BIOSTIMULANT AND MICRONUTRIENT APPLICATIONS IN THE PRODUCTION OF *Acacia mangium* SEEDLINGS

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ABSTRACT

Aims: evaluate the effects of applying biostimulant and micronutrients on *Acacia mangium* Willd seedlings.
Study design: A randomized complete block design was used in a 2x5 factorial scheme, with four replications.
Place and Duration of Study: The experiment was conducted at the *Universidade Federal de Mato Grosso do Sul*, at the Chapadão do Sul Campus, MS, with the geographical coordinates 18º 46' 44" S and 52º 36' 49" W, in a nursery area from October 2015 to January 2016.
Methodology: The treatments consisted of a combination of the presence or absence of a solution containing micronutrients and five doses of biostimulant (0, 7.5, 15.0, 22.5, and 30.0 mL per L of water). The commercial product Stimulate ^{Tm} was used as the biostimulant, and the micronutrient solution was prepared at the concentration of 1.0%. The micronutrients in the solution were in the following formulations: $ZnS0_4$, H_3BO_3 and $CuS0_4$. The seeds were sown in tubes containing the commercial substrate *Carolina Soil do Brasil*, together with the use of Osmocote Plus^{Tm} fertilizer.
Results: The use of biostimulant associated with micronutrients favored the rate of emergency speed. The doses of 24.8, 14.0 and 26.1 mL L^{-1} of biostimulant water provided the highest values for leaf area, plant height and total dry mass, respectively, when associated with the use of micronutrients. The height / diameter ratio decreased with increasing doses of biostimulant, while the Dicson quality index increased in the same condition.
Conclusion: The use of micronutrients and biostimulants were favourable for the production of *A. mangium* seedlings. Only the height/diameter ratio (HDR) reduced for all biostimulant doses.

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Comment [H2]:

Comment [H3]:

Keywords: Stimulate, forest nutrition, phytoregulators, seedling fertilization

1. INTRODUCTION

Among the species belonging to the genus Acacia, Acacia mangium is an exotic tree originating in Australia and widely cultivated in the tropical world, as well as being considered one of the most promising species for reforestation in the tropics [1]. This is a species that reaches its maximum performance in open air areas, as its initial growth rate is proportional to light incidence, thus becoming a fast-growing pioneer legume with facility to establish itself in a wide variety of environmental conditions [2].

The use of growth regulators in seedlings associated with or without fertilization may interfere with the seedling quality after planting, in which their development is physiologically modified as a result of the regulating stimulus [3]. Plant regulators or biostimulants are organic, non-nutrient compounds formed by indolebutyric acid, kinetin and gibberellic acid, which can increase plant growth and development, stimulate cell division and also increase the absorption of water and nutrients by plants, depending on their composition, concentration and proportion of substances employed [4].

In addition to the importance of plant regulators, micronutrients play a major role in plant development, and these have been associated with growth regulators in seed treatment in seeking higher germination values and better plant establishment in the field; moreover, studies on the nutritional requirements of forest species are essential to help ensure proper forest management [5,6]. It was revealed that some essential elements such as macro and micronutrients are essential for vegetable chemical mediation and signaling [4]. In this context, it can be inferred that there must be nutritional and hormonal balance due to minerals participating as mediators and chemical signaling agents.

Comment [H4]: ?

Comment [H5]: My advice

In the literature, there are different studies that show the importance of micronutrients for developing healthy plants, such as [7] with mahogany seedlings, where they observed that the micronutrient limiting their development was Cu^{+2}, as it is unavailable when its content is equal to or below 0.08 mg kg-1 of soil. In the work by [8], the micronutrient content in the leaves of five native forest species in the forest presented a general order of: Fe > Mn > B > Zn > Cu.

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The use of biostimulant promotes plant growth and development, with an emphasis on greater rooting and consequently greater water and nutrient uptake by plants [9]. Its use has optimized the physiological processes of seedling germination and growth in several species [10]. Research on using growth regulators, whether associated with fertilization or not, has been increasingly occurring as a way to improve and accelerate the seed germination process, as well as promoting the growth of young plants, arousing the interest of several researchers in recommending plant regulator use [11, 3].

