TERRA LATINOAMERICANA

Impact of Humic Acids and Chitosan on the Ionic Composition of the Soil Solution and the Nutritional Content of Cabbage (*Brassica oleracea* L.) Under Greenhouse

Impacto de Ácidos Húmicos y Quitosano en la Composición Iónica de la Solución del Suelo y el Contenido Nutrimental de Col (*Brassica oleracea* L.) Bajo Invernadero

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SUMMARY

Soil solution (SS) constitutes the volume from which plant roots extract dissolved nutrients. Despite its importance, the impact of its composition due to the application of organic amendments has been relatively little studied. The objective of this research was to study the impact of humic acids (HA) and chitosan (Cs) on the concentration of minerals in the SS, as well as their effect on the yield of the cabbage crop (*Brassica oleracea* L.). Under greenhouse conditions with calcareous soil, two doses of HA were applied (200 and 500 kg ha^{-1}), as well as 50 and 150 kg ha^{-1} of Cs in a targeted manner. From the SS, 22 samples were taken non-destructively from the root zone once a week. The results of mineral content in the soil solution and mineral content on the biomass did not present significant differences, however, it was noted that the Cs50 treatment stimulated 41% higher plant biomass than the control ($P \leq 0.5$, Tukey). It is concluded that Cs and HA do not significantly impact the ionic content of the SS or the mineral content of the biomass, although they do stimulate the yield.

Index words: *biostimulants, lysimeters, plant nutrition, steiner solution.*

RESUMEN

La solución del suelo (SS) constituye el volumen de donde las raíces de las plantas extraen los nutrientes disueltos. A pesar de su importancia, el impacto de su composición por la aplicación de enmiendas orgánicas se encuentra relativamente poco estudiado. El objetivo de esta investigación fue estudiar el impacto de los ácidos húmicos (AH) y quitosán (Cs) sobre la concentración de los minerales en la SS, así como su efecto en el rendimiento del cultivo de col (*Brassica oleracea* L.). En condiciones de invernadero con suelo calcáreo se aplicaron dos dosis de AH (200 y 500 kg ha^{-1}), así como 50 y 150 kg ha^{-1} de Cs de forma dirigida. De la SS se tomaron 22 muestras en forma no destructiva de la zona de la raíz una vez por semana. Los resultados del contenido mineral en la solución del suelo y del contenido mineral en la biomasa no presentaron diferencias significativas, no obstante, se apreció que el tratamiento Cs50 estimuló 41% la biomasa vegetal superior al testigo ($P \leq 0.5$, Tukey). Se concluye que el Cs y AH no impactan significativamente el contenido iónico de la SS ni el contenido mineral de la biomasa, aunque si estimulan el rendimiento.

Palabras clave: *bioestimulantes, lisímetros, nutrición vegetal, solución Steiner.*

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INTRODUCTION

Organic content is one of the most important aspects that directly affect soil fertility. Humic acids are important organic compounds that can impact the chemical, biological, and physical properties of soil nutrients (Dhaliwal *et al*., 2019). However, in this same system, the "soil solution" (SS) is composed. In SS the solvent is water, and the solutes consist of dissolved substances (ions), free salts, CO_2, O_2, and other gases; we also find dispersed inorganic and organic compounds, called colloids. Understanding how solutes modify the chemical composition of SS is of great importance since crop roots take most of the nutrients for their growth and development from the liquid phase (Llanderal, García, Contreras, Segura, and Teresa, 2019).

In recent years, studies on the dynamics of crop nutrition based on the allometry between nutrient uptake and plant biomass accumulation have led to the hypothesis of the existence of co-regulation in the nutrient uptake rate governed by the concentration of nutrients in the root medium and growth capacity of the plant itself (Devienne-Barret, Justes, Machet, and Mary, 2000). This means that the availability of nutrients in the soil is the result of the functioning of the integrated soil-plant system. The application of biostimulants is decisive for the efficient use of inorganic fertilizers (Shahrajabian, Chaski, Polyzos, Tzortzakis, and Petropoulos, 2021). Recent research has shown that biostimulants reduce the application of inorganic mineral fertilizers, mainly N-P-K (Bulgari, Franzoni and Ferrante, 2019). In the category of biostimulants, we find humic acids, fulvic acids, protein hydrolases, and chitosan, among others. Specifically, humic acids have the ability to form complexes with metal ions, since they contain oxygen, organic acids, phenolic and alcoholic compounds (Chen, Clapp, and Magen, 2004).

