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Effect of chitosan on growth and productive parameters in broccoli plants (*Brassica oleracea* L. var. Calabrese)

Efecto del quitosano sobre parámetros de crecimiento y productivos en plantas de brócoli (*Brassica oleracea* L. var. Calabrese)

Efeito da quitosana no crescimento e parâmetros produtivos em plantas de broccoli (*Brassica oleracea* L. var. Calabrese)

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Crop production

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Abstract

Biostimulants improve the absorption and assimilation of nutrients by plants, making them more tolerant to biotic or abiotic stress, improving their agronomic characteristics. Natural and biodegradable biostimulants such as chitosan have fungal and bactericidal activities and promote growth and crop yield, this is why, to evaluate the effect of chitosan application on growth and productive parameters of broccoli (*Brassica oleracea* L.), three concentrations of chitosan (T_1 = 500 mg.L^{-1}; T_2 = 1000 mg.L^{-1}; and T_3 = 2000 mg.L^{-1}) and a control treatment (T_4 = distilled water), were applied by foliar spray when the true leaves unfolded, using a completely randomized design with 30 repetitions per treatment. The variables height of the plant, number of leaves per plant, diameter of the flowering stalk, diameter of the flowering head, length of the flowering stalk, total length of the flowering stalk, fresh biomass of the flowering head, of the root, and of the aerial part, total dry biomass and yield were measured. All the variables increased ($P<0.05$) as the chitosan dose increased, concluding that the application of chitosan to the broccoli crop is a viable alternative as a substitute for synthetic fertilizers.

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Resumen

Los bioestimulantes mejoran la absorción y asimilación de nutrientes de las plantas, haciendolas más tolerantes al estrés biótico o abiótico, mejorando sus características agronómicas. Bioestimulantes naturales y biodegradables como el quitosano tienen actividades fúngicas, bactericidas y son promotores de crecimiento y rendimiento de los cultivos, es por ello que con el objetivo de evaluar el efecto de la aplicación de quitosano sobre parámetros de crecimiento y productivos del brócoli (*Brassica oleracea* L.), tres concentraciones de quitosano (T_1= 500 mg.L^{-1}; T_2= 1000 mg.L^{-1} y T_3= 2000 mg.L^{-1}) y un tratamiento control (T_4= agua destilada), fueron aplicados mediante aspersión foliar al desplegarse las hojas verdaderas, utilizando un diseño completamente al azar con 30 repeticiones por tratamiento. Se midieron las variables altura de la planta, número de hojas por planta, diámetro del tallo, diámetro de la pella, longitud del tallo del ramo, longitud total del ramo, biomasa fresca de la pella, de la raíz y de la parte aérea, biomasa seca total y rendimiento. Todas las variables se incrementaron (P<0.05) a medida que la dosis de quitosano aumentó, concluyéndose que la aplicación de quitosano al cultivo del brócoli es una alternativa viable como sustituto de los fertilizantes sintéticos.

Palabras clave: bioestimulante, estrés abiótico, promotor de desarrollo

Resumo

Os bioestimulantes melhoram a absorção e assimilação dos nutrientes pelas plantas tornando-as mais tolerantes ao estresse biótico ou abiótico, melhorando suas características agronômicas. Bioestimulantes naturais e biodegradáveis, como a quitosana, possuem atividades fúngica e bactericida e promovem o crescimento e a produtividade das culturas, por isso com o objetivo de avaliar o efeito da aplicação de quitosana nos parâmetros de crescimento e produção de brócolis (*Brassica oleracea* L.), três concentrações de quitosana (T_1 = 500 mg.L^{-1}; T_2 =1000 mg.L^{-1} e T_3 = 2000 mg.L^{-1}) e um tratamento controle (T_4 = agua destilada), foram aplicados por pulverização foliar quando as folhas verdadeiras se abriram, em delineamento inteiramente casualizado com 30 repetições por tratamento. As variáveis altura da planta, número de folhas por planta, diâmetro do cuale, diâmetro do pellet, comprimento do caules do buquê, comprimento total do buquê, biomassa fresca do pellet. Da raíz e da parte aérea, parte biomassa seca total e productividade. Todas as variáveis aumentaram (P<0.05) com o aumento da doce de quitosana, concluindo que a aplicação de quitosana na cultura de brócolis é uma alternativa viável em substituição aos fertilizantes sintéticos.