Considering that a biostimulant associated or not to micronutrients can improve the performance of nursery seedlings, the present work aimed to evaluate the effect of micronutrient application and different biostimulant doses on seed germination and on the initial growth of *Acacia mangium* seedlings.

2. MATERIAL AND METHODS

The experiment was conducted at the *Universidade Federal de Mato Grosso do Sul*, at the Chapadão do Sul Campus, MS, with the geographical coordinates 18º 46' 44" S and 52º 36' 49" W, in a nursery area from October 2015 to January 2016, using *Acacia mangium* seeds purchased from the Biosementes company.

A randomized complete block design was used in a 2x5 factorial scheme, with four replications. The treatments consisted of a combination of the presence and absence of a solution containing micronutrients and five biostimulant doses (0, 7.5, 15.0, 22.5 and 30.0 mL per L of water). The commercial product Stimulate was used as the biostimulant, and the micronutrient solution was prepared at a concentration of 1.0%. The micronutrients in the solution were in the following formulations: $ZnS0_4$, H_3BO_3 and $CuS0_4$. Each plot consisted of eight tubes, with a single seed in each.

Comment [H7]: Contains ? microbes or hormone?

Seed dormancy was broken prior to sowing by soaking the seeds in water for one minute at 100ºC. The seeds were then immersed in the biostimulant solution at the different dosages for 4 hours. After this period, they were placed in plastic trays containing tubes with 50 cm³ capacity which contained Carolina Soil do Brasil commercial substrate presenting the following composition: Sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural gypsum and traces of NPK fertilizer. Substrate fertilization was done using the Osmocote Plus fertilizer (formulation 15-9-12), with controlled release for a period of 3 to 4 months, at the substrate dosage of 6 kg m^{-3}. They were later kept inside a greenhouse and irrigated by micro sprinkling three times a day until 90 days after sowing.

Seedling emergence count began on the seventh day after sowing, continuing for 10 days until stabilization, and the percentage of emerged plants was calculated using the following formula:

$$IVE = \frac{E_1}{N_1} + \frac{E_2}{N_2} + \cdots \frac{E_N}{N_N}$$

In which E_1, E_2, … E_N referred to the number of seedlings emerged on the first, second and n^{th} day, and N_1, N_2, ... $N_{N,}$ number of days after sowing the first, second, and n^{th} counts [12].

Comment [H8]: IVE ?

At 90 days after sowing, the following seedling characteristics were evaluated: a) plant height (cm), obtained from the level of the tube until the height of the apical meristem, using a graduated ruler; b) stem diameter (mm), determined with a digital caliper; c) number of leaves; d) dry mass of the area and total area, determined after drying the material in an air circulation oven at 65ºC, remaining until reaching constant mass; e) leaf area (cm^2), determined by using a leaf area meter (CI-203 CA, Bioscience); f) specific leaf area (SLA) ratio between leaf area and dry leaf mass; g) stem height and diameter ratio (HDR) (cm mm^{-1}); h) shoot dry mass and root dry mass ratio (SRDMR); i) Dickson Quality Index (DQI), consists of a balanced formula that includes the relationships of the morphological parameters such as total dry matter weight (TDMW), shoot dry matter weight (SDMW), root dry matter weight (RDMW), shoot height (H) and collection diameter (CD); j) Chlorophyll - determining the chlorophyll index in the leaves using a chlorophyll meter (ClorofiLOG-CFL1030, Falker), considering one leaf per seedling which was totally exposed to light for measurement.

Comment [H9]: Which parts of plant

Comment [H10]: SDM/RDM

The data were submitted to analysis of variance at 5% probability and there was a significant difference in the regression analysis for the Biostimulant doses.

3. RESULTS AND DISCUSSION

Most of the evaluated characteristics were influenced by the associated use of micronutrients and biostimulant, while the variables of plant height, stem height/diameter ratio (HDR) and Dickson quality index (DQI) were only influenced by the biostimulant application. The variables, number of leaves and shoot dry mass/root dry mass ratio (SRDMR) were not influenced by applying the products alone or in combination.

