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Article

Efficacy and Differential Physiological–Biochemical Response of Biostimulants in Green Beans Subjected to Moderate and Severe Water Stress

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Abstract: Water stress is one of the main factors affecting the development of agricultural crops. An innovative alternative to improve tolerance to water stress is the application of biostimulants. In the present study, the efficacy and physiological and biochemical responses of different biostimulants were evaluated in beans under moderate and severe stress. The treatments consisted of three types of irrigation: FC100, without water stress; FC75, irrigation reduced by 25% (moderate water stress); and FC50, irrigation reduced by 50% (severe water stress). In the treatments with water deficits, foliar biostimulants were applied: zinc oxide nanoparticles plus chitosan, Codasil®, Osmoplant®, Stimplex® and salicylic acid. Foliar application of ZnO + chitosan nanoparticles benefited biomass accumulation and yield under moderate water stress (FC75) and Codasil® and Osmoplant® under severe water stress (FC50). Proline, free sugars and gas exchange were higher with the application of ZnO + chitosan nanoparticles under moderate water stress and with Codasil® and Osmoplant® under severe water stress. Depending on the severity of water stress, ZnO + chitosan nanoparticles, Codasil® and Osmoplant® are viable products to increase tolerance in green bean cv. Strike plants.

Keywords: *Phaseolus vulgaris* L.; nanofertilizers; bioregulators; nanotechnology

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1. Introduction

Water stress is one of the main abiotic factors that reduce the development and yield of plants. Therefore, agricultural productivity and food security are threatened by water deficits [1]. Plants are under water stress if the water supply to the roots is limited or exceeded by the rate of transpiration [2]. Because the fresh biomass of plants is composed of 85% water, drought severely affects physiological and biochemical processes [3]. Some of these alterations include mineral absorption, photosynthesis, respiration rate, gas exchange in leaves and an increase in reactive oxygen species (ROS), among others [4,5].

The physiological and biochemical modifications in response to drought stress in crops are given by the duration and intensity of the period to which they are subjected. In this way, tolerance mechanisms such as osmotic adjustments, cell wall modifications and activation of the antioxidant system arise as a response to stress [6]. Therefore, the degree of tolerance to drought stress in plants depends on the efficacy of the activation of said mechanisms [7].

Although plants can reduce the adverse effects of low water availability, reducing the transpiration cycle through stomatal closure, this also causes an alteration in the photosynthesis process, specifically in the availability of CO_2, which in turn would slow down the production of energy generation and structural biocomposites [8]. Due to this, it is necessary to investigate the use of compounds that are effective in stimulating development and production in plants and that are also capable of enhancing responses against water stress.

One of the innovative proposals that has emerged in recent decades to increase crop growth and production, improve the efficient use of nutrients and promote tolerance against abiotic stress in an environmentally friendly way is the use of biostimulants in plants at low concentrations [9]. Biostimulants have been defined as compounds obtained from different organic or inorganic substances and/or microorganisms which can improve the growth and productivity of plants and reduce the negative effects of stress. Among the most common biostimulants are minerals such as zinc (Zn) and silicon (Si), vitamins, amino acids, poly and oligosaccharides, and some types of plant hormones [10–12]. However, due to the wide range of products cataloged as biostimulants in development and the lack of published studies on the mechanisms of action in metabolism, it is necessary to investigate the effects of these compounds on physiological and biochemical modifications in plants. We hypothesize that the commercial products tested will improve growth variables under moderate and severe stress conditions. Therefore, the objective of this study is to evaluate the efficacy and differential physiological–biochemical response of biostimulants in green beans cv. Strike subjected to moderate and severe water stress.

2. Materials and Methods

2.1. Crop Management

The experiment was conducted at the facilities of the Food and Development Research Center in Delicias, Chihuahua, Mexico, during the months of August and September in 2021. The experiment was established under greenhouse conditions at an average ambient temperature of 30.7 °C. Green bean seeds (*Phaseolus vulgaris* L.) cv. Strike were grown in 13.4 L plastic pots (two plants per pot) in a substrate mixture composed of vermiculite and perlite in a 2:1 ratio. The plants were watered with a complete nutrient solution composed of 6 mM NH_4NO_3, 1.6 mM K_2HPO_4, 0.3 mM K_2SO_4, 4 mM $CaCl_2$, 1.4 mM $MgSO_4$, 5 μM Fe-EDDHA, 2 μM $MnSO_4$, 0.25 μM $CuSO_4$, 0.5 μM H_3BO_3 and 0.3 μM Na_2MoO_4 with a pH of 6.0–6.1. The solution was applied depending on the amount of irrigation (100, 75 and 50%).

2.2. Experimental Design

A completely randomized design with thirteen treatments and six repetitions was used (Table 1). A control (FC100) was used at 100% field capacity (without water stress and without the application of biostimulants). In addition, two water stress treatments were applied: FC75 with a water application restriction of 25%, and FC50 with a water application restriction of 50%. Biostimulants were applied via foliar application to both water stress conditions: ZnO nanoparticles + chitosan, Codasil®, Osmoplant®, Stimplex® and salicylic acid (Table 1) in the doses indicated by the manufacturer (Table 2). Biostimulant treatments were applied 15 days after germination at the appearance of the first true leaves. Thereafter, foliar applications were made every week for two months.

Table 1. Treatment description.

Percentage of Water Applied (FC)	Added Biostimulant	Doses of Added Biostimulant	Code
100	-	-	FC100
75	-	-	FC75
75	Nano ZnO + Chitosan	100 ppm	FC75 + NanoZn + Q
The75	Codasil®	200 ppm	FC75 + Codasil
75	Osmoplant®	200 ppm	FC75 + Osmoplant
75	Stimplex®	200 ppm	FC75 + Stimplex
75	Salicylic Acid	0.1 mM	FC75 + SA

Table 1. *Cont.*

Percentage of Water Applied (FC)	Added Biostimulant	Doses of Added Biostimulant	Code
50	-	-	FC50
50	Nano ZnO + Chitosan	100 ppm	FC50 + NanoZn + Q
50	Codasil®	200 ppm	FC50 + Codasil
50	Osmoplant®	200 ppm	FC50 + Osmoplant
50	Stimplex®	200 ppm	FC50 + Stimlex
50	Salicylic Acid	0.1 mM	FC50 + SA

Table 2. Chemical composition of biostimulants and doses applied on green bean plants cv. Strike under water stress.