Chitosan (Cs) is a biodegradable and biocompatible polysaccharide with an emerging interest due to its properties and possible technological applications (Aranaz *et al*., 2021). The application of this biopolymer in crop production is constantly increasing due to its multidirectional bioactivity (Shahrajabian *et al*., 2021). Cs can regulate mineral uptake by crop roots since its amino and hydroxyl functional groups act as ionic binding sites (Kamari, Pulford, and Hargreaves, 2011); in addition to its chelating capacity (Guibal, 2004). Currently, the possibility of using chitosan as a plant biostimulator in sustainable production systems is being considered (Stasińska-Jakubas and Hawrylak, 2022). Although several research papers demonstrate the beneficial effects of chitosan, they have been applied foliarly and its antimicrobial action has been the main objective (Kahromi and Kahara, 2021; Malerba and Cerana, 2019), likewise, few studies are available about its impact on the chemical constitution of SS and as a biostimulant. The objective of this study was to determine the impact of chitosan and humic acids on the chemical composition of the soil solution, mineral content of leaf tissue, and cabbage crop yield (*Brassica oleracea* L). Cabbage is one of the most important salad vegetables in Mexico and is a source of fiber, minerals, vitamins, and phytochemicals such as carotenoids and other antioxidants (Singh *et al*., 2006).

MATERIAL AND METHODS

Establishment

Cabbage (*Brassica oleracea* L.) seeds were sown in polystyrene trays with 200 cavities. The transplant was carried out in 4 L polyethylene bags with soil as a substrate. The physical-chemical characteristics of the soil were determined and are shown in Table 1. The average greenhouse temperature was 24 °C and the relative humidity was 70 percent.

Treatments

The treatments consisted of the application of 50 and 150 kg ha^{-1} of chitosan (Mv = 200 000, Marine Chemicals, Kerala India) and humic acids (200 and 500 kg ha^{-1}) of commercial origin (Humics-95, Agroscience, México). The applications of AH and Cs were made to the drench. Table 2 presents the characteristics and concentration of the elements of the water. The application of the treatments was carried out one day before the transplant, 40 and 80 days after the transplant (dat).

For irrigation, a solution of Steiner (1961) was used at 25% concentration (EC of 0.75 dS m^{-1}) until the phenological stage of "head formation" (71 dat). Once the formation of the heads was reached, the nutrient solution was applied at 50% (EC of 1.75 dS m^{-1}) of concentration. The pH of the nutrient solution ranged from 5.5 to 6.5 with the addition of nitric, phosphoric, and sulfuric acids.

Soil Solution

To determine the ions content in the soil solution (SS), lysimeters were inserted at a depth of 15 cm in each pot. To generate the vacuum, 75 centibars of pressure were applied to each lysimeter with a suction pump. The collection of the SS was carried out 24 hours after its installation. In total, 22 samplings were carried out with three repetitions per treatment.

Table 1. Physicochemical properties of the soil.

	pH†	EC	Apparent density	Total carbonates	$N\text{-}NO_3^-$	P-Olsen
	H_2O	dS m^{-1}	g cm^{-3}	%	- - - - - - - - - mg L^{-1}	- - - - - - - - -
Soil	7.59	0.58	0.95	29.7	2.72	15
			Exchangeable cations (mg L^{-1})			
	Ca^{2+}	Mg^{2+}	Na^+	K^+	Texture	Organic material %
Soil	6.364	341	101	599	Sandy-Loam	4.90

† pH (1:2 water)

The measurement of pH and EC was carried out with a portable potentiometer (HANNA HI 98130). The concentration of potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), iron (Fe), zinc (Zn^{2+}), copper (Cu^{2+}), and molybdenum (Mo) was determined by the digestion technique (Hernández-Hernández *et al*., 2018). Phosphorus (P) was determined by visible spectrophotometry (AOAC, 1990).

