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Microorganism-Based Biostimulants for Alleviating Water Deficit in 'Formosa' Papaya: Physiological Indices and Growth

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Abstract

Papaya is an economically important crop, but its production in semiarid regions is severely limited by water scarcity. However, microorganism-based biostimulants have been studied as a promising strategy to mitigate water stress and support plant growth. Therefore, the objective of this study was to evaluate the effect of microorganism-based biostimulants on gas exchange, photochemical efficiency, and growth of 'Formosa' papaya under water deficit in a semiarid area. The experimental design was a randomized complete block design with split plots. The plots considered three irrigation depths (100, 75, and 50% of crop evapotranspiration-ETc) and the subplots the application of four biostimulants (control (no biostimulant application); *Trichoderma harzianum*; *Ascophyllum nodosum*; *Bacillus aryabhattai*), with three plants per plot and four replicates. *B. aryabhattai* mitigated the effects of deficit irrigation at 50% ETc on 'Formosa' papaya, increasing transpiration, CO_2 assimilation rate, and instantaneous carboxylation efficiency. Under irrigation at 50% ETc, *T. harzianum* provided beneficial effects on water use efficiency, instantaneous carboxylation efficiency, and photosystem II quantum efficiency. *A. nodosum* stimulated chlorophyll *a* synthesis in 'Formosa' papaya plants irrigated at 75% ETc during the fruiting stage, but reduced the absolute and relative growth rate in stem diameter under 50% ETc. Irrigation at 50% ETc reduced stomatal conductance and growth of 'Formosa' papaya plants 235 days after transplanting. We conclude that the application of *B. aryabhattai* and *T. harzianum* is a viable strategy to increase the tolerance of 'Formosa' papaya to the adverse effects of water deficit in semiarid regions.

Keywords: *Carica papaya* L.; water scarcity; beneficial microorganism; semiarid

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1. Introduction

The papaya (*Carica papaya* L.), originating from Central America and Mexico, holds significant economic potential due to year-round production and its role in generating employment for small farmers, reducing the rural exodus [1]. The fruit is consumed fresh or processed for nutritional properties like vitamins and fiber. In the pharmaceutical industry, it is used for extracting papain, a proteolytic enzyme that acts as an anti-inflammatory agent [2]. In Brazil, the 2023 harvest yielded 1,138,343 tons from 26,839 hectares, averaging 42,414 kg ha^{-1} [3]. However, expanding this crop into semiarid Northeastern Brazil is challenged by water scarcity, resulting from low precipitation and high evaporation, which hinders perennial crop growth [4]. Consequently, growers often resort to alternative water sources (wells, reservoirs), which frequently fail to meet full irrigation demand, leading to the strategy of deficit irrigation to maintain the orchard [5].

Water restriction causes stress in plants, compromising metabolic activities. This triggers abscisic acid signaling for the partial closure of stomata, limiting water and nutrient uptake, and reducing transpiration, the photosynthetic rate, and cell division, which can elevate the concentration of reactive oxygen species (ROS) [6]. The accumulation of ROS in cells promotes oxidative stress and plant senescence, depending on the severity of the water stress and the crop's tolerance [7].

Studies conducted by Souza et al. [8] on papaya irrigated with 60% of the reference evapotranspiration showed that water stress resulted in the inhibition of height, stem diameter, and leaf number. Similarly, Melo et al. [9] found that an irrigation depth of less than 75% of the reference evapotranspiration (ETo) intensified the physiological effects of water stress, reducing stomatal conductance, the photosynthetic rate, and the quantum efficiency of photosystem II during the production phase.

Among strategies to mitigate water stress, the application of biostimulants like *Trichoderma* spp., *Bacillus* spp., and seaweed extracts (*Ascophyllum nodosum*) stands out [10–12]. Each operates via distinct mechanisms: *Trichoderma* spp. primarily enhance drought tolerance by promoting a more extensive root system, improving water and nutrient absorption, and activating the plant's antioxidant defenses [13]. *Bacillus* spp. induce systemic tolerance by modifying root architecture and producing phytohormones, such as auxins, and ACC deaminase to lower stress ethylene, in addition to contributing to regulating osmotic potential through osmolytes like proline [14]. *Ascophyllum nodosum* extracts, rich in bioactive compounds, "prime" the plant by upregulating antioxidant enzymes and osmolytes, which help maintain stomatal conductance and chlorophyll content under stress [15].

The beneficial effects of biostimulants in reducing the impact of water deficit have been observed in tomato treated with *A. nodosum* [16], in maize with *Bacillus* spp. [17], and in wheat using *T. asperellum* [18]. However, in the literature, there is a lack of information regarding the effects of microorganism-based biostimulants in mitigating water deficit in fruit crops [19–21], especially for 'Formosa' papaya under the specific conditions of the Paraíba semiarid region. Thus, it is essential to develop research aimed at establishing strategies for cultivating this fruit crop under water-restricted conditions.

In this context, this study aimed to evaluate the effects of microorganism-based biostimulants in mitigating the negative effects of water deficit on the physiological indices and growth of 'Formosa' papaya in a semiarid region.

2. Materials and Methods

2.1. Site Description and Experimental Conditions

The research was conducted from 28 June 2023, to 17 February 2024, in the Fruit Culture Sector of the 'Rolando Enrique Rivas Castellón' Experimental Farm, belonging to the Center of Agri-Food Science and Technology (CCTA) of the Federal University of Campina Grande

(UFCG), located in São Domingos, Paraíba, Brazil (6°48′51.7″ S, 37°56′13.8″ W, at an average altitude of 190 m).

According to the Köppen–Geiger classification, the region's climate is BSh type (hot and semiarid), with a mean annual temperature exceeding 26.7 °C and average annual precipitation of 800 mm [22]. Meteorological data for the experimental period, including maximum and minimum temperature and mean relative air humidity, were obtained from the São Gonçalo weather station (Sousa—PB) and are presented in Figure 1.

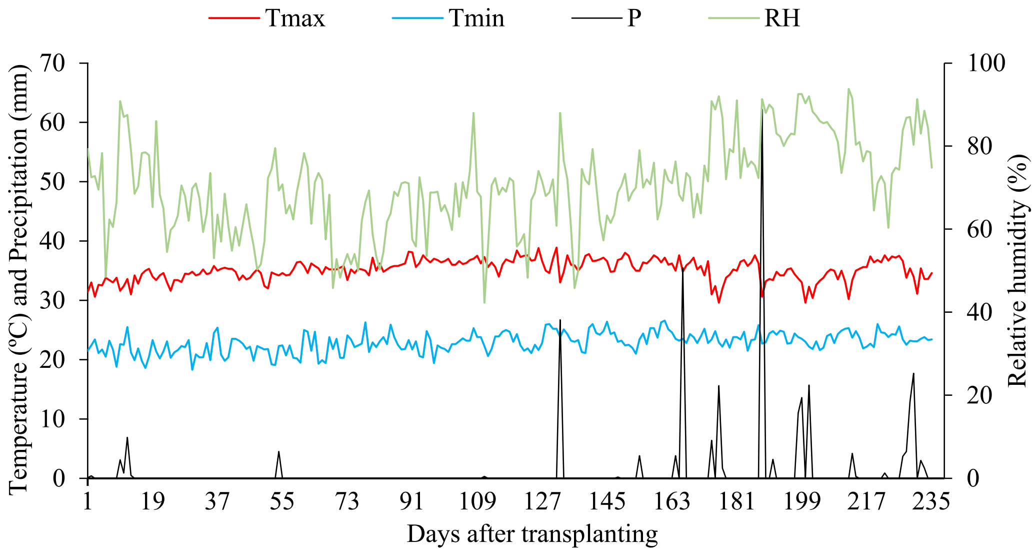

Figure 1. Climate data of maximum temperature—Tmax and minimum temperature—Tmin, precipitation—P, and relative air humidity—RH during the experimental period from 28 June 2023 to 17 February 2024.

2.2. Experimental Design and Treatments

The experiment was laid out in a randomized block design with a split-plot arrangement. The main plots consisted of three irrigation levels (IL), corresponding to 100%, 75%, and 50% of the crop evapotranspiration (ETc). The subplots consisted of four biostimulant applications: a control (C, no biostimulant), *Trichoderma harzianum* (T), *Ascophyllum nodosum* (A), and *Bacillus aryabhattai* (B). The experiment included four replications with three plants per plot, totaling 48 experimental units. Border plants were planted at the four extremities of the experimental area to provide a protective barrier against external influences.

The irrigation levels were based on a study by Melo et al. [9] with papaya. The biostimulant sources were Trichodermil SC 1306® (*T. harzianum*, 2.0 $\times 10^9$ (Maceió, Alagoas, Brazil) viable conidia mL^{-1}), Aryacompost® (*B. aryabhattai*, 4 × 10^8 CFU g^{-1}) (Arapongas, Paraná, Brazil), and Alga 95® (*A. nodosum*, 95% concentration) (Juazeiro, Bahia, Brazil). Biostimulants were applied to the soil around the root system of each plant, 15 cm from the stem, at the rates recommended by the manufacturer: Alga 95® (0.75 kg ha^{-1}), Aryacompost® (0.5 kg ha^{-1}), and Trichodermil SC® 1306® (1.0 L ha^{-1}) (Maceió, Alagoas, Brazil). Applications began five days after transplanting and were repeated at 50-day intervals, for a total of five applications. The application rates for these commercial products, along with an economic overview of their estimated unit prices and total costs per hectare, are detailed in Table 1.

Table 1. Commercial names, manufacturers, application rates, estimated unit costs, and total costs per hectare for the biostimulant treatments used in the experiment.

Biostimulant Treatment	Commercial Name	Recommended Dose (per ha)	Dose per Plant	Total Cost per Application (USD per ha) [1]
T. harzianum	Trichodermil SC 1306®	1.0 L	0.7 ml	25.02
B. aryabhattai	Aryacompost	0.5 kg	0.35 g	35.76
A. nodosum	Alga 95®	0.75 kg	0.52 g	23.16

[1] Estimated costs in USD based on Brazilian market prices at the time of the experiment (2023/2024).

