agronomy

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Enhancing Tomato Growth and Quality Under Deficit Irrigation with Silicon Application

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Abstract: This study aimed to evaluate the effect of two irrigation systems (deficit irrigation (DI)—70% of field capacity—and full irrigation (FI)—100% of field capacity) and a biostimulant formulation (silicon (Si) and calcium (Ca) at four different rates) on the chemical composition and fruit quality of greenhouse-grown tomatoes. Deficit irrigation and biostimulant application influenced the proximate composition of tomato fruits. Fructose and glucose were the main soluble sugars, while malic and citric acids were the predominant organic acids. Free sugar and organic acid content increased under DI and biostimulant applications. In contrast, deficit irrigation combined with biostimulant application decreased α-tocopherol levels. In terms of carotenoids, lycopene and β-carotene concentrations were higher under full irrigation. The main fatty acids were palmitic (C16:0) and linoleic (C18:2n6) acids, with saturated (SFA) and polyunsaturated (PUFA) fatty acids being the main classes. Moreover, biostimulant applications reduced the total phenolic content regardless of the irrigation regime, whereas the flavonoid content increased when biostimulants were applied under FI conditions. Regarding antioxidant activity (assessed by TBARS and OxHLIA assays), a variable response to irrigation and biostimulant application was observed. In conclusion, the application of Si and Ca under DI showed promising results in terms of yield and quality of tomato fruit and it could be considered a sustainable strategy to mitigate adverse effects of climate change on horticultural crops.

Keywords: biostimulants; water stress; bioactive properties; *Solanum lycopersicum* L.; antioxidant activity

Academic Editor: Wei Wu

Received: 15 February 2025
Revised: 26 February 2025
Accepted: 7 March 2025
Published: 12 March 2025

Citation: Añibarro-Ortega, M.; Pereira, A.; Pinela, J.; Liava, V.; Chaski, C.; Alexopoulos, A.A.; Barros, L.; Petropoulos, S.A. Enhancing Tomato Growth and Quality Under Deficit Irrigation with Silicon Application. *Agronomy* **2025**, *15*, 682. https://doi.org/10.3390/agronomy15030682

1. Introduction

Tomatoes (*Solanum lycopersicum* L.) are one of the most important horticultural crops worldwide, and their fruits are highly appreciated for their nutritional and organoleptic characteristics [1,2]. The tomato fruit is a rich dietary source of minerals and vitamins (A, B-complex, C, E, K) and its consumption significantly contributes to fulfilling the average daily intake of essential macro and micronutrients [3–5]. Moreover, tomatoes contain soluble sugars, organic acids (citric, glutamic, malic), carotenoids (lycopene, β-carotene, lutein, violaxanthin), and several phenolic compounds such as homovanillic acid-*O*-hexoside, rutin, quercetin, kaempferol-3-*O*-rutinoside, naringenin, chlorogenic acid, caffeic acid with antioxidant, antiproliferative, antidiabetic, anti-inflammatory, and other health-promoting features [6–8].

The increase in the world population has dramatically raised food demand, putting food security at risk, while climate change and unfavorable weather conditions pose major challenges to crops and, consequently, to food production [9,10]. Moreover, changing climate conditions are often associated with abiotic stressors, such as drought salinity and extreme temperatures, which, alongside the gradual decline in soil fertility, result in significant losses in crop yield and the quality of the final products [11,12]. In particular, drought is considered one of the most hazardous implications of climate change in terms of agroeconomic aspects [13] since it affects plant growth and metabolic processes, leading to reduced production, yield, and quality of crops [14–17]. In addition, drought is often associated with the accumulation of reactive oxygen species (ROS), enzyme inactivation, proline toxicity, hormonal imbalance, and reduced photosynthesis [18]. Therefore, mitigation measures that increase plant tolerance to water stress are urgently needed to support crop productivity and protect food availability and food security [19,20].

The limited availability of irrigation water influences almost all horticultural crops, particularly tomatoes, which have high water requirements, especially at the flowering and fruit development stages. As a result, the tomato crop is highly affected by drought conditions [3,21–23]. Therefore, in order to optimize management and improve the use efficiency of irrigation water, it is essential to determine the growth stages that are critical to ensure the unimpeded development of the tomato plants and a high yield and quality of fruit [24–27]. Sustainable practices should alleviate the impacts of drought stress on horticultural crops and increase the growth and productivity of crops [28]. For this purpose, the application of biostimulants and the adoption of deficit irrigation are promising agronomic practices that can help farmers address challenges of modern crop production [29–31].

Silicon (Si) has biostimulatory properties and it appears in large amounts in soil; however, its uptake by plants is generally low due to its limited solubility, while plant species are categorized as intermediate, high, and non-Si accumulators [32]. Despite being non-essential for plant nutrition, Si possesses a variety of biostimulatory effects on the growth and development of plants [33,34]. For instance, its application can mitigate the effects of biotic and abiotic stresses, especially during drought stress, by enhancing plant tolerance in various species, including those that are classified as non-Si-accumulators [28,35,36]. Especially under drought stress, the application of Si improves water absorption [35] and activates plant defense and phytohormones signaling [37], while it induces biochemical, physiological, and molecular mechanisms [14] and consequently increases plant growth, biomass yield, nutrient uptake, and photosynthesis [19]. However, its effects may vary depending on the application rate and form, as well as the plant species [38].

Similarly, deficit irrigation is a strategy that withholds water at non-critical growth stages, thereby saving water and increasing crop water use efficiency (WUE) [23,31,39]. According to the literature reports, the scheduled use of deficit irrigation may lead to increased fruit quality without compromising crop yield in tomato plants [10,40]. For example, it has been suggested that mild drought stress in tomato plants may have a benefit on irrigation water use efficiency (IWUE) without a statistically significant reduction in yield compared to normally irrigated plants [22]. On the other hand, apart from increased WUE values, deficit irrigation is associated with improved fruit quality due to the accumulation of functional bioactive compounds, such as vitamins, carotenoids, organic acids, and phenolic compounds [5,40,41]. However, the response of tomatoes to deficit irrigation depends on several factors, such as the cultivar, the severity of water stress, the quality of irrigation water, the growth stage of plants when subjected to irrigation withholding, and the climate and soil conditions of the growing area [22,42–44].

Considering the variable effect of biostimulants on horticultural crops reported in the literature, the present study conducted a greenhouse experiment to assess the effect of different rates of a Si-based biostimulant product on yield parameters, chemical composition, and fruit quality of tomato plants grown under deficit irrigation conditions.

2. Materials and Methods

2.1. Experimental Conditions

The study took place during the spring–summer growing season of 2022 in the unheated greenhouse of the University of Thessaly in Velestino, Greece. Tomato seedlings of cv. Benhur were transplanted on 14 May 2022. Plants were planted in rows at a plant density of 25,000 plants/ha (plant distances of 0.8 m between rows and 0.5 m within each row) following the split-plot design by using three rows for each irrigation treatment and six tomato plants for each biostimulant level in the same row (each experimental plot included six plants). The biostimulant formulation was an experimental product that contained CaO and SiO_2, together with a calcium mobilization and translocation agent, as well as trace elements (e.g., Mo, Bo, and Zn). The biostimulant treatments varied as follows: control treatment (no biostimulants added); Tr1: 35% CaO (*w*/*v*), 35% SiO_2 (*w*/*v*) 1% Mo (*w*/*v*), 15% B (*w*/*v*), 30% Zn (*w*/*v*), at 15 L/ha; Tr2: the same as Tr1 but applied at 10 L/ha; Tr3: the same as Tr1 but applied at 7.5 L/ha; Tr4: the same as Tr1 but applied at 2.5 L/ha. All the treatments included the same amount of calcium mobilization and translocation agent (1 L/ha). The application of the biostimulants was carried out by fertigation every 10 days (8 applications throughout the growing season, starting on 26 May), while the control treatment plants received only nutrient solution. The formula of nutrient solution and the fertigation regime was previously described in the study of Fernandes et al. [45]. Throughout the growing period, the plants were trained to a single leader using a vertical plastic string suspended from a horizontal cable above each row of plants and by removing suckers at regular intervals. One month before the last harvest, plants were topped to avoid the development of any further vegetation. Moreover, after each harvest, the lower leaves (the ones below the harvested fruit cluster) were removed. Pollination was supported by bumblebees released in the greenhouse during the flowering period. The mean air temperature (°C), relative humidity (%), and average solar radiation (W/m^2) throughout the growing season were as follows: May: 25.2 °C, 44.2%, and 284 W/m^2; June: 32.3 °C, 48.1%, and 214 W/m^2; July: 31.2 °C, 45.6%, and 223 W/m^2; August: 29.6 °C, 52.3%, and 183 W/m^2; September: 23.7 °C, 63%, and 227 W/m^2; and October: 18.3 °C, 77.7%, and 156 W/m^2. To avoid excessive temperatures within the greenhouse, the cover materials were sprayed with Sunblock (Agrohum S.A., Aspropyrgos, Greece) which is used for shading through UV reflection (38–42% of UV radiation is blocked) and allows the decrease in temperatures. The application of Sunblock was performed on 15 June 2022, while it was removed in September 2022. Seven harvests were conducted throughout the growing season starting on 30 July, while the final harvest was carried out on 22 October 2022, during which quality evaluation and chemical composition analyses of the samples were also carried out.

