



Biofertilizer based on mycorrhizal fungi and rhizobacteria in maize cultivation under two water regimes

Biofertilizante a base de hongos micorrícicos y rizobacterias en el cultivo de maíz bajo dos regímenes hídricos

A. T. R. Felix¹; A. C. Mesquita^{1*}; F. A. F. Moura¹; M. C. C. Alencar¹;
B. G. A. Barros²

¹Departamento de Tecnologia e Ciências sociais/Laboratório Fisiologia Vegetal e Pós-Colheita, Universidade do Estado da Bahia, 48904-711, Juazeiro-Bahia, Brasil

²Departamento de Tecnologia e Ciências sociais/Laboratório de Microbiologia do Solo, Universidade do Estado da Bahia, 48904-711, Juazeiro-Bahia, Brasil

*amesquita@uneb.br

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Biostimulants are able to increase tolerance to water and salt stress and help enhance the absorption of nutrients from the soil, in addition to increasing the extension of roots, they are also effective in the synthesis of plant hormones (such auxins, gibberellins, cytokinins) and in the capacity to make insoluble phosphorus in the soil available to plants, minimizing the effects of biotic and abiotic stresses. The objective of this study was to evaluate the phytotechnical and physiological responses of maize subjected to two water regimes and a biostimulant containing mycorrhizae and rhizobacteria of the genus *Bacillus* spp., through the characterization of the antioxidant response conditioned by this association. The experiment was set up in a 5x2 factorial scheme, with five treatments related to the doses of biostimulant [negative control; 0.250 kg ha⁻¹; 0.500 kg ha⁻¹; 0.750 kg ha⁻¹ and 1 kg ha⁻¹] and two water availability levels (60% and 100% of field capacity), with four replicates, totaling 40 pots. The following growth parameters were analyzed at 15 and 30 days after planting: plant height, stem diameter, number of leaves, root length and root volume, as well as the following biochemical parameters: lipid peroxidation, total peroxidase and catalase of leaf and root. The biostimulant promoted an increase in the growth of the aerial part and root system of corn plants, in addition to an adjustment of antioxidant enzymes, indicating that, up to 30 days after planting (DAP), corn plants show better adaptability to the water regime with 60% of field capacity.

Keywords: *Zea mays*, fertilization, irrigation.

Los bioestimulantes son capaces de aumentar la tolerancia al estrés hídrico y salino, ayudar a mejorar la absorción de nutrientes del suelo, aumentar la extensión de las raíces, eficaces en la síntesis de hormonas (auxinas, giberelinas, citoquininas) y en la capacidad de hacer que el fósforo insoluble del suelo esté disponible para las plantas, minimizando los efectos del estrés biótico y abiótico. El objetivo de este estudio fue evaluar las respuestas fitotécnicas y fisiológicas del maíz sometido a dos regímenes hídricos y un bioestimulante que contenía micorrizas y rizobacterias del género *Bacillus* spp., a través de la caracterización de la respuesta antioxidante condicionada por esta asociación. El experimento se montó en un esquema factorial 5x2, dosis de bioestimulante [control negativo; 0,250; 0,500; 0.750 y 1 kg ha⁻¹] y dos niveles de disponibilidad hídrica (60% y 100% de la capacidad de campo), con cuatro réplicas, totalizando 40 macetas. Se analizaron los siguientes parámetros de crecimiento a los 15 y 30 días después de la siembra: altura de la planta, diámetro del tallo, número de hojas, longitud de la raíz y volumen radicular, así como los siguientes parámetros bioquímicos: peroxidación lipídica, peroxidasa total y catalasa de hoja y raíz. El bioestimulante promovió un aumento en el crecimiento de la parte aérea y el sistema radicular de las plantas de maíz, ajuste de las enzimas antioxidantes, indicando que, hasta 30 días después de la siembra (DAP), las plantas de maíz muestran mejor adaptabilidad al régimen hídrico con 60% de la capacidad de campo.

Palabras clave: *Zea mays*, fertilización, riego.

1. INTRODUCTION

Corn has established itself as the world's largest agricultural crop, being the only one to surpass the mark of 1 billion tons produced annually. [1]. Brazil stands out for being the third largest producer and largest exporter of maize on the planet, with a significant expected growth in production (+10.2%), which will rise to 124.7 million tons [2], making maize an essential crop for the development of Brazilian agriculture in all regions, because in addition to its importance in human and animal food, it is also a source of raw materials for various production chains, such as fuels, beverages, polymers etc. [3].

However, this sector still faces challenges with abiotic stresses and anthropogenic activities that negatively affect the quality of agricultural areas, and water stress is among the main ones. Its damage is greater than the damage caused by other stresses, such as cold, heat, and excess of light or chemicals, making it a problem mainly in the arid and semi-arid regions of the world [4]. Despite unfavorable climatic conditions due to the lack of rainfall, the semi-arid biome in Brazil has a significant production of maize [5].

Biological stimulants composed of mycorrhizal microorganisms and rhizobacteria are able to increase the specific surface of the rhizosphere, improving nutrient absorption and boosting plant growth, in addition to promoting beneficial impacts, including greater absorption of water and nutrients through root development, as well as the production of plant hormones and especially biological nitrogen fixation [6]. The presence of these microorganisms increases tolerance to water and salt stress and also helps enhance the absorption of soil nutrients, including P, K and Si; in addition to increasing the extension of roots, the application of the biostimulant is effective in the synthesis of plant hormones (such as auxins, gibberellins and cytokinins) and in the capacity to make insoluble phosphorus in the soil available to plants [7].

The consequences of water deficit result in changes in plant physiology, biochemistry and anatomy, affecting developmental stages, directly interfering with enzymatic activities and reducing crop yield, resulting in losses for producers [8]. Therefore, it is essential to conduct studies that analyze the regulatory responses of plants to variations in water availability.

Reduced water intake in maize affects yield, height, diameter, and reduces cell expansion due to damage to the photosynthetic apparatus and inactivation of enzymes involved in essential plant metabolism processes, which can cause overproduction of reactive oxygen species (ROS) [9]. This excessive production can result in lipid peroxidation, protein oxidation, and a series of metabolic dysfunctions, with detrimental effects that can lead to plant death [10].

To combat oxidative stress caused by ROS overproduction, plants have a protective shield: the antioxidant system. Antioxidant enzymes (superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.15.1.1), glutathione reductase (GR, EC 1.6.4.2), peroxidase (POD, EC 1.11.1.7), etc.) act in the defense response, seeking to minimize the formation of ROS under stress [11]. The use of microorganisms is an important strategy to maintain the antioxidant potential, preventing the formation of ROS at high levels and increasing the activity of enzymes such as superoxide dismutase, catalase, and peroxidase, bringing benefits to photosynthesis [12].

