



MACROPHYTES AS SOURCE OF NUTRIENTS ON INITIAL GROWTH OF SUNFLOWER PLANTS

Macrófitas como fonte de nutrientes no crescimento inicial de plantas de girassol

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ABSTRACT

The present study assessed the initial growth of sunflower seedlings under different concentrations and species of macrophytes, verifying the viability of their use as source of nutrients. Biometric and vigour variables were assessed through measurements of stem diameter, height of aerial part, number of leaves, relative chlorophyll contents and production of dry matter from roots and shoots. The experimental design was entirely randomized with 4 different materials for substrate composition: *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* and mixed macrophyte compost, and the following concentrations of macrophyte nitrogen in the substrates: 40, 80 and 120 kg N ha⁻¹. Pots containing only sand and pots with sand and commercial worm humus at 80 kg N ha⁻¹ were used as control treatments. The collections were performed at 14 and 21 Days After Sowing (DAS), containing eight repetitions with three plants each. The averages obtained at 14 and 21 DAS were compared by Tukey's test ($P \leq 0.05$) through the statistical program Sisvar 5.6. It was found that the use of macrophytes promoted increases in the variables analyzed, however, the best results were provided by the treatment *Salvinia sp.* at 120 kg N ha⁻¹.

Keywords: Plant Biomass. *Helianthus annuus* L.. Plant Growth Analysis.

RESUMO

O presente trabalho analisou o crescimento inicial de plântulas de girassol submetidas a diferentes concentrações e espécies de macrófitas verificando a viabilidade da utilização como fonte de nutrientes. Avaliaram-se variáveis biométricas e de vigor através das medidas de diâmetro do coleto, altura da parte aérea, número de folhas, teores relativos de clorofila e produção de matérias secas das raízes e partes aéreas. O delineamento experimental foi o inteiramente casualizado com 4 diferentes materiais para composição dos substratos: *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* e composto misto de macrófitas, e as seguintes concentrações de Nitrogênio em macrófitas nos substratos: 40, 80 e 120 kg N ha⁻¹. Foram utilizados como tratamentos controle: vasos contendo somente areia e vasos com areia e húmus de minhoca comercial na proporção de 80 kg N ha⁻¹. As coletas foram realizadas aos 14 e 21 Dias Após a Semeadura (DAS), contendo oito repetições com três plantas cada. As médias obtidas aos 14 e 21 DAS foram comparadas pelo teste de Tukey ($P \leq 0,05$) através do programa estatístico Sisvar 5.6. Verificou-se que a utilização de macrófitas promoveu incrementos nas variáveis analisadas, contudo, os melhores resultados foram proporcionados pelo tratamento *Salvinia sp.* a 120 Kg N ha⁻¹.

Palavras-chave: Biomassa Vegetal. *Helianthus annuus* L.. Análise de Crescimento de Plantas.

Introduction

Most Brazilian water bodies have eutrophication as a major water quality problem (von SPERLING, 2005). Siqueira and Oliveira Filho (2005) attribute the excess of fertilizers and the dumping of domestic and industrial sewage without prior treatment as the main activities responsible for this stand.

The spread of macrophytes is intensified by eutrophication. These plant species act as filters, retaining particles and providing substantial amounts of contact area for adhesion of microorganisms (RAMOS et al., 2009). They can remove nutrients (phosphorus and nitrogen) and eliminate pathogens and inorganic substances (TANAKA et al., 2015). Macrophytes have a potential bioindicator role in water bodies, being expressed and influenced by limnological characteristics of different environments (PEREIRA et al., 2012).

However, the disorder occurs when there is excessive growth of biomass or area of colonization, bringing harm to the multiple uses of the reservoirs and biota, as in water quality due to increased consumption of dissolved oxygen during the decomposition process (POMPÊU, 2017). Thus, removal of excess macrophytes from water bodies is recommended. Another relevant issue is the adequate final disposal of biomass, which, according to Pompêu (2017), the option most used in Brazil is landfill disposal. The author considers that it is more interesting to use the biomass removed, avoiding losses of energy and nutrients accumulated in the plant mass.

