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# Ascophyllum nodosum seaweed extract and mineral nitrogen in Alibertia edulis seedlings<sup>1</sup>

Extrato de alga *Ascophyllum nodosum* e nitrogênio mineral em mudas de *Alibertia edulis* 

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#### HIGHLIGHTS:

*Foliar application of* Ascophyllum nodosum *is promising on physiological and nutritional management of seedling production.* A. nodosum *increased concentration, uptake efficiency, and use of nitrogen in the tissue of* Alibertia edulis *seedlings.* A. edulis *seedlings with 15 and 20 mL* L<sup>-1</sup> *of* A. nodosum *showed greater growth of aerial parts and root systems.* 

**ABSTRACT:** Physiological and nutritional management is an important practice for obtaining high quality seedlings. The use of seaweed extract has emerged as an alternative to fertilizers and biostimulants, although information on this with respect to *Alibertia edulis*, a fruit-bearing species native to the Cerrado region, is scarce. The aim of this study was to evaluate the effects of the *Ascophyllum nodosum* L. seaweed extract (ANE), both with and without the addition of mineral nitrogen, on the growth, nitrogen nutrient efficiency indices, and quality of *A. edulis* seedlings. The seedlings received five doses of ANE (0, 5, 10, 15, and 20 mL L<sup>-1</sup> of water) via foliar application, with and without the addition of mineral N to the soil (50 mg kg<sup>-1</sup> of urea). A randomized block design was used with the treatments arranged in a 5 × 2 factorial scheme. The plant height, stem diameter, chlorophyll index, and number of leaves obtained were analyzed in split-plots over time. Foliar application of 15 and 20 mL L<sup>-1</sup> of ANE contributed to greater growth and seedling quality, regardless of the addition of mineral nitrogen. Seedlings treated with mineral N alongside 13.98 mL L<sup>-1</sup> of ANE showed the greatest leaf area. The highest N use efficiency, N uptake, and nutrient use occurred in seedlings that received *A. nodosum* extract.

Key words: urea, biostimulant, nutritional management, nutrient use efficiency

**RESUMO:** O manejo fisiológico e nutricional é uma prática importante para obtenção de mudas de alta qualidade e o uso de extrato de algas tem se tornado uma alternativa aos fertilizantes e bioestimulantes, mas informações para *Alibertia edulis*, uma espécie frutífera e nativa do Cerrado, são escassas. Objetivou-se com este trabalho avaliar o efeito de doses do extrato de alga *Ascophyllum nodosum* L. (ANE), associadas ou não ao nitrogênio mineral no crescimento, índices de eficiência do nutriente nitrogênio e na qualidade de mudas de *A. edulis*. As mudas receberam aplicação de cinco doses de ANE (0, 5, 10, 15 e 20 mL L<sup>-1</sup> de água) via foliar, sem e com adição de N mineral (50 mg kg<sup>-1</sup> de ureia ao solo). O delineamento utilizado foi o de blocos casualizados, e os tratamentos foram arranjados em esquema fatorial 5 × 2. Os dados de altura, diâmetro do coleto, número de folhas e o índice de clorofila ao longo do tempo foram analisados em parcelas subdivididas no tempo. A aplicação foliar do extrato de *A. nodosum* nas doses de 15 e 20 mL L<sup>-1</sup>, contribuiu para maiores características de crescimento e qualidade de mudas, independente do uso de nitrogênio mineral. Mudas com N mineral e com 13,98 mL L<sup>-1</sup> de ANE tiveram maior área foliar. A maior eficiência e absorção de N e uso de nutrientes ocorreu nas mudas que receberam o extrato de alga *A. nodosum*.

Palavras-chave: ureia, bioestimulante, manejo nutricional, eficiência do uso do nutriente

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### INTRODUCTION

Alibertia edulis (Rich) A. Rich (Marmelo do cerrado, Rubiaceae) is a semi-deciduous dioecious tree belonging to the native fruiting species of the Brazilian Cerrado, and these fruits can be consumed either in natura or after processing (Santos et al., 2020a). Furthermore, the seedlings can be used for reforestation, native forest enrichment, or agroforestry (Santos et al., 2020b). However, rampant extractivism in native areas poses a threat, whilst more research is still required on the management and ex situ cultivation of *A. edulis*, specifically focused on its conservation and sustainable use.

