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MICROALGAE-BASED BIOSTIMULANT AS A MODULATOR OF GAS EXCHANGE AND WATER USE EFFICIENCY IN SOYBEAN UNDER WATER DEFICIT

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ABSTRACT

Climate change has increased rainfall irregularity, making water management critical for soybean productivity. Microalgae-based biostimulants have been proposed to mitigate the effects of water restriction on plant physiology. This study evaluated the effect of foliar application of Scenedesmus obliquus on transpiration and water use efficiency in soybean plants under contrasting water regimes. The experiment was conducted under controlled conditions using the cultivar Embrapa 59 grown in sandy soil (65% sand). A completely randomized design was adopted with four replicates, combining three biostimulant doses (0, 0.3, and 1.2 g L^{-1}) and two water management regimes (with and without deficit). A 30-day water deficit was imposed from the R1 stage. Transpiration rate (E) and water use efficiency (A/E) were measured using an infrared gas analyzer. Microalgae application significantly increased water use efficiency, particularly under water deficit (up to 250%), and helped maintain transpiration compared to the control. The highest dose produced the most favorable responses, indicating the potential of this biostimulant under water-limited conditions, although further studies are needed to standardize application protocols.

Palavras-Chave:
Bioestimulante foliar;
Scenedesmus obliquus
Ondutância estomática
Transpiração

BIOESTIMULANTE À BASE DE MICROALGAS COMO MODULADOR DAS TROCAS GASOSAS E DA EFICIÊNCIA DO USO DA ÁGUA NA SOJA SOB DÉFICIT HÍDRICO

RESUMO

As mudanças climáticas têm intensificado a irregularidade das chuvas, tornando o manejo da água um fator crítico para a produtividade da soja. Bioestimulantes à base de microalgas têm sido propostos como estratégia para mitigar os efeitos da restrição hídrica sobre a fisiologia vegetal. Este estudo avaliou o efeito da aplicação foliar da microalga Scenedesmus obliquus sobre a transpiração e a eficiência de uso da água em plantas de soja submetidas a regimes hídricos contrastantes. O experimento foi conduzido em condições controladas, utilizando a cultivar Embrapa 59 cultivada em solo arenoso (65% de areia). Adotou-se delineamento inteiramente casualizado, com quatro repetições, combinando três doses do bioestimulante (0, 0,3 e 1,2 g L^{-1}) e dois manejos hídricos (com e sem déficit). O déficit hídrico foi imposto por 30 dias a partir do estádio R1. A taxa de transpiração (E) e a eficiência de uso da água (A/E) foram determinadas por analisador de gases infravermelho. A aplicação da microalga aumentou significativamente a eficiência de uso da água, especialmente sob déficit hídrico (até 250%), além de contribuir para a manutenção da transpiração em relação ao controle. A maior dose apresentou as respostas mais favoráveis, indicando o potencial do bioestimulante em condições de limitação hídrica, embora estudos adicionais sejam necessários para a padronização dos protocolos de aplicação

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INTRODUCTION

In the current global scenario, agriculture faces increasing challenges related to water availability and management, particularly in crops of major economic importance such as soybean (JUNPING *et al*., 2020). Soybean (Glycine max L.) is one of the most relevant agricultural crops worldwide, playing a strategic role in both the global economy and food security (STANIAK *et al*., 2023). Brazil currently leads global soybean production and exports, with an estimated production of 166.33 million tons for the 2024/25 growing season (CONAB, 2025), driven by technological advances and adaptation to diverse climatic conditions.

Despite this productive expansion, soybean is highly sensitive to water deficit, and prolonged periods of water restriction can significantly impair plant development, particularly under rainfed cultivation systems (MARQUES *et al*., 2023). According to Melo *et al*. (2024), even moderate water stress conditions can compromise vegetative growth and yield, resulting in losses exceeding 50%. Therefore, effective water management is a key determinant of agricultural sustainability, especially in the context of climate change (JUNPING *et al*., 2020).

In this regard, plant water use efficiency has become a critical factor for maintaining productivity while promoting the rational use of water resources in agricultural systems (GITAU *et al*., 2022). Consequently, strategies aimed at optimizing water use have gained increasing attention, particularly technologies that enhance plant resilience to periods of water limitation.

