

Article

Enhancing Water Productivity, Biomass Production and Drought Tolerance of Passion Fruit (*Passiflora edulis Sims*) Seedlings Using Chitosan and Spirulina Biostimulants Under Deficit Irrigation

Mohamed S. Gawish ^{1,2,*} , Nahed M. Rashed ^{3,*} , Reda Kh. Darwesh ⁴, Raga M. Elzaki ⁵ , Egbal Elmsaad ⁶  and Nardin B. Farag ¹

¹ Horticulture Department, Faculty of Agriculture, Damietta University, New Damietta 34517, Egypt; msagawish@du.edu.eg (M.S.G.); nardin.boulis@du.edu.eg (N.M.R.)

² The Center for Excellence in Research of Advanced Agricultural Sciences (CERAAS), Faculty of Agriculture, Damietta University, New Damietta 34517, Egypt

³ Department of Arid Land Agriculture, College of Agricultural and Food Science, King Faisal University, Al-Ahsa 31982, Saudi Arabia; nrashed@kfu.edu.sa

⁴ Water Requirements and Field Irrigation Department, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza 12619, Egypt; drreda1982@gmail.com.

⁵ Department of Agribusiness and Consumer Science, College of Agricultural Sciences and Food, King Faisal University, Al-Ahsa, Saudi Arabia; rmali@kfu.edu.sa

⁶ Department of Agricultural Systems Engineering, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia; eelmsaad@kfu.edu.sa

* Correspondence: msagawish@du.edu.eg (M.S.G.); nrashed@kfu.edu.sa (N.M.R.); Tel.: +966-557-670-398

Abstract

Water scarcity is a major constraint to sustainable horticultural production, particularly in arid and semi-arid regions. This study evaluated the effectiveness of two natural foliar biostimulants, chitosan and Spirulina extract, in improving water productivity, growth performance, biomass production, and drought tolerance of passion fruit (*Passiflora edulis Sims*) seedlings under deficit irrigation. A factorial experiment was conducted using three irrigation regimes (100%, 85%, and 70% of field capacity, FC) combined with three foliar spray treatments: distilled water (control), chitosan (1 g L⁻¹), and Spirulina extract (1 g L⁻¹). Deficit irrigation significantly reduced chlorophyll content, vegetative growth, biomass production, and water productivity, with the greatest reductions observed at 70% FC. Foliar application of both biostimulants effectively alleviated these adverse effects by enhancing plant growth, biomass production, chlorophyll retention, and water productivity under water-limited conditions. Chitosan consistently outperformed Spirulina, particularly under moderate water deficit, by reducing irrigation water requirements while maintaining seedling growth and physiological performance comparable to those under full irrigation. Multivariate analyses, including Pearson correlation, principal component analysis, and response surface methodology, revealed strong positive relationships among biomass production, chlorophyll content, vegetative growth traits, and water productivity. Among all treatments, foliar application of chitosan at 1 g L⁻¹ combined with 85% FC was identified as the optimum strategy, providing substantial irrigation water savings while maintaining vigorous seedling growth, high biomass production, and superior water productivity. These findings demonstrate that integrating moderate deficit irrigation with chitosan application is an effective and sustainable strategy for improving passion fruit nursery production under conditions of limited water availability.

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Keywords: *Passiflora edulis*; water productivity; drought tolerance; chitosan; *Spirulina* extract; deficit irrigation; biomass production

1. Introduction

Water scarcity is one of the greatest challenges threatening sustainable agricultural production worldwide, particularly in arid and semi-arid regions where freshwater resources are increasingly limited. Agriculture accounts for approximately 70–72% of global freshwater withdrawals, making irrigation management a key component of sustainable crop production [1,2]. Increasing competition among agricultural, industrial, and domestic sectors, together with the growing frequency and intensity of drought events associated with climate change, has intensified pressure on available water resources. Consequently, improving crop water productivity, defined as the amount of biomass or economic yield produced per unit of water consumed, and developing water-efficient production systems have become major priorities for sustainable horticulture [3–6].

Deficit irrigation is a well-established water-saving strategy that improves irrigation efficiency by supplying water below full crop evapotranspiration (ET_c) requirements while maintaining acceptable levels of plant growth and productivity. Depending on crop species and production objectives, deficit irrigation is commonly classified as mild (90–95% ET_c), moderate (approximately 70–85% ET_c), and severe (<70% ET_c). Moderate deficit irrigation has been successfully applied in many horticultural crops to reduce water use for irrigation while maintaining satisfactory growth and improving water productivity. However, its effectiveness depends on several factors, including crop species, developmental stage, stress severity, and the plant's capacity to tolerate water limitation. Therefore, integrating deficit irrigation with complementary approaches that enhance drought tolerance has become an important strategy for improving water-use efficiency under water-limited conditions [7–10].

The passion fruit (*Passiflora edulis* Sims) is an economically important tropical and subtropical fruit crop, due to its unique flavor, high nutritional content and increasing global market demand. The cultivation of passion fruit has been extended in North Africa and the Middle East into newly reclaimed and water-scarce areas in recent years. However, the crop has a shallow root system and is very sensitive to water deficit, especially during the nursery and establishment stages. Previous physiological studies have demonstrated that drought stress reduces leaf expansion, chlorophyll synthesis, stomatal conductance, photosynthesis, nutrient uptake, and biomass production, ultimately limiting plant establishment and productivity [11–14].

Among the possible approaches, plant biostimulants have emerged as environmentally friendly tools to improve crop performance under abiotic stress conditions. Biostimulants are natural substances or microorganisms that can increase nutrient-use efficiency, stress tolerance and plant growth by regulating physiological and biochemical processes. These positive effects are often related to enhanced photosynthetic activity, improved antioxidant defense systems, improved nutrient acquisition and maintenance of cellular water status under stress conditions [15–17].

Chitosan is a biodegradable polysaccharide obtained by the deacetylation of chitin and is widely recognized as a plant biostimulant because it enhances nutrient-use efficiency, stress tolerance, and plant physiological performance [18]. Chitosan, a biopolymer derived from chitin, has been widely reported to improve plant performance under water deficit by enhancing water-use efficiency, maintaining photosynthetic activity, and promoting physiological processes associated with drought tolerance, including im-

proved stomatal regulation, osmotic adjustment, and antioxidant defense [19–21]. Likewise, microalgae-based biostimulants such as *Spirulina* encompass a wide range of bioactive compounds, including amino acids, phytohormones, vitamins, polysaccharides, proteins, and mineral nutrients, resulting in enhanced nutrient uptake, chlorophyll retention, photosynthetic efficiency, and drought tolerance [22–24]. These physiological improvements often lead to higher growth, biomass and water productivity under stressful environmental conditions.

Although chitosan and microalgal biostimulants have been extensively evaluated in several horticultural crops, limited information is available regarding their combined effects on water productivity, drought tolerance, and physiological performance of *Passiflora edulis* seedlings under deficit irrigation. Moreover, previous studies have generally focused on individual growth or physiological responses, whereas comprehensive assessments integrating irrigation water requirements, consumptive water use, biomass production, chlorophyll status, water productivity indices, and multivariate statistical analyses remain scarce. Addressing this knowledge gap is essential for developing sustainable irrigation strategies for passion fruit nursery production under water-limited environments. We hypothesized that foliar application of chitosan and *Spirulina* extract would alleviate drought-induced reductions in growth and physiological performance by improving water-use efficiency, maintaining chlorophyll content, and enhancing biomass production under deficit irrigation. Therefore, this study aimed to evaluate the effectiveness of chitosan and *Spirulina* extract in improving drought tolerance and water-use efficiency of passion fruit seedlings grown under different irrigation regimes. Specifically, the study assessed (a) water use and productivity, (ii) vegetative growth, chlorophyll content, and biomass production, and (iii) the relationships among these traits using multivariate statistical analyses.

2. Materials and Methods

2.1. Experimental Site and Plant Material

The experiment was conducted during the 2024 and 2025 growing seasons at the nursery of the Faculty of Agriculture, Damietta University, Egypt (31°25' N, 31°49' E; 3 m above sea level). The region is characterized by a Mediterranean climate with hot dry summers and mild winters. Meteorological data including temperature, relative humidity, wind speed, reference evapotranspiration (ET₀), and rainfall were obtained from the local meteorological station and are presented in Figure 1.

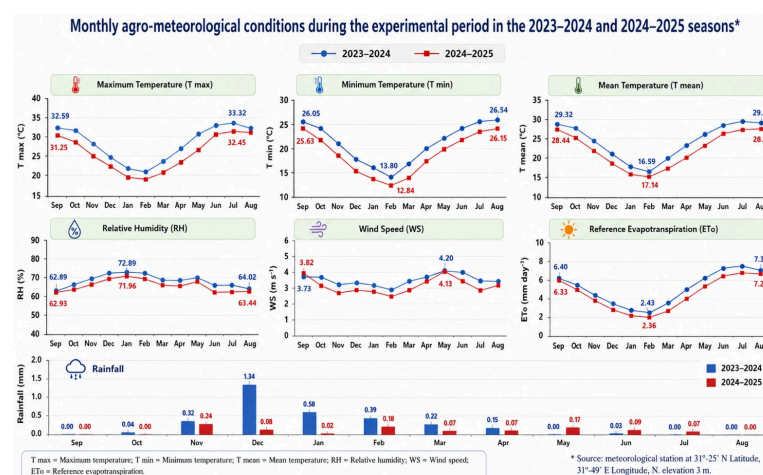


Figure 1. Monthly meteorological conditions during the experimental period.

Uniform passion fruit (*Passiflora edulis* Sims) seedlings were propagated from semi-hardwood stem cuttings collected from healthy, disease-free mother plants grown in a commercial orchard in Ismailia Governorate, Egypt. Stem cuttings, approximately 30 cm in length (measured from the basal cut to the shoot apex) and 1.5 cm in diameter, each containing 3–4 nodes, were prepared by making a straight basal cut immediately below a node and a slanting cut above the uppermost node. The basal 2–3 cm of each cutting was dipped in an aqueous solution of indole-3-butyric acid (IBA) at 3000 mg L⁻¹ for 1 min to promote root initiation. Rooted cuttings of uniform size and vigor were then transplanted into plastic pots (30 cm upper diameter, approximately 12 L capacity) filled with 8 kg of sand:clay:peat moss mixture (2:1:1, v/v/v). The seedlings were acclimatized for 60 days under nursery conditions before imposing the irrigation treatments. During the acclimatization period, all plants received identical cultural practices, including a standard fertilization program consisting of NPK fertilizer (1 g L⁻¹) and humic acid (7 g L⁻¹). At the beginning of the experiment, the seedlings were approximately 60 days old after transplanting. The irrigation and foliar treatments were applied for six months. All growth, biomass production, chlorophyll, and water productivity measurements were recorded at the end of the experimental period.

2.2. Soil Physical and Chemical Characteristics

Before the experiment, representative samples of the growing medium were collected to determine its physical and chemical properties. Particle-size distribution was determined using the hydrometer method, while bulk density was measured using the core sampling method according to Klute [25]. Field capacity (FC), permanent wilting point (PWP), and available water (AW) were determined using a pressure plate apparatus following the procedures described by Klute [25]. Soil chemical analyses, including pH, electrical conductivity (EC), soluble cations (Ca²⁺, Mg²⁺, Na⁺, and K⁺), and soluble anions (CO₃²⁻, HCO₃⁻, Cl⁻, and SO₄²⁻), were performed according to the methods described by Jackson [26]. The physical and chemical characteristics of the growing medium are presented in Tables 1 and 2.

