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The application of a biostimulant based on *Ascophyllum nodosum*, fulvic acid and nutrients mitigates water deficit in soybean

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Abstract

This study was intended to assess the effects of a biostimulant based on *Ascophyllum nodosum* and fulvic acid in the mitigation of water deficit in soybean plants. The experimental design was completely randomized in a 2 x 4 + 1 factorial scheme (two application times, four proportions of biostimulant, and a control. 65 days after sowing, plant height, stem diameter, number of leaves, leaf area, gas exchange, photosynthetic pigments, membrane damage, and dry masses were measured. The proportion of 0.250 kg ha^{-1} before the imposition of water deficit positively increased growth, gas exchange variables, and the relative chlorophyll index ($p < 0.05$). Leaf water potential was less negative ($p < 0.05$) when applying the proportion of 0.100 kg ha^{-1} before or 0.250 kg ha^{-1} after water deficit. The damage was reduced when 0.250 to 0.500 kg ha^{-1} was applied before the imposition of water deficit ($p < 0.05$). The biostimulant mitigated the effects of water stress on soybean, mainly when applied before the imposition of stress.

Keywords: *Glycine max* (L.) Merrill, Abiotic stress, Anti-stress effect.

Abbreviations: IRGA_infrared gas analyzer, DAS_ Days after transplant, H_Height, T_Time, RGR_Relative growt rate, SD_Stem diameter, RV_Root volume, TDM_Total dry mass, NL_number of leaves, LA_Leaf area, RDM_root dry mass, SDM_shoot dry mass, RL_root length, *A*_Photosynthesis, *gs*_stomatal conductance*, E*_transpiration, iCE (*A*/Ci)_instantaneous carboxylation efficiency, EL_electrolyte leakage, Ψw_water potential, ANOVA_analysis of variance, Ad_Additional control, Fact_factorial treatments, P_Proportion of the biostimulant

Introduction

Water stress is a relevant environmental problem faced in agriculture, especially regarding water deficit, which may cause disturbances in plants and negatively affects production. According to Ihuoma and Madramootoo (2017), water stress tends to disrupt the balance of plants, which changes physiological conditions. Therefore, the use of irrigation in agriculture and, consequently, water consumption has grown considerably worldwide.

In this context, in arid and semi-arid regions, rainfall irregularity is one of the main limiting factors for food production. In these regions, the occurrence of prolonged droughts associated with high temperatures cause significant damage to plant tissues, which is intensified according to the duration, intensity and frequency. Therefore, they affect agricultural production of the main crops of interest (Matos et al., 2014; Carrizo et al., 2021).

Among these crops, we can highlight soybeans (*Glycine max* L.), considered the crop of greatest economic importance in Brazil, a title acquired because it has shown constant growth in recent years; with a production of 120.39 million tons in the 2019/2020 harvest (Conab, 2020). Large-scale cultivation of this crop requires high initial investments in fixed capital; however, it shows high liquidity, resulting in several possibilities of obtaining better yields compared to other crops cultivated in Brazil (Carrer et al., 2020).

The increases in soybean productivity are directly related to the technological advances in crop management, use of agricultural machinery, genetic improvement, as well as the greater efficiency of the agricultural products. Among the new technologies used that seek to increase soybean yields, the use of biostimulants has been gaining attention. These substances, which can be synthetic or natural, are easy to apply and can be administered in soil applications, foliar applications, and seed treatments (Dourado Neto et al., 2014).

Biostimulants favor the expression of the genetic potential of plants through changes that take place in vital and structural processes, promoting a greater hormonal balance and stimulating the development of the root system, since it accelerates the process of fungal colonization in the roots (Santos et al., 2020). In plants, these products increase the absorption of water and nutrients, as well as their resistance to stresses and to the residual effects of herbicides in the soil (Rosa et al., 2021).

Despite the increasing number of studies about biostimulants in agriculture, the correlation of their application with adaptive responses to water stress are still poorly known. Hence, this study was intended to assess if the use of a biostimulant based on *Ascophyllum nodosum*, fulvic acid and nutrients helps in the mitigation of water deficit in soybean plants, seeking to understand which morpho-physiological mechanisms are involved in the tolerance of soybean under these conditions.

Results

In the analysis of variance shown, the variable "height" (H) was significant at the level of 1% for the factor "time" (T) and for the interaction between the time and the proportion of the biostimulant (P). On the other hand, the variable "relative growth rate" (RGR) did not have a significant effect ($p>0,05$) on the interaction among individual factors. The variables "stem diameter" (SD), "root volume" (RV), and "total dry mass" (TDM) showed significant effects at the levels of 5 and 1%, respectively, only for the additional control (Ad) x factorial treatments (Fact) interaction ($p < 0.05$). For the variables "number of leaves" (NL), "leaf area" (LA) and root dry mass (RDM), there was a significant effect for the factors "E" and "Ad x Fact". The shoot dry mass (SDM) was significant ($p < 0.05$) only for the factor "proportion" of the biostimulant, while the root length (RL) had no significant effect ($p > 0.05$).

When assessing the mean height values at the different application times (Table 3), it is observed that the application of the product before imposing water deficit provided a greater increase in plant height. For example, when applied 0.100 kg ha^{-1}, a mean of 57.83 cm was obtained, while the same dosage applied after the imposition of deficit, showed a mean of 46 cm. When comparing these means, there is an increase of 25.71%. It is important to highlight that only two treatments were lower than the control, T1 (0 kg ha^{-1} before stress) and T4 (0.100 kg ha^{-1} after stress), which indicates that the application of the biostimulant in some of these proportions can mitigate the effects of water deficit.