However, information on the physiological and nutritional management responses of *A. edulis* is scarce. Nitrogen is one of the most limiting nutrients in plant growth since it plays an important role in photosynthesis in forming part of the chlorophyll molecule. In addition, N is used in other metabolic processes (Faria et al., 2020) and improves seedling nutrition and growth. This is especially important considering that native species generally exhibit slow initial growth (Santos et al., 2020c).

Using biostimulants is a promising practice because they work as activators of plant physiological processes. Among those in the commercial sphere, the seaweed extract *Ascophyllum nodosum* (L.) Le Jolis (ANE), also classified as a Class A organomineral fertilizer (Okolie et al., 2018), contributes to plant growth and improves the antioxidant defense system through regulation of physiological, nutritional, biochemical, and molecular processes (Frioni et al., 2019; Shukla et al., 2019).

However, few studies have tested the isolated use of ANE foliar application, or its combined use with mineral N, regarding efficiency and seedling production in native species. Thus, this study aimed to evaluate the effect of *A. nodosum* seaweed extract doses with and without added mineral nitrogen, on the growth, nitrogen nutrient efficiency indices, and quality of *A. edulis* seedlings.

## **MATERIAL AND METHODS**

Seeds were randomly collected from the fruits of 10 Alibertia edulis matrices (SISGEN Access Registry No. A9CDAAE - CGEN-MMA, of 10/15/2018) in a remnant Cerrado area (18° 07' 03" S, 54° 25' 07" W, 452 m). After identification of the species, an exsiccate was deposited in the DDMS herbarium at the Universidade Federal da Grande Dourados (UFGD) under number 4649. The fruits were manually pulped and the seeds were subsequently selected, washed under running water, and immersed in 1% sodium hypochlorite for 5 min. After this, they were placed in 128cell expanded polystyrene trays with Tropstrato<sup>\*</sup> substrate, in accordance with Santos et al. (2020a). Two seeds were sown per cell, and thinning was performed after a height of 5 cm was reached.

Seedlings were watered frequently according to the needs of the substrate. The substrate used (Tropstrato<sup>\*</sup>) had the following attributes: pH CaCl<sub>2</sub>, 5.75; P, 65.70 mg dm<sup>-3</sup>; K, 1.60 cmol<sub>2</sub> dm<sup>-3</sup>; Ca, 23.80 cmol<sub>2</sub> dm<sup>-3</sup>; Mg, 12.40 cmol<sub>2</sub> dm<sup>-3</sup>; Al,

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 $0.00 \text{ cmol}_{c} \text{ dm}^{-3}$ ; H + Al, 4.20 cmol $_{c} \text{ dm}^{-3}$ ; sum of bases, 39.80 cmol $_{c} \text{ dm}^{-3}$ ; cation exchange capacity, 42.10 cmol $_{c} \text{ dm}^{-3}$ ; and base saturation (V %), 64.80.

When the seedlings reached an average height of 5.0 cm, which occurred 60 days after sowing, they were transplanted to pots with a capacity of 5 dm<sup>3</sup> and filled with Oxisols (Soil Taxonomy, USDA, 2014). The experiment was conducted from March to October 2021 in a nursery with upper and lateral coverage with black nylon mesh providing 50% shade and upper plastic cover protection of 150  $\mu$ m in the Medicinal Plants Nursery at UFGD.

The factors of this study comprised five ANE doses: 0 (without), 5, 10, 15, and 20 mL L<sup>-1</sup> of water based on the work of Santos et al. (2019), with and without the addition of mineral nitrogen (urea). A randomized block design was used for this, with the treatments arranged in a  $5 \times 2$  factorial scheme with three repetitions. Each experimental unit comprised one pot, containing two plants. The plant height, stem diameter, chlorophyll index, and number of leaves obtained were analyzed over time in split-plots.

The ANE employed was Acadian<sup>\*</sup>, and foliar application was carried on the abaxial and adaxial faces. This was performed in the morning period until dripping point (10 mL per plant, based on pre-test), every 30 days, from 20–200 days after transplanting (DAT). The addition of 50 mg kg<sup>-1</sup> of mineral N directly to the soil (based on the work of Santos et al., 2020c) was performed at 30 DAT, using urea (45% N) as a source, diluting the amount of N in distilled water, and adding around the collar of the seedlings.

