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Article

Nano ZnO and Bioinoculants Mitigates Effects of Deficit Irrigation on Nutritional Quality of Green Peppers

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Abstract: Green peppers (*Capsicum annuum* L.) is a fruit vegetable with great culinary versatility, and present important nutritional properties for human health. Water deficit negatively affects nutritional quality of green peppers fruits. This study aimed to investigate the influence of zinc oxide nanoparticles (ZnONPs), associated with plant growth-promoting bacteria (PGPB), on the post-harvest quality nutritional of green peppers subjected to water deficit. In an open-field experiment, two irrigation levels (50 and 100% of crop evapotranspiration - Etc), four treatments composed by combination of ZnONPs, zinc sulfate (ZnSO₄) and PGPB (T1 = ZnSO₄, T2 = foliar ZnONPs, T3 = foliar ZnONPs + PGPB, T4 = ZnSO₄ via soil + PGPB via soil) and a control treatment (Check) were tested. Water deficit or water deficit mitigation treatments did not interfere with the physical-chemical parameters (except vitamin C content) and physical color parameters (except L*) of green pepper. The water deficit reduced the levels of Ca, Mg, P, K, Mn, Fe, B, and Zn in the fruits. The foliar application of ZnONPs in association with PGPB provided better nutritional quality of green pepper in terms of vitamin C and mineral nutrients. Under water deficit, ZnONPs or ZnSO₄ via foliar, associated or not with PGPB, increased the levels of Ca, P, Mg, and Fe in the fruits. Applying Zn via foliar as ZnONPs or ZnSO₄ mitigated the negative effects of water deficit on the quality of pepper fruits that was enhanced by the *Bacillus subtilis* and *B. amyloliquefaciens* inoculation.

Keywords: *Capsicum annuum*; drought stress; fruit quality; mineral nutrients

1. Introduction

Fruit vegetables such as peppers (*Capsicum annuum* L.) are important sources of vitamins and mineral nutrients in the human diet [1,2]. In Brazil, green peppers are among the ten most economically important vegetables, which is predominantly sold *in natura* [3]. In the semi-arid region of Brazil, green peppers are widely cultivated, however, water scarcity generally promotes a decrease in fruit productivity, which can also affect their nutritional quality [4].

Water deficit negatively affects the production and quality of vegetables such as tomatoes and peppers, especially during flowering, fruiting and fruit maturation [5,6]. In some situations, water stress can increase the concentration of soluble solids, titratable acidity and vitamin C, but decreases others quality parameters, such as fruit size, firmness and mineral elements content [6]. The water deficit decreases fruit yield, translocation of photoassimilates and content of minerals such as calcium, potassium, magnesium and iron to the fruits [5]. The decrease in mineral nutrients in fruits also occurs due to the decrease in the diffusion process and mass flow that contribute to the largest portion of the amount of nutrients that reach the roots, and subsequently their redistribution to the fruits [7].

The foliar application of zinc, as zinc oxide nanoparticles (ZnONPs) in some plant species, including fruit vegetables, can contribute to mitigates the negative effects of environmental stresses such as water deficit and saline stress on the plants and improve some aspects of the physical-chemical quality of fruits [8–13]. Due to their high specific surface, the main mode of action of ZnONPs would be through the stimulating effect on plant metabolism, through their interaction with biomolecules, promoting an increase in enzymatic activity and the synthesis of substances that act to mitigates oxidative stress in plants [14,15].

ZnONPs can also act as an efficient source of zinc (Zn) for plants, with slower release than conventional sources, such as zinc sulfate [8,16]. In plants, zinc is involved in the synthesis of several hormones, such as gibberellin, auxin, cytokinin and abscisic acid [17]. In addition, Zn to participate on the synthesis of chlorophyll, the development of chloroplasts and the integrity of the cell membrane, providing protection, stability and structure [17,18]. Furthermore, zinc plays a crucial role in fruiting, seed growth and fruit maturation, increasing resistance to environmental stress, acting as a co-enzyme in enzymatic processes related to protein synthesis, glucose metabolism and DNA synthesis, being also essential for the production of auxins, which regulate root growth, cell expansion and the beginning of flowering [19–21].

Under water stress, several plant species can be favored by the presence of plant growth-promoting bacteria (PGPB) in the rhizosphere region [22–26]. Indirectly, PGPB can alleviate the effects of oxidative stress caused by drought, through the stimulation of enzymes from the antioxidant system such as catalase, superoxide dismutase and peroxidase [27–30]. The plant inoculation with *B. amyloliquefaciens* improved the survival of pepper plants under different stress conditions, resulting in a higher seedling growth rate and better physicochemical characteristics [31]. In the study conducted by [32], it was noted that the application of *B. amyloliquefaciens* to corn resulted in an increase in nutrient absorption and stimulated plant growth mechanisms, reflected in higher concentrations of amino acids, such as tryptophan, isoleucine, alanine, valine, tyrosine, and sugars, such as fructose and glucose. Similarly, for blueberry plants the inoculating the soil with different strains of *B. amyloliquefaciens* and *B. subtilis* increased the foliar chlorophyll content, nutrient absorption and increases the levels of soluble solids, vitamin C and anthocyanins in the fruits [33].

In the present study, two main hypotheses were considered. The first hypothesis is that water deficit alters the physical-chemical quality and mineral composition of green pepper fruits. The second hypothesis is that zinc oxide nanoparticles (ZnONPs) applied via leaves, associated or not with PGPB could mitigate the negative effects of water deficit on physicochemical attributes of quality and mineral composition of green pepper fruits, and that ZnONPs would be more efficient than the conventional source of zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$).