In Figure 1, we have the charts for the variable "RGR", where the effects of substrate proportions and application time were observed. In general, the proportions of 0.100 and 0.250 kg ha^{-1} provided a higher RGR. For the factor "application time", a positive effect was observed when the biostimulant was applied before stress, with a statistically higher mean than the plants that received the product after stress.

In Figure 2, we have the graphs for the variables "SD" and "RV". For these variables, the mean of the control treatments were higher than the means of the factorial treatments, indicating that, under optimal conditions of water availability, soybean adequately performed its metabolic processes, which resulted in plants with greater stem diameter and root volume than stressed plants that received the biostimulant.

In Figure 3A, we have the variable "number of leaves" as a function of the application period. The application of the biostimulant before stress was more efficient as a way to mitigate the harmful effects of water deficit. When comparing the control with the mean of the factorial treatments (Figure 3B), there was a small reduction in the treatments that suffered stress. However, severe effects were mitigated due to the application of the biostimulant, since the plants were under severe water deficit stress.

Analyzing the means for leaf area (Figure 4), the same pattern reported previously can be observed. Again, the application of the biostimulant before stress was more efficient; in this case, the increase was 23% when applied before. When comparing the control and the factorial treatment, there was a decrease of 13.34%, but this reduction in leaf area did not affect plant height (Table 3).

In Figure 5, we can observe that the variables "dry mass" were significantly affected by stress. In Figure 5A, there was a reduction in RDM as a function of the application time, with the application before stress showing the best results. In Figure 5B, we have the factorial treatments *versus* the additional control, with an observed reduction of approximately 50% in RDM. This result is correlated to the variable "RV", which showed a reduction compared to the control. In figures 5C and 5D, the saline treatments were statistically lower than the additional control treatment.

In the analysis of variance shown, only the variables "photosynthesis" (*A*) and stomatal conductance (*gs*) showed a significance of 1% probability by the F test, for all assessed factors, as well as for the interaction between the factors for *A* and *gs*. The variable "SPAD" showed significance for the isolated factor "time" and for the interaction between the factors (T x P) at 5% probability by the F test, where the isolated factor "proportions" was not significant. For transpiration (*E*), a significant effect ($p < 0.05$) was observed for the factor "proportions" and for the T x P interaction, while, for instantaneous carboxylation efficiency (*A/Ci*), a significance ($p < 0.05$) was observed only for the factor "proportions".

Regarding the mean values of the relative chlorophyll index (Table 4), the general mean value of the application before the imposition of water deficit was higher than the absolute control ($p<0,05$), while the general mean value of application after the imposition of water deficit was lower. Concerning the best proportion, we can infer that when 0.100 kg ha^{-1} of the product was applied before the imposition of water deficit, a higher mean was obtained in relation to the others.

The assessment of the SPAD index showed no difference ($p>0,05$) between the application times and their different proportions in comparison to the control (Table 4). In general, the proportion of 0.100 kg ha^{-1} before the imposition of water deficit provided higher values of the absolute means of the SPAD index, which may be related to the higher values of height in this proportion. The results for photosynthesis (Table 5) show that regardless of the application time of the product and the proportion, the mean values were lower than the control. However, when comparing the general means for the factor "time", the application of the biostimulant, before water deficit, resulted in a higher mean (21.42 µmol CO_2 m^{-2} s^{-1}) compared to the treatments applied after stress (18.43 µmol CO_2 m^{-2} s^{-1}). An increase of 16.22% is observed for application before stress compared to application after stress.

When evaluating the best proportion of the biostimulant, the application of 0.250 kg ha^{-1} in the two application times obtained the highest means. When comparing the means of the proportion of 0.250 kg ha^{-1} with the proportion of 0.500 kg ha^{-1}, a reduction of 37.23% and 19.32% is verified for application before stress and after stress, respectively. We highlight that only the treatment before stress in the proportion of 0.250 kg ha^{-1} did not differ statistically from the control.

Analyzing the stomatal conductance (Table 6), regardless of the application time, the means were lower than the control. Comparing the best proportion and the application time it was observed that after the application of 0.250 kg ha^{-1} before the imposition of stress, the *gs* values showed the highest means, which is in line with the observed increases for *A*. It is important to emphasize that only the treatment where the product was applied before stress in the proportion of 0.250 kg ha^{-1} showed no difference compared to the control, proving to be the most efficient proportion to mitigate the effects of the assessed water stress.

When studying transpiration (Table 7), regardless of the application time or the proportion of the biostimulant, the means were lower than the absolute control, with the exception of the proportion 0.250 kg ha^{-1} before the imposition of stress although they did not differ from the control. Comparing the proportions 0.250 kg ha^{-1} and 0.500 kg ha^{-1}, there was an increase of 18.60% when using the lowest proportion.

As shown in Figure 6, the transpiration values (*E*) as a function of the proportions showed higher means when the proportion of 0.250 kg ha^{-1} was used, compared to the proportions of 0 kg ha^{-1} and 0.100 kg ha^{-1}, and no difference was observed comparing to 0.500 kg ha^{-1}. When comparing the application of 0.250 kg ha^{-1} and 0.100 kg ha^{-1}, the means of 7.46 mmol H_2O m^{-2} s^{-1} and 6.19 mmol H_2O m^{-2} s^{-1} were obtained, representing an increase of 20.51% for the proportion of 0.250 kg ha^{-1}.

When assessing the instantaneous carboxylation efficiency (*A/Ci*) (Table 8), regardless of the application time, the means were lower than the absolute control. When comparing the two application times only in the proportion of 0 kg ha^{-1}, significant differences are observed. It is important to emphasize that the treatments with application before stress and after stress in the proportions of 0.100 kg ha^{-1} and 0.250 kg ha^{-1} showed no difference in comparison to the control.