Biostimulant application associated with micronutrient application enabled the emergence speed index (ESI) to always exceed the values observed when the micronutrient solution was not used for all biostimulant doses (Figure 1). The ESI response to micronutrient use is the opposite. Without employing micronutrients, ESI begins to reduce from the control, reaching the lowest value at the dose of 17.1 mL of the solution, assuming an increase from this dose up to the maximum dose of 30 mL L^{-1}. With the use of micronutrients, the ESI increases from the control until the maximum biostimulant dose of 22 mL, then reducing from that point (Figure 1). [13], found a significant effect of the biostimulant doses on the emergence of *Dimorphandra mollis* seedlings, and the emergence speed index showed an

Comment [H11]: R2 of regression trend

Comment [H12]: inverse parabola is caused by experimental error? Please explain estimated peak of 0.9 with a 15 mL dose of the product. On the other hand, [10], observed that there is a tendency to reduce the plant emergence percentage due to regulator use, in which the authors verified a negative influence on the emergence of jatobá seedlings for the higher biostimulant doses, being that a dose of 35 mL may have been excessive, even causing germination inhibition. Also, the reduced volume and root growth of young *Ilex paraguariensis* plants may have been influenced by the lower micronutrient availability to the plants from the liming action observed by the reduced Cu, Zn and mainly Mn leaf content [14].

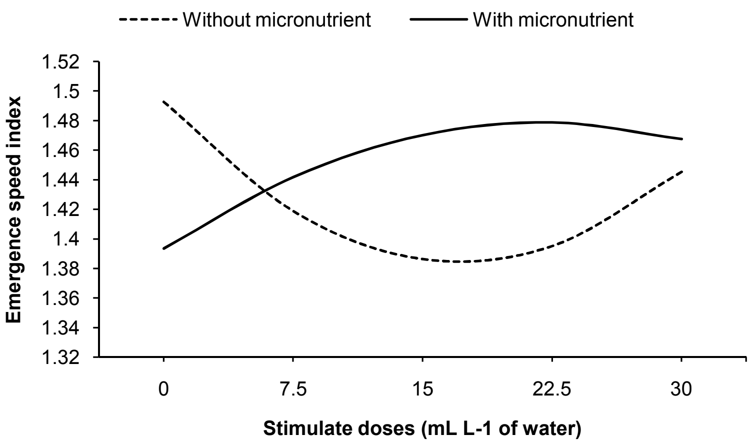

Fig. 1. Emergence speed index for *A. mangium* seeds submitted to biostimulant doses and micronutrient application.

The leaf area of *A. mangium* seedlings was always higher when the micronutrient solution was used, reaching the highest value at the Stimulate dose of 24.8 mL L^{-1}, while the highest value reached without micronutrient application was with the dose of 17.2 mL L^{-1}. Therefore, it is verified that the biostimulant application alone already promotes leaf expansion, but the values were higher when associated with micronutrient use (Figure 2). In evaluating the effects of different biostimulant doses on the emergence and development of '*Roxinho do Kênia*' passion fruit seedlings, [15] related that biostimulant application provided an increase in leaf area at all doses (6, 12, 18, 24 and 30 mL kg^{-1} of seeds) when compared to the control, and the highest leaf area average was verified in the seedlings of the treatment with the dose of 12 mL kg^{-1}.