Among these strategies, biostimulants have emerged as a promising approach. These products, derived from substances or microorganisms, are capable of modulating plant physiological processes and enhancing adaptation to adverse environmental conditions (JUNPING *et al*., 2020). Biostimulants act at multiple physiological levels, promoting nutrient uptake and regulating the synthesis of plant hormones associated with abiotic stress responses (LAMB *et al*., 2023).

In this context, microalgae have been identified as a particularly promising alternative. Species such as Scenedesmus obliquus synthesize a wide range of bioactive compounds, including amino acids, phytohormones, antioxidants, and proteins, which exert beneficial effects on plant physiology, especially with respect to water regulation processes such as transpiration and water use efficiency (YOUSSEF *et al*., 2023). According to Gitau *et al*. (2022), foliar application of microalgal extracts enables direct absorption of these compounds by the leaves, accelerating physiological responses even under critical water deficit conditions (MELO *et al*., 2024).

Despite the recognized potential of microalgae-based biostimulants, significant knowledge gaps remain regarding the standardization of application protocols, including dosage, application frequency, and management practices, which currently limit their broader adoption in agricultural systems (GITAU *et al*., 2022; YOUSSEF *et al*., 2023). Therefore, a deeper understanding of the effects of microalgae application on physiological parameters associated with soybean water performance is essential to enhance their use as effective biostimulants.

Accordingly, the objective of this study was to evaluate the effects of foliar application of a microalgae-based biostimulant on transpiration and water use efficiency in soybean plants subjected to contrasting water management conditions, with and without water deficit.

MATERIAL E METHODS

Experimental Conditions

The experiment was conducted in a greenhouse at the Federal University of Viçosa, Brazil, between September 2024 and January 2025. The greenhouse is located at 20°45′ S and 42°08′52″ W, at an altitude of 670 m above sea level. Soybean seeds (Glycine max L.), cultivar Embrapa 59 were used. This cultivar, released in 1996, is classified as having a determinate growth habit and a semi-early maturity group. It is characterized by high susceptibility to water deficit, which makes it suitable for studies involving water stress (OYA *et al*., 2004; POUDEL *et al*., 2023). The cultivar genealogy is FT-Abyara × BR 83-147, line BR 90-5825 (EMBRAPA, 2004).

Plants were grown in 4 L pots filled with sandy soil (65% sand). The experiment was designed to expose plants to water-limited conditions in order to evaluate physiological responses under extreme water management scenarios.

Experimental design and water management

A completely randomized design was adopted in a 2 × 3 factorial arrangement, with four replicates, totaling 24 experimental units. The factors consisted of two water management regimes (with and without water deficit) and three doses of a microalgae-based biostimulant (0, 0.3, and 1.2 g L^{-1}). Water deficit was imposed at the R1 phenological stage and maintained for 30 days (MARQUES *et al*., 2023). Plants under non-deficit conditions were irrigated every two days, maintaining soil moisture close to 90% of field capacity. During the water deficit period, irrigation was controlled daily to simulate a soil water tension of approximately 900 kPa (YOUSSEF *et al*., 2023; MELO *et al*., 2024).

The irrigation depth was determined gravimetrically based on the difference in pot mass between two consecutive irrigations, allowing the estimation of crop evapotranspiration and the corresponding water replacement requirement, as described by Bernardo *et al*. (2019) and expressed in Equation (1):

$$\mathrm{ETc} = \mathrm{M}_{\mathrm{pot}} i - M_{pot\ i+1} \quad (1)$$

where:

ETc is crop evapotranspiration expressed as water volume (L);

$M_{pot\ i}$ is the pot mass on day i (kg);

$M_{pot\ i+1}$ is the pot mass on the subsequent day (kg).

Microalgae cultivation and biostimulant application

The microalga used in this study, Scenedesmus obliquus (strain BR003), was cultivated at the Biofuels Laboratory of the Federal University of Viçosa in a 4.000 L raceway pond under photoautotrophic batch cultivation, following the methodology described by Rocha *et al*. (2019). The harvested biomass was centrifuged and diluted to obtain the target concentrations and subsequently subjected to ultrasonic treatment for one hour to promote cell disruption.

Biostimulant application was performed once via foliar spraying, one day prior to the induction of water deficit (GITAU *et al*., 2022). A volume of 32 mL per treatment was applied, while control plants received the same volume of water (MELO *et al*., 2024).