In Figure 7, the iCE efficiency values as a function of the proportions showed higher means in the presence of the biostimulant, with the proportions of 0.100 kg ha^{-1}, 0.250 kg ha^{-1} and 0.500 kg ha^{-1} being statistically equal. On the other hand, in the absence of the biostimulant (proportion 0 kg ha^{-1}) we observed the lowest mean. The proportion of 0.250 kg ha^{-1} showed a mean of 0.10 in the instantaneous efficiency of fixing the available CO_2; while, at the proportion of 0 kg ha^{-1}, the value was 0.7.

Regarding the instantaneous carboxylation efficiency (*A/Ci*), the proportions of 0.100 kg h^{-1}, 0.250 kg h^{-1} and 0.500 kg h^{-1} showed the highest means compared to the absolute control. Therefore, the proportion of 0.250 kg ha^{-1} of the biostimulant applied via the leaves before the imposition of water deficit enhances the photosynthetic apparatus and, consequently, the physiological processes of the plant, causing a greater stomatal opening, enhancing transpiration and liquid photosynthesis, as well as instantaneous carboxylation efficiency. In the analysis of variance shown, when we assessed the factor "time", only the variable "electrolyte leakage" (EL) was significant at 1% probability by the F test while, for the factor "proportions", only the variable "water potential" (Ψw) was significant. For the interaction between "time" and "proportions", the two variables obtained significance at 1 and 5% probability by the F test.

When assessing the mean values of the water potential in the pre-dawn (Ψw) (Table 9), all treatments differed from the additional control, showing that the plants were under stress. The application of 0.100 kg ha^{-1} before deficit and 0.250 kg ha^{-1} after the imposition of water deficit provided a lower mean of water potential. Generally, the proportions of the biostimulant that promoted better water savings were 0.100 kg ha^{-1} before deficit and 0.250 kg ha^{-1} after imposing water deficit. Therefore, an application before the occurrence of abiotic stresses, such as water, stimulates plants to improve water saving, minimizing the effects of water deficit.

For membrane damage estimated by electrolyte leakage (Table 10), the treatment with a proportion of 0 kg ha^{-1} after stress had a higher mean than the additional control, reflecting a greater damage to membrane integrity. On the other hand, the treatments using 0.250 and 0.500 kg ha^{-1} before imposing water deficit showed lower means than the additional control, reflecting the beneficial effect of the biostimulant, since the damage caused by stress was reduced. The application of 0.250 and 0.500 kg ha-1 before the imposition of water deficit caused the lowest means of EL, indicating that the effects caused by stress were reduced in the plants.

Discussion

Water is an important environmental factor that regulates the growth and development of a plant. Therefore, plant height is an important indicator of water deficit. Therefore, we have observed that the application of the biostimulant at both times showed different responses, regardless of the proportion, with a higher mean when applied before stress. In general, there were no significant differences comparing to the absolute control. The use of the biostimulant probably provided an improved development, with higher plant height, even under water stress. Accordingly, studies conducted by Bertolin et al. (2010) showed that the use of a biostimulant in the *Vitória* grape cultivar also caused greater plant heights.

Almeida et al. (2014) when evaluating the agronomic performance of common bean, cv. Pérola, with the application of Stimulate®, concluded that the application, in the vegetative stage or at the beginning of the reproductive phase, improved nodulation, root growth, and the contents of soluble sugars and total amino acids. This indicated that the action of this biostimulant must have caused changes in the plant's metabolism that promoted growth, even under water deficit.

In this context, RGR is the most suitable method to assess plant growth, as it represents the amount of biomass produced from a certain amount of pre-existing biomass in a given period. This parameter also depends on the CO_2 assimilation efficiency of the leaves (Lima et al., 2007). Accordingly, the application of the biostimulant in the proportions of 0.100 and 0.250 kg.ha^{-1} before stress caused an improved biomass production, compared to the pre-existing biomass, corroborating with the growth data, where higher means were observed for these proportions.

Stem diameter (SD) is also a widely used variable due to its easy measurement and importance against plant lodging. In addition, it is a biometric aspect that changes according to the physiological aspects and needs of the plant. Generally, a decreased stem diameter is associated with water deficit. The data in this article suggest a reduction in this parameter. The reduction in root volume was 38.63%, which is an effect of deficit. This reduction suggests that there was a lower investment of photoassimilates for the development of the root system, although no significant effect was observed for root length. Similar results were found by Martins et al. (2016) when working with the application of a biostimulant in corn seeds. They found that, at 60 days after sowing, stem diameter and root volume did not show significant responses to the biostimulant. However, the experimental design did not allow them to evaluate the interaction

Table 1. Soil fertility analysis.

C	OM	pH	P	K	Ca	Mg	Na	Al	H+Al	BS	CEC	V	ESP	EC
g kg^{-1}			mg dm^3	mmolc dm^3								%		dS m^{-1}
7.39	12.75	6.6	102	1.24	20.7	6.6	0.33	ND	8.3	28.8	37.1	78	1	0.35

Note. OM – Organic Matter; BS – Base Saturation; CEC – Cation Exchange Capacity; ESP – Exchangeable Sodium Percentage.