Therefore, the objective of this study was to evaluate the phytotechnical and physiological responses of maize (*Zea mays* L.) subjected to two water regimes and a biostimulant containing mycorrhizae and rhizobacteria of the genus *Bacillus* spp., through the characterization of the antioxidant response conditioned by this association. Thus, the aim is to offer a viable alternative for farmers, aiming at better crop management and, consequently, obtaining the best production results under the conditions of the Brazilian semi-arid region.

2. MATERIAL AND METHODS

The experiment was carried out in a greenhouse, in the experimental area of the Department of Technology and Social Sciences – DTCS III, of the State University of Bahia - UNEB, in the municipality of Juazeiro (Latitude 09° 24' 50" S; Longitude 40° 30' 10" W; Altitude of 368 m), from March to July 2023. The local climate is Bswb, semi-arid, according to Köppen's classification, with an average annual rainfall of 540 mm.

The soil for the experiment was collected at 0-20 depth from a Neossolo Flúvico (Fluvent), located at UNEB (Juazeiro, BA), and a 500 g composite sample was sent to the Soil and Water Laboratory (LASAC) of UNEB for analysis of fertility characteristics (Table 1).

Table 1. Chemical and physical attributes of a sample of Fluvisol, belonging to the experimental area located at DTCS/UNEB.

pH	P	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	SB	Al ³⁺	H+ Al	M.O
	mg dm ⁻³	---cmol dm ⁻³ ---		-----cmolc dm ³ -----					
6,67	8	0,54	4	1,60	0,13	7,83	0,05	0,80	11,6

pH – hydrogen ion potential; P – phosphorus; K⁺ – potassium; Ca²⁺ – calcium; Mg²⁺ – magnesium; SB – sum of bases; Al³⁺ – aluminum; H+Al – hydrogen + aluminum; M.O – organic matter.

Basal organic fertilization was carried out as recommended for the crop and according to the Center for Agroecology and Renewable Energies of UNEB (CAERDES). Applications of biofungicides, bioinsecticides and other cultural and management practices were carried out according to crop needs.

The experiment was set up using 5-L pots, filled with a thin layer of crushed stone at the base to facilitate drainage and then completed with soil, planted with 3 seeds of hybrid maize (*Zea mays* L.) of the variety 1051, at 1.5 cm depth. When maize plants had one definitive leaf, thinning was carried out, keeping only one plant per pot.

Irrigation requirement for the crop was determined by conducting the field capacity test, in which the pots were saturated with water by capillarity, covered and left at rest. Initially, the dry weight of the pots and their weight after reaching field capacity were measured. Irrigation was carried out manually on alternate days based on the weighing method, so the pots were weighed before irrigation to obtain the average value of the water depth to be applied, always raising the soil moisture to field capacity.

The biostimulant used has in its formulation mycorrhizal fungi and rhizobacteria specifically combined with chelated microelements and organic elements, so as to provide a better availability of nutrients due to these microorganisms, which colonize the rhizosphere and solubilize minerals such as K, P and Si, thus improving the microbiological composition of the plant/soil system. The product contains three species of mycorrhizae equivalent to 12,500 propagules/g (*Rhizoglyphus irregularis*, *Funneliformis mosseae* and *Funneliformis caledonium*) and rhizobacteria of the genus *Bacillus* spp. equivalent to a concentration of 1x10¹⁰ CFU/g (*Bacillus licheniformis* and *Bacillus mucilaginosus*), besides containing chelated microelements (6.48% iron (Fe); 2.8% manganese (Mn); 0.76% water-soluble boron (B); 0.48% zinc (Zn); 0.24% copper (Cu); and 0.24% water-soluble molybdenum (Mo)) and a nutritional supplement of organic origin (5.42% w/w total nitrogen (N); 0.93% w/w water-soluble phosphorus pentoxide (P₂O₅); 7.5% w/w water-soluble calcium oxide (CaO); and 0.3% w/w water-soluble sulfur trioxide (SO₃)) to boost crops.

The biostimulant was prepared at the Plant Physiology Laboratory (LFV); initially, the respective doses of the product for each treatment were weighed using an analytical balance, and later, these products were dissolved in water and applied to the base of the plant. Negative control was performed

without applying the product, using distilled and deionized water. The treatments were applied when the plants had one definitive leaf.

Two evaluations of plant growth were carried out, at 15 and 30 days after planting (DAP). The following characteristics were evaluated: plant height (PH) in cm, using a graduated ruler; stem diameter (SD) in mm, determined with a digital caliper; and number of leaves (NL) in units, obtained by individual counting of leaves on each plant. At the end, root length was measured using a graduated ruler, and root volume was determined based on the displacement of water in a graduated cylinder.

Leaf and root tissues were collected at 15 and 30 DAP, placed in plastic bags wrapped in aluminum foil, properly identified and frozen to paralyze all metabolic reactions immediately. After collecting the leaf tissue, 300 mg was macerated in a mortar with pestle, with addition of 4 mL of extraction buffer solution (0.1 M potassium phosphate, pH 6.8), and centrifuged at 10000 rpm for 10 minutes at 4 °C to obtain the supernatant, which was used to determine the antioxidant systems. Protein concentration was determined using the method of [13].

Stress characterization was performed through lipid peroxidation analysis (TBAR), by quantifying the content of reactive substances to 0.25% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA), after the sample was homogenized in 5 mL of this solution. Then, the extracted solution was incubated in a water bath at 90 °C for 60 minutes. After cooling, the solution was centrifuged at 10000 rpm for 15 minutes. The supernatant collected from each sample was subjected to absorbance readings in a spectrophotometer at 560 and 600 nm. The molar extinction coefficient of malondialdehyde (155 mmol L⁻¹ cm⁻¹) was used for the calculations, and the analysis was performed according to the technique described by [14].

CAT activity (CAT, EC 1.11.1.6) (μmol H₂O₂ min⁻¹ mg⁻¹ of protein⁻¹) was determined from the mixture of 50 mmol L⁻¹ potassium phosphate buffer, pH 7.0, with the enzyme extract, and the absorbance readings were performed at 240 nm every 20 seconds until the decay stabilized. The molar extinction coefficient (36 mmol L⁻¹ cm⁻¹) was used to calculate the specific activity of catalase, considering the concentration of soluble protein [15].

