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

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7 **Nathália Ferreira Alves¹, Sebastião Ferreira de Lima^{2*}, Ana Paula Leite**
8 **de Lima², Catia Aparecida Simon³, Pedro Paulo Virgilio Damis⁴**

9
10 ¹*Progress of forestry engineering course, UFMS, Chapadão do Sul campus, PO Box, 112*

11 ²*Docentes dos cursos de Agronomia e Engenharia Florestal, UFMS, campus Chapadão do*
12 *Sul, Caixa Postal, 112*

13 ³*PhD student at Esalq / USP, department of soil science, Piracicaba, SP*

14 ⁴*Student of Agronomy, UFMS, Chapadão do Sul campus, PO Box 112*

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19 **ABSTRACT**
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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 18° 46' 44" S and 52° 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 StimulatTM 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: ZnSO₄, H₃BO₃ and CuSO₄. The seeds were sown in tubes containing the commercial substrate *Carolina Soil do Brasil*, together with the use of Osmocote PlusTM 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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22 **Keywords:** StimulatTM, forest nutrition, phyto regulators, seedling fertilization
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* E-mail address: sebastiao.lima@ufms.br.

24 **1. INTRODUCTION**

25

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

32

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

40

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

49

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

56 The use of biostimulant promotes plant growth and development, with an emphasis on
57 greater rooting and consequently greater water and nutrient uptake by plants [9]. Its use has
58 optimized the physiological processes of seedling germination and growth in several species
59 [10]. Research on using growth regulators, whether associated with fertilization or not, has
60 been increasingly occurring as a way to improve and accelerate the seed germination
61 process, as well as promoting the growth of young plants, arousing the interest of several
62 researchers in recommending plant regulator use [11, 3].

63

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

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69 **2. MATERIAL AND METHODS**

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71 The experiment was conducted at the *Universidade Federal de Mato Grosso do Sul*, at the
72 Chapadão do Sul Campus, MS, with the geographical coordinates 18° 46' 44" S and 52° 36'
73 49" W, in a nursery area from October 2015 to January 2016, using *Acacia mangium* seeds
74 purchased from the Biosementes company.

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76 A randomized complete block design was used in a 2x5 factorial scheme, with four
77 replications. The treatments consisted of a combination of the presence and absence of a
78 solution containing micronutrients and five biostimulant doses (0, 7.5, 15.0, 22.5 and 30.0
79 mL per L of water). The commercial product Stimulat™ was used as the biostimulant, and
80 the micronutrient solution was prepared at a concentration of 1.0%. Stimulat™ is a liquid
81 product composed of three plant regulators with 90 mg L⁻¹ (0.009%) of kinetin, 50 mg L⁻¹
82 (0.005%) of gibberellic acid and 50 mg L⁻¹ (0.005%) of indolebutyric acid. The micronutrients
83 in the solution were in the following formulations: ZnSO₄, H₃BO₃ and CuSO₄. Each plot
84 consisted of eight tubes, with a single seed in each.
85

86 Seed dormancy was broken prior to sowing by soaking the seeds in water for one minute at
87 100°C. The seeds were then immersed in the biostimulant solution at the different dosages
88 for 4 hours. After this period, they were placed in plastic trays containing tubes with 50 cm³
89 capacity which contained Carolina Soil™ do Brasil commercial substrate presenting the
90 following composition: Sphagnum peat, expanded vermiculite, dolomitic limestone,
91 agricultural gypsum and traces of NPK fertilizer. Substrate fertilization was done using the
92 Osmocote Plus fertilizer (formulation 15-9-12), with controlled release for a period of 3 to 4
93 months, at the substrate dosage of 6 kg m⁻³. They were later kept inside a greenhouse and
94 irrigated by micro sprinkling three times a day until 90 days after sowing.
95

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

$$ESI = \frac{E_1}{N_1} + \frac{E_2}{N_2} + \dots + \frac{E_N}{N_N}$$

99 In which ESI = emergency, speed an index; E₁, E₂, ... E_N referred to the number of seedlings
100 emerged on the first, second and nth day, and N₁, N₂, ... N_N, number of days after sowing the
101 first, second, and nth counts [12].