Mineral Content of Plants

Three destructive samplings were carried out by collecting three randomly collected plants in each treatment during the experiment (35, 58, and 150 dat). The fresh weight of the heads, leaves, and roots was determined on an analytical balance. The fresh samples were placed in paper bags and dried in a dehydration oven at 80 °C for 72 hours until constant weight. For the determination of carbon (C), hydrogen (H), and nitrogen (N) content we used microchemical method using the CHN 628 elemental analyzer (AOAC, 1990); the carbon content was estimated as 50% of the ash-free dry matter. Ca^{2+}, Mg^{2+}, K^+, Fe, and Zn^{2+} were determined by digesting the dry samples according to the methodology of (Hernández-Hernández *et al*., 2018), with some modifications. 0.5 g of dry sample was weighed, to which 30 ml of concentrated nitric acid (HNO_3) was added and later placed on an electric stove with boiling heat for three hours. The collected volume was filtered with Whatman No. 42 filter paper and made up to 100 ml with deionized water. The mineral content was determined in a flame emission atomic absorption spectrophotometer (Varian AA - 1275). The results were expressed in milligrams per gram of dry weight (mg g^{-1} dry weight) for macro and microelements, respectively.

Table 2. Characteristics and concentration of elements of the irrigation water used in the experiment.

Variable	Unit's values
pH	7.50
EC	1.15 dS m^{-1}
$N\text{-}NO_3^-$	5.74 mg L^{-1}
K^+	3.90 mg L^{-1}
Ca^{2+}	111 mg L^{-1}
Mg^{2+}	29 mg L^{-1}
Na^+	74.1 mg L^{-1}
SO_4^-	77.3 mg L^{-1}
HCO_3^-	461 mg L^{-1}
CO_3^-	0.00 mg L^{-1}
Cl^-	77.0 mg L^{-1}

Statistical Analysis

The experiment was established in a completely randomized block design, with 5 treatments and 43 repetitions. An analysis of variance and means comparison test was performed according to Tukey ($P \leq 0.05$).

RESULTS AND DISCUSSION

Figures 1 show the recorded values of pH, EC, and the ionic content of the soil solution in different samplings. The treatments did not significantly affect the ionic composition of the SS concerning the control. In the six samplings carried out, the ion concentration range was very wide and did not allow us to observe a particular trend.

Figure 2a-f shows the concentrations of C, H, N, and Ca^{2+}, Mg^{2+}, K^{+}, Fe^{++}, Mn, Zn, and Cu in plant tissue in response to HA, Cs, and control treatments. The treatments did not significantly affect the mineral content respecting the control. However, higher Mn content was recorded in the leaves of the plants treated with Cs150 compared to the rest treatment; while the Ca^{2+} content was higher with the AH500 treatment. Also, the K, C, and N content was found in higher concentration in the root of the control plants in contrast to the leaf content.