Biostimulant	Chemical Composition	Doses
Codasil®	Liquid solution with a high concentration of soluble silicon composed of 20% silicon, 4% free amino acids and 11.20% potassium.	2 mL/L (manufacturer's recommendation).
Osmoplant®	Liquid solution composed of 6% free amino acids, 2.4% nitrogen and 3.3% potassium.	2 mL/L (manufacturer's recommendation).
Stimplex®	Liquid solution composed of *Ascophyllum nodosum* algae extract as its active ingredient at 0.34%, with a formulation of total nitrogen 0.1% and soluble potassium (K_2O) 4.0%.	2 mL/L (Manufacturer's recommendation).
Zinc Oxide Nanoparticles	<50 nm, 99.9%	0.1246 g/L (100 ppm) [13].
Chitosan (Poli-D-glucosamine)		2 mL/L [14].
Salicylic acid	$C_7H_6O_3$	0.0138 g/1 L. (0.1 mM) [15].

2.3. Plant Sampling

Once the plants reached the state of physiological maturity at 60 days of crop development, the plant material was harvested. The collected material was washed with distilled water to remove residues and finally separated into organs (root, stem, leaves and fruit). The samples were divided into fresh and dry material. The fresh material was used for in vivo analyses, which included yield, nitrate reductase activity, photosynthetic pigments, amino acids and proteins, and proline, sucrose, glucose and fructose assays. The dry material was used for the quantification of biomass and organic nitrogen content.

2.4. Plant Analysis

2.4.1. Aerial, Foliar, Root and Total Biomass

The production of aerial, foliar, root and total biomass of the plant was evaluated separately from the dry weight of the plant. To quantify the weight, an analytical balance (AND HR-120, San José, CA, USA) was used; the weights are expressed as grams per plant based on dry weight (g plant^{-1} d.w.).

2.4.2. Biomass and Yield

The total fresh weight of fruits per plant was quantified. Ripe pods were harvested, and their weight was recorded using an analytical balance (AND HR-120, San José, CA, USA). The results are expressed as grams of fresh weight per plant (g plant^{-1} f.w.).

2.4.3. Nitrate Reductase Activity In Vivo

Nitrate reductase (NR) activity in vivo (EC 1.6.6.1) was determined using the method proposed by [16]. The foliar disks were cut into 7 mm sections and placed in 10 mL of maceration buffer (100 mM K phosphate buffer, pH 7.5 and 1% (*v*/*v*) propanol). The samples were infiltrated at a pressure of 0.8 bar. They were incubated at 30 °C in the dark for 1 h and finally placed in a boiling water bath to stop NR activity. Then, 1 mL of the enzyme extract was taken and 2 mL of 1% (p/v) sulfanilamide in 1.5 M HCl and 2 mL of 0.02% 1-naphthylenediamine N-dihydrochloride in 0.2 M (p/v) HCl was added. The resulting nitrite concentration was determined by spectrophotometry at 540 nm against a standard curve of NO_{2^-}.

2.4.4. Photosynthetic Pigments

For the quantification of photosynthetic pigments, the methodology described by [17] was followed. From the fresh leaf material, specifically from the leaf blade, 7 mm foliar disks were obtained up to an approximate weight of 0.125 g. Four replicates per treatment were acquired and placed in test tubes. A total of 10 mL of pure methanol (99%) (CH_3COH) was added to each tube and sealed with parafilm tape. It was left to stand in the dark for 24 h. Once this time had elapsed, the samples were shaken and the reading was carried out in a Genesis 10S UV-VIS spectrophotometer (Thermo Scientific, Waltham, MA, USA) at three wavelengths: 666, 653 and 470 nm. Photosynthetic pigment concentrations were expressed as mg g^{-1} f.w. and were calculated using the following formulas:

$$\text{Chl a} = [15.65(\text{A666}) - 7.34(\text{A653})]$$

$$\text{Chl b} = [27.05(\text{A653}) - 11.21(\text{A666})]$$

$$\text{Carotene} = [(1000 \times \text{A470}) - 2.86(\text{Chl a}) - 129.2(\text{Chl b})]/2212.5.7$$

2.4.5. Chlorophyll Index

For the quantification of the chlorophyll index, a Minolta SPAD 502 chlorophyll reader (Konica Minolta Sensing, Inc., Osaka, Japan) was obtained, which measures in situ without causing damage to the leaf. Reading is achieved by projecting light through a sheet. The measurement was made on parts free of ribs. The results are expressed as SPAD units [18].

2.4.6. Amino Acids and Soluble Proteins

For the quantification of amino acids and soluble proteins, 0.5 g of fresh plant material was weighed and homogenized in cold 50 mM KH_2PO_4 buffer at pH 7. The sample was centrifuged at 12,000× g for 15 min. The supernatant obtained was used for the determination of total amino acids by the ninhydrin method, with slight modifications [16]. Total amino acids are expressed as mg g^{-1} f.w. The soluble protein content was measured with the Bradford reagent [19] and is expressed as mg g^{-1} f.w., using bovine serum albumin as the standard.

2.4.7. Proline, Sucrose, Glucose and Fructose Assay

The extraction for the determination of the concentration of proline, sucrose, glucose and fructose was conducted following the methodology proposed by Irigoyen et al. [20], with modifications for the experiment proposed by Sánchez et al. [21]. An amount between 0.25 and 0.5 of fresh plant material was homogenized with 5 mL of 96% ethanol, and subsequently, two rinses with 5 mL of 70% ethanol were applied. The resulting homogenate was centrifuged at 5500 rpm for 10 min, and the resulting supernatant was used to determine the concentrations of proline and soluble sugars. The concentration of proline and soluble sugars is expressed as mg g^{-1} f.w.

