Article

Effectiveness of the application of biostimulants in snap bean under water stress

Karla Ivonne Hernández-Figueroa[1]
Esteban Sánchez-Chávez[1§]
Damaris Leopoldina Ojeda-Barrios[2]
Celia Chávez-Mendoza[1]
Ezequiel Muñoz-Márquez[1]
Alejandro Palacio-Márquez[1]

[1]Delicias Unit-Center for Research in Food and Development. Av. Fourth south 3820, Fracc. Winners of the Desert, Cd. Delicias, Chihuahua, Mexico. ZC. 33089. (hernandez.karla1611@gmail.com; celia.chavez@ciad.mx; emunoz@ciad.mx; alexmarquezmj8@gmail.com). [2]Autonomous University of Chihuahua-Faculty of Agrotechnological Sciences-University *Campus* I. Chihuahua, Mexico. ZC. 31350. (dojeda@uach.mx).

[§]Corresponding author: esteban@ciad.mx.

Abstract

Water stress is one of the main factors that affect both the growth and productivity of agricultural crops. An innovative alternative to improve resistance to this water stress is the application of biostimulants. The objective of this work was to evaluate the effectiveness of biostimulants on the growth, yield, content, and water use efficiency (WUE) at different levels of water stress in the 'Strike' snap bean plant. The experiment was carried out under greenhouse conditions in Delicias, Chihuahua during the August-September period of 2021. A completely randomized experimental design was used and the treatments consisted of three types of irrigation: at 100% of field capacity (CC, for *capacidad de campo*), without water stress and at 75 and 50% of CC, in these treatments with water deficit, the biostimulants: nanoparticles of zinc oxide plus chitosan, Codasil®, Osmoplant®, Stimplex® and salicylic acid, were applied foliarly. The results obtained indicate that the best treatment applied was CC75 + nano Zn + chitosan since it favored the greater accumulation of biomass, fruit production, water content and the efficiency of water use in snap bean plants cv. Strike, which allowed it a better adaptation and tolerance to water stress compared to the treatments CC50 + Stimplex® and CC75 + Stimplex®, that probably the negative effects of water stress were greater than the benefits of the Stimplex® biostimulant applied. Finally, it is concluded that the nanoparticles of zinc oxide plus chitosan was the most efficient biostimulant to relieve and tolerate the effects of water stress, so it is considered an innovative alternative to maintain and improve the growth and production of the crop against water stress problems.

Keywords: *Phaseolus vulgaris* L., abiotic stress, drought, productivity.

Reception date: March 2022
Acceptance date: May 2022

Introduction

The frequency and intensity of drought have increased worldwide and are expected to worsen due to global climate change (Bechtold *et al*., 2018). This is the main factor that reduces crop yields and poses a threat to food security (Sytra *et al*., 2019). To avoid the effects of water deficiency, plants present alterations of several essential physiological and biochemical processes that affect their development and can limit growth and productivity (Farouk and Amany, 2012).

To counteract the harmful effects of drought on crops, the use of certain compounds with the aim of mitigating water stress has been reported, these are the so-called biostimulants, which are defined as compounds of organic or inorganic molecules and microorganism, which, usually applied in low concentrations externally in the plant, stimulate their growth, development and defense against pathogens, but mainly increase stress tolerance (Dalal *et al*., 2019).

Silicon (Si) is a relevant element in the treatment of stress in plants and is considered a biostimulant for its positive effect on the growth and development of different plant species, increasing their tolerance to biotic and abiotic stress (Szulc *et al*., 2019). This element is not considered essential for plants but is beneficial for them as it has been seen to improve primary metabolism by increasing photosynthesis and nutrient absorption, and secondary metabolism by promoting the production of phenolic compounds that favor antioxidant defense (Vega *et al*., 2019).

There is also the report of the alga *Ascophyllum nodosum* as an organism with beneficial effects for the plant, being applied to crops as a biostimulant to face stress, resulting in a higher photosynthetic yield, high levels in the use of water, increasing tolerance to the loss of it (Rosario *et al*., 2021).

Another biostimulant is zinc oxide, which is used for the regulation of plant growth and development at different levels and the improvement of tolerance of biotic and abiotic reactions (Ma *et al*., 2015). Hand in hand with the qualities of zinc oxide is the nanotechnology, in recent years its use has been of great importance in areas, including agriculture where the nanoparticles are commercialized, which, due to their size, offer a better use of agricultural inputs as they are used in smaller quantities since their size gives them greater ease of penetration through biological membranes and a greater use compared to conventional inputs (Razzaq *et al*., 2016).

A biostimulant used together with zinc oxide nanoparticles (Palacio *et al*., 2021), is Chitosan, which is used to protect plants from oxidative stress and stimulate their growth (Farouk *et al*., 2011). This compound is natural, little toxic and biodegradable, its foliar applications resulted in greater vegetative growth and an improvement in fruit quality (Ghoname *et al*., 2010).

