Productivity, morphology and mineral composition of forage palm clones biostimulated with humic substances

Produtividade, morfologia e composição mineral de clones de palma forrageira bioestimulados com substâncias húmicas

Productividad, morfología y composición mineral de clones de palmeras forrajeras bioestimulados con sustancias húmicas

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ABSTRACT
An initial protocol was carried out in pots with forage cactus, in a greenhouse for 90 days and, in this, the dilution of SH_6.25% [1/16 - SH (100%)] of humic substances (HS) was determined. of commercial vermicompost (earthworm humus) as the best dose of stimulus to plants through the integration of dose-response curves. This concentration was then tested in the field when planting cactus. The application of humic substances occurred one week after planting, randomizing the planting lines, so that of the 24 lines implemented, half received application of humic substances and the other half did not receive any treatment after implantation. Morphological and morphogenetic analyzes of cactus pear were carried out and it was observed that the application of humic substances provided greater positive correlations between the morphogenic variables and the production of vegetative biomass since in the treatment with humic substances the plants obtained greater vegetative biomass, height, length, width of cladode perimeter and largest cladode area index. Higher levels of potassium, magnesium, sulfur, iron, manganese and boron were also observed in plants that received humic substances. These responses show that the use of humic substances increases the production of palm biomass and positively affects the morphogenic characteristics of the crop.

Keywords: humic substance, cladodium, forage palm, biomass.

RESUMO
Realizou-se um protocolo inicial em vasos com palma forrageira, em casa de vegetação por 90 dias e, neste, determinou-se a diluição de SH_6,25% [1/16 - SH (100%)] de substâncias húmicas (HS) de vermicomposto comercial (húmus de minhoca) como a melhor dose de estímulo às plantas através da integração das curvas dose-resposta. Em seguida esta concentração foi testada em campo por ocasião do plantio da palma forrageira. A aplicação das substâncias húmicas ocorreu uma semana após o plantio, aleatorizando as linhas de plantio, de modo que das 24 linhas implantadas, metade recebeu aplicação de substâncias húmicas e a outra metade não recebeu nenhum tratamento após a implantação. Foram realizadas análises morfológicas e morfogenéticas da palma forrageira e observou-se que a aplicação de substâncias húmicas proporcionou maiores correlações positivas entre as variáveis morfogênicas e a produção de biomassa vegetativa uma vez que no tratamento com substâncias húmicas as plantas obtiveram maior biomassa vegetativa, altura, comprimento, largura e perímetro dos cladódios e maior índice de área do cladódio. Observou-se também maiores teores de potássio, magnésio, enxofre, ferro,
manganês e boro nas plantas que receberam substâncias húmicas. Estas respostas evidenciam que a utilização de substâncias húmicas aumenta a produção de biomassa de palma e afeta positivamente as características morfogênicas da cultura.

**Palavras-chave:** substância húmica, cladódio, palma forrageira, biomassa.

**RESUMEN**

Se realizó un protocolo inicial en macetas con cactus forrajeros, en invernadero durante 90 días y, en éste, se determinó la dilución de SH_6.25% [1/16 - SH (100%)] de sustancias húmicas (HS) de vermicompost comercial (humus de lombriz) como la mejor dosis de estímulo a las plantas mediante la integración de curvas dosis-respuesta. A continuación, esta concentración se probó en el campo al plantar los cactus. La aplicación de sustancias húmicas se produjo una semana después de la plantación, aleatorizando las líneas de plantación, de forma que de las 24 líneas implantadas, la mitad recibió aplicación de sustancias húmicas y la otra mitad no recibió ningún tratamiento después de la implantación. Se realizaron análisis morfológicos y morfogenéticos del nopal y se observó que la aplicación de sustancias húmicas proporcionó mayores correlaciones positivas entre las variables morfogénéticas y la producción de biomasa vegetativa ya que en el tratamiento con sustancias húmicas las plantas obtuvieron mayor biomasa vegetativa, altura, longitud, anchura w perímetro del cladodio e índice de área del cladodio mayor. También se observaron mayores niveles de potasio, magnesio, azufre, hierro, manganeso y boro en las plantas que recibieron sustancias húmicas. Estas respuestas muestran que el uso de sustancias húmicas aumenta la producción de biomasa de palma y afecta positivamente a las características morfogénicas del cultivo.