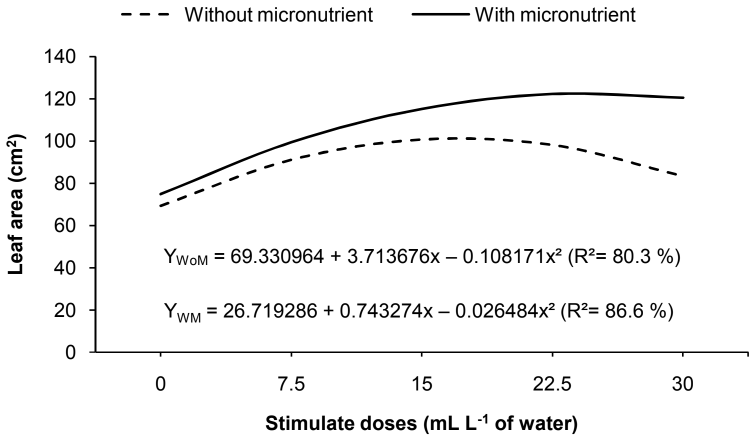

Fig. 2. Leaf area of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

Biostimulant use promoted height growth in the seedlings up to the dose of 14 mL L^{-1}, reaching a mean value of 32 cm (Figure 3). The height gain provided by the best biostimulant dose was 19.8% higher than that of the control, while the highest applied biostimulant dose (30 mL L^{-1}) provided a 25.2 cm seedling; still a lower height than what was observed without the biostimulant dose. This response may have been triggered by the presence of the group of hormones that structure the biostimulant, which has the function of providing stem growth, among other physiological effects. The growth regulators promoted a significant increase in the stem length of '*Roxinho do Kênia*' passion fruit seedlings, in which the seedlings from the biostimulant treatment at the dose of 6 and 24 mL.kg^{-1} presented the longest stem length, while those of the control treatment had shorter length [15].

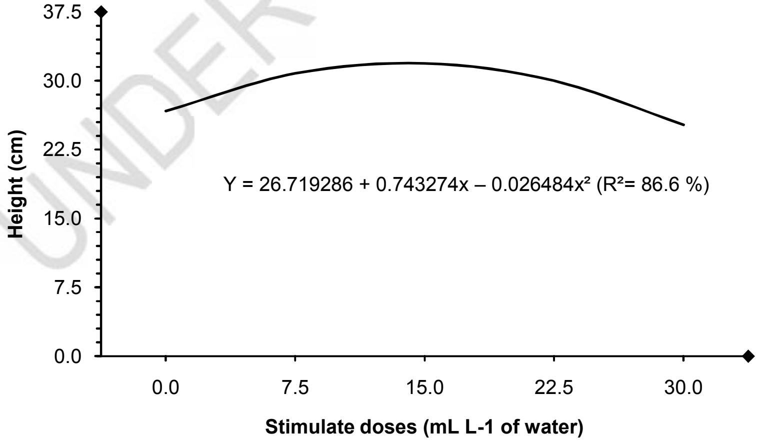

Fig. 3. Height of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

Comment [H13]: R2= 0.866

According to [16], the shoot height of guavira seedlings did not vary significantly between biostimulant application treatments. [10] observed higher *Jatoba* seedling height using 15 mL of Stimulate application to 0.5 kg of seeds. It is known that this biostimulant is constituted by three hormones, with auxin among them, and according to [4], auxin promotes biochemical events within the theory of acid growth, thus increasing the number of cells and the growth of finer root tissues, in turn inferring that the presence of this hormone in the biostimulant formulation may have promoted *A. mangium* seedling growth.

However, the presence of growth regulating hormones can promote stem diameter growth in seedlings (as in this case), in which the plants reached 2.8 mm from the dose of 0.0 to the dose of 23.6 mL L^{-1}, which is 55.5% larger than the diameter obtained without the biostimulant application (Figure 4). In the results described by [17]Cunha et al. (2016), Stimulate application in the furrow and foliar route in corn within 0.45 m spacing enabled larger stem diameter.

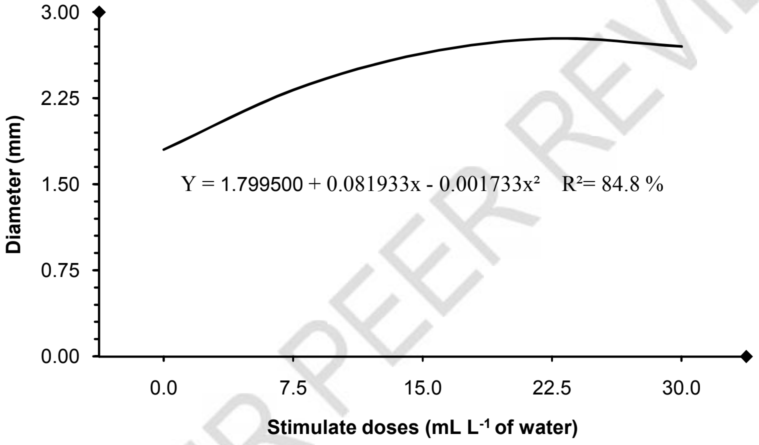

Fig. 4. Stem diameter of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

Comment [H14]: R2=0.848

In evaluating the emergence and initial growth of *Jatoba* seedlings treated with Stimulate and under shading, [10] observed that stem diameter showed linear growth, reaching 3.14 mm at 226 days after sowing. The authors further describe that the biostimulant doses do not alter seedling growth or metabolism after 100 days. Biostimulant application has been tested in forest species, in grains, and also in epiphytes, as in the study by [18], where the authors describe the effect of different biostimulant doses on the development of *Vriesea carinata Wawra* (Bromeliaceae), showing that 4 and 8 mL L^{-1} concentrations promoted significant and positive effects.