2.3. Plant Material and Seedling Production

Seedlings of papaya cv. 'Sunrise Solo' (Formosa group) was produced at the experimental farm's greenhouse under 50% shading. Sowing was performed in 162-cell rigid plastic trays (50 mL per cell), filled with a substrate composed of two parts experimental area soil and one part cured bovine manure (v/v). In each cell, one seed was sown, and trays were manually irrigated daily with water of low electrical conductivity (0.3 dS m^{-1}). The 'Sunrise Solo' (Formosa) cultivar is known for its high productivity, vigor, tall stature, rapid growth, and uniform, oblong fruits with smooth, firm skin and orange, consistent pulp [23].

2.4. Soil Preparation and Characterization

The experimental area was prepared by plowing and harrowing to break up clods and level the soil. A composite soil sample was collected from the 0–0.30 m layer, and its physical and chemical characteristics (Table 2) were determined according to the methodologies described by Teixeira et al. [18]. Planting beds measuring 0.4 × 1.0 × 30 m (height × width × length) were prepared and spaced 3.5 m apart. A foundation fertilization of 8.73 kg ha^{-1} of phosphorus (P) was applied using simple superphosphate (18% P_2O_5), as recommended by Teixeira et al. [24].

Table 2. Physical-hydric and chemical attributes of the soil (0–0.30 m) at the experimental site.

Chemical Characteristics								
pH H_2O)	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
(1:2.5)	g kg^{-1}	(mg kg^{-1})	cmol$_c$ kg^{-1}					
7.19	1.40	5.95	0.49	0.07	4.70	3.63	0	0
Chemical Characteristics				Physical Characteristics				
EC_{se}	CEC	SAR_{se}	ESP	Particle-size fraction—(dag kg^{-1})			Moisture—(dag kg^{-1})	
(dS m^{-1})	cmol$_c$ kg^{-1}	(mmol $L^{-1})^{0.5}$	%	Sand	Silt	Clay	33.42 kPa [1]	1519.5 kPa [2]
0.58	8.89	1.40	0.79	73.5	20.1	6.35	15.8	6.41

Determined attributes: pH—Hydrogen potential, OM—Organic matter: Walkley-Black Wet Digestion; P—Extracted with Mehlich-1; Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; Al^{3+}+H^+ extracted with 0.5 M CaOAc at pH 7.0; ECse—Electrical conductivity of the saturation extract; Estimated attributes: CEC—Cation exchange capacity; SARse—Sodium adsorption ratio of the saturation extract; ESP—Exchangeable sodium percentage. [1] Field capacity; [2] Permanent wilting point

2.5. Crop Establishment and Management

Seedlings were transplanted into the field with three seedlings per planting hole, maintaining a spacing of 15 cm between them. The spacing between planting holes was 2 m within rows and 3.5 m between rows. The root collar of the seedlings was placed at ground level. Sexing was performed at 96 days after transplanting (DAT), leaving only one hermaphrodite plant per hole.

Top-dressing fertilization with nitrogen (N), phosphorus (P), and potassium (K) followed the recommendations of Embrapa [25], with a total of 260, 17.46, and 74.71 kg ha^{-1}

of N, P_2O_5, and K_2O, respectively, applied during the first year. Fertilizers were applied via fertigation at 30, 60, 90, and 120 DAT, and every 15 days from flowering until 360 DAT. The sources used were urea (45% N), potassium sulfate (50% K_2O), and monoammonium phosphate (50% P_2O_5, 11% N). Foliar micronutrient applications were performed every 30 days using Dripsol micro® (composition: 1.2% Magnesium, 0.85% Boron, 3.4% Iron, 4.2% Zinc, 3.2% Manganese, 0.5% Copper, and 0.06% Molybdenum) at a concentration of 0.5 g L^{-1}.

Plant staking was performed to prevent lodging. Cultural practices consisted of removing lateral shoots from the main stem and manual weed control.

2.6. Irrigation System and Management

A drip irrigation system was used, with two 16 mm polyethylene lateral lines per plot. Four self-compensating drippers (10 L h^{-1}) were installed per plant, 15 cm from the stem. The average dripper flow rate in the experimental area was 9.5 L h^{-1}, with a water application uniformity coefficient of 86%. The irrigation water, sourced from an artesian well, had a pH of 7.22 and an electrical conductivity (EC) of 1.032 dS m^{-1}.

Plants were irrigated daily in the morning. Irrigation levels were calculated based on the crop evapotranspiration (ETc). For the 100% ETc treatment, the level was estimated according to Bernardo et al. [26] (Equation (1)). The 75% and 50% ETc levels were calculated based on the 100% ETc value.

$$\text{ETc} = \text{ETo} \times \text{Kc} \tag{1}$$

where ETc is the crop evapotranspiration (mm day^{-1}); ETo is the reference evapotranspiration (mm day^{-1}); and Kc is the crop coefficient (dimensionless).

The ETo was determined daily using the Penman-Monteith method from data collected at the São Gonçalo weather station. The Kc values used were 0.64, 1.16, and 1.19 for the vegetative (46–95 DAT), flowering (96–201 DAT), and fruiting (after 202 DAT) phases, respectively [27]. The irrigation volume was controlled daily by adjusting the irrigation time. When precipitation occurred, the amount was measured and subtracted from the irrigation depth.

2.7. Soil Biological Analyses

To assess soil population density and metabolic activity, a baseline composite sample (0–0.30 m) was collected before the experiment began. At the fruiting stage, new soil samples were collected from the root zone of each treatment. All samples were stored at −4 °C. Before analysis, samples were thawed at room temperature for eight hours to restore microbiological activity.

The total density of bacteria, fungi, and actinomycetes was determined according to Hungria and Araújo [28]. A 10 g aliquot of each sample was subjected to serial dilutions (10^{-1} to 10^{-10}), and the last three dilutions were plated in triplicate on specific culture media. Nutrient agar (NA) was used for bacteria, potato dextrose agar (PDA) for fungi, and PDA with starch for actinomycetes. Plates were incubated at 28 °C. Bacteria were counted as colony-forming units (CFU), while fungi and actinomycetes were estimated using the most probable number (MPN) method with the McCrady table.

Soil microbial respiration (C-CO_2 evolution) was measured according to Mendonça and Matos [29]. C-CO_2 produced from 50 g of soil was trapped in 30 mL of 0.5 M NaOH over 48 h. A 10 mL aliquot of the NaOH was then titrated with 0.25 M HCl. The amount of C-CO_2 was calculated using Equation (2). Results are presented in Table 2.

$$\text{C} - \text{CO}_2(\text{mg}) = (B - V) \times M \times 6 \times \frac{V1}{V2} \tag{2}$$

where: B = volume of HCl used in the blank (mL); V = volume of HCl used in the sample (mL); M = molarity of HCl; 6 = equivalent weight of C in CO_2; $V1$ = total volume of NaOH (mL); $V2$ = volume of NaOH used in titration (mL).

2.8. Plant Physiological and Growth Analyses

The effects of the treatments were evaluated at 235 days after transplanting (DAT). Gas exchange was assessed under natural conditions of air temperature and CO_2 concentration using a portable infrared gas analyzer (IRGA), model LCPro+ Portable Photosynthesis System® (ADC BioScientific Limited, Hoddesdon, UK), with a light radiation of 1200 µmol photons m^{-2} s^{-1} and an airflow of 200 mL min^{-1}. The following parameters were measured: transpiration rate (E), stomatal conductance (gs), CO_2 assimilation rate (A), and internal CO_2 concentration (Ci). From these data, water use efficiency (WUE) and instantaneous carboxylation efficiency (CEi) were estimated. In the same period, chlorophyll *a* fluorescence parameters: initial (F_0), maximum (F_m), variable (F_v), and maximum quantum efficiency of PSII (F_v/F_m)—were determined using a modulated pulse fluorometer (Model OS5p, Opti-Science, Hudson, NY, USA) on leaves that were dark-adapted for 30 min.

To determine plant water status, the relative water content (RWC) was calculated from eight leaf discs (12 mm diameter) per plant. The fresh mass (FM), turgid mass (TM), and dry mass (DM) were recorded, and RWC was determined according to Weatherley [30] using Equation (3).

$$\text{CRA}(\%) = \frac{(\text{MF} - \text{MS})}{(\text{MT} - \text{MS})} \times 100 \tag{3}$$

Membrane integrity was evaluated by measuring electrolyte leakage (EL) from the leaf lamina. Eight leaf discs (113 mm^2 area) were used to determine the initial (C1) and final (C2) electrical conductivity after heat treatment (80 °C for 90 min). EL was then calculated according to Scotti-Campos et al. [31] using Equation (4).

$$\text{EL}(\%) = \frac{\text{C1}}{\text{C2}} \times 100 \tag{4}$$

Photosynthetic pigments were quantified from eight leaf discs extracted in dimethyl sulfoxide (DMSO) for 48 h in the dark. The absorbance of the extracts was analyzed with a spectrophotometer at 665, 649, and 480 nm to determine the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) according to the methodology of Wellburn [32], using Equations (5)–(7).

$$\text{Chl } a = 12.19\text{A}_{665} - 3.45\text{A}_{649} \tag{5}$$

$$\text{Chl}b = 21.99\text{A}_{649} - 5.32\text{A}_{665} \tag{6}$$

$$CAR = (1000A_{480} - 2.14Chl_a - \frac{70.16Chl_b}{220} \tag{7}$$

Plant growth was evaluated by measuring stem height (SH) and stem diameter (SD). From these measurements, taken at 92 and 235 DAT, the absolute growth rate (AGR) and relative growth rate (RGR) of SH and SD were determined according to the methodology of Benincasa [33], using Equations (8) and (9).

$$\text{AGR} = \frac{\text{A2} - \text{A1}}{\text{t2} - \text{t1}} \tag{8}$$

$$\text{RGR} = \frac{\ln \text{A2} - \ln \text{A1}}{\text{t2} - \text{t1}} \tag{9}$$

where AGR = absolute growth rate (cm day^{-1} or mm day^{-1}); RGR = relative growth rate (cm cm^{-1} day^{-1} or mm mm^{-1} day^{-1}); A2 = assessment at time t2 (days); A1 = assessment at time t1 (days).

