

Plant Growth, Antioxidative Enzymes and Lipid Peroxidation in Sunflower Seedlings Supplemented With *Eichhornia crassipes* Organic Fertilizer Under Drought Stress Conditions

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Abstract

In the semiarid region of the Brazilian Northeast, there is still the occurrence of soils with low concentrations of organic mass and nutrients. *Eichhornia crassipes* (water hyacinth) is recognized as one of the top ten endemic herbs in the world. However, its accumulation capacity means it can be an alternative source of nutrients. The objective of this study was to analyse the effects of macrophyte organic residue (ROM) on plant growth, antioxidative enzyme activity and membrane lipid peroxidation in leaves and roots of sunflower seedlings submitted to drought stress conditions. The experiment was conducted under greenhouse conditions at the Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Maracanaú Campus, Brazil. Samples of *E. crassipes* were collected in the Parangaba Lagoon, Fortaleza, Ceará, Brazil. The treatments were: 1) 100% (by volume) sand; 2) sand + fertilizer (following the recommendation of 80 kg of N/ha), and 3) sand + 100% of the recommended nitrogen in macrophytes (RN). In general, the use of ROM caused better seedling growth in relation to the other treatments in all conditions studied. Increases in antioxidative enzyme activity and reductions in the deleterious effects of drought stress on plant growth were observed.

Keywords: drought, *Helianthus annuus*, oxidative stress, water hyacinth

1. Introduction

Water deficit is one of the main agricultural problems that reduces crop yields in arid and semiarid regions of the world, including the Brazilian Northeast (Farooq et al., 2009; Niu et al., 2017). These regions present irregular distribution of precipitation, high evaporation rates, and shallow and nutrient-poor soils (Santos et al., 2010). Despite these limiting factors, irrigation practice is the best way to ensure agricultural production, which together with organic fertilization can make soils more fertile and productive (Nobre et al., 2011; Finatto et al., 2013).

Drought stress can cause morphological, physiological and biochemical changes in plants (Ferrari et al., 2015), as well as the restriction of nutrient and water acquisition (Manivannan et al., 2016), in addition to stomatal closure reducing the rate of evapotranspiration. Consequently, the photosynthetic rate may be reduced due to the lower availability of CO₂, causing damage to the growth and development of the plants (Ghobadi et al., 2013; Cerqueira et al., 2015).

Drought stress can also alter biochemical processes, thus increasing the production of reactive oxygen species (ROS). In excess, ROS are highly toxic to plants, with the following outstanding: superoxide radical ([•]O₂⁻), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂) and hydroxyl ([•]OH) (Demidchik, 2015). However, plants have defence mechanisms to combat this oxidative stress, such as the synthesis of non-enzymatic antioxidant compounds and/or the synthesis and activation of antioxidative enzymes such as superoxide dismutase (SOD),

catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) which aim to reduce ROS concentrations (Gill & Tuteja, 2010; Gonçalves, 2017).

In the semiarid region of the Brazilian Northeast, there is still the occurrence of soils with low concentrations of organic matter and nutrients such as nitrogen and phosphorus (Esteves & Meirelles-Pereira, 2011). In this region, there is also a large presence of floating macrophytes in the water bodies, which may be related to disturbances in their short flood periods and prolonged dry periods (Pedro et al., 2006; Henry-Silva et al., 2010).

Macrophytes are plant organisms distributed throughout the world in numerous humid environments and which have high nutrient storage capacity (Bonanno & Lo Giudice, 2010). They are among the groups that produce organic matter and play an important role in the geochemistry of wetlands. In addition, because of their accelerated decomposition, macrophytes when deposited in the soil can enrich it nutritionally (Dibble, 2005; Vodyanitskii & Shoba, 2015).

