

Application of chitosan in the cultivation of colored fiber cotton 'BRS Jade' under water restriction

Aplicação de quitosana no cultivo do algodoeiro de fibra colorida 'BRS Jade' sob restrição hídrica

Kheila Gomes Nunes^{1*}; Vera Lúcia Antunes de Lima², Geovani Soares de Lima²; Lauriane Almeida dos Anjos Soares³; André Alisson Rodrigues da Silva⁴; Denis Soares Costa¹; Allesson Ramos de Souza¹; Thiago Filipe de Lima Arruda⁵; Mirandy dos Santos Dias⁵; Francisco de Assis da Silva⁶

Highlights

Water restriction reduces the levels of photosynthetic pigments in cotton.

Chitosan can increase seed cotton weight under water restriction.

Chitosan spraying is capable of reducing the effects of water restriction.

Abstract

In the semi-arid region of Northeastern Brazil, temporal and spatial variations in rainfall are common, resulting in water limitations that significantly impact production, especially of cotton. In this context, chitosan may serve as a strategy to minimize the effects of water deficits by enhancing water and nutrient absorption. This study aimed to evaluate the effectiveness of different concentrations of chitosan as a mitigator of water restriction in the cultivation of the naturally colored fiber cotton variety 'BRS Jade'. The plants were cultivated in drainage lysimeters under greenhouse conditions. A completely randomized design was implemented using a 2 × 4 factorial arrangement, which included two levels of irrigation (100% and 50% of the water requirement of the crop) and four chitosan concentrations

¹ Master's Scholar of Agricultural Engineering Course, Center of Technology and Natural Resources, Universidade Federal de Campina Grande, UFCG, Campina Grande, PB, Brazil. E-mail: kheilagomesnunes@gmail.com; deniscosta1313@gmail.com; allesson13@outlook.com

² Profs., Post Graduate Program in Agricultural Engineering, UFCG, Campina Grande, PB, Brazil. E-mail: vera.lucia@professor.ufcg.edu.br; geovani.soares@professor.ufcg.edu.br

³ Profa, Academic Unit of Agricultural Sciences, Center of Agrifood Science and Technology, UFCG, Pombal, PB, Brazil. E-mail: laurispo.agronomia@gmail.com

⁴ Postdoctoral Fellow, Post Graduate Program in Engineering and Management of Natural Resources, UFCG, Campina Grande, PB, Brazil. E-mail: andrealisson_cgpb@hotmail.com

⁵ Doctoral Scholar of Agricultural Engineering Course, Post Graduate Program in Agricultural Engineering, Center of Technology and Natural Resources, UFCG, Campina Grande, PB, Brazil. E-mail: thiago.filipe.la@gmail.com; mirandydias@gmail.com

⁶ Postdoctoral Fellow, Post Graduate Program in Agricultural Engineering, Center of Technology and Natural Resources, UFCG, Campina Grande, PB, Brazil. E-mail: agrofdsilva@gmail.com

* Author for correspondence

(0.0, 0.25, 0.50, and 0.75 g L⁻¹), with three replications and one plant per plot. Water restriction at 50% of the required amount reduced the relative water content, the synthesis of photosynthetic pigments, and seed cotton weight. However, foliar application of chitosan at concentrations between 0.25 and 0.50 g L⁻¹ alleviated the detrimental effects of water restriction on the chlorophyll b content; carotenoid content; stem diameter; leaf area; 100-seed weight; average boll weight; seed cotton weight; total boll dry biomass; and dry biomass of stems, leaves, and shoots of the 'BRS Jade' colored fiber cotton plant.

Key words: *Gossypium hirsutum* L. Acclimation. Biostimulant. Abiotic stress.

Resumo

Na região semiárida do Nordeste brasileiro é comum a ocorrência de variação temporal e espacial de chuvas, resultando em limitações hídricas que afetam a produção, especialmente do algodoeiro. Nesse sentido, a quitosana pode ser uma estratégia para minimizar os efeitos do déficit hídrico, melhorando a absorção de água e nutrientes. Objetivou-se com este estudo avaliar o efeito de concentrações de quitosana como atenuante da restrição hídrica no cultivo do algodoeiro de fibra naturalmente colorida 'BRS Jade'. As plantas foram conduzidas em lisímetros de drenagem sob condições de casa de vegetação. Utilizou-se o delineamento inteiramente casualizado em esquema fatorial 2 × 4, sendo duas lâminas de irrigação (100 e 50% da necessidade hídrica da cultura) e quatro concentrações de quitosana (0,0; 0,25; 0,50 e 0,75 g L⁻¹) com três repetições e uma planta por parcela. A restrição hídrica em 50% da lâmina reduziu o conteúdo relativo de água, a síntese de pigmentos fotossintéticos e a massa de algodão em caroço. Entretanto, a aplicação foliar de quitosana entre as concentrações de 0,25 e 0,50 g L⁻¹ amenizou os efeitos deletérios da restrição hídrica sobre o teor de clorofila b, carotenoides, diâmetro do caule, área foliar, peso de 100 sementes, peso médio de capulho, massa de algodão em caroço, fitomassa seca total de capulhos, fitomassa seca do caule, da folha e da parte aérea do algodoeiro de fibra colorida 'BRS Jade'.

Palavras-chave: *Gossypium hirsutum* L. Aclimação. Bioestimulante. Estresse abiótico.

Introduction

In the context of global climate change, concerns about water availability have intensified, both quantitatively and qualitatively. The unchecked consumption of water has progressively limited this natural resource, which serves various purposes, notably agriculture. This sector accounts for an average consumption of 70% of the total volume extracted (Koch et al., 2019; Medeiros et al., 2019; Tavares et al., 2019). Water is a critical factor influencing

numerous physiological processes, including aspects of plant growth, development, and metabolism (Magalhães et al., 2020).

The semi-arid region of Northeast Brazil faces notable challenges due to its intrinsic water limitations (Soares et al., 2018). This situation is aggravated by significant temporal and spatial variability in rainfall, high rates of evapotranspiration, and consequently complex water management demands (Lacerda et al., 2022; Wanderley et al., 2022). Given these climatic traits, irrigation is often essential to ensure reliable

agricultural production in the area (Lima et al., 2018; Silveira et al., 2019).

The cultivation of colored fiber cotton is crucial in Northeast Brazil. These cultivars have captured the interest of both producers and consumers due to their socioeconomic and environmental sustainability, notably in reducing the generation of toxic waste during fiber production and processing, as they eliminate the need for chemical dyes (Araújo et al., 2019; Barbosa et al., 2019).

Although cotton plants are relatively tolerant to water deficits, the expansion of cotton farming in the Brazilian semi-arid region is constrained by local water conditions. Research on cotton crops under water restrictions has shown that water scarcity, particularly during critical phases like flowering and boll formation, directly impacts plant growth, development, and ultimately the productivity and quality of the fibers (Cordão et al., 2018; Maniçoba et al., 2021; Soares et al., 2023).