2.9. Statistical Analysis

Data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Bartlett's test). Analysis of variance (ANOVA) was performed using the F-test ($p \leq 0.05$). In cases of significance, means for the irrigation levels and biostimulant factors were compared using the Tukey test ($p \leq 0.05$) in the SISVAR 5.8 software [34]. To understand the joint effect of the treatments, multivariate analysis of variance (MANOVA) was performed on the principal components (PCA) using Hotelling's T^2 test ($p \leq 0.05$). A Pearson correlation matrix was also generated. MANOVA, PCA, and correlation analyses were performed using R software (v. 4.3.2) [35].

3. Results

3.1. Soil Biological Attributes

The analysis of the soil's biological attributes, assessed during the flowering and fruiting period, revealed that the treatments influenced the microbial populations and metabolic activity (Table 3). The population density of bacteria (CFU) and fungi (MPN) remained relatively stable across the different irrigation depths and biostimulant applications. However, the total density of actinomycetes was highest in the treatments involving *T. harzianum* at 50% ETc (ID50T) and *B. aryabhattai* at 100% ETc (ID100B) (6.05 Log MPN). A pronounced effect was observed in soil microbial respiration; the C-CO_2 evolution in the 75%A treatment (75% ETc with *A. nodosum*) was markedly higher (36.04 mg) than all other treatments, which generally ranged from 6.15 to 16.12 mg.

Table 3. Logarithm of the means of the Colony Forming Units (CFU) for bacteria, Most Probable Number (MPN) of fungi and actinomycetes, and evolution of C-CO_2 present in the soil in the period between flowering and fruiting.

Treatments	Bacteria	Fungi	Actinomycetes	C-CO_2
	Log_{10} (CFU per g of Soil)	Log MPN		mg
Control	9.12	5.38	5.05	6.15
50%A	10.20	5.05	5.05	12.45
50%B	10.46	5.38	5.72	11.72
50%C	9.52	5.38	5.38	9.82
50%T	9.95	5.38	6.05	12.75
75%A	9.88	5.17	5.17	36.04
75%B	10.52	5.17	5.17	11.87
75%C	9.07	5.17	5.47	12.16
75%T	10.57	5.17	5.17	13.77
100%A	9.83	5.05	5.05	12.45
100%B	9.62	5.05	6.05	10.84
100%C	9.81	5.38	5.05	16.12
100%T	9.69	5.05	5.05	10.26

50% = irrigation level of 50% ETc; 75% = irrigation level of 75% ETc; 100% = irrigation level of 100% ETc; C = control; B = *Bacillus aryabhattai*; T = *Trichoderma harzianum*; A = *Ascophyllum nodosum*.

3.2. Gas Exchange and Stomatal Conductance

There was a significant interaction effect between the factors of irrigation levels and biostimulants for internal CO_2 concentration, transpiration, CO_2 assimilation rate, water use efficiency, and instantaneous carboxylation efficiency of 'Formosa' papaya plants at 235 days after transplanting (Table S1). The irrigation depths significantly influenced stomatal conductance. While the microorganism-based biostimulants did not evidence a significant effect on this parameter.

The internal CO_2 concentration (Figure 2a) of the control plants irrigated with 75% ETc was significantly different from the 50% ETc level, with no difference from 100% ETc. However, the irrigation levels promoted a similar statistical effect with the application of *B. aryabhattai*. Plants under *T. harzianum* irrigated with 75% ETc evidenced the highest internal CO_2 concentration (151 μmol mol^{-1}), in comparison with plants that received 50% and 100% ETc. The supply of *A. nodosum* induced 100% and 75% ETc levels, and increased *Ci* in relation to plants irrigated with 50% ETc. For the biostimulants interacting with the 75% ETc level, it was verified that *T. harzianum* obtained a positive effect in comparison to *B. aryabhattai*, but with an effect similar to the control and *A. nodosum*. The biostimulants at the 100% and 50% levels did not show significant differences among themselves.

Regarding leaf transpiration (Figure 2b), no significant differences were found among the irrigation levels in the control (no biostimulant) treatment. For plants receiving *B. aryabhattai*, the 50% ETc level resulted in significantly higher transpiration (E) compared to the 75% ETc level, but it was not statistically different from the 100% ETc level. In plants treated with *T. harzianum*, the 100% ETc irrigation yielded the highest E value (4.72 mmol H_2O m^{-2} s^{-1}), which was significantly greater than the 75% ETc treatment; however, no significant difference was observed between the 100% ETc and 50% ETc levels.

The CO_2 assimilation rate (Figure 2c) in control plants irrigated with 75% ETc was superior to those subjected to 50% ETc and did not differ statistically from the 100% ETc level. The application of *B. aryabhattai* increased the CO_2 assimilation rate in plants under 75% and 50% ETc, in comparison to 100% ETc. The supply of *T. harzianum* on plants cultivated under the 100%, 50%, and 75% ETc levels did not statistically influence CO_2 assimilation rate. *A. nodosum* provided a higher A with 100% ETc in comparison to 50% ETc, and there was no significant difference with 75% ETc. *B. aryabhattai* and *T. harzianum* under the influence of 50% ETc obtained better results for the CO_2 assimilation rate in comparison to the control and *A. nodosum*. At 100% and 75% levels, the biostimulants did not differ significantly.

As for water use efficiency (Figure 2d), the control and *A. nodosum* under distinct irrigation levels did not promote a significant effect. The application of *B. aryabhattai* at the 75% ETc level improved the WUE of the plants compared to those subjected to 50%; however, the 100% ETc induced a similar result. In plants under *T. harzianum*, 50% and 100% ETc irrigation levels promoted higher efficiency, differing statistically from 75% ETc. Among the biostimulants, *T. harzianum* stood out at the 100% ETc irrigation level. Meanwhile, *B. aryabhattai* obtained a higher WUE than *T. harzianum* and *A. nodosum* at 75% ETc, but there was no distinction from the control. The WUE of plants under *T. harzianum* was superior in comparison to the control and *B. aryabhattai* biostimulant at 50% ETc.

For instantaneous carboxylation efficiency (Figure 2e), it was verified that the irrigation levels in the control and *A. nodosum* treatments did not differ. For *B. aryabhattai*, the 50% and 100% ETc levels differed from each other, with the 50% level promoting a higher CEi value of 0.20 [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-1} s^{-1})$^{-1}$], while the 75% ETc did not differ significantly. In the *T. harzianum* treatment, a significant difference was found between the plants under 50% and 75% ETc irrigation levels, with 50% ETc obtaining the highest CEi, a value of 0.21 [(μmol CO_2 m^{-2} s^{-1}) (μmol CO_2 m^{-1} s^{-1})$^{-1}$], and it did not differ from 100% ETc. The biostimulants did not have a significant effect among themselves under the same conditions of 100% and 75% ETc. *B. aryabhattai* and *T. harzianum* in plants irrigated with 50% ETc did not differ, but obtained superior results to the plants under the control and *A. nodosum* treatments.

The 100% ETc irrigation level resulted in higher stomatal conductance in 'Formosa' papaya plants (Figure 3a), differing from plants cultivated under 50% ETc, while plants under the 75% ETc level did not show differences in relation to the other levels. The biostimulant application (Figure 3b), as a main effect, did not significantly affect stomatal conductance (gs), which showed a mean value of 198.64 mmol H_2O m^{-2} s^{-1}.

