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Effect of biostimulant application on the water footprint of common beans (*Phaseolus vulgaris* L.)

Efecto de la aplicación de bioestimulantes en la huella hídrica del frijol (Phaseolus vulgaris L)

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ABSTRACT: With the aim of estimating the water footprint of beans (*Phaseolus vulgaris* L.) subjected to two irrigation regimes and biostimulant applications. Seeds of the Triunfo 70 black bean variety were used and planted in 18 concrete containers. The treatments consisted of applying 100% of the ETc (crop evapotranspiration) in standard conditions to 9 containers and 75% to the other 9. Before sowing, the seeds in all containers were inoculated with Azofert®-bean (A), and Pectimorf® (P) was added to 12 containers (six from each irrigation variant) together with Azofert®-bean, and six (three from each irrigation variant) were sprayed with Quitomax® (Q) at the beginning of flowering. The treatments in which 75% of the ETc was replaced were covered with transparent polyethylene sheets to prevent rainfall. The results indicate that the application of both Pectimorf® to the seed and Quitomax® as a foliar spray at the beginning of flowering promotes the growth and yield of the bean crop, as well as helping to maintain better water status in the plants. Both products improved the plants' water use efficiency and contributed to reducing the water footprint by almost 20% compared to the control treatment. Quitomax® showed signs of exerting an antitranspirant action on the plants.
Keywords: Soil Moisture, Relative Water Content, Leaf Water Potential, Growth, Yield.

RESUMEN: El trabajo se realizó con el objetivo de estimar la huella hídrica del frijol (*Phaseolus vulgaris* L.) sometido a dos regímenes de riego y aplicaciones de bioestimulantes. Se utilizaron semillas de la variedad de frijol negro Triunfo 70 sembradas en 18 contenedores. Los tratamientos consistieron en aplicar en 9 contenedores el 100 % de la ETc (Evapotranspiración del cultivo) en condiciones estándares y en los otros 9 al 75 %. Antes de la siembra todas las semillas de todos los contenedores fueron inoculadas con Azofert®-frijol (A), a 12 contenedores (seis de cada variante de riego) se le adicionó Pectimorf® (P) junto con el Azofert®- frijol y a seis (a tres de cada variante de riego) se le asperjó el Quitomax® (Q) al inicio de la floración. Los tratamientos en los que se les repuso el 75 % de la ETc fueron cubiertos con mantas de polietileno transparente para evitar la incidencia de las precipitaciones. Los resultados indican que, la aplicación tanto de Pectimorf® en la semilla, como de Quitomax® en aspersión foliar al inicio de la floración favorecen el crecimiento y el rendimiento del cultivo del frijol, así como, contribuyó a mantener un mejor estado hídrico en las plantas. Ambos productos mejoraron la eficiencia en el uso del agua por las plantas y contribuyeron a disminuir la huella hídrica, en casi un 20 % respecto al tratamiento control. El Quitomax®, mostró señales de ejercer una acción antitranspirante en las plantas.
Palabras clave: humedad del suelo, contenido relativo de agua, potencial hídrico foliar, crecimiento, rendimiento.

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INTRODUCTION

Bean cultivation (*Phaseolus vulgaris*) is considered a priority in the grain production program for import substitution carried out by the Cuban government (Cisneros *et al.*, 2020).

It is the most significant legume in human consumption, constituting an indispensable nutritional supplement in the daily diet of more than 300 million people worldwide and an important element in agricultural production systems. Its grain is considered essential, not only for its nutritional and culinary properties, but also for its presence on all five continents and its value for the rural and social development of many economies (Magaña *et al.*, 2015).

It is often grown in low-fertility soils, which reduces crop yields (Beaver *et al.*, 2021). In Cuba, much of the production of this grain takes place during the dry season, which requires water to be supplied through irrigation.

Climate change is one of the most studied phenomena in the current era given the strong impact it can have on agriculture, mainly due to the occurrence of low rainfall (Ottaiano *et al.*, 2021).

Cuba, as a long and narrow archipelago, faces significant limitations in its water reserves, since rainfall is the main source of supply to guarantee the precious liquid required for the nation's development (Girón *et al.,* 2015).

Water stress is the physiological response of plants to water deficit in the soil, and it affects the balance between transpiration and water absorption (Girón *et al.,* 2015). Under conditions of water stress, crop growth decreases in proportion to the severity and magnitude of the stress condition (Rodríguez-Larramendi *et al.*, 2021).