2.4.8. Photosynthetic Activity, Stomatal Conductance, Maximum Fluorescence and Transpiration

Photosynthetic activity and stomatal conductance were measured in the leaves when the plant reached its physiological maturity, in a time range from 10:00 a.m. to 11:00 a.m. A portable meter LI-COR 6400 (Lincoln, NE, USA) was used, and a healthy leaf of homogeneous color and free of damage was selected on each plant. A concentration of 400 µmol per mol CO_2 was used in the reference cell, while the sample cell was maintained at approximately 380 µmol per mL CO_2. The air vapor pressure deficit in the sample chamber was less than 1.5 and the temperature of the block that housed the leaf was 25 °C. Photosynthetic activity is expressed as μM CO_2 $m^2 \cdot s^{-1}$ and stomatal conductance is reported as mM CO_2 $m^2 \cdot s^{-1}$. Transpiration is reported as μmol H_2O $m^{-2} \cdot s^{-1}$ and maximum chlorophyll fluorescence is expressed as (Fm′).

2.5. Statistical Analysis

The data obtained were subjected to an analysis of variance and the significant differences in the means were determined by the test of least significant difference (LSD) ($p < 0.05$) using the statistical software package SAS 9.0.

3. Results and Discussion

3.1. Aerial, Foliar, Root and Total Biomass

One of the key variables when analyzing the physiological state of plants is the accumulation of biomass [7,15]. In the present study, significant differences were found in the production of aerial, foliar, root and total biomass because of the application of water stress and biostimulants (Figure 1). Regarding aerial biomass, the FC75 + NanoZn + Q treatment presented the highest accumulation, with an increase of 15.24% with respect to FC75, placing this combination as a viable biostimulant to reduce the effect of water stress on plants, even surpassing other established commercial biostimulants.

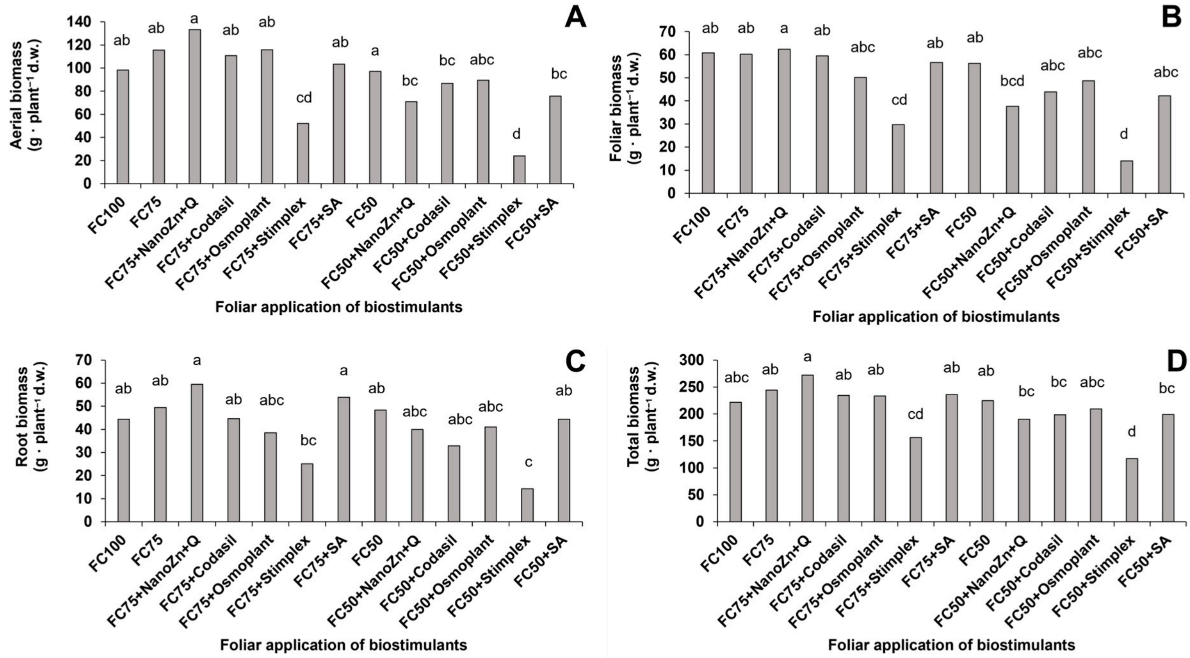

Figure 1. Effect of biostimulant application on aerial (**A**), foliar (**B**), root (**C**) and total (**D**) biomass production in plants of green bean cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

The same trend was recorded in leaf biomass, with FC75 + NanoZn + Q being the best treatment, with an increase of 91.2% with respect to FC75 + Stimplex, which was the treatment that favored this parameter to a lesser extent when irrigation was reduced by 25%. Likewise, when the amount of water was reduced by 50%, the FC50 treatment obtained the highest yield, with an increase three times the value of the FC50 + Stimplex treatment, as the treatment with the lowest accumulation of foliar biomass (Figure 1).

In the case of root biomass, when the amount of irrigation was reduced by 25%, the treatment that obtained the highest value was FC75 + NanoZn + Q, presenting an increase of 136.8% with respect to FC75 + Stimplex, which was the least value treatment. As in the previous sections, when the amount of irrigation was reduced by 50%, the treatment that benefited the most was FC50, presenting an increase of 236.8% with respect to FC50 + Stimplex with the least accumulation (Figure 1).

Finally, in relation to the total biomass, when the amount of irrigation was reduced by 25%, the treatment that favored the accumulation was FC75 + NanoZn + Q, with an increase of 73.7% compared to FC75 + Stimplex, the treatment with the least accumulation. In addition, when the irrigation reduction was 50%, the FC50 treatment presented the highest accumulation, increasing by 91.4% with respect to the treatment with the lowest accumulation, FC50 + Stimplex (Figure 1).

These results agree with Patel et al. [22], who reported an increase in biomass of 23.77% when a combination of zinc and chitosan nanoparticles was added, compared to the control without application, and a decrease when only Zn nanoparticles were added, demonstrating the potential of these compounds together. This joint effect is related to the signaling reactions triggered by zinc nanoparticles and chitosan. These reactions include the generation of hormones such as ABA, which influences root architecture, increasing the adaptation of plants to resist water stress [23].

The application of biostimulants did not favor the accumulation of biomass once the plants were subjected to FC at 50% with respect to the control, highlighting the FC50 + Codasil and FC50 + Osmoplant treatments as viable options when drought stress is severe. This phenomenon was caused by the decrease in stomatal density under severe drought conditions, which in turn affected the photosynthesis cycle, limiting plant growth and decreasing the absorption efficiency of foliar-applied biostimulants [24].

