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The composition of biostimulants applied in seed treatment interferes with the soybean emergence under water deficiency

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ABSTRACT: High physiological quality seeds with high germination and vigor are essential for successful soybean (*Glycine max* L. Merrill) cultivation, ensuring proper establishment and early development. Biostimulants have shown significant potential to enhance soybean performance, especially under environmental stress, such as water deficit, which impairs germination and emergence. This study evaluated the effects of seed treatments with biostimulants and their interaction with insecticide and fungicide on biometrics, nutrition, and biochemical variables during soybean initial development under water deficit conditions. A randomized block design in an 8×2 factorial scheme was employed, comprising eight seed treatments [control (no biostimulant); Imidacloprid and fungicide (Carboxin + Thiram) (IF); three biostimulants (B1, B2, B3); and their combinations with IF] under two water regimes (100% and 50% of pot capacity), with three replicates. Evaluations 21 days after application included germination, emergence speed, root and shoot length, dry matter, and enzymatic activity. Biostimulants, particularly seaweed- and plant extract-based, improved water deficit tolerance, enhancing germination, emergence, biometrics, enzymatic activity (superoxide dismutase, peroxidase, and catalase), and nutrient uptake (P, K, Mg, Cu, Mn, Zn). However, further studies are needed to assess potential adverse interactions between biostimulants, fungicides, and insecticides.
Keywords: *Glycine max* (L.) Merrill; germination; mineral nutrition; water stress.

A composição dos bioestimulantes aplicados em tratamento de sementes interfere na emergência de soja sob deficiência hídrica

RESUMO: Sementes de alta qualidade fisiológica, com elevada germinação e vigor, são essenciais para o sucesso do cultivo de soja (*Glycine max* L. Merrill), garantindo o estabelecimento adequado e o desenvolvimento inicial da cultura. Os bioestimulantes têm demonstrado potencial significativo para melhorar o desempenho da soja, especialmente sob estresse ambiental, como o déficit hídrico, que prejudica a germinação e a emergência. Este estudo avaliou os efeitos do tratamento de sementes com bioestimulantes e sua interação com inseticidas e fungicidas na biometria, nutrição e variáveis bioquímicas durante o desenvolvimento inicial da soja sob déficit hídrico. Foi utilizado delineamento em blocos causalizados, em esquema fatorial 8×2, composto por oito tratamentos de sementes [controle (sem bioestimulante); inseticida Imidacloprido e fungicida (Carboxina + Tiram) (IF); três bioestimulantes (B1, B2, B3); e suas combinações com IF] sob dois regimes hídricos (100% e 50% da capacidade de vaso), com três repetições. As avaliações realizadas 21 dias após a aplicação incluíram germinação, velocidade de emergência, comprimento de raiz e parte aérea, massa seca e atividade enzimática. Bioestimulantes, especialmente à base de extratos de algas e plantas, aumentaram a tolerância ao déficit hídrico, promovendo melhorias na germinação, emergência, biometria, atividade enzimática (superóxido dismutase, peroxidase e catalase) e absorção de nutrientes (P, K, Mg, Cu, Mn, Zn). No entanto, estudos adicionais são necessários para avaliar possíveis interações negativas entre bioestimulantes, fungicidas e inseticidas.
Palavras-chave: *Glycine max* (L.) Merrill; germinação; nutrição mineral; estresse hídrico.

1. INTRODUCTION

Ensuring optimal performance for soybean crops (*Glycine max* L. Merrill) relies heavily on using seeds with high physiological quality and high germination rates and vigor. These seeds play a crucial role in establishing robust initial crop growth in the field, paving the way for elevated production levels (KRZYZANOWSKI et al., 2018).

One of the primary challenges impacting crop productivity stems from abiotic factors, such as water scarcity in certain agricultural regions, which detrimentally affect seed germination (DU et al., 2020; BEGUM et al., 2022).

Water availability significantly influences seed germination, crop growth, and development (TAIZ et al., 2017). According to Pandey (2015), germination and emergence stages are particularly susceptible to the effects of water deficit, requiring a minimum water content for seed establishment. Insufficient water availability during this critical phase can hinder germination, as it is essential for initiating and sustaining seed metabolism (PASZKIEWICZ et al., 2017).

Reductions in germination potential, emergence, and initial development have been observed in several crops under water deficit conditions, including wheat (Khaeim et al., 2022), cotton (Rehman et al., 2022), rapeseed (Xu et al., 2022), spinach (Xu; Leskovar, 2015), lettuce (Lucini et al., 2015), soybean (Ogunkanmi et al., 2022), cucumber (Campobenedetto et al., 2020), sweet corn (Carmo et al., 2021), and corn (AMARO et al., 2023). As a result of sowing under unfavorable environmental conditions or the use of low-vigor seeds, there was a 17% reduction in pod numbers per plant and yield losses ranging from 24% to 35% in soybean crops. These losses are typically attributed to poor plant establishment and the redirection of plant energy to mitigate stress conditions (FRANÇA-NETO et al., 2016).

Seed treatment (ST) comprises applying chemical or biological products, inoculants, macro- and micronutrients, biostimulants, and polymers to seeds (MEDEIROS et al., 2023). The primary aim is to protect seeds against pests and microorganisms between sowing and germination while enhancing efficiency, resistance, and adaptation to environmental adversities (MAJKOWSKA-GADOMSKA et al., 2017). ST typically requires minimal amounts of active ingredients per production area and has been shown to improve germination and growth compared to untreated seeds (ROUPHAEL; COLLA, 2018). Studies by Lauxen et al. (2010) and Cunha et al. (2015) support the efficacy of seed treatment, ensuring adequate plant populations even under unfavorable soil and climatic conditions during sowing, germination, and emergence while preserving genetic, physiological, and sanitary qualities and exerting beneficial effects on crop growth and initial development.

Biostimulants are natural or synthetic products that can be applied directly to plants or as seed treatment. They aim to alter their vital processes and promote improvements in parameters such as production and quality (SILVA et al., 2018). They have a similar action to the groups of plant hormones and favor the balance of these hormones in addition to the expression of the genetic potential of the plants, and act in the formation of enzymes, chlorophyll and in the transport and storage of nitrogen (TAIZ et al., 2017; FRASCA et al., 2020). They are complex mixtures derived from raw materials of different origins and have a broad spectrum of biological activities (BULGARI et al., 2019). Biostimulants have plant regulators, amino acids, polysaccharides, fatty acids, steroids, and polyamines in their composition (GUPTA et al., 2021).

In addition, products that contain nutrients in their formulation, act at different stages of plant development, and participate in fundamental physiological processes, which is a strategy for ensuring proper crop establishment, have also been considered in this category (ROSA et al., 2021; REPKE et al., 2022).

When applied to seeds, biostimulants stimulate root production and growth, helping in the accelerated recovery of seedlings, especially in unfavorable conditions such as soils with low fertility and water deficit, acting on the hormonal and nutritional increase (MANDAL et al., 2023).

Andrade et al. (2018) observed significant plant growth, dry mass, and leaf area enhancements when utilizing biostimulants composed of *Ascophyllum nodosum*, organic carbon, manganese, and zinc in treating corn seeds. They recommended the incorporation of these biostimulants to optimize initial crop development. Similarly, other studies underscore the undeniable advantages associated with biostimulant application, particularly those derived from algae extracts, humic and fulvic acids, microorganisms, amino acids, derivatives of microbial fermentation of cereals, essential oils, and formulations enriched with nutrients, all contributing to improved crop growth and development (VAN OOSTEN et al., 2017).

According to Campobenedetto et al. (2020), biostimulants can be crucial in supporting plant physiological maintenance. Under stress conditions, plant cells are susceptible to damage from free radicals. However, biostimulants can mitigate these radicals' toxicity, bolstering the plant's defense mechanisms and enhancing antioxidant activity levels.

Plants with elevated antioxidant activity exhibit improved root and shoot development, maintaining optimal leaf water content (ZHANG et al., 2020; WANG et al., 2021). Examining the oxidative system's involvement is vital, considering the interplay between water stress and early crop development. For instance, observing the increase of antioxidant activity facilitated by enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Rahman et al., 2021) is crucial.

Moreover, nutrients significantly influence plant physiology, serving as precursors for various secondary metabolites. Bradácová et al. (2016) suggest that the protective effects conferred by biostimulant application may stem from the micronutrients within their composition, acting as cofactors for enzymes. Essential ions such as magnesium and manganese are particularly vital for the proper functioning of enzymes like SOD (IGHODARO; AKINLOYE, 2017).

Given the diverse compositions and mechanisms of action of recommended biostimulants for soybean seed treatment, this study posits that varying biostimulant formulations elicit distinct responses in antioxidant enzyme activity and nutrient dynamics during the soybean germination process under water stress.

Therefore, this study investigated the impact of different biostimulant compositions, either alone or combined with insecticide and fungicide seed treatments, on soybean seed germination, antioxidant enzyme activity, and seedling nutrition under water deficiency conditions.

2. MATERIAL AND METHODS

2.1. Description of the experimental area and plant material

The experiment was carried out in September 2018 in a greenhouse at the Department of Crop Production of the School of Agricultural Sciences of São Paulo State University (UNESP), located in Botucatu, SP, Brazil (22°53'09" S, 48°26'42" W, and 840 m asl).

The soybean cultivar chosen for the experiment was M5917IPRO (Agro Bayer, Brazil). Pots with a capacity of 12 liters (measuring 25 cm in height and 30 cm in diameter) were filled with soil obtained from the arable layer (20 cm deep), classified as dystrophic red Latosol. The physicochemical properties of the soil substrate are detailed in Table 1. Based on the chemical analysis, no soil pH correction was deemed necessary.

Table 1. Soil granulometric parameters, chemical attributes, and micronutrient content of the experimental plots in 0 to 20 cm depth. Botucatu, SP – 2018.
Tabela 1. Parâmetros granulométricos, atributos químicos e teor de micronutrientes do solo das parcelas experimentais na profundidade de 0 a 20 cm. Botucatu, SP – 2018.

Granulometry	$g\ kg^{-1}$
Clay (<0.002 mm)	611
Silt (0.053–0.002 mm)	179
Total sand	210
Coarse sand (2.00–0.210 mm)	70
Fine sand (0.210–0.053 mm)	140
Chemical attributes	
pH ($CaCl_2$)	5.4
OM ($g\ kg^{-1}$)	25
Presin ($mg\ kg^{-1}$)	19
K ($mmol_c\ kg^{-1}$)	7
Ca ($mmol_c\ kg^{-1}$)	36
Mg ($mmol_c\ kg^{-1}$)	15
H+Al ($mmol_c\ kg^{-1}$)	31
Al^{3+} ($mmol_c\ kg^{-1}$)	0
SB ($mmol_c\ kg^{-1}$)	58
CEC ($mmol_c\ kg^{-1}$)	89
V (%)	65
m (%)	0
S–SO_4 ($mg\ kg^{-1}$)	5
Micronutrient	$mg\ kg^{-1}$
Cu (DTPA)	10.6
Fe (DTPA)	12
Zn (DTPA)	2.0
Mn (DTPA)	32.8
B (Hot-water)	0.22

pH = hydrogenionic potential; OM = organic matter; SB = sum of bases; CEC = cation exchange capacity; V% = base saturation; m% = aluminum saturation.

After filling, the pots were placed on benches and left to dry for a week. Subsequently, the substrates were saturated with water, and measurements were taken to determine the pot capacity (PC) and establish the parameters for water supplementation treatments required for the experiment (50% and 100% of PC).

