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FOLIAR APPLICATION OF ASCOPHYLLUM NODOSUM SEAWEED EXTRACT IN SOYBEANS FOR STRESS MITIGATION

APLICAÇÃO FOLIAR DE EXTRATO DA ALGA ASCOPHYLLUM NODOSUM EM SOJA PARA MITIGAÇÃO DE ESTRESSE

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ABSTRACT

The use of seaweed extracts as biostimulants has emerged as a sustainable strategy to mitigate the effects of abiotic stresses in agricultural crops. The aim of this study was to evaluate the effects of foliar application of *A. nodosum* seaweed extract at different doses and application times, and how this product impacts the mitigation of stress caused by temperature and water scarcity.The experiment was conducted at the Federal University of Santa Maria in a completely randomized design with three treatments: control (0 L·ha^{-1}), 2 L·ha^{-1} (recommended dose), and 4 L·ha^{-1} (twice the recommended dose), applied at the V5-V6 and R1 growth stages. Growth, yield, and biochemical variables were evaluated, including total leaf area, grain yield, thousand grain weight, and the activity of antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD), as well as hydrogen peroxide (H_2O_2) content. The results showed no statistically significant differences in growth and yield variables. However, significant increases in H_2O_2 levels and SOD activity were observed in the seaweed extract treatments, indicating activation of the plant antioxidant defense system. These findings suggest that, although agronomic effects were not pronounced under the experimental conditions, *A. nodosum* extract induces biochemical responses associated with stress tolerance. Further studies with a higher number of replications, under different edaphoclimatic conditions and growth stages, are recommended to better assess the biostimulant's effects.

Keywords: soybean; *Ascophyllum nodosum*; stress mitigation.

RESUMO

O uso de extratos de algas marinhas como bioestimulantes tem se destacado como estratégia sustentável para mitigar os efeitos de estresses abióticos em culturas agrícolas. Este estudo teve como O objetivo deste estudo foi avaliar os efeitos da aplicação foliar do extrato da alga marinha A. nodosum em diferentes doses e épocas de aplicação, e como esse produto impacta a mitigação do estresse causado pela temperatura e pela escassez de água. O experimento foi conduzido na Universidade Federal de Santa Maria em delineamento inteiramente casualizados, com três tratamentos: testemunha (0 L·ha^{-1}), 2 L·ha^{-1} (dose recomendada) e 4 L·ha^{-1} (duas vezes a dose recomendada), aplicados nos estádios V5-V6 e R1 da cultura. Foram avaliadas variáveis de crescimento,

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produtivas e bioquímicas, como: área foliar total, produtividade, peso de mil grãos, e atividade das enzimas antioxidantes superóxido dismutase (SOD) e peroxidase (POD) e os teores de peróxido de hidrogênio (H_2O_2). Os resultados demonstraram ausência de diferenças estatísticas significativas nas variáveis de crescimento e produtivas. No entanto, observaram-se aumentos significativos nos teores de H_2O_2 e na atividade de SOD nos tratamentos com o extrato de algas, indicando ativação do sistema antioxidante das plantas. Esses resultados mostram que, embora os efeitos agronômicos não tenham sido expressivos nas condições experimentais, o extrato de A. nodosum induz respostas bioquímicas associadas à tolerância ao estresse. Novos estudos são recomendados, com maior número de repetições, em diferentes condições edafoclimáticas e fases fenológicas, para observar melhor os efeitos do bioestimulante.

Palavras-chave: *soja; Ascophyllum nodosum; mitigação de estresse.*

1 INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a legume originating from East Asia, especially China, where its cultivation dates back more than five thousand years (EMBRAPA, 2020). Over the centuries, soybean expanded to other continents, reaching the Americas in the 19th century and establishing itself as one of the most important agricultural crops in the world, both for its nutritional value, rich in oils and proteins, and for its industrial versatility.

Currently, soybean is cultivated in several countries, such as the United States, Brazil, China, and Argentina, with a global production of 395.91 million tons (USDA/PSD, 2024). Brazil is the largest producer of this crop, producing around 147.35 million tons on 45.98 million hectares, with an average yield of 3,205 kg/ha (CONAB, 2024).