In the Brazilian semi-arid region, it is essential to implement strategies that allow for effective water management, ensuring the replacement of water in the soil in appropriate amounts to maximize productivity and water efficiency in agriculture. The biopolymer chitosan has demonstrated potential in stimulating physiological responses that enhance tolerance to water limitations, offering an alternative method to mitigate these effects (Almeida et al., 2020).

Chitosan, obtained through the deacetylation of chitin, is a polysaccharide abundantly found in nature, primarily derived from the exoskeletons of arthropods and crustaceans, as well as from the cell walls of certain fungi (Moenne & Gonzalez, 2021).

Although chitosan is insoluble in water, it dissolves in dilute acid solutions (Park et al., 2001) and is valued for its non-toxic, biodegradable, bioactive, and biocompatible properties (Coma et al., 2002; Hemantaranjan et al., 2014). The mechanisms by which chitosan affects plant biology are not fully elucidated, but the biological responses it induces depend on its chemical composition and the timing and rate of application (Malerba & Cerana, 2016).

Research has explored using chitosan to counteract water limitations. For instance, Almeida et al. (2020) discovered that foliar application of chitosan at a concentration of 140 mg L⁻¹ enhances the development of the root system in corn plants under water-restricted conditions, specifically when the soil water potential reached approximately -138 kPa.

In the study by Pirbalouti et al. (2017) on basil, exogenous application of chitosan at a concentration of 0.4 g L⁻¹ improved growth parameters in two basil species (*Ocimum ciliatum* and *O. basilicum*) under conditions of water stress (30% of soil field capacity). These findings suggest that chitosan could be a promising agent for alleviating the adverse effects of water stress.

This research is predicated on the hypothesis that foliar application of chitosan can minimize the detrimental effects of water restriction on the growth, physiology, and yield components of the 'BRS Jade' colored fiber cotton plant. The aim of this study is to evaluate the efficacy of various concentrations of chitosan as mitigators of water restriction in the cultivation of naturally colored fiber cotton 'BRS Jade'.

Material and Methods

The research was conducted from April to September 2023 in an arch-shaped greenhouse measuring 30 m in length and 21 m in width, with a ceiling height of 3 m. The greenhouse was covered with a 150 μm low-density polyethylene screen and is part of the

Academic Unit of Agricultural Engineering (UAEA) at the Federal University of Campina Grande (UFCG), located in Campina Grande, Paraíba, Brazil (7°15'18" S, 35°52'28" W, at an average altitude of 529 m). Figure 1 illustrates the daily collected data on maximum and minimum temperatures and relative humidity throughout the experimental period.

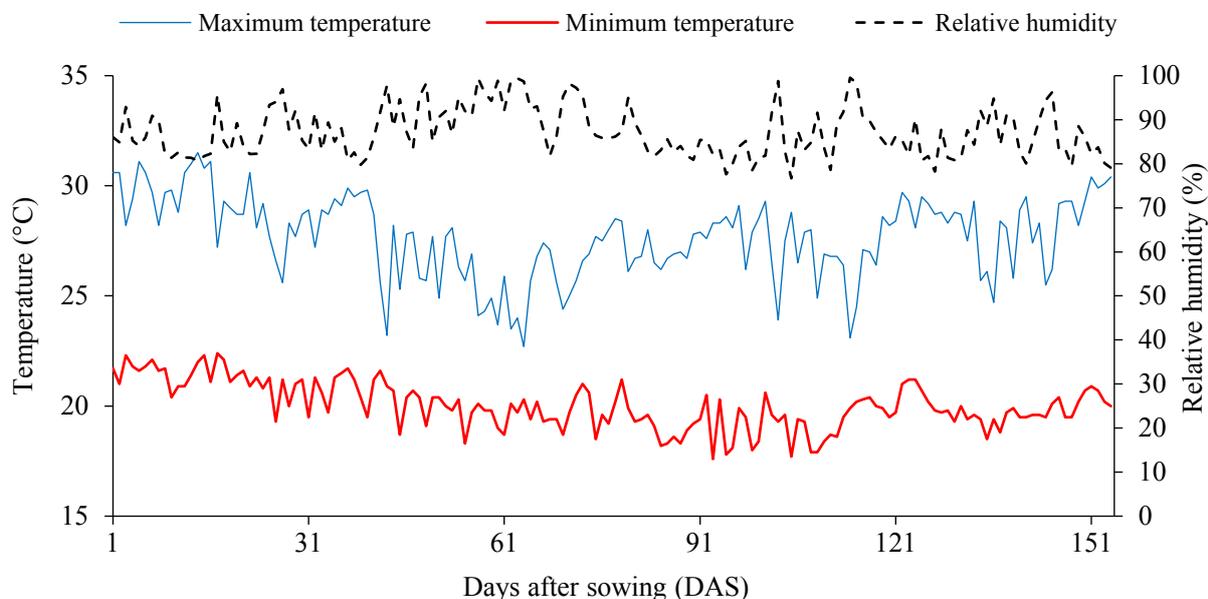


Figure 1. Maximum temperature, minimum temperature, and relative humidity data during the experimental period.

The treatments involved two irrigation levels (100% and 50% of the water requirement of the crop) and four chitosan concentrations (0.0, 0.25, 0.50, and 0.75 g L^{-1}), organized in a 2×4 factorial arrangement. These were distributed in a completely randomized design with three replications and one plant per plot, totaling 24 experimental units. The irrigation amounts were defined based on the study by Soares et al. (2020), and the chitosan concentrations were adapted from Almeida et al. (2020).

Plants were cultivated in plastic containers adapted as drainage lysimeters, each 70 cm high and 60 cm in diameter with a volume of 200 dm^3 . Each lysimeter was fitted with a perforated base to connect two 16-mm diameter hoses, serving as drains linked to two 2-L plastic containers to collect the drained water for later analysis of water consumption by the plants. Inside each lysimeter, a non-woven geotextile blanket (Bidim) was placed over the drain outlets, topped with a gravel layer to prevent clogging from soil material.

The lysimeters were filled with 250 kg of Regolithic Neosol (Entisol) with a sandy clay loam texture (0-20 cm depth), sourced from the rural area of Lagoa Seca, PB, Brazil. The chemical and physical properties of the soil

were analyzed at the Irrigation and Salinity Laboratory (LIS) at UAEA/UFCG, following the methodology described by Teixeira et al. (2017) (Table 1).