After harvest, the fruit weight was measured, and the total yield was calculated as the cumulative yield of each experimental plot from all the harvests. Twenty fruit from each plot were selected for quality assessment, including fruit color (L^*, a^*, b^*) and firmness (two measurements in the vertical (F1) and horizontal axis (F2)), as well as soluble solids (°Brix), pH, electrical conductivity (EC), and acidity of the juice [46]. Additionally, a portion of the samples underwent an air-drying process at 69 °C to determine the percentage of dry matter, while another segment of the samples was stored in a freezer for subsequent chemical analyses.

2.2. Irrigation Treatments

The irrigation was applied according to two different regimes via a drip irrigation system, namely deficit irrigation (DI: plants that received water according to 70% of field capacity (FC)) and full irrigation (FI: plants that received water according to 100% FC). The irrigation dates and the water supply are presented in Figure 1. The total amount of water provided to plants for FI and DI treatments was 8248 m^3/ha and DI = 5871 m^3/ha, respectively. At the beginning of the experiment and after plant transplantation, plants received the same amount of water (until 5 June). Then, the irrigation was scheduled based on the reading of sensors at 40 cm of soil depth as previously described by Liava et al. [30].

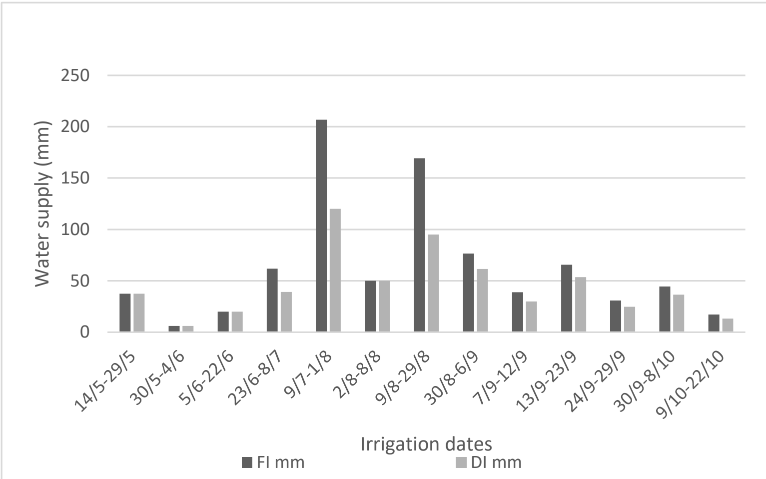

Figure 1. Water supply (mm) during the growing period 2022.

2.3. Chemical Composition Analysis

2.3.1. Proximate Composition and Energy

Lyophilized samples of the tomato fruits were evaluated for protein, fat, and ash contents according to the methods of the Association of Official Analytical Chemists (AOAC) [47]. The energetic value was calculated after converting proteins and carbohydrates to 4 kcal/g and fat to 9 kcal/g (European Parliament & Council of the European Union, 2011). The results were presented as kcal per 100 g fw.

2.3.2. Analysis of Free Sugars and Organic Acids

Free sugars were determined using a high-performance liquid chromatography (HPLC) system (Knauer, Smartline system 1000, Berlin, Germany) coupled to a refraction index (RI) according to the method of Spréa et al. [48]. The identification of free sugars was achieved through chromatographic comparisons with commercial standards, while quantification (g per 100 g fw) was performed using the internal standard method.

The analysis of organic acids took place with an ultra-fast liquid chromatography (UFLC) system (Shimadzu 20A series, Kyoto, Japan) coupled to a photodiode array detector (PDA) according to the protocol of Pereira et al. [49]. The identification of the detected compounds was accomplished by chromatographic comparisons with commercial standards, while quantification (mg per 100 g fw) was performed using calibration curves ($r^2 \geq 0.994$) constructed by the commercial standards of oxalic acid ($y = 8 \times 10^6 x + 331,789$), malic acid (y = 942,562x + 38,506), ascorbic acid ($y = 5 \times 10^7x + 449{,}262$), and citric acid (y = 968,367x − 12,295) (Sigma-Aldrich, St. Louis, MO, USA).

2.3.3. Analysis of Fatty Acids, Tocopherols, and Carotenoids

Fatty acids were determined through the transesterification of fats, and the fatty acid methyl ester (FAME) mixture was determined in a YOUNG IN Chromass 6500 Gas Chromatography System (YL Instruments, Anyang, Republic of Korea) equipped with a flame ionization detector (FID) according to the method of Spréa et al. [48]. The identification of detected fatty acids was accomplished through a chromatographic comparison of the retention times of the sample FAME peaks with those of the standard mixture 47885-U (Sigma-Aldrich, St. Louis, MO, USA). The results for each fatty acid were expressed as relative percentage.

Tocopherols were evaluated in the HPLC system coupled to a fluorescence detector (FP-2020, Jasco, Easton, MD, USA) following the conditions and the procedure described by Pinela et al. [50]. The identification of the detected compounds was conducted through chromatographic comparisons of analyzed samples with authentic commercial standards, while the quantification (mg per 100 g fw) was performed based on the internal standard method.

Lycopene and β-carotene were determined according to the protocol optimized by Nagata and Yamashita [51] and their content was expressed in mg per 100 g fw.

2.4. Evaluation of Bioactive Properties

2.4.1. Preparation of Hydroethanolic Extracts

Lyophilized samples (1 g) were subjected to stirring for 1 h at room temperature using 30 mL of ethanol/water (80:20, *v*/*v*) and, subsequently, they were filtered with a Whatman No. 4 filter paper. The filtrate was collected while the solid residue was extracted using 50 mL of the same ethanol/water solvent. Both filtrates were mixed and then they were evaporated under a reduced pressure and redissolved in the same ethanol/water solvent for the quantification of total phenolics and flavonoids or in phosphate-buffered saline (PBS) and Tris-HCl buffer solutions for the assessment of antihemolytic and lipidic peroxidation inhibition activities, respectively.

2.4.2. Total Phenolic Compounds and Flavonoids Content

The total phenolic content (TPC) was determined by the Folin–Ciocalteu method [6]. Quantification was performed using gallic acid (0.05–0.8 mg/mL) as a standard for constructing the calibration curve y = 2.0372x + 0.043 ($r^2 = 0.9981$). The results were presented as mg of gallic acid equivalents (GAE) per g of extract.

The total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method [6]. Quantification was performed using catechin (0.03125–1 mg/mL) as a standard for constructing the calibration curve $y = 0.8578x + 2 \times 10^{-5}$ ($r^2 = 0.9999$). The results were presented as mg of the catechin equivalents (CE) per g of extract.

2.4.3. Thiobarbituric Acid Reactive Substances (TBARS) Assay

The assessment of TBARS was performed following the protocol of Lockowandt et al. [52]. The results were presented as EC_{50} values (μg/mL).