102 At 90 days after sowing, the following seedling characteristics were evaluated: a) plant
103 height (cm), obtained from the level of the tube until the height of the apical meristem, using
104 a graduated ruler; b) stem diameter (mm), determined with a digital caliper, in the portion of
105 the stem just above the substrate c) number of leaves; d) dry mass of the area and total
106 area, determined after drying the material in an air circulation oven at 65°C, remaining until
107 reaching constant mass; e) leaf area (cm²), determined by using a leaf area meter (CI-203
108 CA, Bioscience); f) specific leaf area (SLA) ratio between leaf area and dry leaf mass; g)
109 stem height and diameter ratio (HDR) (cm mm⁻¹); h) shoot dry mass and root dry mass ratio
110 (SDM/RDM); i) Dickson Quality Index (DQI), consists of a balanced formula that includes the
111 relationships of the morphological parameters such as total dry matter weight (TDMW),
112 shoot dry matter weight (SDMW), root dry matter weight (RDMW), shoot height (H) and
113 collection diameter (CD); j) Chlorophyll - determining the chlorophyll index in the leaves
114 using a chlorophyll meter (ClorofilOG-CFL1030, Falker), considering one leaf per seedling
115 which was totally exposed to light for measurement.
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117 The data were submitted to analysis of variance at 5% probability and there was a significant
118 difference in the regression analysis for the Biostimulant doses.
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120 3. RESULTS AND DISCUSSION

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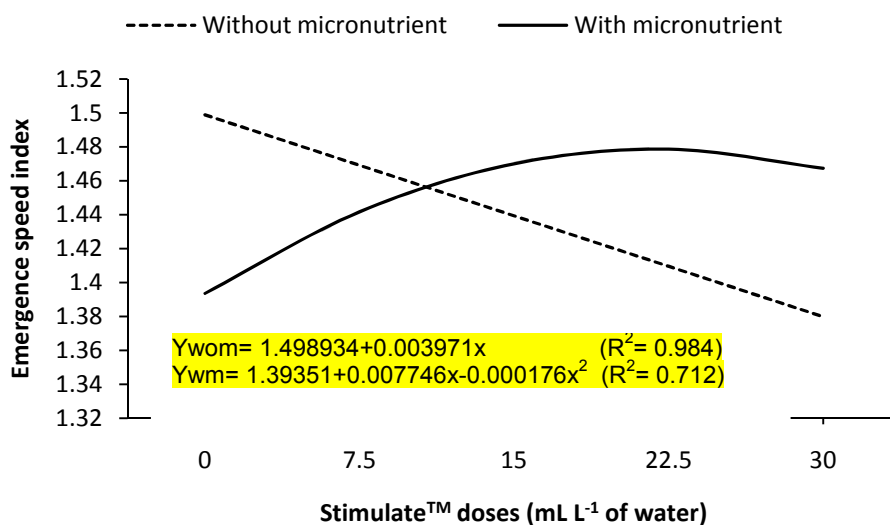
122 Most of the evaluated characteristics were influenced by the associated use of
123 micronutrients and biostimulant, while the variables of plant height, stem height/diameter

124 ratio (HDR) and Dickson quality index (DQI) were only influenced by the biostimulant
125 application. The variables, number of leaves and shoot dry mass/root dry mass ratio
126 (SRDMR) were not influenced by applying the products alone or in combination.

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128 Without the use of micronutrients, the ESI reduced from the control treatment. With the use
129 of micronutrients, the ESI increases from the control until the maximum biostimulant dose of
130 22 mL, then reducing from that point (Figure 1). [13], found a significant effect of the
131 biostimulant doses on the emergence of *Dimorphandra mollis* seedlings, and the emergence
132 speed index showed an estimated peak of 0.9 with a 15 mL dose of the product. On the
133 other hand, [10], observed that there is a tendency to reduce the plant emergence
134 percentage due to regulator use, in which the authors verified a negative influence on the
135 emergence of jatobá seedlings for the higher biostimulant doses, being that a dose of 35 mL
136 may have been excessive, even causing germination inhibition. Also, the reduced volume
137 and root growth of young *Ilex paraguariensis* plants may have been influenced by the lower
138 micronutrient availability to the plants from the liming action observed by the reduced Cu, Zn
139 and mainly Mn leaf content [14].