Salicylic acid is currently considered to be a biostimulant of plant growth (Najafian *et al*., 2009), it is reported to have distinct effects including closing stomata and reducing perspiration (Larqué *et al*., 1978), increase in foliar, root and fruit biomass (Sánchez *et al*., 2011) and its application has also demonstrated adaptive responses in extreme environments, increasing its concentration when plants are subjected to stress conditions (Salinas, 2010). Therefore, the objective of the present work was to evaluate the effectiveness of the application of biostimulants in the cultivation of snap bean *cv* Strike subjected to different levels of water stress.

Materials and methods

Crop management

The experiment was carried out at the facilities of the Center for Research in Food and Development in Delicias City, Chihuahua, Mexico, during August and September 2021. The experiment was established under greenhouse conditions at an ambient average temperature of 30.8 ±4.6 °C. Seeds of snap bean (*Phaseolus vulgaris* L.) cv. Strike were used, which were grown in plastic pots of 13.4 L (two plants per pot) in a substrate mixture composed of vermiculite and perlite in a ratio of 2:1.

Each irrigation applied was carried out 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 $CuSO_4$ and 0.5 μM H_3BO_3, prepared with purified water with a pH of 6-6.1. The levels of the irrigations depended on the water stress to be considered, three levels of irrigation were used, a complete irrigation at 100% considering it as control (without water stress), a second irrigation at 75% per each liter of nutrient solution applied to the irrigation of 100% only 0.75 L was applied, and finally an irrigation at 50% where per each liter applied to the irrigation of 100% 0.5 L was applied. Five biostimulants were used in the experiment: salicylic acid, Stimplex®, Codasil®, Osmoplant® and zinc oxide nanoparticles with Chitosan, each one was applied to the plants with irrigations of 75 and 50% and plants were left with these irrigation levels without application of biostimulants for their function as a control.

Characterization of biostimulants

Five biostimulants were used in this study; the product Codasil® composed of 20% silicon, 4% free amino acids and 11.2% potassium in doses of 2 ml L^{-1} of H_2O (recommended by the manufacturer), the product Osmoplant® composed of 6% free amino acids, 2.4% nitrogen and 3.35% potassium in doses of 2 ml L^{-1} of H_2O (recommended by the manufacturer) , the product Stimplex® composed of *Ascophyllum nodosum* alga extract as its active ingredient at 0.34%, with a total nitrogen formulation 0.1% and soluble potassium (K_2O) 4% in doses of 2 ml L^{-1} of H_2O (recommended by the manufacturer), another biostimulant was zinc oxide nanoparticles (<50 nm, 99.9%) in doses of 0.1246 g L^{-1} of H_2O (100 ppm) together with chitosan (Poly-D-glucosamine) in doses of 2 ml L^{-1} of H_2O and the last compound used as a biostimulant was salicylic acid $C_7H_6O_3$ in doses of 0.0138 g L^{-1} of H_2O (0.1 mM).

Experimental design and treatments

Each experimental unit consisted of a pot with two plants, having a total of thirteen treatments (Table 1). The biostimulant treatments were applied to the experimental units from 15 days after germination and the appearance of the first true leaves, six foliar applications were made, every seven days, in the evening, for a period of two months.

Table 1. Description of treatments applied.

Treatment	Irrigation dose (%) at field capacity	Biostimulant/dose
CC100	100	None
CC75	75	None
CC75 + NPOZn + Chitosan	75	Zinc oxide nanoparticles with Chitosan/100 ppm, 100 ppm
CC75 + Codasil	75	Codasil®/commercial dose
CC75 + Osmoplant	75	Osmoplant®/commercial dose
CC75 + Stimplex	75	Stimplex®/commercial dose
CC75 + AS	75	Salicylic acid/0.1 mM
CC50	50	-
CC50 + NPOZn + Chitosan	50	Zinc oxide nanoparticles with Chitosan/100 ppm, 100 ppm
CC50 + Codasil	50	Codasil®/commercial dose
CC50 + Osmoplant	50	Osmoplant®/commercial dose
CC50 + Stimplex	50	Stimplex®/commercial dose
CC50 + AS	50	Salicylic acid/0.1mM

Plant sampling

At 60 days after germination, when the plants reached their physiological maturity, they were sampled for their analysis. The plants were divided into the aerial and root parts, then weighed, then washed, first with running water and then twice with distilled water, followed by total drying.

Vegetal analysis

Aerial biomass

The aerial biomass was evaluated considering the organs of leaves, stems and fruits of the plant, for the calculations, the fresh and dry mass of the plant was considered. To quantify the weight, an analytical balance (AND HR-120, San José, California, USA) was used. Aerial biomass was expressed in grams of dry mass.