Palavras-chave: bioestimulante, estresse abiótico, promotor de desenvolvimento

Introduction

Broccoli (*Brassica oleracea* L.) is a very perishable climacteric vegetable, rich in minerals, vitamin C, dietary fibers, nutritional antioxidants, glucosinolates, and phenolic compounds (Bhandari *et al*., 2019). The importance of its consumption has increased in recent years because of its significant amount of anticarcinogenic and antioxidant compounds, as well as its multiple vitamins (El-Beltag *et al*., 2022), therefore, the planting areas of this crop have grown, due to the increase in demand in national and international markets. The main destinations of this vegetable are Japan, the United States, and the European Union (Duque and Murillo, 2021).

Broccoli responds significantly to nitrogen fertilization (Lazcano *et al*., 2006), however, the excessive use of fertilizers by farmers increases production costs, deteriorating quality and denaturing soil fertility (Borboa *et al*., 2016). In addition, the excessive use of fertilizers in soils causes erosion and salinity, accumulation of heavy metals, eutrophication of water, and accumulation of nitrates (Rahman and Zhang, 2018), therefore, the search for ecological solutions that increase the efficiency of plant production and decrease the use of synthetic chemicals has become essential (Stasińska-Jakubas and Hawrylak-Nowak, 2022). In recent years, the use of biostimulants has been an alternative to the application of synthetic fertilizers (Nunez *et al*., 2023), since these, in addition to having increased their costs, cause damage to human and animal health and the environment (Torres-Rodriguez *et al*., 2021; Dupouy, 2023). A biostimulant is an agrochemical product formulated with mixtures of natural substances and/or microorganisms that applied to plants, improve the efficiency of mineral nutrition, tolerance to abiotic stress (salinity, drought, high temperatures, heavy metals, among others), and biotic and/or crop yield, or improve the quality characteristics of crops (Rouphael and Colla, 2020; García-Sánchez *et al*., 2022). In general, nine categories of substances that act as biostimulants have been defined: (1) humic substances; (2) complex organic materials (obtained from agro-industrial and urban waste, sludge extracts, compost, and manure); (3) beneficial chemical elements (Al, Co, Na, Se, and Si); (4) inorganic salts (5) algae extracts (brown, red and green macroalgae); (6) antiperspirants (kaolin and polyacrylamide); (7) free amino acids and N-containing substances (peptides, polyamines and betaines); and (8) plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi, and *Trichoderma* spp. (García-Sánchez *et al*., 2022).

Some organic raw materials contain biostimulants or biostimulant components of industrial waste, which are effective in agriculture. These include vermicompost, sewage sludge, protein hydrolysate, and chitin/chitosan derivatives, among others (Xu and Geelen, 2018).

Therefore, biostimulants could represent a sustainable measure to foster the resilience of cropping systems (Antonucci *et al*., 2023). Their positive effects are mainly due to bioactive compounds that stimulate plant growth, such as phytohormones, amino acids, and nutrients (Kisvarga *et al*., 2022). One of the biostimulants used to increase growth, development, and yields in crops is chitosan, due to its biocompatibility, biodegradability, and bioactivity (Reyes-Pérez *et al*., 2020b; Torres-Rodriguez *et al*., 2021). Chitosan is a biopolymer obtained from chitin, which is the second most common natural polysaccharide after cellulose (Muthu *et al*., 2021). The positive effects of chitosan on plants include improvements in physiological mechanisms and growth, as well as an increase in the shelf life of fruits and vegetables (Stasińska-Jakubas and Hawrylak-Nowak, 2022). The application of chitosan improves the assimilation of nutrients in plants (Kahromi and Khara, 2021), increases their growth and development, stimulates seed germination, increases the fresh weight of the roots and the plant, and increases yields in different crops such as tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), pepper (*Capsicum annuum* L.), among others (Zohara *et al*., 2019; Reyes-Pérez *et al*., 2020b; Reyes-Pérez *et al*., 2021a; Rouphael *et al*., 2022). In addition, it is important to control plant pathogens that colonize plants, activating defense responses such as increased callose deposition, production of defense-related enzymes, phytoalexins, and PR proteins (Torres-Rodriguez *et al*., 2021; Loron *et al*., 2023), as well as the increase in chlorophyll content in plants (Holguin-Peña *et al*., 2020; Reyes-Pérez *et al*., 2020c) and the decrease in water loss due to transpiration (Morales *et al*., 2016). In view of the above, the objective of this work was to evaluate the effect of the application of chitosan at different concentrations, on the growth and productive parameters of broccoli cultivation.