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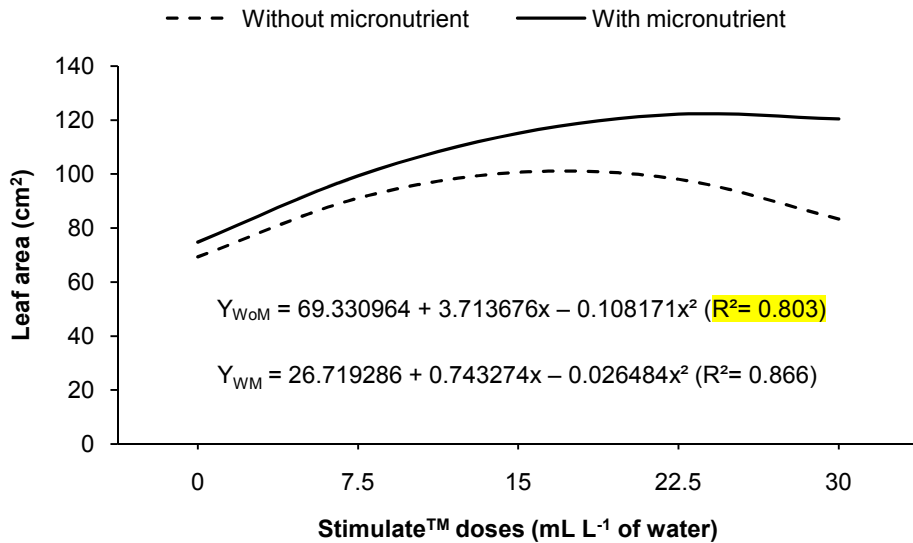
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144 **Fig. 1.** Emergence speed index for *A. mangium* seeds submitted to biostimulant doses and
145 micronutrient application.

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

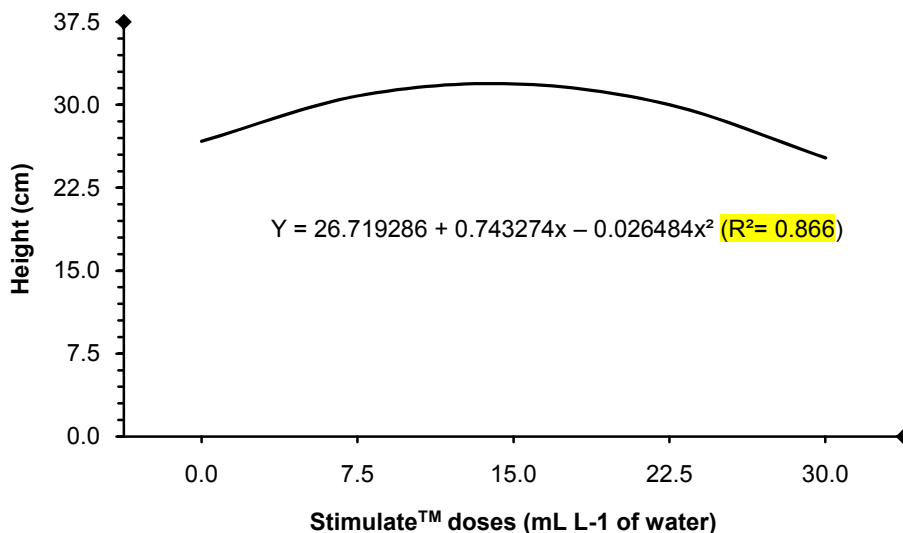
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Fig. 2. Leaf area of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

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].