**Palabras clave:** sustancia húmica, cladodium, palma forrajera, biomasa.

1 **INTRODUCTION**

Espírito Santo has an area of 1.34 million hectares of pastures, occupied by a cattle herd of approximately 2.3 million heads (IBGE, 2016). The dairy farming in Espírito Santo produces, annually, 500 thousand tons of milk, with an average production of 4.1 kg⁻¹ cow⁻¹day⁻¹. The annual gross value of production is around 481 million reais and is among the three main activities with the highest share in the gross value of agricultural production in Espírito Santo (Pedeag 3, 2015).

Dairy farming stands out for providing monthly income throughout the year for producers, which favors the permanence of families in rural areas. The milk and its derivatives produced in the State are mostly destined to supply the domestic consumer market and involve about 18 thousand producers (Pedeag 3, 2015).
The period of drought that occurred in several Brazilian states, such as Espírito Santo, between 2014 and 2016 caused forage cactus to be cultivated again on a large scale by farmers in the dairy basins of Pernambuco, Alagoas, Ceará and Minas Gerais.

This increase in the cultivation of forage cactus in the longer periods of drought is a recurrent practice in these dairy basins, as Domingues, 1963 already mentioned this practice with the objective of increasing the consumption of dry matter in the diets of the animals when supplied mixed with hay, silage, corn, beans, sorghum or even grass.

In the Brazilian semiarid, due to its rusticity, physiological, anatomical and structural adaptations, which provides high production in conditions of low water availability, cactus has become a relevant resource for animal feed as it stands out superior for forage supply, when compared to the native vegetation of that region (Oliveira et al., 2010).

In the literature, however, there are few or no studies that address the cultivation of cactus pear in the state of Espírito Santo and it is in this sense that the purpose of this study is given, to elucidate the productive potential of cactus pear, small variety \( \text{Opuntia ficus-indica} \ (L.)\ P.\ \text{Mill.} \) resistant to carmine cochineal under dry conditions, its responses to organic (humic) fertilization and its mineral composition.

2 MATERIAL AND METHODS

An initial protocol in pots was carried out in a greenhouse. For the liquid organic fertilization of the forage palm, the extraction of humic substances (HS) samples of commercial vermicompost (earthworm humus) was carried out using the classic protocol of extraction and purification of the International Society of Humic Substances (IHSS), using NaOH as solvent.

From the concentrated solution (SH 100%), the pH of the humic solution was adjusted to 5.8-6.0 and then the following dilutions of the material obtained were performed: SH 50.0% [1/2 - SH (100%)]; SH 25.0% [1/4 - SH (100%)]; SH 12.5% [1/8 - SH (100%)]; SH 6.25% [1/16 - SH (100%)]; SH 3.12% [1/32 - SH (100%)]; SH 1.56% [1/64 - SH (100%)].

Clones of forage cactus were grown in pots (in quintuplicate) containing sand and vermiculite in a 1:1 proportion, accommodated in a greenhouse, where 250 mL of different humic concentrations were supplied.
The clones were kept in the treatments for 90 days (different concentrations of SH) and, later, collected for the following root analyses: 1) the root area calculated using the computer program for digital analysis of Delta-T ScanTm® images; 2) mass of fresh roots determined on a precision analytical balance, immediately after collection; and 3) dry root mass determined on a precision analytical balance after 72h in an oven at 75°C.

The number of shoots in each pot was also counted and their measurements were defined (width, length and thickness) using a parking meter.

After regression analysis, SH_6.25% [1/16 - SH (100%)]; as the best dose of stimulus to the plants through the integration of the dose-response curves (Table 1 and Figure 1). Thus, cactus pear was supplied at field level, via liquid fertilization with a knapsack sprayer in the area where the plant intersects with the soil, aiming at the biostimulation of its roots during planting.

Once the best dose was determined, the research was tested in the field, in the large animals sector of the Federal Institute of Education, Science and Technology of Espírito Santo, Campus Santa Teresa. The site's altitude is 150 meters above sea level, with coordinates of 19° 48' south latitude and 40° 40' west longitude of Greenwich.

