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Performance of maize seedlings using biostimulant in seed treatment

Desempenho de plântulas de milho mediante uso de bioestimulante no tratamento de sementes

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Abstract

Biostimulants are used as a strategy to minimize the effects of climatic adversities by allowing the seedlings to express more strongly their metabolic capacity and to have a greater root system development. The objective was to evaluate the effect of seed treatment with different doses of a biostimulant composed of *Ascophyllum nodosum* on the agronomic performance of maize seedlings. The experiment was carried out in a randomized block design with five treatments and four replicates, using five doses of the biostimulant, 0,0; 0.25; 0.50; 0.75; and 1.00 liter of product per 100 kg of seeds. The following were evaluated: vigor, fresh mass of roots and shoots, dry mass of roots and shoots, root length, shoot height, and leaf area. The data were submitted to ANOVA and regression analysis to verify the behavior of the characteristics as a function of the biostimulant doses. In the treatment of seeds, these doses provided increases in vigor, shoot height, fresh and dry mass of shoots, and leaf area. The biostimulant derived from the *Ascophyllum nodosum* extract showed potential use in the treatment of maize seeds, with the doses of 0.50 and 0.75 liters per 100 kg seeds being the most promising for the initial development of maize seedlings.

Additional keywords: root growth; agronomic performance*, Zea mays*.

Resumo

Os bioestimulantes são uma estratégia para minimizar os efeitos das adversidades climáticas permitindo que as plântulas expressem de forma mais acentuada sua capacidade metabólica e tenham maior desenvolvimento do sistema radicular. O objetivo foi avaliar o efeito do tratamento de sementes com diferentes doses de bioestimulante composto de *Ascophyllum nodosum* sobre o desempenho agronômico das plântulas de milho. O ensaio foi conduzido em delineamento de blocos ao acaso, com cinco tratamentos e quatro repetições, empregando-se cinco doses do bioestimulante, 0,0; 0,25; 0,50; 0,75 e 1,00 litro de produto para 100 kg de sementes. Foram realizadas avaliações de vigor, massa fresca da raiz e da parte aérea, massa seca da raiz e parte aérea, comprimento de raiz e parte aérea, além da área foliar. Os dados foram submetidos à análise de variância e análise de regressão para verificação do comportamento das características em função das doses do bioestimulante. Doses estas que, no tratamento de sementes, proporcionaram incrementos no vigor, altura da parte aérea, peso de matéria fresca e seca da parte aérea e área foliar. O bioestimulante derivado do extrato de *Ascophyllum nodosum* apresentou potencial de uso no tratamento de sementes de milho, sendo que as doses de 0,50 e 0,75 litros por 100 kg sementes foram as mais promissoras ao desenvolvimento inicial das plântulas de milho.

Palavras-chave adicionais: crescimento radicular; desempenho agronômico*, Zea mays*.

Introdução

The maize crop plays a fundamental role in the production systems in Brazil and worldwide, being considered one of the most important cereals grown and consumed in the world, possibly due to its high yield potential and nutritive value (Santos et al., 2013). New technologies, such as the use of improved seeds and adequate nutritional management, are applied to increase crop yield, highlighting the use of biostimulants by the possibility of use in seed, foliar, and soil application, which may provide changes in plant metabolism, leading to increased crop yield (Dourado Neto et al., 2014).

In order to increase agricultural production, seed germination and seedling emergence must be rapid and uniform (Rajjou et al., 2012). Factors such as low seed quality, uneven planting/fertilization, low soil fertility, and mainly climatic adversities may limit the development of maize seedlings (Bomtempo et al., 2016). Thus, biostimulants would be a strategy to minimize the effects of low seed quality (Klahold et al., 2008), favoring the expression of the genetic potential of plants, promoting hormonal balance, and stimulating the development of the root system (Silva et al., 2008; Castro & Amaral, 2014).

Among the biostimulants most used in large crops stand out *Ascophyllum nodosum* extract derivatives, due to their characteristically complex composition, consisting of macro- and micronutrients, amino acids, oligosaccharides, cytokinins, auxins, abscisic acid, gibberellins, betaines, and alginates (Mackinnon et al., 2010; Sharma et al., 2014). Among the possible benefits attributed to these compounds is reported the anti-stressing action caused by the increase in chlorophyll content, an effect related to betaines by reducing chlorophyll photodegradation (Zobiole et al., 2010). However, there is still no consensus regarding their physiological implications.