2.2. Experimental design

The experimental design was a randomized block design 8 × 3 factorial scheme, consisting of eight seed treatments: control – without biostimulant application; Imidacloprid insecticide (700 $g\ kg^{-1}$) and Carboxin (200 $g\ L^{-1}$) + Thiram (200 $g\ L^{-1}$) fungicide (IF); biostimulants B1, B2, and B3 applied at a rate of 250 mL commercial product (cp) per 100 kg seeds alone and in combination with Imidacloprid insecticide (700 $g\ kg^{-1}$) and Carboxin (200 $g\ L^{-1}$) + Thiram (200 $g\ L^{-1}$) fungicide. Additionally, two watering regimes were implemented: 100% and 50% of the established pot capacity (PC) since sowing, each with three replicates. The treatment details and their respective compositions can be found in Table 2.

For each seed treatment, 150 grams of seeds were treated with biostimulants and their combinations, with an application spray volume of 5 $mL\ kg^{-1}$ of seeds. The control underwent the same procedure but was only done with distilled water. Fifty seeds per pot were sown.

The amount of water placed in the pots during the implementation of water regimes was determined through a series of steps. Initially, the pots were saturated with water and weighed, followed by draining for 12 hours until reaching pot capacity. Subsequently, a new weighing was conducted. The amount of water required for each treatment was calculated using Equation 1.

$$A = Pcc - Pp \qquad (01)$$

where: A = amount of water to be added to the pot (in mL); Pcc = initial weight of the pot with soil moisture at the PC or 50% of the PC, in grams; Pp = daily weight of the pot, in grams.

The pots were weighed and rehydrated daily throughout the experimental period to maintain the required moisture levels. The experiment spanned 21 days, during which destructive assessments were conducted on 48 of the 96 pots comprising the experiment - two per experimental plot and four per treatment (two subjected to water deficiency and two to regular hydration).

The environmental conditions within the greenhouse were monitored, with a maximum temperature recorded at 25.02 ± 4.3 ºC and a minimum temperature of 13.67 ± 2.4ºC. The minimum relative humidity was measured at 50.89 ± 19.4 mm, while the maximum relative humidity reached 93.44 ± 8.7 mm. These data were sourced from the Agrometeorological and Hydrological Portal of the State of São Paulo (Ciiagro).

2.3. Biometric variables

Daily assessments of emergence were conducted from the day of sowing until the number of emerged seedlings was stable. Seedlings emerged when their cotyledons were above the soil surface and their hypocotyl angle was equal to or greater than 90 degrees (FARIAS et al., 2007).

The final emergence percentage (%EM) was calculated by summing the number of emerged seedlings in each treatment block. The emergence speed index was determined using the formula proposed by Maguire (1962) (Equation 2).

$$ESI = \frac{E1+}{N1+}\frac{E2+\cdots}{N2+\cdots}\frac{En}{Nn} \qquad (02)$$

where: ESI = emergency speed index; E1, E2, En = number of normal seedlings at the first, second, and last count; N1, N2, and Nn = number of days from sowing to first, second, and last count.

At 21 days after sowing (DAS), biometric evaluations, including shoot length (SL), root length (SR), shoot dry matter (SDM), and root dry matter (RDM), were performed. Additionally, the emergence percentage and emergence speed index were assessed.

To determine SDM and RDM, the fresh mass of shoots and roots was initially measured after plant cleaning. It involved a destructive evaluation of one pot within each plot and then washing the plants under running water to remove soil particles and surface debris. The shoot and root samples were then sectioned at the neck and weighed using a digital analytical scale (ELC-6/15/30, Balmak, Santa Bárbara do Oeste, SP, Brazil). Subsequently, the samples were placed in paper bags and dried in a forced air circulation oven at 65°C for 72 hours. Finally, SDM and RDM were determined using the same digital analytical balance.

Table 2. Description of the treatments, compositions, and product doses (mL 100 kg of seeds^{-1}).
Tabela 2. Descrição dos tratamentos, composições e doses dos produtos (mL 100 kg de sementes^{-1}).

Treatments	Description	Dose (cp 100 kg of seeds^{-1})
Control	Without the application of biostimulant	-
IF	Imidacloprid + (Carboxin + Thiram)	250 mL + 250 mL
B1	18 g L^{-1} nitrogen 60 g L^{-1} potassium oxide 0.96 g L^{-1} boron (B) 12 g L^{-1} manganese (Mn) 4.8 g L^{-1} iron (Fe) 12 g L^{-1} sulphur (S) 24 g L^{-1} zinc (Zn) 42 g L^{-1} total organic carbon 0.031% gibberellic acid and indoleacetic acid 0.0083% zeatin combined with hydrolyzed plant extracts	300 mL
B2	50 g L^{-1} nitrogen (N) 12.5 g L^{-1} cobalt (Co) 62.5 g L^{-1} molybdenum (Mo) 75 g L^{-1} total organic carbon complexed with *Ascophyllum nodosum* algae extract and amino acids	250 mL
B3	4.0% nitrogen (N) 5.0% P_2O_5 5.0% K_2O 0.1% molybdenum (Mo) 0.1% zinc (Zn) 0.07% manganese (Mn) 0.04% iron (Fe) Mixer mineral fertilizer with 30% *Ascophyllum nodosum* algae extract enriched with 5% amino acids	400 mL
B1 + IF	-	300 mL+ 250 mL + 250 mL
B2 + IF	-	250 mL + 250 mL + 250 mL
B3 + IF	-	400 mL + 250 mL + 250 mL

cp = comercial product.

2.4. Nutritional variables

After determining the dry matter, the plant samples (including stems, leaves, and roots) were sieved through a 40-mesh stainless steel screen. Subsequently, laboratory analysis was conducted to assess the contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), and boron (B).

For N concentration determination in plant tissues, a sulfuric acid (H_2SO_4) digestion method followed by the semi-micro-Kjeldahl method was employed (MALAVOLTA et al., 1997). Other nutrients were analyzed using nitric acid (HNO_3) and perchloric acid ($HClO_4$) solutions. P, S, and B were quantified using colorimetry from the digested solution, while K, Ca, Fe, Cu, Mg, Mn, and Zn were determined using atomic absorption spectrophotometry (MALAVOLTA et al., 1997).

2.5. Biochemical variables

Ten plants were collected from each pot to perform biochemical evaluations. Each plant was sectioned at the stem upon harvest, and the leaves were promptly submerged in liquid nitrogen before being transferred to an ultra-freezer set at -80°C to preserve the samples until further biochemical analyses.

Soluble protein content was determined using 500 mg of leaf tissue, macerated in 2 mL of 0.1 M phosphate buffer (pH 6.7), and 200 mg of polyvinylpolypyrrolidone (PVPP). The homogenized tissue was then centrifuged at 5000 × g for 10 minutes at 4°C, and the resulting supernatant was collected and stored in an ultrafreezer at -80°C. Subsequently, a 50 µL aliquot of the supernatant dilution (prepared by mixing 250 µL of supernatant with 750 µL of deionized water) was combined with 950 µL of Bradford's solution. Bradford's solution was prepared by dissolving 100 mg of Coomassie brilliant blue dye G-250 in 50 mL of 95% ethyl alcohol, 100 mL of 85% phosphoric acid, and 850 mL of deionized water. This solution was filtered twice using qualitative filter paper. The absorbance readings were performed in a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan) at 595 nm, 15 minutes after stirring the sample. Soluble protein concentrations were calculated based on a standard bovine serum albumin (BSA) curve at a concentration of 1 mg mL^{-1} and expressed in mg g^{-1} fresh matter (FM) (BRADFORD, 1976). It's worth mentioning that this determination was conducted to obtain a substrate for subsequent enzymatic analyses.

To obtain leaf extract to analyze antioxidant enzyme activities, 300 mg of plant tissue was homogenized in 2.0 mL of 0.1 M potassium phosphate buffer (pH 6.8) containing 200 mg of PVPP. The homogenized tissue was then centrifuged at 10,000 ×g for 10 minutes at 4°C, and the resulting supernatant was collected and stored in an ultra-freezer at –80°C. The activity of the enzyme superoxide dismutase (SOD) was determined using a modified method described by Giannopolitis; Ries (1977). In this assay, 50 µL of the ultra-freezer-packed extract was added to 2950 µL of the working solution, which comprised 50 mM potassium phosphate buffer (pH 7.8), 0.1 µM EDTA, 75 µM nitro tetrazolium blue chloride (NBT), 13 mM methionine, and 2 µM riboflavin. The reaction was conducted in 3 mL capacity ELISA plates, placed in a dark room with a 15 W fluorescent light for 10 minutes. After illumination, the formation of formazan blue, resulting from NBT photoreduction, was measured in a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan) at 560 nm. Tubes used as blanks received 3 mL of the working solution, one kept in the dark (to zero the equipment) and the other exposed to light (for 100% photoreduction). One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction. The results were expressed in units of SOD mg^{-1} protein.

To determine catalase enzyme activity (CAT), 50 μL of the ultrafreezer-packed extract was mixed with 950 μL of 50 mM potassium phosphate buffer (pH 7.0) containing 12.5 mM H_2O_2. The reaction was monitored in a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan) at 240 nm, with the absorption variation measured over 60 seconds. Enzyme activity was calculated using the molar extinction coefficient ε= 39.4 mM^{-1} cm^{-1} and expressed in kat μg^{-1} protein (PEIXOTO et al., 1999).

Peroxidase enzyme (POD) activity was determined by combining 100 μL of the ultrafreezer-packed extract with 4.9 mL of 25 mM potassium phosphate buffer (pH 6.8) containing 20 mM Pyrogallol and 20 mM H_2O_2. After a 1-minute incubation, the reaction was stopped with 0.5 mL of H_2SO_4 (15%). The absorbance readings were performed in a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan) at 420 nm. Enzyme activity was calculated using the molar extinction coefficient ε= 2.47 mM^{-1} cm^{-1} and expressed in kat μg^{-1} protein (PEIXOTO et al., 1999).

2.6. Statical analysis

Data underwent the Shapiro-Wilk normality test, and after confirming normal distribution, analysis of variance was conducted using the F-test. Subsequently, means were compared using the Student's test at a significance level of 5%, using the AgroEstat software, version 1.1, Jaboticabal, SP, Brazil (BARBOSA; MALDONADO, 2015). Additionally, standard deviations were calculated based on three replicates per treatment.