Table 1. Best dose of humic substance for forage cactus (%SH).

<table>
<thead>
<tr>
<th>Evaluated Characteristics</th>
<th>Regression equations (y = b2x² + b1x + b0)</th>
<th>R²</th>
<th>SD*</th>
<th>P-value</th>
<th>Great doses (dx/dy): b1 + 2(b2)x = 0</th>
<th>Average = 49.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total root area</td>
<td>y = -0.0045x² + 0.4509x + 6.0704</td>
<td>0.85</td>
<td>1.87</td>
<td>&lt;0.0001</td>
<td>50.01</td>
<td></td>
</tr>
<tr>
<td>Root length</td>
<td>y = -0.0166x² + 1.6445x + 19.299</td>
<td>0.88</td>
<td>1.73</td>
<td>&lt;0.0001</td>
<td>49.53</td>
<td></td>
</tr>
<tr>
<td>Lateral root area</td>
<td>y = -0.0027x² + 0.2667x + 1.634</td>
<td>0.94</td>
<td>0.65</td>
<td>&lt;0.0001</td>
<td>49.39</td>
<td></td>
</tr>
<tr>
<td>Side root length</td>
<td>y = -0.0083x² + 0.7948x + 9.4354</td>
<td>0.83</td>
<td>1.98</td>
<td>&lt;0.0001</td>
<td>47.87</td>
<td></td>
</tr>
</tbody>
</table>

Source: Prepared by the authors
The experimental period lasted 1 year due to the need to form an area with palms varying between 10 and 18 months and the details of the activities and evaluations and their respective methodologies follow.

The ideal time for planting cactus pear is the final third of the dry season (SANTOS et al., 2006), so planting was carried out in September. Planting in the dry season is important to prevent the cladodes from rotting, which, due to excess moisture at the beginning of the rainy season, causes greater contamination by fungi and bacteria. So, in August, the soil was prepared and corrected and, in September, the small variety was planted.

The area formed was 0.8 ha 45 days after the cactus pear was cut for healing (pre-drying in the shade for water loss) to prevent the entry of fungi and bacteria when the plant came into contact with the soil in the time of planting and thus favoring rooting.

The cladodes were buried vertically in the soil at about 2/3 of their length, to ensure better fixation of the future plant, thus avoiding overturning with plant growth. Forage cactus spacing, as a management strategy, was carried out in double rows, following the spacing of 5.0 x 0.3 x 0.1 meters to allow future grazing management with dairy cows.
Cultural treatments (cutting weeds between the crop rows, at the end of the rainy season) were carried out by students linked to the institution. When cultivated in dense spacing, cultural practices are difficult, increasing labor, requiring four weedings during the experimental period.

The application of humic substances took place one week after planting, randomizing the planting lines, so that of the 24 implanted lines, half received application of humic substances and half did not receive any treatment after implantation.

Morphological and morphogenetic analyzes were carried out by evaluating, in a linear meter (1 m) chosen at random from the planting rows, plant height (PH), cladode length (CL), cladode width (CW), cladode thickness (CT), cladode area (CA), cladode area index (CAI), cactus pear at nine months of implantation.

In the measurements of PH, CL and CW, a tape measure was used and the TC measurement was performed with a digital caliper. The CA was determined by the equation proposed by Pinto (2002), where CA = CL * CW * 0.693, and 0.693 is a correction due to the ellipsoid shape of the cladode. The CAI was calculated from the CA multiplied by the number of cladodes contained in the 1 meter of observation, being expressed in m² of cladode per linear meter of plants. Macro and microminerals were obtained by atomic absorption spectrophotometry.

The results were submitted to analysis of variance and the averages of the evaluations were submitted to the Tukey test, at 5% probability.

**3 RESULTS AND DISCUSSION**

A correlation analysis was carried out between vegetative biomass and the morphogenic variables of cactus pear with and without the application of humic substances (Table 2). It is observed that the application of humic substances provides greater positive correlations between the morphogenic variables and the production of vegetative biomass, showing that the use of humic substances improves the productivity of cactus pear (*Opuntia ficus-indica*).
Table 2. Correlation between productive biomass and morphogenic variables of cactus without application of humic substances (A) and with application of humic substances (B).

