stimulate the plant defence system. Among these products, biostimulants are able to act on plant transcription and thus activate secondary metabolism, alter phytohormones concentration and dynamics, improve the biological properties of plants, increase nutrient uptake and modify nutrition, and hence stimulate plant growth (Abdel-Raouf et al., 2012) under salt stress conditions (Jithesh et al., 2019).

Among the biostimulants used in agriculture, *Ascophyllum nodosum* (L.) Le Jolis seaweed-based extracts stood out for containing plant hormones that regulate and stimulate plant growth in addition to macro and micronutrients, pigments, vitamins, amino acids and others osmoprotective compounds (Michalak et al., 2016; Soares et al., 2018; Raghunandan et al., 2019). The attenuating effect of applying *A. nodosum* extract is reported for several crops, as in the case of Rosa et al. (2021) that improved the antioxidant activity of soybean (*Glycine max* L.) increasing productivity under water stress. Popescu (2020) attenuated the effect of salinity on the growth of cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.). Al-Maliki et al. (2019) reduced the effect of water stress on the growth of onion (*Allium cepa* L.).

Thus, this work was carried out to evaluate the effect of *Ascophyllum nodosum* (L.) Le Jolis extract application on phytomass, fluorescence and photosynthetic pigments of *P. peruviana* L. under saline water irrigation.

Materials and Methods

The experiment was carried out in 2019 under greenhouse condition in the Department of Crop and Environmental Sciences from the Center of Agrarian Sciences, Federal University of Paraíba (UFPB), Areia city, Paraíba State, Brazil (6°57'42" S, 35°41'43" W, 573 above the sea level). The climate, according to the Köppen classification, is As' type that is dry and hot summer with rain in winter, with 21.7 °C average temperature and 1.305 mm a year rainfall. The experiment lasted 90 days, being conducted from August to October 2019.

The experimental design was in randomized blocks in an incomplete 5 × 5 factorial scheme with four replications and four plants per plot, where nine treatments were generated through the central composite design (CCD), with the minimum (-α) and maximum (α) values 0.5 and 5.5 dS m⁻¹, respectively, for electrical conductivity of irrigation water (ECw) and 0.0 and 10 mL L⁻¹ for seaweed-based biostimulant doses (BD) (Table 1). The use of CCD allows to evaluate the effect of two or more factors with a smaller number of treatments, optimizing space and work, preserving the efficiency and quality of the results, being the CCD widely used for factorial scheme of up to three factors and suitable for adjustment quadratic surface (Hang et al., 2011).

Seedlings were produced using seeds obtained from healthy and pest-free fruits produced in Pombal, Paraíba, Brazil. Seeds were sown in polystyrene bags of 1.2 dm³ capacity filled with a substrate composed of Latosol (Embrapa, 2018), cattle manure, and washed sand in a 3:1:1 ratio by volume. The substrate has the following chemical characteristics: P = 85.55 mg kg⁻³; K⁺ = 693.60 mg kg⁻³; Na⁺ = 0.23 cmol_c dm⁻³; H⁺ + Al³⁺ = 0.00 cmol_c dm⁻³; Al³⁺ = 0.00 cmol_c dm⁻³; Ca²⁺ = 2.91 cmol_c dm⁻³; Mg²⁺ = 1.59 cmol_c dm⁻³; pH = 7.8; BS = 6.50 cmol_c dm⁻³; CEC = 6.50; O.M. = 22.21 g kg⁻¹.

The saline waters with different electrical conductivities were prepared by adding sodium chloride (NaCl) to the potable water from the UFPB supply system that had 0.5 dS m⁻¹ EC. The desired ECw was obtained by the Equation 1 proposed by Lima et al. (2001).

$$CS = 0.01 \left(\frac{ECw - 0.4}{Pd} \right) \times Weq \quad (1)$$

where CS is the ionic concentration (g L⁻¹), Weq is the equivalent weight and Pd is the purity degree of 97%. ECw was measured using a portable digital conductivity meter (microprocessor, model CD-860, Instrutherm®).

Irrigation was performed daily, and saline water was applied from 10 days after sowing. Water volume applied was determined by the drainage lysimetry method, by the difference between the amount of water applied and drained, which thus keeping the soil at field capacity.

Table 1. Treatments generated through the centre composite design (CCD) matrix.