Table 1
Chemical and physical-water characteristics of the soil used in the experiment

Chemical characteristics								
pH	OM	P	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺	H ⁺
1:2.5	g dm ⁻³	mg dm ⁻³cmolc kg ⁻¹					
6.5	8.1	79	0.24	0.51	14.9	5.4	0	0.0
.....Chemical characteristics.....			Physical characteristics.....				
EC _{se}	CEC	SAR	ESP	Particle fraction (g kg ⁻¹)			Moisture (dag kg ⁻¹)	
dS m ⁻¹	cmolc kg ⁻¹	(mmol L ⁻¹) ^{0.5}	%	Sand	Silt	Clay	33.42 kPa ¹	1519.5 kPa ¹
2.15	16.54	0.16	3.08	572.7	100.7	326.6	25.91	12.96

pH - potential of hydrogen; OM - organic matter, Walkley-Black wet digestion; Ca₂⁺ and Mg₂⁺ extracted with 1 M KCl, pH 7.0; Na⁺ and K⁺ extracted using 1 M NH₄OAc, pH 7.0; Al₃⁺ and H⁺ extracted with 0.5 M CaOAc, pH 7.0; EC_{se} - electrical conductivity of the saturation extract; CEC - cation-exchange capacity; SAR - sodium adsorption ratio; ESP - exchangeable sodium percentage. ^{1,2} referring to field capacity and permanent wilting point.

The experiment used the colored fiber cotton cultivar 'BRS Jade' from the National Cotton Research Center. This cultivar, with a cycle of about 135 days from emergence, is known for its high productive potential in both cerrado and semi-arid environments. In trials conducted in these regions, 'BRS Jade' achieved an average seed cotton yield of 4.47 t ha⁻¹ and an average fiber content of over 40%. It exhibits excellent fiber quality attributes such as length, uniformity, strength, and micronaire, along with other traits desirable for the textile industry (Farias et al., 2017).

Prior to sowing, soil moisture in all experimental units was raised to near field

capacity by drainage lysimetry. Five seeds were then sown per lysimeter at a depth of 2 cm, spaced equidistantly. At 15 days after sowing (DAS), thinning was performed to retain only the most vigorous plant in each container. Soil moisture was maintained near field capacity until the emergence of the third definitive leaf, at which point differentiation of irrigation levels commenced, applying water volumes according to each irrigation level (100% and 50% of the water requirement of the crop), calculated based on the water balance equation (Eq. 1):

$$VI = \frac{(Vp - Vd)}{(1 - LF)} \quad (1)$$

where VI = volume of water for the subsequent irrigation event (mL); Vp = volume applied in the previous irrigation event (mL); Vd = volume drained (mL); LF = leaching fraction of 0.10, applied every 10 days for plants irrigated at 100% of the water requirement.

The chitosan used in the study had the following characteristics: off-white powder appearance; a translucent gel when dissolved in 1% acetic acid; particle size of 40 mesh; a loss due to desiccation of 9.41%; total ash content of 1.31%; pH of 7.4; solubility in acetic acid solution within 11 min; and a degree of deacetylation of 86.12%.

Chitosan solutions for the treatments (0.25, 0.50, and 0.75 g) were prepared by dissolving the respective amounts in 0.1 M acetic acid, followed by dilution with distilled

water and stirring for 11 min using magnetic stirrer equipment, as adapted from the methodology described by Almeida et al. (2020).

To reduce the surface tension of the droplets on the leaf surfaces (both adaxial and abaxial), Wil fix® adjuvant was added at a concentration of 0.5 mL L⁻¹ at the time of application. The applications were conducted at 17:00 h using a manual sprayer operating at a pressure of 35 Psi, with the plant isolated by plastic curtains to prevent solution drift.

Applications commenced 15 DAS and continued through the flowering period, with a spraying frequency of every 30 days, totaling three applications. Table 2 details the average volume applied per plant.

Table 2
Total volume applied per plant for each chitosan concentration

Chitosan concentration (g L ⁻¹)	DAS			Total volume per plant (mL)
	15	45	75	
C1- control	0.0	0.00	0.00	0
C2- 0.25	21.1	104.45	120.00	245.55
C3- 0.50	21.1	104.45	120.00	245.55
C4- 0.75	21.1	104.45	120.00	245.55

DAS – Days after sowing.

Fertilization with nitrogen (N), phosphorus (P₂O₅), and potassium (K₂O) began at 20 DAS and was repeated every 20 days, following the recommendations of Novais et al. (1991). The applied rates were (per kg of soil) 100 mg N, 300 mg P₂O₅, and

150 mg K₂O, distributed over three fertigation events. Calcium nitrate (15% N) was used as the nitrogen source, monoammonium phosphate (MAP; 60% P₂O₅ and 12% N) for phosphorus and supplemental nitrogen, and potassium chloride (60% K₂O) as the

potassium source. Micronutrients were supplied via foliar fertilization using Dripsol micro compound ($Mg_2^+ = 1.1\%$; $B = 0.85\%$; $Cu (Cu-EDTA) = 0.5\%$; $Fe (Fe-EDTA) = 3.4\%$; $Mn (Mn-EDTA) = 3.2\%$; $Mo = 0.05\%$; $Zn (Zn-EDTA) = 4.2\%$) at a concentration of 1 g L^{-1} every 20 DAS. The application was made to both the adaxial and abaxial leaf surfaces using a 20-L capacity knapsack sprayer.

Pest control was conducted using chemical insecticides and acaricides, specifically employing abamectin and chlorfenapyr as active ingredients. These chemicals were used to control aphids (*Aphis gossypii*), cotton mealybugs (*Phenacoccus solenopsis*), and whiteflies (*Bemisia tabaci*) through spraying applications.

The effects of the treatments on cotton 'BRS Jade' were assessed by measuring various growth variables including plant height, stem diameter, number of leaves, and leaf area. Additional assessments included relative water content (RWC), electrolyte leakage (% EL), photosynthetic pigments, chlorophyll a fluorescence, and yield.

The RWC of the cotton plants was determined at 60 DAS (beginning of the flowering phase). This involved using three fully expanded leaves from the middle third of each plant. Initially, the fresh weight (FW) of the leaves was determined. The leaves were then immersed in 50 mL of distilled water for 24 h in beakers. After immersion, excess water was removed with a paper towel, and the leaves were weighed again to obtain the turgid weight (TW). The leaves were then dried in a forced-air oven at $\approx 65 \pm 3 \text{ }^\circ\text{C}$ until they reached a constant weight, and the dry weight (DW) was recorded. The

RWC was calculated using a formula adapted from Weatherley (1950) (Eq. 2):

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100 \quad (2)$$

where RWC = relative water content (%); FW = fresh weight of leaves (g); TW = turgid weight (g); and DW = dry weight (g).

Electrolyte leakage (% EL) was determined by using a copper perforator to obtain five leaf discs of known area. These discs were washed and placed in an Erlenmeyer flask containing 50 mL of distilled water. After sealing with aluminum foil, the flasks were stored at a temperature of $25 \text{ }^\circ\text{C}$ for 24 h. The initial electrical conductivity of the medium (X_i) was measured using a bench conductivity meter. Subsequently, the flasks were subjected to a temperature of $80 \text{ }^\circ\text{C}$ for 120 min in a drying oven. After this period, the contents were allowed to cool, and the final electrical conductivity (X_f) was measured. Electrolyte leakage is expressed as the percentage of conductivity relative to the total conductivity after the 120-min treatment at $80 \text{ }^\circ\text{C}$, according to Scotti-Campos et al. (2013) (Eq. 3):

$$\%EL = \frac{X_i}{X_f} \times 100 \quad (3)$$

where %EL = electrolyte leakage (%); X_i = initial electrical conductivity (dS m^{-1}); and X_f = final electrical conductivity (dS m^{-1}).