Stem development can be promoted by biostimulant application, in which the presence of gibberellic acid, cytokinin and auxin are responsible for cell division, as gibberellic acid promotes stem growth through the differentiation of meristematic cells, and auxin induces differentiation of phloem and xylem [4].

Shoot dry mass accumulation was always superior for micronutrient use, and the maximum accumulation was obtained with the dose of 25.7 mL L^{-1}, reaching 1.4 g. Without micronutrient application, biostimulant application promoted a growing accumulation of shoot dry mass up to the dose of 17.1 mL L^{-1}, reaching 1.3 g (Figure 5). In *Lafoensia pacari* seedlings, shoot dry matter weight presented a maximum point at 47.5 mg Cu kg^{-1} of soil, whereas *Ateleia glazioviana* seedlings showed linear reduction [19].

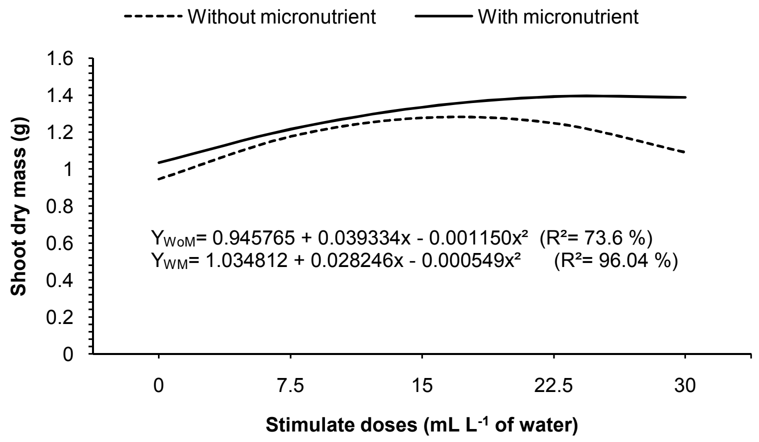

Fig. 5. Shoot dry mass of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

The total dry mass of *A. mangium* seedlings presented similar behaviour to that observed for shoot dry mass, where the micronutrient application favoured dry mass accumulation in the plant, reaching the maximum value at the dose of 26.1 mL L^{-1} with 1.6 g. Without the micronutrient application, the maximum total dry mass accumulation occurred at a dose of 17.5 mL L^{-1} reaching 1.4 g (Figure 6). For [13], no statistical differences were observed between the biostimulant doses applied to seedlings for the dry mass of *Dimorphandra mollis* seedlings, obtaining a mean of 0.134 g.

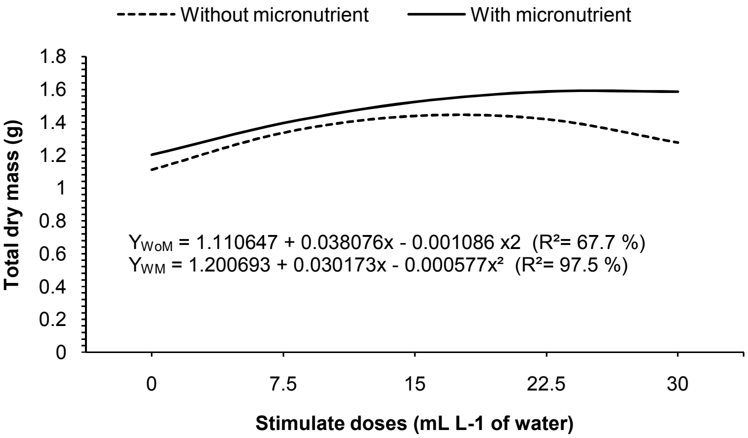

Fig. 6. Total dry mass (root; stem; leaves) of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

[20] observed increases in 100 seed weight and ESI on the dry weight of seedlings when castor seeds were treated with these micronutrients. The authors also conclude that physiological quality of castor seeds is affected by seed micronutrient application. Within the shoot dry weight evaluation in castor bean seeds, it was possible to verify that there was an absorption order among the micronutrients used, being: Mo> Fe = Zn> Co = Mn = H_2O> Bo = control, in ascending order [20]. In this way, it can be inferred that the applied micronutrients influenced the dry mass development of *A. mangium* seedling.

