Revista Thema
v.24 | n.1 | 2025
p.18-27

DOI: http://dx.doi.org/10.15536/thema.V24.2025.18-27.3587
ISSN: 2177-2894 (online)

CIÊNCIAS AGRÁRIAS

Foliar application of Ascophyllum nodosum-derived biostimulant affects the cultivation of Cucurbita pepo

Aplicação foliar de bioestimulante derivado de Ascophyllum nodosum afeta o cultivo de Cucurbita pepo

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ABSTRACT

The application of a biostimulant derived from *Ascophyllum nodosum* (Linnaeus) Le Jolis was hypothesized to positively impact pumpkin development and yield. This study evaluated the effects of foliar application of increasing biostimulant doses (0.0, 150, 200, 250, and 300 mL ha^{-1}) on the growth and production of *Cucurbita pepo* L. The treatments demonstrated significant increases in fruit diameter (9.41%), fruit length (8.80%), fruit fresh weight (30.44%), plant height (15.21%), and plant diameter (16.83%) at the maximum dose of 300 mL ha^{-1}. These results indicate that foliar application of the biostimulant enhances fruit development and overall plant growth, with linear responses observed for most parameters. The findings suggest that biostimulants derived from *A. nodosum* can effectively improve pumpkin cultivation while supporting sustainable agricultural practices.

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Keywords: *Ascophyllum nodosum*; biostimulants; *Cucurbita pepo*; sustainable agriculture; seaweed extracts.

RESUMO

Partindo da hipótese de que a aplicação de um bioestimulante derivado de Ascophyllum nodosum pode ter efeito benéfico no desenvolvimento e produção de abóbora, o presente estudo teve como objetivo avaliar os efeitos de sua aplicação, como pulverização foliar, nas características de crescimento e produção de Cucurbita pepo L. Os tratamentos foram compostos por cinco doses de bioestimulantes derivados de Ascophyllum nodosum (0,0 mL ha^{-1}; 150 mL ha^{-1}; 200 mL ha^{-1}; 250 mL ha^{-1}; 300 mL ha^{-1}) e aplicados como pulverização foliar. Verificamos que houve efeito positivo do aumento das doses nas características de diâmetro do fruto, comprimento do fruto, massa fresca do fruto, diâmetro e altura da planta, com aumento de 9,41%, 8,80%, 30,44%, 16,83% e 15,21%, respectivamente. Assim, concluímos que a aplicação de doses crescentes de bioestimulante derivado de Ascophyllum nodosum, até 300 mL ha^{-1}, aumenta o desenvolvimento dos frutos e o crescimento das plantas de Cucurbita peppo L.

Palavras-chave*: sustentabilidade agrícola; bioprodutos; macroalga marrom; produto ambientalmente amigável; algas marinhas.*

1. INTRODUCTION

The search for environmentally friendly options for increasing agricultural production has been one of the focuses of research on plant science (Shahrajabian *et al.*, 2021; Sousa *et al.*, 2021; Vendruscolo; Lima, 2021; Elnahal *et al.*, 2022). In this sense, the use of algae-derived products is one of the lines of great interest for study, given the benefits that can be obtained through their application to the production system, while these are an option for the search for productions more sustainable agriculture, both from an economic and socio-environmental point of view (Zou *et al.*, 2021; Ammar *et al.*, 2022).

Different species of algae have been studied and some have been identified as beneficial to the development of plant species when applied in the form of biostimulants (Kapoore *et al*., 2021). Among these species is *Ascophyllum nodosum* (Linnaeus) Le Jolis (Ammar *et al.*, 2022). This species has a wide occurrence in the northern hemisphere and has characteristics that allow its use as a raw material for obtaining agricultural inputs, such as nutrients and vitamins (Pereira *et al.*, 2020).