Eichhornia crassipes (water hyacinth) is a free-floating macrophyte native to South America, and one of the most studied for phytoremediation purposes (Gupta et al., 2012; Malignami et al., 2015). This species reproduces sexually and asexually and can quickly colonize new areas (Villamagna & Murphy, 2010). It is present in 65 countries and is recognized as one of the ten main endemic herbs in the world. Its large accumulation can characterize problems such as eutrophication, where this species functions as a bioindicator (Shanab et al., 2010). However, this plant can remove heavy metals, nutrients and sediments from water (Buta et al. 2011). In view of this, *E. crassipes* could be used in the composition of substrates for plants, functioning as an alternative source of nutrients. In addition, it could be an alternative destination for this waste.

The sunflower (*Helianthus annuus* L.) is an oleaginous species that has been gaining ground in the Brazilian Northeast because it has a good tolerance to heat and drought. Among the characteristics of this crop, the following stand out: rusticity, high productivity and the quality of its oil, besides the potential for honey production and use in poultry rations (Lira et al., 2009; Souza et al., 2010; Santos et al., 2015).

Thus, the present work analysed the effects of using macrophyte organic residue (ROM) on the initial growth of sunflower (*H. annuus* L.) plants under conditions of drought stress, evaluating plant growth, antioxidative enzyme activity and membrane lipid peroxidation.

2. Method

The experiment was conducted under greenhouse conditions at Instituto Federal de Educação, Ciência e Tecnologia do Ceará (IFCE), Maracanaú campus, Ceará, Brazil, from October to December 2016, totalling 30 days. The mean values of temperature and relative humidity were 30 °C and 56%, respectively.

Samples of *E. crassipes* (water hyacinth) were manually removed from the Parangaba Lagoon, Fortaleza, Ceará, Brazil, and arranged to dry in the full sun for 20 days. Subsequently, the dry mass was crushed by a mechanical crusher and sent to the Laboratory of Biochemistry and Plant Physiology of the IFCE Maracanaú campus to finalize the drying process in an oven with forced circulation of air at 60 °C.

The material obtained was called ROM. After this process, a sample of the material was sent for analysis in the Laboratory of Soils/Water of the Federal University of Ceará (Table 1), and the results for nitrogen (N) content were used in calculations of amounts of ROM added to the substrates. For the commercial fertilizer treatment, the data of the analyses performed were provided by the manufacturer Terra Vegetal (Table 1).

Table 1. Chemical analysis of the masses of *Eichhornia crassipes* and commercial fertilizer used in the composition of substrates for cultivation of sunflower plants (*Helianthus annuus* L.)

	N-t	Ca	Na	Mg	K	K ₂ O	P	P ₂ O ₅	S	Fe	Cu	Zn	Mn
	g/Kg									mg/Kg			
ROM	19.6	13.3	15.3	16.4	33.2	40.5	3.9	8.9	2.8	19.6	13.3	15.3	16.4
Adubo	2.2	8.43	-	8.10	1.52	1.85	3.9	0.98	-	3.445,5	1.80	89.3	380.1
	*pH	*C.E	R.A.S	C.O.T	C/N	NH ₄	NO ₃ ⁻	NO ₂ ⁻	Cl ⁻	HCO ₃ ⁻	CO ₃ ⁻		
	dS/m			g/kg			--- Formas de N (g/kg) ---			g/Kg			
ROM	6.6	7.52	3.35	453.3	23	14.1	3.9	1.1	10.1	0.4	0.0		
Adubo	7.6	-	-	-	-	-	-	-	-	-	-		

Note. N-t: total nitrogen; T.O.C: Total Organic Carbon; C/N: carbon nitrogen ratio; C.E: Electric conductivity; S.A.R: sodium adsorption ratio. *Relation of (1:10).

Sunflower seeds (cultivar BRS 323) were seeded in plastic vases (5 litres), containing: 1) 100% (by volume) of sand; 2) sand + commercial fertilizer (11.8% N concentrations calculated following the recommendation of 80 kg of N/ha), and 3) sand + 100% of the recommended macrophyte nitrogen (RN) following the recommendation of 80 kg culture of N/ha). The treatments were irrigated at 80% of field capacity, which was calculated based on the total weight of the vase when the substrate was filled with water.