POD activity (EC 1.11.1.7) (μmol of purpurogallin min⁻¹ mg⁻¹ of protein) was determined in a reaction medium of 50 mmol L⁻¹ potassium phosphate buffer, pH 6.5, with the enzyme extract, with addition of 20 mmol L⁻¹ pyrogallol and 5 mmol L⁻¹ hydrogen peroxide (H₂O₂). The tubes were shaken, absorbance readings were performed at 430 nm, and the molar extinction coefficient (2.5 mmol L⁻¹ cm⁻¹) was used to calculate the specific activity of peroxidase, considering the concentration of soluble protein [16].

The experimental design was completely randomized in a 5x2 factorial scheme, consisting of ten treatments related to the doses of biostimulant [T0 (negative control); T1 (0.250 kg ha⁻¹); T2 (0.500 kg ha⁻¹); T3 (0.750 kg ha⁻¹) and T4 (1 kg ha⁻¹)] and two water regimes (60% and 100% of field capacity), with four replicates, totaling 40 pots.

The data obtained were subjected to analysis of variance using the statistical software SISVAR beta version 5.6, comparing the means by Tukey test and regression at 5% probability level [17].

3. RESULTS AND DISCUSSION

According to the statistical results, there were significant effects only for the factors evaluated individually, water availability levels and biostimulant doses, on the following variables: plant height (PH) at 15 DAP, stem diameter (SD) at 15 and 30 DAP, root length (RL) at 30 DAP, root volume (RV) at 30 DAP, lipid peroxidase (MDA) of the leaf tissue at 15 DAP, total peroxidase (TPO) of the root system and leaf tissue at 30 DAP, and catalase (CAT) of the leaf tissue at 15 DAP. These patterns indicate that, in the initial development phases, these variables are mainly regulated by direct effects of water management and biostimulant dose, without simultaneous dependence between the factors.

However, the variables plant height (PH) at 30 DAP, number of leaves (NL) at 15 and 30 DAP, total soluble proteins in leaf tissue and root system at 15 and 30 DAP, lipid peroxidation of leaf tissue at 30 DAP, total peroxidase (TPO) of the root system at 15 DAP, catalase (CAT) of the root system at 15 and 30 DAP and leaf tissue at 30 DAP showed statistically significant results due to the interaction between the factors evaluated. Thus, this behavior shows that, with phenological advancement, the crop responses become more complex, depending on the interaction between soil water availability and the biostimulant dose, reflecting integrated physiological adjustments [18].

For plant height (Figure 1A), at 15 DAP, the best result was obtained with water availability of 100% of field capacity (FC), 24.45 cm; however, there was a slight reduction in the values obtained with 60% FC availability, showing that the maize crop (*Zea mays* L.) was apparently not sensitive to lower water availability, suggesting that the plant possibly has high physiological plasticity, i.e., the ability to grow even under moderate water restriction, possibly due to lower transpiration demand and the use of initial reserves.

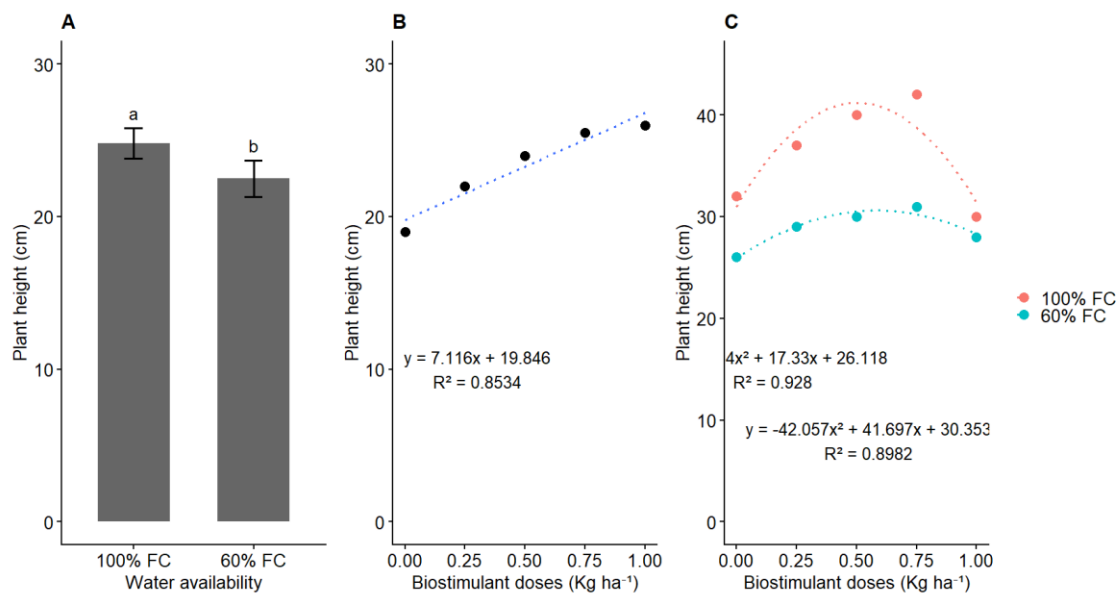


Figure 1. (A) Plant height of maize (*Zea mays* L.) as a function of water availability (FC = field capacity); (B) increasing doses of biostimulant (C) at 15 DAP and 30 DAP.

Figure 1B shows a linear increase in plant height with increasing doses of applied biostimulant. According to Santoyo et al. (2021) [19], mycorrhiza-bacteria interactions in the biostimulant promote increased photoassimilate production and biomass accumulation, enabling greater plant growth, in addition to mitigating abiotic and biotic stress in plants. Similar results were found by Melo et al. (2026) [20], who observed a significant increase in the height and biomass of corn plants treated with microbial biostimulants under water stress, avoiding their action in hormonal regulation and photosynthetic efficiency. According to Taiz et al. (2021) [21] and Silva et al. (2021) [9], water stress reduces photosynthetic activity and causes changes in plant growth, affecting productivity, height and diameter, as well as reducing cell expansion; However, this factor was not yet prominent at 15 DAP, as shown in Figures 1A and 1B.

For plant height evaluated at 30 DAP (Figure 1C), a significant interaction between the factors was observed, and a polynomial regression curve fit the data related to the biostimulant doses. According to the derivatives of the equations, the treatments with 60% and 100% of FC showed maximum height at doses of 0.58 and 0.49 kg ha⁻¹, with values of 31.1 and 40.69 cm, respectively.

This behavior indicates the occurrence of an optimal dose, above which there may be metabolic saturation or hormonal imbalance, reducing the physiological efficiency of the bioinput, since high doses of biofertilizers do not always result in additional increases in plant growth [22].