2.4.4. Oxidative Hemolysis Inhibition (OxHLIA) Assay

The assessment of OxHLIA was performed following the protocol of Lockowandt et al. [52]. The results were analyzed using GraphPad Prism® 8 and presented as IC_{50} values (μg/mL) for a Δt of 60 min.

2.5. Statistical Analysis

The analyses for the chemical compositions were conducted in triplicates, while the analysis of the obtained results was conducted with one-way analysis of variance (ANOVA). The comparison of means was performed with Student's *t*-test $p < 0.05$ when the two means were compared or with Tukey's honestly significant difference (HSD) test at $p < 0.05$ when three or more means were compared. The data were checked to ensure that they followed normal distribution with the Shapiro–Wilk test while the homogeneity of variance was assessed with a Levene test. Tukey's honestly significant difference (HSD) test was used in the case of homoscedastic data ($p > 0.05$), while Tamhane's T2 multiple comparison test was used in the case of heteroscedastic data. All the analyses were conducted with IBM SPSS Statistics software (Version 22.0, IBM Corp, Armonk, NY, USA).

3. Results and Discussion

The two-way ANOVA of the obtained results indicated a significant interaction between the tested factors. Therefore, all the means of the evaluated yield, quality, chemical composition, and bioactive properties parameters were compared at the same time, as described in the following sections.

3.1. Yield Parameters

Yield parameters are presented in Table 1. Deficit irrigation (DI) resulted in the formation of a higher number of fruits compared to full irrigation (FI), especially when it was combined with the highest biostimulant rate (Tr1) where the highest value was recorded. In contrast, the lowest number of fruits per plant was measured for normally irrigated plants that received the highest biostimulant rate (Tr4). On the other hand, FI resulted in higher fruit weight per plant, especially for biostimulant treatments Tr2 and Tr3, thus indicating that plants formed fewer and bigger fruits compared to deficit irrigation conditions. Moreover, the lowest fruit weight per plant was recorded for those submitted to Tr2 and the control under deficit irrigation conditions. Similar trends were observed for total fruit yield, where fully irrigated plants treated with Tr2 and Tr3 treatments showed the highest overall yield (an increase of 7.5% and 7.0% over the control treatment, respectively), while plants grown under deficit irrigation that did not receive biostimulant had the lowest fruit yield. Moreover, the application of Tr2 under deficit irrigation resulted in an increase of 27.9% over the control treatment which corroborates the feasibility and necessity of biostimulant application under water deficit in order to retain high yields. Finally, the tested factors also affected the dry matter of fruit, where Tr3 treatment combined with FI and DI regime had the highest and lowest dry matter content, respectively.

Several literature reports have suggested the significant impact that deficit irrigation may have on tomato fruit yield, both under greenhouse or field conditions, although the yield penalty depended on the severity of water deprivation, as well as on the application schedule (e.g., duration of water stress, plant growth stage, application method, Si form, etc.), the climate conditions, the soil properties, or even the genotype tested [53]. On the other hand, DI resulted in significantly improved water productivity, which is an important aspect in arid and semi-arid regions where irrigation water availability is limited [53]. Similarly to our study, Sani et al. [54] reported that biostimulant application may have a positive impact on tomato yield since it is associated with better nutrient uptake and a higher efficiency of assimilated biosynthesis. According to Peripoli et al. [55], the application of biostimulants had a positive effect on tomato fruit yield compared to the control treatment (no biostimulants added) either on FI or DI, while the same authors suggested that yield increases were positively correlated with application doses. On the other hand, Chakma et al. [28] recorded a variable effect of Si on yield parameters and they suggested that excessive rates (600 kg/ha of monosilicic acid) did not have a positive effect. Moreover, Villa e Vila et al. [56], who studied the combined effect of DI and Si application on lettuce yield, suggested that Si may mitigate the negative effects of water stress up to 80% of crop evapotranspiration (ETc), while at more severe water stress levels (60% ETc), biostimulant application significantly reduced yield penalties compared to the respective treatment where no biostimulants were applied. The positive effects of Si on the growth and productivity of tomato plants has also been indicated in other studies where its application was associated with increased water and nutrients uptake, increased leaf water content, and improved photosynthetic activity, as well as with osmotic adjustments, gas exchange regulation, and the induction of antioxidant defense mechanisms [57–60]. Apart from the impact of Si on biochemical and physiological processes that help plants to cope with water stress, Si may also play an important role as a physical barrier that reduces water losses through leaf stomata in a species-dependent manner when applied with foliage spraying [61,62]. In contrast, Patanè et al. [63] reported a variable effect of biostimulants on two local landraces and two commercial hybrids of tomatoes grown under FI and no irrigation conditions, indicating that climate conditions may also have an impact on biostimulant efficiency. Chakma et al. [57] also suggested an adverse effect of Si on fruit number per plant when applied through seed priming, as was observed in our study, while its application on seeds was not effective in mitigating the negative effect of water stress (50% of FC) on fruit yield.

Table 1. Yield parameters of tomato plants in relation to biostimulant application and irrigation regime (n = 3; mean ± SD).

Treatment	Number of Fruits/Plant	Fruit Weight/Plant (g)	Total Yield (kg/ha)	Dry Matter (%)
Control × DI	18.5 ± 2.1 c	1608 ± 101 f	40,200 ± 1787 g	5.8 ± 0.4 b
Tr1 × DI	22.5 ± 3.1 a	1711 ± 168 e	42,781 ± 1700 f	5.8 ± 0.2 b
Tr2 × DI	20.5 ± 2.4 b	1605 ± 145 f	51,431 ± 1133 d	5.8 ± 0.3 b
Tr3 × DI	20.3 ± 1.5 b	2057 ±112 d	43,402 ± 2806 f	3.6 ± 0.4 d
Tr4 × DI	20.5 ± 2.5 b	1736 ± 119 e	49,583 ± 1988 e	5.4 ± 0.5 b
Control × FI	15.4 ± 1.3 e	2560 ± 160 b	64,000 ± 1500 b	5.0 ± 0.2 c
Tr1 × FI	13.4 ± 2.3 g	2228 ± 146 c	55,709 ± 1158 c	5.1 ± 0.1 c
Tr2 × FI	16.2 ± 2.1 d	2752 ± 180 a	68,794 ± 4492 a	5.0 ± 0.3 c
Tr3 × FI	14.0 ± 1.8 f	2741 ± 162 a	68,521 ± 2541 a	8.3 ± 1.4 a
Tr4 × FI	12.6 ± 1.6 h	2549 ± 125 b	63,718 ± 1532 b	4.9 ± 0.1 c

Means in the same column followed by the same letter are significantly different according to Tukey's HSD test at $p < 0.05$. DI: deficit irrigation; FI: full irrigation; Tr1–4: biostimulant treatments, as described in Section 2.

3.2. Fruit Quality Traits

The results of the assessment of fruit quality parameters are presented in Table 2. Fruit dimensions increased when treated with Tr2 under deficit irrigation or Tr3 under full irrigation, whereas the lowest values were recorded when plants were treated with Tr2 under deficit irrigation conditions. Fruit color was not significantly affected by the studied factors, except for the L^* parameter, where Tr3 × DI treatment differed significantly from Tr4 × FI treatment. Flesh firmness varied among the treatments, with Tr2 × DI showing the highest overall values for firmness at both axes (vertical and horizontal), while the least firm fruit was recorded for Tr2 × FI in the case of the vertical axis and control × DI and Tr4 × FI in the case of the horizontal axis. Similarly, the combined treatment of Tr2 and DI resulted in the highest EC in fruit juice, whereas no significant differences in pH and acidity values were recorded. Finally, the Tr1 and Tr2 treatments applied under DI resulted in the highest °Brix values, while the control treatment under DI and the Tr2 and Tr4 treatments under FI showed the lowest values.

Table 2. Fruit quality parameters of tomato plants in relation to biostimulant application and irrigation regime (n = 20; mean ± SD).