Macrophytes have been analyzed in studies as nutritional alternatives to plant cultivation, and its uses in agriculture have been widely adopted in their final provisions with successful results (SAMPAIO et al., 2007; POMPÊU, 2008; MEES et al., 2009; and BARBOSA et al., 2017). However, there is still a reduced using of plant biomass produced in water bodies where plants need to be removed periodically.

Nevertheless, it is necessary to perform physical-chemical analyses of the residues to assess their effects on the soil and plant development (FERREIRA et al., 2003). The presence of nitrogen in macrophytes justifies its use in plant cultivation, since it is a nutrient required in high proportions by the crops and it develops primary metabolic functions, being a constituent of the chlorophyll molecule, nucleic acids, amino acids, and proteins (TAIZ and ZEIGER, 2006).

Sunflower culture (*Helianthus annuus* L.) stands out in Brazil among the plant species with the greatest potential for biofuel production (FREITAS et al., 2012; and HARRIS et al., 2016), showing constant progress due to its peculiar characteristics of rusticity, drought resistance, oil content and quality (DANTAS et al., 2015).

Thus, it has been sought to establish sunflower cultivation practices that allow its exploitation under rational and economic techniques (DANTAS et al., 2015). Given that the high cost of chemical fertilizers stimulates the search for less costly alternatives, such as the using of organic fertilizer from animal and plant residues (PIRES et al., 2008), the using of macrophytes may provide a nutritional alternative to agricultural crops.

That being said, the present study aimed to analyze the initial growth of sunflower seedlings (*Helianthus annuus* L.) submitted to different species (*Cyperus auriculatus*, *Salvinia auriculata*, *Thalia geniculata* and a mixed macrophyte compound) and macrophyte concentrations in green house conditions, verifying the feasibility of using macrophytes as source of nutrients, using simple and easily reproducible techniques.

Material and methods

Collection and characterization of macrophytes

The macrophytes used in the experiment were obtained from Maracanaú lagoon - Ceará - Brazil, whose coordinates are 3° 52' 45.912" S 38° 37' 46.740" W, through manual and random sampling on the banks of the lagoon. There were two collections: the first on December 6, 2018 and the second on October 1, 2019 (both during the dry period). The collection period is important for understanding the seasonality of the macrophyte species that coexist in the aquatic ecosystem (NEIFF, 1990).

After the collections, the material was washed with water and placed to dry in an oven at 80°C until a constant mass was obtained (approximately 48 hours). Afterwards, the material was crushed, and the samples were sent to the Soil/Water Laboratory of the Federal University of Ceará for macro and micronutrient analysis (Table 1). The results were used in the calculations for the substrates formulations for sunflower plant cultivation.

Experimental conditions, plant material and treatments

The experiment was conducted in a greenhouse located at the Federal Institute of Education, Science and Technology of Ceará - IFCE, in the city of Maracanaú, Ceará, Brazil, in June 2019. The sunflower seeds (*Helianthus annuus* L.), BRS 323 cultivar, were given by EMBRAPA, Produtos e Mercado – Office of Dourados, MS, Brazil. The seeds were sown in 5 L plastic pots filled with fine granulometry sand-based substrates (NBR 6502) and the different macrophytes.

Three species of macrophytes (*Cyperus auriculatus*, *Salvinia auriculata*, *Thalia geniculata*) and a mixed compound with 50% of *Cyperus auriculatus* species, 25% of *Salvinia auriculata* species and 25% of *Thalia geniculata* species were used. The proportion of the mixed compound was based on the relative abundance present in the lagoon. The macrophyte species were identified by herbarium Prisco Bezerra of the Federal University of Ceará. The quantities of organic macrophyte substrates applied in each treatment were defined based on the total nitrogen (N-total) content of the sample after physical-chemical analyses performed in the Soil/Water Laboratory, UFC/FUNCEME (Table 1), and applied proportionally in the vessels corresponding to one hectare under field conditions. The values of macrophyte substrates used were respectively: 40; 80; and 120 kg N ha⁻¹. During the experiment, it was performed a daily watering at 70% of the field capacity of the substrate to replenish the evapotranspiration.