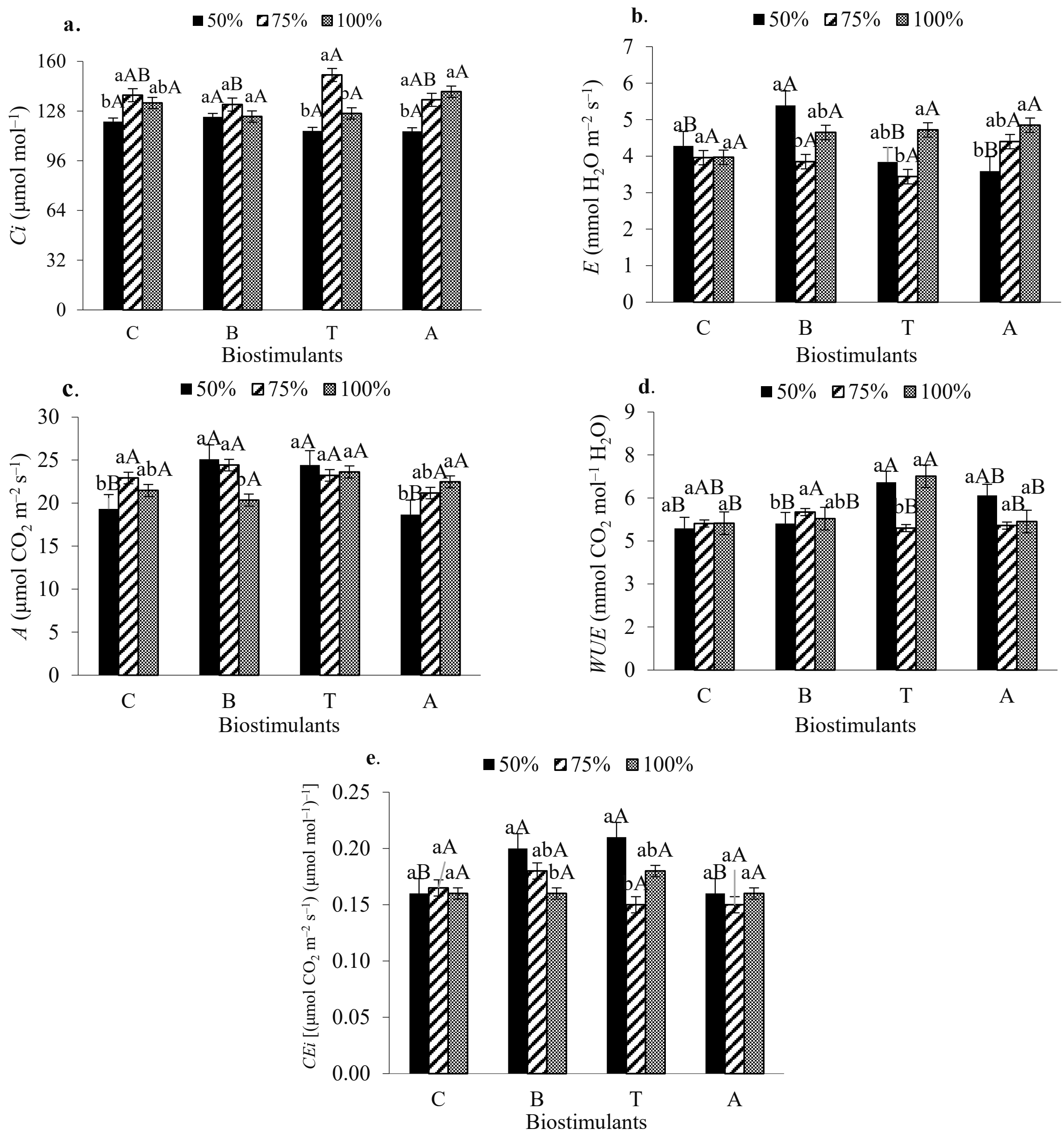

Figure 2. Internal CO_2 concentration—*Ci* (**a**), transpiration—*E* (**b**), CO_2 assimilation rate—*A* (**c**), water use efficiency—*WUE* (**d**), and instantaneous carboxylation efficiency—*CEi* (**e**) of 'Formosa' papaya plants as a function of the interaction between irrigation levels and application of microorganism-based biostimulants, at 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same lowercase letter indicate no significant difference between irrigation levels in the same biostimulant treatment, and bars with the same uppercase letter indicate no significant difference between biostimulants for the same irrigation level, according to the Tukey test ($p \leq 0.05$). Error bars indicate standard error (SE, n = 4).

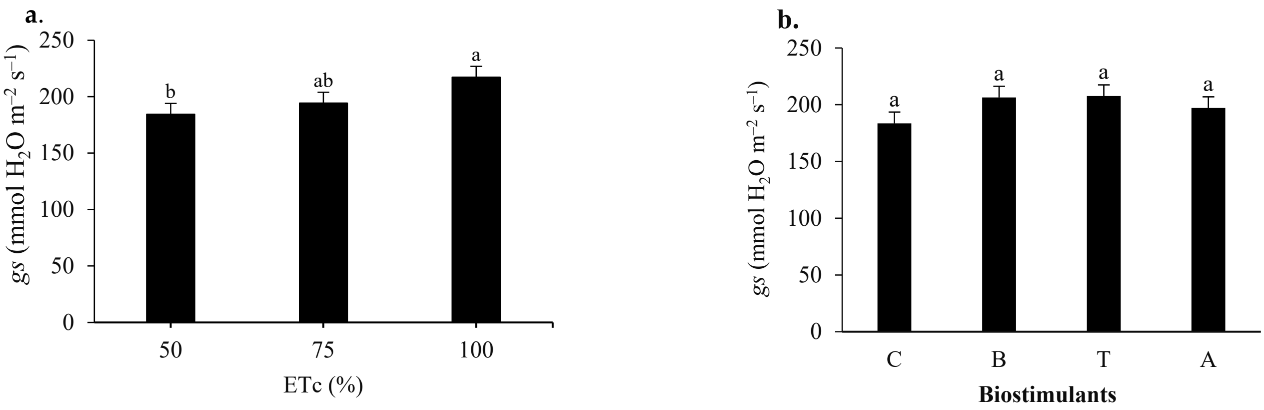

Figure 3. Stomatal conductance—gs of 'Formosa' papaya plants, as a function of irrigation levels (**a**) and application of microorganism-based biostimulants (**b**), 235 days after transplanting. Bars with the same lowercase letter indicate no significant difference between irrigation levels, according to Tukey's test ($p \leq 0.05$). Error bars indicate standard error (SE, $n = 4$).

3.3. Chlorophyll a Fluorescence

The interaction between irrigation level and biostimulant application significantly affected initial (F_0), variable (F_v), and maximum quantum efficiency of PSII (F_v/F_m); the maximum fluorescence (F_m) of 'Formosa' papaya plants was not affected by any source of variation (Table S2).

For initial fluorescence (Figure 4a), no significant difference was found among irrigation levels in plants that received the control, *T. harzianum*, and *A. nodosum* treatments. A significant difference was verified between the 50% and 100% ETc irrigation levels under the application of *B. aryabhattai*, where plants under 100% ETc showed a decrease in F_0 of 11.7% in comparison to the 50% ETc level, but did not differ from 75% ETc. The biostimulants at the distinct irrigation levels had similar effects on the F_0 of 'Formosa' papaya plants.

For variable fluorescence (Figure 4b), the control plants irrigated with 100% ETc differed from 50% ETc, but no significant difference from 75% ETc was observed. Meanwhile, for *B. aryabhattai*, the 75% and 100% ETc irrigation levels maintained the active potential of PSII, differing significantly from 50% ETc. The irrigation levels under the application of *T. harzianum* and *A. nodosum* did not differ from each other. The control treatment did not differ from *B. aryabhattai* but differed from *T. harzianum* and *A. nodosum* under the 100% ETc level. The biostimulants did not differ from each other at the 75% and 50% ETc levels.

The maximum quantum efficiency of PSII (Figure 4c) was superior in control plants under 100% ETc in reference to 50% ETc, and there was no distinction from the 75% ETc level. *B. aryabhattai* and *A. nodosum* promoted a similar effect among the irrigation levels. For plants under *T. harzianum*, the 50% ETc treatment with a value of 0.79 was higher than the result obtained at 100% ETc, and was similar to 75% ETc. The *T. harzianum*-based biostimulant had an influence on increasing the quantum efficiency of PSII in comparison to *B. aryabhattai* and the control at the 50% ETc level, but did not differ from *A. nodosum*. Also, there was no difference among the biostimulants at the 100% and 75% ETc levels. In contrast, maximum fluorescence (F_m) was not significantly affected by any source of variation (Figure 5a,b), with an average value of 149.83.

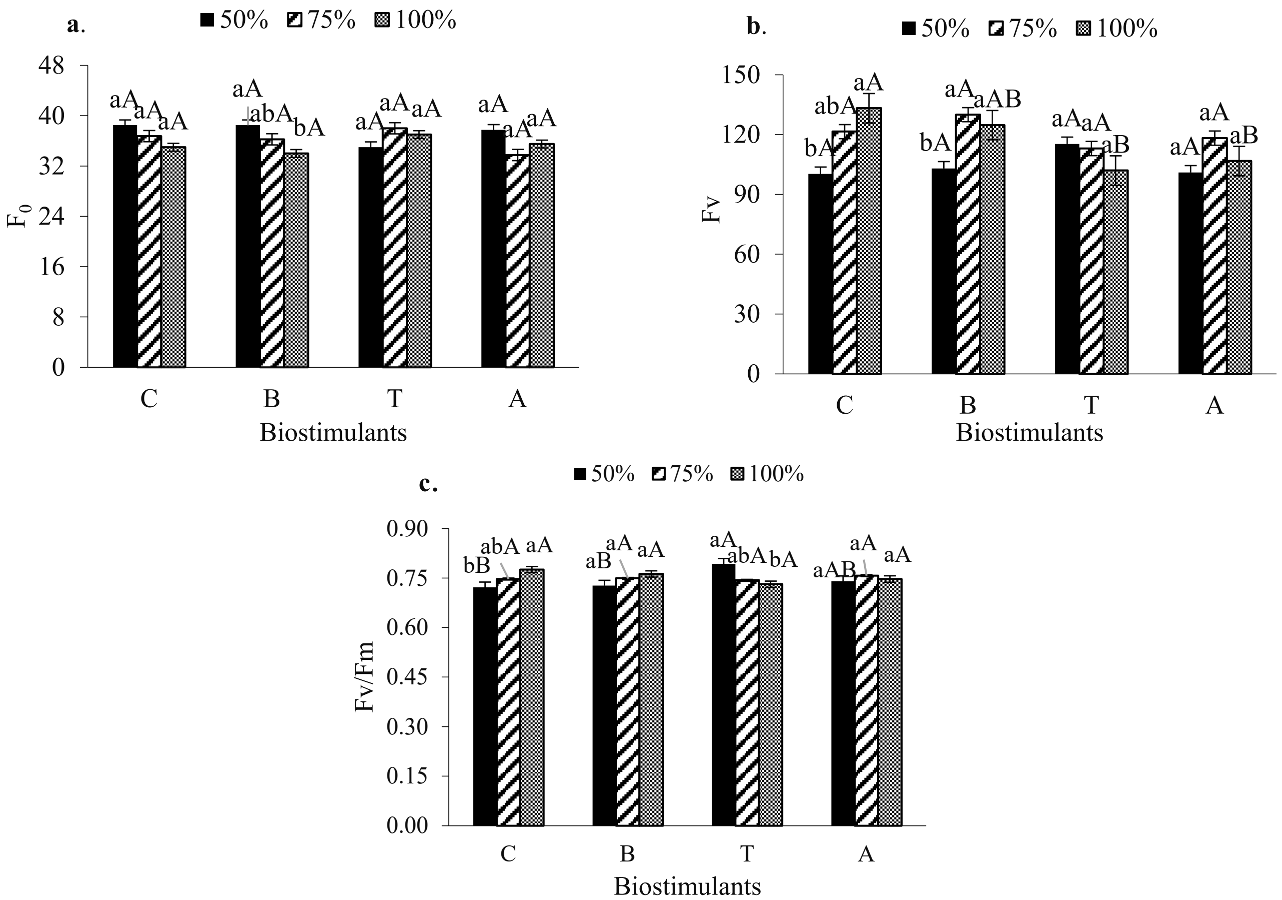

Figure 4. Initial fluorescence—F_0 (**a**), variable fluorescence—Fv (**b**), and quantum efficiency of PSII—Fv/Fm (**c**) of 'Formosa' papaya plants as a function of the interaction between irrigation levels and application of microorganism-based biostimulants, at 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same lowercase letter indicate no significant difference between irrigation levels in the same biostimulant treatment, and bars with the same uppercase letter indicate no significant difference between biostimulants for the same irrigation level, according to the Tukey test ($p \leq 0.05$). Error bars indicate standard error (SE, $n = 4$).