Simultaneously with the assessments of RWC and %EL, the quantification of photosynthetic pigments was conducted. Leaf discs, each with an area of 1.54 cm^2 , were collected from the third fully

expanded leaf from the apical bud. The methodology of Arnon (1949) was used to extract the pigments. The extracts were prepared with 7 mL of 80% acetone, and the photosynthetic pigments were measured

$$\text{Chl } a = (12.25 \times \text{ABS663}) - (2.79 \times \text{ABS647}) \quad (4)$$

$$\text{Chl } b = (21.5 \times \text{ABS647}) - (5.10 \times \text{ABS663}) \quad (5)$$

$$\text{Chl } t = (7.15 \times \text{ABS663}) + (18.71 \times \text{ABS647}) \quad (6)$$

$$\text{Car} = (1000 \times \text{ABS470} - 1.82 \times \text{Chl } a - 85.02 \times \text{Chl } b) / 198 \quad (7)$$

where Chl a = chlorophyll a; Chl b = chlorophyll b; Chl t = total chlorophyll; and Car = carotenoids.

The values obtained for chlorophyll a, b, and total, and carotenoid contents in the leaves were expressed in $\mu\text{g mL}^{-1}$.

At 60 DAS, coinciding with the onset of the flowering phase, chlorophyll a fluorescence was also evaluated using a modulated pulse fluorometer (model OS5p, Opti Science). This was done using the Fv/Fm protocol to determine the fluorescence induction variables: initial fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv), and the quantum efficiency of photosystem II (Fv/Fm). The protocol was carried out after adapting the leaves to darkness for 30 min and measurements were taken at 07:00 h using an equipment clip, ensuring that all primary acceptors were fully oxidized, i.e., the reaction centers were open (Genty et al., 1989).

The growth variables were assessed at 85 DAS, during the transition from the flowering to the fruiting phase. Plant height (PH) was measured using a ruler, stem diameter (SD) with a digital caliper; the

number of leaves (NL) was determined by direct counting, considering only those with a length greater than 3 cm; and the leaf area (LA) was calculated using the methodology proposed by Grimes and Carter (1969) (Eq. 8):

$$\text{LA} = \sum 0.4322X^{2.3002} \quad (8)$$

where LA = leaf area (cm²); and X = length of the main vein of the cotton leaf (cm).

Cotton yield was evaluated based on several parameters: shoot dry biomass (ShDB), seed cotton weight (SCW), harvest index (HI, defined as SCW/ShDB), 100-seed weight (100SW), and average boll weight (ABW). To determine the dry biomass of shoots (including stem, leaves, and bolls without lint), these components were collected, placed in paper bags, labeled, and dried in a forced-air oven at 65 °C until a constant weight was achieved. The materials were then weighed using an analytical balance with a resolution of 0.001 g. Average boll weight was determined following the methodology outlined by Lima et al. (2017).

The collected data underwent normality and homogeneity testing using the Shapiro-Wilk and Levene tests (Levene,

1960; Shapiro & Wilk, 1965). Subsequently, a multivariate analysis was performed using the Principal Components method. This approach reduces the amount of information contained in the original dataset into a smaller number of dimensions through linear combinations of the original variables, based on eigenvalues ($\lambda \geq 1.0$) in the correlation matrix, explaining a percentage greater than 10% of the total variance (Govaerts et al., 2007).

From the dimension reduction, the original data of the variables of each component underwent multivariate analysis of variance (MANOVA) using the Hotelling test (Hotelling et al., 1947) with a significance level of 0.05 for both irrigation amounts and chitosan concentrations, as well as their interaction. Only variables with a correlation coefficient equal to or greater than 0.65 were retained in each principal component (Hair et al., 2009). The analyses were conducted using Statistica software version 7.0 (Statsoft, 2004).

Variables with correlation coefficients below 0.65 were further scrutinized through univariate analysis of variance (ANOVA) with a significance level of $p \leq 0.05$. In cases of significance, linear and quadratic polynomial regression analyses were performed for chitosan concentrations, while Tukey's test was applied for irrigation amounts, utilizing the SISVARESAL statistical software (Ferreira, 2019).

Results and Discussion

The multidimensional space of the original variables was condensed into two principal components (PC1 and PC2) with eigenvalues exceeding $\lambda \geq 1.0$, in line with the criteria proposed by Kaiser (1960). These eigenvalues, along with the percentage of variation explained by each component, collectively accounted for 78.53% of the total variation, with PC1 explaining 43.62% and PC2 explaining 34.91% of the remaining variance (Table 3). The interaction between irrigation amounts and chitosan concentrations (I \times C) significantly influenced both PC1 and PC2 (Table 3).

Variables exhibiting correlation coefficients above 0.65 ($r > 0.65$) were deemed significant. Within PC1, the most influential variables included relative water content (RWC), plant height (PH), leaf area (LA), average boll weight (ABW), 100-seed weight (100SW), seed cotton weight (SCW), stem dry biomass (StDB), boll dry biomass (BDB), and shoot dry biomass (ShDB). Meanwhile, in PC2, chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl t), carotenoids, quantum efficiency of photosystem II (Fv/Fm), stem diameter (SD), number of leaves (NL), and StDB exhibited correlation coefficients exceeding 0.65 (Table 4).

Table 3
Eigenvalues and percentage of total variance explained in multivariate analysis of variance (MANOVA)

	Principal component	
	PC1	PC2
Eigenvalue	7.42	5.93
Total percentage of variance	43.62	34.91
Hotelling test for irrigation amounts (I)	0.004	0.007
Hotelling test for chitosan concentration (C)	0.144	0.097
Hotelling test for the interaction (I × C)	0.049	0.005

PC1 – principal component 1; and PC2 – principal component 2.

Figures 2A and 2B illustrate the biplot projections of treatment effects and variables on the first and second principal components (PC1 and PC2). These components effectively captured the differences stemming from the interaction between irrigation amounts and chitosan concentrations. In PC1, it is evident that the highest values for LA (9763.40 cm²), ABW (7.90 g per boll), and BDB (64.89 g per plant) were attained in cotton plants subjected to the I1C2 treatment (100% irrigation and chitosan concentration of 0.25 g L⁻¹), representing increases of 43.92% (4288.08 cm²), 7.47% (0.59 g per plant), and 12.53% (8.13 g per plant), respectively, compared to plants under the I1C1 treatment (100% irrigation and no chitosan). Additionally, in PC1, it was observed that plants treated with a chitosan concentration of 0.50 g L⁻¹ and receiving 100% irrigation (I1C3) exhibited the highest means for 100SW (10.45 g per plant), leaf dry biomass (134.99 g per plant), and ShDB (287.44 g per plant). These findings suggest a potential role of foliar-applied chitosan in alleviating water stress in cotton

cultivation, as it likely reduces transpiration, thereby enhancing water availability for absorption and consequently promoting plant growth and yield (Pirbalouti et al., 2017).