The experimental design was with four different materials for substrate composition: *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* and mixed macrophyte compounds, and the following concentrations of Nitrogen in macrophytes in the substrates: 40, 80 and 120 kg N ha⁻¹. The following control treatments were used: vessels containing only sand and vessels with commercial earthworm sand and humus at a proportion of 80 kg N ha⁻¹.

Data were collected at 14 and 21 days after sowing (DAS), comprising eight repetitions with three plants each. The means were compared by Tukey's test ($P \leq 0.05$) through the statistical program Sisvar 5.6 (Ferreira, 2018). Graphs were prepared using Sigma Plot 11.0 program.

Growth assessments were performed, and stem diameter (SD) values were determined by means of a digital pachymeter (0.01 mm) 150 mm - Stainless Steel Lee Tools Mod. 684132 with measurement performed at the insertion of the epicotyl-hypocotyl axis; the height of the aerial part (APA) with a ruler graduated in centimeters, measuring from the soil surface to the last node and, the number of leaves (NL) by manual counting. Relative chlorophyll contents were estimated on the first fully expanded leaf from the apex using Chlorophyll Meter SPAD-502 equipment and data were expressed according to the manufacturer's recommendation (in SPAD index).

Harvest of plant material

The plant material was harvested only at 21 DAS, and the plants were separated into roots, stems, and leaves (aerial part) for determination of the Dry Mass of Roots (MSR) and the Shoot Dry Mass (MSPA). The plant material was put in an oven with forced air circulation at 60 °C until constant mass was obtained and then weighed in an analytical balance.

Results and discussion

The chemical attributes of the substrates used in the experiment are shown in Table 1, where it is observed that *Thalia geniculata* species had the highest amount of NPK, and *Salvinia auriculata* species had the highest amount of micronutrients.

Table 1. Chemical characterization of macrophytes used in the experiment.

Treatments	Chemical characterization									
	g.Kg ⁻¹						mg.Kg ⁻¹			
	N	P	K	Ca	Mg	Na	Fe	Cu	Zn	Mn
<i>Cyperus auriculatus</i>	6.4	0.9	9.1	0.5	0.7	4.9	256.4	0.2	6.8	104.8
<i>Salvinia auriculata</i>	7.6	1.3	4.8	0.8	1.5	4	6019.5	23.4	29	341.9
<i>Thalia geniculata</i>	10.4	1.8	19.7	0.4	1.5	12.5	1936.5	3.7	23.3	144.7
Mixed Compound of Macrophytes	9.2	1.4	10.1	0.3	1.6	7.4	3329.4	6.8	20.7	206.5

Figure 1 shows through visual analysis that at 21 DAS, the using of Organic Macrophyte Residues (OMR) as source of Nitrogen in the fertilization of sunflower plants caused increases in growth. The treatment containing *Salvinia auriculata* at 120 kg N ha⁻¹ was the one that promoted highest increments in stem diameter, height of aerial part, number of leaves, relative chlorophyll contents and production of dry matter from roots and shoots. Additionally, *Thalia geniculata* at 80 kg N ha⁻¹ and *Thalia sp.* at 120 kg N ha⁻¹ treatments also caused increments in the growth of the seedlings (Figure 1).