The global average yield may decrease due to climate change, with prolonged droughts and high temperatures. The full development of a crop is impaired when subjected to water and heat stress, reducing CO_2 assimilation rates due to decreased stomatal action, which in turn reduces leaf, stem, and root size, and consequently, productivity (DIAS, 2018).

According to the same author, plants under heat stress release reactive oxygen species (ROS). ROS originate from aerobic metabolism when stress situations occur; they are part of a signalling network indicating that the plant is under stress. The accumulation of these substances is harmful to plant tissues, as they react with biological molecules and cause irreversible damage. Studies have already shown that when ROS are present, antioxidant enzymes are also present, signalling a battle between stress molecules and defence molecules (BARBOSA, 2013). This scenario reinforces the importance of proper crop management and the adoption of technologies such as cultivars tolerant to abiotic stress and the use of biostimulants to mitigate plant stress.

Biostimulants are substances composed of two or more mixtures of plant hormones such as cytokinin and auxin, together with other substances such as humic acids, fulvic acids, amino acids, vitamins, seaweeds, micronutrients, and ascorbic acids (CAVALCANTE *et al.*, 2020). Seaweeds are part of the biostimulants group, with *Ascophyllum nodosum* being the best known. These seaweeds originate from coastal ocean regions, commonly found on the northwestern coast of Europe, eastern Greenland, and the northeastern coast of North America (MOREIRA *et al*., 2017), and they provide benefits for soybean plant development by improving morphological, physiological, and biochemical traits when plants are under stress (KHAN *et al*., 2009).

According to Khan *et al.* (2019), several studies report the benefits of seaweed extract application to plants, such as improved germination and plant establishment, higher productivity, increased stress tolerance, and extended post-harvest shelf life in perishables. Shukla *et al.* (2019) reported that the compounds present in *A. nodosum* seaweed extract mitigated plant stress caused by water scarcity when applied to plants. According to him, there was an improved genetic response in plants, with gene modulation, enhanced antioxidant system activity, production and accumulation of osmolytes, and greater efficiency in gas exchange due to better stomatal regulation.

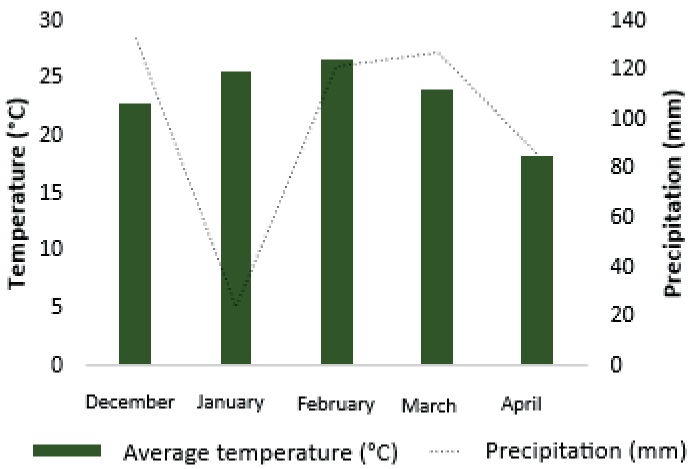
The aim of this study was to evaluate the effects of foliar application of *A. nodosum* seaweed extract at different doses and application times, and how this product impacts the mitigation of stress caused by temperature and water scarcity.

2 MATERIALS AND METHODS

The experiment was conducted at the Federal University of Santa Maria (UFSM), in an area located in the Department of Crop Science, at -29.7 latitude, -53.7 longitude, and 113 meters of altitude. Soybean was sown under wheat residue, and the chosen cultivar was NEO 660 IPRO, with an indeterminate growth cycle. The soil at the site is classified as Argissolo Vermelho Distrófico Arênico, according to the classification of Santos *et al.* (2018). The climate of the region is defined as humid subtropical with hot summers, type Cfa, without a defined dry season, according to Köppen and Geiger's classification (ALVARES *et al*., 2013). Sowing was carried out on December 5, 2024, and after full emergence, 95% of seedlings had emerged. The row spacing was 45 cm.