The mass of the plastic vases was recorded at the beginning of the experiment, and the daily evapotranspiration was replaced with distilled water. At 21 days after sowing (DAS), half of each group of seedlings described above was submitted to irrigation suspension. A collection was performed at 28 DAS (7 days under water drought).

The experimental design was completely randomized, with a 2 (irrigated or non-irrigated) \times 3 (sand, sand + commercial fertilizer, sand + 100% RN in macrophyte) factorial arrangement with seven replicates, each consisting of a vase with two plants. The data were submitted to analysis of variance (ANOVA), and the means were compared by the Tukey test ($P \leq 0.05$) using Assisat 7.7 statistical software; graphs were plotted using Sigma Plot 11.0 software.

The leaf area was measured with a scanner-type meter (model AM350, ADC Bioscientific Ltd.). For the determination of dry mass, the plants were separated into roots, stems and leaves, then placed in an oven with forced circulation of air at 60 °C until a constant mass was obtained. Each sample was then weighed using an analytical balance.

For the determination of antioxidative enzyme activity, fresh leaf and root extracts were prepared according to Nunes Junior et al., (2017). Thus, the activity of the enzymes CAT, GPX, APX and SOD was determined by spectrophotometry.

The activity of GPX was determined by the method of Kar & Mishra (1976), and the reaction was accompanied by an increase in absorbance at 470 nm due to the formation of tetraguaiacol; CAT was determined according to Havir and McHale, (1987) by a decrease in absorbance at 240 nm due to H_2O_2 consumption; the oxidation of ascorbate was measured by a decrease in absorbance at 290 nm, and that of SOD by the method of Beauchamp & Fridovich, (1971), the reaction being measured by an increase in absorbance at 560 nm due to the production of blue formazan resulting from the photoreduction of p-nitroblue tetrazolium (NBT). Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid (TBA) reaction (Buege & Aust, 1978).

The activity of CAT, APX and GPX was expressed in $\mu\text{mol } H_2O_2 \text{ min}^{-1} \text{g}^{-1} \text{MF}$, and that of SOD in $\text{UA g}^{-1} \text{MF}$, where MF represents fresh matter and one UA (unit of enzyme activity) is defined as the amount of enzyme required to cause 50% inhibition of NBT photoreduction. The MDA content was expressed as $\text{nmol MDA g}^{-1} \text{MF}$; leaf area was expressed as $\text{cm}^2 \cdot \text{plant}^{-1}$, and the dry mass as g/plant .

3. Results

In the experimental conditions used, it was verified that the treatment containing ROM caused greater growth of sunflower plants when compared to the sand and commercial fertilizer treatments in control and drought stress conditions. It was observed that the plants of the control group (C) had greater growth than those of the drought stress group (D) at 28 DAS (Figure 1).



Figure 1. Sunflower plants on substrates containing sand, commercial fertilizer and macrophyte organic residue (ROM) under control conditions (C) and drought stress (D) at 28 DAS (7 days under drought stress)

For the variable shoot dry mass (SDM) (Figure 2A), it was verified that at 28 DAS, sunflower plants growing on substrate containing ROM presented higher values when compared to sand and fertilizer treatments in both control and drought stress conditions. It was also observed that, on average, the control treatment containing ROM had values 518 and 184% higher than for the sand and fertilizer treatments, respectively. For treatment under drought stress conditions, SDM was 314 and 94% higher than with sand and fertilizer, respectively. It was found that the SDM for the ROM control treatment was 70% higher than for ROM under drought stress.

For root dry mass (RDM) (Figure 2B), it was observed that plants growing on substrates containing ROM under control conditions had values 300% higher than for sand and fertilizer treatments. Under conditions of drought stress, the RDM for the ROM treatment was 120 and 40% higher than for sand and fertilizer, respectively.