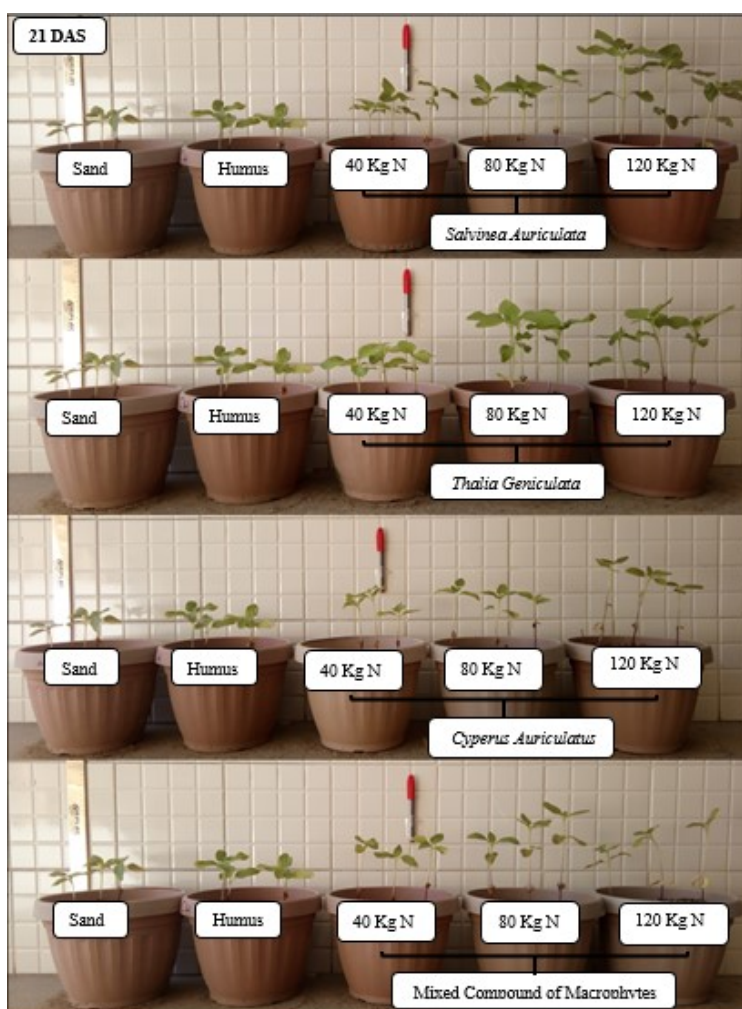


Figure 1. Sunflower seedlings submitted to different types and concentrations of macrophytes as source of N-total at 21 Days After Sowing (DAS).

In general, increases on growth variables were observed for aerial height (figure 2), stem diameter (figure 3), and number of leaves (figure 4) against control treatments (sand and earthworm humus at 80 kg N ha⁻¹) in at least one of the N concentrations employed for each macrophyte-containing treatment. The application of macrophytes caused nitrogen (N) fertilization, which is one of the main macronutrients associated with plant growth, thus, low concentrations directly influence on the number of leaves, collar diameter and height.

All treatments supplemented with different concentrations of *Salvinia auriculata* showed increases in all variables analyzed at 14 and 21 DAS. However, compared to the other treatments, the greatest increases were observed at 21 DAS in the *Salvinia sp.* treatment at 120 kg N (Figure 1). For height of the aerial part there were increases of 66,8 and 56% in relation to sand at 120 Kg N and humus at 120 Kg N, respectively, for the stem diameter there was an increase of 33,6 and 32,5% respectively, and for the number of leaves the percentage increased was 41,7 and 47,8% respectively.

For the height variable (Figure 2), it was found that at 14 and 21 DAS, except for *Salvinia sp.* treatment at 40 Kg N ha⁻¹, all treatments containing OMR presented values higher than those of sand and humus treatment at 80 Kg N ha⁻¹. At 21 DAS the highest averages obtained were from *Salvinia* and mixed treatments at 120 Kg N ha⁻¹, not differing from each other, being 56 and 49% higher than the humus treatment, respectively. With mean values of 13.9 and 13.3 cm respectively.