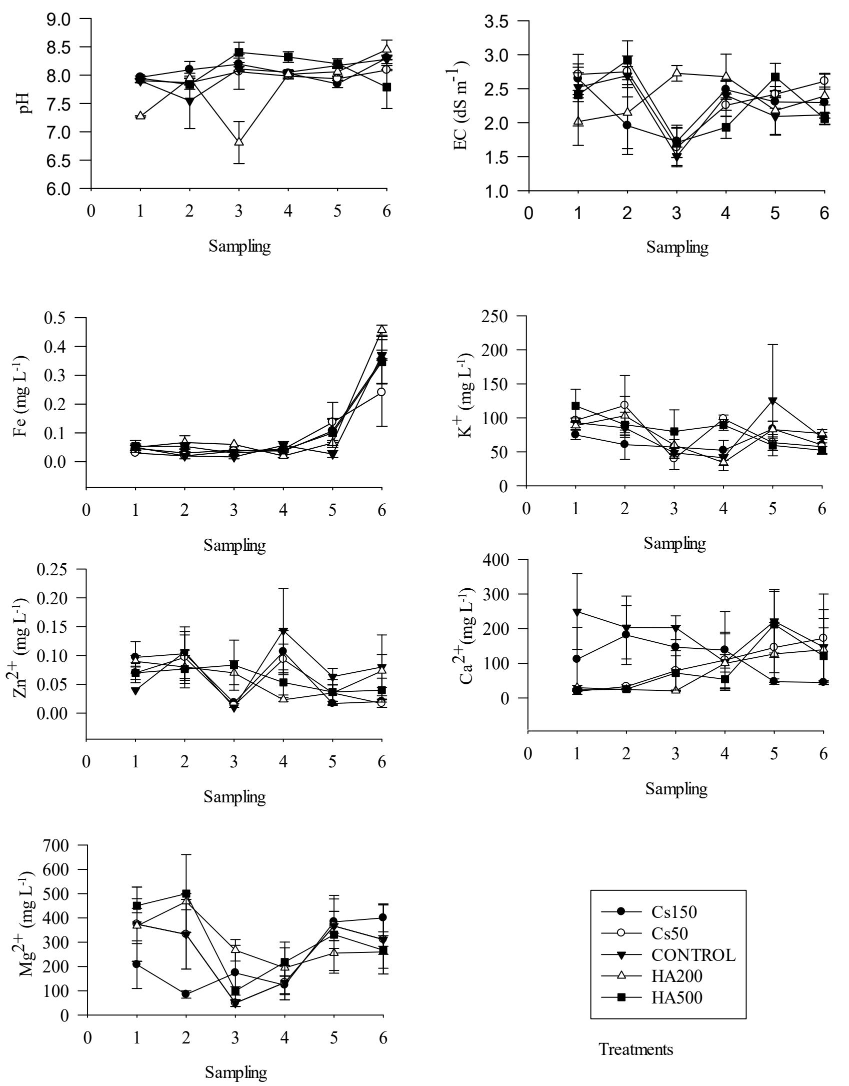

Figure 1. Mean values in pH and EC and in the concentration of Fe, K^{+}, Zn, Ca^{2+}, and Mg^{2+} in the soil solution over time for two types of biostimulants with two soil application rates in cabbage plants. Bars denote standard error.