There was a more pronounced reduction under water availability of 60% of the FC, demonstrating that corn plants at 30 DAP began to show sensitivity to moderate water stress. Bonfim-Silva et al. (2011) [23], when evaluating the growth of irrigated corn with 30% and 60% of the FC, found an even greater reduction; however, under 60% of the FC, the plants showed a height of 25 cm, corroborating the results of Figure 1C, which indicate that the height of the corn plant decreases under water stress. Dias et al. (2022) [24], when inoculating bacteria isolated from the Caatinga in the corn crop, also obtained positive responses against the negative effects of drought. These authors observed an increase of up to 21.2% in height at 45 days after emergence, corroborating the results shown in Figure 1C.

Stem diameter did not show a significant response to the water availability factor, with averages of 6.20 mm and 7.82 mm for 100% field capacity (FC) and 6.28 mm and 7.73 mm for 60% FC, at 15 and 30 days after planting (DAP), respectively. The lack of significance for this factor suggests that stem diameter is less sensitive to water stress in the initial phases, possibly because it is a structural organ with greater physiological stability. For the biostimulant dose factor (Figures 2A and 2B), a polynomial regression curve was fitted to the data; after the derivatives of the equations, the largest diameter found was 6.65 mm at 15 DAP and 8.64 mm at 30 DAP, indicating that the best doses were 0.57 kg ha^{-1} and 0.62 kg ha^{-1} , respectively, with a tendency to decrease from these doses.

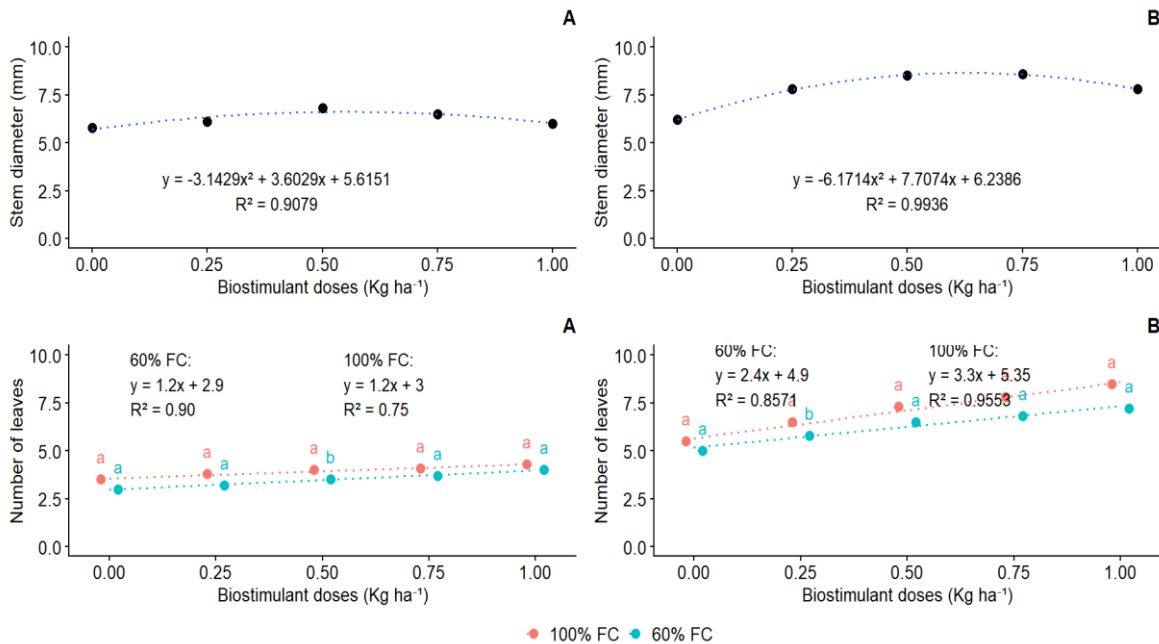


Figure 2. (A) Stem diameter in maize plants (*Zea mays* L.) as a function of increasing doses of biostimulant at 15 DAP (B) and 30 DAP. (C) Number of leaves in maize plants (*Zea mays* L.) subjected to increasing doses of biostimulant under two water availability levels (FC = field capacity) at 15 DAP (D) and 30 DAP. Equal letters for the same biostimulant dose do not differ from each other (Tukey 5%).

Therefore, the biostimulant promoted an increase in the stem diameter of corn plants, corroborating Andrade et al. (2018) [25], who found a diameter of 4.60 mm in the control treatment and 5.53 mm using a biostimulant in reversing glyphosate damage in conventional corn. The stem, also called the stalk, is the part of the plant responsible for supporting the entire aerial part, generally

composed of 4 internodes. The stem diameter influences the increase in productivity, because the larger the diameter, the greater the reserve of sugars and photoassimilates, which will help in filling the grains and will be used by the plant during the senescence period [26, 27].

The occurrence of an optimal dose for stem height and diameter shows that the plant-microorganism interaction has a physiological limit of efficiency. Therefore, higher doses may imply a greater demand for photoassimilates to maintain the root microbial community, reducing the benefit of the symbiosis. Furthermore, high concentrations of biological products can disrupt the hormonal balance of plants, especially auxins and cytokinins produced by rhizobacteria, leading to less efficient responses in terms of vegetative growth [28].

According to the data on the number of leaves on the corn plant, there was a significant interaction between the biostimulant doses and the levels of water availability (Figures 2C and 2D). With increasing doses, a linear increase in the number of leaves was observed at 15 DAP (Figure 2C), and when analyzing the water availability levels considering each applied dose, it was found that only the dose of 0.5 kg ha⁻¹ showed a difference in water availability levels between 60% and 100% of field capacity. This occurs because, in the first few days, the water content needed to increase the internal water potential of the seed corresponds to only 30% to 40% of its mass, without significant interference in water availability until the complete emergence of the fourth leaf (V4). Studies show that water deficit compromises cell expansion and leaf emission, especially in phases of higher metabolic demand [29, 30].

Furthermore, the negative effects of water stress are small, as they do not harm meristematic tissues and other differentiated organs, which are protected below the soil surface at this stage [31]. At 30 DAP (Figure 2D), the control and the 0.75 kg ha⁻¹ dose showed no statistical difference in the number of leaves between water availability levels, unlike the doses of 0.25, 0.50 and 1 kg ha⁻¹, which showed a statistical difference. When analyzing the effect of the applied doses of the biostimulant, a linear regression was observed for water availability levels of 60% and 100% of FC, with a total of 9 and 6 leaves at the maximum applied dose. Water stress at this stage will affect the ability to store photosynthesized products in the stem and reduce plant height, as observed in Figure 1C. This result corroborates those reported by Simões et al. (2020) [32], who observed that, with increased water availability, the number of leaves increased linearly, validating that corn is a crop that needs water replenishment to promote cell expansion, resulting in the emergence of leaves along the stem.