Root biomass

The root biomass was evaluated considering only the root of the plant, for the calculations, the fresh and dry mass of the root was considered. To quantify the mass, an analytical balance (AND HR-120, San José, California, USA) was used. Root biomass was expressed in grams of dry mass.

Pod production

The yield of the plant was expressed as the average weight of the pods per plant and was expressed in grams of fresh mass.

Water content in the plant

The water content in the plant was obtained by calculating the difference between fresh mass and dry mass (Kochhar and Gujral, 2020).

Water use efficiency

Water use efficiency (WUE) was obtained by dividing the mass of dry matter harvested (grams of dry mass per treatment) by the total volume of water applied in each treatment (Trejo, 2006).

Statistical analysis

An analysis of variance of simple classification was performed, with a mean separation test by means of the Tukey method using the SAS 9.4 statistical package.

Results and discussion

Aerial biomass

The accumulated biomass content is one of the most important variables to indicate the correct functioning of the plant (Sánchez *et al*., 2016). In the present study, significant differences were found in the aerial biomass (Figure 1), with the treatment CC75 + NPOZn + Chitosan standing out with an increase of 82% compared to the treatment CC50 + Stimplex, which had the lowest values of aerial biomass.

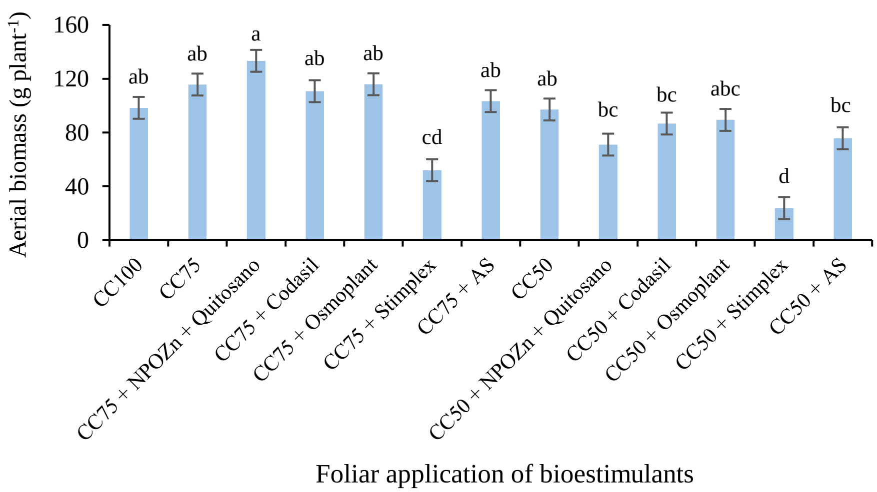

Figure 1. Effect of the application of biostimulants on the production of aerial biomass in snap bean plants *cv* Strike under water stress conditions. Means with equal letters do not differ according to the Tukey test ($p< 0.005$).

Various previous works have indicated the use of zinc oxide nanoparticles as a viable source for biomass production. Palacio *et al*. (2021) reported that the use of zinc oxide nanoparticles with chitosan in snap bean *cv* Strike promoted the higher production of biomass. Burman *et al*. (2013) applied zinc oxide nanoparticles in the chickpea culture, where they reported a biomass increase of 22.8% with respect to their control. Both works coincide with the results obtained in the production of biomass of the present work, attributing to the use of zinc oxide nanoparticles with chitosan to achieve a better production of biomass despite the conditions of water stress.

Root biomass

The results regarding this variable indicated that the treatment without application of biostimulant with irrigation at 75% of its field capacity (CC75) was the one that statistically stands out with respect to the other treatments, having an increase of 89% compared to the treatment CC50 + Stimplex®, which was the one that had the lowest values with respect to the production of root biomass (Figure 2).