According to Sharma et al., (2014) the application of biostimulants derived from *Ascophyllum nodosum* stimulated the dry matter production and root growth in common bean. Later, Araújo (2016) verified that the use of the same extract in treatment of maize seeds did not increase shoot growth, although root growth was increased. Indeed, the mechanism by which biostimulant substances act on plant growth and vigor has not yet been fully elucidated, but much of their effects have been demonstrated by observing morphophysiological responses as a function of the doses employed (Khan et al., 2011; Wally et al., 2013).

For the development of roots and shoots, the ideal concentration of auxins is considered to be 10^{-10} M (Castro & Amaral, 2014; Taiz et al., 2015). Values far below stimulate cytokinin biosynthesis, which promotes the production of branches, and, on the other hand, much higher concentrations stimulate ethylene synthesis, which inhibits root growth (Castro & Amaral 2014; Taiz et al., 2015). This relationship shows that the effects caused by the biostimulant depend on the balance between hormones, being subject to the doses used, concentrations in the plants, edaphoclimatic conditions, and even the hybrid used (Taiz et al., 2015). In view of the above, a result cannot be adopted as a parameter for other situations, even if similar, since small variations can alter the hormonal balance and, consequently, the plant response to the stimuli.

Many of the physiological responses reported demonstrate that the action of biostimulants is related to the presence of plant hormone analogues (Ferreira, 2007; Sharma et al., 2014). These compounds are considered to participate in seed germination, in the formation of adventitious and lateral roots, and in cell division, expansion, and multiplication, besides promoting foliar expansion (Taiz et al., 2015). Thus, seeds treated with biostimulants tend to markedly express their potential, giving rise to seedlings with a higher growth rate, due to the greater translocation of reserves from storage tissues, destined to the growth of the embryonic axis (Leszczynski et al., 2012).

In the maize crop, biostimulants have been providing increases in biometric characteristics, in addition to inferring significant gains in productivity. According to Santos et al. (2013), who observed the use of biostimulant, there was an increase in stem diameter, plant height, leaf area, and leaf dry mass. According to Dourado Neto et al. (2014), also observing the maize crop, biostimulants can increase the number of grains per row and the number of grains per ear, indicating that the response of yield components will depend on the germination capacity of seeds and the metabolic activity of seedlings.

Due to the economic importance of the crop and the limitations imposed during its development, it is important to elucidate the effects of the biostimulant on seed germination and on the initial growth of maize seedlings, as well as on seedling morphology. In this context, the objective is to evaluate the effect of seed treatment with different doses of a biostimulant derived from *Ascophyllum nodosum* extract on the agronomic performance of maize seedlings.

Material e métodos

The experiment was carried out in the southwestern region of Goiás (17°31'21.31" S, 51°13'22.75" W, at an altitude of 890 m), in the municipality of Montividiu, during the second season crop of 2017. The experiment was conducted from February to March 2017.

According to Köppen classification, the climate in the locality in which the experiment was conducted is Aw type, tropical climate with a dry season, characterized by more intense rainfall in summer compared to winter. Rainfall and air temperature data during the conduction of the experiment are shown in Figure 1.