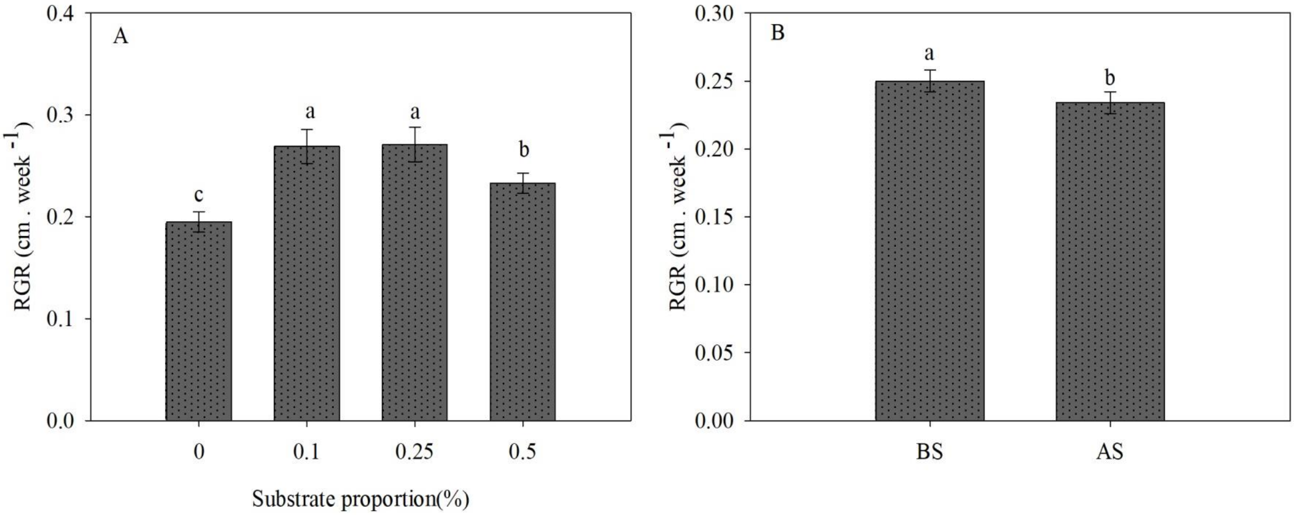

Figure 1. Relative growth rate in soybean plants as a function of additional x factorial treatment and substrate proportion. Means followed by the same letter do not differ by Tukey's test at 5% probability.

Table 2. Biostimulant composition.

	Nutrients - %									Natural compounds - %
	N	Mg	S	B	Cu	Fe	Mn	Mo	Zn	*Ascophyllum nodosum* + Fulvic acid
FH Attivus$^®$	2	1	3.6	0.1	0.04	0.05	0.1	0.1	0.2	15

Note. Source: Rosa et al. 2021.

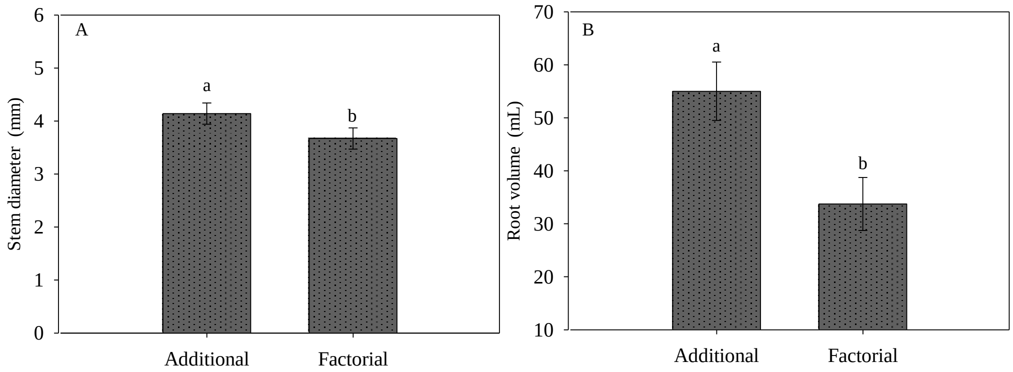

Figure 2. Stem diameter and root volume in soybean plants as a function of additional x factorial treatment. Means followed by the same letter do not differ by Tukey's test at 5% probability.

Table 3. Mean height values (H) in soybean plants subjected to water stress. Two application times and four proportions of biostimulant.

Product application	Proportions kg.ha^{-1}				
	0	0.100	0.250	0.500	Mean
	Plant height				
Before stress	48.62 aB*	57.83 aA	56.37 aA	56.37 aAB	54.41 a
After stress	51.00 aA	46.00 bA*	52.25 aA	52.25 aA	50.06 a
Control	55.05				
CV %	3.84				

Means followed by the same letter (lowercase) in the column and (uppercase) in the row do not differ by Tukey's test at 5% probability. Means containing * differ from additional treatment (control) at 5% probability by Dunnett's test.

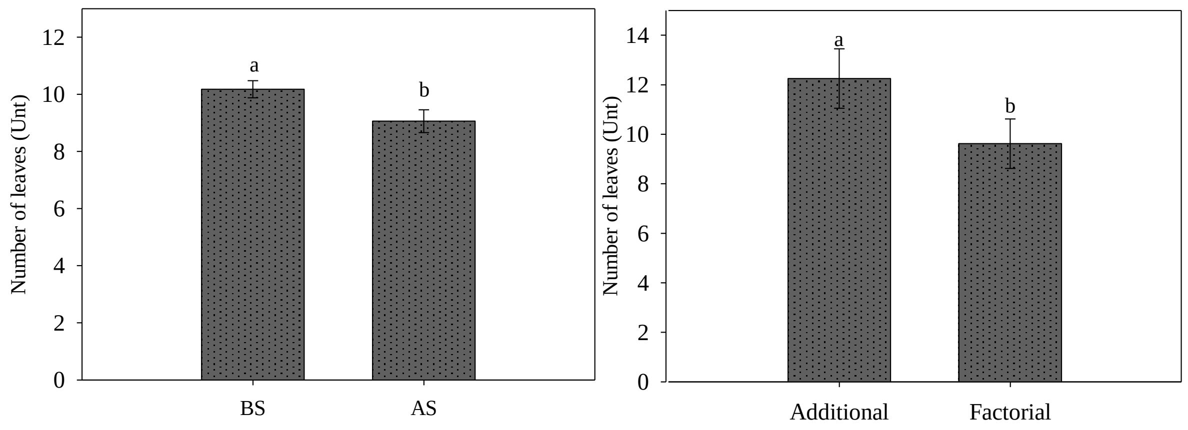

Figure 3. Number of leaves in soybean plants as a function of application times and between additional x factorial treatment. Means followed by the same letter do not differ by Tukey's test at 5% probability.