Treatments	Levels		Dose	
	ECw (dS m ⁻¹)	BD (mL L ⁻¹)	ECw (dS m ⁻¹)	BD (mL L ⁻¹)
T1	-1	-1	1.23	1.45
T2	-1	1	1.23	8.55
T3	1	-1	4.77	1.45
T4	1	1	4.77	8.55
T5	-α	0	0.50	5.00
T6	α	0	5.50	5.00
T7	0	-α	3.00	10.00
T8	0	α	3.00	0.00
T9	0	0	3.00	5.00

ECw = electrical conductivity of irrigation water; DB = biostimulant doses.

The biostimulant doses based on *A. nodosum* seaweed extracts (Acadian®, Agritech, Canada) were applied 20 days after start irrigation with saline water. The extracts had the following characteristics: 8.12, 6.82, 12.00, 1.60, 2.03, and 8.16 g kg⁻¹ N, P, K, Ca, Mg, and S, respectively; 5.74, 13.60, 11.5, 0.04, 24.40, and 20.000 mg kg⁻¹ B, Cu, Fe, Mn, Zn, and Na respectively; potassium hydroxide, with 61.48 g L⁻¹ water-soluble K₂O; 69.60 g L⁻¹ total organic carbon; and 1.16 g dm⁻³ density (Silva et al., 2016). Doses were divided into six foliar applications, performed weekly in the late afternoon by spraying 100 mL solution per plant.

At 75 days after saline application, plant biomass was evaluated. Plants were removed from the pots, separated into leaves, stem, and roots, then stored in paper bags and dried in a forced circulation oven (65 °C; 72 hours) to determine the dry phytomass of each plant organ on a precision analytical scale. Also, shoot (leaves plus stem) and total (leaves plus stem plus roots) phytomass were obtained by the sum of dry phytomass of plant organs, and results were expressed as g per plant.

For plant appraisal, plant height and stem diameter were measured, and values were used to calculate the Dickson Quality Index (DQI) by the Equation 2 (Dickson et al., 1960).

$$DQI = \frac{TDP}{\frac{PH}{SD} + \frac{SDP}{RDP}} \quad (2)$$

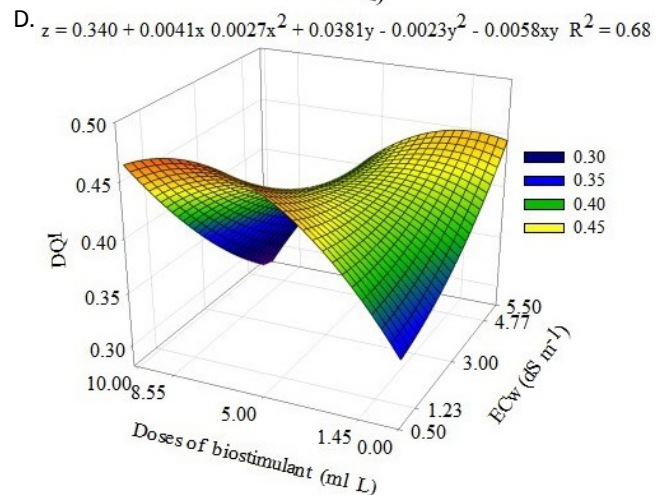
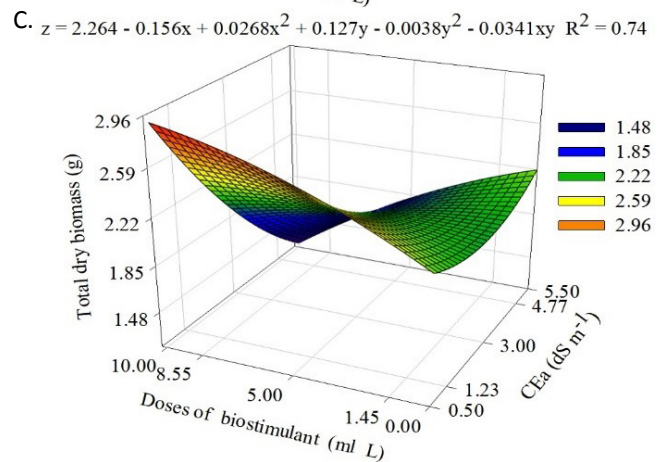
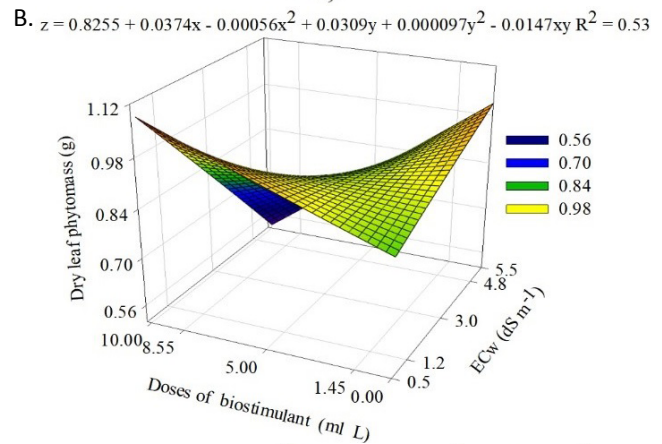
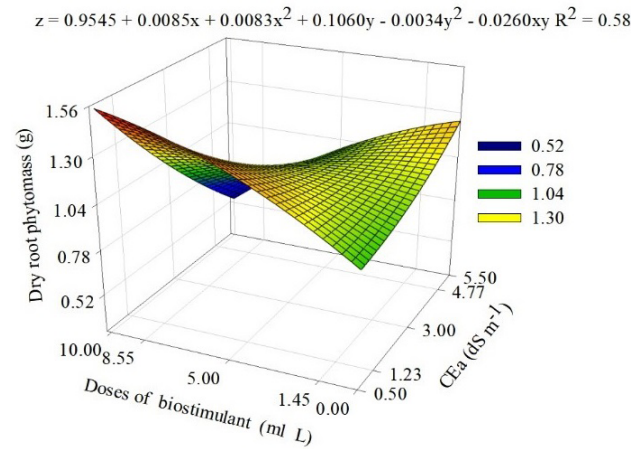
where TDP is the total dry phytomass, PH is the plant height, SD is the stem diameter, SDP is the shoot dry phytomass, and RDP is the root dry phytomass.