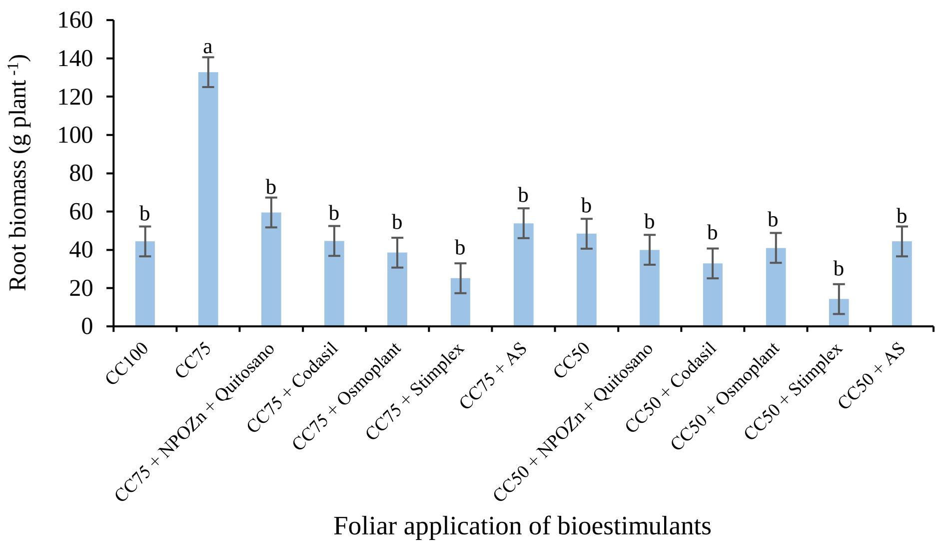

Figure 2. Effect of the application of biostimulants on the production of root biomass in snap bean plants *cv* Strike under water stress. Means with equal letters do not differ according to the Tukey test (p< 0.005).

Authors such as Barrios *et al.* (2014) point out that root growth has a fundamental relationship with aerial biomass and agricultural activities such as tillage can directly affect the production of root biomass and its functional balance with aerial biomass, they report that, under normal conditions of soil moisture, the root activity is higher when tillage is applied than when it is not, however, they point out that in periods with water deficits, this activity is higher when tillage is not done.

These results and observations agree with the present results, where the treatment CC75 (irrigation at field capacity at 75%) has a significant increase compared to the other treatments, which allows us to point out that the water stress applied to the treatment promotes root growth due to the physical and water conditions of the soil, in this case substrate.

Pod production

Significant differences were found in the production of pods due to the effect of the biostimulants applied, it is observed in Figure 3, with the treatment CC75 + Nano Zn + Chitosan standing out with the highest production of pods compared to the treatments CC75 + Stimplex® and CC50 + Stimplex®, which had the lowest values of pod production.

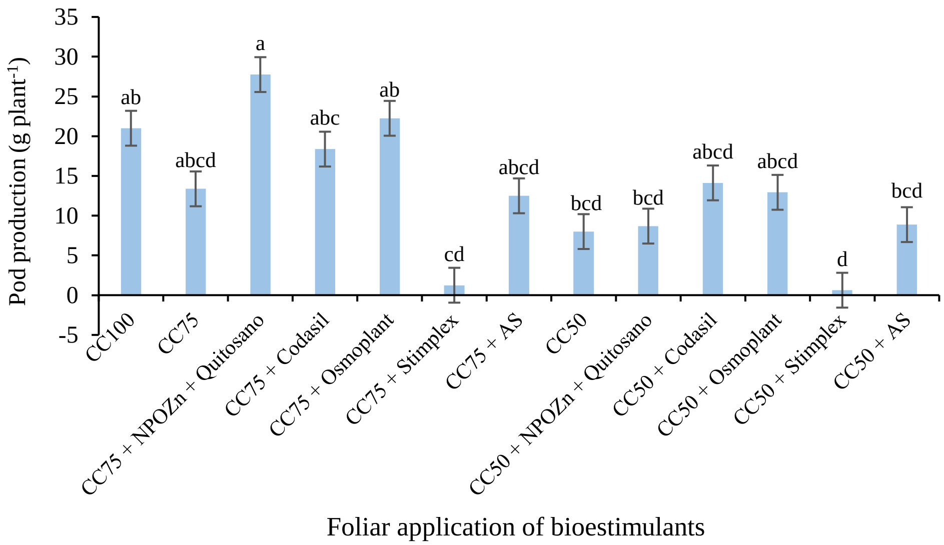

Figure 3. Effect of the application of biostimulants on the production of fruits in snap bean plants *cv* Strike under water stress. Means with equal letters do not differ according to the Tukey test ($p < 0.005$).

In the work carried out by Hassnain *et al*. (2020), it is reported that the use of chitosan increased the production of the tomato culture, both under normal conditions and under water stress, which coincides with the results of the present work. In their work with snap bean, Palacio *et al*. (2021) show favorable results of the use of zinc oxide nanoparticles, especially adding the use of chitosan, they reported that there was an increase of 21.99% when this biostimulant was applied at the dose of 100 ppm, which agrees with the results of the present work, where the treatment of zinc oxide nanoparticles with chitosan shows to be the most favorable treatment for production under water stress, even above the control (CC100).

Arciniegas (2017), in its work with *Ascophyllum nodosum* (Stimplex®) with humic and fulvic acids (Lonite®) on the yield of the rice culture (*Oryza sativa* L.), reported results where it is observed that the best yield was obtained using the two products together, the same as with the work of Sánchez (2019) in corn (*Zea mays* L.), he reported better values using Stimplex with a product together.