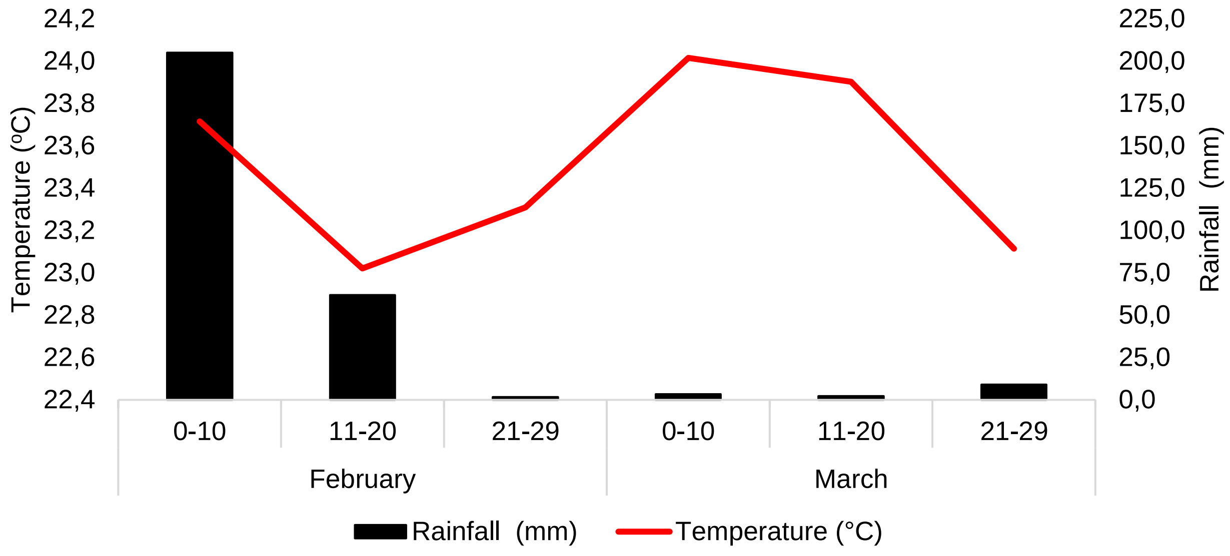

Figure 1 - Average values of rainfall and air temperature during the conduction of the test. Montividiu-GO, 2017 harvest.

The soil of the experimental area was under no-tillage, with soybean as its predecessor crop. A soil sample was collected from 0-20 cm depth, revealing the following characteristics: pH ($CaCl_2$) 4.5; Ca, Mg, K, Al, H + Al, sum of bases, and CEC: 1.73, 0.53, 0.23, 0.12, 4.13, and 2.54, 6.67 $cmol_c$ dm^{-3}, respectively; P: 6.81 mg dm^{-3}; organic matter: 41.8 g kg^{-1}; aluminum saturation (m) and base saturation (v): 4.5 and 38.10%, respectively; clay, silt, and sand: 47%, 8%, and 45%, respectively.

A randomized complete block design was used, with four replicates and five treatments, corresponding to five biostimulant doses in seed treatment, at 0.00; 0.25; 0.50; 0.75; and 1.00 liters per 100 kg of seeds. The biostimulant used is composed of the extract of the seaweed *Ascophyllum nodosum*, containing in its formulation 20% organic carbon (auxins, cytokinins, and gibberellins), 1.5% manganese, and 0.5% zinc. Seed treatment was carried out one hour prior to sowing in a dark plastic container, by adding the seeds and, then, the biostimulant; finally, the container was stirred until homogenization. The mixture was stored inside the container until sowing, keeping the seeds under more adequate environmental conditions, without exposing them to heat.

After treating the seeds with the biostimulant, they received phytosanitary treatment, using 0.15 L ha^{-1} thiamethoxan + 0.20 L ha^{-1} carbendazim + thiram + 0.15 L ha^{-1} lambda-cyhalothrin + chlorantraniliprole for each 100 kg of seeds. This treatment was performed in the same dark plastic containers, adding the products separately, with subsequent stirring of the container until homogenization of the seeds.

The plots contained five 6.0 m-long rows, spaced 0.50 m apart. The useful area was obtained taking into account the two central rows, disregarding 0.5 m of each end, thus presenting 5.0 m^2. Four days before sowing, weeds were desiccated with 3.0 L ha^{-1} glyphosate + 0.40 L ha^{-1} mineral oil + 0.025 L ha^{-1} syrup conditioner, in 58 L ha^{-1} syrup volume. Sowing was performed mechanically on February 28, 2017, using the hybrid PIONNER 4285 YH. In this operation, 375 kg ha^{-1} single superphosphate and 150 kg ha^{-1} potassium chloride were manually broadcasted.