Also, chlorophyll (Chl) fluorescence was analyzed by using a portable modulated fluorometer (Model OS-30p, Sciences Inc., Hudson, USA). Leaf clamps were placed for 30 minutes for leaf adaptation to darkness, then minimum fluorescence (F₀) and maximum fluorescence (F_m) variables were calculated. Also, variable fluorescence (F_v = F_m - F₀), effective quantum yield of energy conversion (F_v / F₀), and maximum quantum yield of PSII (F_v / F_m) were calculated. In turn, pigment concentration (Chl a, Chl b, Chl a + b, and Chl a/b) was determined by using a portable chlorophyll meter (ClorofiLOG®, CFL 1030 model, Falker, Porto Alegre, RS), with values expressed as Falker chlorophyll index (FCI).

The data were submitted to analysis of variance by the F test (p < 0.05), the results being significant for the interaction between the factors presented by means of the response surface and those isolated by means of quadratic or linear polynomial regression, being used the R software (R Core Team 2020).

Results

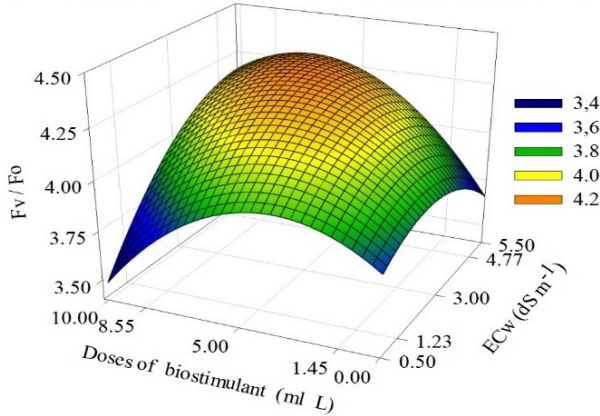
There was a significant interaction between the ECw and BD for root, leaf and total phytomass, DQI, F_v/F₀ and Chl a/b (p < 0.05; Figure 1).