The experiment was conducted in a completely randomized design, consisting of three treatments, totaling 20 plots. Each plot measured 2.25 m in width and 2.24 m in length, and four soybean rows were sown in each plot. Crop management practices such as fertilization, weed control, fungicides, and insecticides were applied as needed. The total precipitation during the crop cycle was 464 mm (INMET, 2025), and a dry spell occurred in January. As shown in Figure 1, the average monthly temperature and precipitation distribution during the crop cycle remained within the range expected for soybean development.

Figure 1 - Average temperature (°C) and total precipitation (mm) in Santa Maria during the crop cycle.



Source: Authors' own elaboration.

Treatment one consisted of the control; treatment two used 2 L ha^{-1} (recommended label dose) of *A. nodosum* seaweed extract; and treatment three used 4 L ha^{-1} (twice the recommended label dose) of *A. nodosum* seaweed extract. Two applications of the extract were carried out: the first at the V5-V6 vegetative stage, on 12/20/2024, and the second at the R1 reproductive stage, on 01/07/2025. Applications were performed using an electric backpack sprayer equipped with a boom containing five nozzles and a flat-fan type tip. The application volume was 200 L ha^{-1}. Table 1 presents the treatments used in the experiment and their respective doses of *A. nodosum* seaweed extract.

Table 1 - Illustration of treatments and their respective doses.

TREATMENTS	DOSE	NUMBER OF PARCELS
Witness	0 L.ha^{-1}	7
T2	2 L.ha^{-1}	6
T3	4 L.ha^{-1}	7

Source: Authors' own elaboration.

For the analyses, leaf area growth and shoot dry mass (DM) were evaluated. For leaf area (LA) analysis, three plants per plot were collected at the grain-filling stage and taken to the laboratory. Approximately 20% of the leaves were detached, scanned at 200 dpi using a scanner (EPSON Expression 11000 equipped with additional TPU light), and analyzed with WinRHIZO© Pro 2007 software (Regent Instruments, Quebec, Canada). From the scans, the leaf area was obtained. Afterwards, both scanned and non-scanned leaves of each plant were dried in an oven at 65 °C separately to obtain DM. Once dried, leaves were weighed, allowing estimation of their total leaf area. For this, the following equation was used:

$$Leaf\ area = \frac{(DM\ of\ scanned\ sheets + DM\ of\ unscanned\ sheets)\ x\ Leaf\ area}{DM\ of\ scanned\ \ \ heets} \quad (1)$$

To obtain shoot dry mass (SDM), stems from the same collected plants were used and dried for 72 hours in an oven at 65 °C. After that, the stems were weighed. SDM was obtained by summing the stem dry mass and leaf dry mass.

For the determination of superoxide dismutase (SOD), peroxidase (POD), and hydrogen peroxide (H_2O_2), the two most recently fully expanded leaves were collected from ten plants per plot at full flowering. Leaves were frozen in liquid N_2 and subsequently stored in an ultrafreezer at -80 °C. Leaf samples (0.5 g) were homogenized in 3.0 mL of 0.05 M sodium phosphate buffer (pH 7.8), according to the method of Zhu *et al.* (2004). The homogenate was then centrifuged, and the supernatant was used for the determination of peroxidase (POD) activity, according to Zeraik, Souza, and Fatibello-Filho (2008), and superoxide dismutase (SOD) activity, according to Giannopolitis and Ries (1977). Hydrogen peroxide content was determined following Loreto and Velikova (2001). The H_2O_2 concentration in the supernatant was evaluated by comparing its absorbance readings with a standard calibration curve at 390 nm. H_2O_2 concentration was expressed as µmol g^{-1} fresh weight.

Lipid peroxidation was determined by TBARS analysis, based on the quantification of malondialdehyde (MDA), according to El-Moshaty *et al.* (1993). Root, stem, and leaf samples (0.5 g) were macerated and homogenized in 3 mL of 0.05 M phosphate buffer (pH 7.8), following Zhu *et al.* (2004). Absorbance was measured at 532 and 600 nm for turbidity correction, and the results were expressed as nmol MDA per mg protein.