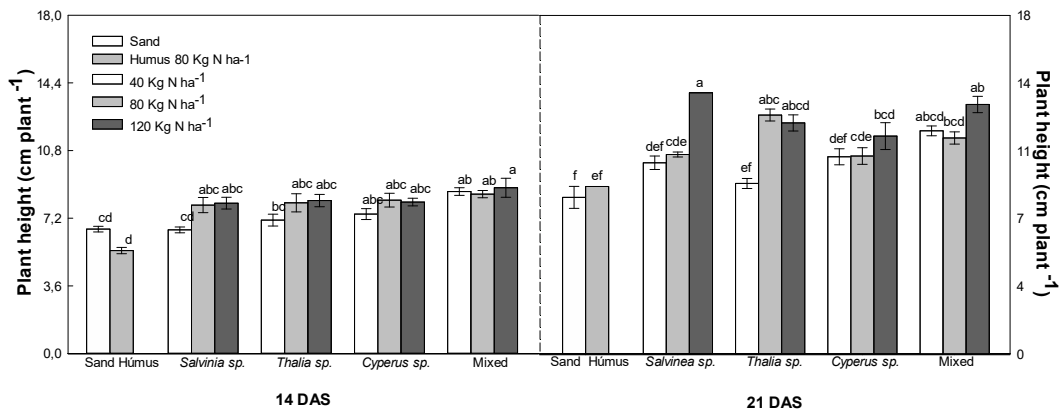


Figure 2. Height of aerial part of sunflower seedlings in substrates containing sand, earthworm humus or increasing concentrations (40, 80 or 120 kg N ha⁻¹) of *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* or mixed macrophytes compound at 14 and 21 Days After Sowing (DAS).

As for the leaf number (Figure 3), at 14 DAS, treatments with OMR of *Salvinia auriculata* and *Thalia geniculata* were higher than those of the other treatments. At 21 DAS, the *Salvinia sp.* at 120 Kg N treatment showed a higher number of leaves and average of 6.8 leaves per plant, representing increase of 42 and 48% respectively when compared to sand and humus at 80 Kg N ha⁻¹ treatments. The results were similar to those of Brito et al. (2018), which proved that at 21 DAS, treatments supplemented with macrophytes residues reached an average of 7.5 leaves per plant, 36% higher than the others.

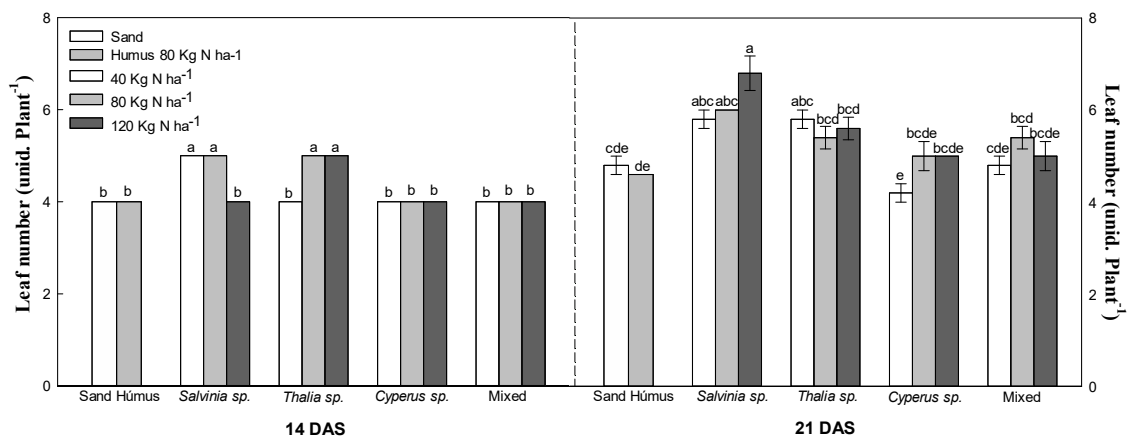


Figure 3. Number of sunflower seedling leaves in substrates containing sand, earthworm humus or increasing concentrations (40, 80 or 120 kg N ha⁻¹) of *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* or mixed macrophytes compound at 14 and 21 days after sowing (DAS).