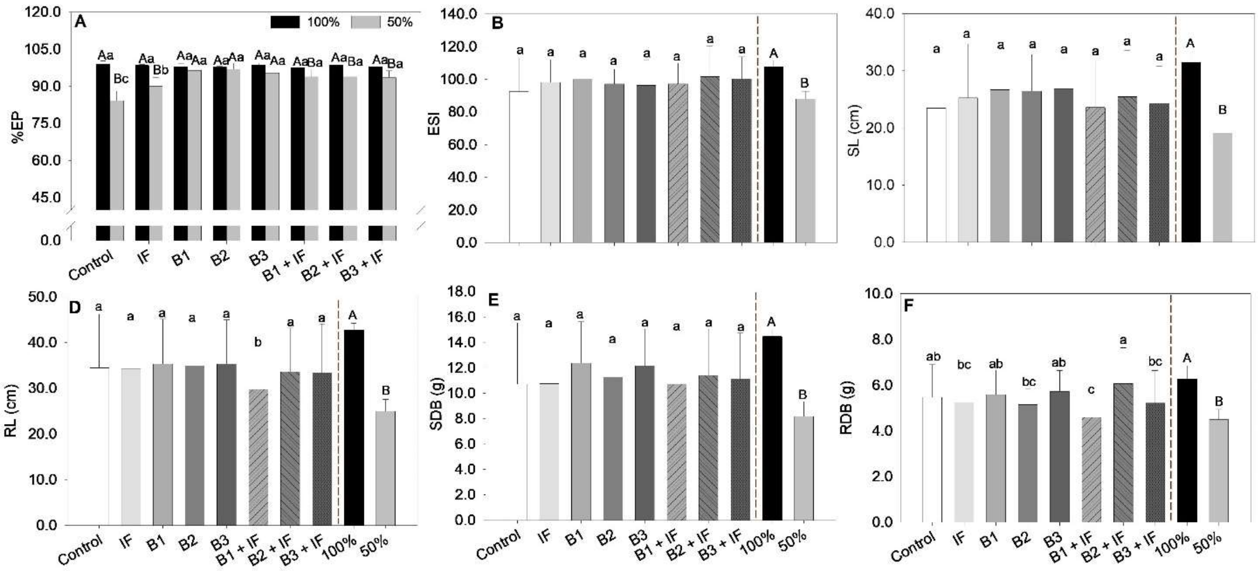
3. RESULTS

3.1. Biometric variables

There was an interaction between seed treatments and water regimes on the emergency percentage variables (%EM). Regarding root dry biomass (RDB) and root length (RL), both factors individually influenced the outcomes. However, shoot dry biomass (SDB), shoot length (SL), and emergency speed index (ESI) were affected by water regimes.

Underwater deficiency (50% PC), B1, B2, and B3 provided higher %EM values (96.4%, 96.9%, and 95.6%, respectively) (Figure 1A). However, combining these biostimulants with fungicide and insecticide decreased the %EM compared to 100% PC, indicating potential incompatibility of the compositions. The most significant reduction induced by water deficiency was observed in control (14.9%), followed by the standard seed treatment IF (8.6%). Conversely, under 100% PC, no significant differences were verified among the treatments, with an average %EM of 98.3%, which represented, on average, 5.6% more than the treatments under water deficiency.

Water deficiency similarly affected the ESI across all soybean seed treatments, resulting in an average of 87.97, representing an average reduction of 22.6% compared to ESI under 100% PC (Figure 1B). Despite the significant reduction induced by water deficiency in all treatments, the lowest reduction was verified under B2 (7.5%), while the highest reductions were observed under control (23.8%) and B2 + IF treatments (24.6%).



Treatments: control; insecticide and fungicide (Imidacloprid + Carboxin + Thiram) (IF); B1 (18 g L^{-1} N, 60 g L^{-1} potassium oxide, 0.96 g L^{-1} B, 12 g L^{-1} Mg, 4.8 g L^{-1} Fe, 12 g L^{-1} S, 24 g L^{-1} Zn, 42 g L^{-1} total organic carbon, 0.031% gibberellic acid and indolacetic acid, 0.0083% zeatin combined with hydrolyzed plant extracts); B2 (50 g L^{-1} N, 12.5 g L^{-1} Co, 62.5 g L^{-1} Mo, 75 g L^{-1} total organic carbon, complexed with *Ascophyllum nodosum* algae extract and amino acids); B3 (Phosphorus chelated cobalt, microencapsulated molybdenum, humic substances, mixed mineral fertilizer with 30% *Ascophyllum nodosum* algae extract enriched with 5% amino acids); B1 + IF; B2 + IF; B3 + IF. Means followed by the same lowercase letter among treatments and within water regimes, and uppercase within each treatment and between water regimes indicate no significant difference according to Student's t-test **($p \leq 0.05$)**.

Figure 1. Emergence percentage (%EM), emergence speed index (ESI), shoot length (SL), root length (RL), shoot dry biomass (SDB), and root dry biomass (RDB).

Figura 1. Porcentagem de emergência (%EM), índice de velocidade de emergência (ESI), comprimento da parte aérea (SL), comprimento da raiz (RL), biomassa seca da parte aérea (SDB) e biomassa seca da raiz (RDB).

Water deficiency significantly impacted SL in all treatments, leading to an average decrease of 39.8% compared to 100% PC (Figure 1C). Under 100% PC, the average SL of all treatments was 31.53 cm. Despite the substantial decrease under water deficiency, B1, B2, and B3 provided the lowest reductions at 31.8%, 35.3%, and 27.6%, respectively, resulting in SL of 21.64 cm, 20.78 cm, and 22.57 cm. In comparison, the most significant differences were found under IF (49.9%), control (47.3%), and B1+ IF (45.6%), resulting in SL of 16.86 cm, 16.21 cm, and 16.62 cm, respectively.

Under 100% PC, the average RL of all seed treatments was 42.72 cm, whereas under 50% PC, it decreased to 25.03 cm, resulting in an average reduction of 40.5%. The control exhibited the highest reduction (47.2%), while B1 + IF and B2 showed the most minor decreases (38.7% and 37.7%, respectively) (Figure 1D).

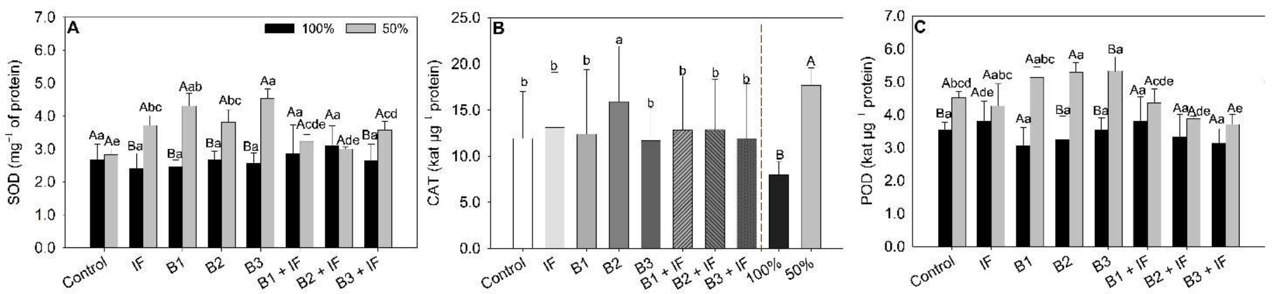
Additionally, under adequate water conditions, the average SDB of soybean seedlings was 14.44 g. Water deficiency reduced SDB accumulation in all seed treatments, with an average reduction of 43.4%. However, B1, B2, and B3 provided the most minor decreases (35.6%, 32.8%, and 35.2%, respectively), while the control and IF exhibited the most significant reductions (47.3% and 49.9%, respectively) (Figure 1E).

RDB was also significantly affected by water deficiency across all seed treatments. Under 100% PC, the average RDB was 6.26 g, whereas under 50% PC, it decreased to 4.50 g, representing an average reduction of 28.1%. B2 + IF provided the highest RDB under adequate water conditions, at 7.13 g, differing from the IF, B2, B1 + IF, and B3 + IF (Figure 1F). Similarly, B2 + IF also resulted in the highest RDB underwater deficiency (5.01 g). The most minor reductions in RDB were observed under B2, B3, and B1 + IF, at 17.1%, 18.6%, and 24.3%, respectively, while the most significant reductions were observed under the control (36.4%) and the B3 + IF (38.3%).

3.2. Biochemical variables

For SOD and POD enzymes, an interaction between seed treatments and water regime was observed, unlike CAT, which was influenced independently by both factors.

The mean values for SOD were higher under water deficiency (3.63 mg^{-1}) compared to the adequate water regime (2.68 mg^{-1}), representing an average increase of 23.8% (Figure 2A). Under 100% PC, the highest SOD activities were observed in B2 and B1 + IF, 3.10 mg^{-1} and 2.87 mg^{-1}, respectively, although they did not statistically differ from the other treatments. Underwater deficiency, B3 and B1 provided the highest values, 4.53 mg^{-1} and 4.31 mg^{-1}, respectively, also exhibiting the highest increases compared to the adequate water regime, at 43% and 43.3%. On the other hand, the control had the lowest value (2.83 mg^{-1}), only 5.9% higher than the adequate water regime, and did not differ from B1 + IF, B2 + IF, and B3 + IF. Only B2 + IF did not lead to an increase in SOD activity under water deficiency.



Treatments: control; insecticide and fungicide (Imidacloprid + Carboxin + Thiram) (IF); B1 (18 g L^{-1} N, 60 g L^{-1} potassium oxide, 0.96 g L^{-1} B, 12 g L^{-1} Mg, 4.8 g L^{-1} Fe, 12 g L^{-1} S, 24 g L^{-1} Zn, 42 g L^{-1} total organic carbon, 0.031% gibberellic acid and indolacetic acid, 0.0083% zeatin combined with hydrolyzed plant extracts); B2 (50 g L^{-1} N, 12.5 g L^{-1} Co, 62.5 g L^{-1} Mo, 75 g L^{-1} total organic carbon, complexed with *Ascophyllum nodosum* algae extract and amino acids); B3 (Phosphorus chelated cobalt, microencapsulated molybdenum, humic substances, mixed mineral fertilizer with 30% *Ascophyllum nodosum* algae extract enriched with 5% amino acids); B1 + IF; B2 + IF; B3 + IF. Means followed by the same lowercase letter among treatments and within water regimes and uppercase within each treatment and between water regimes indicate no significant difference according to Student's t-test **($p \leq 0.05$).**

Figure 2. Activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) enzymes in soybean plants at 21 DAA.
Figura 2. Atividade das enzimas superóxido dismutase (SOD), catalase (CAT) e peroxidase (POD) em plantas de soja aos 21 DAA.

Water restriction resulted in higher CAT activity (17.70 kat µg^{-1}) compared to the 100% PC (7.97 kat µg^{-1}), representing a decrease of, on average, 55% (Figure 2B). B2 exhibited the highest activity of this enzyme, at 20.83 kat µg^{-1} and 10.97 kat µg^{-1}, under 50% and 100% PC, respectively. All seed treatments led to increases in CAT activity, with the highest observed in B1 (67.1%) and B3 + IF (62.1%), while the lowest was in B2 (47.3%) and B3 (40.4%).

Water deficit provided an average increase of 1.13 mg^{-1} in POD concentration, or 23.4%, compared to the adequate water regime (100% PC) (Figure 2C). Under 100% PC, the average of treatments was 3.43 mg^{-1}, with no significant differences between each other. Under 50% PC, B3 exhibited the highest value for POD (5.33 mg^{-1}), not differing from B1 and B2 (5.13 mg^{-1} and 5.30 mg^{-1}, respectively). Conversely, the lowest values of POD activity were found under IF, B1 + IF, B2 + IF, and B3 + IF, indicating a likely incompatibility of the compositions.