Table 1. Physical properties of the growing medium before the initiation of the experiment.

Property	Value
Sand (%)	89.82
Silt (%)	5.67
Clay (%)	4.51
Textural class	Sandy
Bulk density (g cm ⁻³)	1.109
Field capacity (% w/w)	20.48
Permanent wilting point (% w/w)	10.24
Available water (% w/w)	10.24

Table 2. Physicochemical characteristics of the growing medium before the initiation of the experiment.

Cations (mg L ⁻¹)	Value	Anions (mg L ⁻¹)	Value	Property	Value
Ca ²⁺	18	CO ₃ ²⁻	–	pH (1:2.5 soil:water)	7.44
Mg ²⁺	9.6	HCO ₃ ⁻	20.5	EC (dS m ⁻¹)	1.708

Cations (mg L ⁻¹)	Value	Anions (mg L ⁻¹)	Value	Property	Value
Na ⁺	15.53	Cl ⁻	19.53	-	-
K ⁺	10	SO ₄ ²⁻	13.1	-	-

FC = field capacity; PWP = permanent wilting point; AW = available water. CO₃²⁻ was not detected because the soil pH (7.44) was below the threshold for measurable carbonate ions; therefore, alkalinity was present predominantly as HCO₃⁻.

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2.3. Experimental Design and Treatments

The experiment was arranged as a 3 × 3 factorial scheme in a completely randomized design (CRD) with three replications. Each replicate consisted of six uniform passion fruit seedlings, resulting in a total of 162 seedlings (3 irrigation regimes × 3 foliar treatments × 3 replications × 6 seedlings). The first experimental factor consisted of three irrigation regimes based on field capacity (FC): full irrigation (100% FC), moderate deficit irrigation (85% FC), and severe deficit irrigation (70% FC). These levels were selected to simulate practical irrigation scenarios commonly encountered in nursery production under water-limited environments while allowing discrimination between moderate and severe drought responses [27,28]. The second factor consisted of three foliar treatments: (i) distilled water (control), (ii) chitosan at 1 g L⁻¹, and (iii) *Spirulina platensis* extract at 1 g L⁻¹. Chitosan (≥85% degree of deacetylation) was obtained from Sigma-Aldrich (St. Louis, MO, USA), whereas the *Spirulina* extract was supplied by the Algae Production Unit, National Research Centre, Cairo, Egypt. Foliar treatments were applied using a hand-held sprayer until complete leaf wetting (runoff) at two-week intervals throughout the experimental period. The combination of the three irrigation regimes and three foliar treatments resulted in nine treatment combinations.

2.4. Irrigation Management

Field capacity was determined gravimetrically following the procedure described by [29]. Pot capacity was calculated as the difference between the saturated and oven-dried weights of the substrate. Throughout the experiment, pot weights were monitored regularly, and irrigation water was supplied to maintain the target field capacity level for each treatment. Seedlings were maintained under full irrigation during the first 20 days after transplanting to ensure successful establishment. Thereafter, irrigation treatments were imposed and maintained until the end of the experiment.

2.5. Determination of Substrate Field Capacity

Before initiating the irrigation treatments, the field capacity (FC) of the growing medium was determined using the gravimetric method. Each pot containing 8 kg of the substrate mixture (sand:clay:peat moss, 2:1:1, v/v/v) was thoroughly saturated with wa-

ter until drainage occurred through the bottom of the pot. The pots were then allowed to drain freely for 24 h under greenhouse conditions to remove gravitational water. The pot weight after drainage was considered to represent 100% field capacity (FC). The weight of the substrate at 85% FC and 70% FC was subsequently calculated from the weight recorded at 100% FC [30]. Throughout the experimental period, pots were weighed immediately before each irrigation event using a digital balance. The amount of irrigation water required for each treatment was determined as the difference between the actual pot weight and the predetermined target weight corresponding to 100%, 85%, or 70% FC, respectively. The calculated amount of water was then applied to restore the substrate to the assigned field capacity level.

2.6. Applied Irrigation Water

Applied irrigation water (AIW) was recorded as the cumulative volume of water added to each pot throughout the experimental period. At each irrigation event, the amount of water applied was adjusted to restore the substrate moisture content to the predetermined field capacity (100, 85, or 70% FC). The cumulative AIW was expressed as L plant^{-1} .

2.7. Water Consumptive Use

Actual water consumptive use (WCU) was determined using the gravimetric pot-weighing method adapted from Hansen et al. [31]. Because the experiment was conducted in pots, each pot was considered a single homogeneous rooting volume. Pots were weighed immediately before irrigation and again 48 h after irrigation using a digital balance. The reduction in pot weight between two successive measurements was considered as [30] to represent water loss through evapotranspiration and was used to estimate water consumptive use (WCU). The cumulative WCU for each treatment was obtained by summing the water consumed between successive irrigation events throughout the experimental period and was expressed as L plant^{-1} .

2.8. Water Productivity

Two indicators of water productivity were calculated according to Ali & Klein [32]. Water productivity based on applied irrigation water (WPa) was calculated as $\text{WPa} = Y/\text{AIW}$, where Y is total seedling biomass production (g plant^{-1}) and AIW is applied irrigation water (L plant^{-1}). Water productivity based on consumptive water use (WPc) was calculated as $\text{WPc} = Y/\text{WCU}$, where WCU is the cumulative water consumptive use (L plant^{-1}) during the experimental period.

2.9. Vegetative Growth Measurements

Vegetative growth measurements were recorded at the beginning and the end of the experimental period. Plant height (cm), stem diameter (mm), number of shoots per plant, number of leaves per plant, shoot length (cm), and leaf area (cm^2) were measured, and their increases were expressed as percentages relative to the initial values. Leaf area was estimated according to Ahmed and Morsy [33] using the following equation: $\text{leaf area (cm}^2\text{)} = 0.60 (\text{LL} \times \text{LW}) - 1.05$, where LL is leaf length and LW is leaf width. The percentage increase in each vegetative growth parameter was calculated as follows: $\text{Increase (\%)} = [(\text{Final value} - \text{Initial value})/\text{Initial value}] \times 100$. At the end of the experiment, plants were carefully removed from the pots and separated into shoots and roots. Fresh weights of shoots and roots were determined immediately using a digital balance. The samples were then oven-dried at 70°C until constant weight to determine shoot and root dry weights, which were expressed as grams per plant.

At the end of the experimental period, the photosynthetic pigments were quantified to check the effects of deficit irrigation and biostimulant application on the photosynthetic capacity of passion fruit seedlings. Total chlorophyll was measured by the method described by Moran [34] and Wellburn [35]. Pigment concentrations were expressed as mg g⁻¹ fresh weight (FW). Fully expanded young leaves located in the upper third of each seedling were sampled, and five leaf discs (0.30 cm² each) were immersed in 5 mL of N,N-dimethylformamide (DMF) for pigment extraction. Samples were sonicated for 15 min at 4 °C and incubated in darkness for 16 h to allow complete pigment extraction. The extracts were centrifuged at 16,000 rpm for 5 min, and the absorbance of supernatant was determined by a UV-Vis spectrophotometer (UV-1800, Shimadzu, Corporation, Kyoto, Japan). The absorbance was read at 662 and 650 nm for determination of chlorophyll and at 480 nm for estimation of carotenoids. Chlorophyll concentrations were calculated by the following equation according to Moran [34]:

$$\text{Total chlorophyll: } TotalChlorophyll = Chl\ a + Chl\ b$$

2.11. Water-Saving and Drought-Tolerance Indices

To evaluate the effectiveness of deficit irrigation and biostimulant applications in improving water-use efficiency and maintaining seedling growth under water-limited conditions, several water-saving and drought-tolerance indices were calculated.

2.11.1. Water-Saving Percentage (WSP)

The percentage of irrigation water saved relative to the fully irrigated control was calculated as

$$WSP (\%) = [(AIW_{\text{control}} - AIW_{\text{treatment}}) / AIW_{\text{control}}] \times 100$$

where AIW control is the applied irrigation water under 100% field capacity and AIW treatment is the applied irrigation water under each irrigation biostimulant combination.

2.11.2. Relative Growth Maintenance Index (RGMI)

The ability of seedlings to maintain growth under drought stress was estimated according to

$$RGMI (\%) = (\text{Growth under stress} / \text{Growth under full irrigation}) \times 100$$

where Gs is the value of a growth trait under deficit irrigation and Gc is the corresponding value under full irrigation (100% FC).

2.11.3. Water Productivity Improvement (WPI)

The improvement in water productivity resulting from biostimulant application was calculated as $WPI (\%) = \frac{WP_t - WP_c}{WP_c} \times 100$, where (WP_t) is water productivity of the treated seedlings (g L⁻¹), and (WP_c) is water productivity of the untreated control seedlings (g L⁻¹).

2.12. Economic Water Productivity (EWP)

Economic water productivity (EWP) was calculated to assess the economic return generated per unit of irrigation water applied. Since all seedlings were considered marketable and sold as nursery plants, a fixed commercial value (100 EGP seedling⁻¹) was adopted for EWP calculations.

$$EWP = \frac{\text{Economic return } ER}{\text{Water applied } AIW} \quad (1)$$

where EWP is the economic water productivity (EGP L⁻¹), ER is the economic return per seedling (100 EGP plant⁻¹), and AIW is the applied irrigation water (L plant⁻¹).

2.13. Statistical Analysis

Data from the two growing seasons were subjected to combined analysis after confirming the homogeneity of variance. Analysis of variance (ANOVA) was performed using CoStat software (Version 6.303, CoHort Software, Monterey, CA, USA) to evaluate the main effects of irrigation regime, foliar biostimulant treatment, and their interaction. When ANOVA indicated significant treatment effects, Duncan's multiple range test ($p \leq 0.05$) was applied to separate treatment means and identify statistically significant differences among the irrigation and biostimulant treatment combinations.

2.14. Multivariate and Advanced Statistical Analyses

Passion fruit seedlings were treated with different biostimulants and irrigation regimes, and several multivariate statistical analyses were carried out to obtain a complete understanding of the relationships between growth characteristics, water productivity parameters and physiological responses.

2.14.1. Pearson Correlation Analysis

Pearson correlation analysis was performed to quantify the strength and direction of the linear relationships among vegetative growth traits, total chlorophyll content, biomass production, and water productivity parameters. This analysis was used to identify the traits most closely associated with improved water-use efficiency and drought tolerance, thereby providing insight into the physiological responses of passion fruit seedlings to deficit irrigation and foliar biostimulant application. Pearson correlation coefficients (r) were calculated using standardized data, and the resulting correlation matrix was visualized as a heatmap to facilitate the interpretation of positive and negative relationships among the measured variables.

2.14.2. Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed to reduce the dimensionality of the dataset, identify the major sources of variation among treatments, and determine the relationships among growth, physiological, and water productivity traits. The analysis included water productivity based on applied irrigation water (WPa), water productivity based on consumptive water use (WPc), plant height increment, stem diameter increment, leaf number increment, shoot length increment, leaf area increment, total chlorophyll content, and biomass production. Prior to analysis, all variables were standardized (mean = 0, standard deviation = 1) to eliminate differences in measurement scales. PCA was performed using the correlation matrix, and the first two principal components (PC1 and PC2) were retained because they explained the largest proportion of the total variance. A PCA biplot was generated to visualize treatment clustering, identify the variables that most strongly contribute to treatment separation, and evaluate relationships among the measured traits.