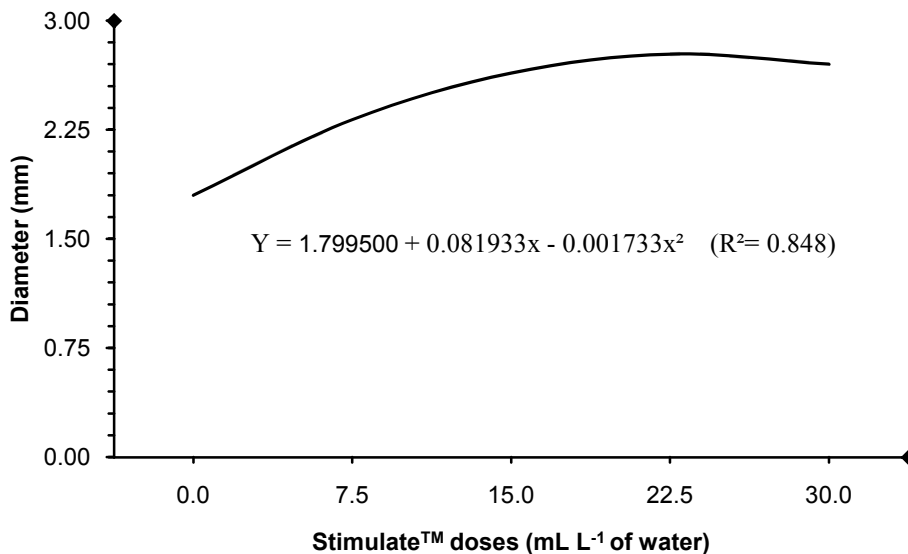
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Fig. 3. Height of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

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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], Stimulate™ application in the furrow and foliar route in corn within 0.45 m spacing enabled larger stem diameter.



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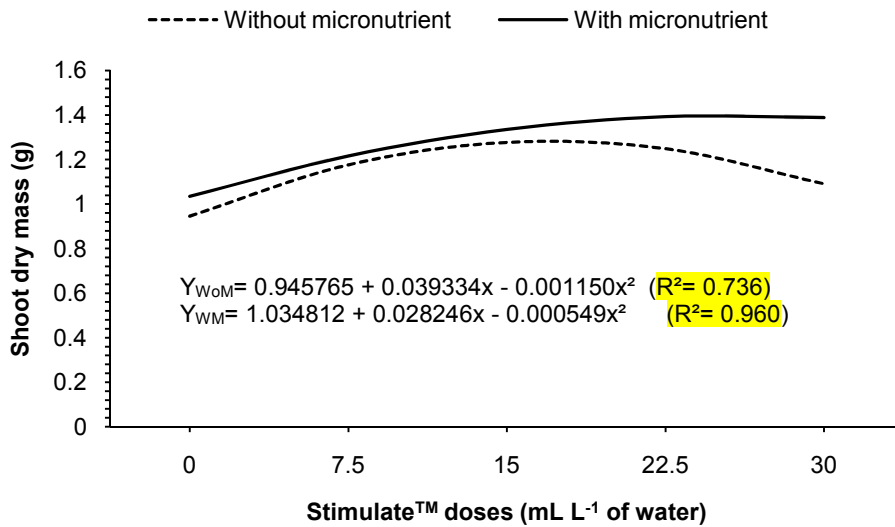
Fig. 4. Stem diameter of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

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].