3.3. Nutritional variables

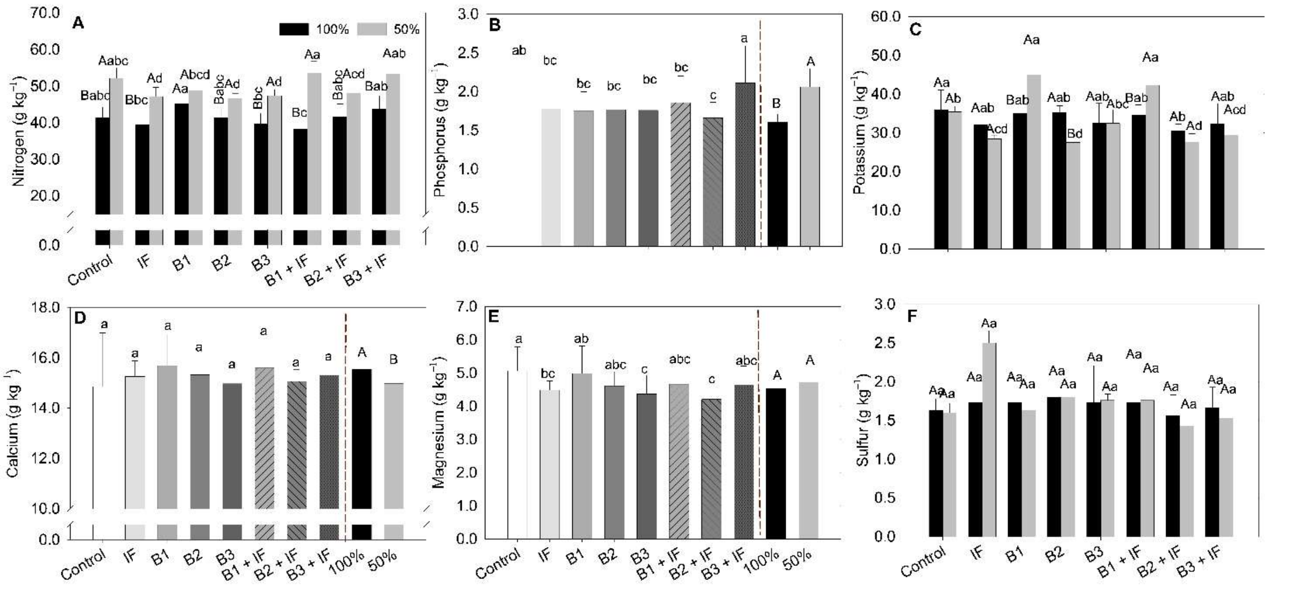
3.3.1. Macronutrients and micronutrients in the shoot

Regarding macronutrients, an interaction was observed between the factors evaluated for N and K; isolated effects of the two factors were observed for P. Seed treatments exhibited effects on Mg, while water regimes influenced Ca, and no effect was observed on S.

For micronutrients, the factors for Cu and Fe interacted. For Mn and Zn, the two factors alone affected them, while B was not affected.

Shoot N content had an overall average of treatments around 49.64 g kg^{-1} under 100% PC and 41.35 g kg^{-1} under 50% PC. Water deficiency, in general, resulted in N content

20.3% lower than adequate hydration. Under 100% PC, N content provided by B1 was the highest (45.29 g kg^{-1}), while B1 + IF was the lowest (38.25 g kg^{-1}). B1 also showed the slightest difference in N content between the water regimes (7.9%). Conversely, under 50% PC, the highest N content was observed under B1 + IF (53.52 g kg^{-1}), followed by B3 + IF (53.39 g kg^{-1}), and control (52.21 g kg^{-1}) (Figure 3A). The highest increments in N content were observed in B1+ IF (15.27 g kg^{-1}), showing a superiority of 39.9%.



Treatments: control; insecticide and fungicide (Imidacloprid + Carboxin + Thiram) (IF); B1 (18 g L^{-1} N, 60 g L^{-1} potassium oxide, 0.96 g L^{-1} B, 12 g L^{-1} Mg, 4.8 g L^{-1} Fe, 12 g L^{-1} S, 24 g L^{-1} Zn, 42 g L^{-1} total organic carbon, 0.031% gibberellic acid and indolacetic acid, 0.0083% zeatin combined with hydrolyzed plant extracts); B2 (50 g L^{-1} N, 12.5 g L^{-1} Co, 62.5 g L^{-1} Mo, 75 g L^{-1} total organic carbon, complexed with *Ascophyllum nodosum* algae extract and amino acids); B3 (Phosphorus chelated cobalt, microencapsulated molybdenum, humic substances, mixed mineral fertilizer with 30% *Ascophyllum nodosum* algae extract enriched with 5% amino acids); B1 + IF; B2 + IF; B3 + IF. Means followed by the same lowercase letter among treatments and within water regimes, and uppercase within each treatment and between water regimes indicate no significant difference according to Student's t-test **($p \leq 0.05$)**.

Figure 3. Shoot macronutrient content of soybean plants at 21 DAA.
Figura 3. Teores de macronutrientes na parte aérea de plantas de soja aos 21 DAA.

Water deficiency also led to higher shoot P contents, with an average increase of 29.4% (Figure 3B). Under 100% PC, the average P concentration in soybean seedlings was 1.60 g kg^{-1}; under water deficiency, the average value was 2.06 g kg^{-1}. Under 100% PC, B3 + IF resulted in the highest P content (1.72 g kg^{-1}), while IF showed the lowest value (1.38 g kg^{-1}). Under 50% PC, B3 + IF showed the highest shoot P content (2.50 g kg^{-1}), representing an increase of 0.80 g kg^{-1} (57.6%) compared to the same treatment under 100% PC. Conversely, B2 + IF exhibited the lowest P content (1.76 g kg^{-1}), representing only a 12.9% increase over 100% PC.

Regarding shoot K content, the means under adequate water regimes and water deficiency were similar (33.56 g kg^{-1} and 33.52 g kg^{-1}, respectively) (Figure 3C). However, under 100% PC, the highest content was observed in the control treatment (36.06 g kg^{-1}), while the lowest was found with the application of B2 + IF (30.61 g kg^{-1}). Under 50% PC, B1 and B1 + IF showed increases of 28.6% and 22.1% in K contents, resulting in 44.99 g kg^{-1} and 42.28 g kg^{-1}, respectively.

The average shoot Ca content of soybean seedlings under an adequate water regime was 15.53 g kg^{-1}, and the biostimulants did not affect this nutrient content (Figure 3D). Water deficiency led to lower Ca accumulation in all treatments except for B1, with an increase of 5.5%. Water deficiency caused the lowest shoot Ca content in the control (13.90 g kg^{-1}), representing a decrease of 13.7% compared to the control under 100% PC.

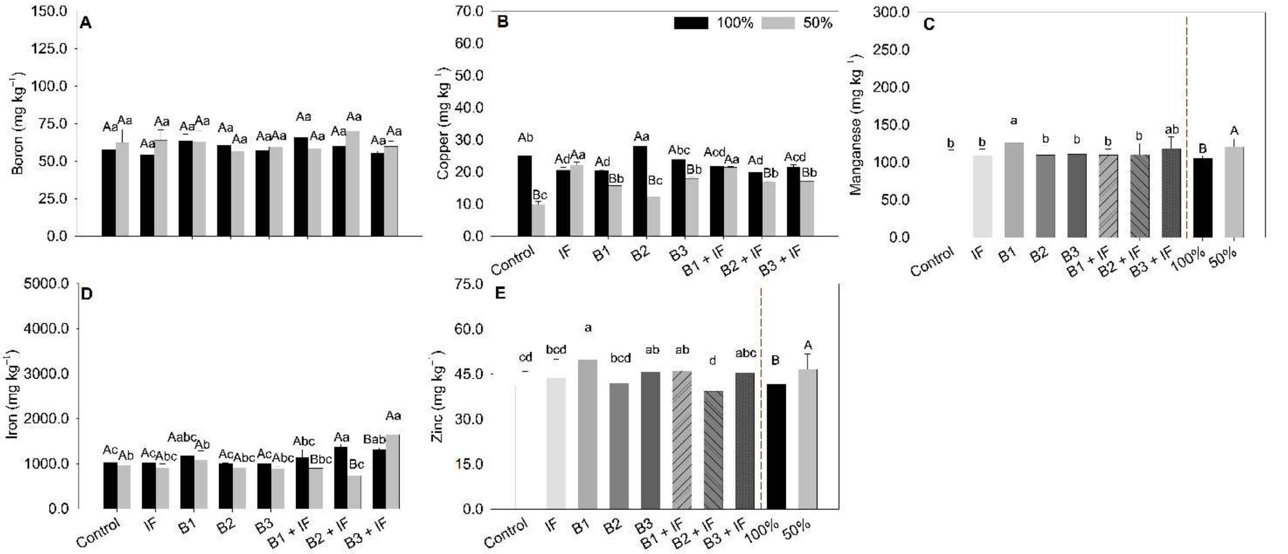
Shoot Mg contents were not influenced by the water regimes, resulting in an average of 4.63 g kg^{-1}. Underwater deficiency, the highest Mg content was observed in the control (5.07 g kg^{-1}), followed by B1 (4.99 g kg^{-1}) and B1 + IF (4.66 g kg^{-1}). These values exceeded those observed for these treatments under an adequate water regime by 16%, 22.7%, and 9.2%, respectively (Figure 3E).

Water regimes and seed treatments did not affect shoot S and B contents, resulting in average contents of 1.73 g kg^{-1} (Figure 3F) and 60.49 g kg^{-1} (Figure 4A).

Shoot Cu contents were affected by water deficiency in seed treatments, except for IF and B1 + IF (Figure 4B). Under 100% PC, the average Cu content was 22.72 mg kg^{-1}, whereas under 50% PC, it decreased to 16.69 mg kg^{-1}, marking a reduction of 26.5%. B2 displayed the highest Cu content under an adequate water regime (28.08 mg kg^{-1}). However, water deficiency led to one of the most significant reductions (56%). Underwater deficiency, IF and B1 + IF provided the highest values (22.19 mg kg^{-1} and 21.45 mg kg^{-1}), respectively. The control exhibited the most substantial reduction due to water deficiency (60.5%).

Similarly to other nutrients, shoot Mn content was influenced by the water regime (Figure 4C). Under 100% PC, the average Mn content was 105.62 mg kg^{-1}; under 50% PC, it increased to 120.98 mg kg^{-1}, marking an average increase of 14.5%. Underwater deficiency, the highest levels were observed under B1 (143.28 mg kg^{-1}) and B3 + IF (130.18 mg kg^{-1}), representing increases of 31.4% and 22.2%, respectively, compared to the adequate water regime. The slightest difference was observed in the control treatment, which was only 1.6% higher under water deficiency.

Similar values were generally observed in the two water regimes for shoot Fe contents, except for B1, B2, and B3 + IF (Figure 4D). Under 100% PC, the mean was 1132.94 mg kg^{-1}, and under 50% PC, 1005.70 mg kg^{-1}, representing a reduction of 11.2%. Underwater deficiency, there was a reduction of 20.7% and 46.5% in Fe contents in B1 + IF and B2 + IF, respectively. Conversely, there was an increase in Fe in B3 + IF, reaching 1653.12 mg kg^{-1}, 45.9% higher than the adequate water regime. Water deficiency increased the shoot Zn contents, except in B2 (Figure 4E). On average, water deficiency promoted an 11.7% increase in Zn contents. Concerning biostimulants, the highest content, 49.74 mg kg^{-1}, was found under B1, without statistically differing from B1 + IF, B3, and B3 + IF (46.03 mg kg^{-1}, 45.82 mg kg^{-1}, and 45.42 mg kg^{-1}, respectively). The highest increases in Zn contents resulted from seeds treated with B1 (31%) and IF (21.5%), while the lowest increases were observed in B2 + IF (2.8%) and B3 + IF (4.8%).