2.14.3. Three-Dimensional Response Surface Analysis

Three-dimensional response surface analysis (RSM) was performed to visualize and interpret the interactive effects of irrigation level and foliar biostimulant application on water productivity based on consumptive water use (WPc) and plant height increment.

The response surfaces were generated from the experimental data using interpolation techniques to illustrate the combined influence of the two experimental factors over the measured response variables. This analysis was used to identify treatment combinations that maximized water productivity while maintaining vigorous seedling growth under deficit irrigation, thereby facilitating the identification of the optimum irrigation–biostimulant combination for sustainable nursery production.

2.14.4. Performance Ranking Heatmap

To provide a comprehensive visual evaluation of the treatment performance across multiple measured traits, a treatment performance ranking heatmap was generated. The evaluated variables were WPa, WPc, plant height increment, stem diameter increment, leaf number increment, shoot length increment, leaf area increment, chlorophyll content and economic water productivity (EWP). Individual trait values were normalized relative to their maximum observed values according to the following equation: $X_n = X_i / X_{max}$, where X_n is the normalized value, X_i is the observed value and X_{max} is the maximum recorded value for the respective trait. Values were normalized to a 0–1 scale with higher values representing better treatment performance. The heatmap was utilized to rank the treatments and identify the best combinations for sustaining growth and water productivity under deficit irrigation conditions. All statistical and graphical analyses were conducted in Python (version 3.x) using Pandas, NumPy, Scikit-learn, SciPy, Matplotlib, and Seaborn packages. **Python (version 3.12.13) with Pandas (version 2.2.2), NumPy (version 2.0.2), Scikit-learn (version 1.6.1), SciPy (version 1.16.3), Matplotlib (version 3.10.0), and Seaborn (version 0.13.2).**

3. Results

3.1. Applied Irrigation Water and Water Consumptive Use

3.1.1. Applied Irrigation Water (AIW)

The irrigation regime and biostimulant application significantly affected the applied irrigation water (Figure 2A). The decrease in total volume of irrigation water applied per seedling from 100% to 70% field capacity was progressive regardless of foliar treatment. The highest values of AIW were recorded under full irrigation (100% FC), ranging from 16.75 to 19.79 L plant⁻¹, while the lowest values were recorded under the treatment of 70% FC, ranging from 12.30 to 14.10 L plant⁻¹. Both chitosan and Spirulina extract decreased the irrigation water requirements at all irrigation levels in comparison to the untreated control. At 100% FC, chitosan application reduced AIW by 15.4% compared to the untreated control, while Spirulina extract decreased AIW by 9.1%. Similar trends were also obtained under deficit irrigation conditions, indicating that the biostimulant-treated seedlings require less irrigation inputs to maintain the targeted moisture level. The results showed that the foliar application of chitosan and Spirulina extract enhanced water-use efficiency and reduced irrigation requirements under both optimal and deficit irrigation conditions.

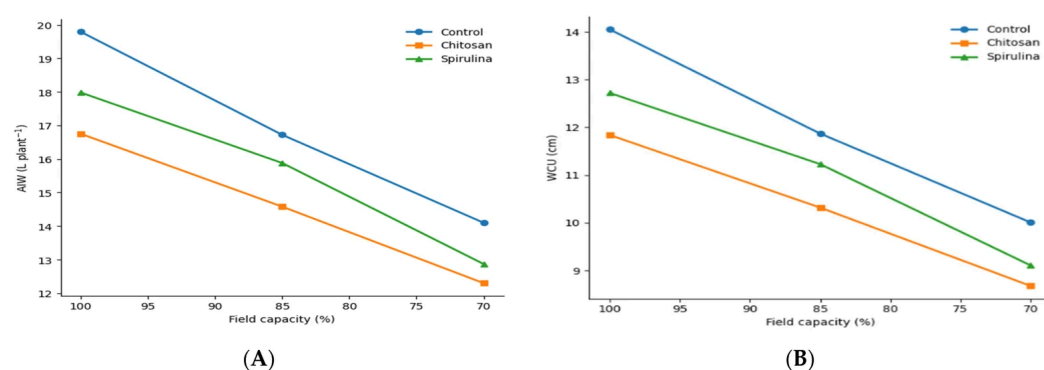


Figure 2. Effects of deficit irrigation and biostimulant applications on water relations of passion fruit seedlings. (A) Applied irrigation water (AIW, L plant⁻¹) and (B) water consumptive use (WCU, cm) under different field capacity levels (100, 85, and 70% FC).

3.1.2. Water Consumptive Use (WCU)

Water consumptive use (WCU) was significantly affected by the irrigation regime and biostimulant application (Figure 2B). WCU gradually decreased as the field capacity decreased from 100% to 70%, indicating that less water was extracted by passion fruit seedlings under deficit irrigation. The highest WCU values were observed under full irrigation (100% FC) followed by severe water deficit (70% FC) under all foliar treatments. The control registered WCU values of 14.05, 11.87 and 10.01 cm under 100%, 85% and 70% FC, respectively. Compared with the control, foliar application of chitosan significantly reduced water consumption at all levels of irrigation, with values of 11.84, 10.32 and 8.68 cm, respectively, under the respective irrigation treatments. Similarly, WCU was reduced by Spirulina extract to 12.72, 11.23 and 9.11 cm under 100%, 85% and 70% FC, respectively. The relative reduction in WCU resulting from chitosan application reached 15.73, 13.06, and 13.29% under 100%, 85%, and 70% FC, respectively, compared with the untreated seedlings. Corresponding reductions achieved by Spirulina extract were 9.47, 5.39, and 8.99%, respectively. These results indicate that chitosan is more effective in reducing water use under both optimal and deficit irrigation scenarios. The interaction between irrigation regime and biostimulant application showed that the combination of 70% FC and chitosan treatment recorded the lowest WCU value (8.68 cm) with a reduction of about 38.2% compared with the fully irrigated untreated control. This implies that biostimulant application increases the ability of passion fruit seedlings to utilize available water more efficiently, thereby reducing the overall water consumption and maintaining plant growth in water-limited conditions. The observed reduction in water consumptive use, particularly in chitosan-treated seedlings, highlights the potential of integrating biostimulant application with deficit irrigation strategies to improve water-use efficiency and enhance the sustainability of passion fruit production under limited water availability.

3.1.3. Water Productivity Based on Applied Irrigation Water (WPa)

The irrigation regime, biostimulant application and the interaction of both factors significantly affected the water productivity based on applied irrigation water (WPa) (Figure 3A). Under all foliar treatments, WPa decreased as irrigation level decreased from 100% to 70% FC. The untreated control exhibited a significant reduction from 1.99 g L⁻¹ in full irrigation to 0.90 g L⁻¹ under severe water deficit, which pointed to a drastic decrease in biomass production per unit of applied water. All irrigation regimes showed significantly higher WPa when treated with foliar application of chitosan and Spirulina extract compared to the untreated seedlings. In full irrigation, the highest WPa value

was achieved in Spirulina-treated plants (3.46 g L^{-1}) followed by chitosan-treated seedlings (3.24 g L^{-1}), showing increments of 73.9% and 62.8%, respectively, compared to the control treatment. Under moderate water deficit (85% FC), chitosan and Spirulina applications increased WPa to 1.82 and 1.79 g L^{-1} , respectively, compared with 1.18 g L^{-1} in untreated seedlings. Similarly, under severe water deficit (70% FC), WPa values of 1.52 and 1.51 g L^{-1} were obtained for chitosan and Spirulina treatments, respectively, whereas the untreated control recorded only 0.90 g L^{-1} . The interaction effect demonstrated that biostimulant-treated seedlings maintained considerably higher water productivity than untreated plants under all irrigation levels. The highest WPa was achieved by Spirulina-treated seedlings under 100% FC (3.46 g L^{-1}), while the lowest value was recorded in untreated plants under 70% FC (0.90 g L^{-1}). These findings indicate that the application of chitosan and Spirulina extract enhanced the efficiency with which passion fruit seedlings converted applied irrigation water into plant biomass, thereby improving overall water-use performance under both adequate and deficit irrigation conditions.

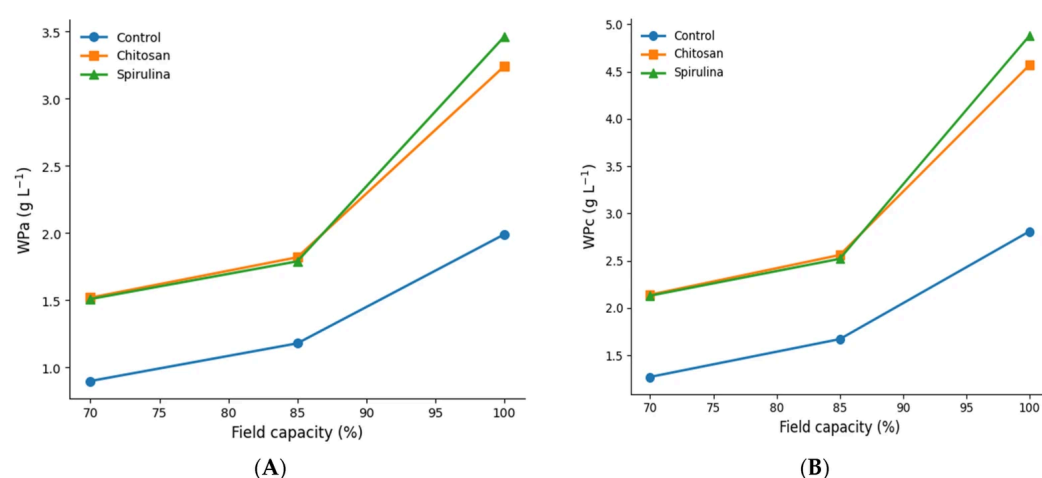


Figure 3. Effect of deficit irrigation and biostimulant applications (chitosan and Spirulina extract) on water productivity based on applied irrigation water (WPa, g L^{-1}) and water productivity based on consumptive water use (WPc, g L^{-1}) of passion fruit seedlings grown under different field capacity levels.

3.1.4. Water Productivity Based on Consumptive Use (WPc)

Irrigation level, biostimulant application and their interaction had a significant effect on water productivity based on consumptive water use (WPc) (Figure 3B). In general, WPc values declined with the increasing water deficit, but the application of chitosan and Spirulina extract considerably improved the efficiency with which passion fruit seedlings transformed consumed water under all irrigation regimes. For the untreated plants, WPc decreased from 2.81 g L^{-1} at 100% FC to 1.67 and 1.27 g L^{-1} at 85% and 70% FC, respectively. This decrease reflects the negative impact of water deficit on biomass production relative to the actual water consumption. In contrast, the foliar application of biostimulants markedly increased WPc in all irrigation levels. The maximum value of WPc was observed in the Spirulina-treated seedlings under full irrigation (4.88 g L^{-1}), followed by chitosan-treated plants (4.57 g L^{-1}), which increased by 73.7 and 62.6%, respectively, over the untreated control. WPc increased from 1.67 g L^{-1} in the untreated seedlings to 2.56 and 2.52 g L^{-1} after the application of chitosan and Spirulina under a moderate water deficit of 85% FC. Similarly, WPc was 2.14 and 2.13 g L^{-1} in chitosan- and Spirulina-treated plants, respectively, under a severe water deficit of 70% FC in comparison to 1.27 g L^{-1} in untreated seedlings. The interaction effect showed that biostimulant

application could compensate for the reduction in water availability by improving the water-use efficiency. Notably, the WPC of chitosan-treated seedlings grown at 70% FC was similar or higher than that of untreated plants grown at 85% FC, demonstrating the potential of biostimulants to sustain productivity under reduced irrigation. The results overall indicate that the use of chitosan and Spirulina extract improved the efficiency of passion fruit seedlings in converting the water used into biomass. The increase in WPC was especially evident under conditions of deficit irrigation, showing that the combination of biostimulant application and controlled water supply can result in a significant increase in water productivity and more sustainable nursery production systems in environments with water scarcity.