Due to the nature of the alga, it could be considered that its qualities as a biostimulant against water stress are of resistance, but not of production, to analyze the quality of the alga other parameters would be necessary, where its qualities against water stress can be observed as reported by Du Jardin *et al*. (2015); do Rosario *et al*. (2021), where they give as an example the photosynthetic activity and the tolerance of the plant to the loss of water.

Water content in the plant

In the present study, significant differences were found in the water content in the plant due to the effect of the application of biostimulants (Figure 4), with the treatment CC75 + Nano Zn + Chitosan standing out with the highest water contents compared to the treatments CC75 + Stimplex® and CC50 + Stimplex®, which had the lowest foliar water contents.

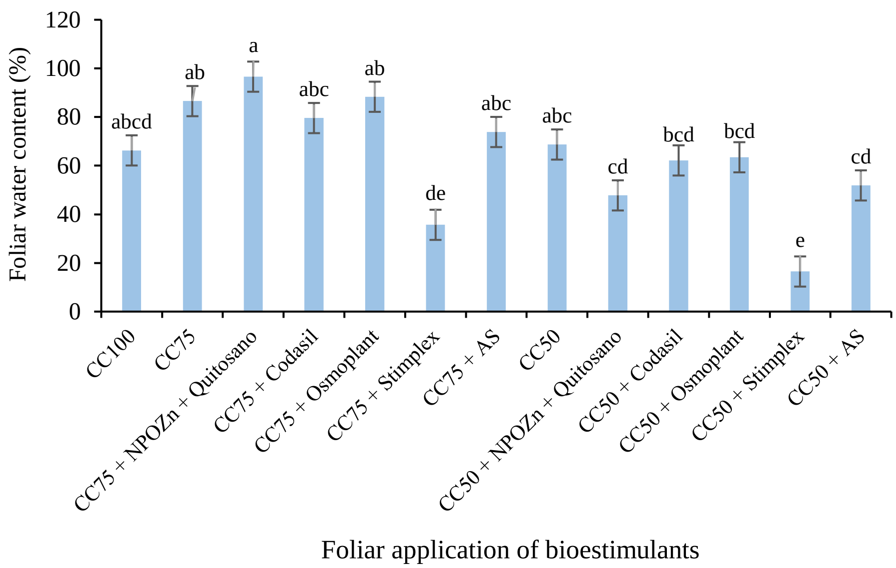

Figure 4. Effect of the application of biostimulants on the water content in aerial organs in snap bean plants *cv* Strike under water stress. Means with equal letters do not differ according to the Tukey test ($p< 0.005$).

Medrano *et al*. (2007) reports that the water content in the plant has a relationship with its optimal yield, since it influences as a positive correlation, the more water is available, despite the environmental resources, the plant has greater size, yield, they mention that for the good water content in the plant, external factors such as temperature, light hours and the availability of nutrients influence, however, they mention that having an optimal availability of water, the plant overcomes obstacles such as the previous ones and achieves the ideal uptake for its development. These results are comparable to those presented in this work, since the production of aerial biomass in the outstanding treatments coincides with the highest values of the analysis of the water content, which would be the treatment CC75 + NPOZn + Chitosan.

With respect to the root water content, significant differences were found due to the effect of the biostimulants applied, it is presented in Figure 5, with the treatment CC75 + Nano Zn + Chitosan standing out with the highest root water content compared to the treatments CC75 Stimplex® and CC50 Stimplex®, which had the lowest root water contents, behaving similarly to the foliar water content (Figure 4).

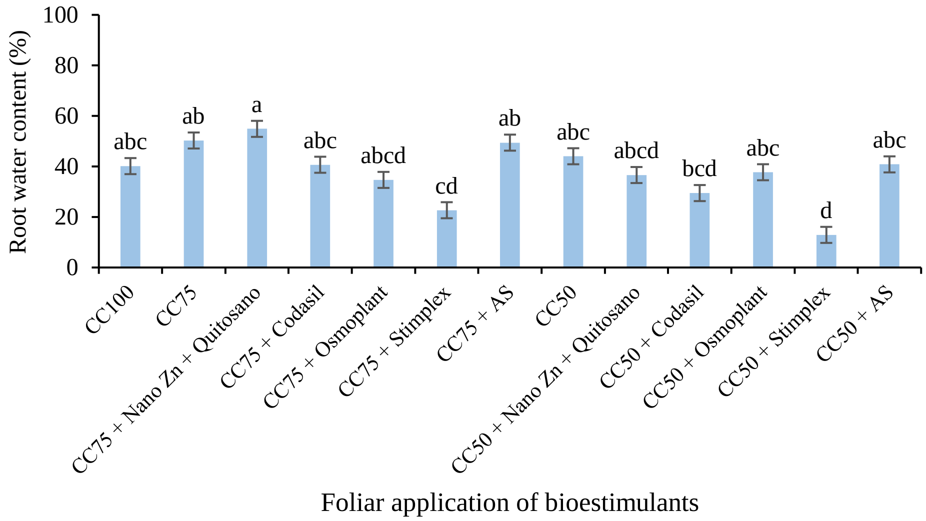

Figure 5. Effect of the application of biostimulants on the root water content in snap bean plants *cv* Strike under water stress. Means with equal letters do not differ according to the Tukey test ($p <$ 0.005).