Pest control was performed initially at 4 DAE (days after emergence), with 1.50 L ha^{-1} methomyl + 0.20 kg ha^{-1} imidacloprid + 0.20 kg ha^{-1} thiodicarb + 0.11 L ha^{-1} of the syrup conditioning adjuvant, plus 0.24 L ha^{-1} tembotrione + 3.00 L ha^{-1} atrazine. Subsequently, at 17 DAE, the following were applied for pest and disease control: 0.40 L ha^{-1} lufenuron + 0.20 kg ha^{-1} imidacloprid + 0.30 kg ha^{-1} thiodicarb + 0.11 L ha^{-1} Good Spray + 0.30 L ha^{-1} of the soybean oil methyl ester adjuvant plus 0.40 L ha^{-1} Priori Xtra (prothioconazole + trifloxystrobin), in 58 L ha^{-1} syrup volume.

Ten days after emergence (DAE) of seedlings, vigor evaluation was performed by observing the development of seedlings based on a subjective grading scale. The pre-established criteria were: plant height, vegetative leaf area, intensity of the green coloration of leaves (using a scale of comparative notes from 1 to 5 in relation to the control, applied by three evaluators, being 1: much lower; 2: lower; 3: equal; 4: higher; and 5: much higher), initial population (counting of the number of plants), and shoot height (measurement from the neck to the curvature of the last developed leaf) (Silva et al., 2016).

Subsequently, at 25 DAE, when the crop was in the V3 stage (third fully expanded leaf), the following characteristics were evaluated:

- Fresh mass of roots and shoots: the seedlings were separated into shoots and roots, exactly at the growth point; with the aid of a precision scale, the mass in grams was determined using five randomly chosen seedlings (Vanzolin & Nakagawa, 2007).

- Dry mass of roots and shoots: the shoots of five randomly selected maize seedlings were packed in paper bags and taken to an oven with forced air circulation at a temperature of approximately 65 °C until constant mass.

- Shoot height and root length: the shoot height of seedlings was determined with the aid of a millimeter ruler, measuring from the growth point to the end of the third fully expanded leaf. Root length was determined by measuring from the growth point to the root end, from five randomly chosen seedlings (Vanzolin & Nakagawa, 2007).

- Leaf area was determined in the V3 stage by means of a LI-COR area meter, model LI-3000 (LI-COR, Inc. Linoln, Nabraska, USA), in five randomly selected seedlings.

The data were submitted to analysis of variance and, when significance was observed for a certain variable, regression analysis was performed to verify the behavior of the characteristics as a function of the biostimulant doses.

Results and discussion

According to the analysis of variance, increasing biostimulant doses in the treatment of maize seeds provided a significant effect ($P<0.05$) on seedling vigor, fresh and dry mass of shoots, shoot height, and leaf area, with no difference for the other evaluated characteristics (Table 1).

Table 1 - Summary of the analysis of variance of the characteristics vigor (VIG), root (RFM) and shoot (SFM) fresh mass, root (RDM) and shoot (SDM) dry mass, shoot height (SH), root length (RL), and leaf area (LA) of seedlings obtained from corn seeds treated with doses of biostimulant. Montividiu-GO. Agricultural crop 2017.

Causes of variation	FD	VIG	RFM	SFM	RDM	SDM	SH	RL	LA
Treatment	4	*	ns	**	ns	**	**	ns	*
CV (%)	--	10,1	15,6	6,9	19,4	10,6	3,1	15,9	7,6

FD: Freedom degree; CV: Coefficient of variation; **; * and ns: significant at 1% and 5%, and not significant at 5% probability by the F test, respectively.

The use of increasing doses of the biostimulant provided increases in seedling vigor up to the dose 0.50 L per 100 kg seeds. According to the adjusted equation, the maximum performance point of the trait was reached with the dose 0.52 L per 100 kg seed (Figure 2). This result was supported by Gehling et al. (2014), where doses up to 0.50 L per 100 kg of seeds of the biostimulant derived from *Ascophyllum nodosum* promoted increases in the emergence rate and dry mass of shoots, increasing the germination capacity of wheat seeds. Thus, doses of up to 0.50 L per 100 kg seeds provided a rapid and uniform emergence, allowing greater development of the adventitious roots and favoring the absorption of nutrients in the period between emergence and evaluation.