Irrigation management determines when and how much to irrigate, based on crop water needs, soil characteristics, and climatic conditions. However, failure to use irrigation scheduling adjusted to climate, soil, and crop characteristics is one of the main causes of excessive irrigation water use (González-Cueto *et al.*, 2020).

The use of biostimulants to increase crop productivity has become established in conventional agricultural practice.

With the aim of making production systems more efficient, various agrochemical industries market different nutrient complexes containing micronutrients, amino acids, plant extracts, and/or phytohormones, which have been termed growth promoters or biostimulants (Winkler *et al.*, 2017; Rouphael y Colla, 2020).

The wide range of biostimulants offers a biotechnological alternative because it promotes plant growth and development, improves their metabolism, and protects them against biotic and abiotic stresses (Van Oosten et al., 2017; Sanches *et al.*, 2019).

Azofert®-bean is an inoculant for legumes that contains native species of rhizobia capable of fixing atmospheric nitrogen, which is used by plants. Unlike other inoculants, it induces high concentrations of nodulation factors in the bacteria, which enhance their nodulation action and their efficiency in biological nitrogen fixation.

Quitomax® is a biostimulant based on a mixture of chitosan polymers, whose active ingredient has been attributed with properties such as antiperspirant Bittelli *et al.* (2001); Iriti *et al.* (2009), responding with particular reference to abiotic stress conditions (Hidangmayum *et al.*, 2019).

Pectimorf® consists of a mixture of pectic oligosaccharides that has been shown to have a positive effect on the development of the root system of plants Posada-Pérez et al. (2016); Sáez-Cigarruista et al. (2024) and has also been shown to have some potential for improving the water use efficiency of plants (Dell Amico et al., 2017).

The water footprint Hoekstra *et al.* (2011), is an indicator that has recently gained importance as a result of the scarcity of fresh water for various activities and processes.

In agriculture, it is expressed as the volume of water used to produce a unit of product ($m^3\ t^{-1}$). Its analysis considers the amount of water applied through irrigation (blue), rainfall (green), and water used to dilute wastewater or fertilizers to levels tolerable by plants (gray).

It consists of three components: the blue water footprint (corresponding to the amount of water applied through irrigation), the green water footprint (amount of water used from rainfall), and the gray water footprint (which considers the fertilizer applied as a pollutant, mainly nitrogen).

Based on the above elements, this study was developed with the aim of estimating the water footprint of bean cultivation under different soil water supply conditions and evaluating the effect of using the aforementioned biostimulants.

MATERIALS AND METHODS

This study was conducted at the National Institute of Agricultural Sciences (INCA, 22º58′00″N and 82°09′00″W and 130 m above sea level). For this purpose, 18 concrete containers measuring 2.60 m long by 0.60 m wide (1.56 m^2) containing leached red ferralitic soil from the province of Mayabeque according Hernández-Jiménez *et al.* (2019), an area that forms part of the Habana-Matanzas karst plain (Magaña *et al.*, 2015).

In each container, Triunfo 70 bean seeds were sown in two rows with a spacing of 0.40 m between rows and 0.10 m between plants (52 plants per container).

The treatments used consisted of applying water corresponding to 100% of the ETc (crop evapotranspiration) in nine containers and 75% in another nine. Before sowing, the seeds in all containers were inoculated with Azofert®- bean (A), which has been considered part of the crop technology. In twelve of them (six from each irrigation variant), Pectimorf (P) was applied together with Azofert®-bean before sowing, and in six of these (three from each irrigation variant), Quitomax®(Q) was applied by foliar spraying at the beginning of flowering. This resulted in the following treatments: 100 A (control); 100 AP; 100 APQ; 75 A (control); 75 AP and 75 APQ.

Each treatment had three replicates (containers) arranged in continuous lines to facilitate irrigation, taking into account the characteristics of the installed system.

Irrigation was applied using an automated micro-sprinkler system, and water delivery was controlled by valves conveniently located on the irrigation sides of each treatment.

The pH and electrical conductivity values of the water applied to the crop during the experiment were 7.8 and 0.58 dS/m, respectively. For this type of soil (leached red ferralitic), classified as category I, there are no limitations on its use for irrigation. The pH reached is within the permissible range for irrigation (4.8 to 8.3) according to Cuban standard (NC 1048, 2014)..