Materials and methods

The present study was carried out in the greenhouse of the Experimental Campus "La María" located in the canton Mocache, province of Los Ríos, at a south latitude of 1°04'48. 6" and west longitude of 9°30'04.2", altitude of 75 m above sea level, humid tropic climate and average annual temperature of 25.3 °C, average annual precipitation of 1587.5 mm; 86 % relative humidity and 994.4 sunshine hours per year. With a loam-loamy soil, pH of 5.5, and a flat topography (INAMHI, 2015). Broccoli seeds of the variety Calabrese were sown continuously without spaces, manually on the plot, subsequently covered with a small sheet of soil (1-1.5 cm) and applying frequent irrigation (50 cm of sheet), as well as carrying out control work of undesirable plants, to achieved plants of good vigor at 35 d after sowing, with heights of 18-20 cm and 6-7 leaves. These plants were transplanted to a plot of 50 m^2 divided into four subplots with 30 plants in each, planted at a distance of 0.50 m between plants and 0.60 m between rows. After the transplant, manual cultural work was carried out such as weeding, and hilling. Light irrigation (50 cm of sheet) was applied so that the crop had moisture throughout its cycle, according to the water needs of the crop. At 15 and 25 days after transplantation, an application (foliar) was made with 250 mL per plot of the following chitosan treatments (Sigma-Aldrech: chitosan high molecular weight / deacetylated chitin ≥ 75%, poly (D-glucosamine)): T_1 = 500 mg.L^{-1}; T^2 = 1000 mg.L^{-1} and T_3 = 2000 mg.L^{-1}), and a control treatment (T_4 = distilled water), taking into account that the plants were in the true leaves unfolding stage. Before closing the field, two manual cleanings were carried out. The plant size (cm) was measured, from the base of the stalk below the first internode to the top of the plant head; Number of leaves; stalk diameter (cm); head diameter (cm); length of the stalk measured from the beginning of the stalk cut to the beginning of the stalk inflorescence); total length of the stalk (measured from the beginning of the cut of the stalk to the cup of the flower head); fresh mass (g) of the head, root, and aerial part; total dry biomass (a recirculating air stove was used ± 5 ºC, where they were maintained for a period of 72 hours at 80 ºC temperature until constant weight and then the dry mass was determined in an analytical balance); yield (extrapolated to kg.ha^{-1}). A descriptive analysis (mean and standard error) of all variables was performed, subsequently, a linear model and a trend analysis by regression were used to establish the effect of the treatments on the behavior of the variables, using the Minitab® statistical software.

Results and discussion

The descriptive statistics (mean and standard error) for each of the variables in the four treatments are presented in Table 1, in which it can be seen that the response to each variable was increasing as the concentration of chitosan increased, it is also appreciated that the greatest responses were presented in the concentration of 2000 mg.L^{-1}. The diameter of the head and the fresh biomass of the stalk were very similar to those reported by Puenayan *et al*. (2009), with doses of 150 kg.ha^{-1} N and 200 kg.ha^{-1} P_2O_5 in broccoli of the variety Italica, and that reported by Borboa *et al*. (2016), for head diameter, with three broccoli hybrids (Avenger, 15.05-15.91 cm; Marathon, 15.03-15.68 cm and Heritage, 14.99-15.32 cm) that received the application of two halobacteria (*Azospirillum halopraeferens* and *Bacillus amiloliquefasciens*) and a control, and higher than those reported by Rivera (2022) who reports head diameter of 13.66 cm and yield of 14.200 kg.ha^{-1} for broccoli variety italica of the hybrid Paraiso, with the application of Kimelgran: 470.63 kg.ha^{-1}, and head diameter of 12.81 cm and yield of 13.25 kg.ha^{-1} with the application of 200-120-60 of fertilizer. However, the yield obtained in our study was lower (20.250 vs 33.440 kg.ha^{-1}) than that reported by Puenayan *et al*. (2009).

Table 1. Descriptive measures (mean and standard error) of the variables of broccoli variety Calabrese in response to the concentration of chitosan.