The combination of characteristics positively influences the physiological parameters, vegetative growth and also the production of horticultural species, when there is the application of products based on *A. nodosum*. Also, Castronuovo *et al.* (2023) verified that the application of a biostimulant derived from *A. nodosum* improved the water conditions of spinach plants when combined with zeolite, as well as increased the number of leaves when applied alone. The benefits of applying this algae in adverse cultivation conditions were also verified for other species of interest, such as soybean (Repke *et al*., 2022) and tomato (Carmody *et al*., 2020), or even in suitable conditions, in which there was a stimulus to obtain higher productivity for tomato (Vila e Vila *et al*., 2023) and increased grape quality (Frioni *et al*., 2018).For fruit-producing species, such as tomatoes, it was verified that the application of biofertilizer based on *A.*

nodosum resulted in significant gains in the quantitative and qualitative characteristics of production, which were also related to the greater photosynthetic capacity of the treated plants (Subramaniyan *et al.*, 2023). Also, for peppers and tomatoes, it was found that in addition to the benefits to growth and fruit production, the biostimulant derived from *A. nodosum* provided better resistance to pathogens, and could also be combined with fungicide treatment to obtain a synergistic effect (Ali *et al.*, 2019).

Despite the benefits achieved with the application of *A. nodosum* in fruit-producing vegetables, its use in cucurbits still lacks information. This is the largest family of vegetables and has high socioeconomic importance in a large part of the world, due to its nutritional and productive characteristics (Rolnik; Olas, 2020; Mukherjee *et al.*, 2022). Brazil is one of the biggest producers of species from this botanical family, with melons, watermelons, cucumbers and pumpkins totaling more than 3.0 million tons of fruit produced annually (IBGE, 2024). There are also specific breeding programs to obtain biofortified fruits, with the aim of improving the population's dietary conditions, as in the case of pumpkins (Moreira *et al*., 2019).

Based on the hypothesis that the application of a biostimulant derived from *A. nodosum* may positively impact on the development and production of pumpkin, the present study aimed to evaluate the effects of its application, as foliar spray, on the growth and production characteristics of *Cucurbita pepo* L.

2. MATERIAL AND METHODS

The experiment was carried out in the field at the Rural Experimental Station of the Goiás Agency for Technical Assistance, Rural Extension and Agricultural Research (Emater), located in Anápolis, in the State of Goiás. The location coordinates are 16° 19′ 43″ South, 48 ° 57′ 12″ West. The highest rainfall in the season occurs between December to March, with the lowest rainfall in June (6.0 mm). The average altitude of the site is 1,000 m. According to Köppen, the climate in the region is tropical with a dry season (Aw) with an average temperature of 22.4° C and an average annual rainfall of 1586 mm (Cardoso *et al*., 2014). The soil in the area is classified as *Latossolo Vermelho* (Santos *et al*., 2018)

Planting fertilization was performed with 1,171 kg ha^{-1} of formulation 03-17-00 and 228.5 kg ha^{-1} of Potassium Chloride (KCl). The implantation of the culture was carried out using seedlings of the Italian zucchini, cultivar PX7051 (Seminis®). The study area consisted of a 50 m by 30 m field, totaling 1500 m², which had previously been cultivated with crambe (*Crambe abyssinica* Hosch.) and had been left fallow for the past three months. For the cultivation of crambe, the experimental area was prepared using a tractor and an intermediate harrow, and the planting fertilization applied was 60 kilograms of NPK 05-25-15 per hectare, which was manually applied and incorporated with a hoe in all treatments.

Approximately fourteen days before transplanting, Roundup Original DI® herbicide was applied at a dosage of 4 L ha^{-1}. The transplanting of seedlings was carried out in the second half of May 2022 in the spacing of 0.7 x 1.0 m between rows, there were daily irrigations in the early morning and late afternoon. During the experiment, manual weeding was carried out to control weeds.

To control the whitefly (*Bemisia tabaci*) two applications of insecticides were carried out. The first was performed with the product Imidacloprid in a dose of 300 g a. i. ha^{-1} right after transplanting and in the treatment the drench technique was used. The second application was carried out with foliar spraying 16 days after transplanting, with the product Acetamiprid and Pyriproxyfen, in a dose of 200 mL ha^{-1}.