Table 4. Mean values of relative chlorophyll index (SPAD) in soybean plants subjected to water stress, two application times and four proportions of biostimulant.

Product application	Proportions kg ha^{-1}				
	0	0.100	0.250	0.500	Mean
	SPAD index				
Before stress	40.95 a	41.80 a	39.90 a	39.35 a	40.50 a
After stress	37.42 b	38.85 b	40.77 a	39.57a	39.15 a
Control	39.82				
CV %	5.02				

Means followed by the same letter (lowercase) in the column and (uppercase) in the row do not differ by Tukey's test at 5% probability. Means containing * differ from additional treatment (control) at 5% probability by Dunnett's test.

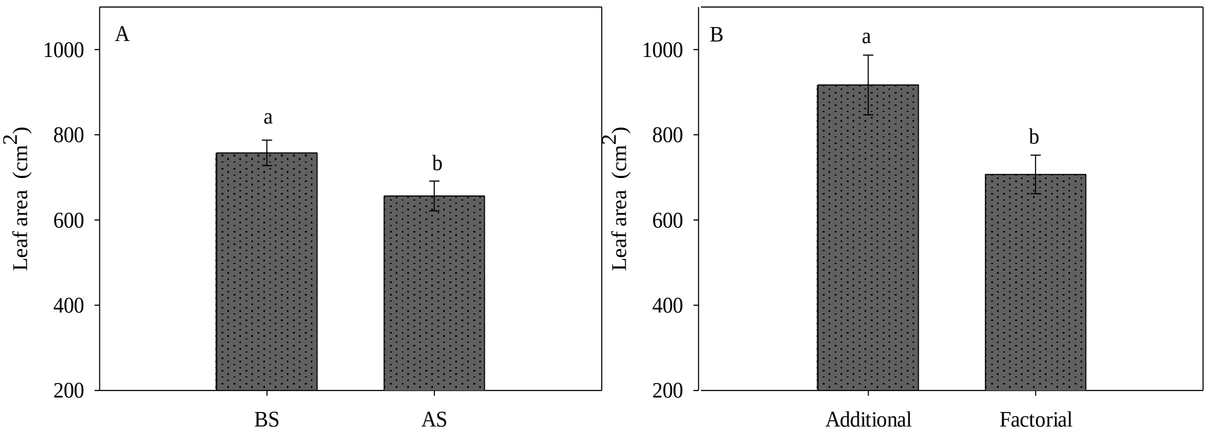

Figure 4. Leaf area in soybean plants as a function of application times and between additional x factorial treatment. Means followed by the same letter do not differ by Tukey's test at 5% probability.

Table 5. Mean values of net photosynthesis (*A*) in soybean plants subjected to water stress, two application times and four proportions of biostimulant.

Product application	Proportions kg.ha^{-1}				
	0	0.100	0.250	0.500	Mean
	A (µmol CO_2 m^{-2} s^{-1})				
Before stress	16.82 aB*	23.72 aB*	26.13 aA	19.04 aA*	21.42 a
After stress	16.11 aB*	19.14 bAB*	20.93 bA*	17.54 aA*	18.43 b
Control	28.52				
CV %	6.77				

Means followed by the same letter (lowercase) in the column and (uppercase) in the row do not differ by Tukey's test at 5% probability. Means containing * differ from additional treatment (control) at 5% probability by Dunnett's test.

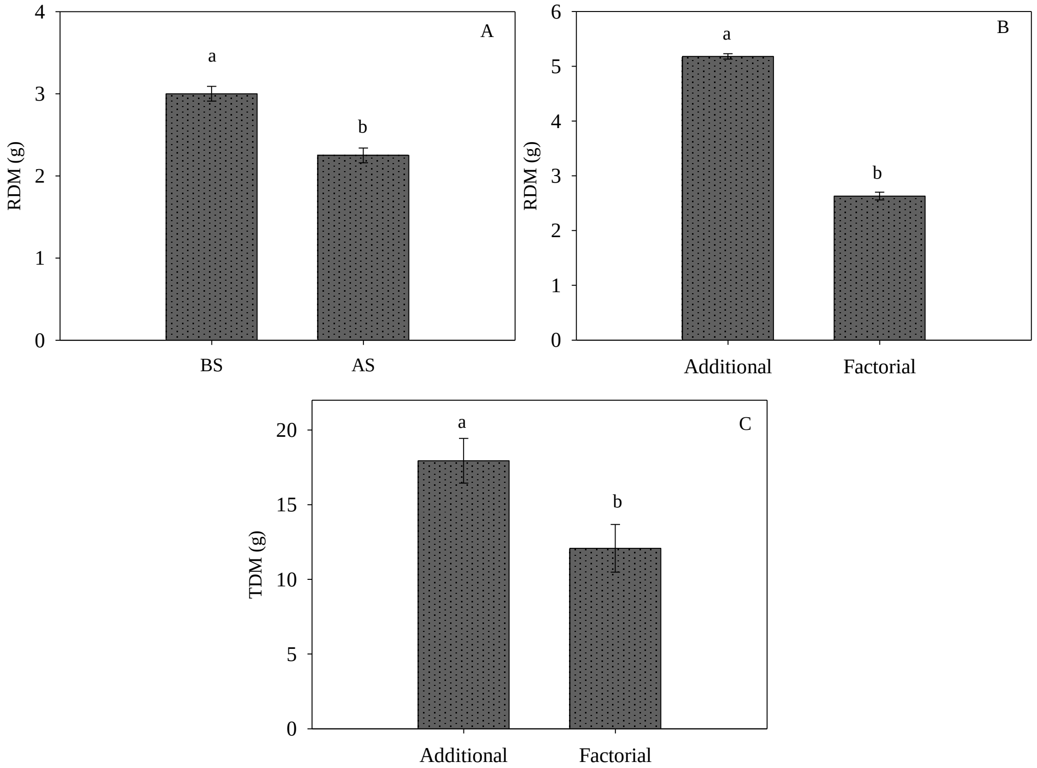

Figure 5. Root dry mass, shoot dry mass, and total dry mass of soybean plants subjected to water deficit. Means followed by the same letter do not differ by Tukey's test at 5% probability.

