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Effect of chitosan on plant growth, flowering, and substrate characteristics of potted Geranium (Pelargonium x hortorum)

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Effect of chitosan on plant growth, flowering, and substrate characteristics of potted Geranium (*Pelargonium x hortorum*)

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Abstract

Modern industrial agricultural practices rely heavily on the use of fertilizers and pesticides to increase crop quality and quality which overtime gradually degrades the quality of cultivated soils and aquatic environments. The purpose of this project is to determine how chitosan polymers with various molecular weights affect the growth and flowering of Geranium plants as well as the water and nutrient retention capacity of the substrate. Three chitosans with different molecular weights (2 kDa, 50 kDa, and 970 Da) were experimented on the geraniums. Each chitosan treatment was applied through a spray system onto the experimental group plants after transplantation into 8-inch square pots with soilless substrate. The medium molecular weight chitosan (T3) was shown to cause the decrease in the amount of applied water and leachate the most, while there was no significant difference found for pH, nitrate, number of inflorescences, SPAD, EC, width, and number of leaves for all treatments.

Introduction

Chitosan is a naturally occurring, biodegradable, and non-toxic biopolymer derived from chitin and composed of a random linear chain of N-acetyl-D-glucosamine units (acetylated unit) and D-glucosamine (deacetylated unit) joined by b-(1-4) linkages (Pandey et al., 2018). Is characterized by its ability to bind to a wide range of cellular components such as cell wall constituents, DNA, and specific receptors, that are involved in defense gene activation, while also stimulating the production of organic molecules that boost adaptation or tolerance towards abiotic stressors (Malerba and Cerana, 2016; Faqir et al 2021). This has allowed it to be used as a biostimulant in the agriculture industry. Biostimulants are naturally occurring substances or microorganisms that stimulate natural processes such as enhancing nutrient uptake, nutrient use efficiency, tolerance to abiotic and biotic stressors, and crop quality and yield. Unlike pesticides and fertilizers, biostimulants do not have a defensive character towards pathogens or contain significant nutrient content (Brown and Saa, 2015; Rouphael and Colla, 2020).

Pelargonium x hortorum are popular fast growing common perennial plants that are most commonly grown for ornamental uses. However, its essential oils (composed of a mixture of volatile phytochemicals) are also used in the pharmaceutical industry for its antifungal, antibacterial, and antiseptic properties (Narnoliya et al., 2019).

Rationale and Objectives

By increasing the rate of plant development and quality through the use of chitosan, it reduces the need for fertilizer and water usage. This can help greenhouse growers and farmers produce more for less while also cutting back on the negative environmental impacts. There is little information on the use of chitosan in the cultivation of pot Geraniums, which is why this research project is aimed at establishing how chitosan polymers with various molecular weights affect the growth and flowering of Geranium plants as well as the water and nutrient retention capacity of the substrate.

Hypothesis

Chitosan biopolymers applied to the foliage and substrate of Geranium pot plants will result in plants with a higher growth rate and a greater number of flowers, as well as a substrate with a greater water retention capacity and less nutrient leaching. Results may vary depending on the molecular weight of the biopolymer used.

Methodology

Plant growing conditions:

Seeds of Geranium (*Pelargonium x hortorum*) were sowed in a 288-plug tray. After 6 weeks of germination, the seedlings were transplanted into 8-inch square pots filled with soilless substrate PROMIX 25 (Premier Tech Horticulture, Québec, Canada) and placed in a polycarbonate greenhouse (located in Agricultural Biotechnology Laboratory) with a heating setpoint of 65°F and a ventilation setpoint of 77°F under natural photoperiod from June to August 2023. The plants were fertilized with 100 ppm N of 13-2-13 once starting 15 days after emergence. The plants were also irrigated to container capacity (CC). CC for Promix BK 25 in an 8-inch pot was determined by rinsing the substrate in four pot replicates until saturation and leaching. Therefore, the substrate was dried until it had a constant weight, and the CC was determined by subtracting the saturated and dry substrate weight.