The physical and chemical specifications of Acadian<sup>\*</sup>, according to the manufacturer, are as follows: appearance = viscous dark brown liquid; odor = marine; water solubility = 100%; pH = 7.4–8.2; organic matter = 13–16%; total N = 0.30–0.60%; available phosphorus ( $P_2O_5$ ) = < 0.1%; soluble potassium ( $K_2O$ ) = 5.00–7.00; S = 0.30–0.60%; Mg = 0.05–0.10%; Ca = 0.10–0.20%; Fe = 30–80 ppm; Cu = 1–5 ppm; Zn = 5–15 ppm; Mn = 1–5 ppm; B = 20–50 ppm; carbohydrates = alginic acid; mannitol, fucoidins, and amino acids = 1.01%.

During the growth cycle, the seedlings were irrigated twice a day, and spontaneous plants were manually removed whenever required. Irrigation was periodically performed according to seedling requirement to maintain 75% water retention capacity in the soil. During the experiment, there was an occurrence of whitefly (*Bemisia tabaci* Genn.) at 170 DAT, and effective control action was taken with the application of 5.0 mL L<sup>-1</sup> of teflubenzuron-based insecticide with a hand sprayer.

Evaluations started 30 days after the first application of ANE (50 DAT) and were repeated every 30 days throughout the following months. Non-destructive characteristics were evaluated. Plant height was measured with a graduated ruler, using the distance between the collar and the uppermost leaf inflection as a standard. Stem diameter was measured using a digital pachymeter inserted 1.0 cm above the substrate level. The number of leaves was counted manually, whilst the chlorophyll index was measured using a Falker CFL 1030 chlorophyll meter on fully expanded leaves in the middle third of the plant. All evaluations were performed between 8:00 and 10:00 am.

ANE/Mineral		pН	P	Ca	K	Mg	AI	H + AI	SB	CEC	V
N		CaCl <sub>2</sub>	(mg dm <sup>-3</sup> )				(mmol <sub>c</sub> dm <sup>-</sup>	-3)			(%)
0 mL	With	4.8	7	36	3.6	13	0	48	52	100	52
	Without	4.6	2	30	2.9	10	2	51	43	94	46
5 mL	With	5.1	6	43	4.0	15	0	45	62	107	58
	Without	4.8	3	34	3.1	11	0	46	48	94	51
10 mL	With	5.1	5	46	4.3	16	0	45	66	111	59
	Without	4.7	3	37	3.2	12	1	58	52	110	47
15 mL	With	5.2	5	47	4.2	17	0	43	68	111	62
	Without	5.2	5	52	4.8	18	0	47	74	122	61
20 mL	With	5.2	6	49	4.7	17	0	43	71	114	62
	Without	5.2	4	44	3.9	14	0	49	62	111	56

Table 1. Soil chemical attributes after application of *Ascophyllum nodosum* extract doses in *Alibertia edulis* seedlings with and without addition mineral N, at 205 DAT

At 205 DAT, seedlings were harvested from the pots, washed, and separated into leaves, stems, and root systems. Moreover, composite samples of the substrate from each treatment were collected to characterize the chemical attributes (Table 1), according to Silva (2009). Leaf area (LA) was measured using an area integrator LI-COR 3100 C. The shoot (leaves and stems) and root dry mass were then weighed on a millesimal precision balance (0.0001 g), placed into Kraft paper bags, and dried in an oven at  $60 \pm 2$  °C for 72 hours.

The leaf area ratio (LAR) (Hunt, 2017) and aerial part:root ratio (APRR) were calculated based on dry mass and leaf area data. The Dickson quality index (DQI) of the seedlings was also calculated, as described by Dickson et al. (1960).

Dry material from the leaves, stems, and roots was ground together, and nitrogen concentration of the seedlings was determined according to Malavolta et al. (1997). Uptake efficiency ( $UE_N$ ) (Swiader et al., 1994) and nutrient use efficiency ( $NUE_N$ ) (Siddiqi & Glass, 1981) were calculated based on the dry mass and N concentration.