3.2. Yield

Yield is linked to biomass production and the physiological state of the plants, since part of the biomass generated is used for the formation of fruits [25]. In the present study, significant differences in yield were found because of the application of water stress and biostimulants (Figure 2). When the plants were subjected to 75% FC, the treatment that favored fruit production was FC75 + NanoZn + Q, with an increase of 107.4% compared to the FC75 treatment and 32.1% compared to FC100 (control). On the other hand, when the plants were subjected to a 50% irrigation reduction, the treatments that were shown to alleviate the effects of stress were FC50 + Codasil and FC50 + Osmoplant, with increases of 76.6% and 59.2%, respectively, compared to FC50. These results show the relationship between total biomass production (Figure 1) and yield (Figure 2). Treatments with higher biomass production were able to translocate the largest amount of assimilates to the fruits. In addition, the results show the decrease in fruit production when the plant is under conditions of moderate (FC75) and severe (FC50) water stress caused by the decrease in growth parameters such as root (Figure 1) and stem elongation.

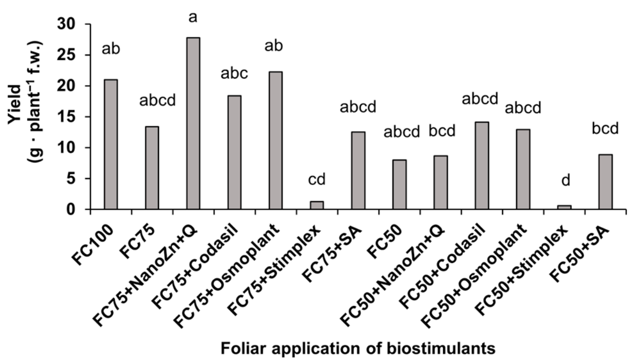

Figure 2. Effect of biostimulant application on yield in green bean plants cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

Finally, the results agree with Etienne et al. [26], who explain that plants subjected to water stress experience an induced deficiency of micronutrients such as Zn, Fe, Mn and Ca, which, in vegetative stages, are necessary for metabolic processes related to growth and development. The application of Zn in the form of nanoparticles, accompanied by chitosan at the recommended doses (100 pm) [27], improved the accumulation of this micronutrient and therefore promoted a higher production through the improvement in the metabolic processes of the plant.

3.3. Nitrate Reductase Activity In Vivo

The enzyme nitrate reductase (NR) is the first step in the nitrate assimilation process in plants, since it reduces the absorbed nitrate into nitrite, which will later be incorporated into nitrogenous metabolites [28]. In the present study, significant differences were found in the NR activity due to the effect of the application of hydric stress and biostimulants (Figure 3). The treatment that presented the highest activity was FC75 + NanoZn + Q, presenting an increase of 490.7% with respect to FC75 + Stimplex, the treatment with the lowest activity when the plants were subjected to moderate stress (FC75). Once the amount of water applied was reduced by 50%, the treatments that benefited the most were FC50 + Osmoplant, FC50 + SA, FC50 + NanoZn + Q and FC50 + Stimplex, with no significant difference between them, with the highest being FC50 +Osmoplant, increasing 6.8 times with respect to FC50.

The data obtained agree with those found in the variables of biomass (Figure 1) and yield (Figure 2), where the most favored treatment was also FC75 + NanoZn + Q. As indicated by Pejam et al. [29], the constant application of Zn nanoparticles tends to modify growth, yield, nutritional status and nitrogen assimilation in plants, making these physiological processes more efficient. These changes are due to the high participation of Zn in signaling, enzymatic cofactor and integral maintenance of DNA. In addition, the results agree with what was found by Ghani et al. [30], who report that the application of ZnO nanoparticles at doses of 25 and 100 ppm foliar-applied to cucumber crops increased growth and biomass accumulation and decreased the effects of water stress. These results place foliar ZnO nanoparticles as a viable option to mitigate the decrease in the nitrogen assimilation process caused by water stress in green bean plants.

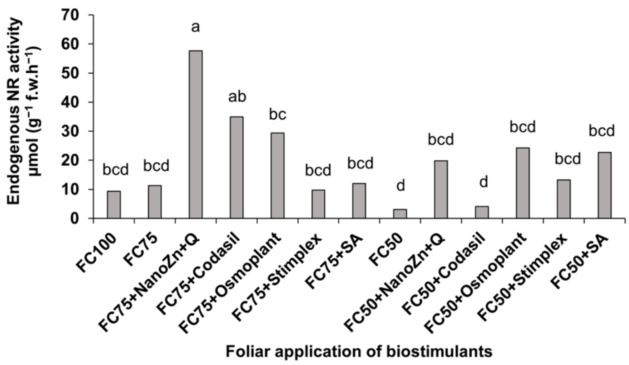

Figure 3. Effect of biostimulant application on NR activity in plants of green bean cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

3.4. Photosynthetic Pigments

The study of the concentration of photosynthetic pigments in plants is a good indicator of the physiological state of crops [31]. In the present study, significant differences were found in the concentration of photosynthetic pigments due to the application of water stress and biostimulants (Figure 4). When subjecting the plants to a water stress of 25% (FC75), the treatment that showed the highest accumulation of total chlorophyll was FC75

+ Stimplex, with an increase of 3.23% compared to FC75, without significant differences between FC75 + Codasil, FC75 + Osmoplant and FC75 + SA. Once water stress increased to 50% (FC50), the FC50 treatment obtained the highest values in total chlorophyll.

The results obtained are similar to those reported by Xu and Leskovar [32], who indicate that a simulated mild stress had no effect on the chlorophyll content in spinach. Other authors mention that in sage plants (*Salvia officinalis* L.) with drought stress induction treated with algae extracts, the concentration of chlorophyll increased [33]. The algae extract used in this experiment had the ability to improve the water relations of the leaves and reduce the closure of the stomata, which reduced the CO_2 fixation capacity and promoted the synthesis of chlorophyll. This treatment could favored the stomatal conductance parameter, facilitating water management through density and stomatal closure. The high concentration of chlorophyll in the FC50 treatment happened due to the development of chlorophylls to make the photosynthetic process more efficient, as explained by Heidari and Golpayegani [34].