Treatments: control; insecticide and fungicide (Imidacloprid + Carboxin + Thiram) (IF); B1 (18 g L^{-1} N, 60 g L^{-1} potassium oxide, 0.96 g L^{-1} B, 12 g L^{-1} Mg, 4.8 g L^{-1} Fe, 12 g L^{-1} S, 24 g L^{-1} Zn, 42 g L^{-1} total organic carbon, 0.031% gibberellic acid and indolacetic acid, 0.0083% zeatin combined with hydrolyzed plant extracts); B2 (50 g L^{-1} N, 12.5 g L^{-1} Co, 62.5 g L^{-1} Mo, 75 g L^{-1} total organic carbon, complexed with *Ascophyllum nodosum* algae extract and amino acids); B3 (Phosphorus chelated cobalt, microencapsulated molybdenum, humic substances, mixed mineral fertilizer with 30% *Ascophyllum nodosum* algae extract enriched with 5% amino acids); B1 + IF; B2 + IF; B3 + IF. Means followed by the same lowercase letter among treatments and within water regimes, and uppercase within each treatment and between water regimes indicate no significant difference according to Student's t-test **($p \leq 0.05$)**.

Figure 4. Shoot micronutrient contents of soybean plants at 21 DAA.
Figura 4. Teores de macronutrientes na parte aérea de plantas de soja aos 21 DAA.

3.3.2. Macronutrients and micronutrients in the roots

Regarding the root macronutrient contents, there was an interaction between the sources of variation in P and Mg, isolated effects of the two factors in K, effects of water regimes on S, and no effect on N and Ca. As for micronutrients, there was an interaction between the water regimes and seed treatments for Cu, Mn, Zn, and B. At the same time, Fe was affected separately by water regimes. The average root contents of N and Ca were 28.82 g kg^{-1} and 1.56 g kg^{-1}, respectively (Figure 5A, 5D).

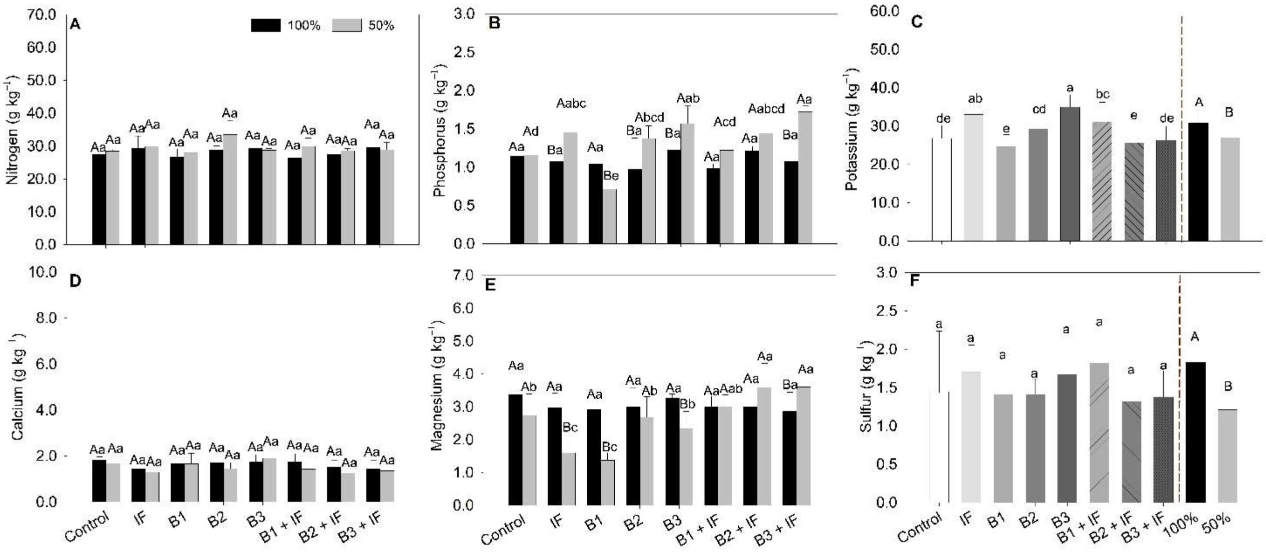
Regarding the root P contents, water deficiency provided values higher than those of the appropriate regime, with an average increase of 22%, except in the control where there was no difference, and treatment B1, which showed a reduction of 32.1%, reaching 0.71 g kg^{-1} (Figure 5B). The highest content, 1.72 g kg^{-1}, was found in B3 + IF, similar to that in B3 (1.57 g kg^{-1}) and B2 + IF (1.44 g kg^{-1}). The highest increase, 58.7%, was promoted by B3 + IF, followed by B2 (40.7%) and IF (35.3%). In contrast, the lowest increases were related to the control (2%).

On average, root K contents were higher by 14.11% in the 100% PC, resulting in 30.88 g kg^{-1} (Figure 5C). Under 50 % PC, B3 promoted the highest root K content (35.80 g kg^{-1}), while B1 resulted in the lowest (26.15 g kg^{-1}). Underwater deficiency, the average K content was 27.06 g kg^{-1}, and B3 provided the highest content, 34.17 g kg^{-1}, similar to IF (32.82 g kg^{-1}). These treatments resulted in the lowest reductions between water regimes. Although the water deficit reduced K concentrations compared to an adequate water regime, B3 and IF provided the lowest reductions (4.6% and 1.4%, respectively).

The average root Mg content under an adequate water regime was 3.05 g kg^{-1}. Water deficiency significantly affected the Mg content, promoting an average reduction of 14.1% (Figure 5E). However, the effects varied among the seed treatments. B2 promoted the lowest decrease in Mg content (10.2%), producing 2.69 g kg^{-1}. In contrast, B3 + IF provided a 25.9% increase in root Mg content under water deficiency.

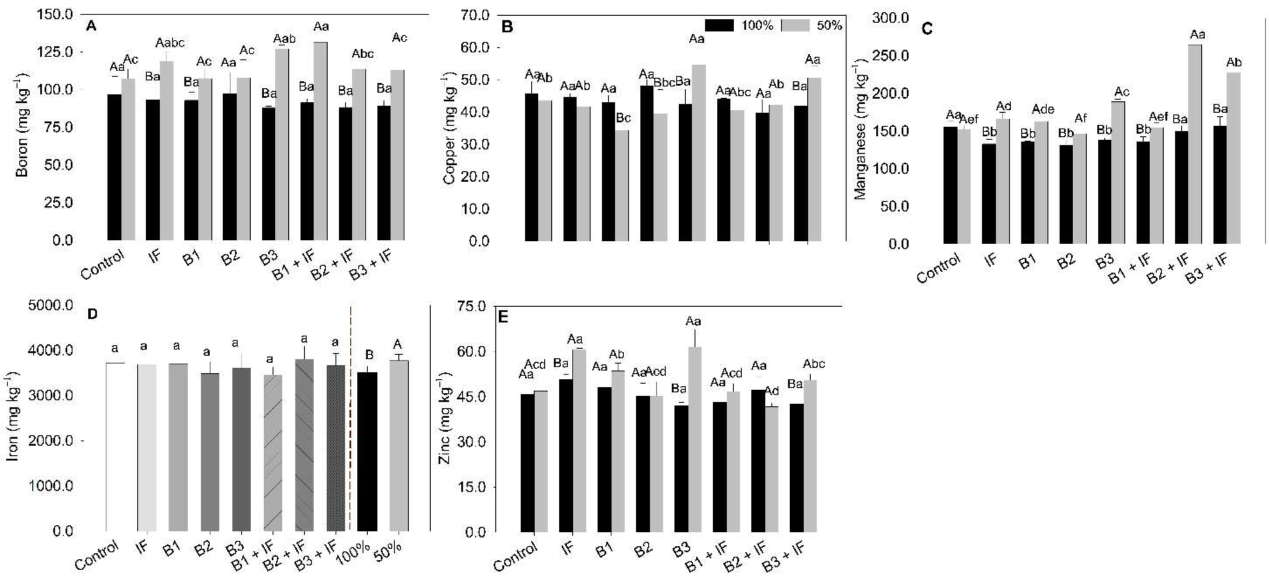
The average root S content was 1.83 g kg^{-1} under 100% PC and 1.21 g kg^{-1} under 50% PC, representing a reduction of 33.1% (Figure 5F). B2 and B1 provided minor reductions, 20.9% and 25.9%, resulting in contents of 1.58 g kg^{-1} and 1.62 g kg^{-1}, respectively, while the most significant decrease, 58%, was observed in control.

B contents in the roots were lower under 100% PC (average of 92.21 mg kg^{-1}), except in the control and B2 (Figure 6A). Water deficiency resulted in an average increase of 20.4% in the root B content of soybean seedlings, and the response varied according to the seed treatment. B1 + IF resulted in the highest B content (131.49 mg kg^{-1}), representing an increase of 43.7%, followed by B3 (127.08 mg kg^{-1}), with a rise of 44.6% compared to the adequate water regime.



Treatments: control; insecticide and fungicide (Imidacloprid + Carboxin + Thiram) (IF); B1 (18 g L⁻¹ N, 60 g L⁻¹ potassium oxide, 0.96 g L⁻¹ B, 12 g L⁻¹ Mg, 4.8 g L⁻¹ Fe, 12 g L⁻¹ S, 24 g L⁻¹ Zn, 42 g L⁻¹ total organic carbon, 0.031% gibberellic acid and indolacetic acid, 0.0083% zeatin combined with hydrolyzed plant extracts); B2 (50 g L⁻¹ N, 12.5 g L⁻¹ Co, 62.5 g L⁻¹ Mo, 75 g L⁻¹ total organic carbon, complexed with *Ascophyllum nodosum* algae extract and amino acids); B3 (Phosphorus chelated cobalt, microencapsulated molybdenum, humic substances, mixed mineral fertilizer with 30% *Ascophyllum nodosum* algae extract enriched with 5% amino acids); B1 + IF; B2 + IF; B3 + IF. Means followed by the same lowercase letter among treatments and within water regimes, and uppercase within each treatment and between water regimes indicate no significant difference according to Student's t-test **($p \leq 0.05$)**.

Figure 5. Root macronutrient contents of soybean plants at 21 DAA.
Figura 5. Teores de macronutrientes nas raízes de plantas de soja aos 21 DAA.



Treatments: control; insecticide and fungicide (Imidacloprid + Carboxin + Thiram) (IF); B1 (18 g L⁻¹ N, 60 g L⁻¹ potassium oxide, 0.96 g L⁻¹ B, 12 g L⁻¹ Mg, 4.8 g L⁻¹ Fe, 12 g L⁻¹ S, 24 g L⁻¹ Zn, 42 g L⁻¹ total organic carbon, 0.031% gibberellic acid and indolacetic acid, 0.0083% zeatin combined with hydrolyzed plant extracts); B2 (50 g L⁻¹ N, 12.5 g L⁻¹ Co, 62.5 g L⁻¹ Mo, 75 g L⁻¹ total organic carbon, complexed with *Ascophyllum nodosum* algae extract and amino acids); B3 (Phosphorus chelated cobalt, microencapsulated molybdenum, humic substances, mixed mineral fertilizer with 30% *Ascophyllum nodosum* algae extract enriched with 5% amino acids); B1 + IF; B2 + IF; B3 + IF. Means followed by the same lowercase letter among treatments and within water regimes, and uppercase within each treatment and between water regimes indicate no significant difference according to Student's t-test **($p \leq 0.05$)**.

Figure 6. Root micronutrient contents of soybean plants at 21 DAA.
Figura 6. Teores de micronutrientes nas raízes de plantas de soja aos 21 DAA.