Physiological measurements

Physiological evaluations were carried out during the water deficit period. Plant physiological responses were inferred based on gas exchange measurements obtained using an infrared gas analyzer (IRGA, LCpro T). Measurements were conducted under a reference CO_2 concentration of 390 μmol mol^{-1} and a photosynthetic photon flux density of 1200 μmol m^{-2} s^{-1}. The recorded parameters allowed the determination of transpiration rate (E) and water use efficiency based on gas exchange (A/E), calculated as the ratio between net photosynthetic rate and transpiration rate.

Statistical analysis

The data were subjected to tests of normality (Shapiro–Wilk) and homogeneity of variances (Bartlett). Analysis of variance was performed using the F test at 1% and 5% significance levels. Mean values were compared using Tukey's test at the 5% significance level. All statistical analyses were conducted using R software (version 4.4.2; R Core Team, 2024).

RESULTS E DISCUSSION

The results obtained for transpiration (E) and water use efficiency (A/E) for each treatment are presented in Table 1. The analysis of variance revealed that both biostimulant dose and water management significantly influenced soybean physiological responses.

Based on the results, it was observed that, regardless of the water management regime adopted, the biostimulant dose of 1.2 g L^{-1} exhibited the highest mean values for the evaluated variables, indicating a positive dose-dependent

Table 1. Mean squares from the analysis of variance for transpiration and water use efficiency (A/E) of soybean as affected by microalgae doses (D) and water management regimes (WM). UFV, 2024-2025

Variable	Mean Square			CV	Dose	Water Management	
	D	MH	D x WM	(%)	(g L^{-1})	Water deficit	No water deficit
Transpiration (mmol H_2Om^{-2} s^{-1})	$3.6x\ 10^{0**}$	$6.0\ x\ 10^{0}$**	$1.5\ x\ 10^{0}$**	13.6	0	0.775 Bb	1.350 Ca
					0.3	0.505 Bb	2.270 Ba
					1.2	1.330 Ab	3.460 Aa
Water use efficiency (A/E)	2.5x 10**	$1.5\ x\ 10^{-2**}$	$1.3\ x\ 10^{-1**}$	6.5	0	0.115 Cb	0.136 Ba
					0.3	0.290 Ba	0.254 Aa
					1.2	0.403 Aa	0.265 Ab

* and ** indicate significance at the 5% and 1% probability levels, respectively. Means followed by the same uppercase letters within columns and lowercase letters within rows do not differ according to Tukey's test ($p < 0.01$)

effect of the microalga on soybean physiological performance.

In general, plants grown under non-water-deficit management showed higher transpiration rates, which is an expected response due to greater soil water availability and the consequent maintenance of stomatal opening. This condition favors water vapor diffusion and CO_2 assimilation (WINCK *et al.*, 2021; SARSEKEYEVA *et al.*, 2024).

Conversely, water-restricted conditions promoted higher water use efficiency values. According to Joan *et al.* (2021), this response reflects a physiological adjustment that enables the optimization of carbon assimilation in relation to water loss, representing a strategic adaptive mechanism of plants under water deficit conditions (ZUNIC *et al.*, 2024).

A detailed analysis of transpiration behavior, presented in Figure 1, demonstrates that, under both water management regimes, the application of the highest biostimulant dose resulted in the greatest transpiration rates. Under water deficit conditions, transpiration reached 1.33 mmol H_2O m^{-2} s^{-1}, whereas under non-restrictive water conditions, values of 3.46 mmol H_2O m^{-2} s^{-1} were recorded. These results indicate that microalgae application contributed to the maintenance of physiological activity even under adverse conditions, partially mitigating the negative effects of water limitation on stomatal functioning and transpiratory flux (SARSEKEYEVA *et al.*, 2024).

The observed values are consistent with the results reported by Lamb *et al.* (2023) and Melo *et al.* (2024), who also documented increases in transpiration rates in plants treated with microalgae-based biostimulants under abiotic stress conditions. This convergence of findings reinforces the consistency of the physiological effects associated with microalgae application, indicating that these biostimulants act as modulators of plant water and gas exchange regulation mechanisms, rather than merely serving as nutritional sources or transient stimulatory inputs (GITAU *et al.*, 2022; ZUNIC *et al.*, 2024).