The biostimulant used derived from *A. nodosum* was Dalgin Mg® (Coda, Lleda, Spain), which is composed of: 61.5 g L^{-1} *Ascophyllum nodosum* seaweed extract, 6.15% w/v (5.0% w/w) water-soluble magnesium oxide (MgO), 12.30% w/v (10.0% w/w) water-soluble sulfur trioxide (SO3), 1.23% w/v (1.0% w/w) water-soluble borum (B), 0.12% w/v (0.10% w/w) water-soluble molybdenum (Mo) and 6.72% w/v (6.0% w/w) free amino acids. The maximum dosage recommended by the manufacturer is 300 mL ha^{-1}, with two applications per crop cycle. The experimental design consisted of four randomized blocks (DBC), containing five treatments. Each experimental plot consisted of four plants, resulting in a total of twenty plants per treatment. The treatments carried out were: (T1): Control (T2): 150 mL ha^{-1}; (T3): 200 mL ha^{-1}; (T4): 250 mL ha^{-1}; (T5): 300 mL ha^{-1}; the dilution was carried out in freshwater.

Two applications were made, the first in pre-flowering 21 days after transplanting and the second at the beginning of the fruit set phase, 31 days after transplanting. Applications were carried out in the afternoon between 5 pm and 6 pm with a 20 L manual pump, with a spray volume of 200 L ha^{-1}.

The evaluations comprised the collection of six fruits and four leaves per plot for evaluations of fruit diameter, fruit length, fruit fresh weight, plant height and plant diameter (Vendruscolo *et al.*, 2023). Also, measurements of length and width of the leaf blade, petiole length, weight of leaf blade dry weight and petiole dry weight were adapted from Lakitan *et al*. (2022).

As a sample selection method, the immature fruits collected should be between 17 and 23 cm in length and not present serious apparent defects such as deformations due to pollination failure, viral symptoms, or general physiological defects. Harvesting started 37 days after transplanting, being carried out three times a week and stopped at the beginning of plant senescence, when vegetative development was paralyzed and the fruits did not present commercial standards according to the Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP).

The fruit diameters were measured with a digital caliper graduated in mm and the fruit length measured with a ruler expressed in cm. The length and width of the leaf were also measured with a ruler in cm and the samples were collected 48 days after transplanting the seedlings, the seventh leaf of each plant was used as a sample selection criterion. The parameters of plant height and plant diameter were performed with a measuring tape also graduated in cm. For the diameter of the plant, the maximum opposite ends were considered, using two means of different directions, and for height, the highest point to the ground was considered.

To obtain the dry weight of the leaves and petioles, the material was placed in paper bags and dried in an oven with forced air circulation for 72 h at a temperature of approximately 65 ºC. The partitioning of the dry weight of the leaf blade and petiole over the total dry weight of the leaf (leaf blade + petiole) was also calculated, the result of this relationship being expressed in percentage.

The data were submitted for analysis of variance and, subsequently, the averages were submitted to regression analysis, emphasizing the results related to linear and quadratic responses. The analysis was performed using the Sisvar statistical software (Ferreira 2019).

3. RESULTS

An increase was observed the application of increasing doses of the biostimulant resulted in linear and positive responses for the diameter, length and fresh mass of the fruits, in addition to plant height and plant diameter. For the other characteristics, no adjustment was found for linear or quadratic regressions (Table 1).

Table 1 – Summary of analysis of variance for the characteristics of fruit diameter (FD), fruit length (FL), fruit fresh weight (FFW), plant height (PH), plant diameter (PD), stem length leaf blade (LBL), leaf blade width (LBW), petiole length (PL), leaf blade dry weight (BDM), petiole dry weight (PDW), partitioning of leaf blade dry weight (PLBW) and partitioning of petiole dry weight (PPW) of *Cucurbita pepo* L. plants sprayed with biostimulant derived from *A. nodosum*.