The overall mean for root Cu contents was 43.61 mg kg⁻¹ (Figure 6B). Among the eight seed treatments, two showed lower values under water restriction than under adequate hydration: B1, with 34.40 mg kg⁻¹, and B2, with 39.65 mg kg⁻

[1], representing reductions of 20.1% and 17.8%, respectively. Conversely, B3 and B3 + IF exhibited the highest values (54.69 mg kg^{-1} and 50.65 mg kg^{-1}), showing increases of 22.3% and 17.1%, respectively, compared to the adequate water regime.

The average root Mn content under an adequate water regime was 142.19 mg kg^{-1}. However, there was a differential response depending on the seed treatments, with the control, B2 + IF, and B3 + IF showing higher values than the others. Water restriction increased Mn contents in the roots, except for the control (Figure 6C). Under these conditions, the highest levels were observed in B3 + IF and B2 + IF (277.44 mg kg^{-1} and 264.52 mg kg^{-1}, respectively), while the lowest levels were verified in control, B2, and B1 + IF (152.45 mg kg^{-1}, 146.56 mg kg^{-1}, and 154.6 mg kg^{-1}, respectively), representing increases of 76.2% and 45.4%, respectively.

Under 100% PC, the root Fe content was 3,508.75 mg kg^{-1}. Except for the control, Fe contents were higher under the 50% PC (Figure 6D), resulting in an average increase of 7.05%. Biostimulant application had no significant effect.

The average root Zn content of all seed treatments under 100% PC was 45.67 mg kg^{-1}, while under 50% PC, it was 50.91 mg kg^{-1}, increasing by 11.5%. Among the eight seed treatments, three of them resulted in higher Zn content under water deficiency: IF (60.74 mg kg^{-1}), B3 (61.55 mg kg^{-1}), and B3 + IF (50.62 mg kg^{-1}), representing increases of 19.7%, 46.6%, and 18.3%, respectively.

4. DISCUSSION

Several compounds exhibit the potential to induce beneficial changes in plants, enhancing their ability to withstand various stressors. A critical aspect of successful germination is adequate seed imbibition; failure in this process can lead to uneven seedling emergence and establishment of suboptimal sowing stands, harming initial development and ultimately reducing crop productivity (STEINER et al., 2017). Notably, water deficiency adversely impacted the assessed biometric parameters. However, seed treatments involving biostimulants (B1, B2, and B3) demonstrated notable benefits, yielding higher %EM and mitigating reductions in ESI, SL, SDB, and RDB.

The effects of water deficiency on seed germination are known. Pereira et al. (2016) reported a reduction in the soybean seed germination subjected to water deficiency, reaching 0% at 21 DAA, attributing it to diminished enzymatic activity and subsequent inhibition of meristematic development.

Moreover, chemical seed treatments during periods of stress have shown potential benefits for seedling emergence and growth. Ludwig et al. (2023) observed that treating soybean seeds with fungicides, insecticides, and biostimulants induced favorable alterations in emergence rates, plant height, chlorophyll levels, and grain yield, thereby enhancing tolerance to water stress.

Generally, biostimulants act on the degradation of seed reserve substance, cell differentiation, division, and elongation (Vasconcelos; Chaves, 2019), leading to enhancements in biometric parameters. However, combining biostimulants with fungicides and insecticides in seed treatments has shown signs of incompatibility, particularly affecting variables such as %EM, ESI, and SA. This phenomenon is corroborated by Carvalho et al. (2022), who observed adverse effects of insecticides and varying water volumes on several commercial seed treatment products, leading to toxicity issues and reduced germination rates in soybean seeds.

Variables such as SDB and RDB exhibited minimal responses to biostimulants but were significantly impacted by water deficiency. The decrease in seedling mass accumulation and growth under water-deficient conditions is attributed to alterations in cell turgidity, protein synthesis, and subsequent inhibition of cell expansion and growth, thereby affecting overall metabolism (MEDEIROS et al., 2015; FAROOQI et al., 2020).

Plant tolerance to water stress has been linked to the accumulation of enzymes that regulate physiological changes, influencing decreases in cellular water potential, stomatal closure, and the initiation of oxidative processes via reactive oxygen species (ROS) formation. The germination and early development stages are particularly susceptible to water deficiency, which triggers an enzymatic defense mechanism involving superoxide dismutase enzymes (SOD), catalase (CAT), peroxidases (POD), and others (TURAN et al., 2021).

Our findings revealed that water deficiency significantly influenced the activities of POD, SOD, and CAT. Biostimulants, particularly B1 and B3, demonstrated an impact on POD and SOD concentrations, while B2 emerged as the most effective composition against water deficiency-induced damage in CAT activity.

The formulations tested comprised compounds such as *Ascophyllum nodosum* (L.) seaweed extract, humic acids, and various macro and micronutrients. These ingredients are known to activate plant metabolism, triggering physiological and biochemical alterations that enhance tolerance to stressful conditions and bolster the efficiency of the antioxidative defense system (VAN OOSTEN et al., 2017). According to Shuckla et al. (2019) and Rosa et al. (2021), the biostimulant components have the potential to enhance antioxidant activity in plants, particularly under water stress, by mitigating the accumulation of reactive oxygen species (ROS).

Campobenedetto et al. (2020) observed that plants treated with biostimulants derived from algae extracts exhibited enhanced activation of antioxidant defenses, coupled with modulation of gene expression encoding enzymes within this system. Furthermore, increases in the enzymatic activities of SOD, POD, and CAT have been documented under water deficiency conditions in various crops such as soybeans (Rosa et al., 2021), beans (Soureshjani et al., 2019), and rice (VAN OOSTEN et al., 2017).

Moreover, water deficiency can induce nutritional limitations beyond the effects above, even in regions with adequate soil fertility levels. This impairs nutrient mobility, uptake, and translocation within plants, subsequently affecting their physiology. However, maintaining optimal levels of macro and micronutrients has been shown to mitigate the impacts of such stressors (TAIZ et al., 2017; BARZANA et al., 2020).

In this context, biostimulants based on seaweed and other plant extracts emerge as pivotal tools for enhancing plant nutrition. They facilitate increased soil nutrient uptake by stimulating root growth, enhancing transpiratory currents, or rendering nutrients more readily available to plants. Consequently, these biostimulants enable plants to withstand stressful conditions and have become integral to modern agricultural practices (BULGARI et al., 2019).

Notably, under water deficiency conditions, biostimulant seed treatments have demonstrated a positive impact on the concentrations of essential nutrients such as P, K, Mg, Cu, Mn, and Zn in both shoot and root tissues, with formulations B1 and B3 exerting significant influence.

Phosphorus is particularly significant in seed germination, as it provides energy to seedlings and promotes the establishment of a robust initial stand (SOARES et al., 2014). Its transport primarily relies on diffusion, closely linked to water availability (FANG et al., 2019). Thus, the observed enhancement in P accumulation, both in root and shoot parts, underscores the potential benefits of biostimulant application in facilitating nutrient uptake.

The higher K content promoted mainly by B1 under water deficiency can be justified by the potassium oxide present in its composition. Some authors associate the reduction of water loss in stress situations with an adequate supply of K due to its relationship with cell turgidity and osmoregulation, as well as stomatal regulation and elimination of ROS (FRANÇA et al., 2022; SILVA et al., 2022).

The positive effect of the biostimulant application found in our research on Mg levels under water deficiency can be explained by its formulation. Humic substances, for example, present in B3, act on the growth and improvement of plant nutrition by increasing nutrient uptake and the soil cation exchange capacity, such as by stimulating H^+ ATPases in the roots (CANELLAS et al., 2014). Plant Mg levels are highly dependent on their concentration in the soil and water availability, which assists in uptake through ion-root contact, root interception, diffusion, or mass flow (ALTARUGIO et al., 2017).

Biostimulant application in seed treatment also increased the concentrations of micronutrients such as Cu, Mn, and Zn. These elements serve as essential co-factors for enzymes like superoxide dismutase (SOD) and catalase (CAT), which play crucial roles in antioxidant defense mechanisms (MARTINS et al., 2022).

One effective strategy to mitigate the adverse effects of water stress involves using micronutrient-based products. These substances aid in detoxifying ROS generated by environmental stresses by facilitating reactions mediated by antioxidant enzymes. They directly and indirectly affect various metabolic pathways and cellular structures (FAGAN et al., 2016). For instance, Bradácová et al. (2016) attributed the protective effects conferred by biostimulants, similar to those evaluated in this study, to the micronutrient components of the formulations, which act as cofactors for antioxidant enzymes.

It is notable that the seed treatments exhibiting higher SOD concentrations, namely B1 and B3, also demonstrated elevated levels of Cu in the root and shoot, respectively. This observation could be attributed to the presence of humic substances in their composition, which are known to enhance O_2 production, augment defense mechanisms against reactive oxygen species (ROS), and are associated with SOD isoforms such as SOD Cu/Zn and SOD Fe found in chloroplasts (BATOOL et al., 2022).

There is a positive correlation between soil Cu content and crucial physiological variables such as photosynthetic rate, internal CO_2 concentration, stomatal conductance, and transpiration. Additionally, Cu is deemed essential for robust crop growth, as it participates in vital processes, including carbohydrate and protein metabolism and lignification, and acts as a catalyst for numerous enzymes. Consequently, its presence is particularly advantageous regarding water deficiency (MIGOCKA; MALAS, 2018).

Manganese (Mn) content was also positively influenced by applying biostimulants (B1, B2, and B3) under water deficiency conditions. Mn is crucial in several physiological processes, such as photosynthesis, respiration, ROS scavenging, and hormone signaling. It is absorbed by the roots primarily in Mn^{2+} ions through diffusion and root interception, aiding plant recovery and maintaining physiological functions during water-stress situations (LIU et al., 2020).

Zinc (Zn) exhibits low mobility in the phloem and requires precise management practices to ensure optimal distribution closer to the root uptake zone. Seed treatment offers an efficient means of supplying Zn (LEMES et al., 2017). The elevated levels of Zn observed under water-deficient conditions in B1 and B3 suggest that plants treated with these micronutrients would be better equipped to withstand stress. Zn serves critical roles in enzymatic activation, amino acid synthesis, and membrane maintenance and acts as a precursor to indoleacetic acid, promoting plant and root growth. This enhances nutrient and water uptake, improving stress tolerance (CHOUDHARY et al., 2019).

Generally, the biostimulant application confers benefits to the initial development of soybean crops under water-stress conditions. These benefits may include enhanced root stimulation, improved water and nutrient uptake, and increased activation or availability of nutrients as co-factors for antioxidative enzymes. The specific mechanisms vary depending on the composition of the biostimulant, which may include nutrients, amino acids, and plant extracts. Given these advantages, biostimulants represent a valuable tool that should be further explored to optimize crop establishment and productivity.

Nevertheless, it is imperative to conduct more thorough investigations into the combinations with insecticides and fungicides, particularly those belonging to the Neonicotinoids group (e.g., Imidacloprid), Carboxynilide (e.g., Carboxin), and Dimethyldithiocarbamate (e.g., Thiram). This is crucial as these chemicals can potentially undermine the expression of beneficial effects.

5. CONCLUSIONS

Applying biostimulants, particularly those derived from seaweed and plant extracts, confers notable advantages to the initial soybean growth under water stress conditions.

Biostimulants in soybean seed treatment enhance the plant's ability to tolerate water deficit, resulting in improved germination, emergence, biometric parameters, and antioxidant enzyme activity.

