

Ecophysiology of *Mentha piperita* under saline stress and biostimulant in the Brazilian semiarid region

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ABSTRACT: Brazilian semiarid region has water with high levels of salts that limit its use in agriculture, necessitating the adoption of strategies to reduce the impact of salinity. The objective of this study was to assess the effect of a biostimulant as a attenuating of mitigate saline stress on the growth, gas exchange, chlorophyll indices, and chlorophyll a fluorescence of *Mentha piperita*. The experiment was performed using a randomized block design, employing a 5 × 5 incomplete factorial schemes. This involved five different electrical conductivities of the irrigation water (EC_w = 0.5, 1.01, 2.25, 3.49, and 4.00 dS m⁻¹) and five doses of the biostimulant (Stimulate® = 0.0, 1.45, 5.00, 8.55, and 10.0 mL L⁻¹). In total, nine combinations were generated using the Central Composite Design. Growth, gas exchange, chlorophyll indices, and chlorophyll a fluorescence were evaluated 45 days after irrigation with saline water. The results indicated that saline stress hindered the growth, gas exchange, chlorophyll indices, and photochemical efficiency of *M. piperita*. However, the biostimulant mitigated the adverse effects of salinity on the growth, gas exchange, and photochemical efficiency of *M. piperita*.

Key words: gas exchange; growth regulators; saline stress

Ecofisiologia de *Mentha piperita* sob estresse salino e bioestimulante no semiárido brasileiro

RESUMO: O semiárido brasileiro apresenta água com elevados teores de sais que limitam o uso na agricultura, sendo necessário a adoção de estratégias para reduzir o efeito da salinidade. O objetivo deste trabalho foi avaliar o efeito de um bioestimulante como atenuador do estresse salino no crescimento, trocas gasosas, índices de clorofila e fluorescência da clorofila a de *Mentha piperita*. O experimento foi conduzido em delineamento de blocos casualizados, em esquema fatorial incompleto 5 × 5, com cinco condutividades elétricas da água de irrigação (CE_a = 0,5, 1,01, 2,25, 3,49 e 4,00 dS m⁻¹) e cinco doses de bioestimulante (Stimulate® = 0,0, 1,45, 5,00, 8,55 e 10,0 mL L⁻¹), totalizando nove combinações geradas através do Central Composite Design. O crescimento, as trocas gasosas, os índices de clorofila e a fluorescência da clorofila a foram avaliados 45 dias após a irrigação com água salina. O estresse salino reduziu o crescimento, as trocas gasosas, os índices de clorofila e a eficiência fotoquímica de *M. piperita*. O bioestimulante atenuou os efeitos nocivos da salinidade no crescimento, trocas gasosas e eficiência fotoquímica de *M. piperita*.

Palavras-chave: trocas gasosas; reguladores de crescimento; estresse salino



Introduction

Mint (*Mentha piperita* L.) is a member of the Lamiaceae family and is extensively cultivated worldwide, primarily for culinary and traditional medicinal purposes. The leaves of this plant contain essential oils with secondary compounds that find applications in the food, pharmaceutical, and cosmetic industries (Alhaithloul et al., 2020).

However, cultivating *M. piperita* in semi-arid regions such as the Brazilian Northeast is challenging due to water scarcity. As a result, farmers have to rely on low-quality water for irrigation, which often contains high salt levels. The elevated salinity levels pose limitations to the plants growth and development. They adversely affect various physiological processes, including gas exchange, nutrient absorption, osmotic balance, and overall plant metabolism (Alavi et al., 2020; Ribeiro et al., 2020). These detrimental effects are a result of reduced water availability caused by decreased osmotic potential, alterations in enzymatic activity, and oxidative stress induced by the production of reactive oxygen species (ROS) and ionic toxicity, particularly from sodium ions (Na^+) and chloride ions (Cl^-) (Khanam & Mohammad, 2018; Faghih et al., 2019; Silva et al., 2019).