Variables	0	500	1000	2000
Plant height (cm)	(22.0 ± 0.58) d	(26.6 ± 0.33) c	(32.0 ± 0.58) b	(39.3 ± 0.88) a
Stalk diameter (cm)	(1.6 ± 0.088) d	(2.2± 0.057) c	(2.7 ± 0.10) b	(3.8 ± 0.03) a
Stalk length (cm)	(6.16 ± 0.17) d	(7.16 ± 0.17) c	(7.76 ± 0.03) b	(8.80 ± 0.11) a
Total length of stalk (cm)	(14.3 ± 0.33) d	(16.8 ± 0.17) c	(18.3 ± 0.33) b	(21.6 ± 0.33) a
Number of leaves	(14.3 ± 0.33) d	(16.6 ± 0.33) c	(20.0 ± 0.57) b	(22.6 ± 0.33) a
Head diameter (cm)	(6.3 ± 0.17) c	(8.0 ± 0.29) c	(12.3 ± 0.33) b	(15.0 ± 0.57) a
Fresh root biomass (g)	(84.7 ± 0.33) d	(96.3 ± 0.88) c	(174.3 ± 2.3) b	(189.0 ± 2.08) a
Fresh biomass aerial part (g)	(835.0 ± 2.9) d	(878.3 ± 6.01) c	(1053.3 ± 4.4) b	(1191.7 ± 7.3) a
Fresh stalk biomass (g)	(154.3 ± 2.3) c	(173.3 ± 1.7) c	(268.3 ± 4.4) b	(400.0 ± 10.4) a
Total dry matter (g)	(127.7 ± 1.45) d	(142.3 ± 1.45) c	(150.0 ± 1.15) b	(163.3 ± 1.67) a
Yield (kg.ha^{-1})	(6772.3 ± 37.5) c	(7763.3 ± 47.8) c	(12010 ± 77.7) b	(20250 ± 608) a

Rows with different literal indicate significant differences (P<0.05)

The response of all variables was directly proportional to the concentration of chitosan, in figure 1 it can be seen that the growth variables; plant height, stalk length, stalk diameter and the number of leaves, increased their response as the concentration of chitosan increased. Increases in plant height in response to chitosan application have been reported in tomatoes (Terry *et al*., 2017; Reyes-Pérez *et al*., 2020a; Chanaluisa-Saltos *et al.*, 2022), turnip (Álvarez *et al*., 2021), rice (Molina *et al*., 2017) and broccoli but using a vegetable protein hydrolysate as a biostimulant (Amirkhani *et al*., 2016).

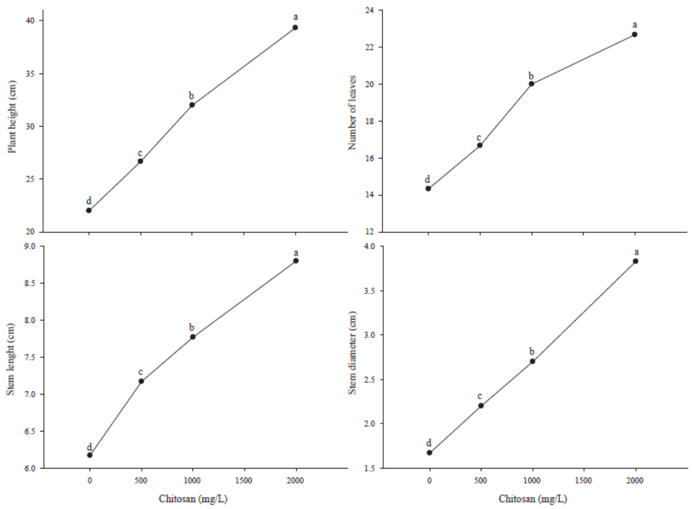

Figure 1. Effect of chitosan concentration on the response of growth variables in the broccoli plant var. Calabrese.

The biostimulant effect of chitosan on crop growth is related to the increased availability and absorption of nutrients and the process of photosynthesis through the accumulation of metabolites and the increase of foliar pigments (Sharif *et al*., 2018). The number of leaves in soybeans was also increased with chitosan applications at doses of 10, 100, and 500 mg.L^{-1} (Costales-Menendez *et al*., 2020), as well as in tobacco crops (González *et al*., 2017), of beans with doses of 600 mg.ha^{-1} of Quitomax® (Morales *et al*., 2016), and potato using doses of 150 mg.ha^{-1} of Quitomax® (Morales *et al*., 2015). The increase in the number of leaves represents a greater leaf surface and as a consequence a higher photosynthetic capacity and an increase in dry matter and yield (Morales *et al*., 2016). An increase in the stem diameter of tomato (Reyes-Pérez *et al*., 2020b; Pincay-Manzaba *et al*., 2021) and tobacco (González *et al*., 2017) was reported in response to the application of chitosan, which stimulates the proliferation of spindle and radial initial cells, which is reflected in an increase in stem diameter (Pincay-Manzaba *et al*., 2021).