These data were submitted to analysis of variance and means of the mineral N application were compared using the F test ( $p \le 0.05$ ). Regression analysis was performed by testing linear and quadratic models for seaweed extract doses ( $p \le 0.05$ ). The data obtained over time were subjected to regression analyses to test linear and quadratic models ( $p \le 0.05$ ).

### **RESULTS AND DISCUSSION**

The height and stem diameter of *Alibertia edulis* seedlings were significantly influenced by the interaction between the doses of *Ascophyllum nodosum* seaweed extract and number of days after application (DAA) (Table 2). The greatest seedling heights were 17.83 and 16.25 cm (Figure 1A) whilst the largest diameters were 3.77 and 3.29 mm (Figure 1B), both with *A. nodosum* seaweed extract (ANE) doses of 15 and 20 mL  $L^{-1}$  of water, respectively. Meanwhile, at 180 DAA, the lowest height values of 9.94 and 9.54 cm occurred with ANE doses of 0 and 5 mL  $L^{-1}$  of water, respectively.

The increased height and diameter results with higher ANE doses were associated with an increase in exogenous hormones in the seaweed extract. According to Khan et al. (2009) and Wally et al. (2013), ANE has a high content of auxin and cytokinins. Auxin promotes cell division and elongation, apical dominance, and root growth (Silva et al., 2021), whereas cytokinins influence cell division, apical development, and root formation, in addition to stimulating meristematic tissue growth and promoting photosynthetic activity (Hartmann et al., 2017; Cruvinel et al., 2019).

Thus, we indicate that there was an effect of ANE on the vegetative growth of *A. edulis* seedlings. The highest growth values at 180 DAA indicated that the species showed gradual growth throughout the crop cycle. In other words, this is a natural response for most tree and arboreal species with a perennial cycle. Similarly, *Solanum lycopersicum* L. (Di Stasio et al., 2018) and *Mangifera indica* L. (Cunha et al., 2022) have been found to have higher growth indicators with ANE compared to those without, demonstrating its beneficial effects for plant nutrition and development.

The number of leaves (NL) was influenced by the mineral  $N \times DAA$  interaction and ANE dosage (Table 2). The highest number of leaves per plant was 13.4, with N at 180 DAA (Figure 1C) and 13.2 leaves with 15.5 mL L<sup>-1</sup> of ANE (Figure 1D). Santos et al. (2020a) observed an increase in this trait in seedlings of the same species throughout the crop cycle at 150

 Table 2. Mean square of plant height, stem diameter, number of leaves and chlorophyll index of *Alibertia edulis* seedlings in function of *Ascophyllum nodosum* doses, with and without mineral N

	-				
Sources of variation	DF	Plant height	Stem diameter	Number of leaves	Chlorophyll index
Block	2	14.32	1.62	4.61	14.87
Nitrogen (N)	1	25.01 <sup>№S</sup>	0.22 <sup>NS</sup>	39.66 <sup>NS</sup>	68.20 <sup>NS</sup>
ANE	4	96.45*	2.43*	67.38*	20.57 NS
$N \times ANE$	4	14.94 <sup>№</sup>	0.43 <sup>NS</sup>	25.47 <sup>№</sup>	22.58 <sup>NS</sup>
Time	5	407.34*	12.85*	133.45*	542.79*
Time $\times$ N	5	7.17 <sup>№</sup>	0.15 <sup>NS</sup>	4.63*	40.25 <sup>NS</sup>
Time $\times$ ANE	20	10.80*	0.35*	3.01 <sup>№</sup>	59.44*
Time $\times$ N $\times$ ANE	20	2.61 <sup>№</sup>	0.11 <sup>NS</sup>	2.78 <sup>NS</sup>	29.02 <sup>NS</sup>
C.V. 1 (%)	-	35.41	32.39	33.20	21.14
C.V. 2 (%)	-	25.50	19.72	15.16	16.42

NS - Non-significant F value at  $p \leq 0.05;$  \* - Significant F value at  $p \leq 0.05;$  DF - Degrees of freedom



\* - Significant at  $p \le 0.05$  by F test; Equal letters in each dose of ANE do not differ statistically regarding mineral N at  $p \ge 0.05$  by F test **Figure 1.** Plant height (A), stem diameter (B) and number of leaves (C and D) of *Alibertia edulis* seedlings in function of *Ascophyllum nodosum* doses, with or without the addition of N addition, as a function of evaluation times

d after transplanting (DAT). Regarding the effect of N on NL, it is one of the most important nutrients in vegetative growth, considering that it comprises molecules of nucleic acids, amino acids, proteins, and chlorophyll (Silva et al., 2020), whilst promoting leaf limb expansion and maximizing photosynthesis.

