Cultivos Tropicales, Vol. 47, No. 3, July-September 2026, e-ISSN: 1819-4087, p-ISSN: 0258-5936, https://ediciones.inca.edu.cu

Cu-ID: https://cu-id.com/2050/v47n3e15

Original article

Chitosan doses and their effect on growth and yield of radish (*Raphanus sativus* L.) under greenhouse conditions

Efectos del quitosano en el crecimiento y rendimiento del rábano (*Raphanus sativus* L.) en invernadero

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ABSTRACT: Chitosan is a biopolymer with biostimulant activity in horticultural crops, although its effect depends on the applied concentration and plant species. The effect of increasing doses of chitosan on the growth and yield of radish (*Raphanus sativus* L.) was evaluated under greenhouse conditions. The experiment was conducted at the "La María" experimental farm, Technical State University of Quevedo, Mocache, Los Ríos, Ecuador. Three chitosan concentrations (1000, 1500, and 2500 mg L^{-1}) plus a distilled water control were tested in a randomized complete block design with five replications. Seedling roots were immersed for one hour in the corresponding solutions prior to transplanting. Morpho-agronomic variables related to vegetative growth and production were evaluated. Data were analyzed using analysis of variance and mean comparison with Tukey's test ($p < 0.05$). The 1000 mg L^{-1} dose stimulated early vegetative growth, with higher values in plant length, leaf number, leaf length, and leaf width at 15 days after transplanting (DAT). The 1500 mg L^{-1} dose produced the best productive response, with higher values in polar and equatorial root diameter, fresh weight, and yield (3885.18 kg ha^{-1}), equivalent to a 159 % increase over the control. It is concluded that chitosan at 1500 mg L^{-1} represents a biostimulant alternative with agronomic potential to improve radish productivity under greenhouse conditions.

Key words: biostimulants, biopolymers, Brassicaceae, plant physiology, agricultural productivity.

RESUMEN: El quitosano es un biopolímero con actividad bioestimulante en cultivos hortícolas, aunque su efecto depende de la concentración aplicada y la especie vegetal. Se evaluó el efecto de dosis crecientes de quitosano sobre el crecimiento y rendimiento del rábano (*Raphanus sativus* L.) bajo condiciones de invernadero. El experimento se realizó en la finca experimental "La María", Universidad Técnica Estatal de Quevedo, Mocache, Los Ríos, Ecuador. Se aplicaron tres concentraciones de quitosano (1000, 1500 y 2500 mg L^{-1}) más un testigo con agua destilada, distribuidos en un diseño de bloques completos al azar con cinco repeticiones. Las raíces de las plántulas fueron sometidas a inmersión durante una hora en las soluciones correspondientes antes del trasplante. Se evaluaron variables morfoagronómicas de crecimiento vegetativo y de producción. Los datos se analizaron mediante análisis de varianza y comparación de medias con la prueba de Tukey ($p < 0,05$). La dosis de 1000 mg L^{-1} estimuló el crecimiento vegetativo temprano, con mayores valores en longitud de planta, número, largo y ancho de hojas a los 15 días después del trasplante (DDT). La dosis de 1500 mg L^{-1} generó la mejor respuesta productiva, con valores superiores en diámetro polar y ecuatorial de raíz, peso fresco y rendimiento (3885,18 kg ha^{-1}), equivalente a un incremento del 159 % respecto al testigo. Se concluye que el quitosano a 1500 mg L^{-1} constituye una alternativa bioestimulante con potencial agronómico para mejorar la productividad del rábano en condiciones de invernadero.

Palabras clave: bioestimulantes, biopolímeros, Crecimiento vegetal, morfoagronómicas, productividad agrícola.

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Received: 20/12/2025

Accepted: 08/06/2026

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Conceptualization: Juan José Reyes-Pérez, Roger Alexander Pincay-Ganchozo. **Investigation:** Juan José Reyes-Pérez, Roger Alexander Pincay-Ganchozo, Mercedes Susana Carranza-Patiño. **Methodology:** Roger Alexander Pincay-Ganchozo, Eduardo Fabian Quinatoa-Lozada. **Supervision:** Juan José Reyes-Pérez, Mercedes Susana Carranza-Patiño. **Writing - original draft:** Roger Alexander Pincay-Ganchozo, Erika Vanessa Castro-Klinger. **Writing - review & editing:** Juan José Reyes-Pérez, Mercedes Susana Carranza-Patiño. **Data curation:** Eduardo Fabian Quinatoa-Lozada, Erika Vanessa Castro-Klinger.