		Control × DI	Tr1 × DI	Tr2 × DI	Tr3 × DI	Tr4 × DI	Control × FI	Tr1 × FI	Tr2 × FI	Tr3 × FI	Tr4 × FI
Dimensions (mm)	Height	40.1 ± 2.7 d	40.2 ± 2.3 d	30.6 ± 3.9 e	45.7 ± 2.7 a	41.3 ± 2.2 cd	45.1 ± 3.0 a	43.0 ± 2.4 bc	46.2 ± 2.0 a	44.3 ± 2.1 ab	42.7 ± 1.9 bc
	Diameter	49.5 ± 2.2 cc	48.0 ± 3.1 d	38.2 ± 2.3 e	55.5 ± 1.7 a	50.5 ± 2.1 c	52.9 ± 2.1 b	50.7 ± 2.4	54.0 ± 3.1 a	52.7 ± 1.4 b	52.2 ± 2.2 b
Color	*L**	39.3 ± 2.9 ab	38.6 ± 2.8 ab	39.7 ± 1.9 ab	37.9 ± 2.5 b	38.5 ± 2.7 ab	40.2 ± 2.5 ab	38.5 ± 2.5 ab	39.4 ± 2.2 ab	39.3 ± 2.0 ab	40.5 ± 2.9 a
	*a**	22.6 ± 2.0 a	21.8 ± 2.8 a	22.6 ± 2.0 a	22.5 ± 2.4 a	22.0 ± 2.5 a	23.8 ± 2.1 a	22.2 ± 2.1 a	23.2 ± 2.4 a	23.5 ± 2.2 a	22.6 ± 3.2 a
	*b**	29.0 ± 2.3 a	29.6 ± 3.4 a	30.3 ± 2.7 a	28.7 ± 3.6 a	29.2 ± 2.9 a	31.6 ± 2.9 a	29.0 ± 3.6 a	30.5 ± 2.6 a	31.2 ± 3 a	31.1 ± 3.2 a
Firmness (Kg)	F1	0.92 ± 0.12 bc	1.22 ± 0.22 ab	1.30 ± 0.21 a	0.97 ± 0.17 bc	0.86 ± 0.14 cd	1.08 ± 0.25 b	0.94 ± 0.12 bc	0.78 ± 0.23 d	0.98 ± 0.31 bc	0.95 ± 0.23 bc
	F2	0.93 ± 0.24 d	1.29 ± 0.31 ab	1.48 ± 0.32 a	0.86 ± 0.13	1.17 ± 0.21 bc	1.03 ± 0.13 c	1.07 ± 0.17 c	1.04 ± 0.31 c	1.10 ± 0.22 c	0.84 ± 0.25 d
EC		2.8 ± 0.1 ab	2.7 ± 0.4 bc	2.9 ± 0.1 a	2.6 ± 0.3	2.8 ± 0.1 ab	2.5 ± 0.1 d	2.8 ± 0.2 ab	2.6 ± 0.1 cd	2.7 ± 0.2 bc	2.5 ± 0.1 d
pH		4.4 ± 0.1 a	4.4 ± 0.1 a	4.4 ± 0.1 a	4.5 ± 0.3 a	4.4 ± 0.0 a	4.4 ± 0.1 a	4.5 ± 0.1 a	4.4 ± 0.1 a	4.4 ± 0.1 a	4.3 ± 0.1 a
Acidity (g citric acid/100 g)		0.03 ± 0.01 a	0.03 ± 0.01 a	0.03 ± 0.01 a	0.02 ± 0.01 a	0.03 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a
°Brix		4.1 ± 0.2 c	4.4 ± 0.3 a	4.4 ± 0.3 a	4.1 ± 0.2	4.2 ± 0.1 bc	4.3 ± 0.4 ab	4.3 ± 0.3 ab	4.1 ± 0.1 c	4.3 ± 0.2 ab	4.1 ± 0.2 c

Means in the same row followed by the same letter are significantly different according to Tukey's HSD test at $p < 0.05$. DI: deficit irrigation; FI: full irrigation; Tr1–4: biostimulant treatments, as described in Section 2.

According to Chakma et al. [28,57], DI significantly reduced tomato fruit dimensions and improved color index and total soluble solids content, whereas Si application had no significant impact on these parameters, a finding which is in contrast with our results. On the other hand, De Souza et al. [64] reported that fertigation with Si may reduce fruit transpiration and water loss and facilitate quality preservation under water stress conditions, while Si application rate may also have an impact on fruit quality parameters [65]. According to Coyago-Cruz et al. [66], the content of total soluble solids is highly affected by the fruit maturity stage at harvest, while Lipan et al. [42] also recorded a variable effect of regulated deficit irrigation depending on the plant phenological state and the water deficit level. Moreover, Plaut et al. [67] suggested that osmotic stress per se is not associated with increased dry matter content, hence increased soluble solids in fruit sap, as a varied response was observed in fruit grown under salinity and water deficit stress. The same authors also suggested that any increase in dry matter content under water stress could be attributed mostly to concentration effects rather than actual increased biosynthesis and accumulation of soluble solids [67]. Therefore, any contradictions between the literature reports could be partly due to the non-uniform criteria for fruit maturity at harvest, as well as to the phenological state of plants when subjected to stress conditions.

3.3. Chemical Composition

3.3.1. Nutritional Value

The energy value and proximate composition of tomato fruit samples are presented in Table 3. The moisture ranged from 93.8 to 95.3 g/100 g fw, while significant differences were detected between the treatments. These values are within the same range of previous reports where the moisture content of different tomato varieties ranged from 92.6 to 96 g/100 g fw [68] since a strong positive correlation between the moisture of tomato fruit and the amount of irrigation water was suggested [69]. Deficit irrigation resulted in lower moisture content for all the biostimulant treatments compared to the respective treatments of FI, except for Tr3, where the opposite trend was recorded. This finding could be due to the functional equilibrium theory, according to which plants tend to accumulate assimilates in the organs that are the most active (e.g., fruit), while, at the same time, they try to increase water and nutrient uptake [70]. Moreover, the application of biostimulants decreased the moisture content of the tomato fruits except for Tr3 and Tr2 treatments in DI and FI, respectively. Similarly to our study, Petropoulos et al. [71] also observed that biostimulant application can decrease the moisture content on bean pods, especially under DI conditions.

Protein content varied among the treatments, although a positive effect for all biostimulant treatments was recorded, regardless of irrigation regime (Table 3). In particular, the highest values were recorded in Tr1 and Tr4 treatments for DI, as well as in Tr3 treatment under FI. Cao et al. [35] also reported that the application of Si increased the content of soluble protein in the roots of the tomato plants, while according to Fernandes et al. [72], DI induced protein accumulation in bean pods. The positive effect of Si application on protein content could be associated with the increased uptake of nutrients that biostimulants may cause, which eventually results in the improved biosynthesis of the assimilates [73]. Crude fat increased under FI for most of the biostimulant treatments compared to the control (except for Tr2 treatment), whereas Tr1 and Tr2 treatments resulted in significantly higher content than the control treatment under DI. According to Agbemafle et al. [69], a negative correlation between the fat content and the amount of irrigation water applied was observed, a finding which was not recorded in our study. Ash content ranged between 0.31 and 0.40 g/100 g fw, whereas Guil-Guerrero and Rebolloso-Fuentes [68] reported a higher range between 0.75 and 1.41 g/100 g fw. Moreover, high and low application rates of biostimulants (e.g., Tr1 and Tr4) resulted in increased ash content values compared to the control treatment under FI, while mid-to-low rates (e.g., Tr3 and Tr4) were the most beneficial under DI conditions. Carbohydrate content and energy values ranged between 3.8 and 5.1 g/100 g fw and 17.8 and 23.6 kcal/100 g fw (for control × RI and Tr2 × DI, respectively). Moreover, DI led to significantly increased carbohydrate content and energy value for all biostimulant treatments, except for Tr3. Similarly to our study, Agbemafle et al. [69] observed a positive effect of DI on carbohydrate content in tomato fruits, while Petropoulos et al. [71] mentioned that DI increased carbohydrates in bean pods. According to Gao et al. [74], this increase could be due to the transportation of carbohydrates from leaves to fruit as part of the osmoregulation mechanism of plants. Moreover, Jalali et al. [75] suggested that the application of high rates of Si (75 mg/L) significantly increased the total carbohydrate content of the tomato fruits, while a positive effect on the total carbohydrate content of the tomato fruits was also recorded for the combined application of Si and Se [76].