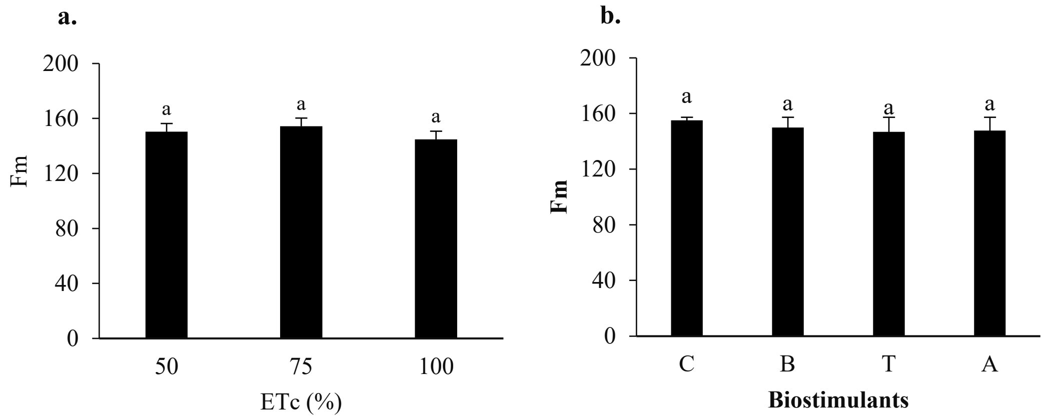

Figure 5. Maximum fluorescence—Fm of 'Formosa' papaya plants as a function of irrigation levels (**a**) and application of microorganism-based biostimulants (**b**), at 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same letter indicate no significant difference between irrigation levels and between biostimulants, according to the Tukey test ($p \leq 0.05$). Error bars indicate standard error (SE, $n = 4$).

3.4. Water Relations, Membrane Integrity, and Photosynthetic Pigments

There was a significant interaction effect between irrigation levels and biostimulant application (ID × Bio) for electrolyte leakage in the leaf lamina, and for chlorophyll a, b, and carotenoid contents of 'Formosa' papaya plants (Table S3). The relative water content was significantly affected by the application of microorganism-based biostimulants.

Irrigation levels did not affect the water content in the leaf blade (Figure 6a), with an average value of 81%. However, plants that received the *T. harzianum*-based biostimulant were superior to the control treatment (no application) (Figure 6b). However, there were no significant differences between *B. aryabhattai* and *A. nodosum*.

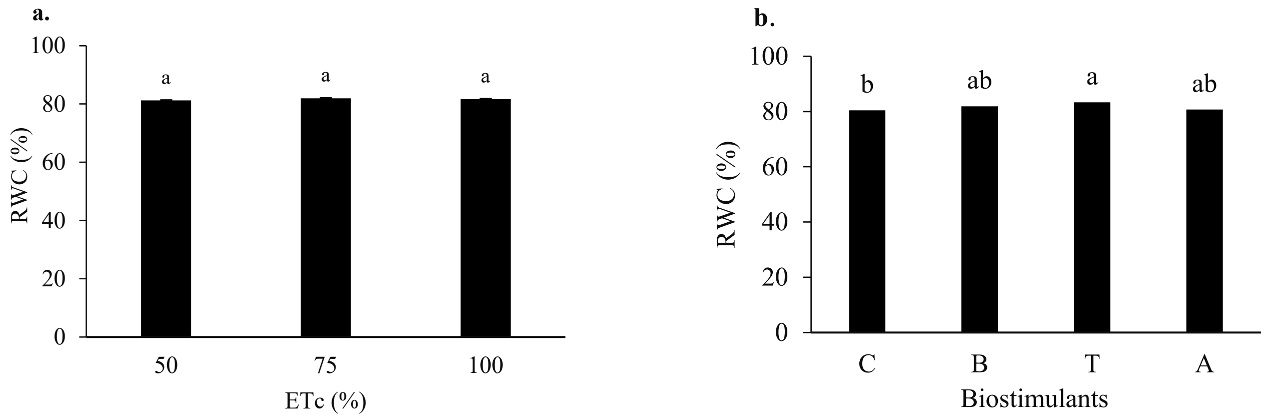

Figure 6. Relative water content—RWC of 'Formosa' papaya plants as a function of irrigation levels (**a**) and application of microorganism-based biostimulants (**b**), at 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same letter indicate no significant difference between irrigation levels and between biostimulants, according to the Tukey test ($p \leq 0.05$). Error bars indicate standard error (SE, n = 4).

In the control and *B. aryabhattai* treatments, electrolyte leakage (EL) did not differ statistically among the irrigation levels (Figure 7a). For the T. harzianum treatment, EL at the 50% and 75% ETc levels differed significantly from the 100% ETc level. Under the application of A. nodosum, the 75% ETc level resulted in a significantly higher EL (33.31%) compared to the 100% (23.43%) and 50% (23.63%) ETc levels. Furthermore, at the 75% ETc level, A. nodosum application led to a greater EL compared to the other biostimulant treatments. However, no significant differences among biostimulants were observed at the 100% and 50% ETc levels.

Regarding chlorophyll *a* content (Figure 7b), in control plants, the 75% and 100% ETc levels resulted in significantly different levels compared to the 50% ETc level. For plants treated with *B. aryabhattai*, irrigation level had no significant effect on Chl *a* content. In plants treated with *T. harzianum*, the 75% ETc level caused a significant reduction in Chl *a* content compared to both the 50% and 100% ETc levels. In contrast, under *A. nodosum* application, the 75% ETc level promoted the highest Chl *a* content (19.98 μg mL^{-1}), which was significantly different from the 50% and 100% ETc levels and also superior to all other biostimulants at that 75% ETc level. Furthermore, *A. nodosum* also resulted in the lowest Chl *a* content at the 100% ETc level, being statistically lower than the control, *B. aryabhattai*, and *T. harzianum* treatments. Finally, at the 50% ETc level, both *T. harzianum* and *A. nodosum* produced superior Chl *a* content compared to the control, but did not differ statistically from *B. aryabhattai*.

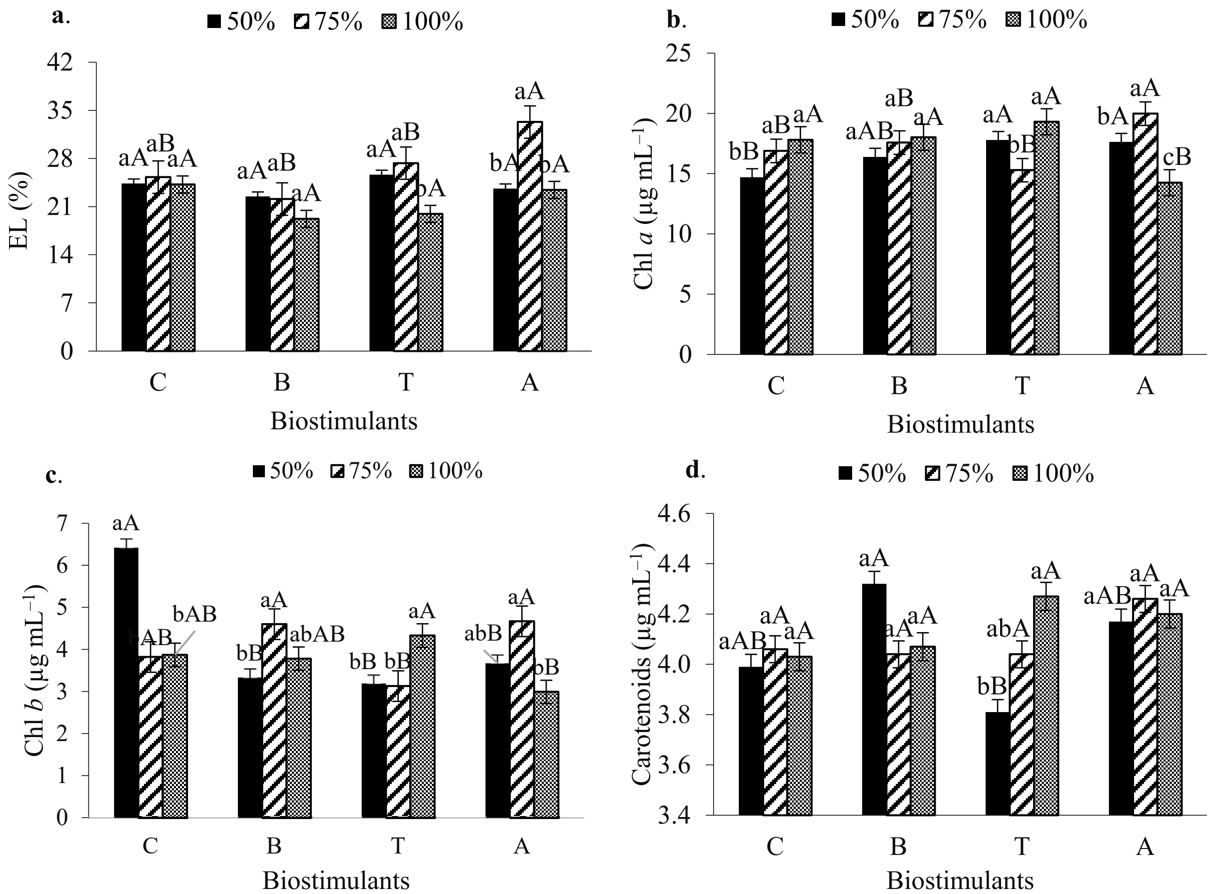

Figure 7. Electrolyte leakage in the leaf blade—EL (**a**), chlorophyll *a*—Chl *a* (**b**), chlorophyll *b*—Chl *b* (**c**), and carotenoids—CAR (**d**) of 'Formosa' papaya plants as a function of the interaction between irrigation levels and application of microorganism-based biostimulants, at 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same lowercase letter indicate no significant difference between irrigation levels in the same biostimulant treatment, and bars with the same uppercase letter indicate no significant difference between biostimulants for the same irrigation level, according to the Tukey test ($p \leq 0.05$). Error bars indicate standard error (SE, $n = 4$).