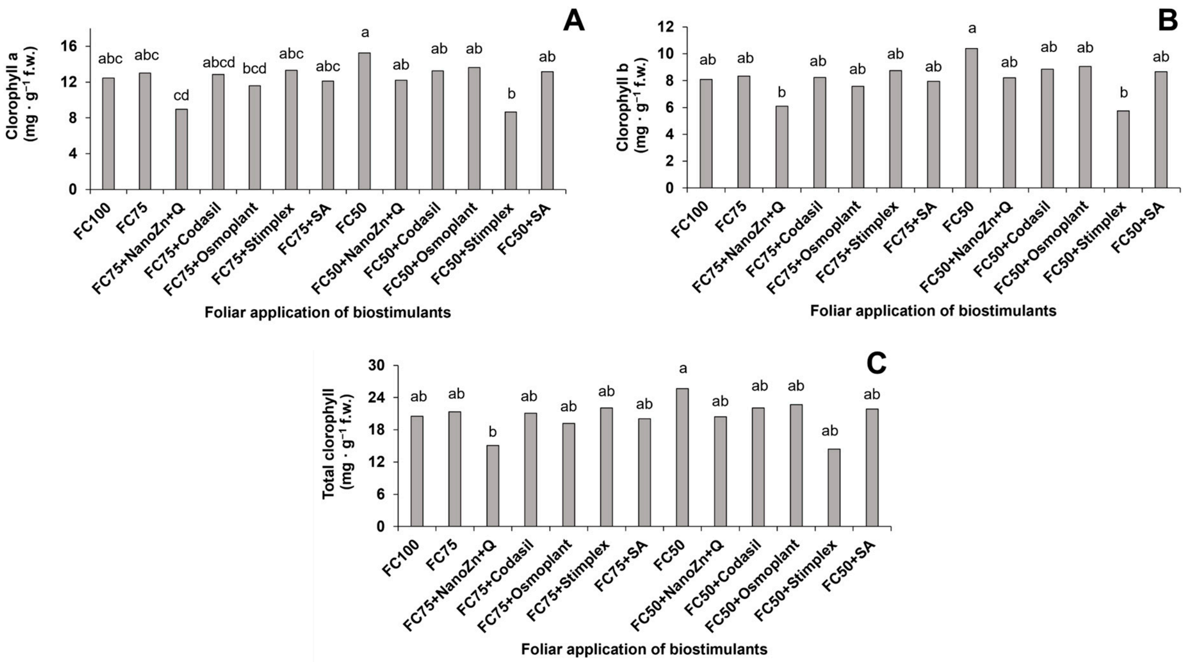

Figure 4. Effect of biostimulant application on chlorophyll a (**A**), chlorophyll b (**B**) and total chlorophyll (**C**) content in green bean plants cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

3.5. Chlorophyll Index

The chlorophyll index (SPAD) is a rapid and non-destructive method to detect chlorophyll levels in plants and determine their nutritional status [18]. In the present study, significant differences were found in the chlorophyll index because of the application of water stress and biostimulants (Figure 5). The treatment that presented the highest SPAD values when irrigation was restricted by 25% (FC75) was FC75 + NanoZn + Q, with an increase of 18.3% compared to FC75 and 10.4% compared to FC100. Similarly, when water stress was 50%, the treatment that accumulated the highest SPAD value was FC50 + NanZn + Q, with an increase of 8.85% with respect to FC50. These values correspond to what was published by [15], who report an increase in SPAD values in bean crops of around 20% when ZnO nanoparticles + chitosan were applied to bean plants. In the same way, ref. [24] reported a similar trend when applying ZnO nanoparticles in eggplant, finding increases in SPAD values with respect to untreated plants with doses of 50 and 100 ppm.

The increase in SPAD values (Figure 5), along with yield (Figure 2) and total biomass (Figure 1) values, can be attributed to the role of zinc and chitosan application in maintaining the integrity of the membranes and the efficiency of the absorption of other micronutrients such as copper and boron, which are key to inhibit the activity of chlorophyllase and maintain the structure of the chloroplasts [35].

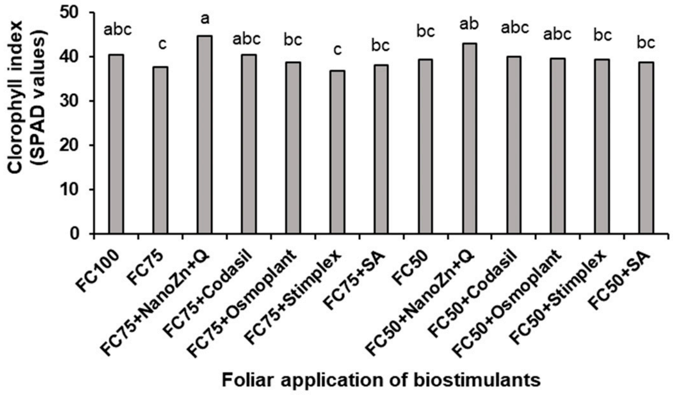

Figure 5. Effect of biostimulant application on the chlorophyll index (SPAD) in green bean plants cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

3.6. Amino Acids and Soluble Proteins

Amino acids are precursors of proteins, and both are part of the nitrogen assimilation process, so they are a biochemical indicator of the effectiveness of the applied treatments [36]. In the present study, no significant differences were found in the concentration of soluble amino acids due to the application of water stress and biostimulants (Figure 6). However, the FC75 + Stimplex treatment favored the accumulation of amino acids with an increase of 13.8% with respect to FC75 when the plants were subjected to a 25% irrigation reduction. When the irrigation reduction was 50%, the treatment that stood out was FC50 + SA, with an increase of 42.3%.

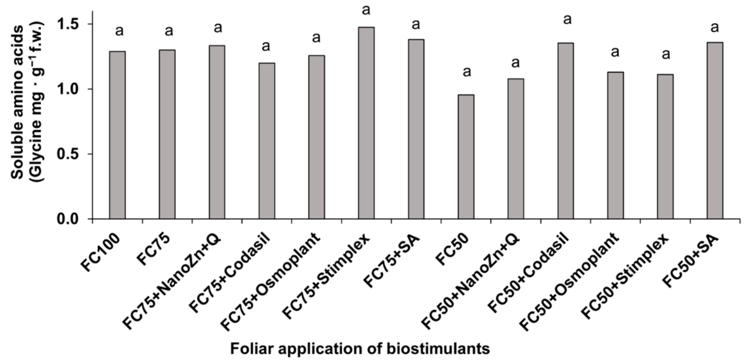

Figure 6. Effect of biostimulant application on soluble amino acid content in green bean plants cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

According to several authors, this effect was probably due to the composition of the seaweed extracts (*Ascophyllum nodosum* L.), which, in addition to containing hormones such as cytokinins and auxins that promote growth, contain protein hydrolysates and free amino acids. In addition to this, the additional source of N provided by the product derived from algae facilitates the assimilation and elaboration of metabolites [37]. Kocira et al. [38]

indicate that compounds such as amino acids and peptides contained in biostimulants are easily absorbed, increasing their concentration in plants and generating a defense against different types of stress.