Notwithstanding, from the dose of 0.50 L per 100 kg seeds, there was a decrease in the response of this characteristic to the doses employed. This is justified because, at high doses, the bioactivity of biostimulants can interact with substances and microorganisms present in the soil, which can generate antagonistic or synergistic effects, leading in some cases to inefficient results (Zandonadi et al., 2014). Moreover, excess nutrients and phytohormones can cause toxicity to the plant, affecting the cellular metabolism and the initial development of seedlings, besides reducing their vigor, as verified in this work (Castro et al., 2017).

It is important to note that doses above the point of maximum technical efficiency caused imbalance in the adequate concentrations and proportions of auxins, cytokinins, and gibberellins. This hormonal imbalance, among the numerous effects, causes a decrease in the quantity and quality of chemical signals, directly interferes with the metabolic capacity of seedlings, and harms the shoot/root relationship causing unequal growth of maize seedlings (Craigie, 2011).

The values of fresh mass of seedlings follow the same trend as in the vigor evaluation, with increases in the value of the trait up to 0.50 L per 100 kg seeds. Higher doses resulted in a reduction of the fresh mass of the plants, with a polynomial behavior of the source of variation, highlighting that the point of maximum technical efficiency was reached with 0.66 L per 100 kg seeds (Figure 3).

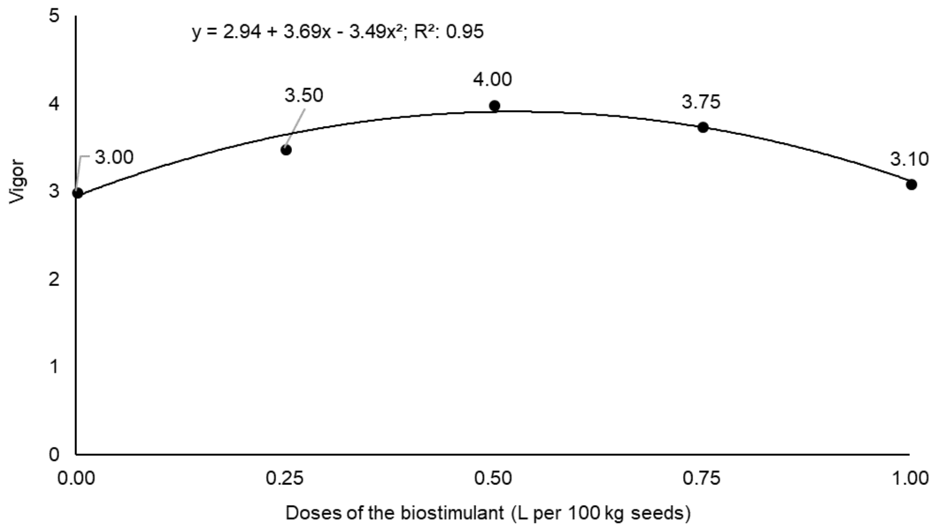

Figure 2 - Vigor of maize seedlings as a function of the doses of the biostimulant. Montividiu GO, 2017.

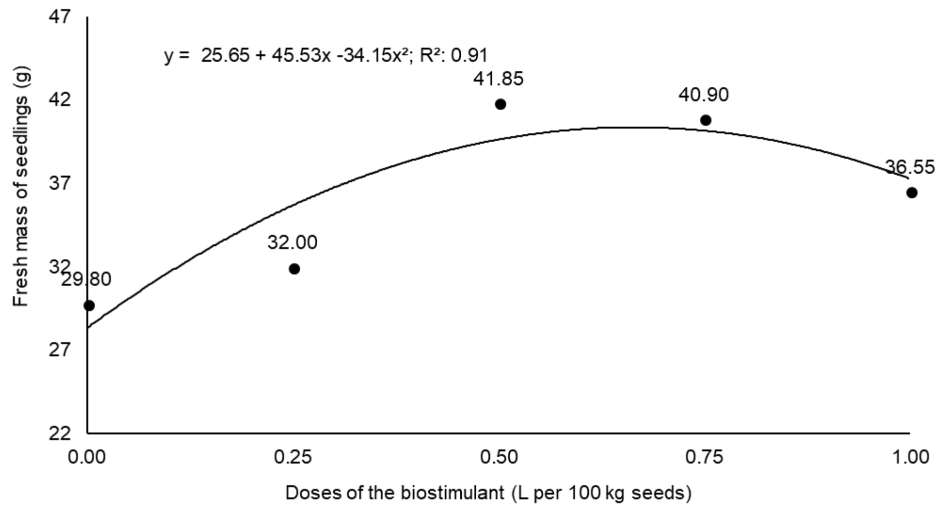

Figure 3 - Massa fresca da parte aérea das plântulas de milho em função das doses do bioestimulante. Montividiu-GO, 2017.