For root length as a function of water availability (Figure 3A) at 30 DAP, it was found that the availability of 100% FC promoted a significant gain compared to the availability of 60% FC, and the means of root length in the initial growth of maize were 32.37 and 27.17 cm at the water availability levels of 100% and 60% FC, respectively. This result differs from that reported by Costa et al. (2020) [33], who evaluated the growth of landrace maize inoculated with arbuscular mycorrhizal fungi and found that the highest mean was 38.2, at 99 days after sowing.

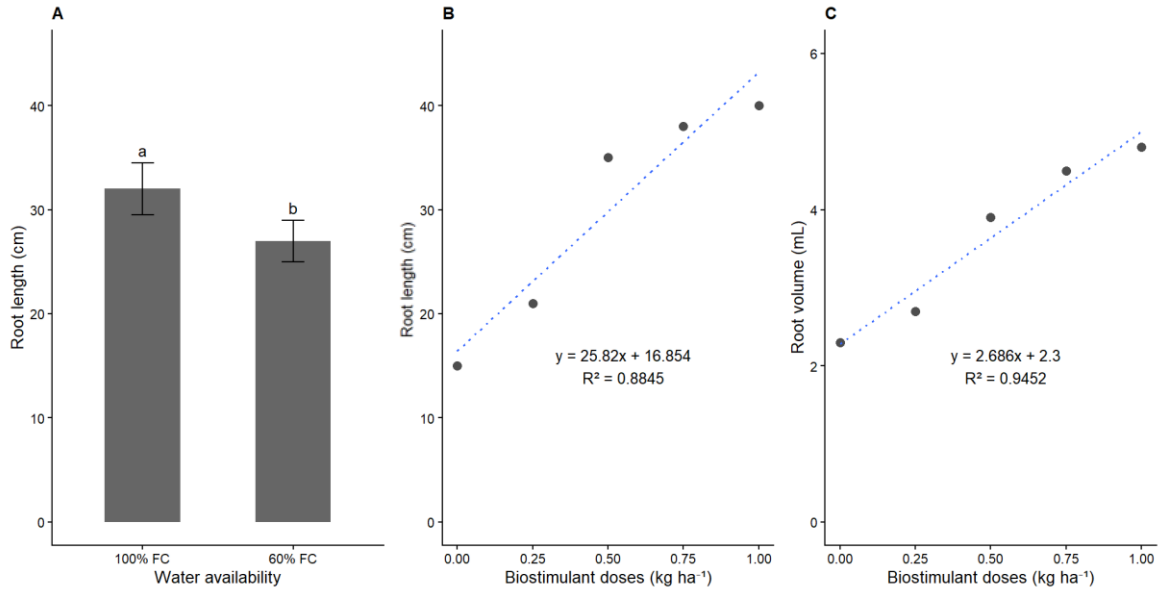


Figure 3. (A) Root length in maize plants (*Zea mays* L.) as a function of two water availability levels (FC = field capacity) and increasing doses of biostimulant (B) at 30 DAP. (C) Root volume in maize plants (*Zea mays* L.) as a function of increasing doses of biostimulant at 30 DAP.

According to Figure 3B, when the doses of the biostimulant were increased, there was a linear increase in the length of the root system, with a value of 39 cm at the maximum dose of the biostimulant. This can be explained by the presence of the compound of mycorrhizal microorganisms and rhizobacteria, which will increase the absorption of water and nutrients from the soil, including P, K, and Si, making insoluble phosphorus in the soil available to plants, in addition to its ability to increase root elongation [7]. Another benefit of the biostimulant is that it synthesizes phytohormones, such as indoleacetic acid (IAA), which is the most commonly found auxin in plant tissues and increases root growth [27]. Backer et al. (2018) [34] demonstrated that microbial biostimulants promote greater root elongation and greater water use efficiency. Furthermore, since the biofertilizer contained two strains of *Bacillus* spp., it is important to point out that these microorganisms have mechanisms to produce biofilm around the roots, which increase water retention in soil particles [35].

Root volume showed a positive linear behavior as a function of the biostimulant doses (Figure 3C), as observed for root length (Figure 3B) and number of leaves (Figures 2C and 2D). These results differ from those found by Costa et al. (2020) [33], who evaluated mycorrhizal colonization and its interaction in landrace maize and observed no influence of the isolates on root length and possibly on root volume.

Figure 4 shows the total soluble protein content, which is a reference indicator to determine the enzymatic activity of catalase and peroxidase, and the interaction between water availability levels and biostimulant doses was significant at 15 DAP (Figures 4A and 4B) and at 30 DAP (Figures 4C and 4D).

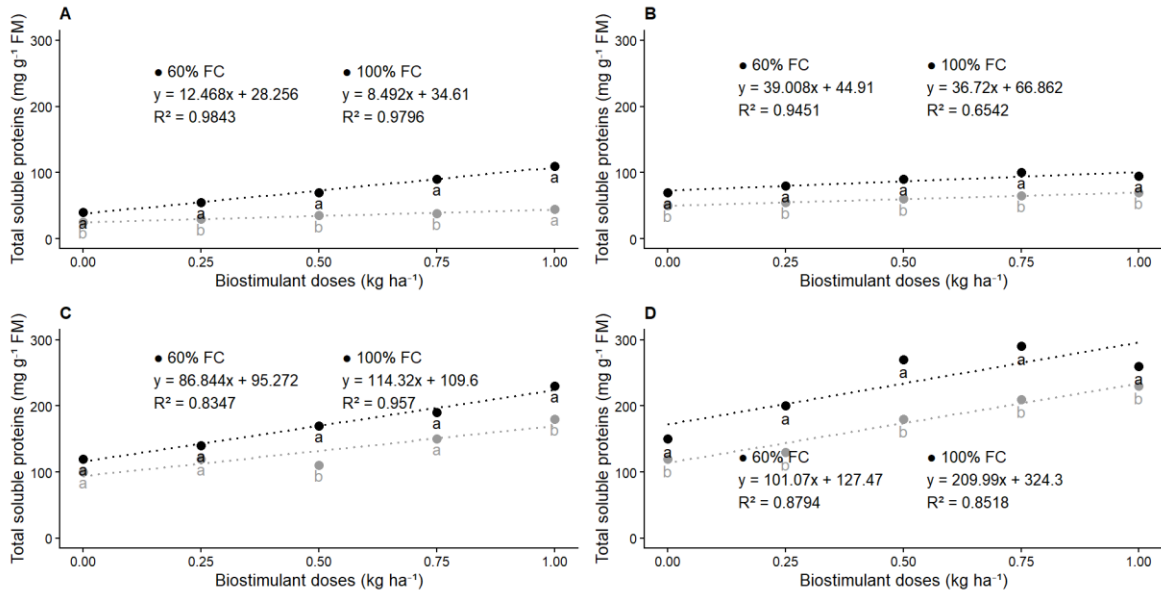


Figure 4. (A) Total protein content of the root system at 15 DAP (A) and 30 DAP (C) and of leaf tissue at 15 DAP (B) and 30 DAP (D) of maize plants (*Zea mays* L.) subjected to increasing doses of biostimulant and two water availability levels (FC = field capacity). Equal letters for the same biostimulant dose do not differ from each other (Tukey 5%).