The objective of this work was to evaluate the effect of applying zinc oxide nanoparticles, associated or not with PGPB, on some physical-chemical parameters and mineral composition of green pepper fruits, from plants grown under two irrigation levels.

2. Materials and Methods

2.1. Characterization of the Experimental Area

The present research was carried out under field conditions, from December 2022 to April 2023, in the Experimental Farm area of the Center for Agro-food Science and Technology at the Federal University of Campina Grande (UFCG), Pombal-PB Campus. The Experimental Farm is located in

the municipality of São Domingos-PB. According to the Köppen classification, the predominant climate is type Aw, hot and humid. Annual rainfall is around 800 mm and the temperature range is always below 5°C. The Experimental Farm is located at an altitude of 190 m, at the following coordinates: Latitude 6° 50' 4" South, Longitude 37° 53' 9" West. According to Gaussen's classification, the Mediterranean type bioclimate, also known as medium-dry northeastern, prevails, with a dry season that lasts between 4 and 6 months.

The preparation of the area consisted of plowing and harrowing with a plow harrow. After this stage, soil sampling was carried out in the area, in the 0 – 20 cm layer for its chemical and physical characterization (Table 1) according to the methodology described in Embrapa [34].

Table 1. Chemical and physical attributes of the soil used in the experiment.

Chemical	Value	Physical	Value
pH (CaCl ₂)	6.20	Sand (g kg ⁻¹)	444
P (mg kg ⁻¹)	291	Silt (g kg ⁻¹)	353
K ⁺ (cmol _c dm ⁻³)	1.19	Clay (g kg ⁻¹)	203
Na ⁺ (cmol _c dm ⁻³)	0.54	BD (g cm ⁻³)	1.36
Ca ²⁺ (cmol _c dm ⁻³)	5.80	PD (g cm ⁻³)	2.59
Mg ²⁺ (cmol _c dm ⁻³)	3.40	TP (m ³ m ⁻³)	0.47
Zn ²⁺ (cmol _c dm ⁻³)	1.69	FC (%)	12.87
H + Al (cmol _c dm ⁻³)	2.30	PWP (%)	5.29
OM (g kg ⁻¹)	6.40	AWC (%)	7.58
V (%)	83.0		
CEC (%)	4.10	-	-

P, K⁺, Na⁺ and Zn²⁺ - Mehlich 1 extractant, H⁺ + Al³⁺ - 0.5 mol L⁻¹ calcium acetate extractant at pH 7, Ca²⁺, Mg²⁺ - 1 mol L⁻¹ KCl extractant, OM - organic matter, V - Base saturation, CEC - cationic exchange capacity, BD - bulk density, PD - soil particle density, TP - total porosity, FC - field capacity, PWP - permanent wilting point, AWC - available water content.

During the experiment period, climatological data on temperature, relative humidity and precipitation (Figure 1) were collected through the Agritempo website [35].

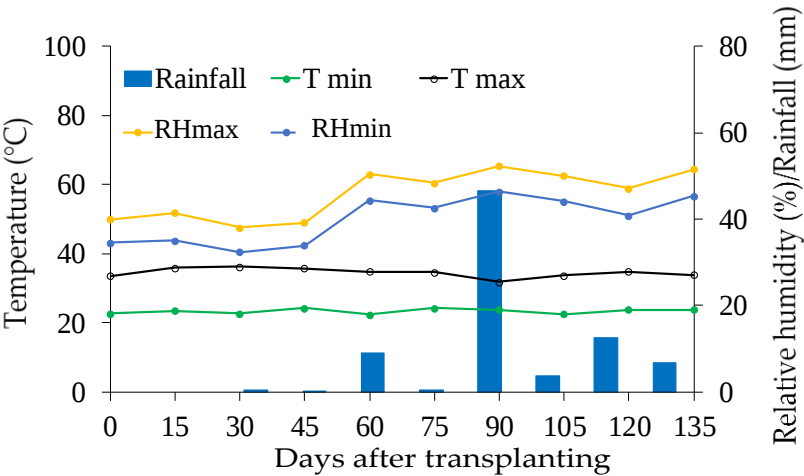


Figure 1. Climatological data on maximum (T max) and minimum (T min) air, maximum (RHmax) and minimum (RHmin) air relative humidity and rainfall during the experimental period in the experiment area.

2.2. Treatments and Experimental Design

The experiment was carried out in a randomized block design, in a split-plot scheme, where the plots were two irrigation levels (50 and 100% of crop evapotranspiration - Etc) and the subplots were four water deficit irrigation mitigation treatments (DHA) composed from nanoparticles of zinc oxide (NPsZnO), zinc sulfate (ZnSO_4) and bioinoculants (PGPB) (T1 = ZnSO_4 , T2 = foliar NPsZnO, T3 = foliar NPsZnO + Bio, T4 = ZnSO_4 via soil + PGPB or via soil), and check treatment, with four blocks, totaling 40 experimental plots.

The total area of the experiment corresponded to 22.8 m in length by 14.8 m in width (Appendix A1). The plants were grown at a spacing of 1.2 m between rows and 0.5 m between plants in the row. Each subplot consisted of four 3.0 m long rows in which 20 plants were grown. The useful plot consisted of an area measuring 3.0 x 2.0 m in the central part of the plot, where 6 plants were evaluated. When installing the experiment, there was a space of 1.2 m between subplots and 2.0 m between blocks (Appendix A1).