3.2. Vegetative Growth Responses

3.2.1. Plant Height, Shoot Length and Stem Diameter

Irrigation regime, application of biostimulant and their interaction significantly affected the percentages of increment in plant height, shoot length and stem diameter (Figure 4A–C). With the decrease in irrigation from 100 to 70% FC, a progressive reduction in vegetative growth was observed. The mean increment of plant height across the foliar treatments decreased from 275.51% under full irrigation to 139.32% under 70% FC, a reduction of about 49.4%. Stem diameter increment decreased as well (63.69 to 54.28%). Shoot length increment decreased significantly (358.72 to 154.60%). The foliar application of chitosan and Spirulina extract significantly increased growth as compared to the untreated seedlings under all irrigation regimes. The maximum increase in average plant height (236.96%) and shoot length (283.08%) was observed in chitosan, while the maximum increase in stem diameter (64.09%) was observed in Spirulina. The interaction effect revealed that the highest stem diameter increment (72.86%) was obtained in Spirulina-treated seedlings at 100% FC, and the highest plant height increment (284.32%) was obtained with chitosan at the same irrigation level. However, untreated seedlings grown under 70% FC exhibited the lowest growth performance, with only a 113.02% increase in plant height and 51.15% increase in stem diameter. Significantly, the vegetative growth of biostimulant-treated seedlings was found to be significantly higher under deficit irrigation than untreated plants, indicating the efficacy of biostimulants in alleviating drought-induced growth depression and maintaining seedling vigor under water stress.

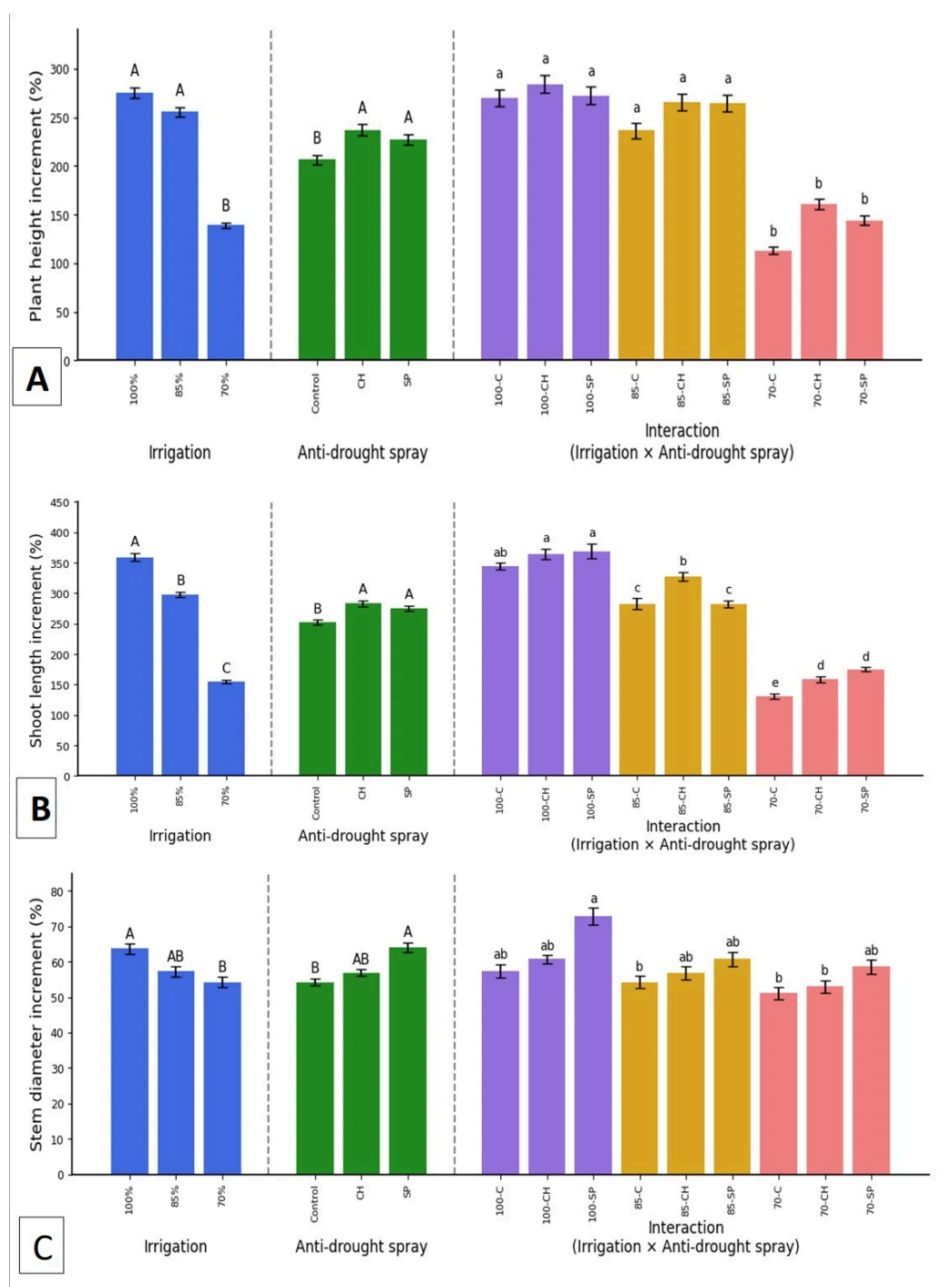


Figure 4. Effects of irrigation regimes, anti-drought spray treatments, and their interaction on plant height increment (%) (A), shoot length increment (%) (B) and stem diameter increment (%) (C) of passion fruit seedlings. Bars represent mean $\pm$ SE. Different letters indicate significant differences according to Duncan's test at $p \leq 0.05$.

3.2.2. Number of Leaves and Leaf Area

The percentage increase in leaf number and leaf area was significantly affected by irrigation regime, biostimulant application and their interaction (Figure 5A,B). Both parameters declined progressively with increasing water deficit, indicating the inhibitory effect of limited water availability on canopy development and leaf expansion. The foliar treatments decreased the number of leaves by $\sim 29.8\%$ (average) with a maximum reduction of 384.18% (100% FC) to 269.77% (70% FC). Likewise, the mean leaf area decreased

from 4.70 under full irrigation to 1.82 under severe water deficit, indicating a decrease of 61.3%. The biostimulant application significantly improved both traits with respect to untreated seedlings. The highest mean increase in leaf number was found in chitosan-treated seedlings (355.84%) followed by Spirulina extract (325.36%), while untreated seedlings recorded only 288.31%. Also, chitosan gave the highest average value of leaf area (3.95) compared to 2.35 and 2.34 for Spirulina-treated and untreated plants, respectively. The interaction effect of irrigation level and foliar treatments showed that the maximum increase in leaf number (404.98%) was recorded in Spirulina-treated seedlings under 100% FC followed by chitosan-treated plants (383.05%). The minimum value (203.10%) was recorded in untreated seedlings at 70% FC. Maximum (7.11) leaf area was recorded in the seedlings treated with chitosan under full irrigation and the minimum (1.46) in the plants treated with chitosan under 70% FC. Both biostimulants greatly enhanced leaf development compared with untreated seedlings under deficit irrigation conditions. The chitosan-treated plants at 70% FC increased the leaf number by 55.4% compared to the untreated control plants, while Spirulina increased the leaf number by 43.1%. These results suggest that biostimulant application partially alleviated the reduction in canopy development caused by drought and contributed to the maintenance of a higher photosynthetically active surface area under water-limited conditions.

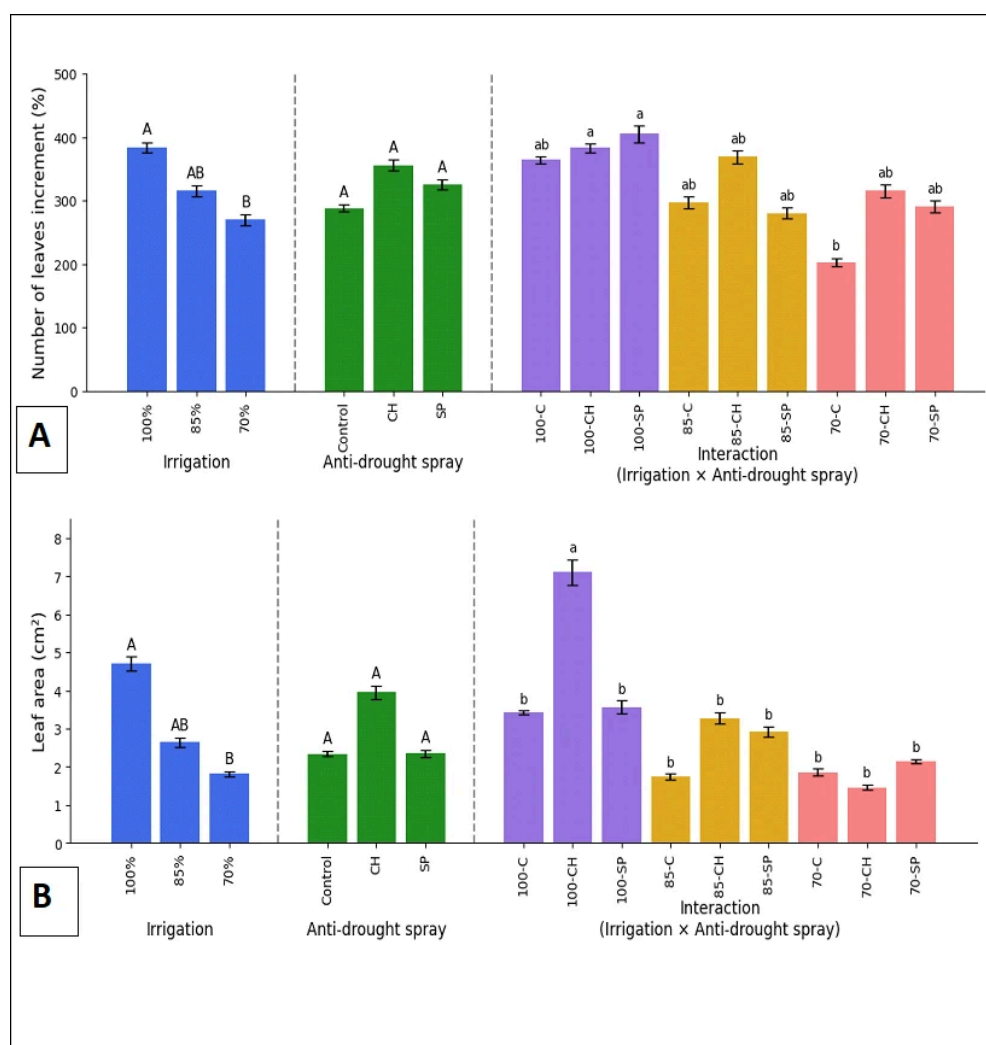


Figure 5. Interaction effects of deficit irrigation and biostimulant applications (chitosan and Spirulina extract) on number of leaves increment (%) (A) and leaf area (cm²) increment % (B) of

passion fruit seedlings grown under different field capacity levels. Bars represent means $\pm$ SE. Different letters indicate significant differences among treatments according to Duncan's test at $p \leq 0.05$.