Regarding relative water content (RWC), an elevation in this parameter was observed with the foliar administration of chitosan at a concentration of 0.75 g L⁻¹, even in conditions of water scarcity, with the highest RWC (76.61%) achieved in treatment I2C4 (50% irrigation and 0.75 g L⁻¹ chitosan) (Table 5). This increase is attributed to chitosan application, as water restriction typically leads to a notable decrease in cellular water content, resulting in cellular damage and hindrance to cell expansion, thus impeding plant growth. The action of chitosan is linked to inducing an antioxidant response, safeguarding plants against oxidative damage, and serving as an antiperspirant under stressful agricultural conditions induced by water scarcity, thereby enhancing water use efficiency and defense against oxidative stress (Silva et al., 2007; Bistgani et al., 2017).

With respect to plant height (PH), it was observed that under water restriction, treatment I2C2 (50% water and 0.25 g L⁻¹ chitosan) displayed comparable average PH to treatment I1C2 (100% irrigation and 0.25 g L⁻¹ chitosan). This suggests that

foliar chitosan application could enhance the height of 'BRS Jade' cotton plant under restricted environmental conditions, as evidenced by the lack of average difference between the two treatments (Table 5).

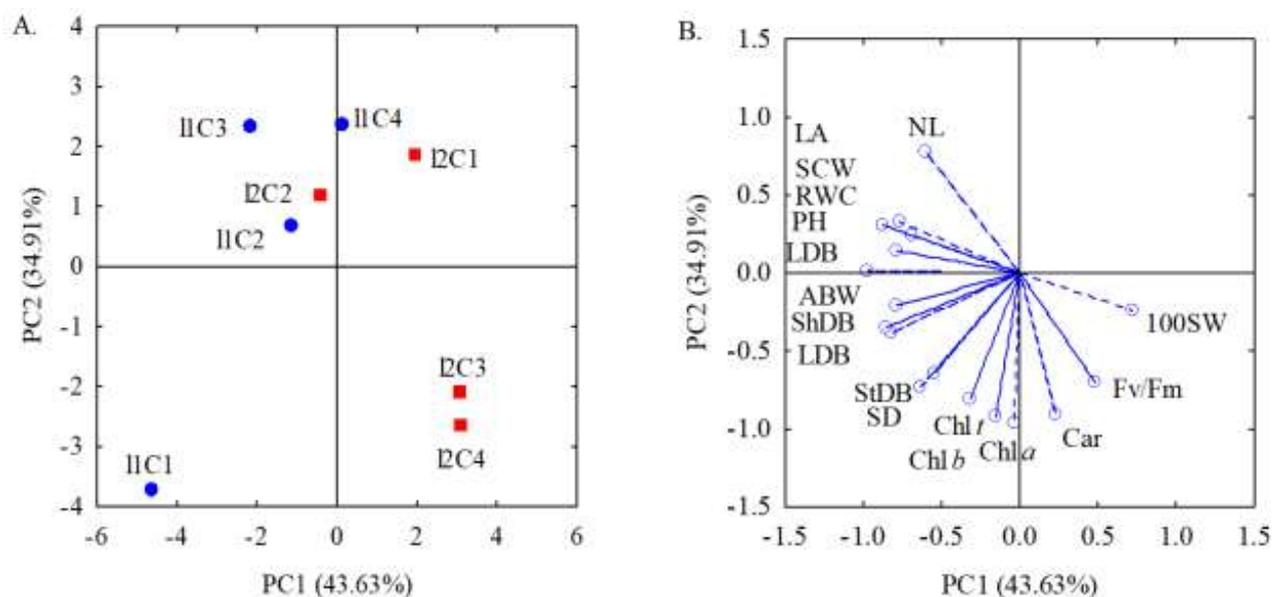


Figure 2. Two-dimensional projection of treatments (A) and analyzed variables (B) into the two principal components (PC1) and (PC2).

Concerning seed cotton weight (SCW), treatment I2C3 emerged with the highest mean, quantitatively totaling 112.01 g. Compared to I2C1, it exhibited a 29.72% increase (25.66 g), indicating that chitosan application at a concentration of 0.50 g L⁻¹ attenuated the impact of water

scarcity on seed cotton production. These findings highlight the potential of chitosan as an antiperspirant in agricultural stress situations, as it demonstrates the capacity to induce water scarcity tolerance, reinforce defense against oxidative stress, and uphold crop productivity (Almeida et al., 2020).

Table 4
Correlation coefficients (r) between original variables and principal components

		Correlation coefficient (r)															
	RWC	Chl a	Chl b	Car	Chl t	Fv/Fm	PH	SD	NL	LA	ABW	SCW	100SW	StDB	LDB	BDB	ShDB
PC1	-0.69	-0.04	-0.31	0.23	-0.15	0.48	-0.79	-0.63	-0.60	-0.77	-0.79	-0.87	0.72	-0.55	-0.82	-0.97	-0.86
PC2	0.25	-0.96	-0.80	-0.91	-0.92	-0.70	0.14	-0.73	0.78	0.33	-0.20	0.31	-0.25	-0.65	-0.38	0.02	-0.35

PC1 – Principal component 1; PC2 – Principal component 2; RWC – Relative water content (%); Chl a – Chlorophyll a ($\mu\text{g mL}^{-1}$); Chl b – Chlorophyll b ($\mu\text{g mL}^{-1}$); Chl t – Total chlorophyll ($\mu\text{g mL}^{-1}$); Car – Carotenoids ($\mu\text{g mL}^{-1}$); Fv/Fm – Quantum efficiency of photosystem II; PH – Plant height (cm); SD – Stem diameter (mm); NL – Number of leaves; LA – Leaf area (cm^2); ABW – Average boll weight (g per plant); SCW – seed cotton weight (g per plant); 100SW – 100-seed weight (g per plant); StDB – Stem dry biomass (g per plant); LDB – Leaf dry biomass (g per plant); BDB – Boll dry biomass (g per plant); and ShDB – shoot dry biomass (g per plant).