It is important to note that this dose, 0.50 L per 100 kg seeds, provided increases of 30% in fresh mass when compared to the control (Table 2), allowing to infer that a suitable dose of the biostimulant favors morphological characteristics of the seedlings. Plant hormone analogues, in an adequate proportion, tend to optimize the transmission of chemical signals, thus enhancing the metabolic activities of the seedlings. This is due to the increase of chlorophyll biosynthesis, which provides increases in the photosynthetic rate, resulting in greater synthesis of metabolites and increased fresh mass (Khan et al., 2009). This process triggers the increase of the transpiration rate, while increasing the osmotic pressure in xylem vessels and allowing plants with higher fresh mass to have a more efficient root system in the uptake of water and nutrients (MacKinnon et al., 2010).

There was increases in the shoot dry mass as a function of the biostimulant doses until 0.70 L 100 kg^{-1} of seeds; from this point, it is observed decrease in the values of this characteristic (Figure 4). This result ratifies that found for fresh mass, and points out the potential of the biostimulant in increasing nutrient translocation, cell division and multiplication (Bomtempo et al., 2016; Martynenko et al., 2016), and, consequently, the dry mass of the seedling. This morphological change provides characteristics of great interest to farmers, such as resistance to water deficit and extreme temperatures, because plants with higher shoot mass (fresh and dry) tend to endure the lack of soil moisture for longer, before entering a permanent wilting point (Vollet, 2006). Furthermore, as a compatible osmolyte, the betaines present in the biostimulant stabilize intracellular macromolecules and contribute to the protection of membranes at high temperatures

(Bergonci et al., 2000; Vollet, 2006; MacKinnon et al., 2010).

The shoot length of maize seedlings presented linear behavior for the sources of variation, so that the increase of the doses of the biostimulant compound provided larger seedlings (Figure 5). However, no effects of biostimulant doses were observed on root length (Table 2).

Table 2 - Vigor (VIG), root (RFM) and shoot (SFM) fresh mass, root (RDM) and shoot (SDM) dry mass, shoot height (SH), root length (RL), and leaf area (LA) of seedlings obtained from corn seeds treated with doses of biostimulant. Montividiu-GO. Agricultural crop 2017.

Dose (L 100 kg^{-1} seeds)	VIG	RFM	SFM	RDM	SDM	SH	RL	LA
	---	--------------- (g) ---------------				---- (cm) ----		(cm^2)
0.00	3.00	3.75	29.80	1.90	13.30	55.15	15.10	111.90
0.25	3.50	3.65	32.00	1.85	16.10	59.10	15.95	135.60
0.50	4.00	4.00	41.85	2.05	20.60	58.50	20.20	142.60
0.75	3.75	4.55	40.90	2.70	20.40	63.15	19.45	151.75
1.00	3.10	4.22	36.55	2.80	18.92	62.75	18.75	148.25
Mean	3.45	4.05	35.60	2.25	17.90	59.75	17.90	138.00