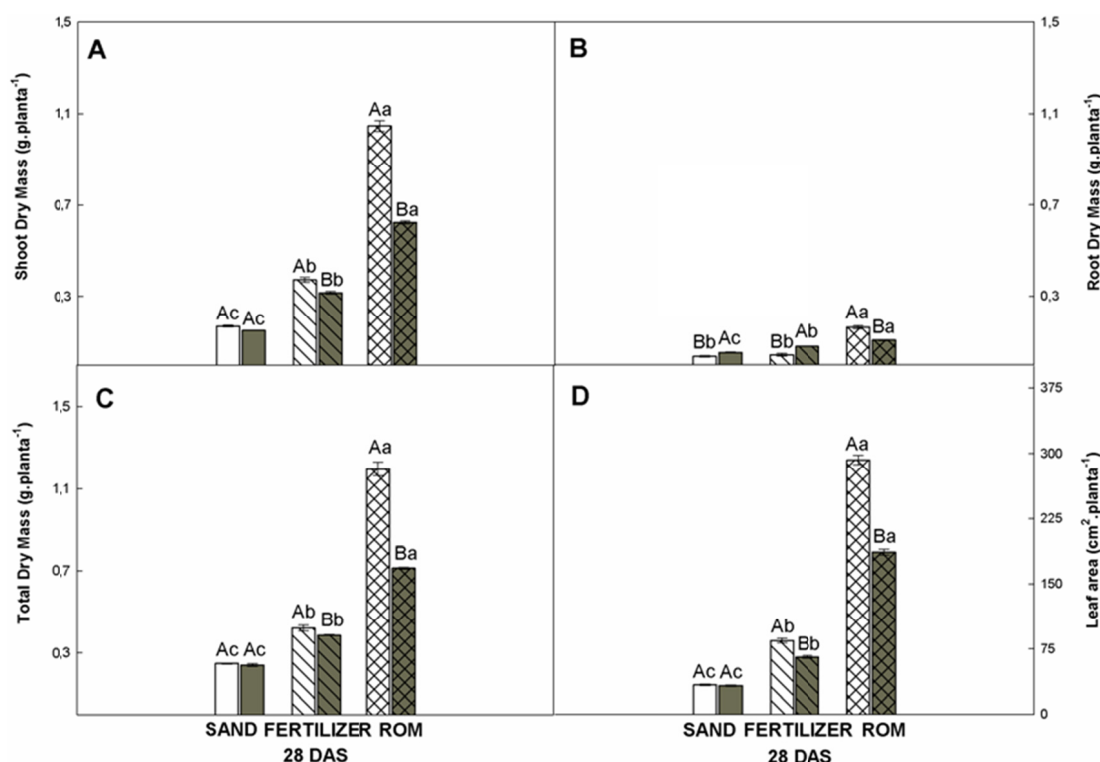


Figure 2. Shoot (A), root (B) and total dry mass (C) and leaf area (D) of sunflower plants 28 days after sowing (7 days after irrigation suspension) on substrates containing sand, commercial fertilizer and macrophyte organic residue (ROM) under control conditions (white bars) or drought stress (gray bars). Different lowercase letters indicate significant differences between the substrates (sand, fertilizer and ROM), and different uppercase letters indicate significant differences between condition types (control and drought stress), according to the Tukey test ($P \leq 0.05$). Bars represent the mean values of seven replicates \pm the standard error

For total dry mass (TDM), at 28 DAS, sunflower plants growing on substrate containing 100% RN in macrophytes presented results 380 and 190% higher, respectively, than for sand and fertilizer treatments; in drought stress conditions, TDM was 200 and 82% higher than sand and fertilizer, respectively. Within the ROM treatment, plants under control conditions had 70% more TDM than those under drought stress.