On April 17, 2025, the experiment was harvested using a Classic Plus plot combine (Wintersteiger®). Three crop rows were harvested, each measuring 2.24 meters, and desiccation was carried out one week prior. The grains were separated into raffia bags and stored in the greenhouse of the Biology Department at the Federal University of Santa Maria.

For biometric analysis, three plants were randomly collected from each plot, and the following measurements were taken: plant height (PH - cm), number of productive nodes (PN), number of branches (NB), number of pods (NPd), grains per pod (GP), and thousand-grain weight (TGW). The average yield per hectare (YLD) was obtained by weighing the grains harvested with the plot combine and estimated according to plot size in soybean bags.

The data obtained were subjected to regression analysis ($p < 0.05$) and mean comparison by Tukey's test ($p < 0.05$) using SISVAR® software (Ferreira, 2011).

3 RESULTS AND DISCUSSION

From the polynomial regression analysis, it was found that there was no statistically significant difference for all biometric variables AFT, SDM, PH, PN, NB, NPd, GP, TGW, YLD, and for the biochemical variable's peroxidase enzyme and TBARS. The growth parameters evaluated in the experiment are presented in Table 2. A statistically significant difference was observed for the variable's hydrogen peroxide and superoxide dismutase enzyme with the application of different doses of the seaweed extract product. The productive variables obtained in the experiment are shown in Table 3.

Table 2 - Comparison of means obtained by Tukey's test for the growth parameters total leaf area (TLA), shoot dry mass (SDM-PA), plant height (PH), number of productive nodes (NP), and number of branches (NR).

Treatment	TLA (cm^2)	SDM-WM (g)	PH (cm)	PN	NB
0 $L.ha^{-1}$	5410,76	88,00	106,28	26,42	6,00
2 $L.ha^{-1}$	7171,03	88,66	105,89	27,16	4,83
4 $L.ha^{-1}$	5620,72	86,14	105,28	26,85	4,85

Source: Author's own work.

Table 3 - Comparison of means obtained by Tukey's test for the growth parameters pod number (NV), grains per pod (GV), thousand grain weight (PMG), and average yield (PT).

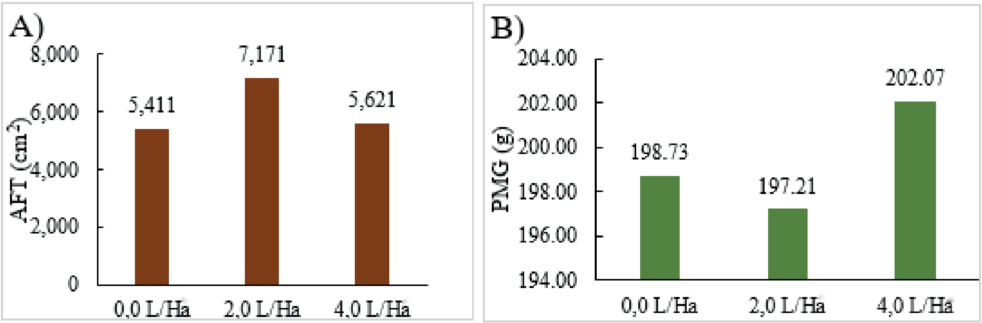
Treatments	NV	GV	PMG(g)	PT (scs)
0 $L.ha^{-1}$	55,14	2,00	198,73	66,19
2 $L.ha^{-1}$	58,00	2,00	197,21	66,65
4 $L.ha^{-1}$	57,42	1,85	202,07	61,34

Source: Author's own work.

The lack of statistically significant difference in the results indicates that the different doses used in the experiment did not influence soybean growth and development in a relevant way compared to the control. This result may be related to the favourable environmental conditions for soybean development, since, as shown in Figure 1, the average monthly temperature did not exceed 30 °C, and the optimal temperature range for soybean development is between 20 °C and 30 °C (MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO, 2020).