Furthermore, the increase in NL with ANE was possibly due to the presence of cytokinins from the seaweed extract, which contribute to cell division. Tissue expansion growth hormones can be identified in the extract and influence the movement of nutrients to the leaf, with an important role in source-sink regulation (Wally et al., 2013).

The characteristics LA, SDM, APRR, DQI, NUE<sub>N</sub>, LAR, and TDM of *A. edulis* seedlings were influenced by the interaction

between ANE doses and the use of mineral N, whereas RDM,  $UE_N$ , and N concentrations were influenced by ANE doses alone (Table 3).

The chlorophyll index in *A. edulis* seedling leaves was influenced by the interaction between ANE doses and DAA (Table 2), with the highest value (42.50) recorded in seedlings grown with ANE doses of 15 mL  $L^{-1}$  at 180 DAA (Figure 2A). This higher value can be associated with the increased capacity of the plant to absorb more nutrients from the soil in the presence of ANE, this being especially beneficial in terms of height and canopy exposure, therefore allowing greater efficiency of light absorption and conversion into chemical energy for photosynthetic metabolism, resulting in

**Table 3.** Mean square of leaf area (LA), shoot dry mass (SDM), root dry mass (RDM), aerial part:root ratio (APRR), Dickson quality index (DQI), N uptake efficiency ( $UE_N$ ), nitrogen utilization efficiency ( $NUE_N$ ), nitrogen concentration (N), leaf area ratio (LAR) and total dry mass (TDM) of *Alibertia edulis* seedlings in function of *Ascophyllum nodosum* doses, with and without mineral N

Sources of variation	DF	LA	SDM	RDM	APRR	DQI
Block	2	64373.42	0.075	1.300	0.073	0.050
Nitrogen (N)	1	15334.10 <sup>NS</sup>	1.917 <sup>№</sup>	0.109 <sup>NS</sup>	0.189 <sup>NS</sup>	0.045 <sup>NS</sup>
ANE	4	70799.71*	1.823*	2.565*	0.444*	0.229*
N x ANE	4	79923.39*	0.330*	0.916 <sup>NS</sup>	0.709*	0.057*
C.V. (%)	-	31.52	27.18	59.86	17.98	36.48
		UE <sub>N</sub>	NUE <sub>N</sub>	N	LAR	TDM
Block	2	0.599	0.007	1.803	0.012	1.767
Nitrogen (N)	1	0.001 <sup>NS</sup>	0.002 <sup>NS</sup>	0.571 <sup>№</sup>	0.012 <sup>NS</sup>	2.942*
ANE	4	3.022*	0.199*	14.543*	0.011 <sup>№</sup>	8.539*
N x ANE	4	0.452 <sup>NS</sup>	0.047*	21.177 <sup>№</sup>	0.027*	2.211*
C.V. (%)	-	38.54	40.14	21.68	12.88	33.01

NS - Non-significant F value at p  $\leq$  0.05; \* - Significant F value at p  $\leq$  0.05 probability; DF - Degrees of freedom



\* - Significant at p  $\leq$  0.05 by F test

**Figure 2.** Chlorophyll index (A), leaf area (B), leaf area ratio – LAR (C) and aerial part:root ratio – APRR (D) of *Alibertia edulis* seedlings in function of *Ascophyllum nodosum* doses, with or without the addition of mineral N

an increased nutrient demand. A higher chlorophyll index is associated with a larger leaf area, which provides better conditions for photosynthetic conversion and increased production of photoassimilates for leaf expansion (Santos et al., 2020c).