3.2.3. Plant Fresh and Dry Weight (g)

Plant fresh weight and dry weight were significantly influenced by irrigation regime, anti-drought spray treatments and their interaction (Figure 6A,B). Full irrigation (100% FC) resulted in the highest fresh and dry biomass production (average values of 176.26 and 52.02 g, respectively), while the most severe water deficit (70% FC) significantly reduced biomass production to 59.63 and 16.96 g, respectively. Plant fresh weight reduction was 66.2%, while plant dry weight decreased by 67.4% in comparison with fully irrigated plants. Chitosan and Spirulina significantly improved biomass production compared to the untreated control in anti-drought spray treatments. Spirulina application gave the highest mean plant fresh weight (122.15 g) and plant dry weight (36.70 g), chitosan had the second highest mean value of plant fresh weight (113.60 g) and dry weight (33.19 g), and untreated plants had the lowest mean values of plant fresh weight (88.73 g) and dry weight (24.00 g). The interaction between irrigation regime and anti-drought sprays also affected biomass production. The highest plant fresh weight (210.10 g) and plant dry weight (62.25 g) were recorded on Spirulina-treated seedlings under full irrigation (100% FC) followed by chitosan-treated plants under the same irrigation level (186.45 and 54.35 g, respectively). In contrast, the lowest values of fresh and dry biomass were obtained in untreated plants subjected to severe water deficit (70% FC) and were 49.40 and 12.72 g, respectively. Application of biostimulant partially alleviated the adverse effects of drought stress under moderate water deficit (85% FC). Plant fresh weight increased by ~10.9% and plant dry weight by 33.8% for chitosan compared to the untreated plants under the same irrigation regime. Similarly, fresh biomass and dry biomass were increased by 4.1 and 43.4%, respectively, for Spirulina treatment. Under severe water deficit (70% FC), chitosan increased plant fresh and dry weights by 23.8% and 47.2%, respectively, as compared to untreated plants, while Spirulina increased these traits by 38.4% and 52.8%, respectively. The results indicated that biomass production in passion fruit seedlings was very sensitive to water availability. Nonetheless, the application of chitosan and Spirulina effectively improved fresh and dry matter production, especially under moderate- and severe-water-deficit conditions, indicating their potential role in improving plant growth and alleviating the drought-induced reduction in biomass.

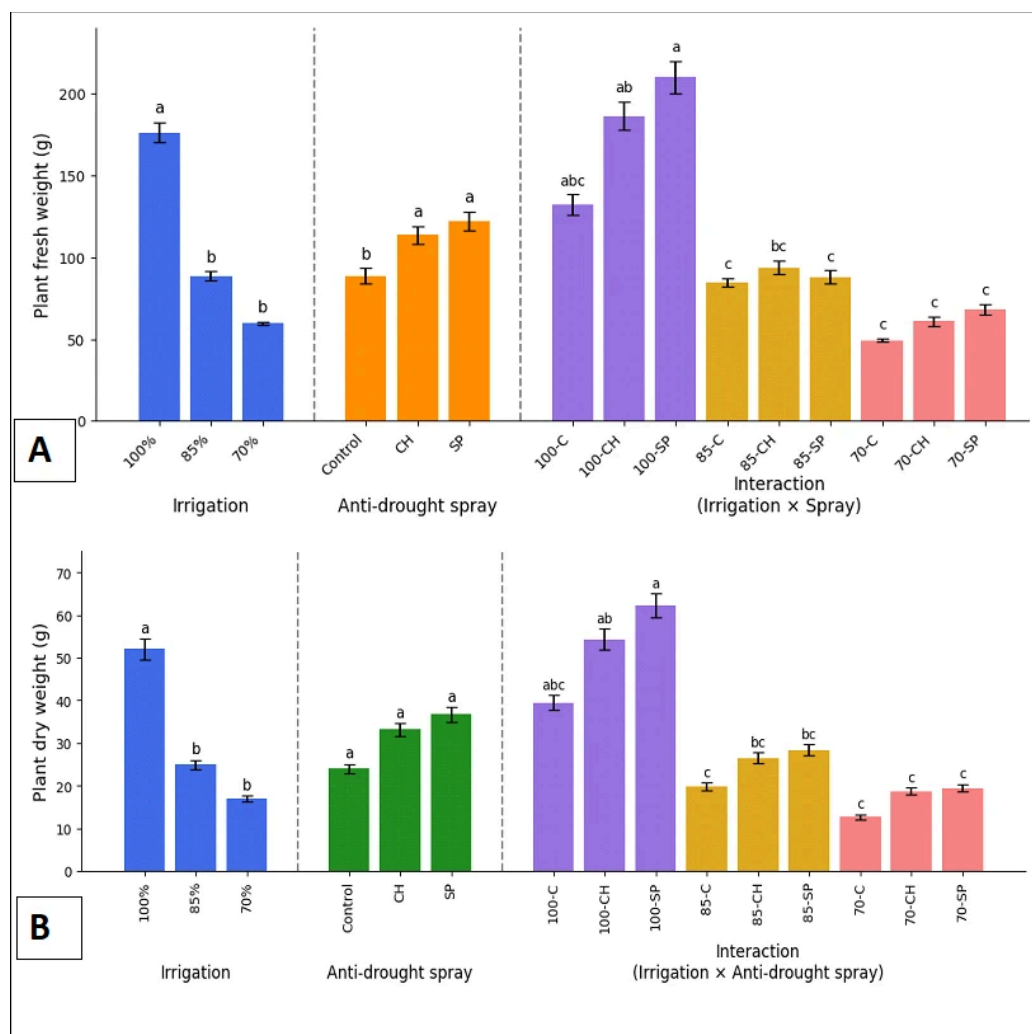


Figure 6. Effects of irrigation regimes, anti-drought sprays, and their interaction on plant fresh weight (g) (A) and plant dry weight (g) (B) of passion fruit seedlings. Bars represent mean $\pm$ SE. Different letters indicate significant differences among treatments according to Duncan's test at $p \leq 0.05$.

3.2.4. Root Fresh and Dry Weight (g)

The root fresh weight and root dry weight were significantly affected by irrigation regime, anti-drought spray treatments and their interaction (Figure 7A,B). The maximum average root fresh weight was recorded under moderate deficit irrigation (85% FC) (60.65 g), followed by full irrigation (51.68 g), while the severe water deficit (70% FC) significantly reduced root fresh weight to 34.96 g. Likewise, root dry weight was maximized under 85% FC (9.62 g) and under 100% FC (8.17 g), while the minimum was recorded under 70% FC (5.09 g). The fresh weight of roots was not significantly affected by the application of either chitosan or Spirulina as anti-drought spray treatment, with mean values ranging from 48.43 to 50.40 g. However, the dry weight of roots was significantly affected by the biostimulant treatments; the application of chitosan showed the highest mean value (8.09 g), followed by Spirulina (7.91 g), while untreated plants had the lowest root dry weight (6.88 g). The interaction between irrigation level and anti-drought sprays showed significant differences between treatments. The maximum fresh weight of roots (68.25 g) and dry weight of roots (11.53 g) were observed in seedlings treated with chitosan under moderate water deficit (85% FC), indicating better root de-

velopment under this irrigation regime. On the contrary, the minimum root fresh weight (26.70 g) and root dry weight (3.70 g) were observed in untreated plants under severe water deficit (70% FC). Within the significant irrigation $\times$ biostimulant interaction, seedlings treated with chitosan under 85% FC exhibited a root fresh weight approximately 7.4% higher than the untreated control and root dry weight 34.9% higher than the untreated control under the same irrigation regime. Similarly, Spirulina application enhanced root dry weight by 2.6% over the untreated control under 85% FC. Chitosan application increased root fresh and dry weights by 32.6 and 55.1%, respectively, while Spirulina application increased root fresh and dry weights by 60.3 and 57.6%, respectively, compared to untreated plants under severe water deficit (70% FC). Importantly, moderate deficit irrigation (85% FC) increased root biomass production compared to full irrigation, particularly within the significant irrigation $\times$ biostimulant interaction. This response showed a shift in biomass allocation to the development of roots under moderate water deficit, allowing seedlings to absorb more water and to develop in lower-water-content soils, but at the expense of shoot growth. Severe drought stress (70% FC) caused a strong reduction in root biomass, but was positively modulated by the application of the biostimulant. Finally, the results obtained showed that the combined application of moderate deficit irrigation and chitosan treatment was the most efficient strategy to improve root development and biomass production, while severe water deficit significantly inhibited root growth in passion fruit seedlings.

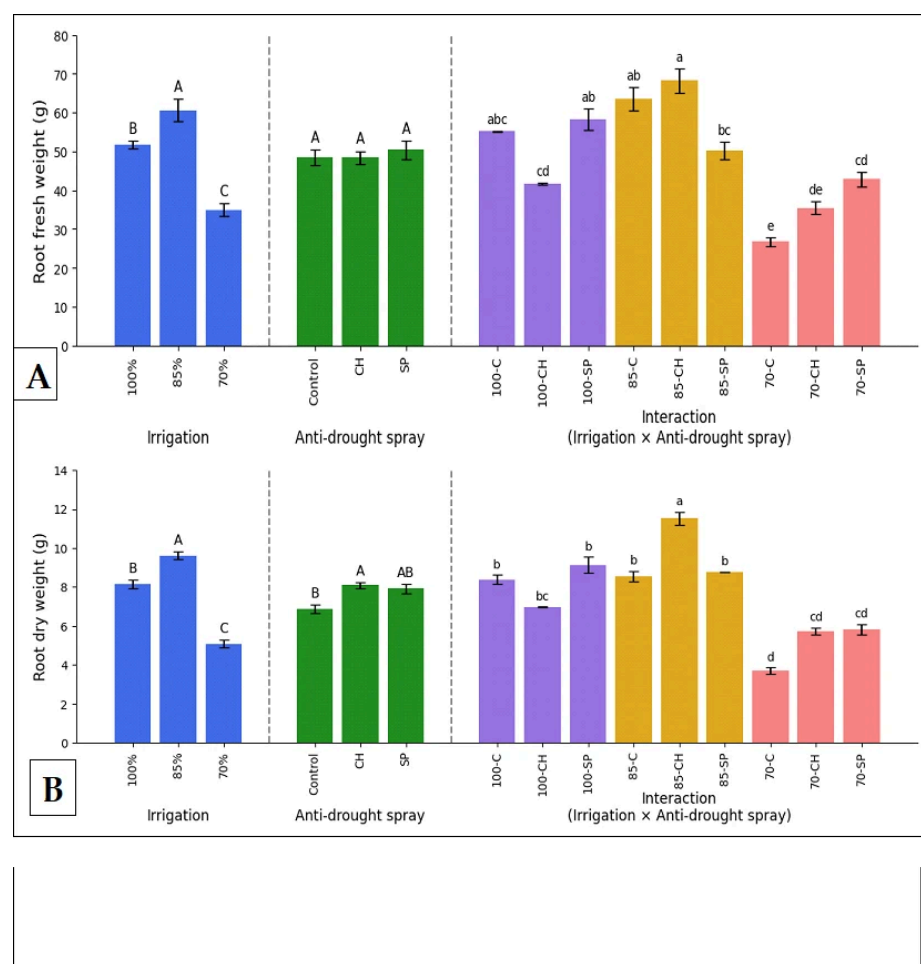


Figure 7. Effects of irrigation regimes, anti-drought sprays, and their interaction on root fresh weight (g) (A) and root dry weight (g) (B) of passion fruit seedlings. Bars represent mean $\pm$ SE.