Table 3. Proximate composition and energy value of tomato fruit samples in relation to biostimulant application and irrigation regime (n = 3; mean ± SD).

	Moisture	Proteins	Crude Fat	Ash	Carbohydrates	Energy
	(g/100 g fw)	(g/100 g fw)	(g/100 g fw)	(g/100 g fw)	(g/100 g fw)	(kcal/100 g fw)
Control × DI	94.6 ± 1.1 b	0.65 ± 0.01 d	0.049 ± 0.003 cd	0.35 ± 0.02 cd	4.4 ± 0.2 c	20.4 ± 0.8 c
Tr1 × DI	93.9 ± 1.3 d	0.78 ± 0.01 a	0.052 ± 0.003 bc	0.40 ± 0.02 a	4.9 ± 0.2 ab	23.1 ± 0.8 a
Tr2 × DI	93.8 ± 1.0 d	0.72 ± 0.01 b	0.057 ± 0.004 a	0.36 ± 0.01 bc	5.1 ± 0.2 a	23.6 ± 0.8 a
Tr3 × DI	95.0 ± 1.6 a	0.70 ± 0.03 bc	0.048 ± 0.001 cde	0.32 ± 0.01 ef	3.9 ± 0.2 d	19.0 ± 0.8 d
Tr4 × DI	94.0 ± 0.8 d	0.76 ± 0.05 a	0.048 ± 0.003 de	0.38 ± 0.01 ab	4.8 ± 0.2 ab	22.7 ± 0.8 a
Control × RI	95.3 ± 1.3 a	0.57 ± 0.01 f	0.047 ± 0.001 de	0.31 ± 0.02 f	3.8 ± 0.2 d	17.8 ± 0.8 d
Tr1 × RI	94.5 ± 0.7 b	0.69 ± 0.01 c	0.054 ± 0.003 ab	0.33 ± 0.02 de	4.4 ± 0.2 c	20.9 ± 0.8 bc
Tr2 × RI	95.1 ± 1.4 a	0.60 ± 0.02 e	0.045 ± 0.003 e	0.31 ± 0.02 f	4.0 ± 0.2 d	18.6 ± 0.8 d
Tr3 × RI	94.1 ± 0.9 cd	0.76 ± 0.01 a	0.056 ± 0.004 a	0.39 ± 0.02 a	4.7 ± 0.2 bc	22.3 ± 0.8 ab
Tr4 × RI	94.4 ± 1.0 bc	0.73 ± 0.01 b	0.052 ± 0.002 bc	0.38 ± 0.01 ab	4.4 ± 0.2 c	21.1 ± 0.8 bc

Means in the same column followed by the same letter are significantly different according to Tukey's HSD test at $p < 0.05$. DI: deficit irrigation; FI: full irrigation; Tr1–4: biostimulant treatments, as described in Section 2.

3.3.2. Free Sugars and Organic Acids

The main free sugars determined in the studied tomato fruit samples are cited in Table 4. Fructose and glucose were the major compounds and ranged from 2.05 to 3.10 g/100 g fw and 0.55 to 1.12 g/100 g fw, respectively, while sucrose content was detected in lesser amounts with a content of 0.01 g/100 g fw for all the studied samples. In most cases, DI increased individual fructose and glucose content, except for Tr3 treatment, where FI resulted in higher amounts for both compounds. Moreover, the highest values of the sugars were recorded in Tr2 × DI treatment, while the lowest ones were recorded in the control × FI treatment. In previous studies, fructose content in tomato fruit ranged from 1.33 to 1.67 g/100 g fw and glucose from 1.46 to 1.78 g/100 g fw [77], while, similarly to our study, Huang et al. [78] also detected higher amounts of fructose than sucrose. In contrast, Li et al. [79] reported higher amounts of glucose than fructose; however, the authors express their data on a dry weight basis, which does not allow direct comparison with our study. These contradictory findings could be associated with differences in fruit ripening at harvest, since, according to Davies et al. [80], glucose is the predominant sugar at the beginning of fruit development but its content is significantly decreased at the onset of ripening due to transformation of glucose to fructose. Moreover, the higher content of free sugars under DI conditions could be attributed to the dilution effect of soluble solids when the plants are fully irrigated [10], as well as to osmoregulatory functions since plants tend to accumulate osmolytes such as soluble sugars under water deficits to maintain osmotic balance [20,81]. Regarding the effect of biostimulant application, Karagiannis et al. [82] reported that Si application increased the concentration of fructose and sorbitol in the flesh and peels of apple fruit and further suggested that Si may promote the synthesis of precursor compounds in sugar metabolism. On the other hand, Liava et al. [30] recorded a variable effect on soluble sugars content in processing tomato fruit when biostimulant application was combined with FI, while biostimulants did not mitigate the negative effect of DI on free sugars content. This contradiction could be due to different cropping systems (e.g., field vs. greenhouse conditions), genotype, and severity of stress.

Table 4. Free sugars and organic acids composition of tomato fruit samples in relation to biostimulant application and irrigation regime (n = 3; mean ± SD).

	Free Sugars (g/100 g fw)				Organic Acids (mg/100 g fw)				
	Fructose	**Glucose**	**Sucrose**	**Total**	**Oxalic Acid**	**Malic Acid**	**Ascorbic Acid**	**Citric Acid**	**Total**
Control × DI	2.6 ± 0.2 c	0.79 ± 0.05 c	0.0100 ± 0.0005 a	3.4 ± 0.2 d	57 ± 4 bc	468 ± 28 bc	14.4 ± 0.1 d	423 ± 16 e	962 ± 48 c
Tr1 × DI	2.85 ± 0.07 b	1.02 ± 0.01 b	0.010 ± 0.001 a	3.88 ± 0.08 b	62 ± 3 a	519 ± 19 a	15.7 ± 0.2 b	518 ± 11 a	1115 ± 34 a
Tr2 × DI	3.10 ± 0.07 a	1.12 ± 0.03 a	0.010 ± 0.001 ab	4.2 ± 0.1 a	56 ± 3 c	539 ± 30 a	17.1 ± 0.3 a	480 ± 19 bc	1092 ± 8 a
Tr3 × DI	2.3 ± 0.2 d	0.61 ± 0.03 e	0.010 ± 0.001 ab	2.9 ± 0.2 e	47 ± 3 d	422 ± 7 d	12.5 ± 0.2 g	397 ± 6 f	879 ± 17 e
Tr4 × DI	2.8 ± 0.2 b	1.03 ± 0.08 b	nd	3.8 ± 0.3 b	57 ± 2 bc	539 ± 22 a	16.0 ± 0.2 b	495 ± 22 b	1107 ± 47 a
Control × FI	2.05 ± 0.09 e	0.55 ± 0.04 f	0.010 ± 0.001 b	2.61 ± 0.05 f	42 ± 1 e	301 ± 5 f	7.4 ± 0.1 i	340 ± 8 h	690 ± 8 g
Tr1 × FI	2.78 ± 0.08 b	0.74 ± 0.05 cd	0.0100 ± 0.0004 ab	3.5 ± 0.1 cd	59 ± 4 abc	476 ± 22 bc	13.9 ± 0.4 e	459 ± 7 d	1007 ± 33 b
Tr2 × FI	2.40 ± 0.02 d	0.63 ± 0.05 e	0.010 ± 0.001 b	3.03 ± 0.07 e	44 ± 2 de	355 ± 10 e	9.3 ± 0.1 h	360 ± 6 g	769 ± 17 f
Tr3 × FI	2.91 ± 0.03 b	0.72 ± 0.05 d	0.010 ± 0.001 ab	3.63 ± 0.03 c	61 ± 3 ab	482 ± 26 b	14.7 ± 0.3 c	476 ± 7 c	1033 ± 36 b
Tr4 × FI	2.42 ± 0.04 d	0.61 ± 0.01 e	0.010 ± 0.001 ab	3.04 ± 0.05 e	59 ± 3 abc	450 ± 25 c	12.8 ± 0.1 f	401 ± 5 f	923 ± 33 d

Means in the same column followed by the same letter are significantly different according to Tukey's HSD test at $p < 0.05$. DI: deficit irrigation; FI: full irrigation; Tr1–4: biostimulant treatments, as described in Section 2; nd: not detected.