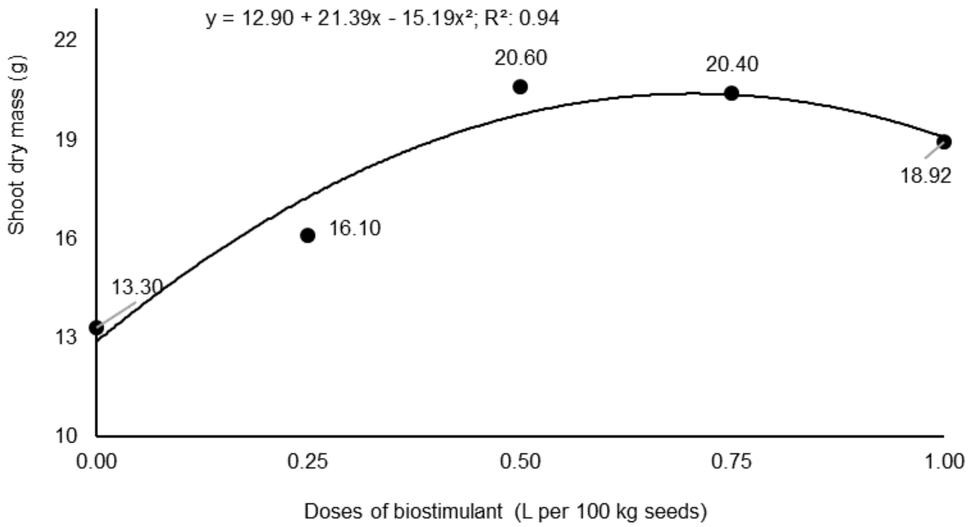

Figure 4 - Shoot dry mass of maize seedlings as a function of the doses of the biostimulant. Montividiu GO, 2017.

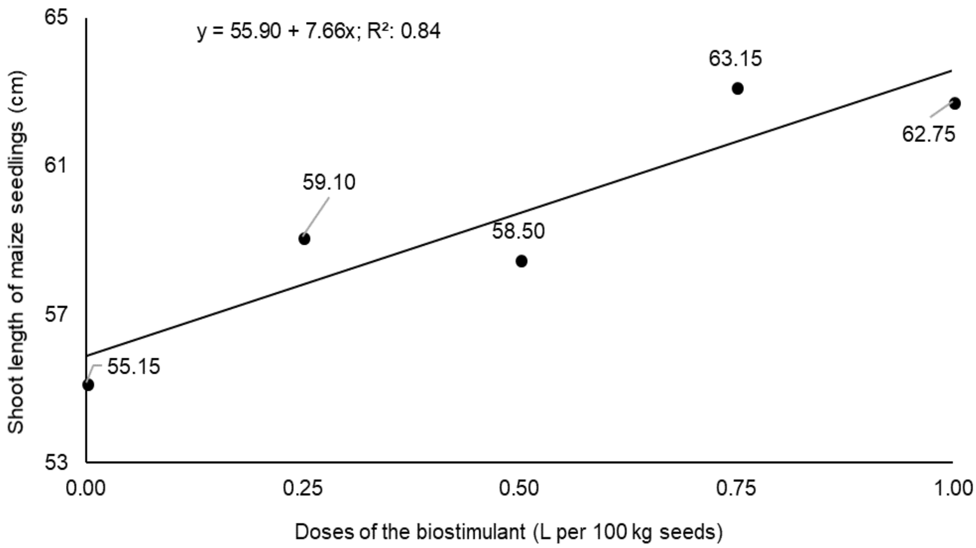

Figure 5 - Shoot length of maize seedlings as a function of the doses of the biostimulant. Montividiu GO, 2017.

Ascophyllum nodosum extracts have traces of auxins, cytokinins, and gibberellins; nonetheless, the development of plants depends on some relationships, such as that between auxins and cytokinins (Castro & Amaral 2014). Cytokinins act by stimulating cell division and both participate in cell cycle regulation. However, despite acting synergistically to stimulate cell division, these hormonal classes act antagonistically in the control of branch initiation (promoted by cytokinins) and root initiation (promoted by auxins), as well as in the establishment of apical dominance (Salisbury & Ross, 2012).

It is believed that the good fertility of the experimental area, with emphasis on the levels of Ca, Mg, P, besides the CEC, has increased the availability of these elements in the soil solution, maintaining adequate levels in the intracellular spaces. In addition to allowing greater uptake by the cells, the nutritional status of the soil stimulated cytokinin biosynthesis and, consequently, induced shoot growth (Raven et al., 2007; Salisbury & Ross, 2012; Taiz et al., 2015). It is possible that the increase in cytokinin biosynthesis inhibited IAA production, which, together with the pH of the slightly acidic soil, may have been determinant for the results of dry and fresh mass and root length (Binsfield et al., 2014).