Under water deficit conditions, as expected, transpiration was reduced compared to treatments under adequate irrigation, reflecting an adaptive strategy adopted by plants to limit water loss and preserve tissue water status (GITAU *et al.*, 2022). Transpiration, which is responsible for water loss in the form of vapor, together with stomatal conductance, is intrinsically linked

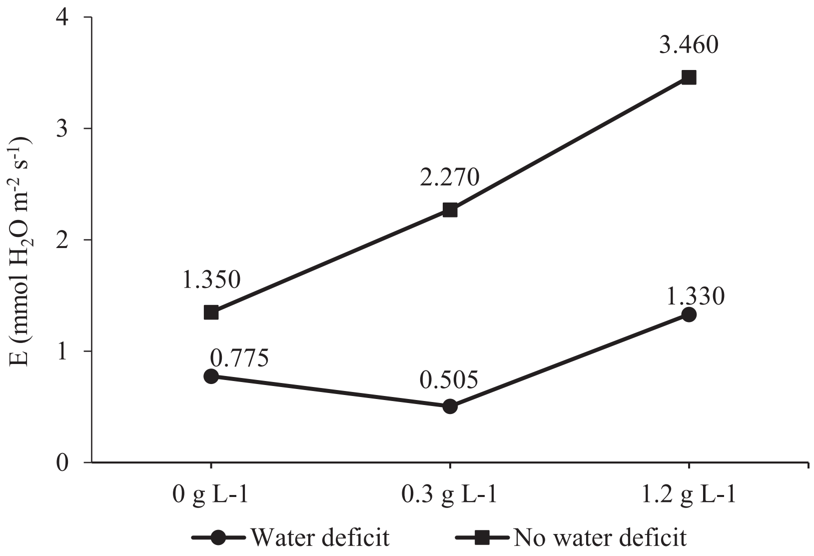

Figure 1. Soybean transpiration as a function of biostimulant dose under different water management conditions

to photosynthesis, since both processes are regulated by stomatal aperture. Stomatal opening simultaneously enables CO_2 diffusion into the leaf and the release of water vapor to the atmosphere (YOUSSEF *et al*., 2023). As highlighted by Zade *et al*. (2023), excessive reductions in transpiration resulting from stomatal closure tend to constrain carbon assimilation and compromise the photosynthetic balance, with negative consequences for crop growth and productivity.

Within this context, considering the functional integration of gas exchange processes, it can be inferred that, in addition to contributing to the maintenance of transpiration, microalgae application exerted positive effects on photosynthetic activity and stomatal conductance (STANIAK *et al*., 2023; SARSEKEYEVA *et al*., 2024). Stomatal conductance refers to the ease with which gases diffuse through the stomata and represents a key parameter in the simultaneous regulation of transpiration and photosynthesis (MARQUES *et al*., 2023).

Accordingly, higher stomatal conductance values indicate greater stomatal aperture, allowing increased CO_2 influx for photosynthesis while simultaneously regulating water loss through transpiration (MELO *et al*., 2024). Thus, the maintenance of relatively high transpiration rates under water deficit treatments suggests that microalgae application may have promoted a more efficient stomatal adjustment, preventing excessive stomatal closure and preserving the balance between carbon assimilation and water conservation (JOAN *et al*., 2021; ZUNIC *et al*., 2024).

Several authors attribute the positive effects observed on gas exchange processes to the biochemical composition of microalgae, which is directly associated with the presence of biologically active compounds. According to Junping *et al*. (2020) and Youssef *et al*. (2022), the presence of phytohormones, amino acids, and antioxidants can enhance stomatal regulation and stabilize photosynthetic processes, thereby promoting greater physiological efficiency under abiotic stress conditions. These compounds act on metabolic pathways related to hormonal signaling and protection of the photosynthetic apparatus, enabling plants to maintain higher levels of gas exchange without a proportional increase in water loss (LAMB *et al*., 2023). Consequently, the use of microalgae-based biostimulants may promote greater physiological resilience, allowing soybean plants to respond more efficiently to limiting water availability conditions (LAMB *et al*., 2023; ZUNIC *et al*., 2024).

In contrast, water use efficiency (A/E) exhibited an inverse pattern relative to transpiration in response to water management. As shown in Figure 2, the highest efficiency values were recorded under water deficit conditions, partially challenging simplistic assumptions that directly associate greater soil water availability with higher plant physiological efficiency (MARQUES *et al*., 2023; MELO *et al*., 2024).