Treatments and experimental design:

The experiment was structured in a complete randomized design with four treatments and twelve replicates per treatment. The treatments were constituted by T1: control without application of chitosan, T2: application of a chitosan solution of low molecular weight (2 kDa), T3: medium molecular weight (50 kDa) and T4: high molecular weight (970 kDa). The biopolymers were obtained from ChitoLytic. Application of method was through spraying 100mls of the selected treatments onto each experimental plants' leaves and shoots.

Preparation of Chitosan stock solutions:

Analytical powder chitosan was obtained from Sigma-Aldrich Inc. (St. Louis, MO) in three different molecular weights. The stock solutions were prepared according to the methodology reported by Li et al. (2013). A chitosan stock solution (5 mg mL^{-1}) was prepared in 1% acetic acid for each type of molecular weight chitosan, with pH adjusted to 6.0 with NaOH. After stirring (160 rpm) for 24 h at room temperature, the stock solution was filtered and stored.

Application of chitosan

A group of plants in the same experimental conditions were used to evaluate two product concentrations. According to some doses reported for other ornamental plants in scientific publications, concentrations of 0.5 and 1 mg mL^{-1} were tested. Since the plants did not present any toxicity symptoms, the concentration of 1 mg mL^{-1} was chosen. The product was applied twice during the experimental period as a spray at a dose of 60 mL per pot.

Measured variables:

The height of the plant, the width of the canopy, the number of leaves, inflorescences and flowers, and the leaf chlorophyll content were measured every other day for the first 3 weeks after transplant. From there, the variables were measured every two days. The values collected were used to establish the influence of chitosan on plant growth. Furthermore, the shoot dry matter was also considered at the end of the experiment.

The leaf chlorophyll content was indirectly estimated by a handheld chlorophyll meter, model SPAD 502 Plus chlorophyll meter (Spectrum Technologies, Inc., IL). This measurement was obtained for three fully expanded leaves from each plant. The shoot and root dry matter was determined by putting the aerial part of the plant and the roots harvested at the end of the experiment in paper bags and placed inside an oven at 72°C for them to dry for 24 hours. Then they were weighed using a digital balance to take the dry weight.

To evaluate the water retention capacity of the substrate, the amount of leachate of the pots was tracked. For this purpose, the pots were placed in a 1 gal. bucket which leaves a space between the bottom of the pot and the bottom of the bucket to collect the leachate. After each irrigation, the amount of leachate was collected and measured using a probe to obtain a value in ml of leachate (Figure 1).

Figure 1. Setting and random distribution of plants in the greenhouse. 8-inch pot with Geranium plants placed on 1-gal buckets to collect the leachate.

The pH, electrical conductivity (EC), and nitrate (NO_3^-) in the leachate media were measured every week using specific probes for the Orion Star Meter (Thermo Fisher Scientific

Inc., MA). 30 ml of the leachate of each pot were taken and put inside a 50 ml Falcon tube. The leachate was filtered using a 0.45 µm pore diameter filter and a vacuum system, then placed in a freezer at 37°F prior to measurements. The nitrate determination was done through the ultraviolet spectrophotometric detection method, adapted from the book "Standard methods For the Examination of water and wastewater 23th edition" (Eaton et al., 2017). The chemical reagents necessary to carry out the standard curve to determine the nitrate in the samples are Potassium Nitrate 99%, Hydrochloric Acid and Chloroform.