Moreover, these biostimulants are crucial in facilitating vital nutrient uptake, such as P, K, Mg, Cu, Mn, and Zn. These nutrients are essential for maintaining optimal photosynthetic efficiency and serving as co-factors for antioxidative enzymes, thereby shielding plants from stress-induced damage.

6. REFERENCES

ALTARUGIO, L. M.; LOMAN, M. H.; NIRSCH, M. G.; SILVANO, R. G.; ZAVASCHI, E.; CARNEIRO, L. M.

S.; VITTI, G. C.; LUZ, P. H. C.; OTTO, R. Yield performance of soybean and corn subjected to magnesium foliar spray. **Pesquisa Agropecuária Brasileira**, v. 52, n. 12, p. 1185-1191, 2017. https://doi.org/10.1590/S0100-204X2017001200007

AMARO, H. T. R.; FERNANDES, H. M. F.; ALMEIDA, P. M. A.; PORTO, E. M. V.; DAVID, A. M. S. S. Tratamento de sementes com bioestimulantes e disponibilidade hídrica no desenvolvimento inicial do milho. **Magistra**, v. 33, p. 1-9, 2023.

ANDRADE, C. L. L.; SILVA, A. G.; CANTÃO, V. C. G.; MELLO, G. B.; SIQUEIRA, G. G. C.; RODRIGUES, R. L. S. Performance of maize seedlings using biostimulant in seed treatment. **Científica**, v. 46, n. 3, p. 274-282, 2018.

BARZANA, G.; RIOS, J. J.; LOPEZ-ZAPLANA, A.; NICOLAS-ESPINOSA, J.; YEPES-MOLINA, L.; GARCIA-IBAÑEZ, P.; CARVAJAL, M. Interrelations of nutrient and water transporters in plants under abiotic stress. **Physiologia Plantarum**, v. 171, n. 4, p. 595-619, 2020. https://doi.org/10.1111/ppl.13206 ppl.13206.

BATOOL, R.; UMER, M. J.; HUSSAIN, B.; ANEES, M.; WANG, Z. Molecular mechanisms of superoxide dismutase (SODs)-mediated defense in controlling oxidative stress in plants. In: AFTAB, T.; HAKEEM, K. R. (Eds). **Antioxidant defense in plants**. Singapore: Springer, 2022. p. 157-179. https://doi.org/10.1007/978-981-16-7981-0_8

BEGUM, N.; HASANUZZAMAN, M.; LI, Y.; AKHTAR, K.; ZHANG, C.; ZHAO, T. Seed germination behavior, growth, physiology and antioxidant metabolism of four contrasting cultivars under combined drought and salinity in soybean. **Antioxidants**, v. 11, e498, 2022. https://doi.org/10.3390/antiox11030498

BRADÁCOVÁ, K.; WEBER, N. F.; MORAD-TALAB, N.; ASSIM, M.; IMRAN, M.; WEINMANN, M.; NEUMANN, G. Micronutrients (Zn/Mn), seaweed extracts, and plant growth-promoting bacteria as cold-stress protectants in maize. **Chemical and Biological Technologies in Agriculture**, v. 3, p. 1-10, 2016. https://dx.doi.org/10.1186/s40538-016-0069-1

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, p. 248-254, 1976. https://doi.org/10.1006/abio.1976.9999

BULGARI, R.; FRANZONI, G.; FERRANTE, A. Biostimulants application in horticultural crops under abiotic stress conditions. **Agronomy**, v. 9, n. 6, e306, 2019. https://doi.org/10.3390/agronomy9060306

CAMPOBENEDETTO, C.; GRANGE, E.; MANNINO, G.; VAN ARKEL, J.; BEEKWILDER, J.; KARLOVA, R.; GARABELLO, C.; CONTARTESE, V.; BERTEA, C. M. A biostimulant seed treatment improved heat stress tolerance during cucumber seed germination by acting on the antioxidant system and glyoxylate cycle. **Frontiers in Plant Science**, v. 11, e386, 2020. https://doi.org/10.3389/fpls.2020.00836

CANELLAS, L. P.; OLIVARES, F. L.; AGUIAR, N. O.; JONES, D. L.; NEBBIOSO, A.; MAZZEI, P.; PICCOLO, A. Humic and fulvic acids as biostimulants in horticulture. **Scientia Horticulture**, v. 196, p. 15-27, 2014. https://doi.org/10.1016/j.scienta.2015.09.013

CARMO, M. A. P.; CARVALHO, M. L. M.; SANTOS, H. O.; ROCHA, D. K.; OLIVEIRA, J. A.; SOUZA, V. F.; GUARALDO, M. M. S.; MESQUITA, C. A. M. Biostimulants applied to seeds and sweet corn plants under abiotic stress conditions. **Brazilian Journal of Development**, v. 7, n. 3, p. 31727-31741, 2021. http://dx.doi.org/10.34117/bjdv7n3-747

CARVALHO, E. R.; ROCHA, D. K.; FRAGA JÚNIOR, E. F.; PIRES, R. M. O.; OLIVEIRA, T. F.; PENIDO, A. C. Soil water restriction and performance of soybean seeds treated with phytosanitary products. **Brazilian Journal of Agricultural and Environmental Engineering**, v. 26, n. 1, p. 59-66, 2022. https://doi.org/10.1590/1807-1929/agriambi.v26n1p59-66

CHOUDHARY, S. K.; JAT, M. K.; MATHUR, A. K. Effect of micronutrient on yield and nutrient uptake in sorghum. **Journal of Pharmacognosy and Phytochemistry**, v. 6, n. 2, p. 105-108, 2017

CUNHA, R. P.; CORRÊA, M. F.; SCHUCH, L. O. B.; OLIVEIRA, R. C.; ABREU JUNIOR, J. S.; SILVA, J. D. G.; ALMEIDA, T. L. Diferentes tratamentos de sementes sobre o desenvolvimento de plantas de soja. **Ciência Rural**, v. 45, p. 1761-1767, 2015. http://dx.doi.org/10.1590/0103-8478cr20140742

DU, Y.; ZHAO, Q.; CHEN, L.; YAO, X.; XIE, F. Effect of drought stress at reproductive stages on growth and nitrogen metabolism in soybean. **Agronomy**, v. 10, e302, 2020. https://doi.org/10.3390/agronomy10020302

FAGAN, E. B.; ONO, E. O.; RODRIGUES, J. D.; SOARES, L. H.; DOURADO NETO, D. **Fisiologia vegetal: metabolismo e nutrição mineral**. São Paulo: Andrei Editora, 2016. 305p.

FANG, Y.; YABUSAKI, S. B.; AHKAMI, A. H.; CHEN, X.; SCHEIBE, T. D. An efficient three-dimensional rhizosphere modeling capability to study the effect of root system architecture on soil water and reactive transport**. Plant and Soi**l, v. 441, n. 1-2, p. 33-48, 2019. https://link.springer.com/article/10.1007/s11104-019-04068-z

FARIAS, J. R. B.; NEPOMUCENO, A. L.; NEUMAIER, N. **Ecofisiologia da soja**. Londrina: Embrapa Soja, 2011. 8p. (Circular Técnica, 48)

FAROOQI, Z. U. R.; AYUB, M. A.; REHMAN, M. Z. U.; et al. Regulation of drought stress in plants. In: TRIPATHI, D. K.; SINGH, V. P.; CHAUHAN, D. K.; SHARMA, S.; PRASAD, S. M.; DUBEY, N. K.; RAMAWAT, N. (Eds.). **Plant Life Under Changing Environment**. Academic Press, 2020. p. 77-104. https://doi.org/10.1016/B978-0-12-818204-8.00004-7

FRANÇA, G. M.; FREIRE, A. L. O.; BATISTA, A. W.; FERREIRA, C. D. O potássio atenua os efeitos do déficit hídrico em mudas de pereiro (*Aspidosperma pyrifolium* Mart. & Zucc.). **Conjecturas**, v. 22, n. 2, p. 266-277, 2022. https://dx.doi.org/10.53660/CONJ-662-701

FRANÇA-NETO, J. B.; KRZYZANOWSKI, F. C.; HENNING, A. A.; PÁDUA, G. P.; LORINI, I.; HENNING, F. A. **Tecnologia da produção de semente de soja de alta qualidade**. Londrina: Embrapa Soja, 2016. 82p. (Documentos, 380)

FRASCA, L. L. M.; NASCENTE, A. S.; LANNA, A. C.; CARVALHO, M. C. S.; COSTA, G. G. Bioestimulantes no crescimento vegetal e desempenho agronômico do feijão-comum de ciclo superprecoce. **Agrarian**, v. 13, n.

47, p. 27-41, 2020. https://doi.org/10.30612/agrarian.v13i47.8571

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutase I. Occurrence in higher plants. **Plant Physiology**, v. 59, n .2, p. 309-314, 1997. https://doi.org/10.1104/pp.59.2.309

GUPTA, S.; KULKARNI, M. G.; WHITE, J. F.; STIRK, W. A.; PAPENFUS, H. B.; DOLEZAL, K.; ÖRDÖG, V.; NORRIE, J.; CRITCHLEY, A. T.; VAN STADEN, J. Categories of various plant biostimulants – mode of application and shelf-life. In: GUPTA, S.; VAN STADEN, J. (Eds.). **Biostimulants for crops from seed germination to plant development.** Academic Press, 2021. p. 1-60. https://doi.org/10.1016/B978-0-12-823048-0.00018-6

IGHODARO, O. M.; AKINLOYE, O. A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. **Alexandria Journal of Medicine**, v. 54, n. 4, p. 287-293, 2017. https://doi.org/10.1016/j.ajme.2017.09.001

KHAEIM, H.; KENDE, Z.; BALLA, I.; GYURICZA, C.; ESER, A.; TARNAWA, Á. The effect of temperature and water stresses on seed germination and seedling growth of wheat *(Triticum aestivum* L.). **Sustainability**, v. 14, e3887, 2022. https://doi.org/10.3390/su14073887

KRZYZANOWSKI, F. C.; FRANÇA-NETO, J. B.; HENNING, A. A. **A alta qualidade da semente de soja: fator importante para a produção da cultura**. Londrina: Embrapa Soja, 2018. 24p. (Circular Técnica, 136)

LAUXEN, L. R.; VILLELA, F. A.; SOARES, R. C. Desempenho fisiológico de sementes de algodão tratadas com tiametoxan**. Revista Brasileira de Sementes**, v. 32, n. 3, p. 61-68, 2010. https://doi.org/10.1590/S0101-31222010000300007

LEMES, E. S.; MENDONÇA, A. O.; DIAS, L. W.; BRUNES, A. P.; OLVEIRA, S.; FIN, S. S.; MENEGHELLO, G. E. Tratamento de sementes de soja com zinco: efeito na qualidade fisiológica e produtividade. **Coloquium Agrariae**, v. 13, n. 2, p. 76-86, 2017.