The HDR value decreased linearly with the biostimulant doses, mainly as a function of the lower stem diameter value in the lower doses of the product. The HDR values found in the control and in the highest biostimulant dose were 14.78 and 9.45 mL L^{-1}, representing a variation of 56.4% (Figure 7). However, it can be observed that the values are higher than the range considered adequate for this relationship, which is from 5.4 to 8.1 [21], corroborating the results obtained by [19] for the *Lafoensia pacari* species.

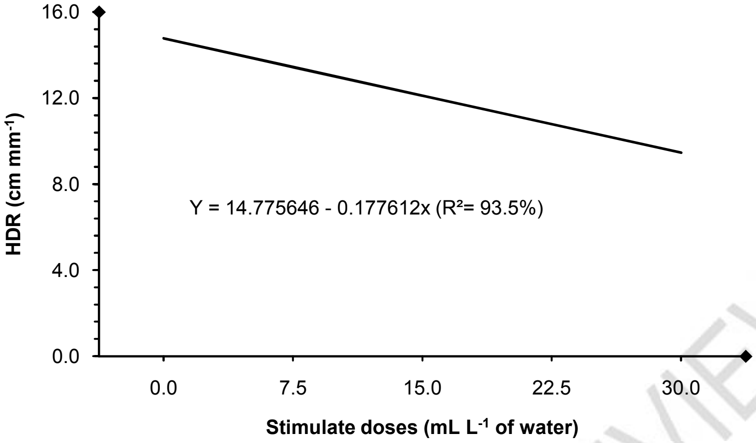

Fig. 7. Height/diameter ratio (HDR) of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

[10] found a higher HDR value for *Jatoba* seedlings with the biostimulant dosage of 35 mL. The effect of micronutrients may affect the development of different species, as verified by [19] in evaluating the effect of copper doses on the growth and quality of *Timbó* seedlings (*Ateleia glazioviana*) and *Dedaleiro* (*Lafoensia pacari*), where they observed that there was no significant difference for the height/diameter ratio.

The specific leaf area presented similar behaviour to the response of the biostimulant doses for the presence and absence of micronutrients. Specific leaf area values were higher in the absence and initial biostimulant doses without micronutrients compared to micronutrient application, reaching the highest dose at 14.1 mL L^{-1}, with 140.6 g (Figure 8).

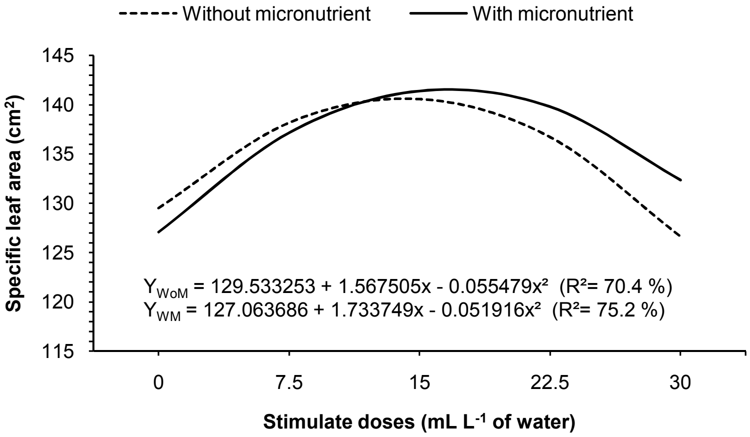

Fig. 8. Specific Leaf Area of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

With the micronutrient application, the specific leaf area values were higher for the higher biostimulant doses, reaching the maximum value with the dose of 16.7 mL L^{-1}, with 141.5 g. This indicates that plants with higher micronutrient rates tend to be more demanding in relation to growth hormones, requiring higher doses in relation to the less fertilized plants to reach better development in this parameter. The results of specific leaf area in the study of *Caesalpinia ferrea* seedlings showed greater leaf blade expansion without a corresponding increase in leaf dry mass, and showing reduced leaf thickness as the luminosity decreases [22].