The quest for products that can mitigate the adverse effects of saline stress on plants is growing, and the use of biostimulants has gained significant attention in recent years. Biostimulants are composed of bioactive substances, including phytohormones, which regulate plant metabolism and enhance the plants ability to tolerate saline stress (Sanchez et al., 2019; Malik et al., 2021). Furthermore, biostimulants play a crucial role in signaling genes associated with defense mechanisms and hormonal balance (Hadia et al., 2020).

Considering the immense importance of utilizing biostimulants to enhance vegetable production, the objective of this study was to evaluate the impact of a biostimulant as a means to attenuate saline stress on the growth, gas exchange, chlorophyll indices, and chlorophyll a fluorescence of *M. piperita*.

Materials and Methods

Experiment site

The research was performed in a greenhouse, located at the Centro de Ciências Agrárias, Universidade Federal da Paraíba, municipality of Areia, state of Paraíba, Brazil. The predominant climate of the experiment site is As' type with dry and hot summer and winter rain, according to Köppen classification.

Plant material

Mint seedlings were produced from 5 cm cuttings, obtained from healthy parent plants free from pest attack. The cuttings were transplanted in polyethylene bags with a capacity of 1.2 dm³ containing a mixture of soil (Latosoil – Embrapa, 2018), tanned cattle manure, and washed sand

(3:1:1, v/v), with the characteristics chemical: pH = 7.8; P = 85.5 mg kg⁻¹; K⁺ = 693.6 mg kg⁻¹; Na⁺ = 0.23 cmol_c dm⁻³; H⁺Al⁺³ = 0.0 cmol_c dm⁻³; Al⁺³ = 0.0 cmol_c dm⁻³; Ca⁺² = 2.9 cmol_c dm⁻³; Mg⁺² = 1.59 cmol_c dm⁻³; SB = 6.5; CEC = 6.5 g kg⁻¹; OM = 22.2 g kg⁻¹. Two cuttings were transplanted into each bag and maintained for 15 days until establishment. Subsequently, plant thinning was performed, retaining only the most robust plant.

Treatments and statistical design

A randomized block design was employed, following an incomplete factorial scheme of 5 (electrical conductivities of irrigation water: 0.5, 1.01, 2.25, 3.49, and 4.00 dS m⁻¹) × 5 (doses of biostimulant: 0.0, 1.45, 5.00, 8.55, and 10.0 mL L⁻¹). The experiment consisted of four replications with two plants per plot, resulting in a total of nine treatments (Table 1). The treatments were generated using the Central Composite Design method (Hang et al., 2011).

Different salinity levels in the water were achieved by adding sodium chloride (NaCl) to the supply system water (initial electrical conductivity, EC_w = 0.5 dS m⁻¹) until the desired conductivities were reached. The electrical conductivity of the water (EC_w) was measured using an Instrutherm[®] microprocessor-based portable conductivity meter (model CD-860).

Irrigation with saline water commenced 10 days after the mint cuttings were established. The biostimulant (Stimulate[®] - Stoller) application began 10 days after irrigation (DAI) with saline water, with a weekly application of 100 mL plant⁻¹ for a duration of six weeks. The biostimulant contains 0.09 g L⁻¹ of kinetin (cytokinin), 0.05 g L⁻¹ of gibberellic acid (gibberellin), and 0.05 mg L⁻¹ of indolebutyric acid (auxin analog).

Table 1. Combinations and factors used in the experiment.

Treatments	Levels		Doses	
	EC _w	DB	EC _w (dS m ⁻¹)	DB (mL L ⁻¹)
1	-1	-1	1.01	1.46
2	-1	1	3.49	1.46
3	1	-1	1.01	8.55
4	1	1	3.49	8.55
5	-α	0	2.25	0.00
6	α	0	2.25	10.00
7	0	-α	4.00	5.00
8	0	α	0.50	5.00
9	0	0	2.25	5.00

EC_w - electrical conductivity of irrigation water; DB - doses of biostimulant. α = distance between each axial point and the center in a central composite design. n = 4.