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Continuation of Figure 1

E. $z = 3.745 + 0.134x - 0.027x^2 + 0.088y - 0.013y^2 + 0.017xy$ $R^2 = 0.79$



F. $z = 5.073 - 0.170x - 0.012x^2 - 0.108y - 0.0028y^2 + 0.037xy$ $R^2 = 0.60$

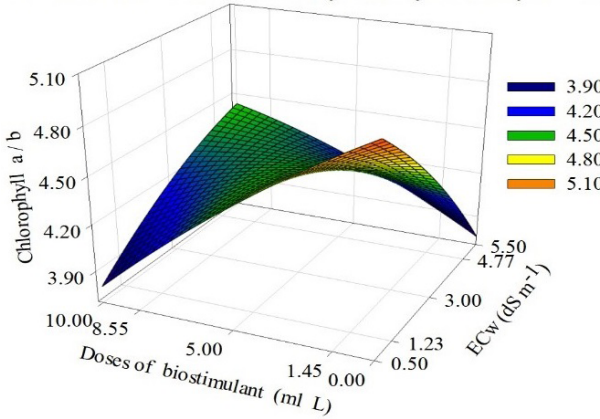


Figure 1. Dry root mass (A), leaves (B) total (C), Dickson quality index - DQI (D), F_v/F_0 (E) ratio and chlorophyll a/b (F) ratio *Physalis peruviana* L., submitted to electrical conductivity of irrigated water and doses of *Ascophyllum nodosum* based biostimulant.

Plants showed maximum root (1.54 g, Figure 1A) and leaf (1.08 g, Figure 1B) phytomass when they were irrigated with water of estimated 0.52 dS m⁻¹ and treated with 9.9 mL L⁻¹ biostimulant. But maximum total phytomass was observed with an application of 9.7 mL L⁻¹ biostimulant (Figure 1C). In this sense, maximum DQI (0.48) was reached in plants irrigated with water of 0.50 dS m⁻¹, but when a lower biostimulant dose was applied (7.63 mL L⁻¹) (Figure 1D). Above these concentrations, DQI declined.

In contrast, F_v/F_0 increased (up to 4.32) with increasing salinity of irrigation water (up to 4.38 dS m⁻¹) and an application of 6.3 mL L⁻¹ biostimulant (Figure 1E). Whereas Chl a/b strongly decreased due to salinity, but it was stimulated by seaweed extract application (Figure 1F). Moreover, regardless of biostimulant use, increased ECw negatively affected shoot dry phytomass accumulation as well as it reduced F_0 , F_v , F_m and F_v/F_m Chl fluorescence variables (Figure 2). Plants accumulated 26.7% less phytomass when plants were irrigated with water of 5.5 dS m⁻¹ compared with 0.5 dS m⁻¹ (Figure 2A). Similarly, F_0 , F_v and F_m were 4.3 (Figure 2B), 8.2 (Figure 2C) and 7.7% (Figure 2D) lower in plants irrigated with water of 5.5 dS m⁻¹. Thereby, F_v/F_m ranged from 0.76 to 0.86 electrons quantum⁻¹ depending on ECw used (Figure 2E).

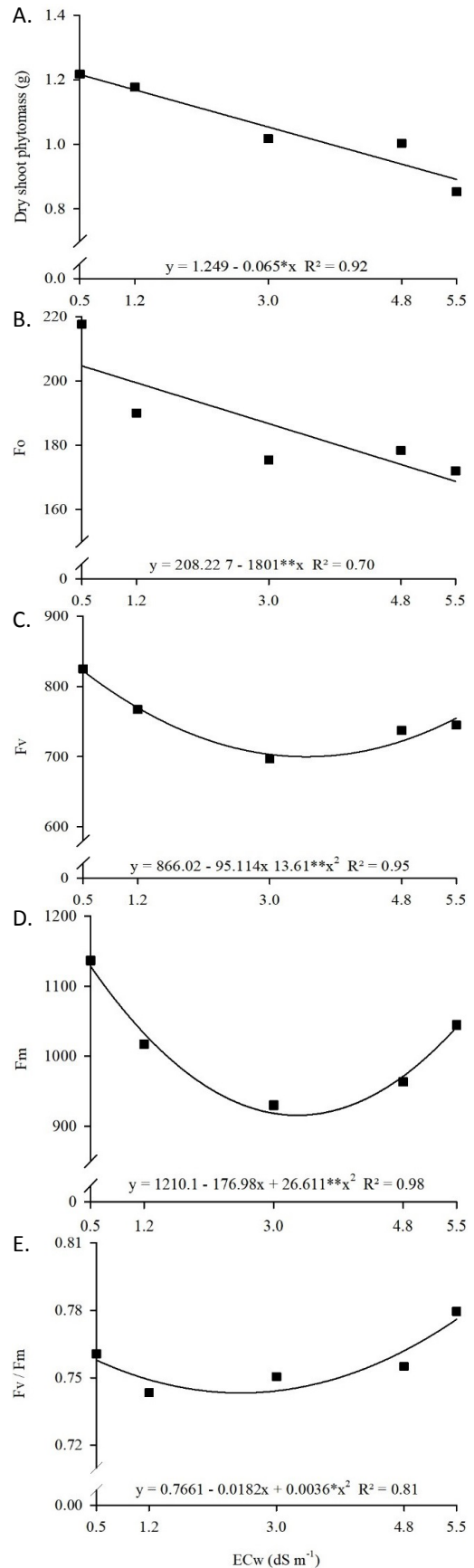


Figure 2. Dry shoot weight (A), initial fluorescence – F_0 (B), variable fluorescence – F_v (C), maximum fluorescence – F_m (D) and FSII quantum yield – F_v/F_m (E) from *Physalis peruviana* L., plants submitted to electrical conductivity of irrigation water.