Different letters indicate significant differences among treatments according to Duncan's test at $p \leq 0.05$.

3.3. Total Chlorophyll Content

Total chlorophyll content was significantly affected by irrigation regime, anti-drought spray treatments and their interaction (Figure 8). Full irrigation (100% FC) had the highest chlorophyll content, with an average value of 12.01 mg 100 g⁻¹ FW, while moderate- and severe-water-deficit treatments (85 and 70% FC) significantly reduced the chlorophyll concentration to 6.31 and 5.25 mg 100 g⁻¹ FW, respectively. Biostimulant application significantly increased chlorophyll accumulation compared to the untreated control. The maximum mean chlorophyll content was observed in chitosan treatment (11.75 mg 100 g⁻¹ FW) followed by spirulina extract (8.18 mg 100 g⁻¹ FW), while the minimum value was observed in untreated plants (3.64 mg 100 g⁻¹ FW). The interaction between irrigation level and anti-drought spray showed significant differences between the treatments. The maximum value of chlorophyll concentration (16.43 mg 100 g⁻¹ FW) was achieved in the seedlings treated with chitosan under full irrigation (100% FC) followed by Spirulina-treated plants under the same irrigation regime (11.55 mg 100 g⁻¹ FW). On the other hand, the minimum chlorophyll content (0.82 mg 100 g⁻¹ FW) was observed in untreated plants under severe water deficit (70% FC). Application of chitosan increased chlorophyll content by about 405% as compared with untreated plants under moderate water deficit (85% FC). Spirulina improved chlorophyll concentration by about 218%. Similarly, under severe water deficit (70% FC), chlorophyll content increased by approximately 930% and 690% by application of chitosan and Spirulina, respectively, when compared to the untreated control. In general, the results indicate that water deficit caused a significant reduction in chlorophyll accumulation in passion fruit seedlings. But both chitosan and Spirulina were effective in overcoming the adverse effects of drought stress. Chitosan had the highest capability to preserve chlorophyll content under both moderate and severe water deficit.

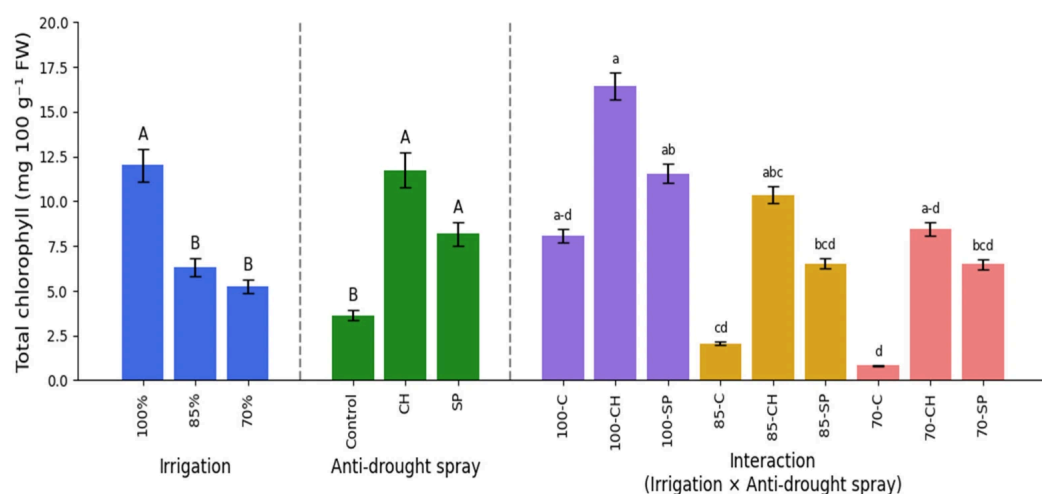


Figure 8. Effects of irrigation regimes, anti-drought spray treatments, and their interaction on total chlorophyll content (mg 100 g⁻¹ FW) of passion fruit seedlings. Bars represent mean $\pm$ SE. Different letters indicate significant differences according to Duncan's test at $p \leq 0.05$.

3.4. Water-Saving and Drought-Tolerance Indices

3.4.1. Water-Saving Percentage (WSP) and Relative Growth Maintenance Index (RGMI)

The water-saving percentage (WSP) and relative growth maintenance index (RGMI)

clearly showed the beneficial effects of biostimulant application under deficit irrigation conditions (Figure 9A,B). The maximum values of WSP were found for chitosan-treated seedlings under all irrigation regimes (15.36% under full irrigation and around 12.8% under deficit irrigation treatments). The calculated RGMI values indicated that both biostimulants increased the passion fruit seedlings' potential to maintain growth under water-deficit conditions. RGMI values were 93.53 and 97.09% in chitosan- and Spirulina-treated plants under moderate water stress (85% FC) and only 87.57% in untreated seedlings. Chitosan maintained 56.51% of the growth under full irrigation under severe water deficit (70% FC), while untreated plants maintained only 41.88%. These results show that the use of biostimulants reduces the need for water irrigation, while at the same time increasing the ability of the seedlings to sustain growth under conditions of water scarcity. The water savings combined with growth maintenance emphasize the potential of combining chitosan and Spirulina extract with deficit irrigation strategies to improve the sustainability of passion fruit nursery production.

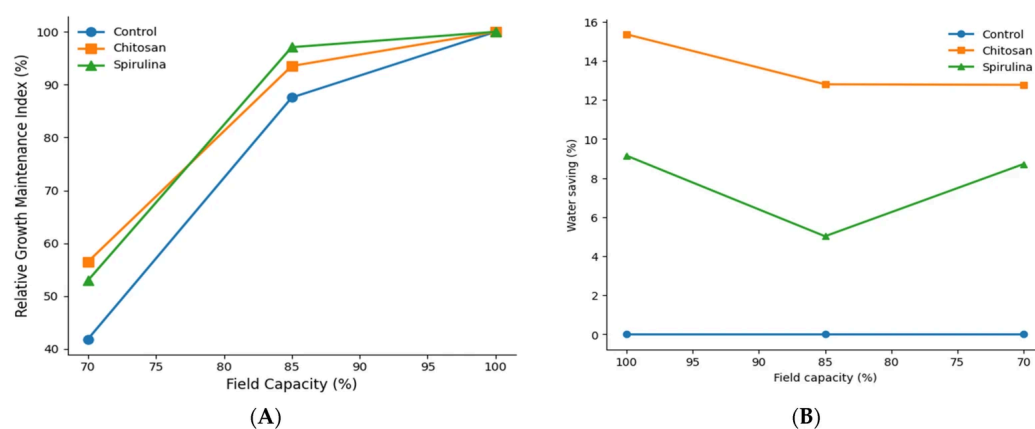


Figure 9. Effects of deficit irrigation and biostimulant applications on (A) water-saving percentage (WSP) and (B) relative growth maintenance index (RGMI) of passion fruit seedlings grown under different field capacity levels (100, 85, and 70% FC). Chitosan and Spirulina extract were applied as foliar sprays at 1 g L⁻¹.

3.4.2. Water Productivity Improvement (WPI)

The water productivity improvement (WPI) values clearly indicated that the application of biostimulant was efficient in improving the water utilization efficiency under both full and deficit irrigation conditions (Figure 10). Water productivity was significantly increased by foliar application of chitosan and Spirulina as compared to untreated seedlings, and thus, the seedlings that received treatment produced more biomass per unit of water consumed. The improved WPI observed could be due to the positive impacts of biostimulants on physiological and biochemical processes related to drought adaptation. Chitosan has been reported to improve stomatal regulation, water retention, nutrient uptake and antioxidant defense systems, thus improving plant performance under water-limited conditions. Spirulina extract also contains amino acids, vitamins, polysaccharides and growth-promoting substances that stimulate metabolic activity and enhance the plant's tolerance to abiotic stress. In the present study, Spirulina had the maximum WPI value under full irrigation while chitosan had high WPI values under severe-water-deficit conditions. This finding reveals that chitosan can be used more efficiently for water productivity maintenance under water stress conditions. The ability of treated seedlings to sustain relatively high productivity under reduced irrigation confirms the beneficial role of biostimulants in improving water-use efficiency and mitigating drought-induced growth reductions. In general, the higher WPI values obtained in

this study confirm that chitosan and Spirulina extract are effective tools for improving water productivity of passion fruit seedlings and support their use as sustainable management practices for nursery production under conditions of limited water availability.

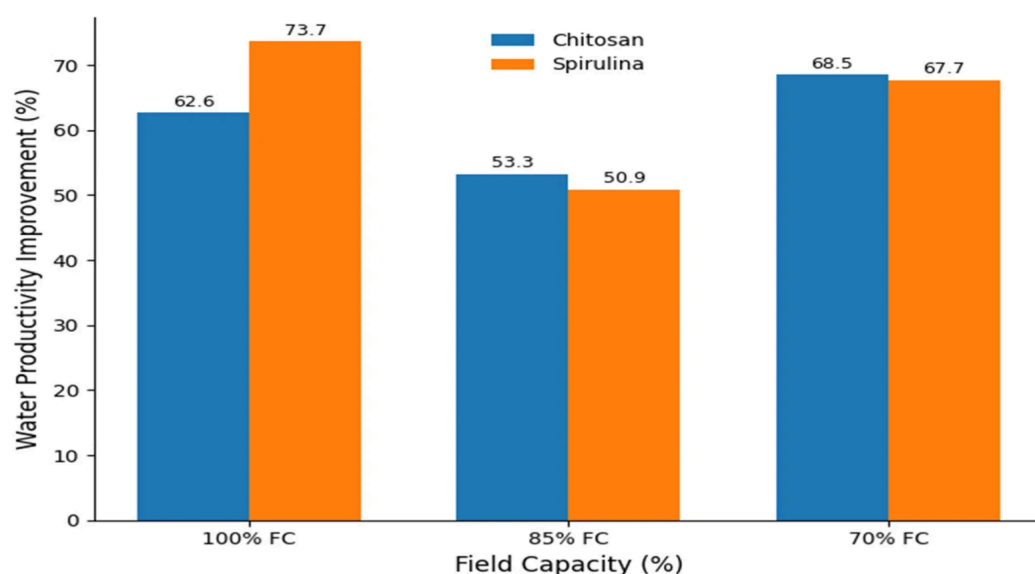


Figure 10. Water productivity improvement (WPI) of passion fruit seedlings treated with chitosan and Spirulina extract under deficit irrigation conditions.

3.5. Economic Water Productivity (EWP)

Economic water productivity (EWP) increased gradually with decreases in the level of irrigation (Table 3). The highest values of EWP were recorded at 70% FC in the case of seedlings treated with chitosan (8130 EGP m⁻³) and Spirulina extract (7769 EGP m⁻³). EWP ranged from 5053 to 5970 EGP m⁻³ under full irrigation. Application of chitosan significantly improved EWP in all irrigation regimes, which demonstrated the efficiency of chitosan in decreasing irrigation requirements with acceptable seedling growth. Chitosan increased EWP by 18.1, 14.7 and 14.6% as compared to the untreated control under 100, 85 and 70% FC, respectively. These results indicate that the combination of deficit irrigation and biostimulant application can considerably improve the economic benefit per unit of irrigation water used.