For chlorophyll *b* content (Figure 7c), in control plants (no biostimulant), irrigation with 50% ETc resulted in a higher content, differing statistically from the 75% and 100% ETc levels. With the application of *B. aryabhattai*, the 75% ETc level promoted an increase in Chl *b* content compared to 50% ETc, but was similar to 100% ETc. In plants treated with *T. harzianum*, the 100% ETc level was superior to both 75% and 50% ETc. Plants cultivated with *A. nodosum* at the 75% ETc level obtained the highest Chl *b* content, which was significantly higher than at the 100% ETc level but similar to the 50% ETc level. A comparison among biostimulants showed that at the 100% ETc level, *A. nodosum* application resulted in the lowest Chl *b* content, differing significantly from *T. harzianum* but not from the control or *B. aryabhattai*. At the 75% ETc level, both *B. aryabhattai* and *A. nodosum* resulted in higher Chl *b* content compared to *T. harzianum*, but were similar to the control. Furthermore, at the 50% ETc level, the control treatment differed significantly from all three biostimulant-treated groups.

For the carotenoid content (Figure 7d), the irrigation levels did not differ from each other in the control, *B. aryabhattai*, and A. *nodosum* treatments. A significant difference was observed between the 100% and 50% ETc levels under the application of *T. harzianum*. The biostimulants did not show differences under the 100% and 75% ETc levels. However, *B.*

aryabhattai differed from *T. harzianum* under the 50% ETc condition, showing similarity with the control and A. *nodosum*.

3.5. Plant Growth

The interaction between irrigation levels and biostimulant application (ID × Bio) significantly influenced stem height, and the absolute and relative growth rates of stem diameter of 'Formosa' papaya plants in the period from 93 to 235 DAT (Table S4). Irrigation levels significantly affected stem diameter, and the application of biostimulants had a significant effect on stem diameter and the absolute growth rate of stem height of 'Formosa' papaya.

For stem height, it was verified that plants cultivated under 50% ETc were smaller in comparison to plants cultivated with 75% and 100% ETc under the control and *B. aryabhattai* treatments (Figure 8). Likewise, 75% ETc increased stem height in comparison to plants under 50% and 100% ETc with the use of *T. harzianum*. The irrigation levels did not differ from each other in the *A. nodosum*-based biostimulant. Regarding the effect of biostimulants, the control treatment differed significantly from *T. harzianum* and *A. nodosum* when they received 75% and 100% ETc, and did not promote a difference with *B. aryabhattai*. The biostimulants did not differ statistically at the 50% ETc level.

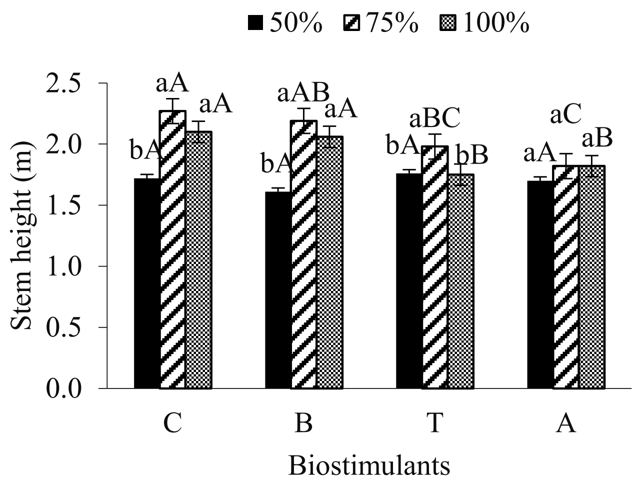

Figure 8. Stem height—SH of 'Formosa' papaya plants as a function of the interaction between irrigation levels and application of microorganism-based biostimulants, at 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same lowercase letter indicate no significant difference between irrigation levels in the same biostimulant treatment, and bars with the same uppercase letter indicate no significant difference between biostimulants for the same irrigation level, according to the Tukey test ($p \leq 0.05$). Error bars indicate standard error (SE, $n = 4$).

In Figure 9a, the absolute growth rate of stem diameter, under the influence of irrigation levels, did not differ when the plants were subjected to the control and *T. harzianum* treatments. It is noted that the 50% and 100% ETc levels increased the AGRsd with the application of *B. aryabhattai*, in relation to 75% ETc. A higher AGRsd was observed in plants irrigated with 100% ETc under the application of *A. nodosum*, differing from those that received 50% and 75% ETc. There was also a significant difference between *A. nodosum* with the other biostimulants in plants irrigated with 50% ETc. Furthermore, there was no significant difference among the biostimulants in the 100% ETc irrigation. The control and *T. harzianum* were superior to *B. aryabhattai* and *A. nodosum* at the 75% ETc level. For the biostimulants at 50% ETc, it was verified that *A. nodosum* did not attenuate the effect of water deficit in comparison to the other biostimulants.

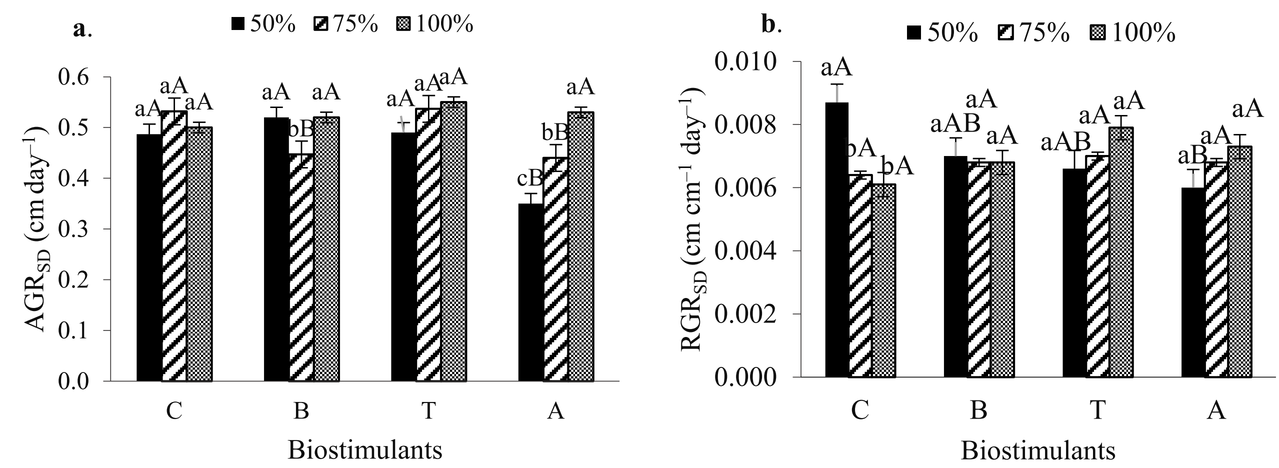

Figure 9. Absolute growth rate—AGR$_{SD}$ (**a**) and relative growth rate—RGR$_{SD}$ (**b**) of stem diameter of 'Formosa' papaya plants as a function of the interaction between irrigation levels and application of microorganism-based biostimulants, in the period from 93 to 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same lowercase letter indicate no significant difference between irrigation levels in the same biostimulant treatment, and bars with the same uppercase letter indicate no significant difference between biostimulants for the same irrigation level, according to the Tukey test ($p \leq 0.05$).

An increase in the relative growth rate of the stem (Figure 9b) was observed in the control at the 50% ETc deficit level in comparison to 75% and 100% ETc. In the other biostimulant treatments, the irrigation levels did not show a difference among themselves. For the effect of biostimulants at each irrigation level, it was found that *A. nodosum* reduced the RGRs$_D$ under the 50% ETc level, showing significant differences in relation to the control, but with no significant difference with the other biostimulants. The biostimulants did not differ under the 100% and 75% ETc irrigation levels.

The stem diameter (Figure 10a) of plants under 100% ETc irrigation was superior (on average 6.47%) to those cultivated with 75% and 50% ETc. However, there were no significant differences between the plants irrigated under the deficit levels.

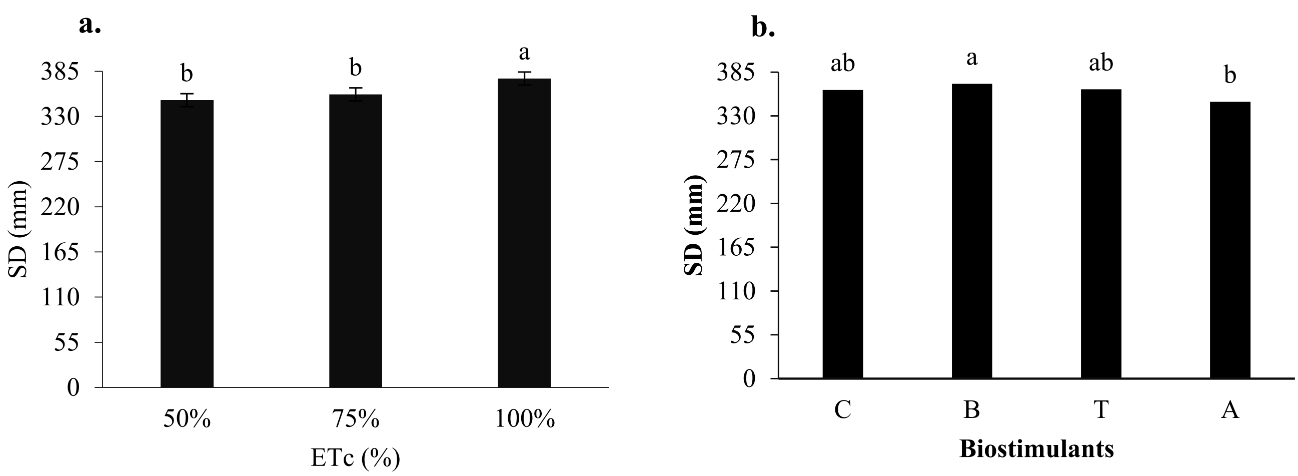

Figure 10. Stem diameter—SD (**a**) of 'Formosa' papaya plants as a function of irrigation levels (**a**) and application of microorganism-based biostimulants (**b**), at 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same letter indicate no significant difference between irrigation levels and between biostimulants, according to the Tukey test ($p \leq 0.05$). Error bars indicate standard error (SE, n = 4).