According to the results found, the increase in the number of leaves, especially at 21 DAS, has proven the relevance of the macrophytes over the photosynthetic process, especially when associated to increase in leaf area (KARADOĞAN and AKGÜN, 2009).

For stem diameter (Figure 4), it was found that between 14 and 21 DAS, there were no significant changes for the variable. However, the highlight is the treatment *Salvinia sp.* at 120 kg N ha⁻¹

at 21 DAS, which differed from sand and humus treatments at 80 kg N ha⁻¹. It presented an average of 3,30 mm and a growth superior to humus 80 Kg N ha⁻¹ in 33%.

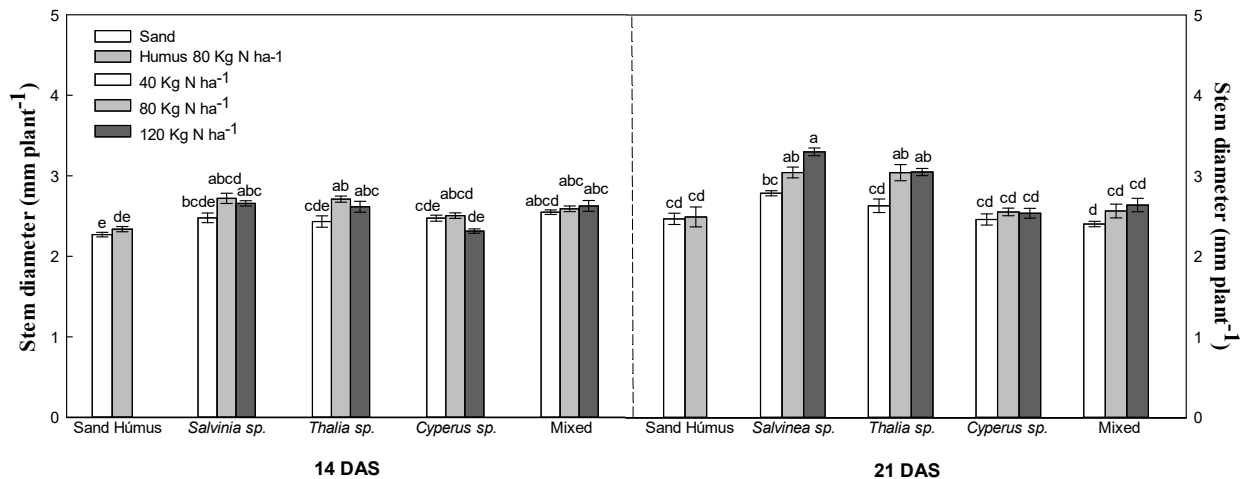


Figure 4. Stem diameter of sunflower seedling collar in substrates containing sand, earthworm humus or increasing concentrations (40, 80 or 120 kg N ha⁻¹) of *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* or mixed macrophytes compound at 14 and 21 days after sowing (DAS).

Similarly, Brito et al. (2018), Barbosa et al. (2017) and Junior et al. (2016) found, at 21 DAS, growth increments for the variable. Barbosa et al. (2017) and Brito et al. (2018), who studied the use of organic waste from *Eichhornia crassipes* and seaweed, respectively, as source of nutrients to analyze the initial growth of sunflower plants, observed increments in the diameters and heights of the plants. Souza et al. (2019), using different macrophyte species, also observed increases in sunflower plant growth variables, especially with *Salvinia auriculata* at 150% nitrogen recommendation (NR).

Santos et al. (2019) state that stem diameter is an important characteristic for sunflower culture, as chapters with larger diameters are indicative of larger number of hairs. It can be an indicator of development and productivity for sunflower plants (DALCHIAVON et al., 2016).