The DQI, which takes into account the shoot, roots and total dry matter production, as well as the height and diameter of the seedling collection, increased linearly with the biostimulant doses (Figure 9). The Dickson quality index presented a significant reduction in *Timbó* seedlings with the application of copper doses, resulting in values below those of the proposed minimum index [19].

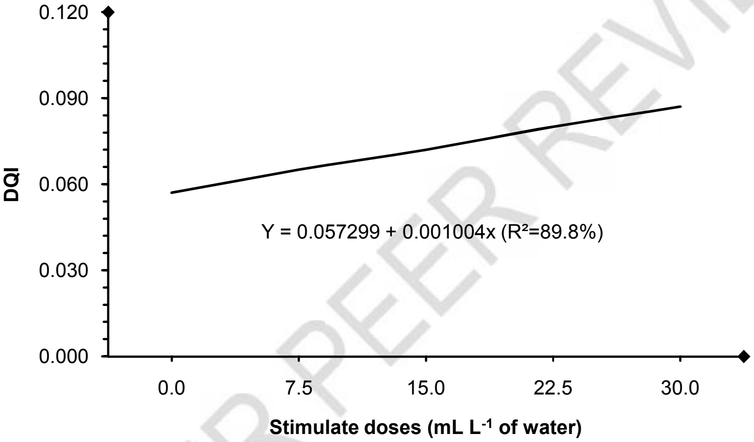

Fig. 9. DQI for *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

One tool that can identify the proper development and if the seedlings have the maximum potential for survival after transplantation to the field is to evaluate the quality of the tree seedlings still in the nursery using indexes that express relationships between the growth parameters [19].

In testing biostimulant application on the basis of seaweed extract in the production of *Anonna glabra* L. rootstocks, [23] showed that there was an increase with the dose of 2 mL L^{-1} of the seaweed extract, resulting in an increase of more than 105% in comparison with the absence of the biostimulant.

For the chlorophyll content, micronutrient application always maintained values above those observed without micronutrient application, and the maximum development point for the seedlings that received micronutrients and those that did not receive application had the same value of 14.8 mL of Stimulate (Figure 10). The best chlorophyll content of the seedlings corresponded to the same dose with the micronutrient solution application. Among the various components present in the environment, light is a prime factor for plant growth, not only for providing energy for photosynthesis, but also for providing signals that coordinate their development through spectral quality, light receptors sensitive to different intensities and polarization state. According to [22], the higher the luminosity degree, the higher the growth rate, the dry mass accumulation rate and the net photosynthesis of *Caesalpinia ferrea* seedlings.

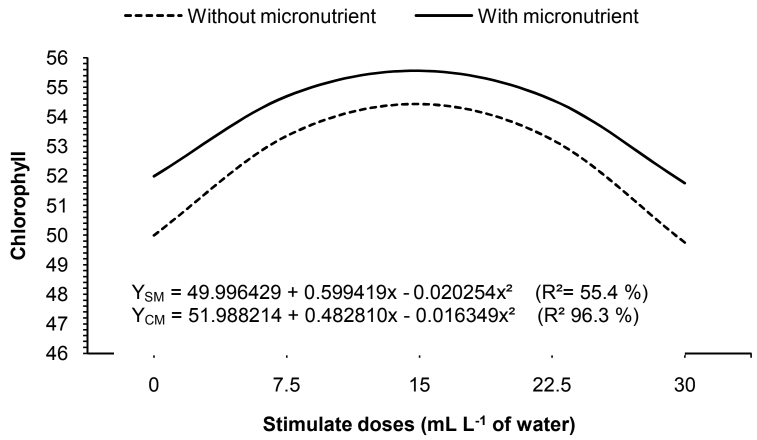

Fig. 10. Chlorophyll content for *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

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In studying the effect of the Stimulate biostimulant on *Jatoba* seedlings, [10] observed higher chlorophyll content in 30% light conditions compared to plants that developed in full sunlight.

4. CONCLUSION

The use of micronutrients and biostimulants were favourable for the production of *A. mangium* seedlings. Only the height/diameter ratio (HDR) reduced for all biostimulant doses.

Comment [H16]: Which doses, your recommendations, highlight, These should be emphasized

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