<table>
<thead>
<tr>
<th>Vegetative biomass</th>
<th>Height</th>
<th>Lenght</th>
<th>Width</th>
<th>Thickness</th>
<th>Estimated perimeter</th>
<th>Cladode Area Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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<th>Cladode Area Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Source: Prepared by the authors

Thus, Table 3 shows the significant effects on the morphogenic characteristics and, consequently, on the production of vegetative biomass of cactus pear treated with humic substances when compared to those that did not receive humic substances. These effects may be related to the ability of humic substances to stimulate the synthesis and activity of H+-ATPase, enhancing cell division and elongation, and to acting on different metabolic routes (photosynthesis and cellular respiration) of plants, in addition, to the effects caused in the soil such as the formation of organic complexes, increased cation exchange capacity, nutrient availability and moisture retention (Baldotto and Baldotto, 2014).

The main effects of the use of humic substances were observed for plant height and cladode perimeter, which were higher by 45.72% and 21.93% respectively, than plants not treated with humic substances.

Another significant variable for the greater production of vegetative biomass can be explained by the higher cladode area index observed in plants that received humic substances, since the cladode area index of cactus clones presents a high and positive correlation with the number total number of cladodes (Pinheiro et al., 2014) which, consequently, results in greater vegetative biomass. In the study by Santos et al. (2018) with the forage cactus variety of the genus Gliricidia, found similar effects (greater length, width and number of cladodes) in 1.0 x 0.5 m plots fertilized with humic substances. Differently, Lima et al. (2018) for the genera Opuntia and Nopalea did not find significant differences (height, length, cladode width and productivity) when compared to the control, possibly justified due to the doses used.
Although the use of humic substances with cactus has not yet been widely evaluated in the Brazilian scientific community, studies with tropical forages such as *Urochloa brizantha* and *Stylosanthes sp*. (Burak et al., 2012), *Mucuna deeringiana*, *Cajanus cajan* and *Crotalaria juncea* (Rosa et al., 2017), *Megathyrsus maximus* (Santos, 2019) and *Panicum maximum* and *Brachiaria brizantha* (Deminicis et al., 2022) found similar responses to those observed in this study. Thus, the main physiological effects of humic substances focus on increasing plant growth (root and aerial), however, it depends on the species tested and the source and concentration of the humic material used (Canellas and Olivares, 2014).

### Table 3. Comparison between productive biomass and morphogenic variables of forage cactus without application of humic substances and with application of humic substances.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without humic substances</th>
<th>With humic substances</th>
<th>CV (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative biomass (kg linear meter⁻¹)</td>
<td>15.80b</td>
<td>17.88a</td>
<td>30.16</td>
<td>0.006</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>36.42b</td>
<td>53.07a</td>
<td>27.17</td>
<td>0.003</td>
</tr>
<tr>
<td>Cladode length (cm)</td>
<td>21.93b</td>
<td>23.52a</td>
<td>5.30</td>
<td>0.004</td>
</tr>
<tr>
<td>Cladode width (cm)</td>
<td>8.00b</td>
<td>8.73a</td>
<td>7.21</td>
<td>0.007</td>
</tr>
<tr>
<td>Cladode thickness (cm)</td>
<td>11.26a</td>
<td>11.76a</td>
<td>11.24</td>
<td>0.358</td>
</tr>
<tr>
<td>Cladode perimeter (cm)</td>
<td>10.99b</td>
<td>13.40a</td>
<td>12.82</td>
<td>0.010</td>
</tr>
<tr>
<td>Cladode Area Index</td>
<td>0.25b</td>
<td>0.33a</td>
<td>13.16</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the line do not differ statistically using the Tukey test for α = 0.05.

Source: Prepared by the authors

In ecophysiological studies with tropical forages, growth rates are usually evaluated as a parameter to estimate tissue flow and establish the adopted management strategy; however, in studies with cactus cactus, restricted information is commonly observed, as most of the studies only evaluate dry mass production in studies carried out only after the 2nd year of implementation. Therefore, knowledge of growth rates can be a fundamental tool in the study of cladode development. Since it is an important forage resource during periods of drought, mainly in the Brazilian semi-arid region, and increasing the potential for biomass production is necessary (Ramos et al., 2015).