An interaction was observed between the ANE doses and mineral N for leaf area (LA) (Table 3), with the highest value (541.36 cm<sup>2</sup> per plant) being observed in seedlings grown with added mineral N alongside 13.98 mL L<sup>-1</sup> of ANE (Figure 2B). In contrast, the values in those that did not receive mineral N did not fit adequately the regression model ( $\hat{y} = 330.8526 +$  $3.5008^*x$ ,  $R^2 = 0.53$ ). The greater LA in seedlings grown with N was observed because N is a precursor to protein biosynthesis and the chlorophyll molecule (Rasteiro et al., 2021), whilst higher ANE doses stimulated greater nutrient uptake (Shukla et al., 2019).

The leaf area ratio (LAR) was influenced by the interaction between ANE dosage and mineral N (Table 3), with the highest value being 0.62 cm<sup>2</sup> g<sup>-1</sup> without ANE and without mineral N (Figure 2C). However, seedlings with added mineral N did not fit the mathematical models ( $\hat{y} = 0.5231 + 0.0102^*x - 0.0004^*x^2$ ,  $R^2 = 0.48$ ). These results demonstrated that higher ANE doses favored physiological efficiency in *A. edulis* seedlings. In other words, there was an increase in the net assimilation rate, considering that the lower the LAR value, the lower the leaf area unit required to increase biomass by 1 g, correlating positively with higher chlorophyll index (Figure 2A) and N uptake efficiency (Figure 4B). Therefore, ANE foliar application positively contributed to foliar metabolism because of the higher N content in the seedlings. When absorbed, N can be assimilated into the root itself or transported to the leaves where it is then assimilated (Bredemeier & Mundstock, 2000). ANE is composed of 0.30–0.60% of N, so foliar spraying with this biostimulant provides nitrogen directly into the nutrient-assimilating organ. Nitrogen in plant tissues is associated with the sugars forming amino acids and proteins, therefore a lack of N can lead to sugar accumulation in the plant system (Faria et al., 2020; Mattar et al., 2021).

In general, the higher values of the characteristics of grown and nutrition of *A. edulis* seedlings with ANE were associated with improvements in the chemical attributes of the substrate compared to substrates without ANE (Table 1). With the observed increase in the dose of the biofertilizer, there was also an increase in the levels of nutrients, cation exchange capacity, organic matter, and saturation by bases, which consequently favors the nutrition of the seedlings.

The highest aerial part: root ratio (APRR) was 1.52, observed in seedlings grown with 4.52 mL L<sup>-1</sup> of ANE without the addition of mineral N (Figure 2D). These results did not fit the mathematical models on seedlings with mineral N ( $\hat{y} = 1.1639 + 0.0578^*x - 0.0025^*x^2$ , R<sup>2</sup> = 0.55). The shoot dry mass (SDM) and the total dry mass (TDM) were influenced by the interaction between ANE and mineral N (Table 3). The highest SDM and TDM values were 1.89 and 3.79 g per plant, respectively, in seedlings grown with

15.86 and 20 mL  $L^{-1}$  of ANE, both with added mineral N (Figures 3A and B). On the other hand, those without added N showed linear SDM and TDM were 1.63 and 3.98 g per plant, respectively, with 20 mL  $L^{-1}$  of ANE. Higher values of these traits were observed due to increased growth (Figure 1), resulting from higher photoassimilate production and biomass allocation.

The dry mass of the root system was only influenced by the ANE dose (Table 3), with the highest value (1.92 g per plant) being recorded in seedlings that received 20 mL  $L^{-1}$  of ANE (Figure 3C). This increase was associated with both increased N uptake efficiency (Figure 4B) and the influence of the extract on hormone synthesis, especially auxin and cytokinins, which stimulate root formation and growth (Cruvinel et al., 2019; Silva et al., 2020).

Additionally, this was impacted by to the nutrients in ANE, which favor both root length and volume for rhizosphere exploitation. Other species, such as *Brassica oleracea* var. viridis (L.) (Silva et al., 2012) and *Passiflora actinia* (Hook) (Gomes et al., 2018), have also previously shown increased root mass



\* - Significant at  $p\leq 0.05$  by F test; Equal letters in each dose of ANE do not differ statistically regarding mineral N at  $p\leq 0.05$  by F test

**Figure 3.** Shoot (A), root system (B) and total (C) dry mass of *Alibertia edulis* seedlings in function of *Ascophyllum nodosum* doses, with or without addition of mineral N

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when receiving *A. nodosum*-based extract. Increased root volume corresponds with a greater water and nutrient uptake, resulting in improved plant development and, consequently, greater metabolic efficiency (Neumann et al., 2017).