The main organic acids detected in studied fruit samples were malic acid (301 to 539 mg/100 g fw) and citric acid (340 to 518 mg/100 g fw), whereas oxalic and ascorbic acid were identified in lesser amounts (Table 4). These findings are in accordance with those of Fernandes et al. [45] and Distefano et al. [83], who also detected the same major organic acids in tomato fruits. Moreover, a varied response to the irrigation regime and biostimulant application was recorded in terms of total and individual organic acid content. However, the observed trend was similar with free sugars, where DI increased individual and total organic acid content, except for the Tr3 treatment, where FI resulted in higher amounts. Similarly, Fernandes et al. [45] suggested that DI may increase organic acid concentration in tomato fruits regardless of the biostimulant treatment, while Jin et al. [84] and Alordzinu et al. [85] suggested that the increase in organic acid content under water stress conditions is associated with the osmoregulatory and antioxidant functions of these compounds. Moreover, Jiang et al. [86] associated the increased content of ascorbic acid under water stress with the increased amounts of free sugars which are the precursors of vitamin C, while Reyes-Pérez et al. [65] reported a dose-dependent increase in vitamin C content in tomato fruits treated with various Si rates. From a nutritional point of view, organic acids may contribute to fruit taste, although oxalic acid is undesirable when consumed in high amounts since it diminishes calcium bioavailability [68]. However, considering the low values of oxalic acid found in this study, the application of biostimulants and deficit irrigation was not associated with a decrease in fruit quality. Therefore, it seems that the application of deficit irrigation not only leads to water saving and improved water productivity but also to improved organoleptic traits and taste of the tomato fruit [44,87].

3.3.3. Tocopherols and Carotenoids

Tomato fruits contained all isoforms of vitamin E, namely α-, β-, γ-, and δ-tocopherol, as presented in Table 5. The main tocopherol of the fruit samples was α-tocopherol, followed by γ-tocopherol and β-tocopherol in descending order, as mentioned previously by Pék et al. [88], while δ-tocopherol was detected only in specific samples. Quadrana et al. [89] and Saini et al. [90] also mentioned that α-tocopherol was the most abundant compound, while its content gradually increased during ripening. Moreover, Kalogeropoulos et al. [91] reported a similar profile, with α-tocopherol being the predominant compound and δ-tocopherol being detected in very low amounts both in whole fruit and byproducts of processing tomatoes. Regarding the effect of biostimulant application and irrigation regime, a significant decrease in α-tocopherol content was observed under DI compared to the control (no biostimulants added) and the respective treatments of FI. In contrast, the use of biostimulants under FI induced α-tocopherol accumulation, with the highest values being recorded in Tr1 and Tr3. In terms of β- and γ-tocopherol, a variable response to the studied factors was observed, while the highest overall values were measured in Tr3 × DI and Tr2 × FI for β- and γ-tocopherol, respectively. Moreover, under DI, the Si application significantly increased β-tocopherol regardless of the application rate, while the same trend was observed for β-tocopherol, except for the case of high Si rates (Tr1) where the lowest overall values were measured. Finally, δ-tocopherol was detected in lesser amounts and only in four samples (Tr2 × DI, control × FI, Tr1 × FI and Tr4 × FI). Total tocopherol content also increased under full irrigation, while the highest overall value was recorded for the highest rate of Si (Tr1 treatment). In previous studies, regular irrigation resulted in decreased total and individual tocopherol content [88,92], a finding which is in contrast to our study, probably due to differences in irrigation regimes and cropping systems (rainfed conditions in the field vs. DI in the greenhouse in our study). Moreover, Fernandes et al. [45] reported that FI (100% FC) increased α- and γ-tocopherol content which were the only isoforms of vitamin E detected in the tomato fruits. The same authors also suggested that biostimulant application resulted in increased tocopherol content under FI, a finding that is similar to our study. The contradictory results in the literature reports could be due to differences in cropping systems and stress severity, since excessive and prolonged stress may disrupt the protective mechanism that involves the biosynthesis of tocopherols and induce the accumulation of other antioxidant compounds [72].

Table 5. Tocopherols and carotenoids composition of tomato fruit samples in relation to biostimulant application and irrigation regime (n = 3; mean ± SD).

	Tocopherols (μg/100 g fw)					**Carotenoids (μg/100 g fw)**	
	α-Tocopherol	**β-Tocopherol**	**γ-Tocopherol**	**δ-Tocopherol**	**Total**	**Lycopene**	**β-Carotene**
Control × DI	206 ± 6 d	9.3 ± 0.6 h	84 ± 1 f	nd	299 ± 7 e	533 ± 14 h	401 ± 14 e
Tr1 × DI	192 ± 2 e	19.6 ±0.5 f	75 ± 3 g	nd	287 ± 6 e	607 ± 8 g	450 ± 9 d
Tr2 × DI	220 ± 6 c	29 ± 1 d	96 ± 4 cd	5.3 ± 0.3 b	351 ± 12 c	632 ± 5 f	491 ± 7 c
Tr3 × DI	188 ± 7 e	39 ± 2 a	92 ± 2 e	nd	318 ± 11 d	734 ± 8 d	515 ± 8 c
Tr4 × DI	197 ± 4 e	27 ± 1 de	94 ± 2 de	nd	318 ± 7 d	496 ± 6 i	387 ± 6 e
Control × FI	208 ± 5 d	31 ± 2 c	103 ± 1 b	5.0 ± 0.2 c	347 ± 9 c	941 ± 9 b	609 ± 22 b
Tr1 × FI	273 ± 6 a	37 ± 2 b	105 ± 1 b	4.8 ± 0.3 d	419 ± 9 a	624 ± 15 fg	456 ± 23 d
Tr2 × FI	230 ± 3 b	11 ± 2 g	114 ± 2 a	nd	355 ± 6 c	841 ± 11 c	521 ± 14 c
Tr3 × FI	269 ± 15 a	27.3 ±0.4 de	98 ± 4 c	nd	395 ± 19 b	714 ± 9 e	491 ± 21 c
Tr4 × FI	211 ± 8 d	26 ± 2 e	84 ± 2 f	6.4 ± 0.3 a	328 ± 11 d	1000 ± 6 a	650 ± 8 a

Means in the same column followed by the same letter are significantly different according to Tukey's HSD test at $p < 0.05$. DI: deficit irrigation; FI: full irrigation; Tr1–4: biostimulant treatments, as described in Section 2; nd: not detected.

Lycopene and β-carotene were the main carotenoids identified in our study, while a varied response to the tested factors was recorded (Table 5). Lycopene was the main carotenoid with values that ranged from 496 to 1000 μg/100 g fw for Tr4 × DI and Tr4 × FI, respectively. On the other hand, β-carotene content ranged between 387 and 650 μg/100 g fw (Tr4 × DI and Tr4 × FI, respectively). According to the literature reports, the effect of the irrigation regime on carotenoid content was not as expected, since Lahoz et al. [87] and Takács et al. [93] observed an increase in lycopene concentration under deficit irrigation, whereas Jiang et al. [86] mentioned that deficit irrigation could reduce the accumulation of lycopene. The later authors attributed this reduction to the stage when deficit irrigation was applied (at flowering or fruit development) and the severity of water shortage, both of which may significantly impact fruit quality. Moreover, Patel et al. [19] suggested that the reduction in carotenoid content could be associated with the overproduction of ROS under drought conditions at levels that the antioxidant defense mechanisms of plants cannot cope with. The effect of biostimulant application varied, although moderate to high Si rates (Tr1, Tr2, and Tr3) resulted in increased content of pigments under deficit irrigation compared to the control treatment. In contrast to this finding, Francesca et al. [94] reported that the application of protein hydrolysates did not increase carotenoids and lycopene content under deficit irrigation compared to untreated plants (no biostimulants added), whereas similar or higher levels of pigments were recorded compared to fully irrigated plants that did not receive biostimulants. Moreover, Hu et al. [76] indicated the beneficial effects of Si and Se on carotenoids in tomato plants grown under deficit irrigation. The Si application rate may also have an impact on carotenoid content, since, according to Wang et al. [95], low to moderate Si rates increase the expression of genes related to carotenoid biosynthesis.