INTRODUCTION

Radish (*Raphanus sativus* L.) is a short-cycle horticultural crop belonging to the Brassicaceae family, whose commercial value lies in the thickened root, its main harvest organ. Its rapid development and ease of management make it a relevant species in small- and medium-scale horticultural systems (1). The growth and quality of the storage organ depend on the plant's physiological response to management conditions, so variables such as leaf morphometry, root diameter, and fresh root weight constitute sensitive indicators of its morphoagronomic performance (2).

Horticultural production faces the need to increase productivity through strategies compatible with agricultural sustainability and the reduction of synthetic input use. In this context, plant biostimulants have gained relevance due to their capacity to modulate physiological processes, improve nutrient uptake, and strengthen plant tolerance to environmental stress (3, 4). Unlike conventional fertilizers, these compounds act on metabolic pathways and intracellular signaling to optimize plant development without directly supplying nutrients (5).

Among biostimulants of natural origin, chitosan stands out for its multifunctional properties. This biopolymer, derived from the deacetylation of chitin found in crustacean exoskeletons, insects, and fungal cell walls, acts as an elicitor of defense responses, growth stimulator, and modulator of tolerance to biotic and abiotic factors (6, 7). Its effect on root development, photosynthetic activity, and nutrient use efficiency has been documented in various economically important horticultural crops, such as *Solanum lycopersicum* (8), *Cucumis sativus* (9), and *Capsicum annuum* (10). However, its effect is not linear; it may vary between positive, neutral, or inhibitory responses depending on concentration, molecular weight, degree of acetylation, and the plant's phenological stage (7, 11).

Information on the effect of chitosan applied via root immersion on radish cultivation under greenhouse conditions is scarce, which limits the definition of technically validated doses. Therefore, the objective of the present study was to evaluate the effect of increasing chitosan doses on the growth and yield variables of *Raphanus sativus* L. under greenhouse conditions.

MATERIALS AND METHODS

Location of the experiment

The experiment was conducted in the greenhouse of the "La María" experimental farm, at the State Technical University of Quevedo, located at km 7.5 on the Quevedo-Mocache road, Mocache canton, Los Ríos province, Ecuador (1°04'48.6" west longitude; 01°03'18" south latitude; 75 m a.s.l.). The site's annual average temperature was 24.4 °C, annual rainfall was 3029.30 mm, and relative humidity was 88 %.

Treatments and experimental design

Three chitosan concentrations were evaluated. Chitosan was commercially obtained as a deacetylated chitin polymer, D-glucosamine (Aldrich). T1 consisted of 1000 mg L^{-1}, T2 of 1500 mg L^{-1}, and T3 of 2500 mg L^{-1}, along with a control treatment with distilled water. These were arranged in a randomized complete block design with five replications. Each experimental unit (EU) comprised 12 plants, with planting distances of 33 cm between plants and 43 cm between rows (1.70 m^2 per EU), for a total of 240 plants in the experiment.

Seedlings were produced in polyethylene trays with a substrate composed of soil and river sand in a 3:2 (v:v) ratio. At 20 days after sowing, the seedling roots were immersed for one hour in the chitosan solutions corresponding to each treatment, or in distilled water for the control. After immersion, one seedling was transplanted per pot containing 2 kg of the same substrate used in the nursery phase.

Agronomic management

Weed control was carried out manually. From 15 DAT (Days after transplanting) onwards, azoxystrobin was applied weekly for preventive and curative management of fungal diseases, and methomyl for insect pest prevention. No fertilizers of any kind were applied.

Variables evaluated

Morphoagronomic variables were evaluated on six plants per experimental unit. Vegetative growth variables were recorded at 8, 15, 22, and 30 DAT; productive variables were recorded at harvest time.

Plant height (cm) was measured with a tape measure from the base of the stem to the vegetative apex. Leaf length (cm) was determined from the base of the blade to the leaf tip, and leaf width (cm) at the region of greatest laminar expansion. Leaf number was obtained by direct counting of fully developed leaves.