Doses	**FD (mm)**	**FL (cm)**	**FFW (g)**	**PH (cm)**	**PD (cm)**	**LBL (cm)**
0	42.38	16.36	170.65	29.85	82.35	17.41
150	43.77	17.35	194.60	32.12	84.94	17.69
200	44.05	17.06	190.96	31.58	85.70	17.75
250	44.77	17.17	194.61	32.38	90.18	17.88
300	46.37	17.80	222.60	34.88	94.88	18.00
CV%	1.70	3.40	6.59	6.64	2.75	6.75
L.R.	**	**	**	*	**	ns
Q.R.	ns	ns	ns	ns	ns	ns

Doses	**LBW (cm)**	**PL (cm)**	**BDW (g)**	**PDW (g)**	**PLBW (%)**	**PPW (%)**
0	21.50	25.31	3.97	8.30	0.30	0.64
150	21.63	26.16	3.97	8.77	0.30	0.60
200	22.78	26.22	4.58	9.13	0.30	0.62
250	22.22	25.59	4.20	8.47	0.31	0.62
300	22.50	25.53	5.44	8.24	0.36	0.55
CV%	6.53	6.36	26.44	17.83	15.65	11.59
L.R.	ns	ns	ns	ns	ns	ns
Q.R.	ns	ns	ns	ns	ns	ns

CV – coefficient of variation; L.R. – linear regression; Q.R. – quadratic regression; **, * and ns – significant at 1%, 5% and not significant, respectively.

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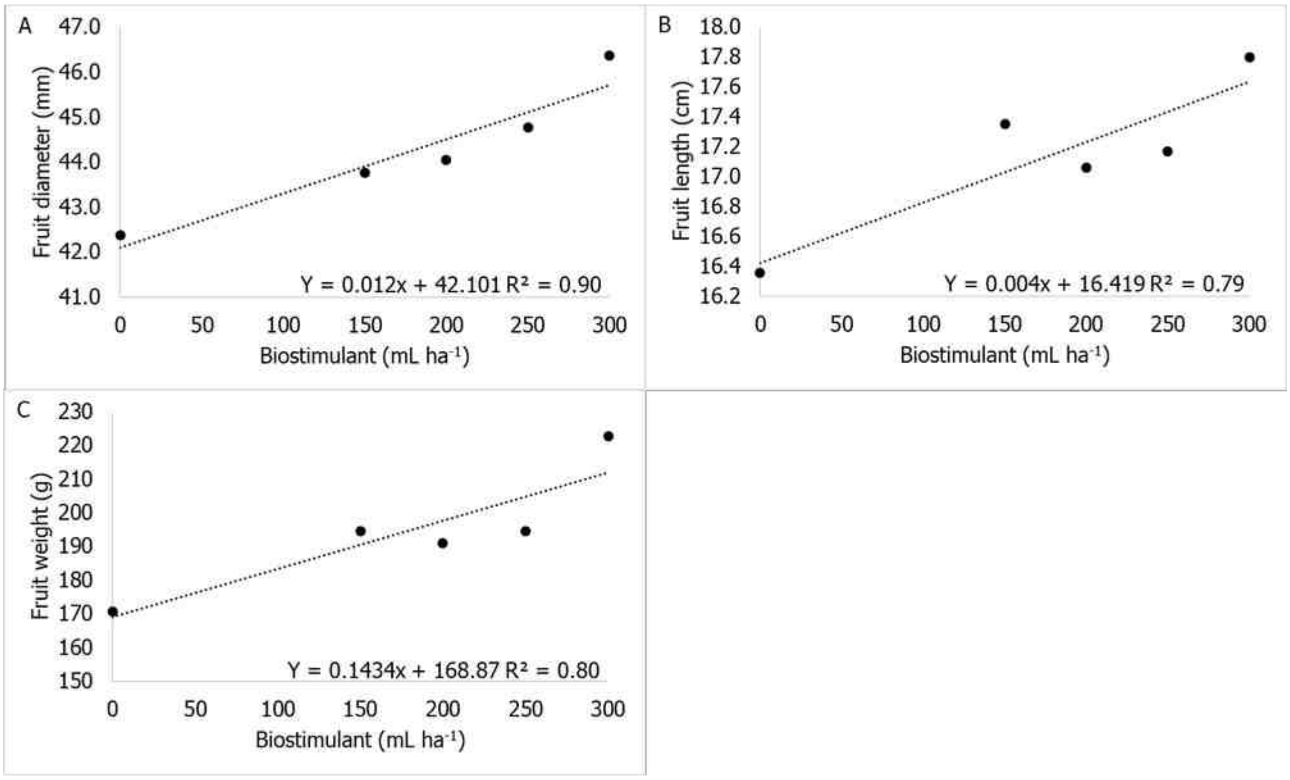
For fruit diameter, fruit length and fruit fresh weight, a linear and positive response was verified, with an increase in the characteristics as the biostimulant doses were increased up to the maximum dose studied, of 300 mL ha^{-1} (Figure 1). At this dosage, there was an increase of 9.41%, 8.80% and 30.44% in the characteristics of fruit diameter, fruit length and fruit fresh weight, respectively. Also, an average gain of these same characteristics of 1.2 mm, 0.4 cm and 14.34 g was estimated for each 100 mL ha^{-1} of biostimulant applied.