2.3. Seedlings Transplanting and Soil Fertilization

Pepper seedlings (*Capsicum annuum* L.) cv. 'Kolima' were produced in expanded polystyrene trays (Isopor®), filled with coconut fiber as a substrate. The transplant was carried out when the seedlings had 4 to 6 true leaves. The seedlings were transplanted in ridges measuring 0.4 m wide and 0.30 m high. Based on the soil analysis (Table 1), planting and supplementary fertilization were carried out, carried out according to the recommendations of the Fertilization Recommendation Manual for the State of Pernambuco [36]. For planting fertilization, the formula NPK 10-10-10 (10%N, 10% P_2O_5 and 10% K_2O) was applied at a dose of 150 g per row, in a single dose and incorporated into the soil when lifting the planting rows. Nitrogen and potassium fertilizations were carried out via fertigation, according to the technical recommendations for the crop, with the first application being made with 39.2 kg ha^{-1} of urea and 15.8 kg ha^{-1} of KCl carried out at 15 days after transplanting (DAT), the second with 39.2 kg ha^{-1} of urea and 15.8 kg ha^{-1} of KCl at 30 days after transplantation, the third with 39.2 kg ha^{-1} of urea and 15.8 kg ha^{-1} of KCl at 45 DAT and the fourth with 39.2 kg ha^{-1} of urea and 15.8 kg ha^{-1} of KCl at 60 DAT.

2.4. Application of Treatments

As ZnONPs, the product nano-zinc oxide (NZnO, P.A.) from the Sigma-Aldrich® brand was used, which has 97% purity, particle size smaller than 100 nm and specific surface area of 10.8 m^2g^{-1} . NPsZnO suspensions and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (P.A) solution (referred in the text only ZnSO_4) were prepared at concentrations of 0.125 g L^{-1} (100 mg Zn L^{-1}) and 4.54 g L^{-1} (1,000 mg Zn L^{-1}), respectively. The treatments referring to bioinoculation (PGPB) consisted of the combination of two commercial products, one containing *Bacillus subtilis* BV-09 at a dosage of 1.0×10^8 CFU mL^{-1} and the other containing *B. amyloliquefaciens* at a dosage of 1.0×10^9 CFU mL^{-1} . Each product was applied at a rate of 3 L ha^{-1} , totaling 6 L ha^{-1} . At the time of application, 45 mL of each PGPB source were mixed with 10 L of water.

The foliar application of the suspensions ZnONPs or ZnSO_4 solution for each treatment (T1, T2 and T3) was carried out with the aid of a knapsack sprayer with a capacity of 20 liters, with an average of 35 mL applied per plant. Neutral detergent it was used as adjuvant in the proportion of 1 mL L^{-1} in all foliar treatments. Regarding soil application, the treatments were applied close to the root zone of the plants using a beaker. For the T4 treatment, 50 mL of the ZnSO_4 solution was applied per plant, corresponding to a dose of 2.42 kg of ZnSO_4 per hectare. The mixture of bioinoculants was applied around 30 mL per plant. The treatments were applied 25 days after transplanting the seedlings, after homogenizing the plants in the field plots.

2.5. Irrigation Levels Application

After transplanting, the plants were irrigated by drip, with drippers spaced 0.20 m apart and with a nominal flow of 1.6 L h^{-1} . After emergence and standardization of the number and size of

plants in the subplots, the plants were irrigated following different levels of water depth, which ran 32 days after transplanting.

The total irrigation required (TIR) was calculated using the following expression:

$$TIR = [(FC - WP) \times Z \times BD \times f] / 10$$

On what:

TIR - corresponds to the initial total depth of water applied in mm;

FC – Soil moisture corresponding to field capacity, %;

WP – Soil moisture corresponding to wilting point, %;

Z - Effective depth of the pepper root system (30 cm);

BD – bulk density, g cm⁻³;

f – water availability factor (0.5).

The water irrigation uniformity test was carried out according to the Christiansen Uniformity Coefficient (CUC) evaluation methodology, proposed by Christiansen [37].

Control of the volume of water supplied to each irrigation level was carried out daily, at a standardized time, according to the ratio of the flow rate of the drippers to the time to reach the reference evapotranspiration proportions (ET_o). As the time interval for each volume of the respective irrigation level was reached, the drip tapes corresponding to each irrigation level were successively disconnected. The irrigation depth corresponding to 100% of ET_c was obtained by calculating ET_c, according to the expression from Jensen [38].

$$ET_c = K_c \times ET_o$$

On what:

ET_c - Crop evapotranspiration, mm day⁻¹;

ET_o - Reference evaporation, mm day⁻¹;

K_c - Crop coefficient (dimensionless).

The K_c values adopted for each crop depending on their phenological phases, and the daily ET_o values were obtained according to the FAO Penman-Monteith model [39]. During the experiment, climatological data were obtained from the automatic meteorological station in the municipality of São Gonçalo, Paraíba, as it is the closest to the experiment site, through the AgriTempo platform [35].

The daily supply of irrigation depth was carried out through the irrigation time considering the characteristics of the cultivation system and the irrigation system according to the following expression:

$$Ti = \frac{Eto \times Kc \times A}{Ea \times n \times q}$$

Where:

Ti – Irrigation time, h;

Eto – Reference evaporation, mm day⁻¹;

Kc – Crop coefficient, dimensionless;

A – Area occupied by one plant, m²;

Ea - Application efficiency (0.90);

n - Number of emitters per plant; and

q - Emitter flow rate, L h⁻¹.

2.6. Cultivation Practices and Phytosanitary Control

To avoid any interference from phytosanitary chemicals on the action of products containing PGPB, weed and pests control was carried out mechanically, manually and, or with the use of hand hoes. During experimental period, not diseases were detected. In addition, horizontal staking was carried out using wooden stakes measuring an average of 1.60 m in length. The stakes were spread across the lines, being placed every 5 meters. The ribbons were tied to the poles before the fruiting period to prevent the plants from tipping over due to the weight of the fruits.