To avoid the effect of rainfall or dew, in the treatments with 75% of the ETc, a transparent polyethylene blanket was placed over the plants without making contact with them.

Irrigation consisted of replenishing the crop evapotranspiration (ETc) accumulated between each irrigation, which was carried out three times a week (Monday, Wednesday, and Friday).

Before sowing, 300 kg $^{ha^{-1}}$of 23-12-17-5 fertilizer was applied. Other cultural practices were carried out as established in the Technical Guide for Bean Cultivation in Cuba (Alfonso, 2000).

The reference evapotranspiration ETo (mm) and crop evapotranspiration ETc (mm) were obtained using the CropWat.8.0 program. This program was updated with a 34-year historical series of meteorological data (1990-2024) from the Tapaste weather station, which belongs to the National Institute of Meteorology and is located about 200 m from the experimental site. Monthly average values were used to calculate ETo and ETc.

Effective precipitation was determined according to the USDA (United States Department of Agriculture, Soil Conservation Service) method, as this is the method most recommended by the FAO. The crop coefficients (Kc, initial = 0.15, Kc, average = 1.10, and Kc, final = 0.65) proposed for the region (Allen *et al.*, 2006). were used.

To estimate the blue footprint, the amount of water applied through irrigation was considered; in the case of the green footprint, rainfall contributions were considered according Hoekstra *et al.* (2011); and for the gray footprint, only the pollutant load of the fertilizer applied was considered.

The evaluations consisted of determining soil moisture using the gravimetric method, the relative water content before 7 solar hours according Turner (1981), the relative chlorophyll content with an SPAD (Soil Plant Analysis Development) meter, for which 15 samples were taken per treatment, and the leaf water potential measured at 11 hours with a Scholander pressure chamber (9 replicates per treatment). These variables were determined before applying replacement irrigation. The length and diameter of the stems, the dry mass of the stems and leaves, the leaf area (18 plants per treatment), the number of pods per plant and grains per pod, and the mass of 100 grains were also measured to estimate yield. The results obtained were used to estimate the water footprint of each of the treatments used.

Data analysis was performed using the Statgraphics Plus 5 statistical package, and means were compared using Tukey's multiple range test. Sigma Plot 11 software was used to graph the data.

RESULTS AND DISCUSSION

When evaluating the behavior of plants under semi-controlled conditions treated with different biostimulants and different water supplies through irrigation (Table 1), differences were found between the treatments studied. It can be seen that the treatments to which the two biostimulants were applied generally showed higher values than those achieved by the plants in the control treatments. Similarly, when Quitomax® was added, the highest values were achieved in each of the soil water supply conditions. This response is associated with the better water status of the plants when this biostimulant was added, which has been attributed, among other properties, with improving plant growth.

When Pectimorf® was applied, improved plant growth was also observed, which is consistent with the positive effect of this biostimulant on root system development according Sáez-Cigarruista et al. (2024),which promotes the absorption of water and nutrients available in the soil.

These results reaffirm those reported by other authors in studies conducted on grapevine cultivation (*Vitis vinifera* L.) when analyzing the morphological and physiological behavior of plants treated with chitosan under conditions of soil water deficit according Khalil y Badr Eldin (2021), in the vegetative growth of *Reutealis trisperma* under water deficiency conditions Irawati *et al.* (2019), as well as in *Gliricidia sepium* (Valverde-Otárola y Arias, 2020).

Table 1. Components of bean plant growth treated with biostimulants and subjected to two irrigation regimes. Different letters indicate significant differences between treatments for α ≤ 0.05 according to Tukey's multiple range test.

Treatments	Stem length (cm)	Stem diameter (mm)	Stem dry weight (g)	Dry mass of leaves (g)	Leaf area (cm²)
100 A (control)	24.8 a	4.31 b	1.19 ab	1.91 ab	4336.76 b
100AP	21.4 ab	4.86 ab	1.27 ab	2.05 a	5279.31 ab
100 APQ	23.6 a	4.92 a	1.46 to	2.16 a	6450.91 a
75 A (control)	18.8 b	4.33 b	1.12 b	1.59 b	3429.36 b
75 AP	24.4 a	4.57	1.26 ab	1.94 ab	4895.85 ab
75 APQ	24.0 a	4.97 a	1.38 ab	2.11 a	5167.84 ab
ESx	1.28*	0.26	0.14	0.15	701.66

Soil moisture (Figure 1), like growth, showed the highest values when chitosan was applied, followed by the treatment that received Pectimorf® at the time of sowing, but without statistical difference between them. The treatment to which Pectimorf®) was applied and the controls showed the same behavior, although the most noticeable response was between the plants in the control treatments and those that received the application of Quitomax®.