Variables analyzed

Plant height (PH) was measured using a graduated ruler, starting from the base of the soil and extending to the last leaf insertion. The measurements were expressed in centimeters (cm). The number of leaves (NL) and branches (NS) were determined by counting all the leaves and branches on each plant. Stem diameter (SD) was measured using a digital caliper, and the values were expressed in millimeters (mm). The number of branches (NS) and leaf area (cm²) (Benincasa, 2003) were assessed at 45 days after irrigation (DAI). To

measure the fresh mass of the root (RFM), stem (StFM), leaves (LFM), shoot (ShFM), and total (TFM), a precision scale with an accuracy of 0.001 g was used. After weighing, the samples were placed in Kraft paper bags and dried in an oven with forced air circulation at 65 °C until a constant mass was achieved. The dry mass of the root (RDM), stem (StDM), leaves (LDM), shoot (ShDM), and the shoot-to-root dry mass ratio (ShDM/RDM) were determined. Both fresh and dry mass values were expressed in grams per plant.

Gas exchanges were measured on the fourth leaf from the apex to the base, between 9:00 and 10:00 a.m., with the infrared gas analyzer - IRGA (model LI-6400XT, LI-COR®, Nebraska, USA) with airflow of 300 mL min⁻¹, humidity between 50-60%, 400 μmol CO₂ and 1000 μmol m⁻² s⁻¹ coupled light source. The stomatal conductance (gs - mol H₂O m⁻² s⁻¹), photosynthesis (A - μmol CO₂ m⁻² s⁻¹), intercellular CO₂ concentration (Ci - μmol CO₂ mol air⁻¹), transpiration (E - mmol H₂O m⁻² s⁻¹), instantaneous water use efficiency (WUE = A/E), intrinsic water use efficiency (iWUE = A/g_s), intrinsic carboxylation efficiency (iCE = A/Ci) and leaf temperature (LT) were evaluated.

The chlorophyll a fluorescence was measured with a modulated fluorometer (Sciences Inc. - Model OS-30p, Hudson, USA). The leaves were subjected to dark adaptation with leaf tweezers for 30 minutes. Initial fluorescence (F₀), maximum fluorescence (F_m), variable fluorescence (F_v = F_m - F₀), F_v/F₀ ratio and quantum yield of photosystem II (PSII = F_v/F_m) were evaluated.

Chlorophyll a (Cha), b (Chb) and total (Tch) indices, and chlorophyll a/b ratio (Cha/b) were determined by the non-destructive method with a portable chlorophyll meter (ClorofiLOG®, model CFL 1030, Porto Alegre, LOL). The chlorophyll indices were expressed as the Falker Chlorophyll Index (FCI).

Statistical analysis

Data were subjected to principal component analysis (PCA) to study the interrelationship between variables and evaluated factors. The statistical program R (R Core Team, 2021) was used to perform the statistical and graphical analyses.

Results

Principal component analysis (PCA) of the mean scores for plant height (PH), stem diameter (SD), number of leaves (NL), number of branches (NS), and leaf area (LA) of *M. piperita* demonstrated a variability of 88.31% (Figure 1). The foliar application of 5.0 mL L⁻¹ of the biostimulant effectively mitigated the detrimental effects of saline stress (ECw of 2.25 dS m⁻¹) on stem diameter (SD). A dose of 1.41 mL L⁻¹ significantly reduced the negative impact of saline stress (ECw of 1.01 dS m⁻¹) on the number of leaves and leaf area. Salinity levels exceeding 3.49 dS m⁻¹ resulted in decreased plant height and a reduced number of branches (NS).