In general, drought stress caused a reduction in leaf area in all treatments when compared to control groups (Figure 2D). Under control conditions, the ROM treatment in relation to the sand and fertilizer treatments provided 770 and 250% more leaf area, respectively. Even in conditions of drought stress, leaf area was 466% higher than for sand and 190% higher than for fertilizer. Additionally, it was verified that the plants given the treatment containing ROM in the control conditions presented a leaf area 61% higher than for those under drought stress.

Figure 3 presents the activity of the enzymes SOD, CAT, APX and GPX in leaves of sunflower seedlings at 28 DAS (7 days after suspension of irrigation).

CAT activity in leaves (Figure 3A) was higher in treatments supplemented with ROM. In addition, control seedlings supplemented with ROM differed by 16% in relation to those submitted to stress and showed, on average, activity 162 and 51% higher than for sand and fertilizer treatments, respectively, under control conditions, and 455 and 112% higher under irrigation suspension conditions.

SOD activity in leaves of sunflower seedlings (Figure 3B) was higher in treatments supplemented with ROM. In addition, control seedlings supplemented with ROM differed by 4% in relation to those submitted to stress and showed, on average, activity 7 and 5% higher than for sand and fertilizer treatments, respectively, under control conditions, and 11 and 5% higher under drought stress conditions.

For APX activity in the leaves (Figure 3C), plants growing on substrate containing 100% RN in macrophytes presented results 35 and 25% higher than for sand and fertilizer treatments, respectively, under control conditions. Even under conditions of drought stress, the treatment containing ROM resulted in superior APX activity, by 65 and 54% compared to sand and fertilizer, respectively. Within the ROM treatment, the plants under control conditions had APX activity superior by 30% when compared to those under drought stress.

The GPX activity in the leaves (Figure 3D) was higher in plants supplemented with ROM. In addition, control seedlings supplemented with ROM differed by 43% in relation to those submitted to stress and had, on average, GPX activity 43 and 65% higher than for the sand and fertilizer treatments, respectively, in the control conditions, and 56 and 26% higher under irrigation suspension conditions.