2.7. Variables Evaluated

2.7.1. Physicochemical Analysis

At the time of the first fruit harvest, which occurred 70 days after transplanting, physical attributes of color, ascorbic acid (vitamin C), pH, soluble solids (SS), titratable acidity (TA) and SS/TA ratio was evaluated.

The physical attributes of fruit color were measured using a Konica Minolta CR-400™ digital colorimeter, using the CIELAB system, which defines a three-dimensional color space with three axes in rectangular coordinates (L^* , a^* , b^*). These coordinates represent lightness (L^*), tones from red to green (a^* positive to $-a^*$ negative) and tones from yellow to blue (b^* positive to $-b^*$ negative). Furthermore, it defines cylindrical coordinates (L^* , C^* , H°), and the a^* and b^* values were converted into Hue angle (H°), representing the intensity of the color, and Chroma (C^*), indicating the purity of the color, according to [40]. The analysis was carried out outside, with one reading per fruit for each experimental plot.

The pH was measured using a digital benchtop pH meter, with direct readings taken from the homogenized pulp, according to [41]. The determination of vitamin C was carried out using the Tillman method. The results of the analyzes were expressed in mg per 100 g of the sample [42]. Titratable acidity was determined following the methodology recommended by the [42]. The results were expressed in g of citric acid per 100 g of the sample. Soluble solids content was determined directly in the homogenized pulp using a digital refractometer (model PR-100, Palette, Atago Co., LTD., Japan). The results were expressed in °Brix [42]. The SS/TA ratio was calculated as the ratio between soluble solids and titratable acidity (SS/TA).

2.7.2. Mineral Nutrient Content

Six commercial fruits were separated from each subplot to take a sample consisting of 100g of fresh fruit mass. These samples were dried in a forced air oven (65-70 °C) until constant weight, to later obtain the dry mass and water content. Subsequently, this material was crushed in a Wiley-type mill and then sulfuric digestion, according to the methodology described in [43]. Digestion was obtained in a Kjeldahl microdigester block, using samples of 0.2g of dry plant material in 3 mL of concentrated sulfuric acid and 1 mL of hydrogen peroxide, with an initial temperature of 180°C and a final temperature of 360°C. In the sulfuric digestion stratum, the levels of the macronutrients calcium (Ca), potassium (K), phosphorus (P) and magnesium (Mg) and the micronutrients boron (B), manganese (Mn), iron (Fe) and zinc were determined by using of the ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) technique. The element contents obtained in terms of dry mass content were converted to fresh mass contents, correcting the values by the water content of the fruits.

2.7.3. Overall quality Index

A general fruit quality index (FQI) was generated with physical-chemical variables, physical color variables and macro and micronutrient content in the fruits. Initially, all values of the studied variables (X) were converted into percentages ($X_i\%$) using the following expression:

$$qi = \frac{X_t}{X_c} \cdot 100$$

Where q_i represents the value of a quality attribute X originating from treatment t and X_c represents the average value of attribute x obtained in the control treatment (check).

FQI values were obtained according to [44], by using the geometric mean of the physical-chemical attributes and the mineral nutrient contents evaluated, according to the following expression:

$$FQI = (\prod_{i=1}^n qi)^{1/n}$$

3.8. Statistical Analyzes

The data were previously subjected to the normality test using the Shapiro-Wilk test [45]. The data obtained were subjected to analysis of variance, followed by the comparison of means using the Tukey test ($p \leq 0.05$). These statistical analyzes were performed using R statistical software R [46].

3. Results

3.1. Physicochemical and Physical Color Parameters

Irrigation depths and water deficit mitigation treatments (DHA) did not influence fruit quality parameters pH, SS, TA, SS/TA, C* and Hue angle (Table 2). In most of the variables evaluated, the average values obtained on the 50% Etc irrigation level were quite similar to those obtained on the 100% Etc, with variations of less than 5%. Among the DHA treatments, the T4 treatment (ZnSO₄ via leaf) provided an increase of 10.45% in the C* value and the T3 treatment (ZnSO₄ via soil+PGPB) increased the SS/TA ratio by 17.79%, both under the 100% ETc.

The interaction of irrigation depth and DHA treatments influenced the ascorbic acid content (Figure 2a) and color luminosity (L*) (Figure 2b). The 50% Etc irrigation level reduced the ascorbic acid content by approximately 30.4% in the control treatment and 33.8% in the T4 treatment (ZnSO₄ via leaf). Under the 50% Etc irrigation level, the T3 treatment (ZnSO₄ via soil+PGPB) promoted a 40.8% increase in the ascorbic acid content, in relation to the control, while the T1, T2 and T4 treatments had similar performance to the control. Under the 100% Etc irrigation level, the T3 treatment increased the ascorbic acid content by 24.3% in relation to the control, while in the other treatments, the values of this variable were similar. The 50% Etc irrigation level increased the L* value in treatment T1 (NPZnO via leaf) and decreased it in treatments T3 and T4. Under water restriction, DHA treatments did not change the L* value, while under 100%ETc, the T1 treatment decreased the value of this parameter.

Table 2. pH values, soluble solids (SS), titratable acidity (TA), SS/TA ratio, chromaticity (C*), Hue angle (°Hue) of green peppers fruits as a function of irrigation levels and deficit irrigation treatments mitigating (DHA).