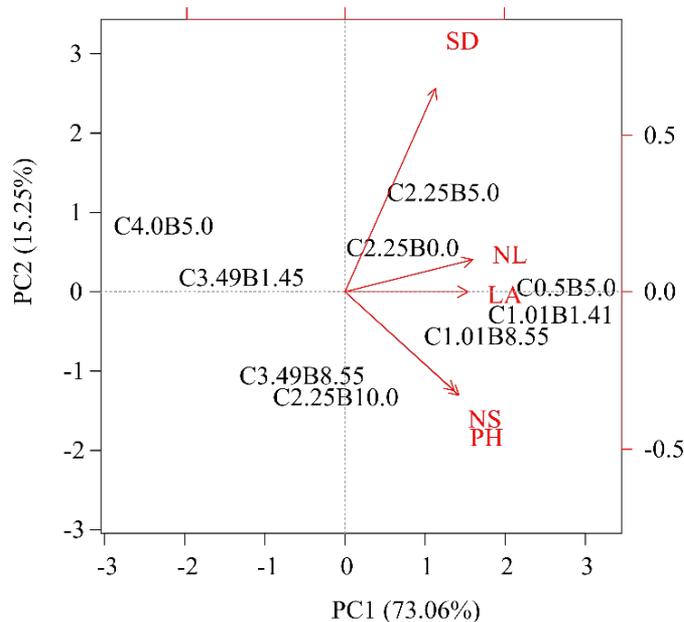


Figure 1. Principal component analysis for plant height (PH), stem diameter (SD), number of leaves (NL), number of branches (NS), and leaf area (LA) of *Mentha piperita* under saline stress and biostimulant application. Treatments: T1 = C1.01B1.41; T2 = C3.49B1.45; T3 = C1.01B8.55; T4 = C3.48B8.55; T5 = C2.25B0.00; T6 = C2.25B10.0; T7 = C4.0B5.0; T8 = C0.5B5.0; T9 = C2.25B5.0.

The principal component analysis (PCA) of the first two mean scores for the fresh and dry masses of *M. piperita* exhibited a variability of 91.4% (Figure 2). The application of 1.45 mL L⁻¹ of the biostimulant proved effective in attenuating the negative effects of electrical conductivity of irrigation water (ECw) up to 1.01 dS m⁻¹ on leaf fresh mass (LFM), total dry mass (TDM), leaf dry mass (LDM), and root dry mass (RDM). Similarly, the application of 1.45 mL L⁻¹ of the biostimulant mitigated the harmful effects of ECw up to 3.49 dS m⁻¹ on leaf dry mass (SDM), shoot dry mass (ShDM), stem fresh mass, stem dry mass (StFM and StDM), and the shoot/root dry mass ratio (ShDM/RDM).

The principal component analysis (PCA) of the first two mean scores for gas exchange of *M. piperita* revealed a variability of 87.13% (Figure 3). The application of 5.0 mL L⁻¹ of the biostimulant resulted in the highest values of stomatal conductance (gs) and transpiration (E) up to an electrical conductivity of irrigation water (ECw) of 2.25 dS m⁻¹. Furthermore, the application of 8.55 mL L⁻¹ of the biostimulant mitigated the negative impact of ECw up to 3.49 dS m⁻¹ on net photosynthesis (A) and intrinsic carboxylation efficiency (iCE). However, the application of the biostimulant did not affect water use efficiency (WUE) and intrinsic water use efficiency (iWUE).

Principal component analysis of the first two mean scores for chlorophyll a fluorescence of *M. piperita* had a variability of 99.44% (Figure 4). The application of 5.0 mL L⁻¹ of biostimulant attenuated the harmful effects of ECw up to 4.0 dS m⁻¹ on the quantum yield of photosystem II (F_v/F_m).