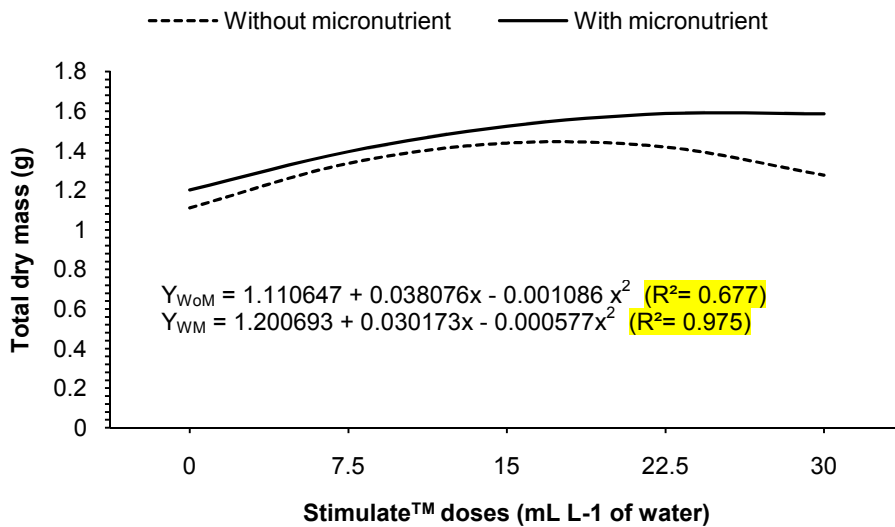
Shoot dry mass accumulation was always superior for micronutrient use, and the maximum accumulation was obtained with the dose of 25.7 mL L⁻¹, reaching 1.4 g. Without micronutrient application, biostimulant application promoted a growing accumulation of shoot

212 dry mass up to the dose of 17.1 mL L⁻¹, reaching 1.3 g (Figure 5). In *Lafoensia pacari*
 213 seedlings, shoot dry matter weight presented a maximum point at 47.5 mg Cu kg⁻¹ of soil,
 214 whereas *Ateleia glazioviana* seedlings showed linear reduction [19].
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 217
 218 **Fig. 5.** Shoot dry mass of *A. mangium* seedlings submitted to different biostimulant doses
 219 and micronutrient application.
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221 The total dry mass of *A. mangium* seedlings presented similar behaviour to that observed for
 222 shoot dry mass, where the micronutrient application favoured dry mass accumulation in the
 223 plant, reaching the maximum value at the dose of 26.1 mL L⁻¹ with 1.6 g. Without the
 224 micronutrient application, the maximum total dry mass accumulation occurred at a dose of
 225 17.5 mL L⁻¹ reaching 1.4 g (Figure 6). For [13], no statistical differences were observed
 226 between the biostimulant doses applied to seedlings for the dry mass of *Dimorphandra*
 227 *mollis* seedlings, obtaining a mean of 0.134 g.
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230 **Fig. 6.** Total dry mass (root; stem; leaves) of *A. mangium* seedlings submitted to different
231 biostimulant doses and micronutrient application.

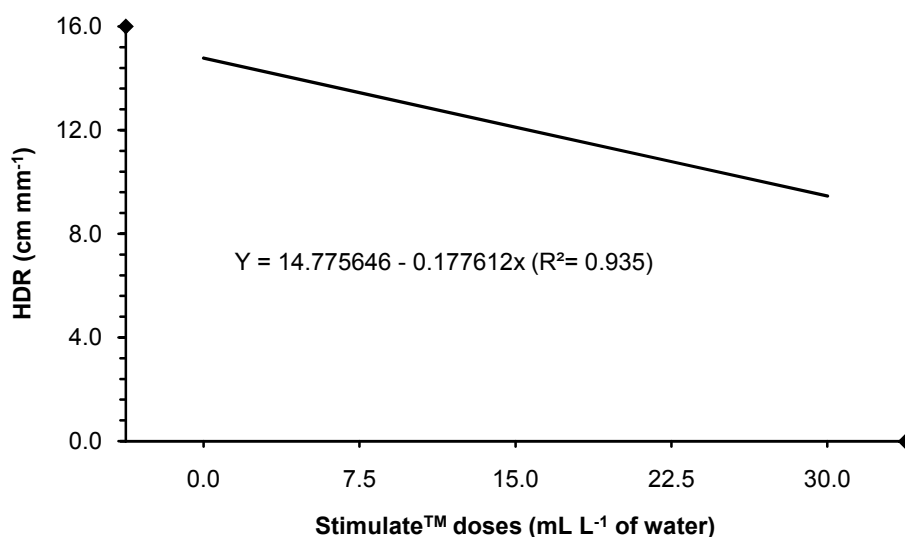
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233 [20] observed increases in 100 seed weight and ESI on the dry weight of seedlings when
234 castor seeds were treated with these micronutrients. The authors also conclude that
235 physiological quality of castor seeds is affected by seed micronutrient application. Within the
236 shoot dry weight evaluation in castor bean seeds, it was possible to verify that there was an
237 absorption order among the micronutrients used, being: Mo > Fe = Zn > Co = Mn = H₂O > Bo
238 = control, in ascending order [20]. In this way, it can be inferred that the applied
239 micronutrients influenced the dry mass development of *A. mangium* seedling.