Regarding the content of soluble proteins (Figure 7), in the same way, the FC75 + Stimplex treatment promoted accumulation when a hydric stress of 25% of water was applied, having an increase of 32.1% with respect to FC75. Similarly, when the field capacity was at 50%, the FC50 + Stimplex treatment increased by 37.8% compared to FC50, being the best treatment. These results agree with those obtained by Latique et al. [39], who report an increase in protein content of 25% compared to the control in snap beans cv. Paulista when algae extracts (*Fucus spiralis* and *Ulva rigida*) were applied foliarly. Several authors have reported the relationship between the application of algae extracts by the foliar route and improvements in the assimilation of growth hormones and other metabolites, in turn improving nitrate reductase activity and chlorophyll and protein content [40].

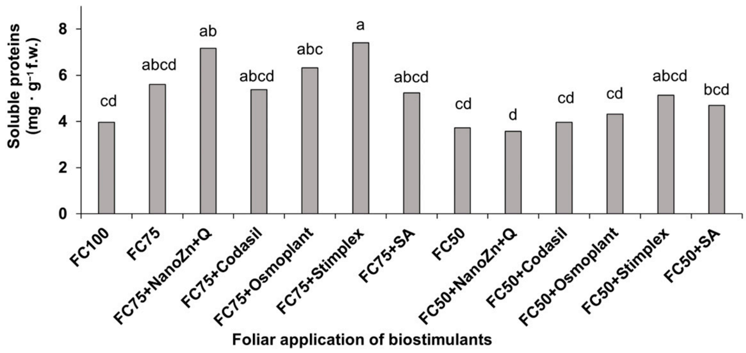

Figure 7. Effect of biostimulant application on soluble protein content in green bean plants cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

3.7. Content of Proline, Sucrose, Glucose and Fructose

One of the adaptive responses to abiotic stress in plants is the accumulation of compatible osmolytes such as sugars (sucrose and glucose) and amino acids (proline) that help protect cells [41]. In the present study, significant differences were found in the contents of proline, sucrose, glucose and fructose because of the application of hydric stress and biostimulants (Figure 8). Regarding the proline content, the treatment with the highest content was FC75, with an increase of 48.6% compared to FC100, evidencing the state of stress of the plants with a decrease in water to 25%. This increase was 13.9% when the water application was reduced to 50% (FC50) with respect to FC100 (control without water stress and without application of biostimulants).

When the amount of water was reduced by 25%, the treatment that decreased the proline content the most was FC75 + Osmoplant, with a reduction of 36% compared to FC75. This treatment did not present significant differences ($p = 0.05$) with respect to FC100, which could indicate a better adaptation to stress in this group of plants. When irrigation was reduced by 50%, the treatment that reduced proline content the most was FC50 + Codasil, with a reduction of 2.5%.

These results are similar to those of Jungklang et al. [42], who indicate that the concentration of proline was higher in plants treated with commercial osmoregulator products compared to those not treated. When stress is induced by salinity or water deficits, the osmotic adjustment is the main response factor [43]. The accumulation of proline in tissues and leaves could play a protective role in drought conditions, together with osmoregulation. On the other hand, products such as Osmoplant® could mitigate water stress since the treated plants did not accumulate proline in the leaves.

The accumulation in the plant of non-structural carbohydrates such as glucose and its derivatives are a good indicator of the physiological state and stress, because it leads to a decrease in photosynthesis and respiration, which ultimately leads to carbon depletion [44]. In the present study, the treatment that favored the accumulation of sucrose, glucose and fructose when the field capacity was at 75% was FC75 + Osmoplant, with increases compared to FC75 of 198% in the three parameters (Figure 6). The treatment that benefited the most in these variables when irrigation decreased by 50% was FC5 + NanoZn + Q, which increased by 205.9% compared to FC50.

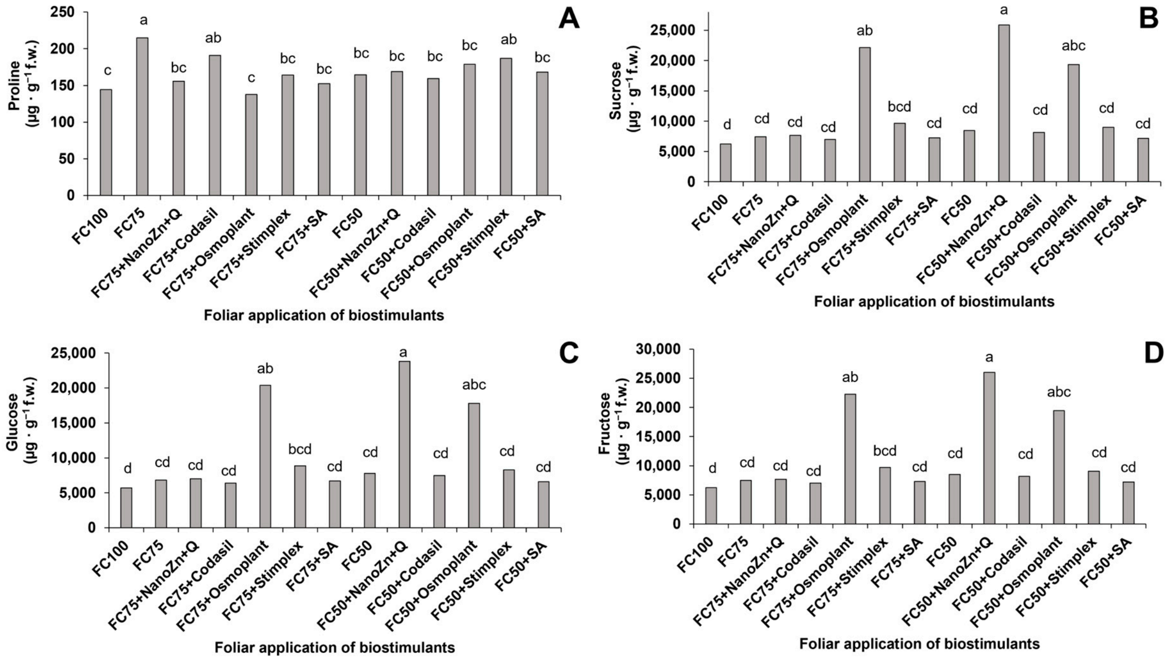

Figure 8. Effect of biostimulant application on proline (**A**,) sucrose (**B**), glucose (**C**) and fructose (**D**) content in green bean plants cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