A lower protein content was observed with water availability of 60% of field capacity, but as the biostimulant doses increased, there was an increase in protein content in both tissues (leaf and root), explained by the microorganisms present in the biostimulant, which promote increases in protein content. In addition, there was an increase in protein at 30 DAP (Figures 4C and 4D). These results corroborate those reported by Galvão et al. (2019) [36], who found that biofertilizers composed of silicon and the mycorrhizal fungus *Rhizophagus irregularis* led to the best results for total soluble protein and catalase activity in soybean.

Protein production can be affected by the production of stress enzymes, since the plant limits protein synthesis and increases the activity of enzymes such as catalase and peroxidase, which means that, under stress conditions, the plant stops performing hydrolysis to combat ROS [37]. The increase in protein content in response to biofertilizer indicates greater metabolic activity and better physiological adjustment under stress. Di Sario et al. (2025) [38] reported similar results, associating biostimulants with increased antioxidant activity and metabolic stability in plants under water stress.

The lipid peroxidation variable was expressed as MDA content, which is indicative of membrane damage. Figure 5A showed a higher MDA content at 15 DAP under the water availability of 60% FC, indicating that this treatment increased the damage to the plant, while a lower MDA content was observed under the water availability of 100% FC.

Cell damage resulting from water deficit is measured by lipid peroxidation level, and such damage can cause programmed cell death and lead to lower growth and reduced plant production. Anjum et al. (2017) [39] obtained similar results, observing cell membrane damage in maize hybrids subjected to water stress at depths equivalent to 100, 80, 60 and 40% FC.

When analyzing Figure 5B, it can be seen that there was a negative linear trend; with the increase in biostimulant doses, there was less damage to the membrane, so the biostimulant may have increased the tolerance to stress, reducing the accumulation of MDA. Increase in MDA content in maize leaves as a function of water stress was also reported by Rabelo et al. (2019) [40], who evaluated the effects of the application of chitosan and its derivatives on increasing the tolerance to water deficit in maize.

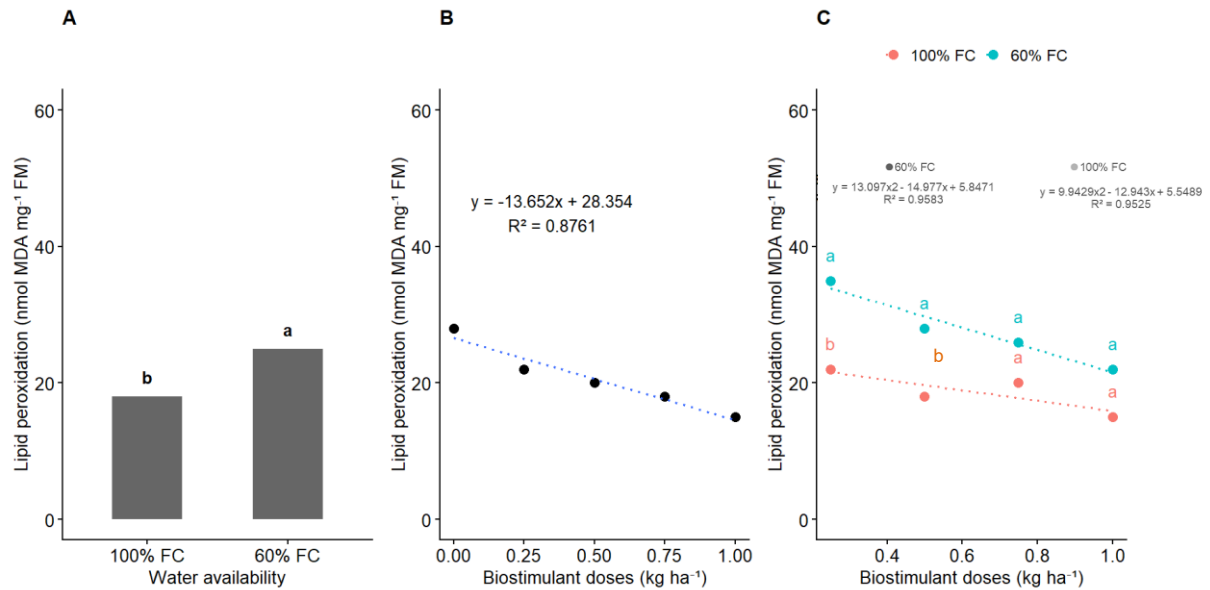


Figure 5. (A) Lipid peroxidation of leaf tissue in maize plants (*Zea mays* L.) as a function of water availability (FC = field capacity) (A) and increasing doses of biostimulant at 15 DAP (B) and at 30 DAP (C). Equal letters for the same biostimulant dose do not differ from each other (Tukey 5%).

In Figure 5C, at 30 DAP, the behavior was negative linear, indicating that the MDA content decreases with the increase in the biostimulant doses applied. Plants subjected to stress induce the synthesis of antioxidant enzymes to minimize the negative effects caused by ROS, which indicates that the biostimulant helps to minimize stresses, as shown in Figure 5B.

When analyzing the influence of the applied depth considering each dose, it can be observed that the water availability of 60% FC did not differ statistically from the water availability of 100% FC at doses above 0.50 kg ha⁻¹. Similar results were found by Portugal et al. (2021) [41], who observed that the MDA level decreases considerably with the exogenous application of the mixture of trehalose derivatives and, as water stress was reduced, there was also a decrease in MDA concentration in all treatments.

Total peroxidase (POD) showed a negative linear trend in the leaf tissue at 15 DAP (Figure 6A) and a significant response to the interaction between water availability levels and biostimulant doses in the root system (Figure 6B). After derivatives of the equations, the treatments with 60% and 100% FC had lower POD content at the doses of 0.57 and 0.65 kg ha⁻¹, respectively, with values of 1.56 and 1.33 μmol of purpurogallin min⁻¹ mg⁻¹ of proteins.