Table 3. Economic water productivity (EWP) of passion fruit seedlings as affected by irrigation level and anti-drought treatments.

Irrigation Level	Control	Chitosan (1 g L ⁻¹)	Spirulina Extract (1 g L ⁻¹)	Mean
100% FC	5053	5970	5562	5528
85% FC	5981	6859	6297	6379
70% FC	7092	8130	7769	7664
Mean	6042	6986	6543	6524

EWP = economic water productivity (EWP, EGP m⁻³).

3.6. Multivariate Analysis

3.6.1. Correlation Analysis and Principal Component Analysis (PCA)

Pearson correlation analysis and principal component analysis (PCA) were performed to assess the relationships between vegetative growth traits, biomass production,

chlorophyll content, and water productivity parameters of passion fruit seedlings under different irrigation regimes and biostimulant treatments. The correlation matrix showed that the measured variables were mostly positively related (Figure 11A). The water productivity parameters (WPa and WPC) were strongly positively correlated with plant height, shoot length, plant fresh weight, plant dry weight, chlorophyll content and leaf number, indicating that improved water-use efficiency was closely related to improved plant growth and biomass production. Similarly, chlorophyll content was positively correlated with most growth and productivity traits, indicating that maintenance of photosynthetic pigments contributed to improved plant performance under water-deficit conditions. The relationships observed in the correlation analysis were further confirmed by the principal component analysis (Figure 11B). The first two principal components explained 88.87% of the total variation, with PC1 explaining 72.10% and PC2 explaining 16.77%. The large amount of variance explained suggests that the PCA was successful in summarizing treatment responses and interactions among variables. The PCA biplot clearly separated the treatments based on their growth performance and drought adaptation. Treatments applied with Spirulina and chitosan under full irrigation (100% FC) were positioned on the positive side of PC1 and had a strong association with chlorophyll content, water productivity parameters, biomass production, and vegetative growth traits. On the other hand, untreated seedlings under severe water deficit (70% FC) were on the negative side of PC1 and were negatively associated with most of the measured parameters. Interestingly, root fresh weight and root dry weight were important contributors to the discrimination of treatments in PC2. The closeness of the 85% FC + chitosan treatment to root-related variables indicates that chitosan enhanced root development and increased the acquisition of water under moderate-water-deficit conditions. Both analyses showed that chlorophyll content, biomass production, vegetative growth and water productivity were all closely interrelated and, in combination, contributed to the improved drought tolerance of the biostimulant-treated seedlings.

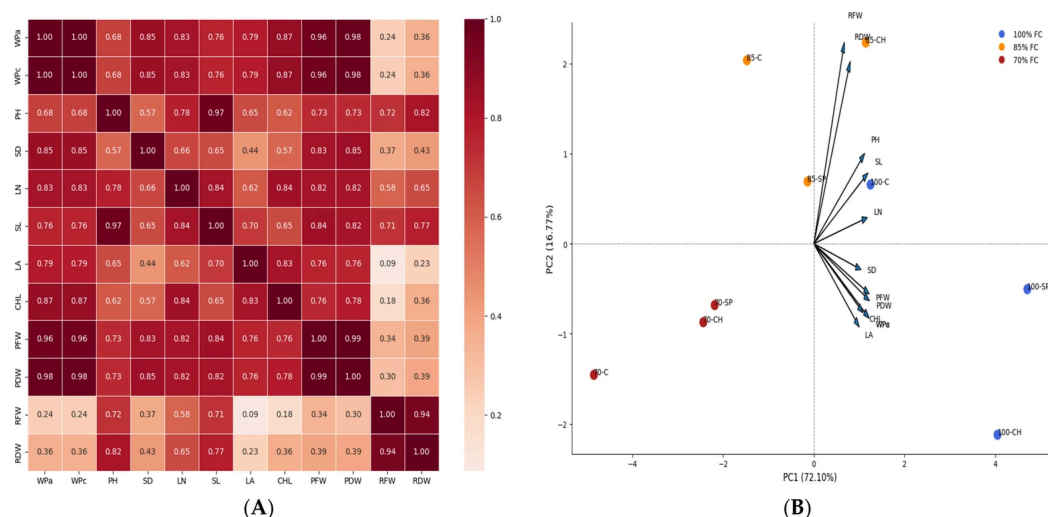


Figure 11. Pearson correlation heatmap and principal component analysis (PCA) biplot illustrating the relationships among water productivity, growth, physiological, and biomass traits of *Passiflora edulis* seedlings grown under different irrigation regimes and foliar biostimulant treatments. Warm colors indicate positive correlations, whereas cool colors indicate negative correlations. The first two principal components (PC1 and PC2) explained 72.10% and 16.77% of the total variance, respectively. Variables included water productivity based on applied irrigation water (WPa), water productivity based on consumptive water use (WPC), plant height increment (PHI), stem diameter increment (SDI), shoot length increment (SLI), leaf number increment (LNI), leaf area increment

(LAI), total chlorophyll content (TChl), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW).

3.6.2. Performance Ranking Heatmap

A performance rating heatmap was generated to summarize the treatment response of parameters related to vegetative growth, biomass production, chlorophyll content, and water-use efficiency (Figure 12). Treatments were ranked according to the average normalized score. The heatmap graphically represented the treatment efficiency for the measured parameters. In general, the seedlings treated with Spirulina or chitosan under full irrigation (100% FC) had the highest normalized scores, indicating better growth, biomass production, chlorophyll content, and water-use efficiency. On the other hand, untreated seedlings grown under severe water deficit (70% FC) had the lowest scores for most variables, indicating significant decrease in plant performance under drought stress. Under moderate water deficit (85% FC), biostimulant-treated plants showed relatively high scores for some traits, especially biomass production, chlorophyll content and water productivity. The chitosan-treated plants responded positively and consistently across a wide range of variables, whereas spirulina treatment improved several growth parameters despite reduced irrigation availability. The ranking analysis further supported that the biostimulant-treated seedlings outperformed untreated controls and that both chitosan and Spirulina were effective in alleviating drought-induced reductions in growth and productivity.

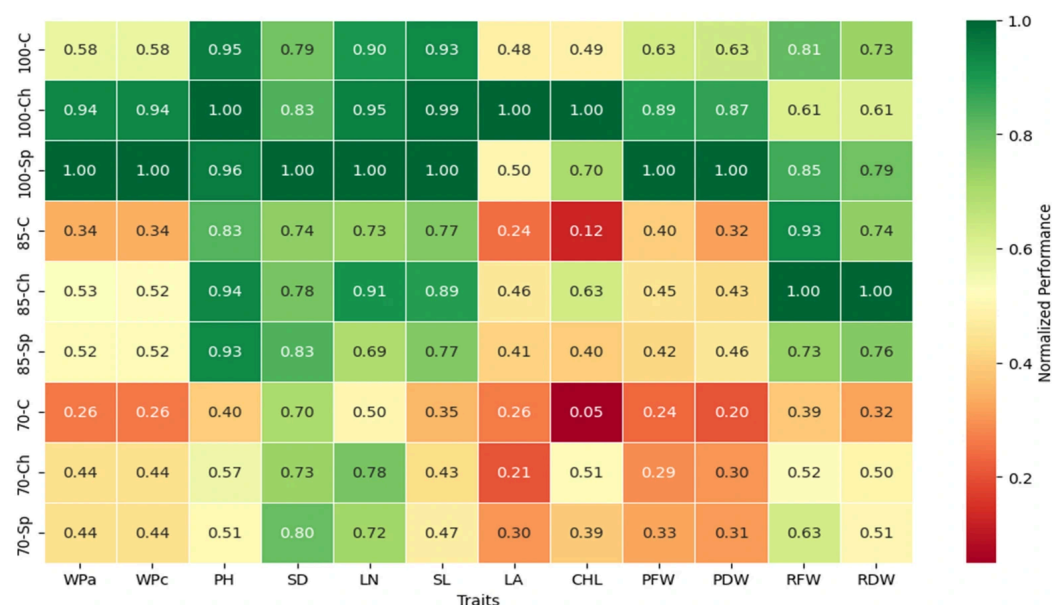


Figure 12. Performance ranking heatmap of *Passiflora edulis* seedlings subjected to different irrigation regimes and foliar biostimulant treatments. Values represent normalized scores (0–1), where higher values indicate superior performance for the corresponding trait. Green colors denote favorable responses, whereas red colors indicate lower performance. The evaluated traits were water productivity based on applied irrigation water (WPa), water productivity based on consumptive water use (WPc), plant height increment (PH), stem diameter increment (SD), leaf number increment (LN), shoot length increment (SL), leaf area (LA), total chlorophyll content (CHL), plant fresh weight (PFW), plant dry weight (PDW), root fresh weight (RFW), and root dry weight (RDW). Treatment combinations were 100-C (100% field capacity + distilled water), 100-Ch (100% field capacity + chitosan), 100-Sp (100% field capacity + Spirulina extract), 85-C (85% field capacity + distilled water), 85-Ch (85% field capacity + chitosan), 85-Sp (85% field capacity + Spirulina extract), 70-C (70% field capacity + distilled water), 70-Ch (70% field capacity + chitosan),

and 70-Sp (70% field capacity + Spirulina extract).

3.7. Three-Dimensional Response Surface Analysis of Consumptive Water Use (WPc) and Plant Height Increment

The response surface visualization clearly showed that both factors and their interaction influenced WPc (Figure 13A,B). Maximum values of WPc were obtained with full irrigation by applying biostimulant, especially in treatments with Spirulina extract and chitosan, which reached 4.88 and 4.57 g L⁻¹, respectively. On the contrary, untreated seedlings showed significantly lower values of WPc under all levels of irrigation. WPc decreased gradually when the irrigation level was lowered from 100 to 70% FC, but the reduction was less clear in biostimulant-treated seedlings than in the untreated control. This response suggests that chitosan and Spirulina application improved water-use efficiency under deficit irrigation conditions. The response surface also indicated that the positive effect of biostimulants was increasingly evident in the conditions with limited water, where the treated seedlings maintained relatively high values of WPc even with a reduction in the amount of irrigation water supplied. These results indicate the potential of biostimulants as complementary tools to enhance water productivity and improve drought resilience in passion fruit nursery production systems. The three-dimensional response surface plot showed significant interaction between the level of irrigation and biostimulant application in plant height increment (Figure 9). Plant height showed a gradual increase with the increase in irrigation level and was further enhanced by biostimulant treatments; the maximum response was observed in full irrigation with chitosan application, while the minimum plant height increment was observed in untreated seedlings at 70% FC. The response surface also indicated that biostimulant application partially alleviated the adverse impact of water deficit, allowing the maintenance of better growth under reduced-irrigation conditions. In general, response surface visualization confirmed the possibility of improving water-use efficiency without compromising satisfactory seedling performance by combining moderate water deficit and biostimulant application, thus contributing to more sustainable irrigation management practices.