LIU, Y.; XUE, Y.; XIE, B.; ZHU, S.; LU, X.; LIANG, C.; TIAN, J. Complex gene regulation between young and old soybean leaves in responses to manganese toxicity. **Plant Physiology and Biochemistry**, v. 155, p. 231-242, 2020. https://doi.org/10.1016/j.plaphy.2020.07.002

LUCINI, L.; ROUPHAEL, Y.; CARDARELLI, M.; CANAGUIER, R.; KUMAR, P.; COLLA, G. The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. **Scientia Horticulturae**, v. 182, p. 124-133, 2015. https://dx.doi.org/10.1016/j.scienta.2014.11.022

LUDWIG, E. J.; NUNES, U.L R.; SANTOS, C. V.; ZINI, P. B.; KLEINPAUL, J. A.; BASTIANI, G. G. Water stress on the physiological quality of soybean seeds with tiamethoxam and polymers. **Research, Society and Development**, v. 12, n. 10, e44121043427, 2023. http://dx.doi.org/10.33448/rsd-v12i10.43427

MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. **Crop Science**, v. 2, n. 1, p. 176-177, 1962. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

MAJKOWSKA-GADOMSKA, J.; FRANCKE, A.; DOBROWOLSKI, A.; MIKULEWICZ, E. The effect of selected biostimulants on seed germination of four plant species. **Acta Agrophysica**, v. 24, n. 4, p. 591-599, 2017.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. **Avaliação do estado nutricional das plantas: princípios e aplicações**. Piracicaba: Potafós, 1997. 319p.

MANDAL, S.; ANAD, U.; LÓPEZ-BUCIO, J.; RADHA, KUMAR, M.; LAL, M. K.; TIWARI, R. K.; DEY, A. Biostimulants and environmental stress mitigation in crops: A novel and emerging approach for agricultural sustainability under climate change. **Environmental Research**, v. 23, e116357, 2023. https://doi.org/10.1016/j.envres.2023.116357

MARTINS, M. C. C.; OLIVEIRA, A. S. S. S.; SILVA, L. A. A.; SILVA PRIMO, M. G.; LIRA, V. B. C. Biological indicators of oxidative stress [malondialdehyde, catalase, glutathione peroxidase, and superoxide dismutase] and their application in nutrition. In: PATEL, V. B.; PREEDY, V. R. (Eds). **Biomarkers in Disease: Methods, Discoveries and Applications.** Springer, Cham, 2022. p. 1-25. https://doi.org/10.1007/978-3-030-81304-8_49-1

MEDEIROS, D. S.; ALVES, E. U.; SENA, D. V. A.; SILVA, E. O.; ARAÚJO, L. R. Desempenho fisiológico de sementes de gergelim submetidas a estresse hídrico em diferentes temperaturas. **Semina: Ciências Agrárias**, v. 36, n. 5, p. 3069-3076, 2015. https://doi.org/10.5433/1679-0359.2015v36n5p3069

MEDEIROS, J. C.; CARVALHO, E. R.; ANDRADE, D. B.; MORAES, L. F. S.; LIMA, J. M. E.; MASSA, M. A. F. Quality of corn seed industrial treatment (IST) and on-farm treatment (OFT) in Brazilian agribusiness. **Journal of Seed Science**, v. 45, e202345017, 2023. http://dx.doi.org/10.1590/2317-1545v45268856

MIGOCKA, M.; MALAS, K. Plant responses to copper: molecular and regulatory mechanisms of copper uptake, distribution and accumulation in plants. In: HOSSAIN, M. A.; KAMIYA, T.; BURRITT, D. J.; TRAN, P. L.-S., FUJIWARA, T. (Eds.). **Plant micronutrient use efficiency: molecular and genomic perspectives in crop plants**. London: Academic Press, 2018. p. 71-86. https://doi.org/10.1016/B978-0-0-12-812104-7.00005-8

OGUNKANMI, L.; MACCARTHY, D. S.; ADIKU, S. G. K. Impact of extreme temperature and soil water stress on the growth and yield of soybean (*Glycine max* (L) Merrill). **Agriculture**, v. 12, n. 1, e43, 2022. https://doi.org/10.3390/agriculture12010043

PANDEY, P.; RAMEGOWDA, V.; SENTHIL-KUMAR, M. Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. **Frontiers in Plant Science**, v. 6, e723, 2015. https://doi.org/10.3389/fpls.2015.00723

PASZKIEWICZ, G.; GUALBERTO, J. M.; BENAMAR, A.; MACHEREL, D.; LOGAN, D. C. *Arabidopsis* seed mitochondria are bioenergetically active immediately upon imbibition and specialize via biogenesis in preparation for autotrophic growth. **Plant Cell**, v. 29, n.

1, p. 109-128, 2017. https://doi.org/10.1105/tpc.16.00700

PEIXOTO, P. H. P.; CAMBRAIA, J.; SANT'ANNA, R.; MOSQUIM, P. R.; MOREIRA, M. A. Aluminium effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. **Revista Brasileira de Fisiologia Vegetal**, v. 11, n. 3, p. 137-143, 1999.

PEREIRA, L. C.; GARCIA, M. M.; BRACCINI, A. L.; PIANA, S. C.; FERRI, G. C.; MATERA, T. C.; FELBER, P. H.; MARTELI, D. C. V. Efeito da adição de biorregulador ao tratamento industrial sobre a qualidade de sementes de soja (*Glycine max* (L.) Merr.) aos sessenta dias de armazenamento convencional. **Revista Colombiana de Investigaciones Agroindustriales**, v. 3, n. 2, p. 15-22, 2016. https://doi.org/10.23850/issn.2422-0582

RAHMAN, M. A.; ALAM, I.; SHARMIN, S. A.; KABIR, A. H.; KIM, Y.-G.; LIU, G.; LEE, B.-H. Physiological and proteomic analyses reveal the protective roles of exogenous hydrogen peroxide in alleviating drought stress in soybean plants. **Plant Biotechnology Reports**, v. 15, p. 805-818, 2021. https://doi.org/10.1007/s11816-021-00719-9

REHMAN, T.; TABASSUM, B.; YOUSAF, S.; SARWAR, G.; QAISAR, U. Consequences of drought stress encountered during seedling stage on physiology and yield of cultivated cotton. **Frontiers in Plant Science**, v. 13, e906444, 2022. https://doi.org/10.3389/fpls.2022.906444

REPKE, R. A.; SILVA, D. M. R.; SANTOS, J. C. C.; SILVA, M. A. Increased soybean tolerance to high-temperature through biostimulant based on *Ascophyllum nodosum* (L.) seaweed extract. **Journal of Applied Phycology**, v. 34, p. 3205-3218, 2022. http://dx.doi.org/10.1007/s10811-022-02821-z

ROSA, V. R.; SANTOS, A. L. F.; SILVA, A. A.; SAB, M. P. V.; GERMINO, G. H.; CARDOSO, F. B.; SILVA, M. A. Increased soybean tolerance to water deficiency through biostimulant based on fulvic acids and *Ascophyllum nodosum* (L.) seaweed extract. **Plant Physiology and Biochemistry**, v. 158, p. 228-243, 2021. https://doi.org/10.1016/j.plaphy.2020.11.008

ROUPHAEL, Y.; COLLA, G. Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture. **Frontiers in Plant Science**, v. 9, e1655, 2018. http://dx.doi.org/10.3389/fpls.2018.01655

SHUCKLA, P. S.; MANTIN, E. G.; ADIL, M.; BAJPAI, S.; CRITCHLEY, A. T.; PRITHIVIRAJ, B. *Ascophyllum nodosum*-based biostimulants: sustainable applications in agriculture for the stimulation of plant growth, stress tolerance and disease management. **Frontiers in Plant Science**, v. 10, e665, 2019. https://doi.org/10.3389/fpls.2019.00655

SILVA, A. M. P.; OLIVEIRA, G. P.; NERES, D. C. C. Germinação e Vigor de Sementes de soja Submetidas ao Tratamentos com Substâncias Bioativas. **Caderno de Publicação Univag**, n. 8, e795, 2018. https://doi.org/10.18312/cadernounivag.v0i08.795

SILVA, L. M.; BERTI, M. P. S. Manganese in soil and plants: a review. **Scientific Eletronic Archives**, v. 15, n. 3, p. 21-25, 2022. http://dx.doi.org/10.36560/15320221512

SOARES, M. M. ; SILVA, L. J. ; OLIVEIRA, G. L. ; SEKITA, M. C. ; DIAS, D. C. F. S. Endogenous level of phosphorous in soya bean seeds and the relationship with physiological quality, oil and protein content. **Seed Science and Technology**, v. 42, n. 3, p. 433-443, 2014. http://dx.doi.org/10.15258/sst.2014.42.3.09

SOURESHJANI, H. K.; NEZAMI, A.; KAFI, M.; TADAYON, M. Responses of two common bean (*Phaseolus vulgaris* L.) genotypes to deficit irrigation. **Agricultural Water Management**, v. 213, p. 270-279, 2019. https://doi.org/10.1016/j.agwat.2018.09.038

STEINER, F.; ZUFFO, A. M.; ZOZ, T.; ZOZ, A.; ZOZ, J. Drought tolerance of wheat and black oat crops at early stages of seedling growth. **Revista de Ciências Agrárias**, v. 40, n. 3, p. 576-586, 2017. http://dx.doi.org/10.19084/RCA16118

TAIZ, L.; ZEIGER, E.; MØLLER, I. M.; MURPHY, A. **Fisiologia e Desenvolvimento Vegetal**. Artmed: Porto Alegre, 2017. 858p.

TURAN, M.; EKICI, E. Y. M; ARGIN, S. Effect of biostimulants on yield and quality of cherry tomatoes grown in fertile and stressed soils. **American Society for Horticultural Science**, v. 56, n. 4, p. 414-423, 2021. http://dx.doi.org/10.21273/HORTSCI15568-20

VAN OOSTEN, M. J.; PEPE, O.; PASCALE, S.; SILLETTI, S.; MAGGIO, A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. **Chemical and Biological Technology in Agriculture**, v.4, n.5, 2017. DOI: https://chembioagro.springeropen.com/articles/10.1186/s40538-017-0089-5.

VASCONCELOS, C. F.; CHAVES, H. G. Biostimulants and their role in improving plant growth under abiotic stresses. In: MIRMAJLESSI, S. M.; RADHAKRISHNAN, R. (Eds.). **Biostimulants in Plant Science**. IntechOpen, 2020. https://doi.org/10.5772/intechopen.88829

WANG, B.; ZHANG, J.; PEI, D.; YU, L. Combined effects of water stress and salinity on growth, physiological, and biochemical traits in two walnut genotypes. **Physiologia Plantarum**, v. 172, n. 1, p. 176-187, 2021. https://doi.org/10.1111/ppl.13316

XU, C.; LESKOVAR, D. Effects of *A. nodosum* seaweed extracts on spinach growth, physiology and nutrition valued under drought stress. **Scientia Horticulturae**, v. 183, p. 39-47, 2015. https://doi.org/10.1016/j.scienta.2014.12.004

XU, Q. Q.; SAMI, A.; ZHANG, H.; JIN, X. Z.; ZHENG, W. Y.; ZHU, Z. Y.; WU, L. L.; LEI, Y. H.; CHEN, Z. P.; LI, Y.; YU, Y.; ZHANG, F. G.; ZHOU, K. J.; ZHU, Z. H. Combined influence of low temperature and drought on different varieties of rapeseed (*Brassica napus* L.). **South African Journal of Botany**, v. 147, p. 400-414, 2022. https://doi.org/10.1016/j.sajb.2022.02.003

ZHANG, Y. B.; YANG, S. L.; DAO, J. M.; DENG, J.; SHAHZAD, A. N.; FAN, X.; LI, R. D.; QUAN, Y. J.; BUKHARI, S. A. H.; ZENG, Z. H. Drought-induced alterations in photosynthetic, ultrastructural and biochemical traits of contrasting sugarcane genotypes. **PLoS ONE**, v. 15, n. 7, e0235845, 2020. https://doi.org/10.1371/journal.pone.0235845

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