Root polar diameter and equatorial diameter were measured with a Vernier caliper (mm); the former corresponded to the maximum longitudinal distance of the root and the latter to the zone of greatest transverse expansion. Root fresh weight (g) was determined on an analytical balance immediately after harvest. Agricultural yield (kg ha^{-1}) was estimated from the total fresh weight of roots per experimental plot (1.70 m^2), proportionally extrapolated to one hectare (10,000 m^2).

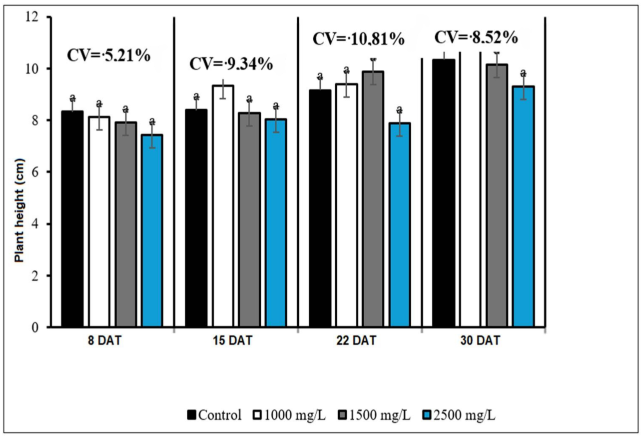
Statistical analysis

Data were subjected to residual normality tests (Shapiro-Wilk) and homogeneity of variance tests (Bartlett), whose results confirmed compliance with parametric assumptions. Analysis of variance (ANOVA) for a randomized complete block design was applied, and for variables with significant differences among treatments, means were compared using Tukey's test ($p < 0.05$). Analyses were performed with R software version 4.4.1 (R Core Team, 2024).

RESULTS AND DISCUSSION

Vegetative growth: plant length

Plant length showed no statistical differences among treatments at 8, 22, and 30 DAT; however, at 15 DAT a significant effect was recorded, with the greatest elongation observed in the 1000 mg L^{-1} treatment and the lowest averages in the 2500 mg L^{-1} dose across all evaluation dates (Figure 1). This temporal pattern indicates that the effect of chitosan on stem elongation is concentrated during the early establishment stages and attenuates as the crop cycle progresses.



Different letters above the bars indicate statistical differences among treatments (Tukey, $p < 0.05$). DAT: days after transplanting

Figure 1. Effect of chitosan doses on plant length of radish under greenhouse conditions

The differential response between doses is consistent with the dose-dependent behavior of chitosan, in which moderate concentrations activate anabolic pathways linked to growth, such as auxin synthesis and cell expansion regulated by the cell wall, while high concentrations may exert an adverse osmotic effect, saturating membrane receptor binding sites and reducing the efficiency of the biostimulant signal (11, 12). Some authors documented positive effects of chitosan on *Raphanus sativus* biomass with clearly concentration-dependent responses (14), while others observed that high concentrations applied as a soil amendment reduced certain growth parameters in lettuce, results consistent with those of the present study (13).

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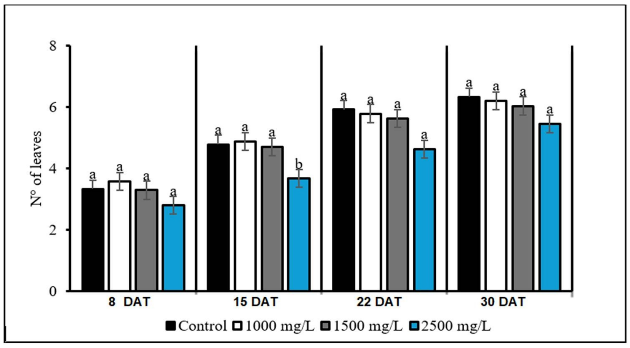