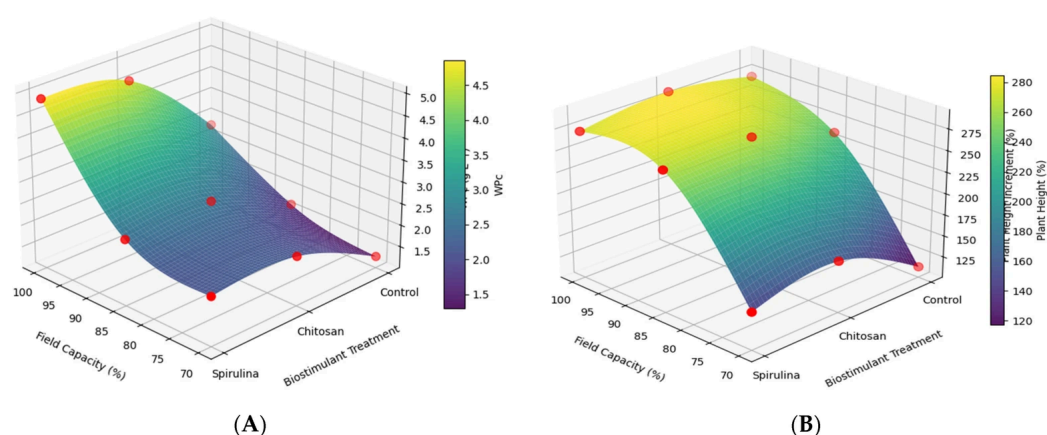


Figure 13. Three-dimensional response surface visualization plot illustrating the interactive effects of irrigation level and biostimulant application on (A) water productivity based on consumptive water use (WPc) and (B) plant height increment of passion fruit seedlings. Red points represent observed experimental values, whereas the surface represents the interpolated response.

4. Discussion

4.1. Deficit Irrigation Alters Water Relations and Seedling Performance

Deficit irrigation significantly affected the water relations, physiological status, and

vegetative development of *Passiflora edulis* seedlings, confirming their high sensitivity to water limitation during the nursery stage. Reducing irrigation from 100% to 70% field capacity (FC) significantly decreased applied irrigation water (AIW), water consumptive use (WCU), water productivity (WPa and WPc), chlorophyll content, vegetative growth, and biomass production. These findings indicate that although water deficit reduced irrigation inputs, severe water restriction impaired the physiological processes required to sustain normal seedling growth. The reductions in plant height, shoot length, leaf area, and biomass production can be attributed to decreased cell turgor, restricted cell expansion, and partial stomatal closure under drought conditions. Reduced stomatal conductance limits CO₂ diffusion into leaves, suppressing photosynthetic carbon assimilation and consequently reducing the production of assimilates required for growth and biomass production. Similar physiological responses have been reported in passion fruit and other horticultural crops subjected to drought stress, where reduced photosynthesis and carbon allocation resulted in lower plant growth and productivity [27,28,36].

The marked decline in total chlorophyll content under severe water deficit further indicates impairment of the photosynthetic apparatus. Drought stress accelerates chlorophyll degradation and disrupts chloroplast function, thereby reducing light-harvesting efficiency and photosynthetic performance. Since chlorophyll content is closely associated with biomass production and water productivity, its reduction contributed directly to the decline in seedling growth observed under severe irrigation deficit. Comparable responses have been reported in several fruit crops exposed to water stress [13,14,37,38]. Interestingly, severe deficit irrigation did not improve water productivity despite reducing water. Biomass production declined more rapidly than water consumption, leading to lower WPa and WPc values. This finding demonstrates that maximizing water productivity depends on maintaining an appropriate balance between water saving and physiological performance rather than simply minimizing irrigation inputs. Similar conclusions have been reported in recent deficit irrigation studies, where moderate water restriction enhanced irrigation efficiency without causing substantial reductions in plant growth [2,10,39]. Overall, the results demonstrate that moderate water deficit is more suitable than severe irrigation restriction for passion fruit seedling production, as it provides meaningful water savings while preserving chlorophyll content, vegetative growth, and biomass production. These findings provide a physiological basis for integrating deficit irrigation with biostimulant application to improve nursery performance under water-limited conditions.

4.2. Physiological Response Underlying the Protective Effects of Chitosan and Spirulina

Foliar application of both chitosan and Spirulina extract effectively alleviated the adverse effects of water deficit, resulting in significant improvements in water productivity, chlorophyll content, vegetative growth, and biomass production compared with untreated seedlings. These findings demonstrate that both biostimulants enhanced drought tolerance by maintaining physiological activity under limited water availability. However, chitosan consistently produced greater improvements than Spirulina, particularly under moderate deficit irrigation (85% FC), indicating a stronger capacity to sustain seedling growth while reducing irrigation inputs. The superior performance of chitosan is likely associated with its multiple physiological functions in drought-stressed plants. Although these physiological responses were not directly evaluated in the present study, previous research has demonstrated that chitosan may enhance drought tolerance through improved stomatal regulation, osmotic adjustment, membrane stability, and antioxidant defense, which could explain the improved growth and water productivity observed in the present experiment [40–43].

Spirulina extract also improved seedling performance under water-limited conditions, although its effects were generally less pronounced than those of chitosan. The beneficial effects of Spirulina are mainly attributed to its rich composition of amino acids, vitamins, proteins, minerals, phytohormones, and other bioactive compounds that stimulate plant metabolism and stress adaptation. These compounds enhance nutrient assimilation, support chlorophyll biosynthesis, and improve photosynthetic activity, thereby promoting vegetative growth and biomass production. Previous studies have similarly reported that microalgal biostimulants improve drought tolerance by enhancing antioxidant capacity, osmotic regulation, and metabolic activity in horticultural crops [44–46].

A particularly important finding of the present study is that the combination of chitosan with moderate deficit irrigation (85% FC) maintained seedling performance at levels comparable to full irrigation while requiring less irrigation water. This response indicates that improvements in water productivity were achieved through enhanced physiological water-use efficiency rather than increased water consumption. The ability of chitosan-treated seedlings to maintain chlorophyll content, vegetative growth, and biomass production under moderate water deficit highlights the potential of this biostimulant to compensate for reduced irrigation through improved physiological resilience. Overall, these findings demonstrate that integrating foliar biostimulants with moderate deficit irrigation represents an effective strategy for enhancing drought tolerance in passion fruit seedlings. Among the evaluated treatments, chitosan showed the greatest potential for improving water-use efficiency while maintaining seedling vigor, supporting its application as a sustainable management practice for nursery production under water-limited conditions.

4.3. Multivariate Analyses Reveal the Physiological Basis of Improved Water Productivity

The multivariate analyses provided a comprehensive understanding of the physiological relationships underlying the responses of passion fruit seedlings to deficit irrigation and biostimulant application. Pearson correlation analysis revealed strong positive associations among chlorophyll content, vegetative growth traits, biomass production, and both water productivity indices (WPa and WPC), indicating that maintenance of photosynthetic capacity was closely linked to improved biomass production and more efficient water utilization. These relationships emphasize that enhanced water productivity under moderate deficit irrigation was achieved primarily through improved physiological performance rather than reduced water consumption alone [38,47].

The PCA further supported these findings by clearly separating the treatment combinations according to their overall physiological performance. Seedlings treated with chitosan or *Spirulina*, particularly under moderate deficit irrigation (85% FC), were closely associated with chlorophyll content, biomass production, and water productivity variables, reflecting superior physiological status and greater adaptation to water-limited conditions. In contrast, untreated seedlings subjected to severe water deficit (70% FC) were positioned opposite these variables, indicating that drought-induced reductions in photosynthetic activity were accompanied by declines in growth and biomass production. This clear separation demonstrates that the combined effects of irrigation regime and biostimulant application were sufficient to distinguish contrasting physiological responses among treatments [48,49].

The performance ranking heatmap complemented the correlation and PCA analyses by providing an integrated assessment of treatment performance across all measured traits. Chitosan combined with 85% FC consistently ranked among the highest-performing treatments, whereas untreated seedlings under 70% FC exhibited the lowest overall

performance. The close agreement among the correlation analysis, PCA, and performance heatmap strengthens the reliability of the observed treatment responses and confirms that chlorophyll preservation, sustained vegetative growth, and biomass production are the principal determinants of improved water productivity in passion fruit seedlings. Collectively, these multivariate analyses demonstrate that moderate deficit irrigation combined with foliar biostimulant application, particularly chitosan, enhances water productivity through coordinated improvements in photosynthetic efficiency, vegetative growth, and biomass production. These findings provide robust statistical support for the physiological response discussed above and reinforce the potential of this integrated management strategy for improving drought resilience during passion fruit nursery production.

4.4. Agronomic Significance and Practical Implications

From an agronomic perspective, the present findings demonstrate that integrating moderate deficit irrigation with foliar biostimulant application provides an effective strategy for improving water-use efficiency without compromising the quality of passion fruit seedlings. Among the evaluated treatments, chitosan applied under 85% field capacity (FC) achieved the most favorable balance between irrigation water savings and maintenance of physiological performance, indicating that moderate water restriction can be implemented without substantial reductions in seedling vigor when supported by an appropriate biostimulant.

The improvement in water productivity observed under this treatment has important implications for nursery production, where irrigation management directly influences both production costs and transplant quality. Maintaining higher chlorophyll content, vegetative growth, and biomass production under reduced irrigation suggests that biostimulant application enhances the physiological efficiency with which seedlings utilize available water rather than merely compensating for water deficit. Consequently, this integrated approach may reduce irrigation requirements while producing vigorous seedlings with greater potential for successful establishment after transplanting [15,50,51].

The combined evidence from water productivity indices, growth measurements, and multivariate analyses further supports the adoption of moderate deficit irrigation as a practical water-saving strategy in regions facing increasing water scarcity. Rather than maximizing irrigation inputs, the results indicate that optimizing the interaction between irrigation management and plant biostimulants can achieve more sustainable resource utilization while maintaining seedling performance. This approach is particularly relevant for arid and semi-arid environments, where improving irrigation efficiency has become a priority for sustainable horticultural production under climate change [42,43,52,53].

Overall, the present study demonstrates that foliar application of chitosan, particularly when combined with irrigation at 85% FC, represents a practical and environmentally sustainable strategy for passion fruit nursery production. The integration of moderate deficit irrigation with natural biostimulants not only improves water productivity and drought tolerance but also contributes to reducing irrigation water demand while maintaining seedling quality. These findings provide a scientific basis for developing climate-resilient nursery management practices and support the wider adoption of eco-friendly biostimulants as components of sustainable horticultural production systems.

5. Conclusions

Deficit irrigation significantly affected the physiological performance, vegetative

growth, biomass production, and water productivity of *Passiflora edulis* seedlings, with the greatest reductions observed under severe water deficit (70% FC). Foliar application of chitosan and Spirulina extract effectively mitigated these adverse effects by improving chlorophyll content, vegetative growth, biomass production, and water-use efficiency. Among the tested biostimulants, chitosan consistently showed superior performance, particularly under moderate deficit irrigation (85% FC), maintaining seedling growth while reducing water irrigation requirements. The positive relationships among chlorophyll content, biomass production, vegetative growth, and water productivity, supported by multivariate analyses, indicate that improved physiological performance was the main factor contributing to enhanced water-use efficiency under water-limited conditions. Overall, foliar application of chitosan at 1 g L⁻¹ combined with 85% field capacity was identified as the optimum treatment, providing substantial irrigation water savings while maintaining high-quality seedlings. This integrated strategy offers a practical and sustainable approach for producing passion fruit in nurseries located in arid and semi-arid regions and may contribute to improving water-use efficiency under increasing water scarcity.

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