Irrigation depth				
(%)	Treatment DHA	pH	SS (°Brix)	TA (g per 100g)
50% Etc	Check	5.443±0.464	4.858±0.175	0.109±0.018
	T1 (NPZnO via leaf)	5.432±0.265	4.975±0.325	0.109±0.001
	T2 (NPZnO via leaf)+PGPB	5.370±0.204	4.767±0.639	0.130±0.018
	T3 (ZnSO ₄ via soil+PGPB)	5.443±0.252	4.792±0.382	0.119±0.022
	T4 (ZnSO ₄ via leaf)	5.437±0.388	5.289±0.615	0.103±0.011
	Mean	5.425	4.936	0.114
100% Etc	Check	5.392±0.292	5.233±0.183	0.119±0.013
	T1 (NPZnO via leaf)	5.438±0.263	4.992±0.185	0.114±0.011
	T2 (NPZnO via leaf)+PGPB	5.350±0.479	5.054±0.042	0.109±0.001
	T3 (ZnSO ₄ via soil)+PGPB	5.282±0.342	5.183±0.745	0.114±0.021
	T4 (ZnSO ₄ via leaf)	5.246±0.138	5.042±0.203	0.125±0.021
	Mean	5.341	5.101	0.116

		SS/TA	C*	Hue
50% Etc	Check	42.792±4.477	20.581±0.910	50.673±4.500
	T1 (NPZnO via leaf)	45.820±2.991	20.809±1.900	53.451±5.700
	T2 (NPZnO via leaf)+PGPB	37.420±4.065	21.514±0.842	50.670±3.060
	T3 (ZnSO ₄ via soil)+PGPB	42.878±2.473	21.276±2.196	50.859±6.110
	T4 (ZnSO ₄ via leaf)	46.971±5.969	21.161±1.872	50.692±4.560
	Mean	43.176	21.068	51.269
100% Etc	Check	44.246±5.600	20.607±2.472	50.311±1.222
	T1 (NPZnO via leaf)	44.029±3.802	21.164±1.079	50.755±5.330
	T2 (NPZnO via leaf)+PGPB	46.549±3.840	21.911±2.09]	50.959±4.060
	T3 (ZnSO ₄ via soil)+PGPB	53.822±2.628	22.170±0.629	49.863±1.485
	T4 (ZnSO ₄ via leaf)	41.123±6.286	23.012±2.622	50.230±2.891
	Mean	45.954	21.773	50.424

The interaction of irrigation levels and DHA treatments influenced the ascorbic acid content (Figure 2a) and color luminosity (L*) (Figure 2b). The 50% Etc Irrigation level increased the ascorbic acid content by approximately 43.8% in the control treatment and 51.0% in the T4 treatment (ZnSO₄ via leaf). Under the level of 50% Etc, treatments T3 (ZnSO₄ via soil+PGPB) and T4 (ZnSO₄ via leaf) promoted an increase of 18.1% in ascorbic acid content, in relation to the control, while treatments T1, T2 had similar performance to the control. Under the 100% Etc irrigation level, the T3 treatment increased the ascorbic acid content by 72.7% in relation to the control. For this variable, treatments T1, T2 and T4 were also superior to the control, but T2 was similar to the treatment T3. The 50% Etc irrigation level increased the L* value in treatment T1 (NPZnO via leaf) and decreased it in treatments T3 and T4. Under water restriction, DHA treatments did not change the L* value, while under 100%ETc, the T1 treatment decreased the value of this variable.

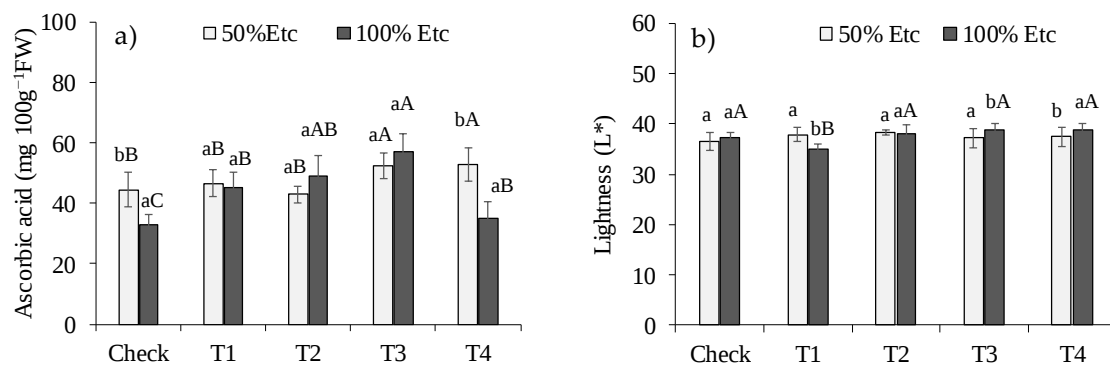


Figure 2. Ascorbic acid content (a) and luminosity (*L) of color (b) in green pepper fruits, as a function of irrigation depths and water deficit alleviating treatments. Control - no product application, T1 - ZnSO₄ foliar, T2 - NPZnO foliar, T3 - NPZnO foliar + PGPB, T4 - ZnSO₄ soil + PGPB. Columns with the same lowercase letters do not differ for water stress, and bars with the same uppercase letters do not differ for DHA treatments by Tukey's test ($p \leq 0.05$). Vertical bars represent the standard error.

3.2. Mineral Nutrient Contents

The effect of DHA treatments on mineral nutrient content in green pepper fruits was dependent on irrigation depths ($p < 0.05$). The water deficit (50%Etc) reduced the levels of Ca (Figure 3a), K (Figure 3b) and P (Figure 3d) in the DHA control and T1 treatments, but did not change the levels of these nutrients in the other treatments. The 50%Etc Irrigation level reduced the Mg (Figure 3c) and Fe (Figure 3f) contents in the control treatments, T1 and T4, the B contents (Figure 3e) in the T4

treatment and the Fe contents in the control treatments, T1 and T4. The water deficit reduced the levels of Mn (Figure 3g) and Zn (Figure 3h) in treatments T1 and T4.