Table 5
Mean values of the variables analyzed by treatment

TREATMENT	Mean values																
	RWC	Chl a	Chl b	Car	Chl t	Fv/Fm	PH	SD	NL	LA	ABW	SCW	100SW	StDB	LDB	BDB	ShDB
I1C1	72.63	1288	398.78	159.63	1686.76	0.80	117.00	15.29	69	5475.54	7.31	94.23	8.06	40.01	54.51	56.76	151.28
I1C2	71.26	1287	372.40	171.83	1659.22	0.82	114.00	18.40	75	9763.40	7.90	88.69	6.79	56.77	80.67	64.89	202.33
I1C3	69.82	415	425.17	184.03	839.79	0.83	115.50	16.20	62	7655.66	7.35	79.14	10.45	111.42	134.99	41.03	287.44
I1C4	66.91	691	117.77	132.05	809.24	0.82	115.00	15.41	77	5210.00	7.37	93.88	9.64	73.05	100.76	52.63	226.44
I2C1	71.99	710	151.16	137.25	861.16	0.82	113.00	14.82	69	5707.44	7.54	86.35	8.79	63.67	60.57	41.67	138.82
I2C2	61.83	701	184.54	132.03	885.54	0.81	114.00	14.22	78	6205.52	7.20	78.82	10.32	54.3	73.83	47.15	167.53
I2C3	69.86	626	180.00	139.51	805.77	0.82	112.00	14.45	86	6205.52	6.89	112.01	9.22	49.2	84.72	54.39	188.31
I2C4	76.61	715	226.69	121.31	941.49	0.88	114.00	13.84	69	5437.78	7.33	63.38	5.93	31.9	70.04	28.44	130.38

PC1 – Principal component 1; PC2 – Principal component 2; RWC – Relative water content (%); Chl a – Chlorophyll a ($\mu\text{g mL}^{-1}$); Chl b – Chlorophyll b ($\mu\text{g mL}^{-1}$); Chl t – Total chlorophyll ($\mu\text{g mL}^{-1}$); Car – Carotenoids ($\mu\text{g mL}^{-1}$); Fv/Fm – Quantum efficiency of photosystem II; PH – Plant height (cm); SD – Stem diameter (mm); NL – Number of leaves; LA – Leaf area (cm^2); ABW – Average boll weight (g per plant); SCW – Seed cotton weight (g per plant); 100SW – 100-seed weight (g per plant); StDB – Stem dry biomass (g per plant); LDB – Leaf dry biomass (g per plant); BDB – Boll dry biomass (g per plant); and ShDB – shoot dry biomass (g per plant).

In PC2, cotton plants irrigated with 100% water and treated with a chitosan concentration of 0.50 g L⁻¹ (I1C3) outperformed other treatments, exhibiting the highest levels of Chl b at 425.17 µg mL⁻¹, carotenoids at 184.03 µg mL⁻¹, and StDB at 111.42 g. Comparing results between treatment I1C3 and I1C1 (100% water and 0 g L⁻¹ chitosan), percentage-wise increases of 6.21% (26.40 µg mL⁻¹), 13.26% (24.40 µg mL⁻¹), and 64.09% (71.71 g) were observed for Chl b, carotenoids, and StDB, respectively. These outcomes may be attributed to chemical resemblance of chitosan to cellulose, albeit possessing positive ionic charges unlike vegetable fiber, which enables it to chemically bind to negatively charged macromolecules (Katiyar et al., 2015). This characteristic suggests that the interaction between chlorophyll and chitosan may occur through its protonated amino group, supporting the observed chlorophyll maintenance (Rizzi et al., 2016).

As for chlorophyll a and total, it was noted that as the chitosan concentration increased, coupled with 100% irrigation, there was a reduction in these variables, resembling the behavior of plants receiving only 50% irrigation. This phenomenon may stem from diminished synthesis of the main pigment and protein complexes, which safeguard the photosynthetic apparatus from oxidative damage to chloroplast lipids and proteins (Pirbalouti et al., 2017).

The highest quantum efficiency of photosystem II (Fv/Fm) was achieved in plants treated with I2C4 under water scarcity and foliar application of 0.75 g L⁻¹ chitosan,

averaging 0.88. This responsiveness likely resulted in the maintenance of the variable and maximum fluorescence ratio, indicating an intact photosynthetic electron transport chain and an uncompromised photosynthetic apparatus. According to Kalaji and Guo (2008), Fv/Fm values should remain below 0.70 to avoid being detrimental to the photosynthetic system. Values below this threshold indicate challenges in CO₂ fixation in leaf tissue and inefficiency in utilizing photochemical radiation when all PSII reaction centers are open, serving as an excellent indicator of plant stress (Peripolli et al., 2021; Marques et al., 2020).

In terms of stem diameter, the highest mean was recorded when cotton plants received 100% irrigation and a chitosan concentration of 0.25 g L⁻¹, totaling 18.40 mm. Regarding the number of leaves, it was notable that even under water restriction, there was an increase in this parameter with chitosan application. Specifically, plants subjected to 50% irrigation and sprayed with 0.50 g L⁻¹ chitosan (I2C3) exhibited the highest leaf count, totaling 86 leaves.

Electrolyte leakage, initial fluorescence, variable fluorescence, maximum fluorescence, and harvest index displayed correlation coefficients below 0.65, leading to their exclusion from the analysis and subsequent univariate examination. As evidenced by the summary of analysis of variance (Table 6), neither the interaction between irrigation levels and chitosan concentrations nor the individual factors significantly influenced any of the analyzed variables ($p > 0.05$).

Table 6

Summary of analysis of variance for electrolyte leakage (%EL), initial (F0), maximum (Fm), and variable (Fv) fluorescence at 60 DAS, and harvest index (HI) in the cultivation of naturally colored fiber cotton 'BRS Jade' subjected to water restriction and foliar application of chitosan concentrations

Source of variation	Mean square					
	DF	%EL	F0	Fm	Fv	HI
Irrigation amount (I)	1	165.37 ^{ns}	0.67 ^{ns}	15.04 ^{ns}	3.37 ^{ns}	.042 ^{ns}
Chitosan (C)	3	361.26 ^{ns}	13.83 ^{ns}	1817.93 ^{ns}	1186.04 ^{ns}	.153 ^{ns}
Linear regression	1	25.20 ^{ns}	38.53 ^{ns}	4308.0 ^{ns}	2990.01 ^{ns}	.208 ^{ns}
Quadratic regression	1	10.53 ^{ns}	2.667 ^{ns}	975.375 ^{ns}	442.04 ^{ns}	.042 ^{ns}
Interaction (I × C)	3	290.37 ^{ns}	9.22 ^{ns}	472.93 ^{ns}	534.37 ^{ns}	.042 ^{ns}
Linear regression	1	2.816 ^{ns}	3.750 ^{ns}	5320.42*	4335.00*	.067 ^{ns}
Quadratic regression	1	520.08 ^{ns}	2.083	444.08 ^{ns}	225.33 ^{ns}	.000 ^{ns}
Residual	16	361.83	10.37	904.46	810.95	.125
CV (%)		32.45	3.97	6.92	8.06	0.41

^{ns}, **, * not significant and significant at $p \leq 0.01$ and $p \leq 0.05$, respectively; DF – Degrees of freedom; CV – Coefficient of variation.

The findings of this study reinforce the hypothesis that foliar chitosan application, within appropriate concentrations, can mitigate the adverse effects of water restriction on the physiology, growth, and yield components of cotton plants. Notably, chitosan application in concentrations ranging from 0.25 to 0.50 g L⁻¹ led to enhancements in chlorophyll b, carotenoids, stem diameter, leaf area, 100-seed weight, average boll weight, seed cotton weight, total boll dry biomass, number of bolls, and dry biomass of stem, leaf, and shoots of colored fiber cotton 'BRS Jade'.