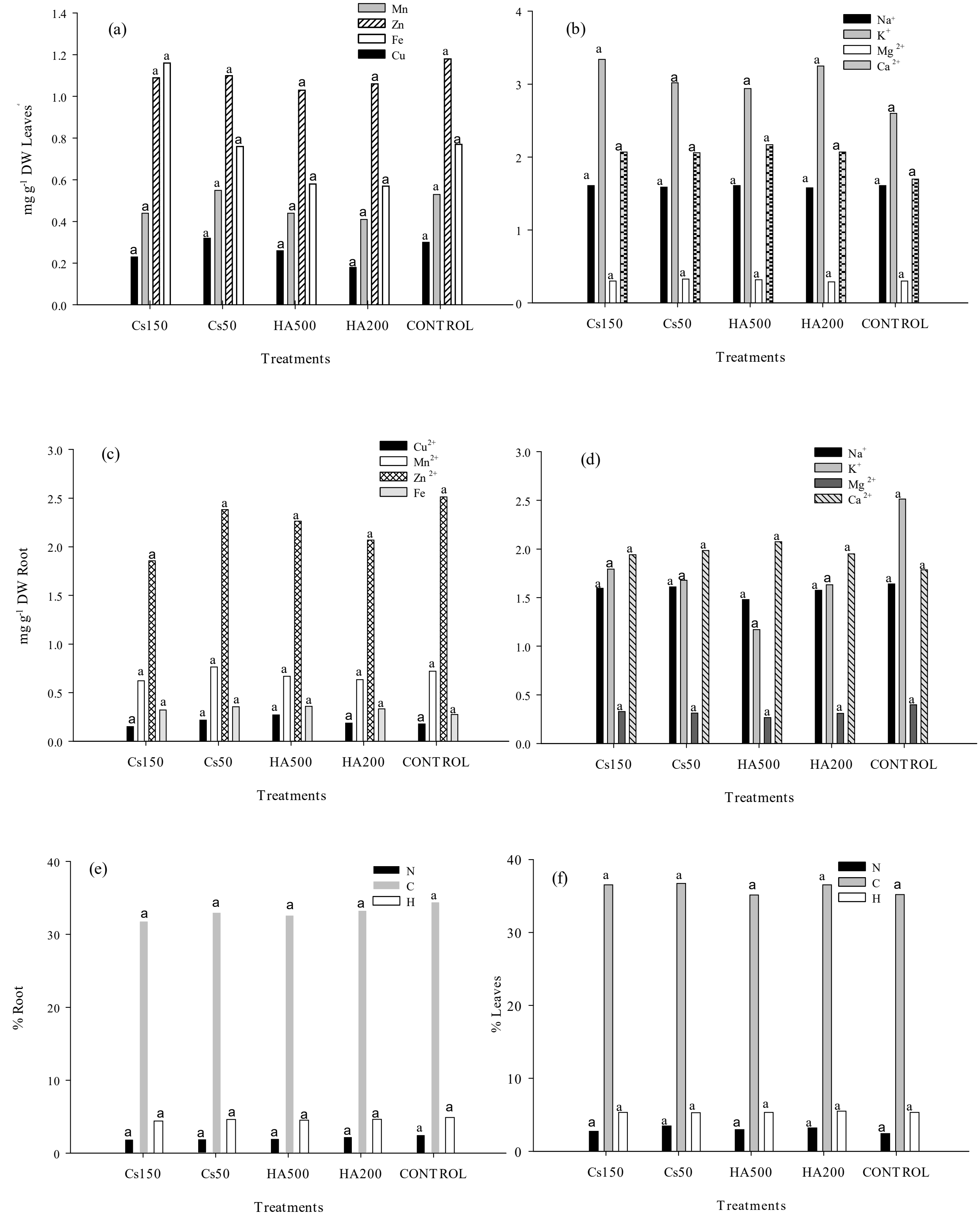

Figure 2. Effect of the application of humic acids and chitosan on nutrient content of cabbage biomass. Letters in common indicate that there are no significant dierences (*P* ≤ 0.05, Tukey). DW: Dry weight.

Regarding the yield per plant, in Figure 3 it can be seen the stimulation of biomass by both amendments. The highest mean values were presented with the Cs50 treatment with 1286 g, which represents an increase of 41% compared to the control that registered 908 grams.

There are several plausible reasons for the different impacts of chitosan on nutrient uptake by plants, including differences in application methods, application timing, and the complexity of biostimulation in different plant species (Malerba and Cerana, 2019). Chitosan foliar sprays on *Dracocephalum kotsyi* plants significantly increased the content of Ca^{2+}, Mg^{2+}, and K^{+} (Kahromi and Khara, 2021). Chitosan applied as a soil amendment increased the height, canopy diameter, and leaf area of chili peppers (Xu and Mou, 2018). The amendment with chitin and chitosan improved the germination and growth of *Solanum lycopersicum* L., *Capsicum annum* L., and *Solanum melongena* L. at low concentrations (Amine, Abla, Mohammed, and Khadija, 2020). Ahmad, Jaleel, Shabbir, Khan, and Sadiq (2019), mention that the positive effects of chitosan may be due to its contribution to amino acids. In our research, chitosan had no significant effect on the ionic composition of the soil solution and mineral content in *Brassica oleracea,* however, the effect on yield can be seen. Boonlertnirun *et al*. (2006) pointed out that Cs mineralizes organic nutrients and improves their availability to roots, while Agbodjato, Noumavo, Adjanohoun, Agbessi, and Baba (2016) reported that Cs has a positive effect on symbiosis with growth-promoting rhizobacteria, which triggers a higher rate of nitrogen fixation and improves plant micronutrient uptake.