In a study carried out with *Ascophyllum nodosum* extract, Santos et al. (2013) observed no significant difference for root dry mass (RDM), and the same was verified at 27 days after emergence of the seedlings, when RDM was 1.97 g, corroborating the result found by the cited authors. On the other hand, Càto (2006), observing the influence of a biostimulant based on auxin (50 mg L^{-1}), cytokinins (90 mg L^{-1}), and gibberellin (50 mg L^{-1}) on the initial development of sorghum, reported that the doses did not promote differences in root length, although shoot dry mass was increased. In this case, the higher concentration of cytokinins possibly promoted the inhibition of auxin synthesis, which, in turn, resulted in greater shoot development, as observed in this study.

Increasing biostimulant doses also promoted increases in leaf area, with the dose of 0.75 L per 100 kg seeds (Figure 6) providing a 35% increase in relation to the control (Table 2).

Due to the fact that maize is widely cultivated as a second-season crop, when climatic conditions are not always favorable, it is necessary to adopt strategies to minimize the effects of environmental stresses, since maize seedlings with larger leaf area have larger contact surface for light absorption and, consequently, their photosynthetic activity will be potentiated, resulting in greater metabolic activity, and greater production and translocation of carbohydrates, providing greater resistance to environmental stresses (Kolling et al., 2016).

It is worth mentioning that the evaluations were carried out at 10 and 20 days after emergence (DAE) of the seedlings, when they were in the V3 stage, at the beginning of the determination of possible leaves and ears (Magalhães et al., 2007). For this reason, it is necessary that during this period the producers seek technologies that allow the development of seedlings under adverse conditions, providing the development of some morphological characteristics that can contribute for the hybrids to express more sharply their yield potential (Conceição et al., 2008).

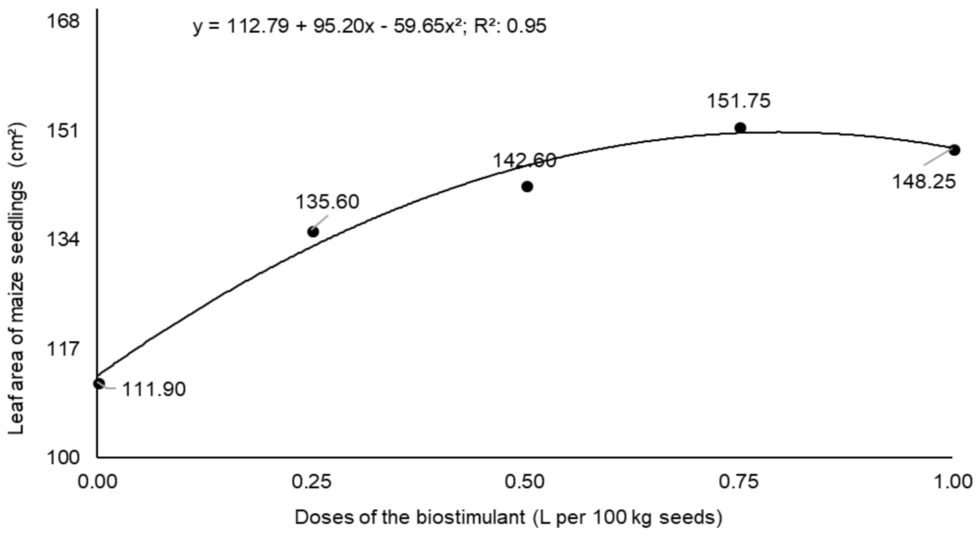

Figure 6 - Leaf area of maize seedlings as a function of the doses of the biostimulant. Montividiu GO, 2017.

Thus, it is believed that maize seedlings with higher yield potential have higher shoot fresh and dry mass. Larger seedlings and those with larger leaf area will be more efficient in water use and nutrient absorption, with expanded photosynthetic capacity and, consequently, greater potential for leaf and ear determination.

Conclusões

The biostimulant derived from *Ascophyllum nodosum* has potential for use in seed treatment as it provides better initial development of maize seedlings. Increasing biostimulant doses provide increases in plant vigor, shoot fresh and dry mass, shoot height, and leaf area. It is recommended the doses of 0.50 and 0.75 liters per 100 kg seeds as the most appropriate to the initial development of maize seedlings.

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