Regarding chlorophyll relative content (Figure 5), both at 14 DAS and 21 DAS were the highest values in treatments containing *Salvinia auriculata*. At 14 DAS, *Salvinia sp.* at 120 kg N ha⁻¹ treatment presented higher mean compared to the others, with 17 and 20% higher than humus at 80 kg N ha⁻¹ and sand, respectively.

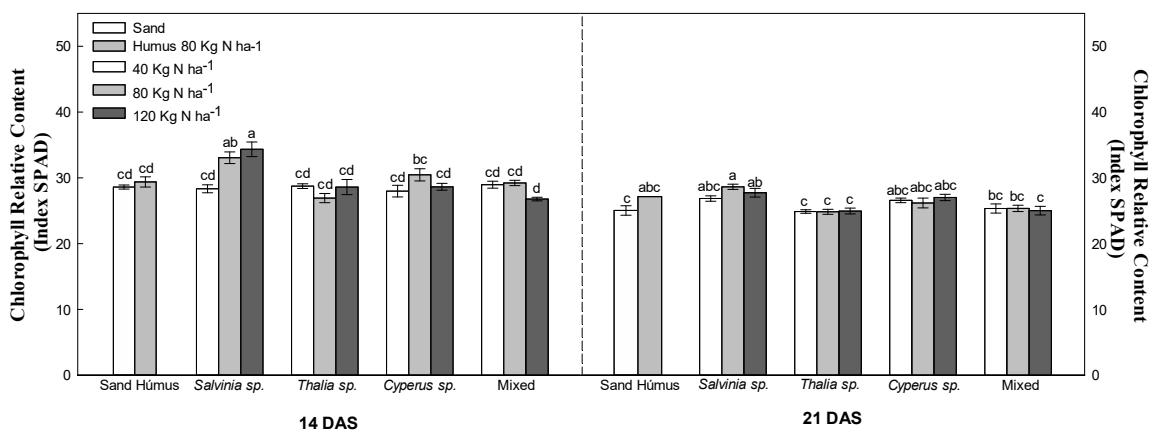


Figure 5. Relative Chlorophyll contents of sunflower seedlings in substrates containing sand, earthworm humus or increasing concentrations (40, 80 or 120 kg N ha⁻¹) of *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* or mixed macrophytes compound at 14 and 21 days after sowing (DAS).

At 21 DAS, *Salvinia sp.* 80 Kg N ha⁻¹ treatment presented higher mean compared to the others, with mean of 28.6 and increase of 5.5% compared to humus 80 Kg N ha⁻¹, however, it did not differ from the other treatments with *Salvinia sp.*, *Cyperus sp.* and humus at 80 Kg N ha⁻¹.

It is believed that the highest levels of chlorophyll found in treatments with *Salvinia auriculata* have occurred due to the high concentration of iron (Fe) present in the species, verified in Table 1. According to (FAQUIN, 2005) Fe, besides being an enzymatic component, it is also involved in chlorophyll synthesis and when in deficit it reduces the chlorophyll content and the number of chloroplasts.

Regarding Shoot dry mass and dry mass of root (Figure 6A and 6B), *Salvinia sp.* at 120 Kg N ha⁻¹ treatment was the one that presented the higher accumulation of mass at the end of the evaluation period against the other treatments, presenting differences of Total Dry Mass (TDM) against sand and humus 80 Kg N ha⁻¹ of approximately 269 and 201% respectively. This variable becomes essential because the amount of nutrients required is a function of their content in the plant material and the total dry matter produced (FAQUIN, 2005).