On the other hand, biostimulant increased by 40% the phytomass accumulation in stem in plants treated with 10 mL L⁻¹ compared to non-treated plants (0.0 mL L⁻¹) (Figure 3A). However, phytomass accumulation in shoot decreased by 20.2% (Figure 3B). Differently, F₀, F_v and F_m increased by 8.2 (Figure 3C), 10.7 (Figure 3D) and 13.4% (Figure 3E) respectively in plants treated with 3.6, 4.0 and 4.1. mL L⁻¹ biostimulant.

Discussion

The application of the doses of the biostimulant of seaweed extracts from *A. nodosum* mitigated the deleterious effects of salt stress, with a beneficial effect on the biomass of the root, leaf and total of *P. peruviana* plants. The beneficial effect on biomass yield and accumulation has been previously reported by Yildiztekin et al. (2018) in pepper plants (*Capsicum annuum* L.). Attenuating effect of biostimulant is associated with growth-promoting substances contained in this seaweed extract. Such compounds affect the physiological state of cells and, consequently, of plant tissues, causing positive and different responses in all plant growth stages (Neumann et al., 2017).

Other authors also observed higher phytomass accumulation in plants after they were treated with seaweed extracts. As reported by Silva et al. (2016), pond apple (*Annona glabra* L.) plants also treated with *A. nodosum* extract accumulated 85.0 and 87.39% more phytomass in shoot and root, respectively, than non-treated plants. Other seaweed extracts also increased plant biomass. Rouphael et al. (2017) found that application of 3 mL L⁻¹ *Ecklonia maximus* extract increased by 17.4% the plant shoot biomass in zucchini (*Cucurbita pepo* L.) plants under salinity conditions. Moreover, results showed that biostimulant application attenuated salt stress damage on the photosynthetic apparatus since ECw above 4.38 dS m⁻¹ reduced F_v/F₀ and hence the plant photochemical conversion. Thus, osmoprotectants contained in the *A. nodosum* extracts exerted photoprotective action on energy dissipation and excitation as well as on electron transport in plants under stress conditions (Demming-Adams et al., 2017).

The seaweed extract also contain amino acids and phytohormones, substances capable of mitigating damages caused by saline and oxidative stress in plants by increasing cell membrane as well as antioxidant and enzyme activity. Because of this, application of these extracts improved plant performance under saline stress conditions and favouring the plant vegetative growth (Raghunandan et al., 2019).

The Chl a / b ratio suffered a positive effect of the biostimulant in *P. peruviana* plants, where under stress conditions, the plants reduced Chl a / b, indicating that the application of *A. nodosum* extract increased the content of Chl a and biogenesis chloroplasts, delaying the degradation of chlorophyll, as previously reported by Jannin et al. (2013) in rapeseed plants (*Brassica napus* L.) in agricultural environments managed with salt. A decrease in photosynthetic pigment concentration may result in changes in excitation energy efficiency by light-harvesting complexes and damages in the photosynthetic reaction centre, as well as it affects the