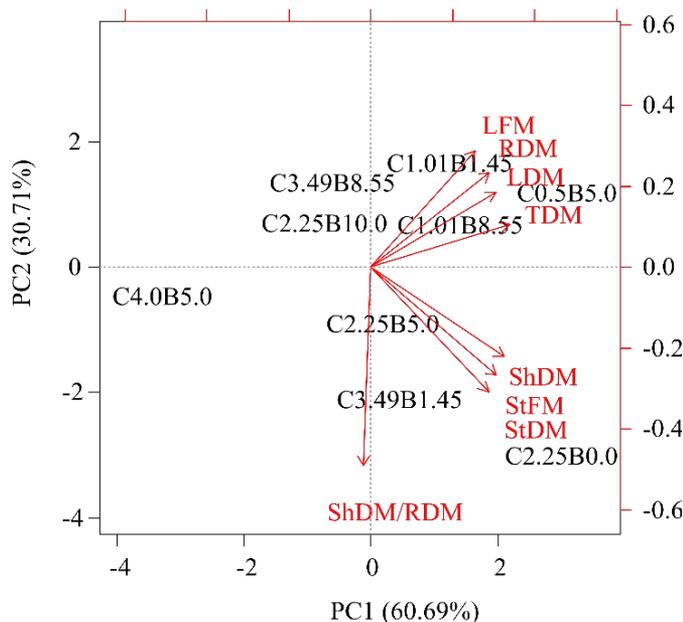


Figure 2. Principal component analysis for leaves fresh mass and leaves dry mass (LFM and LDM), stem fresh mass and stem dry mass (StFM and StDM), root dry mass (RDM) and shoot dry mass (ShDM) and shoot/root dry mass ratio (ShDM/RDM) of *Mentha piperita* under saline stress and biostimulant application. Treatments: T1 = C1.01B1.41; T2 = C3.49B1.45; T3 = C1.01B8.55; T4 = C3.48B8.55; T5 = C2.25B0.00; T6 = C2.25B10.0; T7 = C4.0B5.0; T8 = C0.5B5.0; T9 = C2.25B5.0.

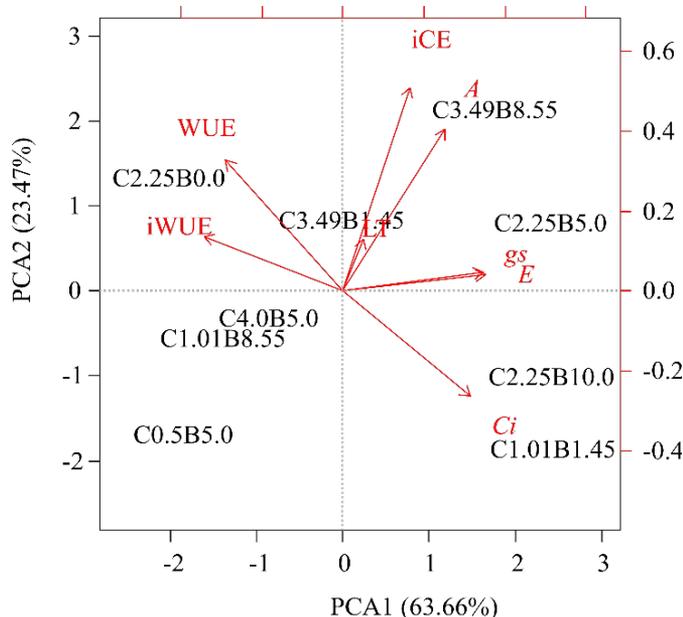


Figure 3. Principal component analysis for stomatal conductance (gs), net photosynthesis (A), intercellular CO₂ concentration (Ci), transpiration (E), water use efficiency (WUE), intrinsic water use efficiency (iWUE), intrinsic carboxylation efficiency (iCE) and leaf temperature (LT) of *Mentha piperita* under saline stress and biostimulant application. Treatments: T1 = C1.01B1.41; T2 = C3.49B1.45; T3 = C1.01B8.55; T4 = C3.48B8.55; T5 = C2.25B0.00; T6 = C2.25B10.0; T7 = C4.0B5.0; T8 = C0.5B5.0; T9 = C2.25B5.0.