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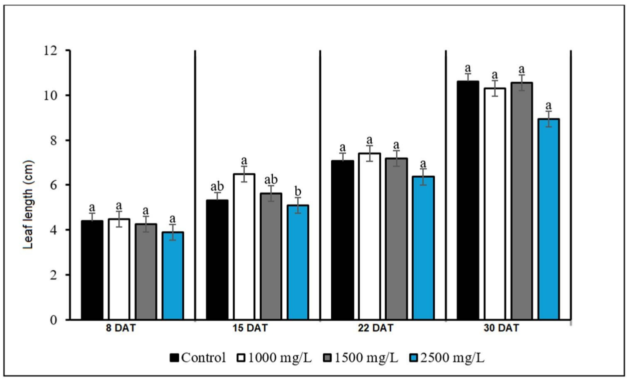
Figure 2. Effect of chitosan doses on leaf number of radish under greenhouse conditions



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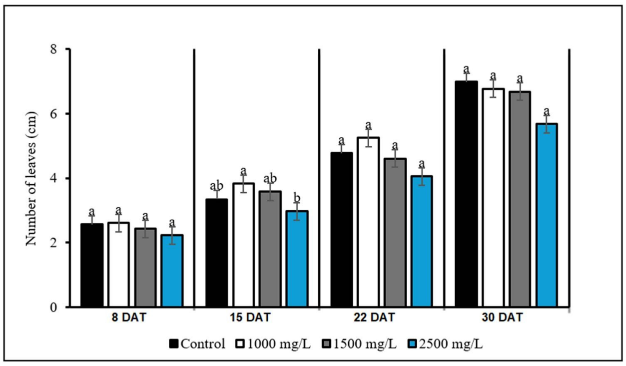
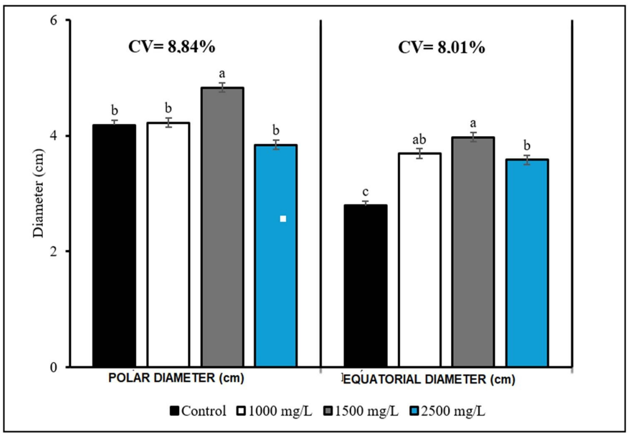
Root polar and equatorial diameter

Root polar and equatorial diameters showed statistical differences among treatments (Figure 5). The 1500 mg L^{-1} dose produced the highest values for both variables, while the control treatment recorded the smallest measurements. The 2500 mg L^{-1} dose presented intermediate values, higher than the control but lower than the 1000 and 1500 mg L^{-1} doses, confirming the inhibition pattern at high concentrations observed for vegetative variables.



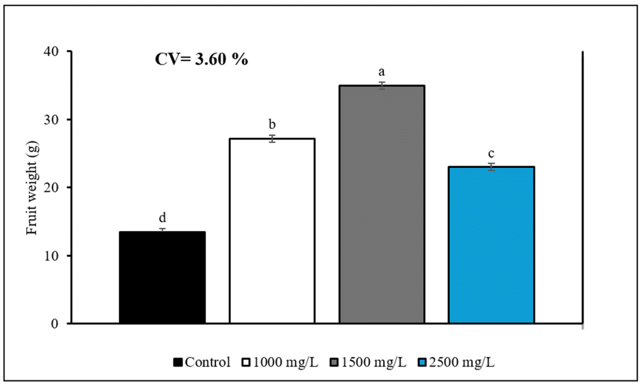
Different letters above the bars indicate statistical differences among treatments (Tukey, $p < 0.05$). DAT: days after transplanting

Figure 4. Effect of chitosan doses on leaf width of radish under greenhouse conditions