Figure 1 – Fruit diameter (A), fruit length (B) and fruit fresh weight (C) of *Cucurbita pepo* L. plants treated with *A. nodosum*-derived based biostimulant.



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Similarly, for plant diameter and plant height, a linear and positive response was obtained, with an increase in characteristics as the biostimulant doses increased up to a dose of 300 mL ha^{-1} (Figure 2) in which there was an increase in characteristics in 16.83% and 15.21%, respectively. Also, an average gain of 3.85 cm, and 1.41 cm, respectively, was estimated for each 100 mL ha^{-1} of biostimulant applied.

Pearson's linear correlation revealed a positive correlation between the fruit characteristics with those related to plant size and between each other, except for the fruit length and plant height correlation, which was not significant. For the characteristics of leaves, only the leaf blade dry weight partitioning had a positive correlation with plant diameter. In addition, the other leaf characteristics had a positive correlation with each other (Table 2).

Figure 2 – Plant diameter (A) and plant height (B) of *Cucurbita pepo* L. plants treated with *A. nodosum*-derived biostimulant.

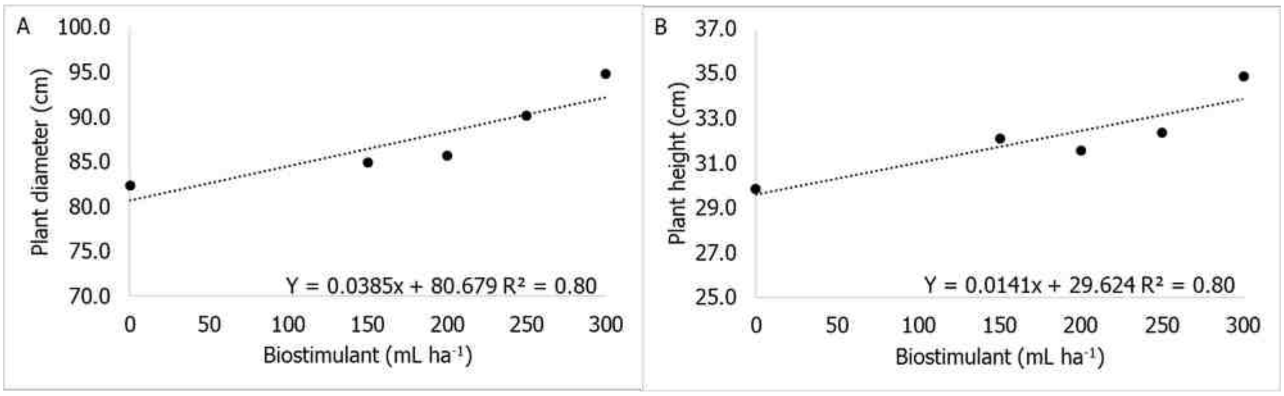

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Table 2 – Estimate of Pearson's linear correlation coefficient between characteristics of fruit diameter (FD), fruit length (FL), fruit fresh weight (FFW), plant height (PH), plant diameter (PD), stem length leaf blade (LBL), leaf blade width (LBW), petiole length (PL), leaf blade dry weight (BDM), petiole dry weight (PDW), leaf blade dry weight partitioning (PLBW) and partitioning of petiole dry weight (PPW) of *Cucurbita pepo* L. plants treated with *A. nodosum*-derived biostimulant.