Water use efficiency (A/E) expresses the plant's ability to maximize carbon assimilation relative to water lost through transpiration (GITAU *et al*., 2022). It represents an instantaneous physiological indicator that quantifies the relationship between CO_2 fixation and water consumption and is widely used to assess plant responses to water deficit and the balance between carbon assimilation processes and water-saving strategies (MELO *et al*., 2024; WINCK *et al*., 2024). From this perspective, higher A/E values reflect a more efficient physiological adjustment among stomatal aperture, photosynthetic activity, and control of water loss (ZADE *et al*., 2023).

As observed, although the non-deficit water management promoted higher absolute transpiration rates, the highest A/E values were recorded under water restriction, particularly at the highest biostimulant dose, which reached values of 0.403 and 0.265 under deficit and non-deficit conditions, respectively. This pattern suggests that, under limited water availability, soybean plants were able to adjust their physiological mechanisms to reduce excessive water losses while maintaining, albeit at moderate levels, CO_2 assimilation, resulting in improved efficiency in the use of water resources (MARQUES *et al*., 2023).

According to Gitau *et al*. (2022), this physiological adjustment occurs mainly as a consequence of reduced transpiration associated with partial stomatal closure, which limits water loss from the leaves. Consequently, despite a concomitant restriction in carbon assimilation, the balance between fixed CO_2 and transpired water becomes more favorable, allowing plants to use water more rationally and to maximize productivity per unit of water consumed (RACHIDI *et al*., 2021; SOLOMON *et al*., 2023).

Similar results were reported by Lamb *et al*. (2023) and Melo *et al*. (2024), who observed water use efficiency values exceeding 1.48 in soybean plants subjected to water deficit and treated with microalgae-based biostimulants, thereby corroborating the role of these inputs in modulating physiological responses under abiotic stress conditions.

As emphasized by Rachidi *et al*. (2021) and Zade *et al*. (2023), water use efficiency does not depend exclusively on the amount of water available within the soil–plant–atmosphere continuum, but rather on the plant's capacity to

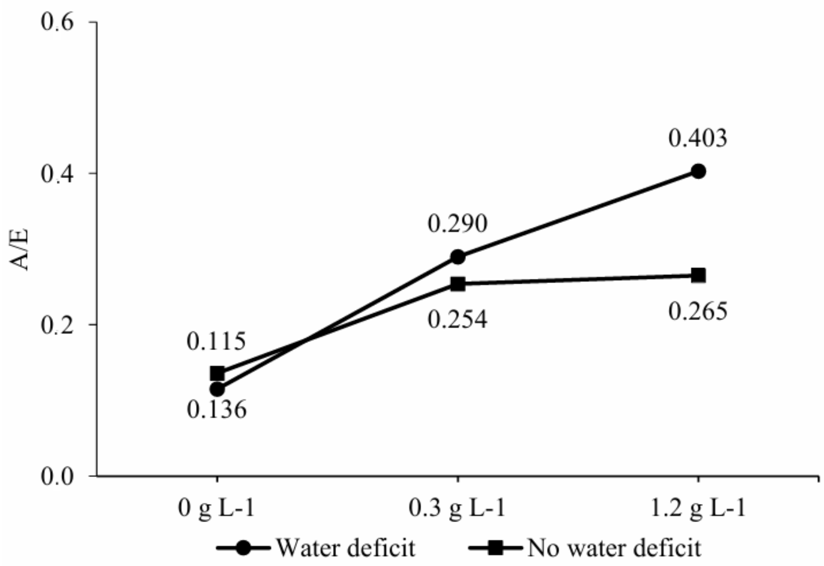

Figure 2. Water use efficiency as a function of biostimulant dose under different water management conditions

optimize the balance between CO_2 assimilation and transpiration. Under water deficit conditions, a controlled reduction in stomatal conductance may limit water loss proportionally more than carbon assimilation, resulting in increased water use efficiency (STANIAK *et al*., 2023).

The integrated analysis of the results further indicates that, even at the lowest dose (0.3 g L^{-1}), the application of the microalgae-based biostimulant exerted a positive effect on the evaluated physiological variables. The percentage-based analysis presented in Table 2 highlights the magnitude of the biostimulant effects relative to the control treatments, which consistently exhibited the lowest transpiration and water use efficiency values under both water management regimes. An exception was observed for the 0.3 g L^{-1} dose with respect to transpiration under water deficit conditions, suggesting the existence of a minimum physiological response threshold to product application (RACHIDI *et al*., 2021; MELO *et al*., 2024).