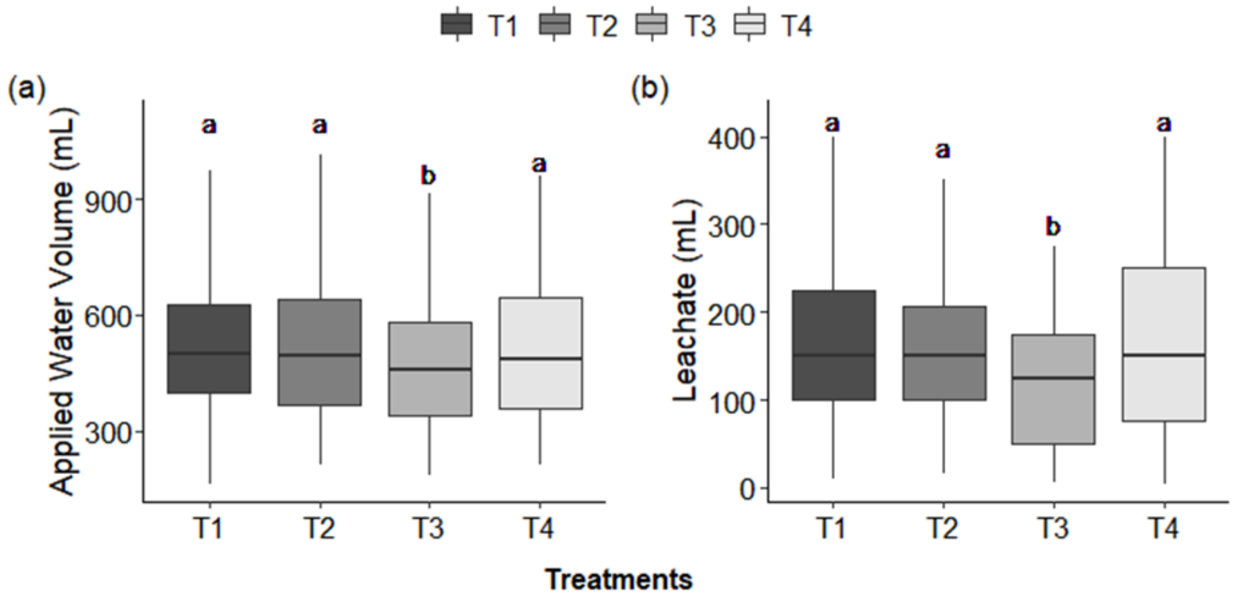
Results

Irrigation and Leachate

Figure 2 shows the values obtained for the volume of water applied and the amount of leachate that was collected for each treatment. There was a statistical difference ($p < 0.05$) between the treatments for the volume of water applied. Treatment 3, in which the medium molecular weight chitosan solution was applied, had less irrigation requirement to be at container capacity than the other treatments. At the same time, this treatment also presented the least amount of leachate. For this treatment, those results could be associated with greater water retention in the substrate or efficient use of water by plants.

Considering the volume of water applied vs. the volume leached from the pots, for T1, T2, and T4, around 35% of water was leached, and 65% was kept in the substrate. For T3, around 29% of water was leached, and 71% was kept in the substrate. This result supports the idea that for T3, there could have been greater water retention in the substrate.

Figure 2. (a) Applied water volume (mL) per irrigation episode and (b) leachate collected from Geranium pot plants growth under chitosan treatments.