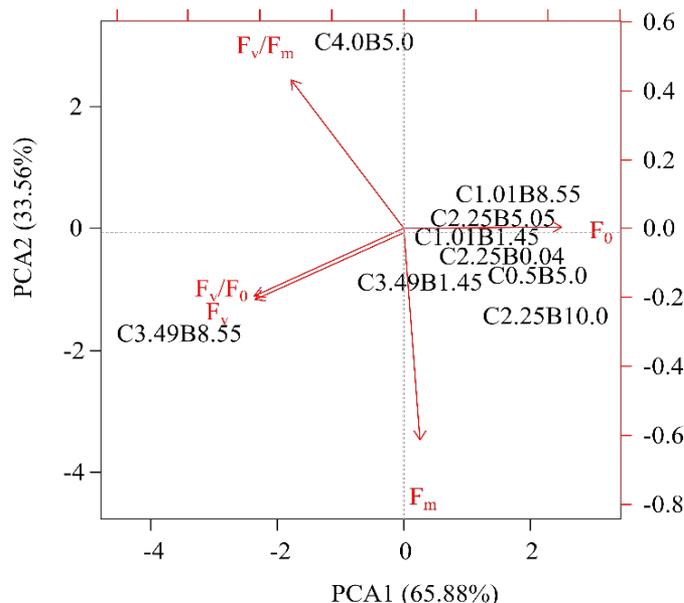


Figure 4. Principal component analysis for initial fluorescence (F₀), maximum fluorescence (F_m), variable fluorescence (F_v), F_v/F₀ ratio and quantum yield of photosystem II (F_v/F_m) of *Mentha piperita* under saline stress and biostimulant application. Treatments: T1 = C1.01B1.41; T2 = C3.49B1.45; T3 = C1.01B8.55; T4 = C3.48B8.55; T5 = C2.25B0.00; T6 = C2.25B10.0; T7 = C4.0B5.0; T8 = C0.5B5.0; T9 = C2.25B5.0.

The dose of 8.55 mL L⁻¹ of biostimulant reduced the negative effects of saline stress on F_v and F_v/F₀ up to ECw 3.49 dS m⁻¹.

The principal component analysis of the first two mean scores for the chlorophyll indices of *M. piperita* had a variability of 99.31% (Figure 5). The application of 8.55 mL L⁻¹

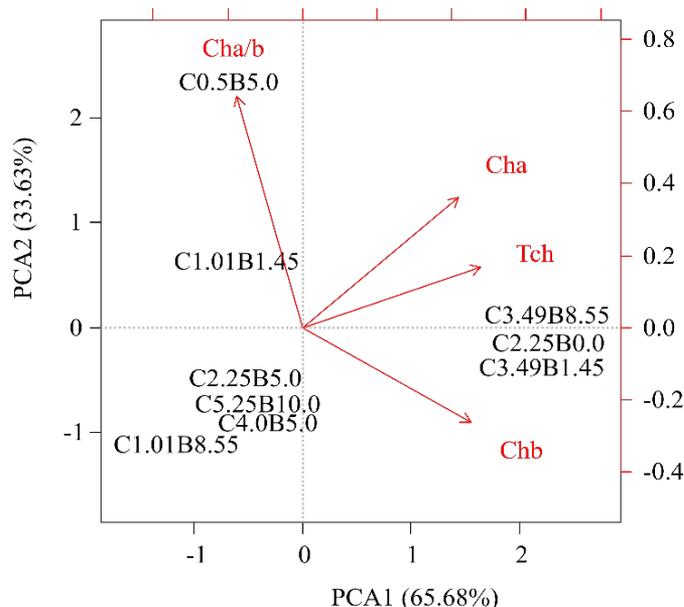


Figure 5. Principal component analysis for chlorophyll a (Cha), b (Chb), total (Tch) indices and chlorophyll a/b ratio (Cha/b) of *Mentha piperita* under saline stress and biostimulant application. Treatments: T1 = C1.01B1.41; T2 = C3.49B1.45; T3 = C1.01B8.55; T4 = C3.48B8.55; T5 = C2.25B0.00; T6 = C2.25B10.0; T7 = C4.0B5.0; T8 = C0.5B5.0; T9 = C2.25B5.0.

of biofertilizer attenuated the harmful effects of EC_w up to 3.49 dS m⁻¹ on the chlorophyll a (Cha) and total (Tch) indices and 1.45 mL L⁻¹ on the chlorophyll b (Chb) index. The highest chlorophyll a/b (Cha/b) ratio was observed in the EC_w of 0.5 dS m⁻¹ and 5.0 mL L⁻¹ of biofertilizer.