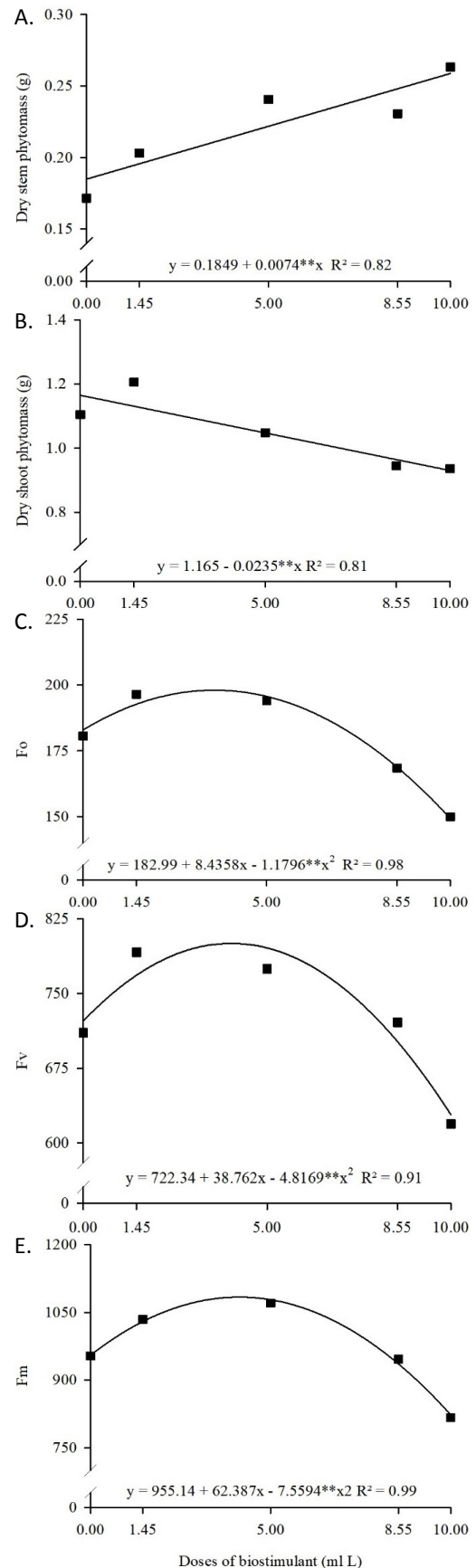


Figure 3. Phytomass dry stem (A), dry shoot (B), initial fluorescence - F₀ (C), variable fluorescence - F_v (D) and maximum fluorescence - F_m (E) from *Physalis peruviana* L. plants submitted at doses of *Ascophyllum nodosum* based biostimulant.

splitting of water and hence limits electron transferring for photochemical process continuity (Tatagiba et al., 2014; Melo et al., 2017). However, similar responses of plants for chlorophyll content and fluorescence in our results demonstrates that the photosynthetic apparatus was little affected by salinity when plants were treated with the seaweed extract.

The deleterious effect of salinity on the photosynthetic apparatus is common in Solanaceae plants. For instance, in bell pepper (*C. annuum* L.) plants, F_o , F_v and F_m reduced significantly above the estimated EC_w level of 3.98 dS m⁻¹ (Cavalcante et al., 2019). Such response was also observed in tomato (*S. lycopersicum* L.) plants under increased salinity of irrigation water (Tatagiba et al., 2014). Similarly, Silva et al. (2015) found that F_v and F_m reduced in eggplant (*S. melongena* L.) plants under water restriction.

Our results suggest biostimulant application may increase biomass accumulation and reduce Chl fluorescence in *P. peruviana* plants by providing and balancing growth regulators in the plant, which favour cell division and improve stem biomass production concerning shoot. Phytohormones, such as cytokine, jasmonate, salicylic acid and ethylene, as well as betaine, polyamines and mineral nutrients contained in the biostimulant may act in plant growth regulation and therefore favouring biomass accumulation (Jannin et al., 2013; Ye & Murata, 2016; Kałużewicz et al., 2017). At the concentrations used in this study, biostimulant preserved the photosynthetic apparatus thereby keeping light transfer to reaction centres which thus resulted in higher energy conversion and photosystem activity. Also, it increased Chl content by providing nitrogen to plant cells thereby improving photosynthetic activity and hence photoassimilates production (Sosnowski et al., 2019).

Conclusions

The salinity of irrigation water reduces the phytomass production and the fluorescence and chlorophyll indices of *Physalis peruviana* plants.

The 9.9 mL L⁻¹ dose of *A. nodosum* extract-based biostimulant attenuates the effect of salt stress on *P. peruviana* biomass production.

Biostimulant application up to 4.1 mL L⁻¹ increases chlorophyll fluorescence rates in *P. peruviana* plants.

Acknowledgements

The Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq) for the granting of scholarships to graduate students of the work.

Compliance with Ethical Standards

Author contributions: Conceptualization: JSN, RTF; Data curation: JTAF, FRAF, WCF; Formal analysis: JSN, RTF; FRAF;

WCF; Investigation: JTAF, FJSP, MFM; Methodology: JSN, TJD, RTF; Project administration: JSN, RTF; Resources: JSN, FJSP, TJD; Supervision: TJD; Validat; Writing – original draft: JSN, MFM, TJD; Writing – review & editing: JSN, TJD.

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

Funding: This work has no financial funding.

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