These results agree with those obtained by Abdel-Latef et al. [45], who reported that the application of ZnO nanoparticles at a dose of 100 mg·kg^{-1} in pea plants increased the sucrose content by 1.8 times; however, no increases in reducing sugars such as glucose and fructose were recorded. The possible explanation for the increase in the concentrations of non-structural carbohydrates in both FC75 + Osmoplant and FC50 + NanoZn + Q is due to the participation of these compounds in the response to stress. The plants that showed the best adaptation were those treated with Osmoplant® and ZnO nanoparticles + chitosan, and it has been reported that sucrose, glucose and fructose can contribute to stress-related signaling pathways [46]. Finally, it should be noted that these results are related to those obtained in fluorescence and photosynthetic activity (Figure 9). Previously, it was mentioned that water stress caused a decrease in photosynthesis efficiency, which would explain why the highest fluorescence values were obtained in the FC75 + Osmoplant treatment, since the highest rate of electron exchange occurred in this treatment, which in turn triggered the biosynthesis of antistress signaling metabolites such as reducing sugars.

3.8. Photosynthetic Activity, Stomatal Conductance, Fluorescence and Transpiration

To obtain biomass and production, a correct balance between carbon assimilation and transpiration is necessary. In this process, gas exchange mediated by stomatal closure is decisive for the supply of CO_2, ATP and NADPH [47]. In the present study, significant differences were found in photosynthetic activity, stomatal conductance, fluorescence and transpiration due to the application of water stress and biostimulants (Figure 9). The highest photosynthetic activity occurred when the amount of irrigation was reduced by

25% in the FC75 + SA treatment, with an increase of 13.6% compared to FC75; however, the increase was not significant. On the other hand, when irrigation was reduced by 50%, the treatment that stood out was FC50 + Osmoplant, increasing photosynthetic activity by 21.2% compared to FC50 (Figure 9). Regarding stomatal conductance, when irrigation was reduced by 25%, the treatment that benefited most was FC75 + SA, with an increase of 38.8% compared to FC75. By supplying a 50% irrigation, the 27.8% increase in the FC50 + SA treatment with respect to FC50 positioned it as the best treatment.

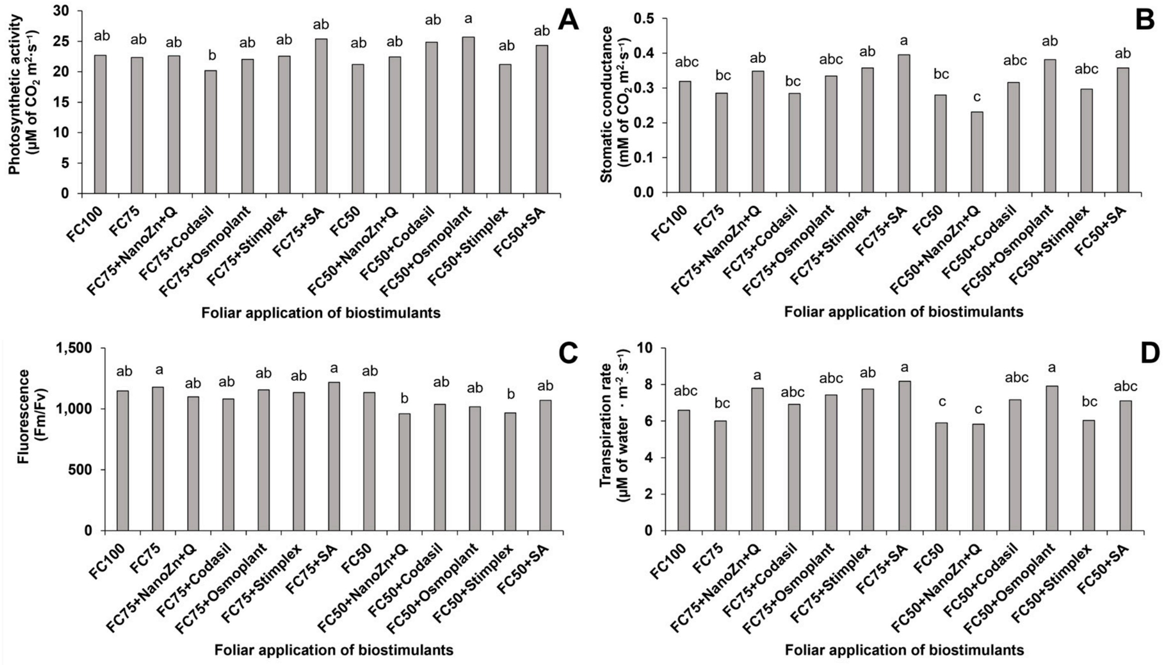

Figure 9. Effect of biostimulant application on photosynthetic activity (**A**), stomatal conductance (**B**), fluorescence (**C**) and transpiration (**D**) in green bean plants cv. Strike under water stress conditions (FC75 and FC50) and a control (FC100, without water stress and without biostimulant application). Means with different letters indicate significant differences according to LSD test ($p < 0.05$).

As the literature indicates, one of the processes most affected by drought is photosynthetic activity due to the reduction in the photosynthetic area, stomatal closure, the reduction in the synthesis of chlorophyll pigments and the deterioration of the photosynthetic machinery [48]. Within the mechanisms of action of commercial products called osmoregulators is the increase in the efficient use of water through restricting transpiration, closing the stomata and reducing evaporation from the surface of the leaf to avoid dehydration and preserve turgor [49]. In the same way, the photoprotective effect of salicylic acid could be the reason that photosynthetic activity remains stable even though the plant is under drought conditions.