Water use efficiency (WUE)

The WUE was expressed in g of pod production between the L of water applied, the treatment with field capacity of 100% was applied 31.5 L in total during the 60 days of cultivation, the treatments with irrigation doses of 75% was applied 23.63 L in total and the treatments subjected to an irrigation of 50% were applied 15.75 L. In the present study, significant differences were found in the WUE due to the effect of the biostimulants applied (Figure 6), with the treatments CC75 + Stimplex® and CC75 + Nano Zn + Chitosan standing out with the greater WUE compared to the treatments CC75 Stimplex® and CC50 Stimplex®, which had the lowest WUE, behaving similarly to the water content in the plant (Figure 4 and 5).

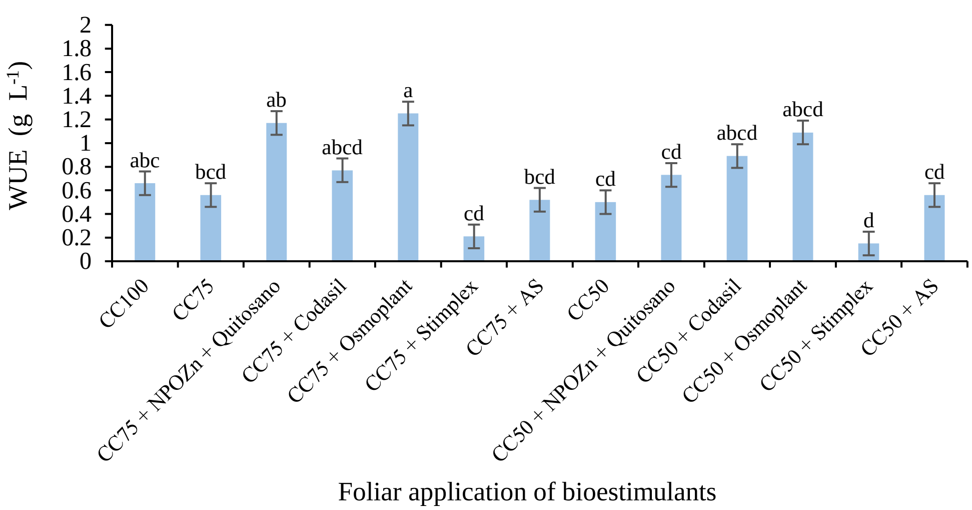

Figure 6. Effect of the application of biostimulants on water use efficiency (WUE) in snap bean plants *cv* Strike under water stress. Means with equal letters do not differ according to the Tukey test ($p <$ 0.005).

WUE involves different plant processes, both physiological and metabolic. Regarding the physiological ones, the levels of water use depend on the production of biomass, on the availability found in the soil, its storage capacity, the density, and depth of the root system directly influence, other factors mentioned above depend on this. Faced with the problem of water stress, the plant employs constitutive behaviors of the species (adaptations), such as the synthesis of abscisic acid, the decrease in stomatal conductance, which in turn leads to limitations of photochemical reactions, in the Calvin cycle and the transport of assimilates (Medrano *et al*., 2007).

It can be seen in Figure 6 that the treatments under irrigation of CC 75% with application of biostimulants (CC75 + Stimplex® and CC75 + Nano Zn + Chitosan) are the ones that have better efficiency in terms of the use of irrigation water compared to the treatments CC50% with application of biostimulants, especially the treatments CC75 Stimplex® and CC50 Stimplex®, which are less efficient in the use of water, because they are subject to greater water stress and the mechanisms used are not sufficient to tolerate such stress; that is, they use their physiological and metabolic processes to survive as a basic measure for resistance to the detriment of biomass accumulation and pod production.

Previous studies have shown that the application of biostimulants play an important role in tolerance to water stress (Colla and Rouphael, 2015). In the present study, the treatment of CC75 + Nano Zn + Chitosan was the one that improved the accumulation of biomass, production of pods, water content in the plant, as well as the efficiency of water use in snap bean plants cv. Strike, compared to the treatments CC50 + Stimplex® and CC75 + Stimplex®, which had the lowest values and were therefore the least effective treatments in tolerating water stress.