Conclusions

The foliar application of chitosan within the concentration range of 0.25 to 0.50 g L⁻¹ mitigates the adverse impacts of water restriction on chlorophyll b synthesis,

carotenoid content, stem diameter, leaf area, 100-seed weight, average boll weight, seed cotton weight, total boll dry biomass, stem dry biomass, leaf dry biomass, and shoot dry biomass of colored fiber cotton 'BRS Jade'. These results validate the hypothesis that foliar chitosan application alleviates the detrimental effects of water restriction in colored fiber cotton cultivation. Nonetheless, further investigations are warranted to elucidate the mechanisms underlying how chitosan acts to mitigate water restriction, including biochemical and enzymatic analyses, alongside field validation studies.

Acknowledgments

Thanks are extended to the Graduate Program in Agricultural Engineering at the Federal University of Campina Grande for providing infrastructure

support. Thanks are also due to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support; National Cotton Research Center (CNPA) of Embrapa Cotton; and ORIGINALIS BIOTECH for supplying chitosan.

References

- Almeida, L. G., Silva, E. M. da, Magalhães, P. C., Karam, D., Reis, C. O. dos, Gomes, C. C., & Marques, D. M. (2020). Root system in maize plants cultivated under water deficit and application of chitosan. *Revista Brasileira de Milho e Sorgo*, 19(1), e1131. doi: 10.18512/1980-6477/rbms 2020.v19.e1131
- Araújo, W. P., Pereira, J. R., Zonta, J. H., Guerra, H. O. C., Cordão, M. A., & Lima, R. F. (2019). Production components and water efficiency of upland cotton cultivars under water deficit strategies. *African Journal of Agricultural Research*, 14(20), 887-896. doi: 10.5897/AJAR2019.13907
- Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24(1), 1-15. doi: 10.1104/pp.24.1.1
- Barbosa, J. L., Nobre, R. G., Souza, L. de P., Veloso, L. L. de S., Silva, E. L. da., & Guedes, M. A. (2019). Crescimento de algodoeiro colorido cv. BRS topázio em solos com distintas salinidades e adubação orgânica. *Revista de Ciências Agrárias*, 42(1), 201-210. doi: 10.19084/RCA17294
- Bistgani, Z. E., Siadat, S. A., Bakhshandeh, A., Pirbalouti, A. G., & Hashemi, M. (2017). Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of thymus daenensis celak. *The Crop Journal*, 5(5), 407-415. doi: 10.1016/j.cj.2017.04.003
- Coma, V., Martial-Gros, A., Garreau, S., Copinet, A., Salin, F., & Deschamps, A. (2002). Edible antimicrobial films based on chitosan matrix. *Journal of Food Science*, 67(2), 1162-1169. doi: 10.1111/j.1365-2621.2002.tb09470.x
- Cordão, M. A., Araújo, W. P., Pereira, J. R., Zonta, J. H., Lima, R. F. de, & Ferreira, F. N. (2018). Cultivares de algodoeiro herbáceo sob déficit hídrico aplicado em fases fenológicas. *Revista Verde de Agroecologia e Desenvolvimento Sustentável*, 13(3), 313-321. doi: 10.18378/rvads.v13i3.5933
- Farias, F. J. C., Morello, C. de L., Pedrosa, M. B., Suassuna, N. D., Silva, J. L. da, Fº., Carvalho, L. P. de, & Ribeiro, J. L. (2017). BRS jade: nova cultivar de algodão colorido de dupla aptidão para o cerrado baiano e para o semiárido nordestino. *Anais do Congresso Brasileiro de Algodão*, Maceió, AL, Brasil, 11.
- Ferreira, D. F. (2019). Sisvar: a computer analysis system to fixed effects split plot type designs. *Revista Brasileira de Biometria*, 37(1), 529-535. doi: 10.28951/rbb.v37i4.450
- Genty, B., Briantais, J. M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica*

- et Biophysica Acta (BBA) - General Subjects*, 990(1), 87-92. doi: 10.1016/S0304-4165(89)80016-9
- Govaerts, B., Sayre, K. D., Lichter, K., Dendooven, L., & Deckers, J. (2007). Influence of permanent planting in high bed and residue management on physical and chemical soil quality in rainfed corn/wheat systems. *Plant and Soil*, 291(1), 39-54. doi: 10.1007/s11104-006-9172-6
- Grimes, D. W., & Carter, L. M. (1969). A linear rule for direct nondestructive leaf area measurements. *Agronomy Journal*, 61(3), 477-479. doi: 10.2134/agronj1969.00021962006100030048x
- Hair, F. J., Black, W. C., Babin, B. J., Anderson, R. E., & Tatham, R. L. (2009). In A. S., Sant'Anna (Trad.), *Análise multivariada de dados* (6a ed.). Porto Alegre.
- Hemantaranjan, A., Katiyar, D., Singh, B., & Bhanu, A. N. (2014). A future perspective in crop protection: chitosan and its oligosaccharides. *Advances in Plants & Agriculture Research*, 1(1), 1-8. doi: 10.15406/apar.2014.01.00006
- Hotelling, H., Eisenhart, C., Hastay, M. W., & Wallis, W. A. (1947). *Multivariate quality control. Techniques of Statistical Analysis*. John Wiley & Sons.
- Kaiser, H. F. (1960). The application of electronic computers to factor analysis. *Educational and Psychological Measurement*, 20(1), 141-151. doi: 10.1177/001316446002000116
- Kalaji, M. H., & Guo, P. (2008). Chlorophyll fluorescence: a useful tool in barley plant breeding programs. *Photochemistry Research Progress*, 29(12), 439-463.
- Katiyar, D., Hemantaranjan, A., & Singh, B. (2015). Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Indian Journal of Plant Physiology*, 20(1), 1-9. doi: 10.1007/s40502-015-0139-6
- Koch, G., Rolland, G., Dauzat, M., Bédiée, A., Baldazzi, V., Bertin, N., Guédon, Y., & Granier, C. (2019). Leaf production and expansion: a generalized response to drought stresses from cells to whole leaf biomass a case study in the tomato compound leaf. *Plants*, 8(10), 1-17. doi: 10.3390/plants8100409
- Lacerda, C. N. de, Lima, G. S. de, Soares, L. A. dos A., Fátima, R. T. de, Gheyi, H. R., & Azevedo, C. A. V. de. (2022). Morphophysiology and production of guava as a function of water salinity and salicylic acid. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 26(6), 451-458. doi: 10.1590/1807-1929/agriambi.v26n6p451-458.
- Levene, H. (1960). Robust tests for equality of variances. In I. Olkin, H. Hotelling (Eds.), *Essays in Honor of Harold Hotelling*. (p. 278-292). Stanford University Press.
- Lima, G. S. de, Dias, A. S., Gheyi, H. R., Soares, L. A. dos A., & Andrade, E. M. G. (2018). Saline water irrigation and nitrogen fertilization on the cultivation of colored fiber cotton. *Revista Caatinga*, 31(1), 151-160. doi: 10.1590/1983-21252018v31n118rc
- Lima, G. S. de, Dias, A. S., Gheyi, H. R., Soares, L. A. dos A., Nobre, R. G., Pinheiro, F. W. A., & Silva, A. A. R. da. (2017). Gas exchanges and production of colored cotton under salt stress and nitrogen fertilization. *Bioscience Journal*, 33(6), 1495-1505. doi: 10.14393/BJ-v33n6a2017-37109