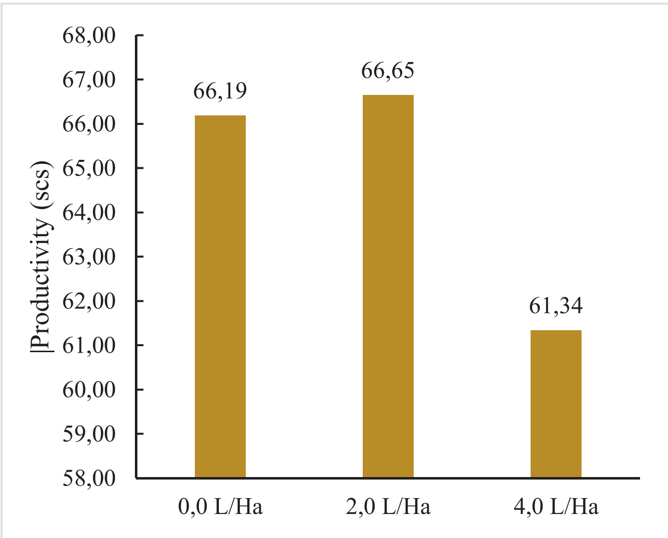
Some trends can be observed and considered, even though they were not statistically significant. T2 (2 L ha^{-1}) achieved the highest AFT (7171.03 cm^2) and total yield (66.65 bags), as shown in Figures 2 and 3, while T3 obtained the highest TGW (202.07), as shown in Figure 4. Although not statistically different, these differences may be important from an agronomic perspective and reflect the need for further studies with larger sample sizes and under different environmental conditions, as they may demonstrate a small response to the use of different doses of the *A. nodosum*-based product. This aligns with previous studies. (CAVALCANTE *et al.*, 2022) used different doses (0.0 L ha^{-1}, 0.5 L ha^{-1}, 1.0 L ha^{-1}, 1.5 L ha^{-1}, and 2.0 L ha^{-1}) of an *A. nodosum* seaweed extract-based product and obtained positive results on plant metabolism, influencing an average increase of one ton per hectare in yield across the different doses, with 2 L ha^{-1} being the most effective. There was also an increase in the number of nodes and leaves according to the increase in dosage. Figures 2 and 3 illustrate the behaviour of total leaf area and grain yield according to the treatments applied. Similar results were reported by Khan *et al.* (2009), demonstrating that the effects of seaweed extracts can vary according to environmental conditions. Thus, the stage of crop development and the level of stress can limit significant agronomic responses, particularly in crop productivity under favourable growing conditions.

Figure 2 - Graph of total leaf area (TLA) and thousand grain weight (MGW) in relation to treatments.



Source: Author's own work.

Figure 3 - Graph of yield (scs) in relation to treatments.



Source: Author's own work.

Different responses according to the applied dose may occur because seaweed-based biostimulants act through complex physiological pathways, and their effectiveness depends on concentration, application timing, and crop physiological status. Another study by (CAVALCANTE *et al.*, 2020), in which different biostimulants (amino acids, seaweed extract, fulvic acids, phytohormones, and nutrients) were compared in soybean, showed that all biostimulants had a significant effect on yield, with seaweed extract in particular promoting a 20.05% increase. In other crops, there are also reports of improved plant development after the application of *A. nodosum*-based products. According to Souza *et al.* (2023), different doses (0, 50, 100, 150, and 200 mL ha^{-1}) of a product containing seaweed were used in maize. Applications were carried out 30 days after sowing, when plants were at the V4 stage. All doses promoted grain yield increases compared to the 0 dose (control), with 200 mL ha^{-1} showing the highest productivity, as well as increases in biometric variables such as plant height, leaf length and width, and thousand-grain weight.

The data obtained from biochemical analyses (Table 3) support the hypothesis that soybean plants did not experience stress levels severe enough to cause yield losses or differences between treatments, since the TBARS analysis, which numerically estimates lipid degradation due to oxidative stress, did not show statistical differences. As observed in Table 4, the increase in hydrogen peroxide content was accompanied by higher superoxide dismutase activity. The increase in superoxide dismutase activity observed in the present study may indicate the activation of the antioxidant defence system, as reported by Barbosa *et al.* (2014), who described SOD as one of the main enzymes involved in the detoxification of reactive oxygen species under stress conditions.