Analyzing the main effects of the biostimulants, stem diameter (Figure 10b) was significantly greater in plants treated with *B. aryabhattai* compared to those treated with *A. nodosum*, but neither differed statistically from the control or *T. harzianum*. For the absolute growth rate of stem height (AGRSH), there was no significant effect of irrigation levels (Figure 11a), which showed a mean value of 0.73 cm day^{-1}. However, for the main effect of biostimulants (Figure 11b), control plants differed significantly from those that received *T. harzianum*, but not from the *B. aryabhattai* and *A. nodosum* treatments. The relative growth rate of stem height (RGRSH) was not significantly affected by either factor, showing a mean value of 0.0058 cm cm^{-1} day^{-1} (Figure 11c,d).

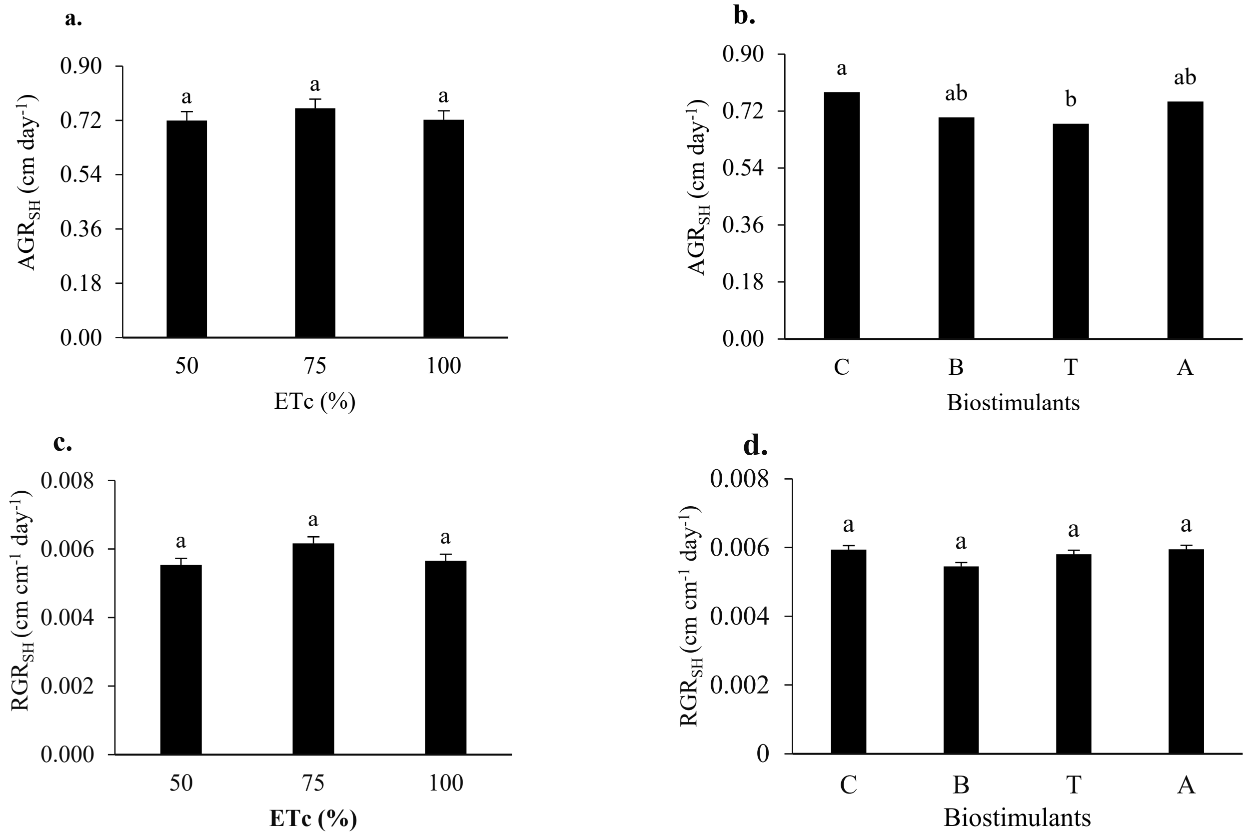

Figure 11. Absolute and relative growth rate in stem height—AGR$_{SH}$ and RGR$_{SH}$, of 'Formosa' papaya plants as a function of irrigation levels (**a**,**b**) and the application of microorganism-based biostimulants (**c**,**d**) in the period from 93 to 235 days after transplanting. C—control (without application of biostimulant); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Bars with the same letter indicate no significant difference between irrigation levels and between biostimulants, according to the Tukey test ($p \leq 0.05$). Error bars indicate standard error (SE, $n = 4$).

3.6. Multivariate Analysis

The principal components (PC1 and PC2) represented 55.6% of the total variation for the interaction of irrigation levels and microorganism-based biostimulants (Figure 12), where PC1 and PC2 explained 31.8% and 23.8% of the variance, respectively. Group 1 included the treatments ID100C, ID100B, ID75B, and ID50T, having an influence on the variables SH, F_v, F_v/F_m, Chl *a*, and SD. For group 2, the correlation of treatments ID50B, ID100T, and ID100A was verified for E and gs. In group 3, the treatments ID75T, ID50A, ID75C, and ID75A related the effects to the variables RGR$_{SH}$, EL, and AGR$_{SH,}$ while group 4 corresponded only to ID50C.

In Figure 13, the correlation between the physiological and growth variables with the irrigation levels and microorganism-based biostimulants showed strong positive correlations between the variables F_m with F_v/F_m (0.64), AGR_{SH} (0.69), and SH (0.77), while F_v was correlated with F_v/F_m (0.65), SH (0.84), and F_m (0.89). Strong negative correlations were observed between the variables F_0 and F_v/F_m (−0.76), and CEi and RGR_{SH} (−0.68).

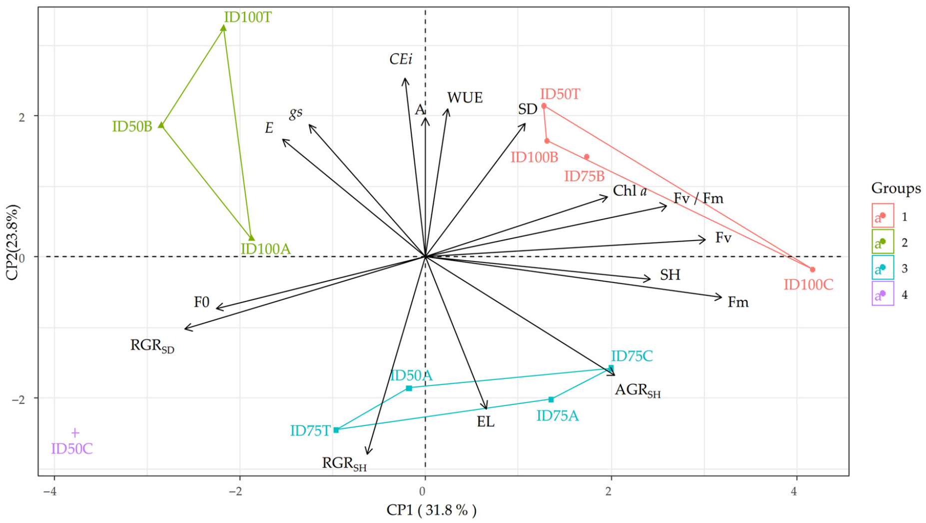

Figure 12. Two-dimensional projection of the principal component scores for the irrigation level and biostimulant factors, and variables analyzed in the two principal components (CP1 and CP2), for 'Formosa' papaya plants at 235 days after transplanting. ID100 = irrigation level of 100% of ETc; ID75 = irrigation level of 75% of ETc; ID50 = irrigation level of 50% of ETc; C—control (without biostimulants); B—*Bacillus aryabhattai*; T—*Trichoderma harzianum*; A—*Ascophyllum nodosum*. Stomatal conductance (gs), transpiration (*E*), water use efficiency (WUE) and instantaneous carboxylation efficiency (CEi), initial fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv) and quantum efficiency of PSII (Fv/Fm), electrolyte leakage (EL), chlorophyll *a* (Chl *a*), carotenoids (CAR), stalk height (SH), stalk diameter (SD), absolute growth rate of stalk height (AGR_{SH}), relative growth rate of stalk height (RGR_{SH}) and relative growth rate of stalk diameter (RGR_{SD}).