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

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

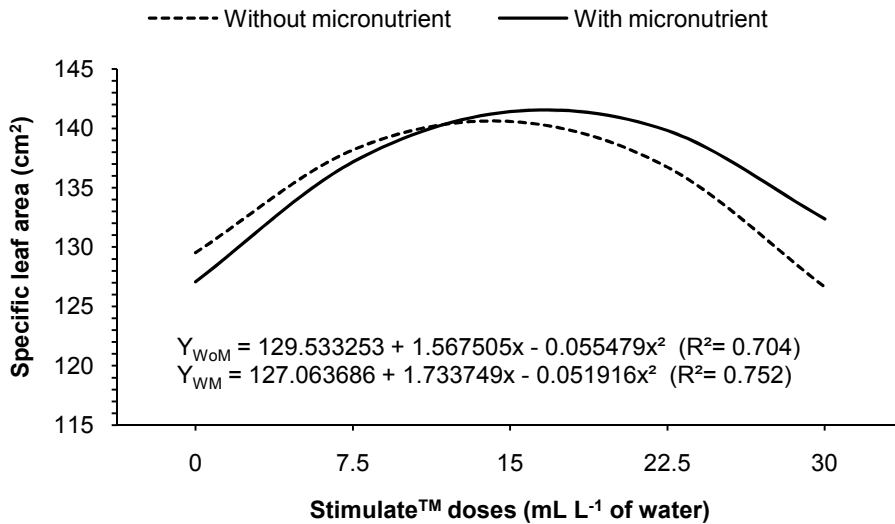
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252 [10] found a higher HDR value for *Jatoba* seedlings with the biostimulant dosage of 35 mL.
253 In this case, the authors used the Stimulate™ product containing 0.005% indolebutyric acid,
254 0.009% kinetin and 0.005% gibberellic acid. The effect of micronutrients may affect the
255 development of different species, as verified by [19] in evaluating the effect of copper doses
256 on the growth and quality of *Timbó* seedlings (*Ateleia glazioviana*) and *Dedaleiro* (*Lafoensia*
257 *pacari*), where they observed that there was no significant difference for the height/diameter
258 ratio.

259

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

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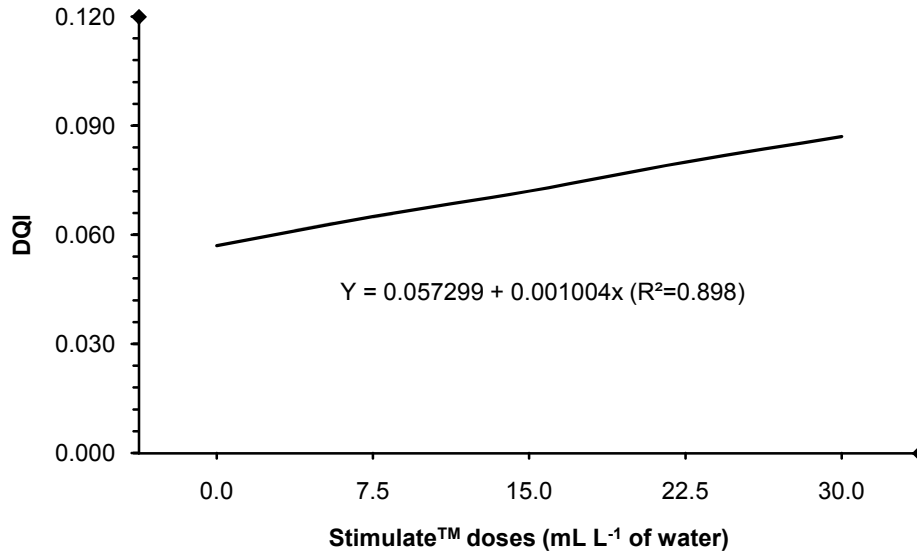