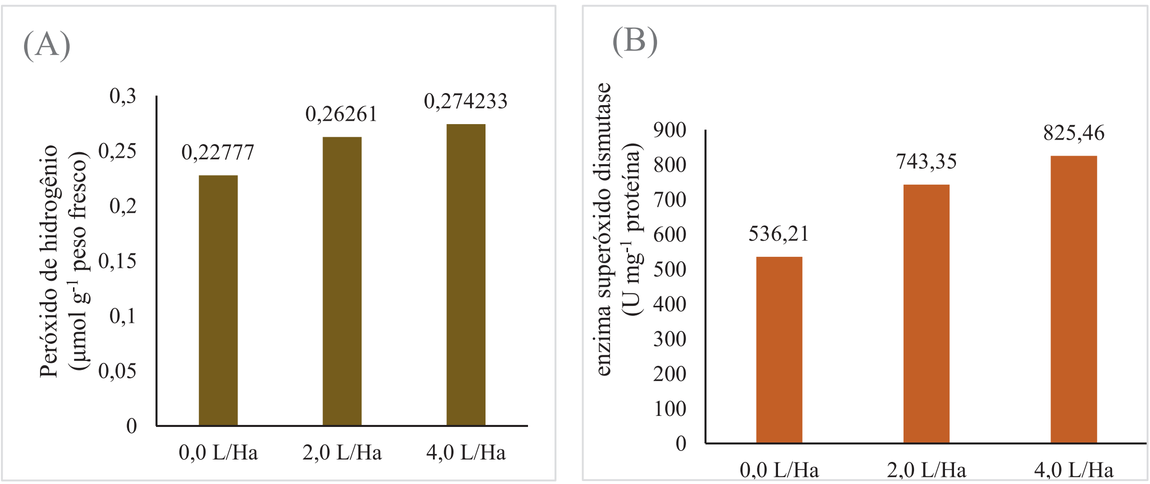
Table 4 - Comparison of means obtained by Tukey's test for the biochemical variables.

Treatments	Hydrogen peroxide	Superoxide dismutase enzyme	Peroxidase enzyme	TBARS
0 $L.ha^{-1}$	0,22 b	536,21 b	10,75 a	0,29 a
2 $L.ha^{-1}$	0,26 a	743,35 a	11,83 a	0,31 a
4 $L.ha^{-1}$	0,27 a	825,46 a	12,30 a	0,36 a

Source: Author's own work.

The variables hydrogen peroxide and superoxide dismutase enzyme increased according to the increase in seaweed extract dose, as shown in Figure 5. According to Nunes *et al.* (2015), superoxide dismutase enzyme activity is related to hydrogen peroxide production. The superoxide dismutase enzyme is responsible for converting superoxide (highly toxic) into hydrogen peroxide (less toxic) (SANCHES *et al.*, 2024), thereby mitigating stress effects. This may have occurred because plants that received the product had a stronger stimulus to combat stress and, therefore, a greater production of these compounds, considering that all plants, regardless of treatment, were under the same field conditions. Figure 4 shows the response of hydrogen peroxide content and superoxide dismutase activity according to the doses of *A. nodosum* extract.

Figure 4 - Graph of hydrogen peroxide (µmol g-1 fresh weight) and superoxide dismutase (U mg-1 protein) in relation to treatments



Source: Author's own work.

4 CONCLUSIONS

The foliar application of *A. nodosum* seaweed extract at different doses in soybean did not result in statistical differences among the evaluated parameters, such as total leaf area, shoot dry mass, plant height, number of productive nodes, number of branches, number of pods, grains per pod, thousand-grain weight, and average yield per hectare.

Although no statistical difference was observed, a trend was noted in some parameters, such as an increase in total leaf area and average yield in treatment 2 (2 L ha^{-1}), and an increase in thousand-grain weight in treatment 3 (4 L ha^{-1}). These differences, although not statistically significant, may be relevant from an agronomic perspective.

Therefore, further studies are recommended, with more replications, additional dosages, and conducted in different locations and application periods.

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