N concentration and uptake efficiency (UE<sub>N</sub>) in *A. edulis* seedlings were both influenced by ANE dosage (Table 3), with higher values of 11.01 g kg<sup>-1</sup> and 1.92 g g<sup>-1</sup> were observed when receiving applications of 6.54 and 20.0 mL L<sup>-1</sup> of ANE, respectively (Figures 4A and B). Regarding nutrient use efficiency (NUE<sub>N</sub>), there was an interaction between the factors in this study, with linear adjustment both in the presence and absence of mineral N as a function of ANE dose. The highest value (0.57 g g<sup>-1</sup>) was observed in seedlings grown without N and with 20 mL L<sup>-1</sup> of ANE (Figure 4C).

The response of N concentration in the seedlings to ANE doses demonstrated a reflection of the nutrient dilution effect due to the higher SDM production under the same growing conditions. Furthermore, owing to the occurrence of higher  $UE_N$  and  $NUE_N$ , we suggest that foliar-applied ANE may be an alternative for higher uptake and use-efficiency of this nutrient. Therefore, when using a biostimulant based on the *A. nodosum* seaweed extract in nursery practices, it is possible to decrease costs with fertilizers, since the TDM values are similar, regardless of the addition of mineral N, at the highest doses of ANE.

The Dickson quality index (DQI) was influenced by the mineral N × ANE interaction (Table 3), which showed linear adjustment with higher values of 0.63 and 0.61 with and without mineral N, respectively, both of these being with 20 mL L<sup>-1</sup> of ANE (Figure 4D). Theses DQI may also have been associated with the amino acids in the seaweed extract. Amino acids are fundamental in protein synthesis and are involved in several functions that can influence metabolic activity, such as antioxidant enzyme activity as a protective mechanism, leaf nutrition and metabolic processes (Chrysargyris et al., 2018).

Plants can synthesize amino acids from the elements absorbed by the root or leaf systems and transform them into enzymes and proteins directly involved in biomass production (Moreno-Hernández et al., 2020). Furthermore, we emphasize that higher DQI values reflect higher N use efficiency and concentrations in *A. edulis* seedling tissues, therefore contributing to their robustness.

Based on these results, it can be concluded that the application of ANE is a promising practice in physiological and nutritional management of *A. edulis* seedling production. This is because it favors the increase in vigor and quality based on the initial growth visual aspect (Figure 5), both in aerial parts and the length and volume of the root system.

However, added mineral N contributed little to seedling growth and quality standards, possibly because the seedlings' need for N at this growth stage was supplied by that already available from the ANE. Further studies should be conducted to test the *A. nodosum* seaweed extract, such as in terms of determining gas exchange, antioxidant enzyme activity, and leaf analysis for other nutrients.



\* - Significant at  $p \le 0.05$  by F test; Equal letters in each dose of ANE do not differ statistically regarding mineral N at  $p \le 0.05$  by F test **Figure 4.** Concentration (A), uptake efficiency (B), nitrogen utilization efficiency (C) and Dickson quality index (D) of *Alibertia edulis* seedlings in function of *Ascophyllum nodosum* doses, with or without the addition of N mineral



*Ascophyllum nodosum* algae extract (mL L<sup>-1</sup>) **Figure 5.** Visual aspect of *A. edulis* seedlings cultivated with doses of *Ascophyllum nodosum*, without and with the addition of mineral N, at 205 days after transplantation

#### CONCLUSIONS

1. Foliar application of *Ascophyllum nodosum* extract contributed to higher growth characteristics, physiological indices, biomass production, and nitrogen concentration in the plant tissues of *Alibertia edulis* seedlings.

2. Application of 15 and 20 mL  $L^{-1}$  of *A. nodosum* extract favored the production of high-quality *A. edulis* seedlings, regardless of added mineral nitrogen.

3. The highest efficiency of N uptake and nutrient use occurred in seedlings that received 20 mL  $L^{-1}$  A. nodosum extract.

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