- Magalhães, I. D., Melo, A. S. de, Fernandes, P. D., Queiroz, M. F. de, Arriel, N. H. C., Ferraz, R. L. de S., Suassuna, J. F., Maia, S. de O., Jr., Medeiros, A. de S., Viegas, P. R. A., Costa, F. da S., & Costa, P. da S. (2020). Gas exchange, photochemical efficiency, and yield of jatropha curcas irrigated with saline water. *Australian Journal of Crop Science*, 14(5), 802-809. doi: 10.3316/informit.260507589787346
- Malerba, M., & Cerana, R. (2016). Chitosan effects on plant systems. *International Journal of Molecular Sciences*, 17(7), e996. doi: 10.3390/ijms17070996
- Maniçoba, R. M., Espínola Sobrinho, J., Zonta, J. H., Cavalcante, E. G. Jr., Oliveira, A. K. S. de, & Freitas, E. A. da S. (2021). Resposta do algodoeiro à supressão hídrica em diferentes fases fenológicas no semiárido brasileiro. *Irriga*, 26(1), 123-133. doi: 10.15809/irriga.2021v26n1p123-133
- Marques, R. F., Pinheiro, G. H. R., Araújo, P. P. dos S., Souza, R. M. de, & Marchi, S. R. de. (2020). Efeito de sub doses de 2,4-d sal colina na eficiência quântica do fotossistema II do algodoeiro. *Colloquium Agrariae*, 16(2), 60-71. doi: 10.5747/ca.2020.v16.n2.a359
- Medeiros, A. de S., Maia, S. de O., Jr., Gonzaga, G. B. M., Santos, T. C. dos, Queiroz, M. M. F. de, Araújo, R. A. de, Neto, Magalhães, I. D., Ferraz, R. L. de S., Costa, P. da S., Andrade, J. R. de, & Pereira, M. de O. (2019). Growth of okra under nitrogen rates and wastewater in the brazilian semiarid region. *Journal of Agricultural Science*, 11(5), 408-418. doi: 10.5539/jas.v11n5p408
- Moenne, A., & González, A. (2021). Chitosan-, alginate-carrageenan-derived oligosaccharides stimulate defense against biotic and abiotic stresses, and growth in plants: a historical perspective. *Carbohydrate Research*, 503(1), e108298. doi: 10.1016/j.carres.2021.108298
- Novais, R. F., Neves, J. C. L., & Barros, N. F. (1991). Ensaio em ambiente controlado. In A. J. Oliveira, *Métodos de pesquisa em fertilidade do solo* (pp. 189-253). Brasília.
- Park, S. Y., Lee, B. I., Jung, S. T., & Park, H. J. (2001). Biopolymer composite films based on κ-carrageenan and chitosan. *Materials Research Bulletin*, 36(3-4), 511-519. doi: 10.1016/S0025-5408(01)00545-1
- Peripolli, M., Dornelles, S. H., Lopes, S. J., Tabaldi, L. A., Trivisiol, V. S., & Rubert, J. (2021). Application of biostimulants in tomato subjected to water deficit: physiological, enzymatic and production responses. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 25(2), 90-95. doi: 10.1590/1807-1929/agriambi.v25n2p90-95
- Pirbalouti, A. G., Malekpoor, F., Salimi, A., & Golparvar, A. (2017). Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. *Scientia Horticulturae*, 201(15), 114-122. doi: 10.1016/j.scienta.2017.01.
- Rizzi, V., Fini, P., Semeraro, P., & Cosna, P. (2016). Detailed investigation of ROS arisen from chlorophyll a/chitosan

- based-biofilm. *Colloids Surfaces B-Biointerfaces*, 142(1), 239-247. doi: 10.1016/j.colsurfb.2016.02.062
- Scotti-Campos, P., Pham-Thi, A. T., Semedo, J. N., Pais, I. P., Ramalho, J. C., & Céu Matos, M. do. (2013). Physiological responses and membrane integrity in three Vigna genotypes with contrasting drought tolerance. *Emirates Journal of Food and Agriculture*, 25(13)1002-1013. doi: 10.9755/ejfa.v25i12.16733
- Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 52(3/4), 591-611. doi: 10.2307/2333709
- Silva, M. de A., Jifon, J. L., Silva, J. A. G. da, & Sharma, V. (2007). Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. *Brazilian Journal of Plant Physiology*, 19(1), 193-201. doi: 10.1590/S1677-04202007000300003
- Silveira, R. N. C. M., Peixoto, F. da S., Costa, R. N. T., & Cavalcante, I. N. (2019). Efeitos da seca em perímetros irrigados no semiárido brasileiro. *Anuário do Instituto de Geociências*, 41(2), 268-275. doi: 10.11137/2018_2_268_275
- Soares, L. A. dos A., Dias, K. M. M., Nascimento, H. M., Lima, G. S. de, Oliveira, K. J. A. de, & Silva, S. S. da. (2020). Estratégias de manejo do déficit hídrico em fases fenológicas do algodoeiro colorido. *Irriga*, 25(4), 656-662. doi: 10.15809/irriga.2020v25n4p656-662
- Soares, L. A. dos A., Felix, C. M., Lima, G. S. de, Gheyj, H. R., Silva, L. de A., & Fernandes, P. D. (2023). Gas exchange, growth, and production of cotton genotypes under water deficit in phenological stages. *Revista Caatinga*, 36(1), 145-157. doi: 10.1590/1983-21252023v36n116rc
- Soares, L. A. dos A., Fernandes, P. D., Lima, G. S. de, Suassuna, J. F., & Pereira, R. F. (2018). Gas exchanges and production of colored cotton irrigated with saline water at different phenological stages. *Revista Ciência Agronômica*, 49(2), 239-248. doi: 10.5935/1806-6690.20180027
- Statsoft, I. N. C. (2004). *Programa computacional Statistica 7.0*. E. A. U.
- Tavares, V. C. de A., Paulino, I. R., & Silva, D. G. da. (2019). Desertificação, mudanças climáticas e secas no semiárido brasileiro: uma revisão bibliográfica. *Geosul*, 34(70), 385-405. doi: 10.5007/2177-5230.2019v34n70p385
- Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). *Manual de métodos de análise de solo* (3a ed.). EMBRAPA solos.
- Wanderley, J. A. C., Azevedo, C. A. V. de, Brito, M. E. B., Ferreira, F. N., Cordão, M. A., & Lima, R. F. de (2022). Gas exchange in yellow passion fruit under irrigation water salinity and nitrogen fertilization. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 26(2), 135-141. doi: 10.1590/1807-1929/agriambi.v26n2p135-141
- Weatherley, P. (1950). Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytologist*, 49(1), 81-97. doi: 10.1111/j.1469-8137.1950.tb05146.x