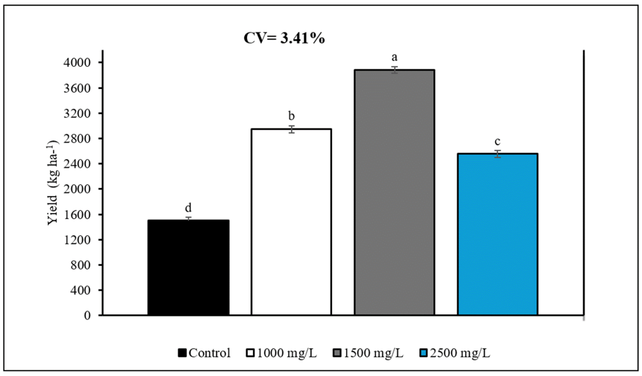
Different letters above the bars indicate statistical differences among treatments (Tukey, $p < 0.05$)

Figure 5. Effect of chitosan doses on polar and equatorial root diameter of radish under greenhouse conditions



Different letters above the bars indicate statistical differences among treatments (Tukey, $p < 0.05$)

Figure 6. Effect of chitosan doses on root fresh weight of radish under greenhouse conditions



Different letters above the bars indicate statistical differences among treatments (Tukey, $p < 0.05$)

Figure 7. Effect of chitosan doses on radish yield under greenhouse conditions

The increase in root diameters with 1500 mg L^{-1} can be attributed to greater physiological efficiency during the filling phase of the storage organ. Chitosan at intermediate concentrations stimulates the activity of enzymes related to reserve carbohydrate synthesis and improves root hydraulic conductivity, which favors the translocation of assimilates toward the thickening root (7). In tomato, some researchers reported significant increases in fruit polar and equatorial diameters with chitosan applications, a behavior that suggests a common physiological mechanism related to the mobilization of photoassimilates toward the harvest organ, regardless of the species (8, 15).

Fresh weight and agricultural yield

Root fresh weight and agricultural yield showed statistical differences among treatments (Figures 6 and 7). The 1500 mg L^{-1} dose produced the highest fresh weight, followed by 1000 mg L^{-1} (27.17 g), 2500 mg L^{-1} (23.00 g), and the control (13.50 g). In terms of yield, the 1500 mg L^{-1} dose reached 3885.18 kg ha^{-1}, equivalent to a 159 % increase over the control (1500 kg ha^{-1}); the 1000 mg L^{-1} dose obtained 2945.55 kg ha^{-1} (96 % higher than the control).

The marked productive increase with 1500 mg L^{-1} shows that this concentration represents the optimal biostimulation threshold under the evaluated conditions. At the physiological level, chitosan at intermediate doses increases root fresh and dry weight, modifies root architecture, and improves water and nutrient uptake (7), enhancing biomass accumulation in the commercial organ. Some studies have pointed out that chitosan activates plant growth-promoting microorganisms in the rhizosphere and regulates signaling pathways that translate into higher productivity, a mechanism that could explain part of the observed increases (6).

In contrast, the 2500 mg L^{-1} dose produced the lowest yield among the chitosan treatments. This inhibition can be attributed to the saturation of chitosan receptors on the plasma membrane, an increase in reactive oxygen species production above the cellular antioxidant capacity, or an adverse osmotic effect induced by the high concentration of the solution during root immersion (7, 11). The non-linear dose-dependent response of chitosan has been documented in multiple horticultural crops (8, 13), and the results of the present study confirm the existence of an optimal concentration range outside which biostimulant effects revert to inhibitory ones.

The yield increases obtained with chitosan at 1500 mg L^{-1} are comparable with those reported for other management sources in radish. Other studies documented improvements in productivity with organic fertilization (16); with integration of mineral fertilizers and vermicompost (17); and with the combination of organic fertilization and chemical fertilizer reduction (19, 20). Chitosan at the optimal dose positions itself as a biostimulant alternative with a favorable environmental safety profile and productive efficiency comparable to that of organic inputs.

CONCLUSIONS

Chitosan exerts a biostimulant-like effect, with differentiated impacts on radish growth and yield, showing concentration-dependent and phenological stage-dependent responses. The 1000 mg L^{-1} dose stimulates early vegetative growth, with significant responses in leaf morphometry and plant length at 15 DAT.

The 1500 mg L^{-1} concentration constitutes the dose with the greatest agronomic potential, reaching the highest values in root polar and equatorial diameter, fresh weight, and yield (3885.18 kg ha^{-1}, 159 % higher than the control). The 2500 mg L^{-1} dose shows an inhibitory effect, consistent with the non-linear dose-dependent behavior of chitosan.

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