3.3.4. Fatty Acids

The main fatty acids detected in tomato fruit are presented in Table 6. The most abundant compounds were palmitic acid (C16:0; values ranged from 34.0% (Tr3 × FI) to 41.0% (Tr3 × DI)) followed by linoleic acid (C18:2n6; 32.0% (Tr2 × FI) to 37.0% (control × FI)). Moreover, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), and monounsaturated fatty acids (MUFA) ranged from 45.0% to 55.0%, 38.0% to 49.0%, and 3.9% to 10.0%, respectively, while the PUFA/SFA and n6/n3 ratios ranged between 0.72 and 1.10 and 2.21 to 2.70, respectively. A variable response to the irrigation regime and biostimulant application was observed, where increasing or decreasing trends for specific compounds were recorded depending on the irrigation and biostimulant treatments. These results are in accordance with previous reports where the main fatty acids of table tomato fruit were palmitic and linoleic acid [45], while Liava et al. [30] detected higher amounts of linoleic than palmitic acid in processing tomato fruit Moreover, a variable effect of drought stress on fatty acid composition has been reported both in processing and table tomato fruits [30,45], as well as in black cumin, where a significant increase in SFA and PUFA was detected [96], in cottonseed oil, where unsaturated fatty acids content decreased [97], and in common beans, where no clear trends were suggested [72]. These findings indicate the complexity of the effects of the biostimulant and irrigation regimes on fatty acid composition since several factors are involved, including the species and the cultivar, the cropping system, the severity of stress, and the growing conditions which regulate the role of fatty acids as protective compounds against stressors or as precursors of other bioactive compounds [98].

Table 6. The main fatty acids composition of tomato fruit samples in relation to biostimulant application and irrigation regime (n = 3; mean ± SD).

	Fatty Acids									Categories				
	C14:0	**C16:0**	**C18:0**	**C18:1n9**	**C18:2n6**	**C18:3n3**	**C20:0**	**C23:0**	**C24:0**	**SFA**	**MUFA**	**PUFA**	**PUFA/SFA**	**n6/n3**
Control × DI	0.98 ± 0.02 e	36 ± 1 cde	5.4 ± 0.4 a	2.9 ± 0.1 f	33.3 ± 0.4 cd	13 ± 1 bc	1.35 ± 0.04 b	0.64 ± 0.05 c	2.2 ± 0.1 bc	49 ± 1 cd	3.9 ± 0.2 g	47 ± 1 abc	0.94 ± 0.03 cd	2.6 ± 0.1 ab
Tr1 × DI	1.16 ± 0.07 cd	36 ± 2 cde	5.3 ± 0.2 ab	3.2 ± 0.1 de	33.3 ± 0.6 cd	14 ± 1 b	1.31 ± 0.04 b	0.63 ± 0.03 cd	1.79 ± 0.06 d	48 ± 2 cde	4.28 ± 0.03 f	47 ± 2 abc	0.98 ± 0.05 bc	2.4 ± 0.1 c
Tr2 × DI	1.20 ± 0.04 c	35 ± 1 de	5.0 ± 0.4 c	3.2 ± 0.1 de	34 ± 2 bc	14 ± 1 b	1.06 ± 0.03 b	0.55 ± 0.04 e	2.5 ± 0.2 a	47 ± 1 e	4.5 ± 0.1 def	48 ± 1 ab	1.02 ± 0.03 b	2.5 ± 0.2 bc
Tr3 × DI	1.36 ± 0.07 b	41 ± 2 a	5.5 ± 0.1 a	3.6 ± 0.2 d	34 ± 1 bc	nd	1.33 ± 0.04 b	0.59 ± 0.02 de	2.11 ± 0.05 c	55 ± 2 a	6.1 ± 0.2 c	39 ± 1 e	0.72 ± 0.03 e	-
Tr4 × DI	1.43 ± 0.07 b	38 ± 2 bc	4.6 ± 0.1 d	3.5 ± 0.1 d	33 ± 2 cd	12.0 ± 0.1 c	1.11 ± 0.05 b	0.68 ± 0.04 b	2.3 ± 0.2 b	50 ± 2 c	4.67 ± 0.01 de	46 ± 2 cd	0.92 ± 0.05 cd	2.7 ± 0.1 a
Control × FI	1.10 ± 0.01 d	37 ± 1 bcd	nd	5.4 ± 0.4 b	37 ± 1 a	nd	13 ± 1 a	0.69 ± 0.06 b	nd	52 ±2 b	10.0 ± 0.7 a	38 ± 1 e	0.72 ± 0.03 e	-
Tr1 × FI	1.2 ± 0.1 c	38 ± 2 bc	5.1 ± 0.2 bc	3.1 ± 0.2 ef	33 ± 1 cd	13 ± 1 bc	1.12 ± 0.04 b	0.45 ± 0.04 f	1.73 ± 0.04 d	49 ± 2 cd	4.5 ± 0.1 def	46 ± 2 cd	0.94 ± 0.05 cd	2.60 ± 0.04 ab
Tr2 × FI	1.5 ± 0.1 a	38 ± 1 bc	4.4 ± 0.1 d	4.1 ± 0.3 c	32 ± 1 d	13 ± 1 bc	1.1 ± 0.1 b	0.90 ± 0.01 a	2.4 ± 0.1 a	50 ± 1 c	4.9 ± 0.3 d	45 ± 1 d	0.89 ± 0.03 d	2.49 ± 0.05 bc
Tr3 × FI	1.17 ± 0.01 cd	34 ± 1 e	5.4 ± 0.1 a	6.5 ± 0.3 a	34 ± 1 bc	15 ± 1 a	1.3 ± 0.1 b	0.60 ± 0.04 de	1.8 ± 0.1 d	45 ± 1 f	6.5 ± 0.3 b	49 ± 2 a	1.10 ± 0.05 a	2.21 ± 0.03 d
Tr4 × FI	0.97 ± 0.04 e	37 ± 2 bcd	5.5 ± 0.2 a	4.2 ± 0.1 c	35 ± 1 b	13 ± 1 bc	nd	0.54 ± 0.04 e	2.22 ± 0.03 bc	47 ± 2 de	4.20 ± 0.04 f	48 ± 2 ab	1.02 ± 0.06 b	2.66 ± 0.09 a

Means in the same column followed by the same letter are significantly different according to Tukey's HSD test at $p < 0.05$. DI: deficit irrigation; FI: full irrigation; Tr1–4: biostimulant treatments, as described in Section 2; nd: not detected.