Discussion

The damage caused by saline stress on the growth of *M. piperita* plants was reduced by the application of the biostimulant. Possibly, the presence of phytohormones increased the plants' osmotic adjustment capacity, improving their defense mechanisms against saline stress. In this sense, cytokinin and gibberellin present in the biostimulant act in gene signaling and expression, inducing the osmoprotectors production, increasing the tolerance of plants to salinity ([Hadia et al., 2020](#)).

Furthermore, the application of the biostimulant resulted in increased biomass accumulation in *M. piperita* plants, suggesting that the presence of growth regulators attenuated the effects of salinity. Hormone accumulation triggers changes in gene expression, promoting increased tolerance to stress conditions, and gibberellins act as elicitors in signaling the plants defense mechanisms against saline stress ([Khan et al., 2020](#)). Additionally, cytokinins and gibberellins, at low concentrations, can stimulate cell division, expansion processes, and overall cell functions ([Small & Degenhardt, 2018](#)), enabling biomass accumulation even under adverse conditions.

The biostimulant effectively reduced the damage caused by saline stress on gas exchange parameters, resulting in increased rates of stomatal conductance (gs), transpiration (E), net photosynthesis (A), and intrinsic carboxylation efficiency (iCE). This improvement can be attributed to the presence of cytokinins and gibberellins in the biostimulant composition, which play a role in regulating physiological processes in plants, including photosynthesis ([Gururani et al., 2015](#)). These phytohormones, at specific levels, contribute to the regulation of genes involved in the biosynthesis of compounds that enhance plant tolerance to saline stress ([Fahad et al., 2015](#); [Arif et al., 2020](#)).

The application of the biostimulant resulted in the stimulation of chlorophyll a and total indices, indicating that the plant regulators present in its composition played a role in responding to saline stress. These regulators contribute to the synthesis of enzymes and osmolytes that help alleviate the damage caused by salinity, and these metabolites are involved in growth and photosynthetic characteristics ([Khanam & Mohammad, 2018](#)). Cytokinins and gibberellins are hormones that participate in cell division and expansion processes, as well as inducing the synthesis of proteins and photosynthetic pigments ([Taiz et al., 2017](#)).

The application of the biostimulant resulted in the stimulation of chlorophyll a fluorescence and the quantum yield of photosystem II, effectively reducing the damage caused by saline stress and enhancing the photochemical

efficiency of *M. piperita* plants. Biostimulants have a significant impact on the physiology of plants under stress conditions, as they regulate the synthesis of growth regulators that participate in various processes, including gas exchange and chlorophyll a fluorescence ([Malik et al., 2021](#)). The presence of these phytohormones likely improved the cellular osmoregulation process and the production of osmoprotective compounds, thereby reducing the effects of oxidative stress ([Khan et al., 2018](#)).

Conclusions

The foliar application of the biostimulant effectively mitigated the damage caused by saline stress, resulting in improvements in growth, gas exchange, chlorophyll index, and photochemical efficiency of *Mentha piperita*.

Specifically, the foliar application of 8.55 mL L⁻¹ of the biostimulant reduced the negative effects of saline stress up to an EC_w of 3.49 dS m⁻¹, particularly on chlorophyll a and total indices, as well as on photosynthesis and phytomass production of *M. piperita*.

Furthermore, the application of 1.45 mL L⁻¹ of the biostimulant attenuated the impact of salinity on the phytomass production of *M. piperita* up to an EC_w of 3.49 dS m⁻¹.

Compliance with Ethical Standards

Author contributions: Conceptualization: JSN, FRAF; Data curation: LVS, RTF; Formal analysis: TIS; Investigation: JSN, TIS, LVS, JESR, FRAF, RTF; Methodology: JESR; Project administration: TJD; Resources: TJD, RLAB; Supervision: RLAB; Writing – original draft: JSN, TIS, JESR, FRAF; Writing – review & editing: JSN, TIS, JESR, TJD, RLAB.

Conflict of interest: The authors declare that they have no conflict of interest.

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