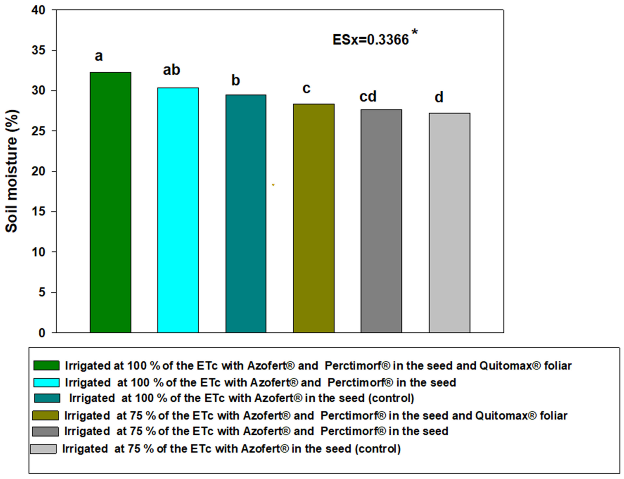

Figure 1. Soil moisture in bean plants treated with biostimulants and subjected to two irrigation regimes. Different letters indicate significant differences between treatments for α ≤ 0.05 according to Tukey's multiple range test.

The differences between the levels of water supply applied to the soil are noteworthy, ensuring the presence of two very different treatments.

The unequal behavior between treatments with the same water supply level is due, first of all, to the ability of Pectimorf® to stimulate root system growth Sáez-Cigarruista et al. (2024), which allows it to absorb water from areas where unstimulated plants cannot reach it, or also through the lower transpiration of plants treated with chitosan according Bittelli *et al.* (2001) e Iriti *et al.* (2009), which leads to lower water absorption by plants.

The relative chlorophyll content (Figure 2) showed no significant differences between the treatments studied, demonstrating that the plants maintained a very similar nutritional status between the treatments used, particularly with regard to nitrogen, which ensures that this factor did not cause any variation in plant behavior.

Determinations of relative chlorophyll content are currently widely used to quickly and non-destructively determine the chlorophyll levels present in plant leaves. These measurements are closely related to their nutritional status, mainly with regard to nitrogen (Lopez-Bellido et al., 2004).

Most of the nitrogen (N) in leaves is incorporated into chlorophyll. Therefore, quantifying the chlorophyll content provides an indirect measure of the N level in the leaves (Loayza *et al.*, 2022).

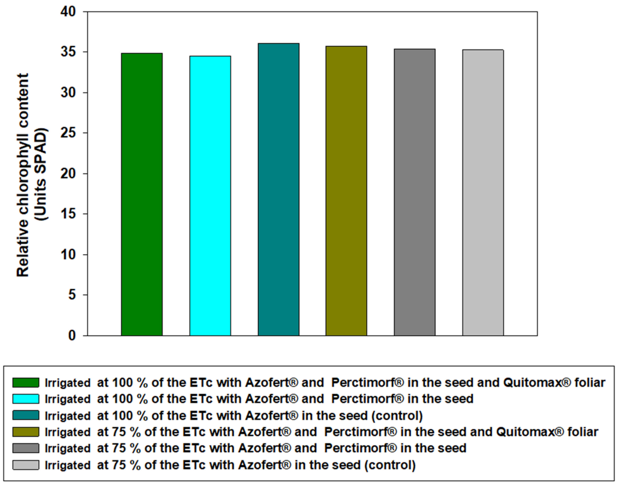

Figure 2. Relative chlorophyll content in bean plants treated with biostimulants and subjected to two irrigation regimes. Different letters indicate significant differences between treatments for α ≤ 0.05 according to Tukey's multiple range test.

This is a very important element for plant development. It is used to produce the amino acids that form part of proteins, enzymes, and chlorophylls. It is essential in cell constitution and is one of the basic components of DNA.