Table 6. Mean values of stomatal conductance (*gs*) in soybean plants subjected to water stress, two application times and four proportions of biostimulant.

Product application	Proportions kg.ha^{-1}				
	0	0.100	0.250	0.500	Mean
	gs (mol.m^{-2}.s^{-1})				
Before stress	0.35 aB*	0.44 aB*	1.00 aA	0.45 aB*	0.56 a
After stress	0.32 a*	0.40 a*	0.49 b*	0.44 a*	0.41 b
Control	1.25				
CV %	6.77				

Means followed by the same letter (lowercase) in the column and (uppercase) in the row do not differ by Tukey's test at 5% probability. Means containing * differ from additional treatment (control) at 5% probability by Dunnett's test.

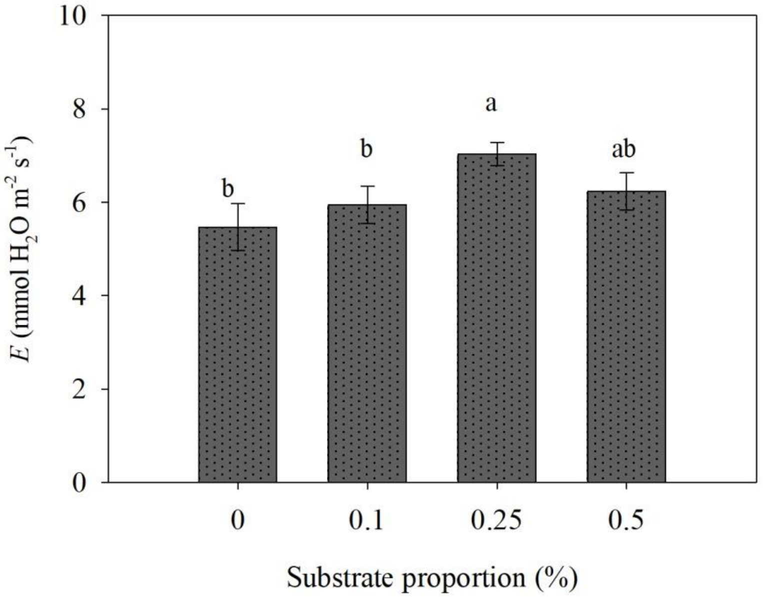

Figure 6. Soybean plant transpiration as a function of the applied proportions. Means followed by the same letter do not differ by Tukey's test at 5% probability.

Table 7. Mean values of transpiration (*E*) in soybean plants subjected to water stress, two application times and four proportions of biostimulant.

Product application	Proportions kg.ha^{-1}				
	0	0.100	0.250	0.500	Mean
	E (mmol H_2O m^{-2} s^{-1})				
Before stress	5.28 aA*	6.19 aA*	7.46 aA	6.29 aA*	6.30 a
After stress	5.66 aA*	6.69 aA*	6.61 aA*	6.18 aA*	6.28 a
Control	8.12				
CV %	22.14				

Means followed by the same letter (lowercase) in the column and (uppercase) in the row do not differ by Tukey's test at 5% probability. Means containing * differ from additional treatment (control) at 5% probability by Dunnett's test.

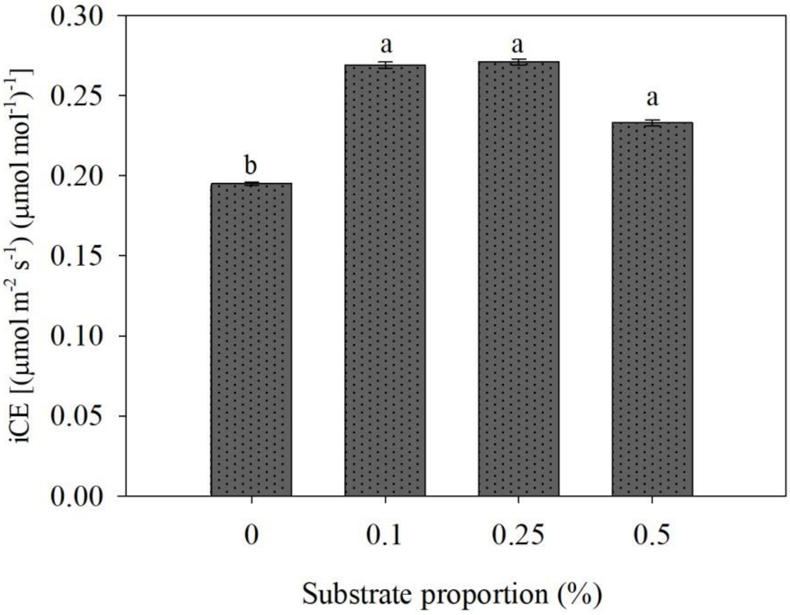

Figure 7. Instantaneous carboxylation efficiency (iCE; *A/Ci*) of soybean plants as a function of the applied proportions. Means followed by the same letter do not differ by Tukey's test at 5% probability.