3.4. Bioactive Properties

Total Phenolic and Flavonoid Contents and Antioxidant Activity

The total phenolic and flavonoid contents of tomato fruits are presented in Table 7. The general tendency showed that biostimulant application decreased the total phenolic content in both irrigation regimes, while the highest and lowest values were recorded in control × FI and Tr1 × DI treatments, respectively. In terms of total flavonoid content, the biostimulant application had a positive effect under DI and the opposite trend was indicated under FI, while the highest and lowest values were recorded in Tr2 × DI control × DI treatments, respectively. Similarly to our study, Fernandes et al. [72] and Liava et al. [30] also suggested that the content of total phenolic compounds in beans and tomato fruits, respectively, increased under DI conditions. Moreover, Coyago-Cruz et al. [66], who evaluated the effect of regulated DI (at 25% and 50% of FI) on the chemical composition of black tomato fruit, reported a significant decrease in the content of total phenolic compounds compared to the control treatment, regardless of the DI level, while Barbgalio et al. [99] also suggested an increase in total phenolic compounds and no effects on flavonoid content in tomato fruit grown in the field under FI and no irrigation (rain-fed plants). Pernice et al. [100] and Tallarita et al. [101] suggested a compound-specific effect of DI on phenolic compounds in tomatoes, while the latter authors reported that the severity of stress may also differentiate the response of total phenols and total flavonoid content. In the same vein, Bogale et al. [102] recorded increasing trends in total phenolic compounds of the fruit of two tomato cultivars under DI and partial root zone drying, both at 50% of FI. Finally, Pék et al. [88] reported that rain-fed conditions resulted in an increased content of flavonoids, phenolic acids, and total phenolics in tomato fruits, regardless of the sampling date. Regarding biostimulant application, Kalisz et al. [103] observed that the application of Si nanoparticles decreased the total phenolic compounds and flavonoid contents of lettuce by 17% and 14%, respectively, a finding which was also recorded in our study for total phenolic compounds content under both irrigation regimes, as well as for total flavonoids content under FI. The same authors mentioned that this negative effect is due to changes in certain metabolic pathways, and plant genotype may result in a variable response to stress conditions [103]. On the other hand, Reyes-Pérez et al. [65] suggested that increasing rates of Si directly applied to the soil resulted in a significant increase in both phenolic compounds and flavonoids, a finding which is in contrast with our study. Xu et al. [104] also indicated that the application method of Si (root or foliar) may also have an impact on antioxidant enzymes and non-enzymatic antioxidants such as polyphenols. Therefore, the contradictory results among the literature reports suggest that several other factors, such as the growing conditions, the irrigation regime, the genotype, the water stress level, as well as the silica form and the application method, may be involved in the induction of polyphenols biosynthetic pathways [100].

The antioxidant activity was assessed with TBARS and OxHLIA and the results are presented in Table 7. Regarding the TBARS assay, the lowest values and, therefore, the highest antioxidant activity were recorded under DI for the control, Tr3, and Tr4 treatments, while the control treatment of FI also showed promising results. Moreover, biostimulant application had a negative impact on antioxidant activity under FI compared to the control treatment, while no clear trends were recorded for biostimulant application under DI. In contrast, FI resulted in significantly higher antioxidant activity for the OxHLIA assay compared to DI, regardless of Si application rate, while the highest activity was measured for Tr4 treatment without being significantly different from the control and Tr1 (all under FI). However, in all the studied samples, the antioxidant activity for both assays was lower than Trolox, which was used as a positive control. These findings are in accordance with the results of Fernandes et al. [45] who also observed a variable response of the tomato fruits to different assays. Moreover, Pinedo-Guerrero et al. [105] suggested that the application of Si nanoparticles (K_2SiO_3 and SiO_2) increased the antioxidant activity for ABTS assay in tomato leaves either under NaCl stress or no stress, whereas only K_2SiO_3 increased antioxidant activity compared to the control treatment in the DPPH assay under NaCl stress. The varied response between the different antioxidant activity assays could be attributed to several bioactive compounds such as lycopene, β-carotene, α-tocopherol, organic and fatty acids, and polyphenols, which contribute to the overall antioxidant activity of tomato samples with a different mechanism owing to their lipophilic and hydrophilic nature [30,68,101].

Table 7. Total phenolic and flavonoid contents and the antioxidant activity of the tomato fruit samples in relation to biostimulant application and irrigation regime (n = 3; mean ± SD).

	Total Phenolics	Total Flavonoids	TBARS	OxHLIA
	(mg GAE/g Extract)	(mg QE/g Extract)	(EC_{50}, μg/mL)	(IC_{50}, μg/mL)
Control × DI	16.6 ± 0.8 f	2.14 ± 0.03 g	315 ± 6 f	397 ± 16 b
Tr1 × DI	12.5 ± 0.5 i	2.7 ± 0.1 c	434 ± 19 d	783 ± 26 a
Tr2 × DI	15.3 ± 0.6 gh	2.9 ± 0.1 a	553 ± 20 c	181 ± 12 c
Tr3 × DI	15.7 ± 0.2 g	2.3 ± 0.1 f	322 ± 2 f	376 ± 26 b
Tr4 × DI	14.8 ± 0.2 h	2.9 ± 0.1 b	312 ± 2 f	115 ± 5 d
Control × FI	21.4 ± 0.3 a	2.7 ± 0.1 c	339 ± 11 ef	69 ± 4 ef
Tr1 × FI	17.6 ± 0.3 e	2.3 ± 0.1 f	697 ± 27 a	64 ± 4 f
Tr2 × FI	20.0 ± 0.3 b	2.3 ± 0.1 f	598 ± 24 b	160 ± 10 c
Tr3 × FI	18.1 ± 0.8 d	2.5 ± 0.1 e	354 ± 9 e	97 ± 6 de
Tr4 × FI	19.3 ± 0.3 c	2.6 ± 0.1 d	549 ± 10 c	46 ± 2 f
Trolox	-	-	5.4 ± 0.3	21.8 ± 0.3

Means in the same column followed by the same letter are significantly different according to Tukey's HSD test at $p < 0.05$. DI: deficit irrigation; FI: full irrigation; Tr1–4: biostimulant treatments, as described in Section 2.

4. Conclusions

Our results indicate the significant impact of irrigation regime and Si application rate on the yield and chemical composition parameters of tomato fruits. The combined application of Si and deficit irrigation significantly increased carbohydrates, energy, fructose, glucose, malic acid, ascorbic acid, and citric acid, especially for the Tr2 treatment. Tocopherols (α-, β-, and γ-tocopherol) and carotenoids showed a variable response in the tested factors, although the lowest rates of biostimulants enhanced the accumulation of lycopene and β-carotene, regardless of the irrigation regime. Interestingly, total phenolic compound content decreased in the combined application of deficit irrigation and biostimulants, whereas the opposite trend was recorded for flavonoid content under full irrigation and biostimulant application. Finally, the irrigation regime and biostimulant application rate did not have a clear impact on the antioxidant activity of tomato fruits, showing a variable response depending on the assay. In conclusion, despite the variable effect on quality traits, the application of the studied Si-based biostimulant showed promising results when combined with deficit irrigation, thus it could be suggested as an eco-friendly and sustainable agronomic practice aiming to save water resources while maintaining fruit quality and minimizing yield penalties in greenhouse tomato cultivation due to water stress. However, further research is needed including more genotypes to evaluate the response of various cultivars to Si application under deficit irrigation, while of particular interest is the comparison of cultivars with contrasting responses to drought stress (e.g., drought stress resistant and sensitive genotypes) to reveal the actual mechanisms of Si effects.

Author Contributions: Conceptualization, L.B. and S.A.P.; methodology, M.A.-O., A.P., J.P., V.L., C.C. and A.A.A.; validation, M.A.-O., A.P. and J.P.; formal analysis, M.A.-O., A.P., J.P., V.L. and C.C.; investigation, M.A.-O., A.P., J.P., V.L. and C.C.; resources, L.B. and S.A.P.; data curation, M.A.-O.,

A.P., J.P., V.L., C.C. and A.A.A.; writing—original draft preparation, J.P., V.L., A.A.A. and S.A.P.; writing—review and editing, L.B. and S.A.P.; visualization, L.B. and S.A.P.; supervision, L.B. and S.A.P.; project administration, S.A.P.; funding acquisition, S.A.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH—CREATE—INNOVATE (project code: T2EDK-05281).

Data Availability Statement: All data are included in the manuscript.

Acknowledgments: The authors would like to thank the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC): CIMO UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020), SusTEC LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020), and National funding by FCT through the scientific employment program-contract with L.B. (DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002) and the individual research grant (DOI: 10.54499/2020.06297.BD) of M.A.O.

Conflicts of Interest: The authors declare no conflicts of interest.

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