The relative water content represents the degree of water saturation of the plant under a given soil water supply condition. As is well known, it is closely related to processes such as transpiration and photosynthesis, especially transpiration, which determines the absorption of water and nutrients into the plant, promoting the other processes involved in plant physiology.

Figure 3 shows the behavior of this variable in each of the treatments used. It shows differences between the treatments with the best water supply and those that received only 75% of the ETc, as well as between the variants that were treated with the two biostimulants and those that were only treated with Pectimorf® and the controls.

This response coincides with what has been proposed about the possible effects of the chitosans used and their antitranspirant capacity by Berliana *et al.* (2020) and Jawad & Al-Shammari (2023) which allowed the plants to maintain a better water status compared to the control.

Measuring water potential is a useful tool in irrigation planning, as it is directly associated with the water status of the plant. Knowing its critical threshold is a tool for determining when and how much to irrigate.

Figure 4 shows the behavior of water potentials measured in uncovered leaves of bean plants. It can be seen that the potential was lower in plants that were subjected to a water deficit in the soil, a condition that leads to a reduction in water potential in plant tissues (Avila *et al.*, 2020).

It can also be seen that the leaf water potential, like the relative water content, indicates differences in water status when the plants were treated with biostimulants, with marked superiority in those sprayed with Quitomax®.

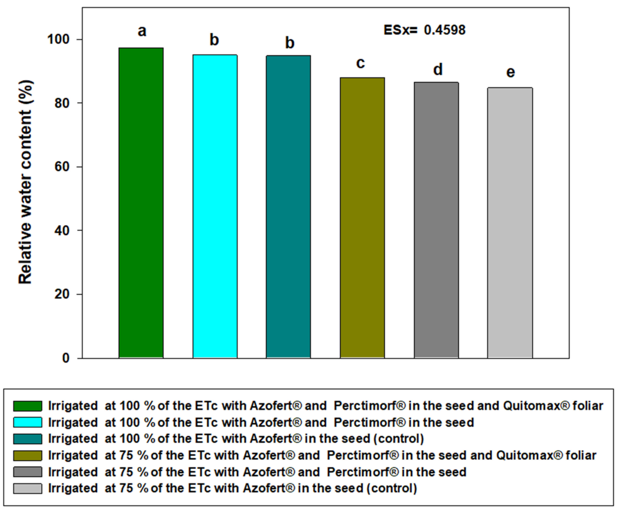

Figure 3. Relative water content in bean plants treated with biostimulants and subjected to two irrigation regimes. Different letters indicate significant differences between treatments for α ≤ 0.05 according to Tukey's multiple range test.

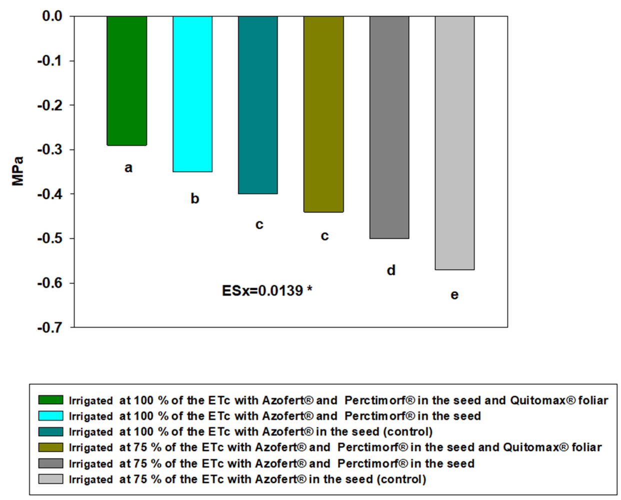

Figure 4. Leaf water potential in bean plants treated with biostimulants and subjected to two irrigation regimes. Different letters indicate significant differences between treatments for α ≤ 0.05 according to Tukey's multiple range test.

This response is associated with its ability to elicit defense mechanisms in response to abiotic stress conditions. In this regard, it has been noted that treatment with chitosan stimulates the photosynthetic rate and stomatal closure through the synthesis of ABA according Hidangmayum *et al.* (2019), a mechanism that controls water loss by the plant through transpiration (García-León et al., 2019).

This observation may help to understand the above-mentioned antitranspirant capacity of chitosan found by other authors Bittelli *et al.* (2001); Iriti *et al.* (2009); Berliana *et al.* (2020); Jawad y Al-Shammari (2023).