Table 8. Mean values of instantaneous carboxylation efficiency (*A/Ci*) in soybean plants subjected to water stress, two application times and four proportions of biostimulant.

Product application	Proportions kg.ha^{-1}				
	0	0.100	0.250	0.500	Mean
	(A/Ci)				
Before stress	0.08 aB*	0.10 aA	0.10 aA	0.08 aB*	0.09 a
After stress	0.07 bB*	0.10 aA	0.10 aA	0.08 aB*	0.08 a
Control	0.12				
CV %	15.41				

Means followed by the same letter (lowercase) in the column and (uppercase) in the row do not differ by Tukey's test at 5% probability. Means containing * differ from additional treatment (control) at 5% probability by Dunnett's test.

Table 9. Mean values of water potential (Ψw) in soybean plants subjected to water stress, two application times and four proportions of biostimulant.

Product application	Proportions kg.ha^{-1}				
	0	0.100	0.250	0.500	Mean
	Ψw				
Before stress	-0.58 bC*	-0.44 aA*	-0.47 aAB*	-0.55 aBC*	-0.51 a
After stress	-0.49 a*	-0.55 b*	-0.44 a*	-0.50 a*	-0.49 a
Control	-0.085				
CV %	37.7				

Means followed by the same letter (lowercase) in the column and (uppercase) in the row do not differ by Tukey's test at 5% probability. Means containing * differ from additional treatment (control) at 5% probability by Dunnett's test.

Table 10. Mean values of electrolyte leakage (EL) in soybean plants subjected to water stress, two application times and four proportions of biostimulant.

Product application	Proportions kg.ha^{-1}				
	0	0.100	0.250	0.500	Mean
	EL (%)				
Before stress	21.64 aAB	23.52 aA	18.97 aB*	18.90 aB*	20.75 a
After stress	26.52 bB*	19.12 bA	21.65 aAB	19.38 aB	21.66 b
Control	21.09				
CV %	9.95				

Means followed by the same letter (lowercase) in the column and (uppercase) in the row do not differ by Tukey's test at 5% probability. Means containing * differ from additional treatment (control) at 5% probability by Dunnett's test.

between additional x factorial treatment. The variables "number of leaves" and "leaf area" are closely correlated because as the number of leaves increases, there is an exponential increase in leaf area. According to Yokoyama et al. (2018), the leaf area is directly related to the development and productivity of soybean, since it contributes to greater light interception, photoassimilates production and, consequently, the accumulation of biomass in the plant and in the grains (Taiz et al., 2017). Therefore, the increased number of leaves and leaf area following the application of the biostimulant, before imposing the stress, indicate that the product is more responsive during the early phase of soybean development, positively influencing the yield.

Additionally, the reduction in shoot dry mass in plants subjected to water stress is partly due to the restriction in water absorption. As a result, the reduction in biomass values may reflect the metabolic cost of energy to maintain the water flow in the soil-plant system (Oliveira et al., 2016). However, the effects of water deficit may have been minimized, since there was a mitigation of the harmful effects of drought for the physiological parameters.

Table 4 shows the means for the SPAD index. Some studies correlate the relative chlorophyll index to the concentrations of some essential nutrients for plant development, such as copper, sulphur, iron, manganese and, especially, nitrogen in the leaf (Martínez et al., 2017). Thus, these results corroborate with those of growth, since the greatest increment observed for the SPAD index was also observed for other variables, such as plant height and RGR.

The values of *A*, *gs,* and *E* when plants received 0.250 kg ha^{-1}, before the imposition of water deficit, showed a positive correlation among their means, but did not differ from the control probably due to the positive effect of the biostimulant in the early mitigation of water deficit symptoms. Similarly, studies conducted by Rosa et al. (2021) found that the biostimulant based on seaweed extract *Ascophyllum nodosum (L.)* and fulvic acids induced a better response of soybean plants to water deficit, providing an increase in stomatal conductance and photosynthetic activity. In addition, it is known that, when there is a higher incidence of stress in the plant, the values of *A*, *gs* and *E* decrease. In this sense, Fioreze et al. (2013) mentioned in a study with the soybean crop that the variables *A*, *gs* and *E* decrease at the same intensity as water deficit increases.

Osakabe et al. (2014) found that water stress decreased water potential in leaves and stomata closure, which consequently decreases the regulation of genes associated to photosynthesis and to the availability of CO_2 in the plant.

The iCE value is closely related to the intracellular concentration of CO_2 and the rate of CO_2 assimilation. So, the higher values of *A*, *gs,* and *E* provided a higher iCE at the proportion of 0.250 kg ha^{-1}, which may be directly related to the superiority in the variables *A*, *gs,* and *E* in this proportion (Silva et al., 2015; Silva et al., 2021).

Studies show that EL is an indicator of damage to cell membranes, which are impaired due to the formation of free radicals, responsible for greater electrolyte leakage under water restriction, due to increased membrane damage (Fioreze et al., 2013; Jungklang et al., 2017). Accordingly, we can state that the use of 0.250 or 0.500 kg ha^{-1} caused less electrolyte leakage, thus reducing the stress on plants.

In general, the proportions of the biostimulant that promoted greater membrane integrity were 0.250 and 0.500 kg ha^{-1} applied before deficit, probably because the biostimulant applied before the imposition of stress provided better conditioning, inducing the plant to develop membranes that are more resistant.

Materials and Methods

Experiment location

The research was conducted from September to November 2019, lasting 65 days, in the city of Fortaleza-Ceará, Brazil, located in the coastal zone at 15.49 m altitude, 3°43'02" south latitude and 38°32'35" west longitude, in a greenhouse in the experimental area of the Center for Agricultural Sciences (CCA, as per its Portuguese acronym), Pici Campus, at Federal University of Ceará (UFC, as per its Portuguese acronym).