The explanation of why the treatment CC75 + Nano Zn + Chitosan was better than the treatments CC50 + Stimplex® and CC75 + Stimplex® could be due to the physiological and biochemical mechanisms and responses related to tolerance to water stress. In the case of physiological responses, these involve: 1) loss of turgor and osmotic adjustment; 2) reduced transpiration rate due to stomatal closure (low stomatal conductance); 3) reduced internal CO_2 concentration; 4) reduced photosynthetic rate; and 5) reduction in growth (Onaga and Wydra, 2016).

With respect to biochemical responses, these include: i) decrease in photochemical efficiency; ii) decrease in Rubisco activity; iii) accumulation of metabolites such as glutathione, proline, glycine betaine and polyamines; iv) increase in antioxidant enzymes: and v) decrease in the accumulation of reactive oxygen species (Onaga and Wydra, 2016). It is considered that, in the case of the best treatment applied, such is the case of CC75 + Nano Zn + Chitosan, it was probably favored in the physiological and biochemical responses, which allowed it a better adaptation and tolerance to water stress compared to the treatments CC50 + Stimplex® and CC75 + Stimplex®, that the negative effects of water stress were probably greater than the benefits of the Stimplex® biostimulant applied.

Conclusions

The results obtained indicate that the best treatment applied was CC75 + Nano Zn + Chitosan since it favored the greater accumulation of biomass, fruit production, water content and water use efficiency in snap bean plants cv. Strike, which allowed it a better adaptation and tolerance to water stress compared to the treatments CC50 + Stimplex® and CC75 + Stimplex®, that the negative effects of water stress were probably superior to the benefits of the Stimplex® biostimulant applied. Finally, it is concluded that the nanoparticles of zinc oxide plus chitosan was the most efficient biostimulant to relieve and tolerate the effects of water stress, so it is considered an innovative alternative to maintain and improve the growth and production of the crop against water stress problems.

Cited literature

Arciniegas, B. S. R. 2017. Efecto de la aplicación de *Ascophyllum nodosum* con ácidos húmicos y fúlvicos, sobre el rendimiento del cultivo de arroz (*Oryza sativa* L.), bajo riego, en la zona de Babahoyo. Universidad Técnica de Babahoyo. Facultad de Ciencias Agropecuarias. 30-31 pp.

Barrios, M. B.; Buján, A.; Debelis, S. P.; Sokolowski, A. C.; Blasón, Á. D.; Rodríguez, H. A.: López, S. C.; De Grazia, J.: Mazo, C. R. y Gagey, M. C. (2014). Relación de raíz/biomasa total de Soja (*Glycine max*) en dos sistemas de labranza. Terra Latinoam. 32(3):221-230.

Bechtold, U. and Field, B. 2018. Molecular mechanisms controlling plant growth during abiotic stress. J. Exp. Bot. 69(11):2753-2758.

Burman, U.; Saini, M. and Kumar, P. 2013. Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. Toxicol. Environ. Chem. 95(4):605-612.

Colla, G. and Rouphael, Y. 2015. Biostimulants in horticulture. Sci. Hortic. 196(30):1-134.

Dalal, A.; Bourstein, R.; Haish, N.; Shenhar, I.; Wallach, R. and Moshelion, M. 2019. Dynamic physiological phenotyping of drought-stressed pepper plants treated with 'productivity-enhancing' and 'survivability-enhancing' biostimulants. Front. Plant Sci. 905(10):2-3.

Farouk, S.; Mosa, A. A.; Taha, A. A.; Ibrahim, H. M. and Gahmery, A. M. 2011. Protective effect of humic acid and chitosan on radish (*Raphanus sativus* L. var. Sativus) plants subjected to cadmium stress. J. Stress physiol. Biochem. 7(2):99-116.

Farouk, S. and Amany, A. R. 2012. Improving growth and yield of cowpea by foliar application of chitosan under water stress. Egypt. Acad. J. Biol. Sci. 14:14-26.

Ghoname, A. A.; Nemr, M. A.; Abdel, M. A. M. R. and Tohamy, W. A. 2010. Enhancement of sweet pepper crop growth and production by application of biological, organic, and nutritional solutions. Res. J. Agric. Biol. Sci. 6(3):349-355.

Hassnain, M.; Alam, I.; Ahmad, A.; Basit, I.; Ullah, N.; Alam, I. and Shair, M. M. 2020. Efficacy of chitosan on performance of tomato (*Lycopersicon esculentum* L.) plant under water stress condition. Pak. J. Agric. Res. 33(1):27-41.

Du Jardin, P. 2015. Plant biostimulants: definition, concept, main categories and regulation. Sci. Hortic. 196(30):3-14.

Kochhar, S. L. and Gujral, S. K. 2020. Plant physiology -theory and applications. 2nd (Ed.). Cambridge University Press. Cambridge, United Kingdom. 702 p.