The analysis of yield and its components reaffirms the results obtained in the variables evaluated above. Plants that received 100% and were under the effect of biostimulants showed higher yields than the rest of the treatments. This response is due to the water supply conditions to which they were exposed, together with the biostimulating effect that Pectimorf® and Quitomax® have on crops.

In other studies, conducted on this crop using these biostimulants, yield increases have been obtained compared to those achieved in plants that were not treated with them, demonstrating their potential to stimulate the mechanisms that contribute to yield formation (Dell Amico et al., 2017; Morales-Guevara et al., 2017; Romero-Félix *et al.*, 2019).

In studies conducted on other crops under conditions of soil water deficiency, increases in yields and improvements in quality have also been found with the application of chitosan (Tawaha *et al.*, 2020). In this regard, it has been suggested that this compound induces tolerance to water deficit because it causes regulations in the process of photosynthesis and the production of primary metabolites, osmoregulators, and antioxidants (Ávila *et al.*, 2023).

As can be seen in the table, the blue water footprint accounts for the largest share, indicating that rainfall was insufficient to meet the crop's needs.

It should be noted that in treatments not covered with polyethylene sheets, effective rainfall contributed 25% of the water required by the crop.

It is also noteworthy that the application of Pectimorf® to the seed and the subsequent addition of Quitomax® by foliar spraying at the beginning of flowering contributed to an 18% reduction in the water footprint compared to the respective controls.

Table 2. Yield and its components in bean crops treated with biostimulants and subjected to two irrigation regimes. Different letters indicate significant differences between treatments for α ≤ 0.05 according to Tukey's multiple range test.

Treatments	Pods per plant	Beans per pod	Beans per plant	Mass of 100 grains (g)	Estimated estimated (t ha^{-1})
100 A (control)	10.81 bc	5.63 bc	62.24 b	18.56 ab	1.65 b
100 AP	11.36 b	5.77 ab	65.13 b	18.28 abc	1.71 b
100 APQ	12.59 a	6.04 a	76.13 a	18.59 a	2.03 a
75 A (control)	9.45 d	5.40 c	52.00 c	18.17 bc	1.33 c
75 AP	9.78 cd	5.66 bc	59.26 bc	17.08 d	1.52 bc
75 APQ	11.08 bc	5.82 ab	63.00 b	18.01 c	1.62 b
ES	0.46*	0.14	3.14	0.14	0.12

Table 3. Estimated water footprint in bean cultivation treated with biostimulants and subjected to two irrigation regimes.

Treatments	Irrigation allocation (m^3 ha^{-1})	Effective rainfall (m^3 ha^{-1})	Yield (t ha^{-1})	Blue water footprint (m^3 t^{-1})	Green water footprint (m^3 t^{-1})	Gray water footprint (m^3 t^{-1})	Total water footprint (m^3 t^{-1})
100 A (control)	3413	1144	1.65	2068	693	44	2806
100 AP	3413	1144	1.71	1996	669	42	2707
100 APQ	3413	1144	2.03	1681	564	35	2280
75 A (control)	2560	0	1.33	1925	0	54	1979
75 AP	2560	0	1.52	1684	0	47	1731
75 APQ	2560	0	1.62	1580	0	44	1624

On the other hand, it was observed that the use of both Pectimorf® and Quitomax® improved water use efficiency, with the best results in the treatment that received a foliar application of chitosan, a product that has been shown, like other chitosan derivatives, the property of not only promoting yield but also causing a decrease in the transpiration rate Jafari *et al.* (2025), caused by the accumulation of abscisic acid (ABA), a compound that stimulates the partial closure of stomata (Hidangmayum *et al.*, 2019).

CONCLUSIONS

From the results obtained, it can be concluded that the application of both Pectimorf® to the seed and Quitomax® as a foliar spray at the beginning of flowering promotes the growth and yield of the bean crop, as well as helping to maintain better water status in the plants.

The use of both products improved the efficiency of water use by the plants and contributed to reducing the water footprint by almost 20% compared to the control treatment.

Quitomax®, whose active ingredient is a mixture of chitosan polymers, has shown signs of exerting, like other commercial products containing chitosan, an antitranspirant action in plants, allowing them to maintain better water status in their tissues.

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