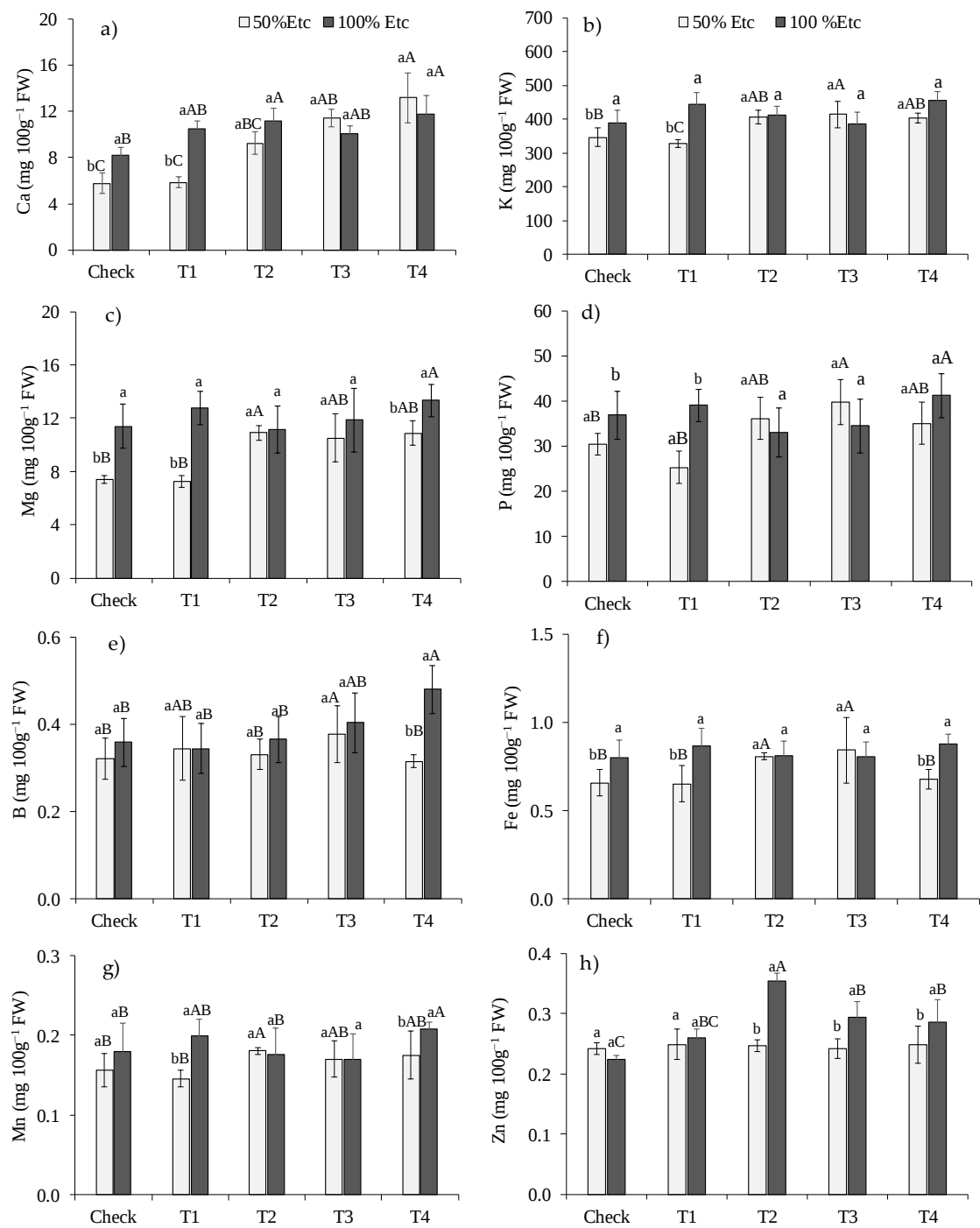


Figure 3. Contents of calcium (a), potassium (b), magnesium (c), phosphorus (d), boron (e), iron (f), manganese (g) and zinc (h) in green pepper fruits, in function of irrigation depths and treatments to alleviate water deficit. Control - no product application, T1 - ZnSO₄ foliar, T2 - NPZnO foliar, T3 - NPZnO foliar + PGPB, T4 - ZnSO₄ soil + PGPB. Columns with the same lowercase letters do not differ for water stress, and bars with the same uppercase letters do not differ for DHA treatments by Tukey's test ($p \leq 0.05$). Vertical bars represent the standard error.

Within each irrigation level, the most prominent DHA treatments were T2 and T3. Under the 50% Etc irrigation level, the T2 treatment increased the contents of K, Mg, Fe and Mn, the T3 treatment increased the contents of Ca, K, Mg, P, B and Fe and the T4 treatment increased the Ca contents in G.

Under 100% Etc water supply, the T2 treatment increased the Ca and Zn contents and the T4 treatment increased the Ca and Mn contents. Compared to the control treatment, the most notable increases occurred for Ca (69%) with the T4 treatment, under the level of 50%Etc and for zinc (57%), with the T2 treatment, under 100%Etc.

There was a significant interaction between irrigation depths and DHA treatments, for the general fruit quality index (FQI) (Figure 3). The water deficit decreased the FQI in the control, T1 and T4 treatments, while in the other DHA treatments, there was no difference between the irrigation levels for this variable. At the irrigation level of 50%Etc, treatments T2 and T3 increased the FQI value by 8 and 12%, respectively, in relation to the in FQI in relation to the control, however, this treatment was similar to the T1, T2 and T3 treatments.