Regarding the nutritional aspect of cactus pear, shown in Table 4, significant differences were found in the levels of magnesium, potassium, iron, boron, manganese and sulfur, being higher in plants that received humic substances. These differences were greater, 145.77%, 109.42%, 64.79%, 52.48%, 49.77% and 23.11% respectively, when compared to the values found for plants that did not receive humic substances.
The use of humic substances promotes an increase in the absorption of nutrients by plants and in the dynamics and availability of macro and micronutrients in the soil (Baldotto et al., 2017; Rosa et al., 2017), due to the influence on the permeability of the cell membrane and the power chelating agents, which directly alter the biochemical metabolism of plants and, consequently, can benefit growth and development and enhance the absorption and translocation of nutrients in plants (Vaccaro et al., 2015; Gomes Júnior et al., 2019).

| Table 4. Comparison between the macro and microminerals of cactus without the application of humic substances and with the application of humic substances. |
|----------------|----------------|----------------|----------------|----------------|
| Variable       | Without humic substances | With humic substances | CV (%) | P-value |
| Nitrogen (g kg\(^{-1}\)) | 17.58a | 18.71a | 35.07 | 0.6659 |
| Phosphorus (g kg\(^{-1}\)) | 1.40a | 1.66a | 24.42 | 0.1015 |
| Potassium (g kg\(^{-1}\)) | 11.04b | 23.12a | 66.53 | 0.0106 |
| Calcium (g kg\(^{-1}\)) | 8.74a | 11.02a | 55.83 | 0.1225 |
| Magnesium (g kg\(^{-1}\)) | 6.27b | 15.41a | 53.57 | 0.0014 |
| Sulfur (g kg\(^{-1}\)) | 2.25b | 2.77a | 22.85 | 0.0358 |
| Iron (mg kg\(^{-1}\)) | 63.16b | 104.08a | 53.64 | 0.0359 |
| Copper (mg kg\(^{-1}\)) | 11.42a | 15.08a | 40.06 | 0.1047 |
| Zinc (mg kg\(^{-1}\)) | 24.83a | 32.08a | 69.19 | 0.3768 |
| Manganese (mg kg\(^{-1}\)) | 32.83b | 49.17a | 40.53 | 0.0249 |
| Boron (mg kg\(^{-1}\)) | 62.06b | 94.63a | 24.99 | 0.0005 |

Means followed by the same letter in the line do not differ statistically using the Tukey test for \(\alpha = 0.05\). Source: Prepared by the authors

In the literature, Silva et al., (2012) and Dubeux Júnior et al., (2010) evaluated the mineral composition of cactus, however, based on different levels of nitrogen (NPK and NP), phosphate and potassium (P and K). In both cases, they showed a decrease in magnesium content and did not influence the levels of micronutrients (iron, boron and manganese), unlike the results of this study, which were increased by the use of humic substances in the tissues of cactus cladodes.

However, in the evaluation of the nutritional composition of cactus (Opuntia sp.) based on the application of organic compounds Donato et al. (2016) (cattle manure), Azevedo Júnior et al. (2019) (treated domestic sewage) and Lédo et al. (2020) (cattle manure) showed an influence on the average magnesium content, which varied between 11.0 and 19.6 g kg\(^{-1}\), within the sufficiency range of 9.5-14.3 g kg\(^{-1}\) for cactus pear (Donato et al., 2017), as well as found for plants that received humic substances (15.41 g kg\(^{-1}\)). According to Botero et al. (2009) this effect may be related to the large amount of oxygenated groups present in humic substances, which favors complexation with magnesium. In the soil, humic substances perform the function of
retaining metals, especially magnesium and calcium in the form of ions, thus making them available to plants (Trevisan et al. 2010; Baldotto and Baldotto, 2014).

4 CONCLUSION

The use of humic substances increases the production of cactus biomass and positively affects the morphogenic characteristics of the crop.

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