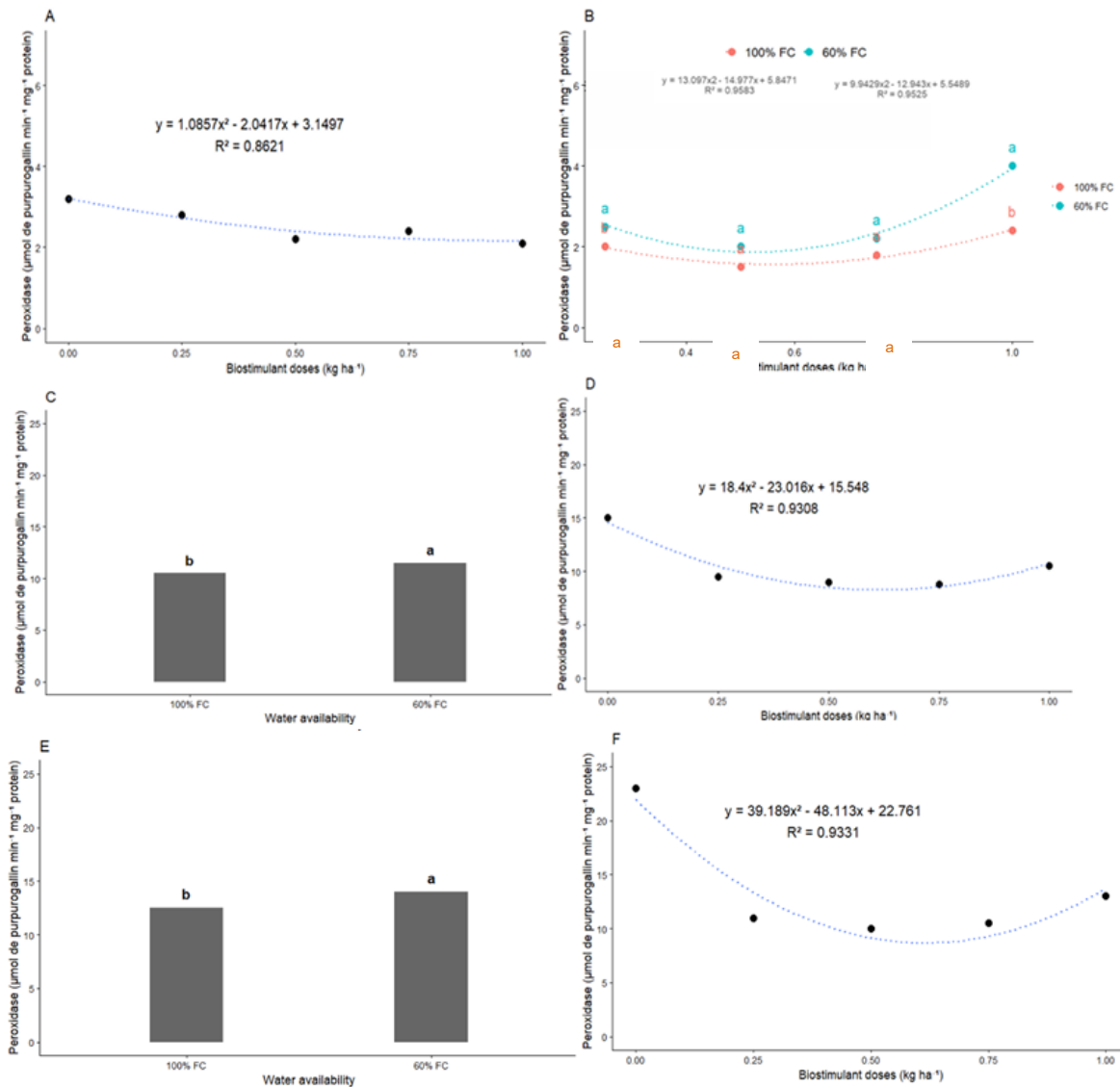


Figure 6. (A) Peroxidase of leaf tissue in maize plants (*Zea mays* L.) as a function of increasing doses of biostimulant at 15 DAP (A) and two water availability levels at 30 DAP (C and D) and peroxidase of root system in maize plants (*Zea mays* L.) subjected to increasing doses of biostimulant and two water availability levels (FC = field capacity) at 15 DAP (B) and 30 DAP (E and F). Equal letters for the same biostimulant dose do not differ from each other (Tukey 5%).

Only the treatment with the biostimulant dose of 1 kg ha⁻¹ led to differences between the water availability levels, increasing the enzymatic activity, probably because the high concentrations of the biostimulant can cause stress in plants. Peroxidases are fundamental in plant metabolism and peroxide oxidation, as they act as hydrogen acceptors, being of paramount importance in the defense metabolism. In Figures 6C and 6D, at 30 DAP in the leaf tissue, the highest rates of enzymatic activity occurred at the water availability of 60% FC, indicating that maize plants increased enzyme activity under conditions of moderate water stress.

Peroxidase data (Figure 6F) were described by a polynomial regression curve, and after performing the derivatives of the equation, the lowest enzymatic activity obtained in the root system was 7.99 μmol of purpurogallin min⁻¹ mg⁻¹ of protein, while in the leaf tissue (Figure 6D) the lowest

enzymatic activity was $8.35 \mu\text{mol}$ of purpurogallin $\text{min}^{-1} \text{mg}^{-1}$ of protein, both at a biostimulant dose of 0.61 kg ha^{-1} . Similar results were found by Shafiq et al. (2021) [42], who observed activity of this enzyme of approximately $8 \mu\text{mol min}^{-1} \text{mg}^{-1}$ protein in the roots and $3 \mu\text{mol min}^{-1} \text{mg}^{-1}$ protein in the shoots, after exposure of maize to aluminum and sodium nitroprusside (SNP).

For catalase in leaf tissue, Figure 7A, there was higher enzymatic activity under the water availability of 60% FC, as observed for peroxidase in Figures 6C and 6E, demonstrating that catalase and peroxidase are induced by stress and participate in the antioxidant and cellular protection mechanisms, and catalase removes H_2O_2 in an energy-efficient manner. Similar results were observed by Magalhães et al. (2016) [43], who obtained higher catalase content in maize under 65% water availability than under 100% water availability.

Figure 7B shows a negative linear behavior of catalase as a function of biostimulant doses, indicating that the biostimulant reduces the specific activity of this enzyme and, consequently, the need for stress recovery.

The enzyme catalase in plants is divided into three classes: class I, which is most of the activity of the enzyme, produced in photorespiration and photosynthetic tissues, contained within peroxisomes and cytosol, participating in the removal of H_2O_2 produced during photorespiration; class II is contained in vascular tissues; and class III is contained in the leaf mesophyll, abundant in young seedlings, but almost absent in advanced stages of development. CAT I of maize is one of them, but in maize CAT III is found in mitochondria [44].