Larqué, S. A. 1978. The antitranspirant effect of acetylsalicyc acid on *Phaseolus vulgaris*. Physiol. Plant. 43(2):126-128.

Ma, Y.; Zhang, P.; Zhang, Z.; He, X.; Li, Y.; Zhang, J. and Zhao, Y. 2015. Origin of the different phytotoxicity and biotransformation of cerium and lanthanumoxide nanoparticles in cucumber. Nanotoxicology. 9(2):262-270.

Medrano, H.; Bota, J.; Cifre, J.; Flexas, J.; Ribas, C. M. and Gulías, J. 2007. Eficiencia en el uso del agua por las plantas. Investigaciones Geográficas. (43):63-84.

Najafian, S.; Khoshkhui, M.; Tavallali, V. and Saharkhiz, M. J. 2009. Effect of salicylic acid and salinity in thyme (Thymus vulgaris L.): Investigation on changes in gas exchange, water relations, and membrane stabilization and biomass accumulation. Australian J. Basic Appl. Sci. 3(3):2620-2626.

Onaga, G. and Wydra, K. 2016. Advances in plant tolerance to abiotic stresses. Plant Genomics. 10(9):229-272.

Palacio, M. A.; Ramírez, E. C. A.; Gutiérrez, R. N. J.; Sánchez, C. E..; Ojeda, B. D. L.; Chávez, M. C. and Sida, A. J. P. 2021. Efficiency of foliar application of zinc oxide nanoparticles versus zinc nitrate complexed with chitosan on nitrogen assimilation, photosynthetic activity, and production of green beans (*Phaseolus vulgaris* L.). Sci. Hortic. 288(10):110297.

Rosário, R. V.; Santos, A. L.; Silva, A. A.; Sab, M. P. V.; Germino, G. H.; Cardoso, F. B. and Almeida, S. M. 2021. Increased soybean tolerance to water deficiency through biostimulant based on fulvic acids and *Ascophyllum nodosum* (L.) seaweed extract. Plant physiol. Biochem. 158(11):228-243.

Razzaq, A.; Ammara, R.; Jhanzab, H. M.; Mahmood, T.; Hafeez, A. and Hussain, S. 2016. A novel nanomaterial to enhance growth and yield of wheat. J. Nanosci. Technol. *2*(1):55-58.

Sánchez, B. J. J. 2019. Efecto del *Ascophyllum nodosum* combinado con ácidos húmicos y fúlvicos, sobre el rendimiento del cultivo de maíz (*Zea mays* L.). Universidad Técnica de Babahoyo, Facultad de Ciencia Agropecuarias. 29-32 pp.

Sánchez-Chávez, E.; Barrera-Tovar, R.; Muñoz-Márquez, E.; Ojeda-Barrios, D. L. y Anchondo-Nájera, Á. 2011. Efecto del ácido salicílico sobre biomasa, actividad fotosintética, contenido nutricional y productividad del chile jalapeño. Rev. Chapingo. Ser. Hortic. 17(SPE1):63-68.

Sánchez, C. E.; Ruiz, J. M. and Romero, M. L. 2016. Compuestos nitrogenados indicadores de estrés en respuesta a las dosis tóxicas y deficientes de nitrógeno en frijol ejotero. Nova Sci. 8(16):228-244.

Sánchez, C. E.; Barrera, T. R.; Muñoz, M. E.; Ojeda, B. D. L. y Anchondo, N. Á. 2011. Efecto del ácido salicílico sobre biomasa, actividad fotosintética, contenido nutricional y productividad del chile jalapeño. Rev. Chapingo. Ser. Hortic. 17(1):63-68.

Sytar, O.; Kumari, P.; Yadav, S.; Brestic, M. and Rastogi, A. 2019. Phytohormone priming regulator for heavy metal stress in plants. J. Plant Growth Regul. 38(2):739-752.

Szulc, W.; Rutkowska, B.; Hoch, M.; Ptasinski, D. and Kazberuk, W. 2019. Plant available silicon in differentiated fertilizing conditions. Plant, Soil Environ. 65(5):233-237.

Trejo, J. A. M.; Monsivais, A. O. G.; Ramírez, J. O.; González, A. Z.; Cerda, E. R.; Hernández, M. F. y Nuncio, R. A. 2006. Efecto de tres profundidades de cinta de riego por goteo en la eficiencia de uso de agua y en el rendimiento de maíz forrajero. Rev. Mex. Cienc. Pec. 44(3):359-364.

Vega, I.; Nikolic, M.; Pontigo, S.; Godoy, K.; Mora, M. D. L. L. and Cartes, P. 2019. Silicon improves the production of high antioxidant or structural phenolic compounds in barley cultivars under aluminum stress. Agronomy. 9(7):388-403.