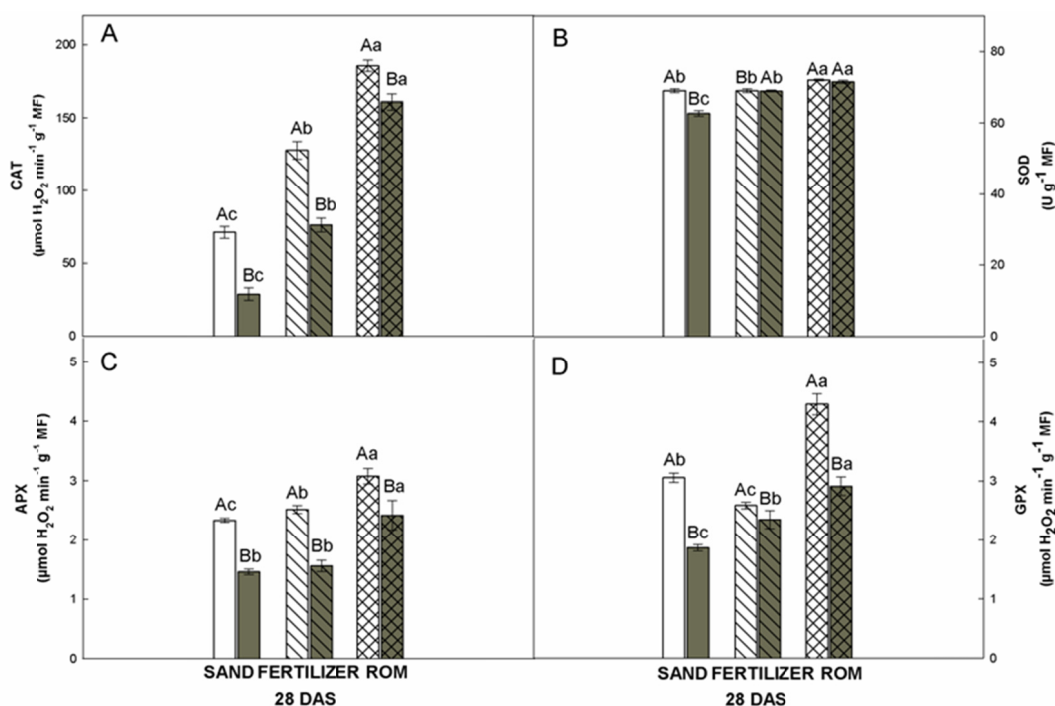


Figure 3. Activities of catalase-CAT (A), superoxide dismutase-SOD (B), ascorbate peroxidase-APX (C) and guaiacol peroxidase-GPX (D) in leaves of sunflower plants at 28 days after sowing (7 days after irrigation suspension) on substrates containing sand, fertilizer commercial and Macrophyte Organic Residue (ROM) under control conditions (white bars) or drought stress (gray bars). More details in the legend of Figure 2

In the present work, at 28 DAS (7 days after the suspension of the irrigation), no CAT activity was observed in the roots of the sunflower seedlings. Thus, in Figure 4, the activity of SOD, APX and GPX are presented. No CAT activity was observed in other studies with sunflowers under drought stress either, such as that of Nunes Junior et al. (2017) using landfill percolation as a substrate, and that of Braga et al. (2017) who tested organic shrimp residue.

SOD activity in the roots (Figure 4A) was higher in plants under control conditions supplemented with ROM. Additionally, there were no statistical differences between control conditions and drought stress within the ROM treatment.

For APX activity in the roots (Figure 4B), sunflower plants growing on substrate containing ROM presented results higher by 20 and 15%, respectively, than for sand and fertilizer treatments under control conditions. Even under conditions of drought stress, the treatment containing ROM resulted in APX activity 10 and 14% higher than for sand and fertilizer, respectively. Within the ROM treatment, plants under control conditions had 33% higher APX activity when compared to those under drought stress.