The morphological variables of fresh biomass of the root, of the aerial part of the stalk, and the total dry matter (figure 2), as well as the total length of the stalk and diameter of the head, also increased as the concentration of chitosan increased (figure 3).

An increase in the fresh biomass of tomato fruit and root was reported in response to the application of chitosan (Chanaluisa-Saltos *et al*., 2022; Rivas-García *et al*., 2021). The diameter of the broccoli head variety italica, also increased in response to chitosan (Yildirim *et al*., 2011), in contrast, Kałużewicz *et al*. (2018) found no effect on this variable in response to the application of an amino acid as a biostimulant in broccoli of the variety Tiburon.

The yield increased as the concentration of chitosan increased (figure 4). Yield increases in response to chitosan application were reported in broccoli variety italica (Yildirim *et al*., 2011) and tomato (Rivas-García *et al.,* 2021; Chanaluisa-Saltos *et al*., 2022).

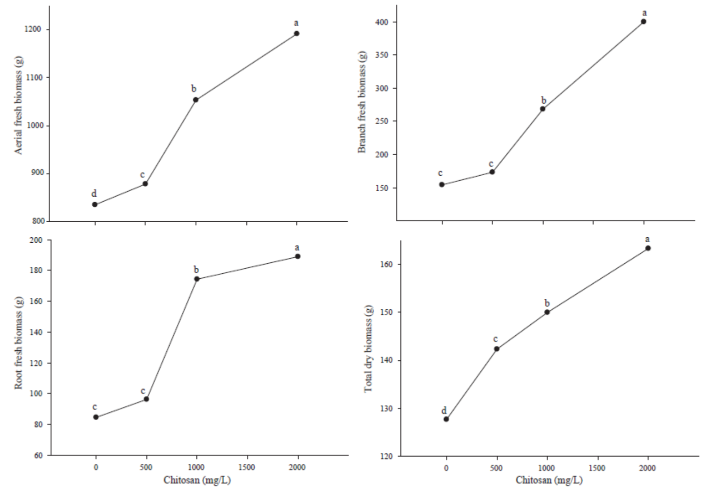

Figure 2. Effect of chitosan concentration on the response of morphological variables in the broccoli plant var. Calabrese.

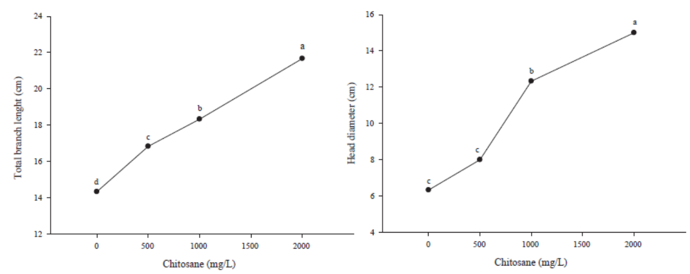

Figure 3. Behavior of stalk length and head diameter in response to chitosan concentration.

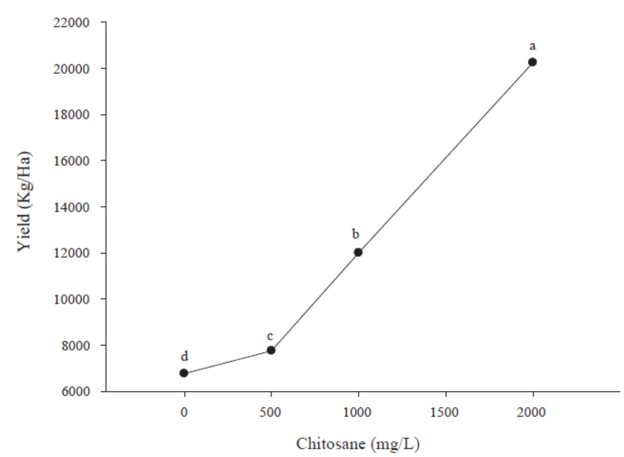

Figure 4. Yield of broccoli in response to chitosan concentration.

Conclusions

The greatest response in all the variables studied was presented when a concentration of 2000 mg chitosan was used, however, the trend study showed that the response could be increased at even higher concentrations. The application of chitosan to the cultivation of broccoli variety Calabrese is an alternative to reduce the use of synthetic fertilizers, since not only the responses to growth and productive variables of this crop are increased, but also considering that its application contributes to the control of phytopathogens because it increments callose deposits, the production of enzymes related to plant defense, phytoalexins, and PR proteins, which makes its use a viable biostimulant alternative for use in this crop.

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