Overall, when compared with untreated plants, those receiving the highest biostimulant dose (1.2 g L^{-1}) exhibited increases exceeding 70% in transpiration under water deficit and more than 150% under non-deficit conditions. For water use efficiency, the gains were even more pronounced, reaching increases of up to 250% under water deficit and 94% under adequate water management.

According to Winck *et al*. (2021), the favorable effects observed with product application may be associated, among other factors, with the action of extracellular polysaccharides (EPS) present in microalgae. These compounds contribute to maintaining the water status of the soil–plant system and stimulate the synthesis of antioxidant compounds, thereby mitigating physiological damage induced by water deficit (SUCHITHRA *et al*., 2022).

Studies have shown that microalgae application supplies metabolites such as glycine, betaine, and free amino acids, which play a central role in chlorophyll biosynthesis and in the metabolic regulation associated with stress tolerance (JOAN *et al*., 2021; MARQUES *et al*., 2023; SOLOMON *et al*., 2023). Taken together, these mechanisms indicate that microalgae exert a multifunctional effect, simultaneously promoting the maintenance of photosynthetic capacity and the activation of antioxidant defense systems, thereby favoring plant physiological adaptation under adverse conditions (YOUSSEF *et al*., 2022).

From a physiological standpoint, the modulation driven by compounds present in microalgae may have resulted in increased or maintained stomatal conductance at adequate levels, enhancing CO_2 assimilation without excessively intensifying water loss through transpiration (JUNPING *et al*., 2020). This regulation among stomatal opening, photosynthesis, and transpiration enables plants to maximize water use efficiency under both

Table 2. Percentage increase in transpiration and water use efficiency relative to the control treatment under different water management regimes and biostimulant doses

Variable	Dose ($g\ L^{-1}$)	Water Management	
		Water deficit	No water deficit
		Increase (%)	
Transpiration ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	0	-	-
	0.3	-34.84	68.15
	1.2	71.61	156.30
Water use efficiency (A/E)	0	-	-
	0.3	152.17	86.76
	1.2	250.43	94.85

favorable conditions and water restriction (JOAN *et al*., 2021). Accordingly, the intensification of physiological activity observed in this study in response to biostimulant application suggests an improved capacity of plants to optimize resource use and sustain high physiological performance across contrasting water availability scenarios (LAMB *et al*., 2023).

Despite the demonstrated potential of microalgae use in soybean cultivation, uncertainties remain regarding the optimal application protocol, including biostimulant dose, frequency, and method of delivery. In addition, factors such as phenological stage, intensity of water deficit, and environmental conditions may influence the magnitude of the observed physiological responses (GITAU *et al*., 2022; MELO *et al*., 2024). Therefore, further studies are essential to advance knowledge and to consolidate microalgae-based biostimulants as a sustainable strategy for soybean management.

CONCLUSIONS

- Based on the results obtained, foliar application of the microalgae-based biostimulant proved effective in mitigating the adverse effects of water deficit on soybean physiology, contributing to improved physiological regulation, particularly at the dose of 1.2 g L^{-1}.
- Microalgae application enhanced the regulation of gas exchange, supporting the maintenance of transpiration under water deficit conditions, with increases of up to approximately 70% compared to untreated plants.
- Furthermore, the use of the biostimulant improved plant water use efficiency, resulting in increases of up to approximately 250% under water-restricted conditions, thereby promoting more efficient utilization of available water resources.
- Overall, the application of microalgae-based biostimulants represents a promising strategy for improving soybean performance under water-limited conditions and contributes to the development of more sustainable agricultural systems.

AUTHORSHIP CONTRIBUTION STATEMENT

PINHEIRO, K. D. R.: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft; **CUNHA, F. F.:** Conceptualization, Methodology, Supervision, Writing – review & editing; **ROCHA, D. N.:** Conceptualization, Methodology, Supervision, Validation; **MARTINS, M. A.:** Funding acquisition, Resources, Visualization; **NIÑO, J. N. R.:** Investigation, Visualization.

DECLARATION OF INTEREST

The authors declare that they have no financial or personal interests that could influence the work reported in this article.

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