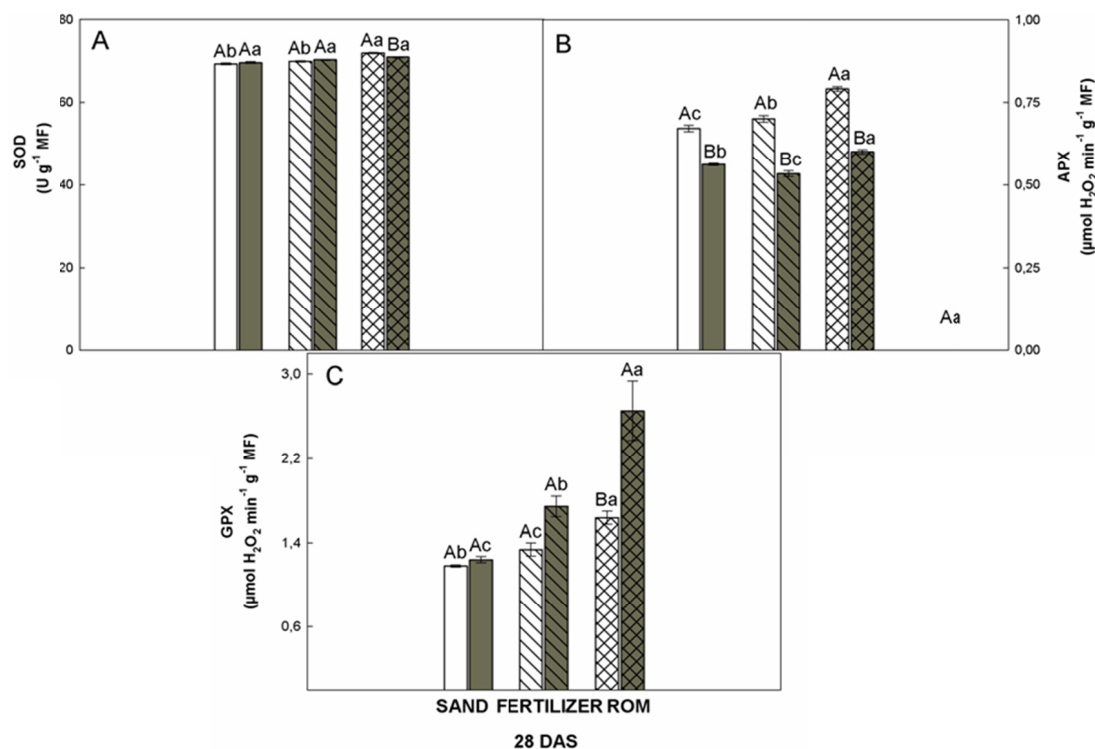


Figure 4. Activities of superoxide dismutase-SOD (A), ascorbate peroxidase-APX (B) and guaiacol peroxidase-GPX (C) in roots of sunflower plants at 28 days after sowing (7 days after irrigation suspension) on substrates containing sand, fertilizer commercial and Macrophyte Organic Residue (ROM) under control conditions (white bars) or drought stress (gray bars). More details in the legend of Figure 2

The GPX activity in the roots (Figure 4C) was higher in plants supplemented with ROM under drought stress, differing by 63% in relation to the control plants of the same treatment, and showed, on average, activity higher by 114 and 52% than for the sand and fertilizer treatments, respectively.

In general, plants subjected to drought stress presented the highest levels of MDA, especially for sand and fertilizer treatments, which had 93 and 50% more MDA, respectively, when compared to the treatment containing 100% RN. Thus, plants supplemented with ROM, both under control conditions and drought stress, had the lowest MDA content.

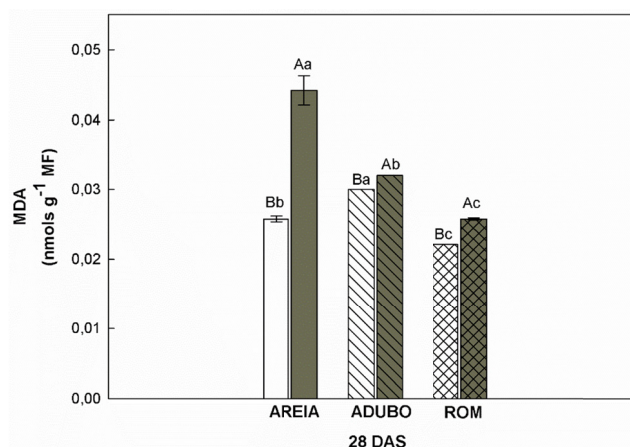


Figure 5. Malondialdehyde (MDA) contents in leaves of sunflower plants at 28 days after sowing (7 days after irrigation suspension) on substrates containing sand, commercial fertilizer and Macrophyte Organic Residue (ROM) under control conditions (white bars) or drought stress (gray bars). More details in the legend of Figure 2

4. Discussion

In general, a reduction in plant growth was observed due to the applied water restriction (Figures 1 and 2). However, the use of ROM in the substrate minimized this damage. This is due to plant growth depending on cell division and expansion, the latter being conditioned to the turgescence pressure, a process affected by poor availability of water in the soil or by excessive transpiration (Santos et al., 2014).

This behaviour was like that observed in other studies, such as that of Carneiro (2011) in which young sunflower plants were sensitive to water deficit; to minimize deleterious effects, the plants reduce stomatal opening, maximizing the efficiency of their use of water.