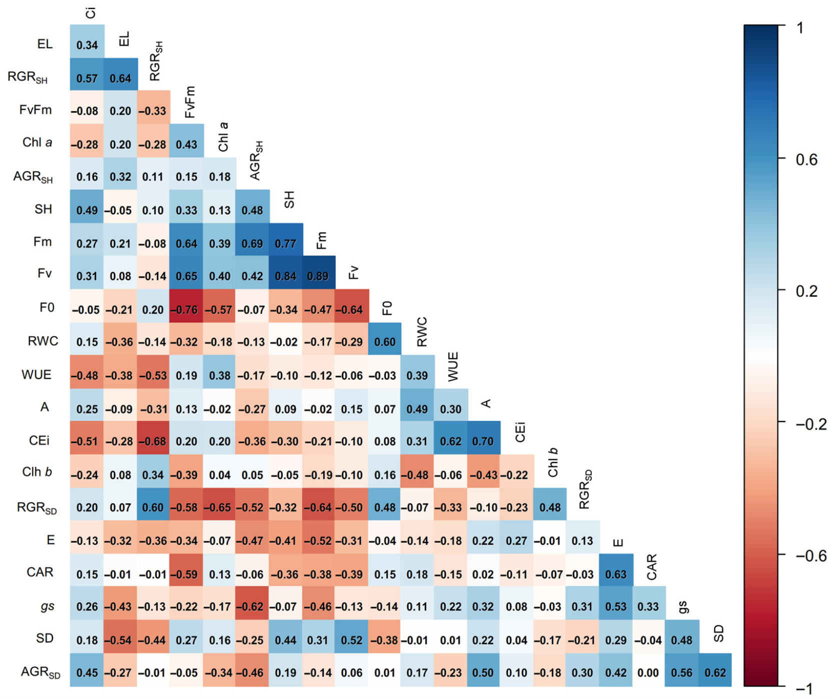

Figure 13. Pearson correlation matrix for the physiological, biochemical, and growth variables of 'Formosa' papaya under the irrigation levels and application of microorganism-based biostimulants, 235 days after transplanting.

4. Discussion

Water deficit induced a classic stomatal closure response in 'Formosa' papaya plants, evidenced by the reduction in stomatal conductance (gs) under the 50% crop evapotranspiration (ETc) level. This is a primary water-conservation strategy to maintain cell turgor under water-restricted conditions [6,18]. However, the main contribution of this study lies in demonstrating that microorganism-based biostimulants can significantly modulate this response, optimizing physiological processes even under severe stress.

Bacillus aryabhattai mitigated the impact of deficit irrigation (50% ETc), enhancing both transpiration (E) and CO_2 assimilation (A), indicating effective plant acclimation. This response likely resulted from integrated physiological adjustments induced by the bacterium. *B. aryabhattai* produces phytohormones like auxins (IAA), which stimulate root proliferation, particularly lateral root development, thus expanding the root surface area for improved water and nutrient acquisition [14]. Concurrently, the bacterium or the inoculated plant synthesizes compatible osmolytes such as proline, glycine betaine, and sugars, facilitating osmotic adjustment to maintain cellular turgor and water potential despite reduced soil water availability [36]. This maintenance of turgor, potentially coupled with hormonal signals, likely permitted sustained stomatal conductance, supporting continued CO_2 influx for photosynthesis (higher A) and evaporative cooling through transpiration (higher E), which are vital drought tolerance strategies [21,37]. Furthermore, the observed increase in instantaneous carboxylation efficiency (CEi) suggests that *B. aryabhattai* might also have enhanced photosynthetic carbon fixation by optimizing the activity or activation state of the RuBisCO enzyme, possibly through modulation of the plant's hormonal balance or intracellular redox environment [14,38].

Trichoderma harzianum acted distinctly. Under 50% ETc, it increased water use efficiency (WUE) and carboxylation efficiency (CEi). As a recognized root growth promoter [13], it improves water acquisition and, possibly along with stomatal regulation, enhances WUE [10]. *Trichoderma* also induces antioxidant defenses [18], mitigating oxidative stress and protecting the photosynthetic apparatus (PSII), thus explaining the maintenance of Fv/Fm and CEi [13]. The unexpected increase in internal CO_2 (Ci) at 75% ETc, typically indicating non-stomatal limitations [39], may stem from a transient imbalance under moderate stress. The fungus potentially maintained relatively high stomatal conductance (due to better water/hormonal status), while carboxylation capacity was temporarily reduced by stress, leading to Ci accumulation before acclimation stabilized [10,13].

Comparatively, *B. aryabhattai*, being a bacterium, appears to optimize shoot physiology via hormonal/osmotic regulation to maintain gas exchange [14]. In contrast, *T. harzianum*, a filamentous fungus, forms hyphal networks exploring soil and penetrating roots [13], focusing on enhancing the root-soil interface for water acquisition and activating antioxidant defenses [10,11]. This difference in inferred primary mechanisms (bacterial systemic action vs. fungal root/defense action) likely explains the distinct physiological outcomes observed at 50% ETc, where *B. aryabhattai* increased A and E, while *T. harzianum* increased WUE and maintained Fv/Fm.

The less pronounced effects of *B. aryabhattai* and *T. harzianum* under 75% ETc might be attributed to this level representing only moderate stress for 'Formosa' papaya under these conditions. It is plausible that under moderate stress, the plant's intrinsic defense mechanisms, such as osmotic adjustments and antioxidant system activation, remain relatively effective in maintaining physiological homeostasis [15]. Biostimulants like *Bacillus* and *Trichoderma* often enhance plant resilience by further modulating phytohormone levels, boosting antioxidant capacity, or improving resource acquisition [14,18]. Consequently, their significant beneficial impact may become most evident primarily when the stress intensity surpasses the plant's endogenous adaptive capacity, as observed under the more severe stress scenario (50% ETc).

The integrity of the photosynthetic apparatus, assessed by chlorophyll *a* fluorescence, was also positively influenced by the biostimulants. An increase in initial fluorescence (F_0) is an indicator of damage to the reaction center of photosystem II (PSII) [40]. The fact that plants treated with *B. aryabhattai* under full irrigation (100% ETc) showed lower F_0 suggests a protective effect, improving the capacity for energy dissipation and preventing the oxidation of photosynthetic complexes [36]. Under stress, both *B. aryabhattai* and *T. harzianum* helped to maintain the maximum quantum efficiency of PSII (F_v/F_m), indicating that the electron transfer was not compromised [41]. This effect is aligned with the ability of these microorganisms to activate plant defense systems that detoxify reactive oxygen species (ROS) and protect thylakoid membranes against photoinhibition [38,39].

The *Ascophyllum nodosum* extract exhibited a more complex response. On one hand, it stimulated chlorophyll *a* synthesis in plants under moderate water deficit (75% ETc), which is consistent with studies reporting its role in reducing pigment degradation due to its composition rich in antioxidants and hormones [11,42]. However, this same biostimulant was unable to mitigate the effects of severe water deficit (50% ETc) on growth, reducing the absolute and relative growth rates of the stem diameter. This negative result may indicate that, under intense water deficit, the benefits of *A. nodosum* in pigment protection were insufficient to elevate the carbon assimilation required for growth, suggesting a probable investment in secondary metabolism due to the limitations imposed by stomatal closure [12,19].

Finally, the growth responses corroborate the superiority of *B. aryabhattai* in stress mitigation. Plants treated with this bacterium showed a larger stem diameter compared to those treated with *A. nodosum*. This effect is a direct reflection of the observed physiological benefits, such as the maintenance of photosynthesis and the probable production of indole-3-acetic acid, which stimulates cell division and elongation [14]. The ability of *T. harzianum* to increase the relative water content, a direct indicator of improved plant water status, also aligns with its role as a resilience promoter, enhancing membrane stability and overall morphological activity [13].

It should be noted that although the experiment was conducted in a region classified as semiarid, the experimental period coincided with the occurrence of precipitation. The additional air humidity may have allowed the plants to recover their water potential and maintain metabolic activity, attenuating the characteristic environmental conditions, which could have partially minimized the effects of the biostimulants. Continuous and more severe water and heat stress, typical of periods without rain, would likely have made the benefits of the microorganisms even more evident. Therefore, future studies under more adverse climatic conditions are recommended to validate the maximum potential of these biostimulants in promoting the resilience of papaya to water deficit. Furthermore, evaluating the economic viability and efficacy under field conditions, including cost–benefit analyses compared to conventional water management practices, will be crucial to ascertain the practical value and adoption of these biostimulant technologies in agriculture.

5. Conclusions

Water deficit imposed by irrigating with 50% of the crop evapotranspiration (ETc) reduced the stomatal conductance and growth of 'Formosa' papaya plants. However, biostimulants based on *Bacillus aryabhattai* and *Trichoderma harzianum* conferred beneficial effects, improving the physiological indices of plants under deficit irrigation. This makes the application of these biostimulants a viable strategy to increase plant tolerance to the adverse effects of water deficit in semiarid regions. In contrast, while *Ascophyllum* *nodosum* stimulated chlorophyll *a* synthesis in papaya plants irrigated with 75% ETc, it did not mitigate the negative effect of severe water deficit on plant growth. We conclude that the application of *B. aryabhattai* and *T. harzianum* is a viable strategy to increase the tolerance of 'Formosa' papaya to the adverse effects of water deficit in semiarid regions. However, further studies are recommended to fully elucidate the specific physiological and biochemical mechanisms involved in stress mitigation by these microorganisms.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11111348/s1, Table S1. Summary of analysis of variance for internal CO_2 concentration (Ci), stomatal conductance (gs), transpiration (*E*), CO_2 assimilation rate (*A*), water use efficiency (*WUE*), and instantaneous carboxylation efficiency (*CEi*) of 'Formosa' papaya plants grown under irrigation levels (IL) and application of biostimulants (Bio), at 235 days after transplanting. Table S2. Summary of the analysis of variance for initial fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv) and quantum efficiency of PSII (Fv/Fm) of 'Formosa' papaya plants grown under irrigation levels (ID) and application of biostimulants (Bio), at 235 days after transplanting. Table S3. Summary of the analysis of variance for relative water content (RWC), electrolyte leakage (EL), and chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (CAR) contents of 'Formosa' papaya plants grown under irrigation levels (ID) and application of biostimulants (Bio), at 235 days after transplanting. Table S4. Summary of the analysis of variance for stem height (SH) and stem diameter (SD) at 235 days after transplanting, and absolute and relative growth rates of stem height (AGRSH, RGRSH) and stem diameter (AGRSD, RGRSD) of 'Formosa' papaya plants grown under irrigation levels (ID) and application of biostimulants (Bio), in the period from 93 to 235 days after transplanting.

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