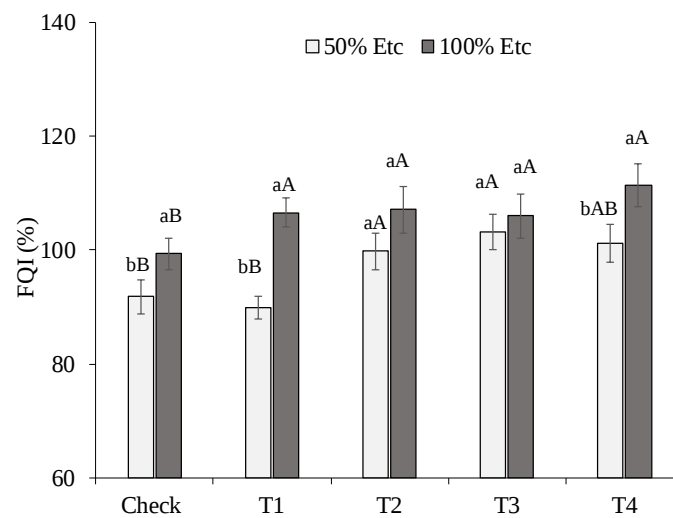


Figure 4. General fruit quality index (FQI) of green pepper, as a function of irrigation depths and water deficit alleviating treatments. Control - no product application, T1 - ZnSO₄ foliar, T2 - NPZnO foliar, T3 - NPZnO foliar + PGPB, T4 - ZnSO₄ soil + PGPB. Columns with the same lowercase letters do not differ for water stress, and bars with the same uppercase letters do not differ for combinations involving zinc oxide nanoparticles (NPZnO) or bioinoculants (PGPB) by Tukey's test ($p \leq 0.05$). Vertical bars represent the standard error.

4. Discussion

4.1. Physicochemical Attributes and Color Parameters

Among the physical-chemical attributes (ascorbic acid content, pH, titratable acidity -TA, soluble solids -SS and SS/TA ratio) and physical color parameters (L^* , C^* and Hue angle), only the acid content ascorbic acid and the L^* parameter were affected by water deficit and DHA treatments. Water deficit increased the ascorbic acid content in mango fruits [47]. Ascorbic acid plays crucial roles in plant metabolism, such as protecting cells against oxidative damage and acting as a cofactor for enzymes involved in the production of flavonoids and phytohormones [51]. The increase in ascorbic acid content under water stress, therefore, is a plant response to adjust to the stress condition, playing an important role in detoxifying reactive forms of oxygen (ROS) and thus avoiding damage to the plant's general metabolism [49].

Contrary to the results obtained in the present study, an increase in the levels of SS, TA and the SS/TA ratio and changes in the color parameters C^* and Hue angle were expected in fruits whose plants were subjected to water deficit, such as observed in other works [52,53]. The increase in SS content, TA titratable acidity and color parameters in fruits under water deficit is generally attributed to the increase in the synthesis of metabolites, and increased activity of hexokinase enzymes, due to the senescence process [54] and the loss of water by the fruit [53,55]. However, in eggplant, water restriction (50%Etc) decreased ascorbic acid levels, and the C^* parameters and Hue angle [56].

According to these authors, this decrease was probably due to the decrease in the photosynthetic rate and consequently in photoassimilates and pigments. In the present study, under water restriction, the triggering processes to increase the levels of SS, TA and the SS/TA ratio and changes in the C* parameters and Hue angle were probably counterbalanced by the decrease in the production of photoassimilates, with the decrease in the photosynthetic rate. In this sense, an increase or decrease observed in the levels of metabolites (SS, organic acids, ascorbic acid and carotenoids) in tomato fruits grown under water deficit (60% Etc) was dependent on the stage of fruit maturation (green or ripe) and the metabolites considered [48].

The DHA treatments affected only the ascorbic acid levels and slightly, the fruit luminosity (L^*). As already highlighted, ascorbic acid levels were the attribute most sensitive to water deficit. For this attribute, the similarity of the values obtained between the irrigation regimes (50% Etc and 100% Etc) in treatments T1 (foliar $ZnSO_4$), T2 (foliar NPZnO) and T3 (foliar NPZnO + PGPB) indicates that Zn applied as $ZnSO_4$ or ZnONPs possibly attenuates the negative effects of water deficit on ascorbic acid biosynthesis, which was enhanced by inoculation of plants with BPCP. ZnONPs at a dose of 500 mg L^{-1} increased the antioxidant activity in peppers in the germination phase [8] and ascorbic acid content in bell pepper fruits applied at a dose of 30 mg L^{-1} via the leaves [12]. The application of Zn as $ZnSO_4$ also increased SS contents in red pepper [29]. In plants, Zn performs important functions such as activating enzymes that protect against oxidative stress (such as superoxide dismutase), synthesizing tryptophan, which is a precursor of IAA (indole acetic acid), acting on cell division and integrity of the cell membrane [7,18,21]. In turn, the synergistic effect of PGPB with Zn may be due to the increase in the biological activity of the soil and/or production of plant growth-stimulating substances in the rhizosphere which, such as organic acids, hormones, enzymes, which can favor the photosynthetic rate and photoassimilates [25,26,57].