Cultivation conditions and treatments

The cultivation was performed in plastic pots containing 6 kg of medium-textured soil (Table 1). Field capacity (FC) was determined as described by Souza et al. (2000), considering the difference between the weight of wet soil after saturation and free drainage and the weight of air-dried soil.

The treatments were arranged in a completely randomized design (CRD), in a 2 x 4 + 1 factorial scheme (two application times and four biostimulant proportions applied via foliar application) and an absolute control (no biostimulant application and no stress), with 9 treatments and 4 repetitions. The proportions of the biostimulant were: 0 kg.ha^{-1} (T1); 0.100 kg.ha^{-1} (T2); 0.250 kg.ha^{-1} (T3); 0.500 kg.ha^{-1} (T4) applied on the day of the imposition of water stress (V3; 25 DAS) and 0 kg.ha^{-1} (T5); 0.100 kg.ha^{-1} (T6); 0.250 kg.ha^{-1} (T7); 0.500 kg.ha^{-1} (T8) applied after the imposition of water stress, in addition to the absolute control (T9), which did not undergo the imposition of water stress or application of the biostimulant.

The water deficit was imposed at stage V3 (25 Days After Sowing - DAS) and continued until 65 DAS, when the evaluations were performed. To induce water deficit, the corresponding treatments were conducted at 40% of field capacity, while the other treatments were conducted at 80% of field capacity.

The biostimulant used in this work was FH Attivus® (Heringer Fertilizers) whose formulation is based on fulvic acids, algae extract, and nutrients (Table 2).

Sowing and product application

The Faedo Company donated soybean seeds, hybrid Monsoy 8349 IPRO. The seeds were sanitized with a 1% sodium hypochlorite solution for 3 minutes and then washed with distilled water until the product was completely removed. Five seeds were sown per pot, with thinning performed 10 days after sowing (DAS), maintaining one plant per pot.

Maintenance of FC was performed daily in all pots, weighing them and replacing the volume of water lost by evapotranspiration, leaving them with 80% of FC using a digital scale with a capacity of 20 kg until the imposition of water stress. For deficit induction, plant irrigation was suspended and pot mass monitored throughout the day until it reached 40% of FC moisture in the treatments under deficit. The control was maintained at 80% of FC from the beginning.

The application of the biostimulant took place at stage V3, 25 days after sowing (DAS), for treatments from T1 to T4 at 7:00 a.m. on the day before the imposition of water deficit. As for the treatments T5 to T8, the application took place after the imposition of deficit when the plants were in the full flowering stage (R2), at 35 DAS. The application of the biostimulant was done using a pressure sprayer, maintaining the same volume applied for each treatment, and a syrup in the proportion 1.0 kg ha^{-1} was prepared, where the respective dilutions were made for each treatment. In the proportion 0 kg ha^{-1}, distilled water was applied.

Biometric and physiological assessments

At 65 DAS, the following biometric variables were assessed: stem diameter, with the aid of a digital caliper; number of leaves, by counting the trefoils; aerial part height, using a graduated ruler and leaf area, using a leaf area integrator. For the physiological analysis, there were determined: relative chlorophyll index, using a portable SPAD-type chlorophyllometer (Soil Plant Analysis Development, Minolta SPAD-502 model); gas exchange, using an infrared gas analyzer (IRGA); leaf water potential, with the aid of a Scholander-type pressure pump, and membrane damage through electrolyte leakage.

Leaf water potential (Ψw) was measured to determine plant water status at pre-dawn (05:00 to 06:00 a.m.), when the plants are in equilibrium with the soil, using a pressure pump. Scholander-type (Scholander et al., 1965). The level of membrane damage caused by water stress on the leaves was determined through electrolyte leakage. Leaf discs (about 100 mg) were removed, placed in test tubes containing 10 mL of deionized water and maintained at room temperature for 2 hours. Then, the initial electrical conductivity (EC_1) was determined using a benchtop conductivity meter. Subsequently, the tubes containing the leaf discs were heated in a water bath at 95º C for 30 minutes to obtain the final electrical conductivity (EC_2). Electrolyte leakage (EL) was estimated using the equation: $EL = (CE_1/CE_2) \times 100$.

The reading of gas exchange took place at 65 DAS, between 08:00 and 10:00 a.m., where net CO_2 assimilation (*A*), leaf transpiration (*E)*, stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), and instantaneous carboxylation efficiency (iCE; *A/Ci*) were assessed. The readings were taken under saturating light (1200 µmol m^{-2} s^{-1}), with CO_2 concentration and room temperature. To this end, an infrared gas analyzer was used (IRGA, LCi model, ADC BioScientific, England).

Statistical analysis and chart design

The results were subjected to analysis of variance (ANOVA) and the Shapiro-Wilk normality and homogeneity test when significant by the F test, and were subjected to mean comparison analysis by the Tukey's test, through the RStudio computer program. The additional control was compared to other treatments by Dunnet's test at 5% probability ($p \leq 0.05$). The charts were designed using the SigmaPlot program, version 11.0.

Conclusions

The Biostimulant based on *Ascophyllum nodosum*, fulvic acid, and nutrients was effective in the mitigation of the effects of water deficit, showing positive effects both on the biometric characteristics of growth and on the physiological variables, especially when applied before the imposition of water deficit. The proportion of 0.250 kg ha^{-1} increased physiological variables, such as: net photosynthesis, stomatal conductance, transpiration, and instantaneous carboxylation efficiency. In addition, it promoted better water saving and less membrane damage in the presence of water stress. The application of the biostimulant (0.250 kg ha^{-1}), before the imposition of stress, showed the best responses in plants, being, therefore, the recommended proportion and time for the soybean crop under water restriction conditions.

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