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Fig. 8. Specific Leaf Area of *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

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].



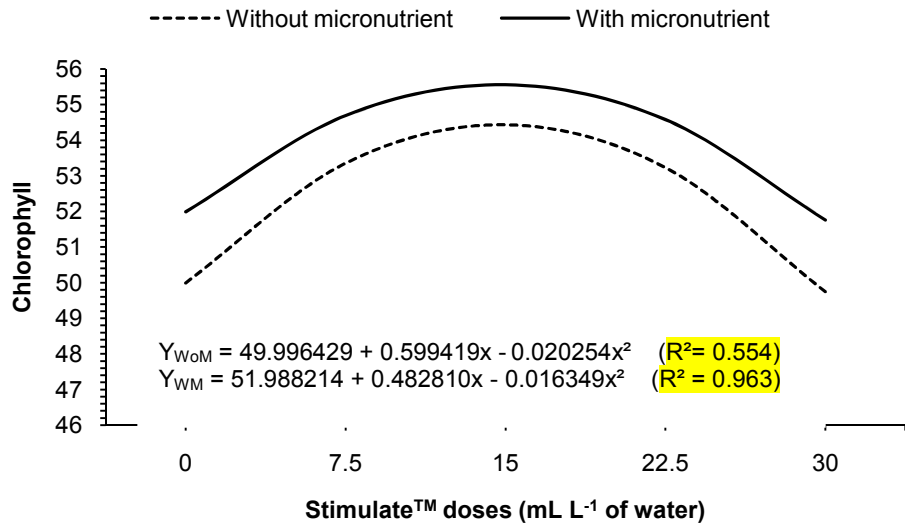
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Fig. 9. DQI for *A. mangium* seedlings submitted to different biostimulant doses and micronutrient application.

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.



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

313
314 In studying the effect of the biostimulant (Stimulate™) on *Jatoba* seedlings, [10] observed
315 higher chlorophyll content in 30% light conditions compared to plants that developed in full
316 sunlight.

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318 **4. CONCLUSION**

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320 The use of micronutrients and biostimulants were favourable for the production of *A.*
321 *mangium* seedlings. Only the height/diameter ratio (HDR) reduced for all biostimulant doses.
322

323 The associated use of micronutrients with biostimulant provided increase of chlorophyll and
324 specific leaf area in the doses of Stimulate™ of 14.8 and 16.7 mL L⁻¹ of water. For the
325 variables leaf area, shoot dry mass and total dry mass, the highest values were obtained
326 with the doses of stimulate™ in the range of 24.8 to 26.1 mL L⁻¹ of water.

327 Regardless of micronutrient use, Stimulate™ doses of 14.0 and 23.6 mL L⁻¹ of water,
328 provided higher values for plant height and stem diameter. Dickson's quality score increased
329 with Simulate™ dose
330

331 **COMPETING INTERESTS**

332

333 “Authors have declared that no competing interests exist.”.

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335 **AUTHORS’ CONTRIBUTIONS**

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337 The authors Nathália Ferreira Alves and Pedro Paulo Virgilio Damis participated in the
338 elaboration, conduction, collection of experimental data and writing of the first version of the
339 manuscript. Authors Sebastião Ferreira de Lima, Ana Paula Leite de Lima and Cátia
340 Aparecida Simon performed the statistical analyzes, corrections and adjustments in the
341 manuscript, as well as the management of the available bibliography. All authors read and
342 approved the final manuscript.

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