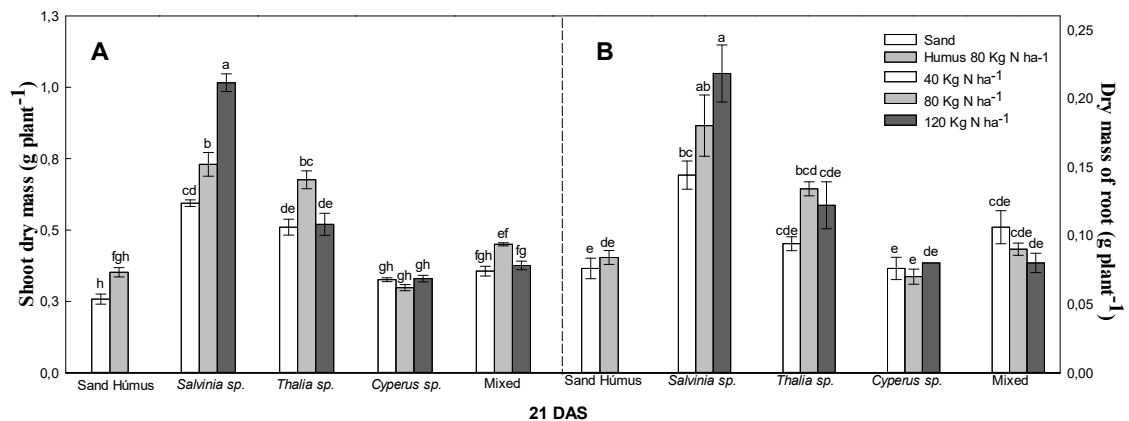


Figure 6. a) Shoot Dry Mass and b) Dry Mass of Root of sunflower seedlings in substrates containing sand, earthworm humus or increasing concentrations (40, 80 or 120 kg N ha⁻¹) of *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* or mixed macrophytes compound at 21 days after sowing (DAS).

The results found do not differ from those described by Barbosa et al. (2017) who observed that the use of OMR at 50 and 100% of the N recommendation for sunflower promoted higher growth, Britto et al. (2018) who observed that the use of Organic Algal Bloom Residues (OABR), especially at 50% of the NR in OABR, led to improvement in growth variables compared to other treatments and Braga et al. (2010) who observed higher photosynthetic efficiency of ornamental pot sunflower plants with increased nitrogen doses.

Pompêo (2017) points to the great potential of macrophyte biomass for use in agriculture as fertilizer; similarly, Silva et al. (2011) mention that experiments under field conditions showed that organic fertilization with aquatic macrophytes (*Egeria densa*) resulted in higher corn production.

For sunflower cultivation, Lobo et al. (2011) state that the range for fertilization goes from 40 to 80 kg N ha⁻¹. Oliveira et al. (2012) found that 80 kg ha⁻¹ dose of N had the best results of sunflower mass production. Thus, as the highest values are presented by the treatment of 120 Kg N ha⁻¹, in most of the analyzed variables, it is possible to apply higher concentrations of macrophytes in the physical-chemical conditions and stage of development of the plants used in this work as a recommended nutritional source for the species.

The increasing and differential increases observed in treatments with different macrophyte concentrations, in particular *Salvinia sp.* at 120 Kg N ha⁻¹, during the experimental period suggest the possibility of using macrophytes as source of N and other macronutrients as nutritional stimulus for sunflower crops. Thus, macrophytes appear as possible alternatives to the problem of destination and disposal of waste with decreased environmental liabilities.

Conclusions

The using of the macrophyte species *Salvinia auriculata*, *Thalia geniculata*, *Cyperus auriculatus* (and the mixed compound) in the fertilization of sunflower seedlings promoted increments in most of

the variables analyzed against those of plants with only sand (control) or sand + earthworm humus at 80 kg N ha⁻¹.

The highest values of the analyzed growth variables (Plant height, stem diameter, Leaf number, chlorophyll relative content and production of dry mass) were observed in *Salvinia sp.* at 120 Kg N ha⁻¹ treatment, thus constituting the best treatment employed.

The work shows the potential of using macrophytes as a practical and less expensive alternative for the fertilization of sunflower culture, reconciling sustainable values with current agricultural practices.

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