	FD	FL	FFW	PH	PD	LBL	LBW	PL	BDW	PDW	PLBW	PPW
FD		0.712	0.879	0.626	0.757	-0.092	-0.015	-0.261	0.055	-0.275	0.156	-0.313
FL	**		0.870	0.321	0.493	-0.200	-0.142	-0.143	-0.012	-0.224	0.097	-0.333
FFW	**	**		0.461	0.620	-0.110	-0.080	-0.246	0.067	-0.255	0.198	-0.319
PH	**	ns	*		0.651	-0.022	-0.030	-0.109	0.005	-0.261	0.151	-0.299
PD	**	*	**	**		0.167	0.155	-0.052	0.424	0.037	0.533	-0.191
LBL	ns	ns	ns	ns	ns		0.818	0.582	0.727	0.755	0.503	-0.030
LBW	ns	ns	ns	ns	ns	**		0.772	0.786	0.743	0.544	-0.277
PL	ns	ns	ns	ns	ns	**	**		0.701	0.657	0.570	-0.448
BDW	ns	ns	ns	ns	ns	**	**	**		0.768	0.894	-0.191
PDW	ns	ns	ns	ns	ns	**	**	**	**		0.542	0.228
PLBW	ns	ns	ns	ns	*	*	*	**	**	*		-0.170
PPW	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	
					p≤0.01	p≤0.05	p≤0.05	p≤0.01				

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4. DISCUSSION

The present study revealed a direct relationship between the increase in doses of biostimulant derived from *A. nodosum* and the increase in fruit and plant vigor (Fig. 1 and 2). The results point to a continuous increase in the application of doses higher than those studied, of up to 300 mL ha^{-1}.

This gain in fruit growth is directly related to the gain in plant vigor and development (Table 2). This is a key point for obtaining higher yields, since the productivity of pumpkin plants is directly related to fruit mass (Pokluda *et al.*, 2018). This factor is also related to the availability of nutrients and the photosynthetic capacity of plants, which influences the production of photoassimilates and the consequent accumulation of reserves in fruits (Ors *et al.*, 2016; Walters, 2020).

Despite the scarce results from the application of *A. nodosum* in pumpkin crops, it is verified that the effects of the application of products derived from this alga positively influence characteristics of importance for the production and growth of fruits (Vendruscolo *et al*., 2024). In this sense, the application of a biostimulant derived from *A. nodosum* positively increased the activity of gas exchange, the vegetative growth and the productive parameters of tomato when applied in the form of soil drenching (Subramaniyan *et al.*, 2023).

The increases in gas exchange capacity and development, influenced by the application of *A. nodosum*, are related to the expression of genes that regulate hormone production, increase in protective capacity, metabolism of nutrients such as nitrogen, calcium and sulfur, production of photosynthetic pigments, cell division, among other functions (Saeger *et al.*, 2020; Dell'aversana *et al.*, 2021). These characteristics also give plants a greater capacity for protection when subjected to abiotic stresses in the growing environment, such as water deficit, high salinity levels and high temperatures (Shukla *et al.*, 2019; Carmody *et al.*, 2020).

In addition to the verified effects, the form of application of the biostimulant also interferes with their action. Complementarily, the foliar application carried out in the present study may also have contributed to obtaining the positive results, since there is evidence that the application on the vegetative organs results in a greater accumulation of sugars in the leaves and, consequently, in a higher rate of atmospheric CO_2 assimilation, when compared to soil application (Frioni *et al.*, 2021).

As well as the results verified in the literature cited above, that addresses the effect of applying different products derived from *A. nodosum*, point to numerous advantages of its use aimed at increasing production, as well as a tool for the search for more sustainable production systems. However, the variability in management aspects, as well as the diversity of environmental conditions and species of interest, imply the need for continuous generation of information that provides a basis for optimizing the use of *Ascophyllum nodosum* in modern agriculture.

5. FINAL CONSIDERATION

The application of increasing doses of biostimulant derived from *Ascophyllum nodosum*, up to 300 mL ha^{-1}, increases fruit development and growth of *Cucubita peppo* L. plants.

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Submetido em: **29/10/2023**

Aceito em: **18/09/2024**