4.2. Mineral Nutrient Content

Among the minerals evaluated, green pepper exhibited higher levels of K, P, Mg and Ca, in that order, followed by Fe, B, Zn and Mn. Depending on the ADH treatments, the levels of these minerals were considerably reduced by the water deficit. Water deficit (80% Etc) reduced the contents of K and Ca in pepper [58], decreased the contents of Mg, K, Cu and Zn in tomato under 50% Etc [59] and Ca and B in strawberry under 70% Etc [60]. The process of nutrient accumulation in fruits depends on factors such as the genetic potential of the species, supply via soil or leaves and nutrient mobility in the phloem [7]. Nutrient supply via soil depends on many factors, including soil moisture [60]. Mass flow and diffusion are the two main mechanisms that deliver mineral nutrients to plant roots [7]. The decrease in mineral nutrient content in pepper fruits was possibly due to the lower amount of these nutrients in contact with the plant's roots, since Ca, Mg, K, Fe, Mn, B and Zn reach the roots, mainly by mass flow, while K and P, by diffusion [7,61].

Under water deficit, treatments T2 (NPZnO via leaf +PGPB) and T3 ($ZnSO_4$ via soil+PGPB) had a greater influence on the mineral composition of pepper fruits, since they increased the levels of K, Ca, Mg, P, Fe, B and Mn. On the other hand, when there was no water restriction, these treatments (except for Ca and Zn) did not have the same performance in relation to the control, that is, only the T4 treatment ($ZnSO_4$ via leaf) increased the Mn and B contents. Highlights it is clear that treatments T2, T3 and T4 increased Zn levels to the level of 100%ETc, but this did not occur under water deficit. Foliar application of ZnONP increased the contents of K, Ca, P, Mg and Zn in pepper fruits [12]. The role of Zn in cell division and the growth of fine roots contributes to greater absorption of water and nutrients by the pepper plants and their subsequent translocation to the fruits [62]. The presence of PGPB in treatments T2 and T3 enhanced the beneficial effect of zinc on the accumulation of nutrients in pepper fruits. The role of beneficial microorganisms such as *Bacillus subtilis* and *B. amyloliquefaciens* in mitigating water stress in various crops appears to be due to their direct effects on plants and indirectly on the soil [24–26]. These bacteria possibly contributed to increasing the absorption of water and nutrients by peppers, due to the increase in the photosynthetic rate with better adaptation of metabolism and osmotic adjustment under water deficit [63,64] and/or, due to increase of solubility

of low soluble forms of nutrients in the soil as well as by increasing the biological activity of the soil [26].

The FQI index as a measure of general fruit quality revealed, in a summarized way, the negative effect of water deficit on the quality of pepper fruits and the role of Zn as attenuator, both in the form of ZnONPs and in the form of ZnSO_4 , as well as PGPB. This effect was more pronounced in treatments T2 and T3, in which no significant differences were observed between the irrigation levels tested. On the other hand, the superiority of treatments T2, T3 and T4 for the FQI index revealed the stimulating effect of Zn and BPCP, even under adequate irrigation (100%ETc).

Finally, it is worth highlighting that the first hypothesis considered in this work was confirmed for the ascorbic acid content and the mineral element content. The second hypothesis can be considered confirmed based on the mineral nutrient content and the general quality index (FQI) and considering that the dose of Zn supplied via ZnONsP was 10 times lower than the dose of Zn via $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, thus proving, the highest efficiency of ZnONPs. Although water deficit increased the ascorbic acid content in fruits, lack of water decreased the overall quality of fruits, especially in terms of mineral nutrients, especially when considering the role of these nutrients in human nutrition [65].

5. Conclusions

Water deficit increased ascorbic acid levels in green pepper fruits, without altering soluble solids levels, color parameters (C^* and Hue angle) and titratable acidity, but decreased mineral nutrient levels. The application of Zn via foliar, in the form of zinc oxide nanoparticles (ZnONPs) or zinc sulfate (ZnSO_4), mitigated the negative effects of water deficit on the quality of pepper fruits. The ZnONPs source was more efficient than the ZnSO_4 source. The water deficit alleviating effect of both zinc sources was enhanced when the plants were inoculated with *Bacillus subtilis* and *B. amyloliquefaciens*.

The findings of the present work, together with other research, are important in terms of new technological perspectives for adapting plants to water deficit, whether in a semi-arid climate or in climates that are already suffering from the effects of climate changes.

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Data Availability Statement: The data used to support the findings of this study are included within the article.

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

Appendix A

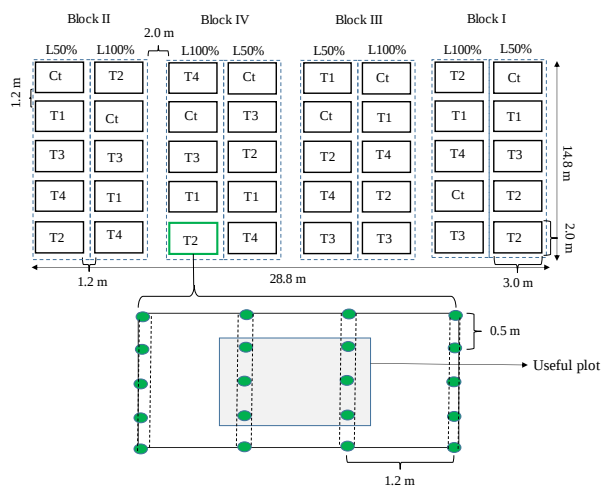


Figure A1. Experimental design used in the research showing the distribution of blocks, combination of irrigation levels with treatments from subplots, delimitation of experimental plots and useful plot, as well as spacing between blocks, plots, planting rows and plants.



Figure A2. Partial view of the experimental area showing the installation of the drip irrigation system (a) and the and plants in the fruit production phase (b).



Figure A3. Harvest (a), sanitization process and classification (b) of green pepper fruits before carrying out physical-chemical analyzes.

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