Nobre et al. (2017) also observed reductions of SDM in sunflower plants under stress. Guedes Filho et al. (2011) observed that nitrogen concentration and water content in the soil positively influenced all variables in a sunflower crop (cv. EMBRAPA 122/V-2000) corresponding to dry phytomass production. Similarly, Dutra et al. (2012) observed significant differences in the dry mass of sunflower plants under control and drought stress conditions; they attributed this behaviour to the addition of water by irrigation.

The results for growth were also reflected in the leaf area (Figure 2). In general, irrigated and nitrogen-supplemented treatments were superior. Similar results were found by Nobre et al. (2017); they concluded that a higher nitrogen content causes an increase in the leaf area of sunflower plants (cv. EMBRAPA 122/V-2000) under stress, and Barbosa et al. (2018) showed that sunflower plants supplemented under different concentrations of macrophyte residue had a high leaf area.

A decrease in leaf area under drought stress was also observed by Nascimento et al. (2011). A reduction in leaf area represents an important mechanism for acclimatization to drought stress, as it reduces water losses through stomatal flow and contributes to the maintenance of a high water potential in the plant.

In the present study, the use of ROM caused high leaf area, which could indicate a better efficiency of water use, and the existence of a larger area available for photosynthesis.

The high growth of the plants (Figures 1 and 2) supplemented with ROM in comparison to the other treatments can also be explained by the high activity of antioxidant enzymes (Figures 3 and 4) as well as reductions in lipid peroxidation (Figure 5).

SODs are metalloenzymes that are considered the first line of defence against ROS in which they catalyse the dismutation of two $\cdot\text{O}_2$ radicals, generating H_2O_2 and O_2 (Barbosa et al., 2014). CAT is one of the most effective enzymes in defence against oxidative processes (Akçay et al., 2010), and is still believed to be the major H_2O_2 -killing enzyme (Barbosa et al., 2014).

In general, in the leaves, the CAT activity for the irrigated treatments was superior to that under drought stress. However, treatments containing ROM had significantly higher CAT activity than the other treatments. Thus, the data corroborate those of Eyidogan and Oz (2007) who demonstrated that an increase in CAT activity causes a reduction in H_2O_2 accumulation with consequent reduction of lipid peroxidation.

In addition, both the leaves and roots of plants treated with ROM showed higher APX and GPX activity when compared to the other treatments. This may be associated with the behaviour of these species which function as a secondary mechanism in the elimination of ROS when compared to CAT, which first contributes to H₂O₂ detoxification (Bhatt & Tripathi, 2011).

In addition to these defence mechanisms, it is important to quantify lipid peroxidation, which produces MDA which is used to determine process intensity in the lipids of plant cell membranes. Thus, an increase of this substance is directly associated with indications of oxidative stress (Hendges et al., 2015).

It should be noted that in both stress and control conditions, the treatments containing ROM had the lowest MDA levels. Moreover, similar results were found in other studies such as that of Messchmidt et al. (2015) who observed high levels of MDA in *Prunus* spp. under conditions of drought, and Silva (2010) who found a significant increase in the concentrations of MDA in sugarcane plants also submitted to drought.

Maia et al. (2012) concluded that increases in the activity of enzymes such as SOD, APX and CAT are associated with a reduction of lipid peroxidation (MDA) in plants under conditions of drought stress. Thus, it is suggested that the use of ROM causes greater antioxidative enzyme activity and provides a reduction in lipid peroxidation and the deleterious effects of drought stress.

5. Conclusions

In the present experimental conditions, the use of ROM caused an increase in plant growth (SDM, RDM, TDM and leaf area) and antioxidant enzyme activity (SOD, CAT, APX and GPX) which contributed to a reduction of membrane lipid peroxidation (MDA) and the deleterious effects of drought stress, both under control conditions and drought stress, when compared to the other treatments.

Thus, the use of dried and crushed *E. crassipes* to add nutrients and organic matter to the soil is suggested.

Subsequent studies should be carried out to verify the use of this residue on a large scale and to act as an environmentally correct destination.

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