Humic acids have been reported as one of the best plant growth stimulants (Yang and Antonietti, 2020). HA increased access to phosphorus and various nutrients and significantly increased the yield of wheat (*Triticum aestivum*) plants (Dinçsoy and Sönmez, 2019). The HA applied as a soil amendment significantly increased the content of ascorbic acid and lycopene, and increased the yield in Cherry tomato plants (Deng, Luo, Su, Wu, and Zhao, 2021). The stimulating effect of AH at low concentrations has been attributed to the direct effect on plants, mainly in stimulating hormones, together with the indirect effect on soil microorganisms, the dynamics of nutrient absorption, and the physical conditions of the soil (Maji, Misra, Singh, and Kalra, 2017). The combination of biofertilizers based on beneficial microorganisms with humic acids stimulated growth and increased yield in broccoli plants (*Brassica oleracea* var. italica) (Al-Taey, Al-Shareefi, Mijwel, Al-Tawaha, and Al-Tawaha, 2019). In this study, the treatment with HA at an application of 500 kg ha^{-1} did not promote the uptake of nutrients in cabbage (*Brassica oleracea* var. capitata). Although no direct response was observed in the mineral content of cabbage, it is possible to see effects on biomass yield compared to control. Hemati *et al*., (2022) reported no effects on *Brassica napus* plants.

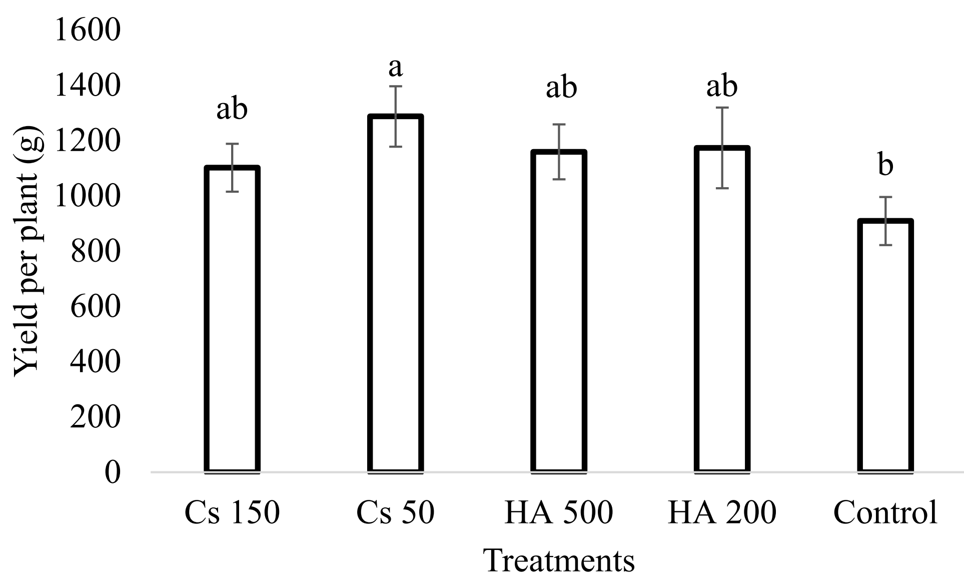

Figure 3. Mean yield values quantified in the crop cycle for two types of biostimulants with two application doses in cabbage plants. Letters in common indicate that there are no significant dierences ($P \leq 0.05$, Tukey).

CONCLUSIONS

In this study, no significant effect was observed on yield, mineral content on soil solution, and biomass by chitosan and humic acid amendment. Although no direct response was observed in the mineral content of cabbage, it is possible to see effects on biomass yield compared to control, so it will be necessary for later works to carry out studies on other variable growth.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

The data sets generated or analyzed during the study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FINANCING

Not applicable.

AUTHORS' CONTRIBUTIONS

Methodology: J.E.C.A. Formal analysis and writing: N.F.F. Conceptualization: H.O.O. Draft preparation and editing: A.J.M. Supervision, project administration and funding acquisition: A.B.M.

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