On the other hand, these results, together with those obtained in total biomass (Figure 1), yield (Figure 2), total chlorophyll (Figure 4) and chlorophyll index (Figure 5), lead to the conclusion that the application of ZnO nanoparticles + chitosan has a beneficial effect on photosynthetic efficiency, which ultimately leads to maintaining this process despite stress conditions due to reduced irrigation and allows for plants root development and the accumulation of aerial biomass to finally obtain a high yield.

Maximum relative fluorescence quantification (Fm′) is another essential element when considering photosynthetic efficiency. The energy that is absorbed by the photosynthetic pigments is sent to the reaction centers of photosystems I (PSI) and II (PSII) for transformation to photochemical energy or dissipated as fluorescence [50]. In the present study, the highest value when the plants were subjected to a 25% decrease in irrigation was presented in the FC75 + SA treatment, with an increase of 3.4% with respect to FC75. On the other hand, once the amount of water decreased to 50%, the treatment with the highest fluorescence value was FC50, followed by the FC50 + SA treatment (Figure 9).

These results agree with what was reported by [51], who found that the application of salicylic acid at a dose of 0.5 mM reduced the adverse effects of drought and increased photosynthetic efficiency. Other authors have indicated that although the exogenous application of salicylic acid in broad bean plants improved CO_2 assimilation and chlorophyll content, it did not have significant effects on photoprotective compounds such as chlorophyll fluorescence [52]. The values obtained can be related to the effect of salicylic acid in the protection of the photosynthetic machinery, since, during the light reactions in the leaves, one of the main processes of energy dissipation for the absorption of light by light-harvesting pigments is the fluorescence emission of chlorophyll.

The treatments with the highest values in transpiration were FC75 + SA, when irrigation was applied at 75%, with an increase of 36.2%, and FC50 + Codasil, when irrigation was applied at 50% (Figure 9). These data agree with what was mentioned above and with the production values obtained. Although the treatment with salicylic acid improved the response to water stress through an improvement in photosynthetic efficiency, it did not reach the highest values in production and biomass due to its high rate of transpiration.

Finally, the treatment with zinc nanoparticles plus chitosan is listed as the best treatment against moderate stress, and the treatments with Codasil and Osmoplant as the best treatments against severe stress in bean plants (Figure 10).

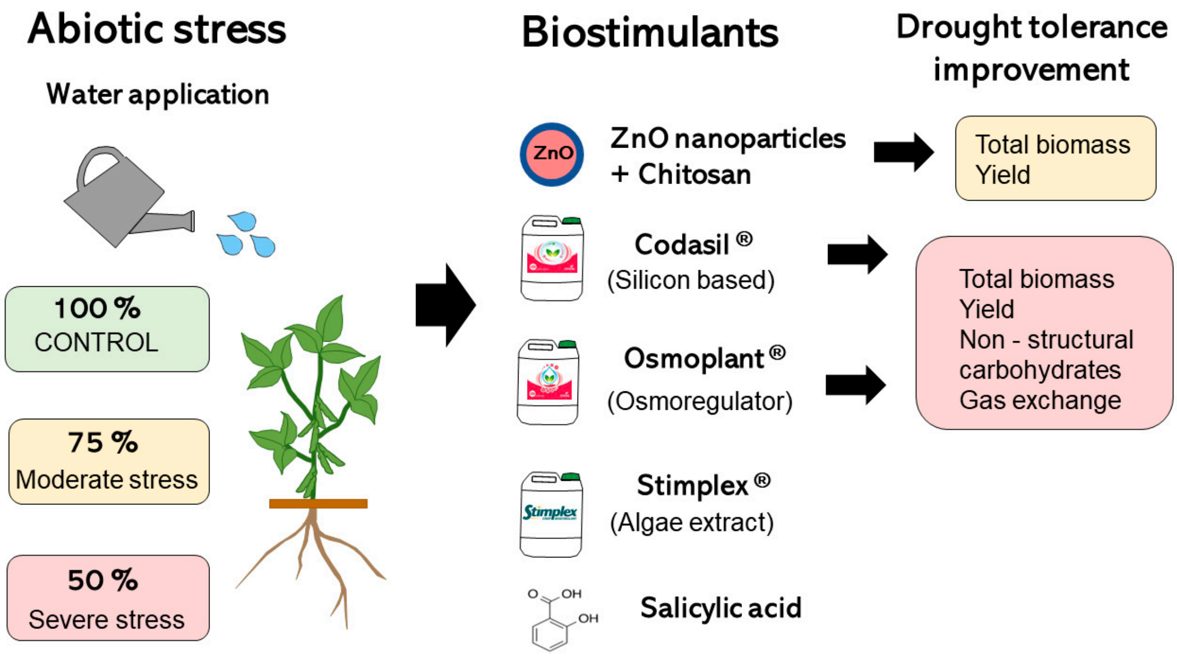

Figure 10. Graphical summary of the treatments applied and the effect on the main variables measured under moderate (FC75) and severe (FC50) stress in green bean plants cv. Strike.

4. Conclusions

According to the results obtained, it was concluded that the foliar application of biostimulants with zinc oxide nanoparticles + chitosan was the foliar treatment that most benefited biomass accumulation and yield in green bean plants cv. Strike under a moderate level of water stress (FC75). In contrast, when water stress was severe (FC50), the application of Codasil® and Osmoplant® were the treatments that benefited yield and biomass accumulation. The commercial product Stimplex® increased the accumulation of proteins and amino acids in the plant; however, the content of free sugars had a greater response with the application of zinc nanoparticles and Osmoplant®. A similar trend was found in the gas exchange parameters, where, in general, the application of zinc nanoparticles + chitosan and Osmoplant® were the treatments that stood out. These results could indicate that the use of ZnO nanoparticles accompanied by chitosan can increase tolerance to moderate water stress in green bean plants. In addition, when water stress is severe, products that focus on osmotic balance such as Osmoplant® and Codasil® make them a viable alternative to increase tolerance to this type of stress.

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Data Availability Statement: The authors declare that all data discussed in the study are available in the manuscript.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The data shown in this publication are used for academic and research purposes; therefore, none of the co-authors are related to the products or the companies that provided them.

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