It is possible to observe in Figure 7C that catalase activity in the leaf tissue at 30 DAP was affected by the interaction between biostimulant doses and water availability levels, with a negative linear behavior, as well as at 15 DAP (Figure 7B). These results corroborate those reported by Guimarães et al. (2017) [45], who found a linear decrease in catalase in the 1011-IPA and 2502-IPA varieties of grain sorghum (*Sorghum bicolor* (L.) Moench) irrigated with saline water under semi-arid conditions.

For catalase in the root system (Figures 7D and 7E), the interaction between water availability levels and biostimulant doses was significant, causing a negative linear behavior, as observed in the leaf tissue (Figures 7B and 7C); however, there were higher levels of enzymatic activity, so it can be inferred that the root system suffered greater oxidative stress than the leaf tissue. These results differ from those reported by Fortes et al. (2022) [46], who found higher catalase content in the shoots and reduced the specific activity of catalase using an aqueous extract of *Inga marginata* in maize seedlings.

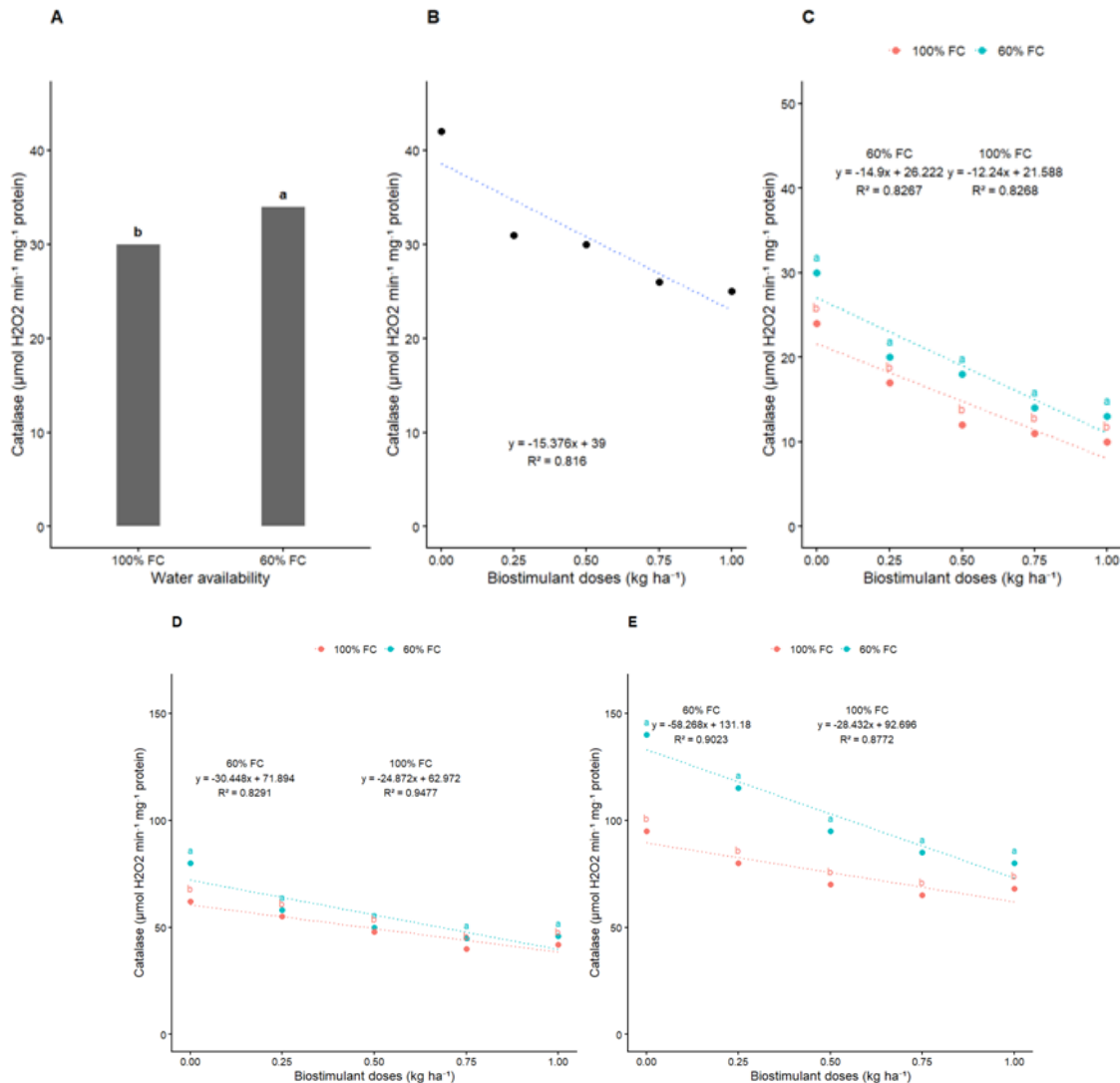


Figure 7. (A) Catalase of leaf tissue in maize plants (*Zea mays* L.) as a function of water availability (FC = field capacity) (A) and increasing doses of biostimulant at 15 DAP (B) and at 30 DAP (C). Catalase of root system in maize plants (*Zea mays* L.) subjected to increasing doses of biostimulant and two water availability levels (FC = field capacity) at 15 DAP (D) and 30 DAP (E). Equal letters for the same biostimulant dose do not differ from each other (Tukey 5%).

The interaction between vegetative and biochemical parameters shows that the increase in root length and volume favored greater absorption of water and nutrients from the soil, which may have reduced the intensity of oxidative damage to the cells, revealed by the lower concentration of MDA at the higher doses of the biofertilizer. In parallel, the activity of the enzymes catalase and peroxidase suggests that the microorganisms acted positively in adjusting the plant's antioxidant system [47].

Therefore, the results obtained in our study demonstrate that the application of biofertilizers based on rhizobacteria and arbuscular mycorrhizal fungi positively modulates the morphological, enzymatic, and biochemical parameters of maize under different levels of water availability, highlighting the importance of dose adjustment and the plant's developmental stage. This evidence applies current knowledge by integrating growth responses and antioxidant metabolism, contributing to the efficient use of bio-inputs under conditions of lower water availability in the Brazilian semi-arid region.

4. CONCLUSION

The use of biostimulants promoted an increase in the growth of the aerial part and root system of the plant at a dosage of 0.75 kg/ha¹ under conditions of lower water availability; however, the ideal dose of biostimulant varies according to the water regime applied. In addition to adjusting antioxidant enzymes, at doses lower than those applied up to 30 days after planting (dap), the use of biostimulants can be a good adaptation strategy to mitigate the effects of lower water availability in corn.

Further work under field conditions is essential to validate this technology in terms of productivity.

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