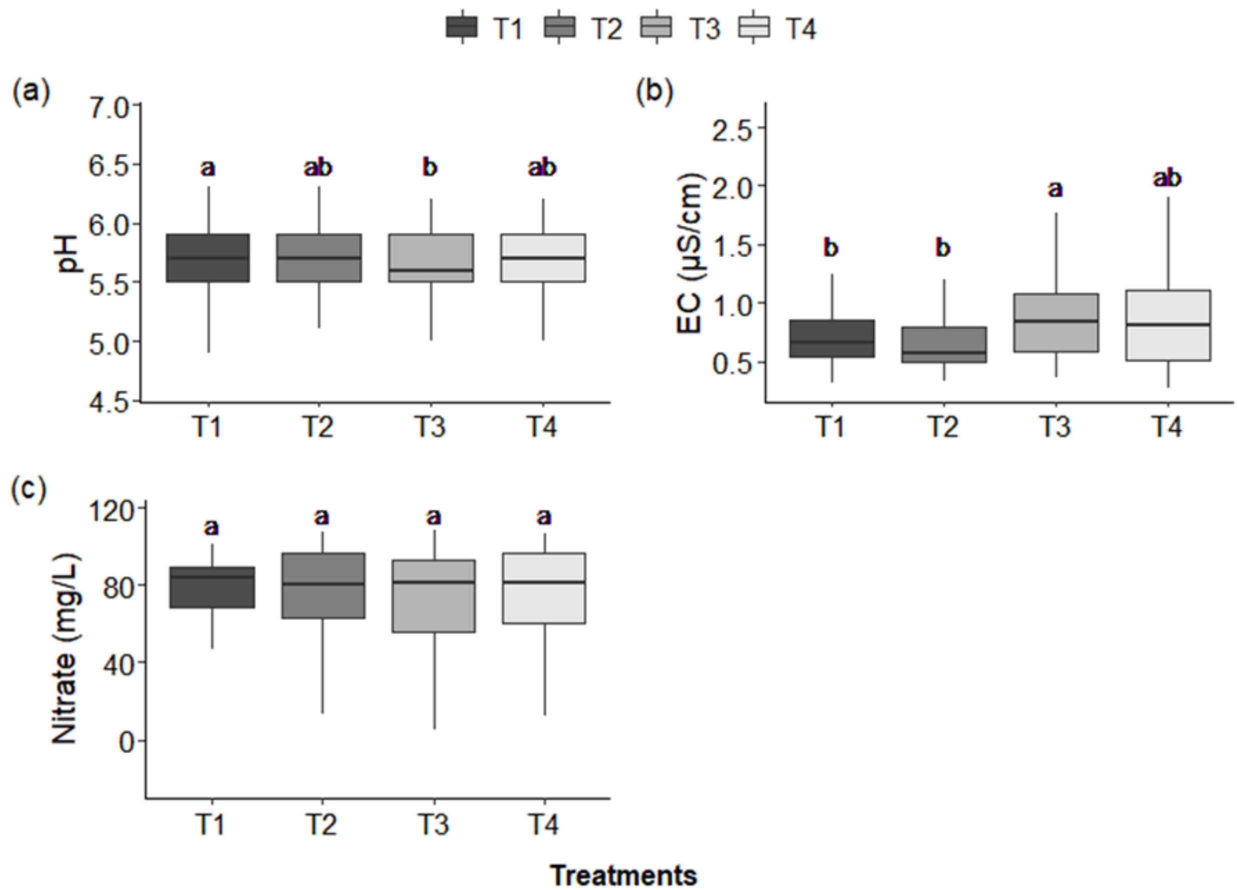


Note. T1, control group without application of chitosan; T2, application of a chitosan solution of low molecular weight (2 kDa); T3, application of a chitosan solution of medium molecular weight (50 kDa); and T4, application of a chitosan solution of high molecular weight (970 kDa). Means with the same letters are not significantly different according to Tukey's Studentized range honestly significant difference separation test at $P \leq \alpha = 0.05$ ($n = 12$)

Leachate Nitrate, pH, and EC

Figure 3 shows the results obtained for the leachate's pH, electrical conductivity, and nitrate content. There were statistical differences between treatments ($p < 0.05$) for pH and EC, without differences for nitrate. The pH, which refers to a measurement of the hydrogen ion concentration (how acidic or basic a solution is), is an important factor influencing plant growth. Plant roots absorb nutrients best when in an environment with the right pH level (5.5-6.5). The pH of the leachate for all treatments varied mainly between 5.5 and 6, which is within the desirable range for most crops to grow satisfactorily.

Figure 3. (a) pH, (b) electrical conductivity – EC (μS/cm), and (c) nitrate concentration (mg/L) of the leachate collected after irrigation of Geranium pot plants growth under chitosan treatments.

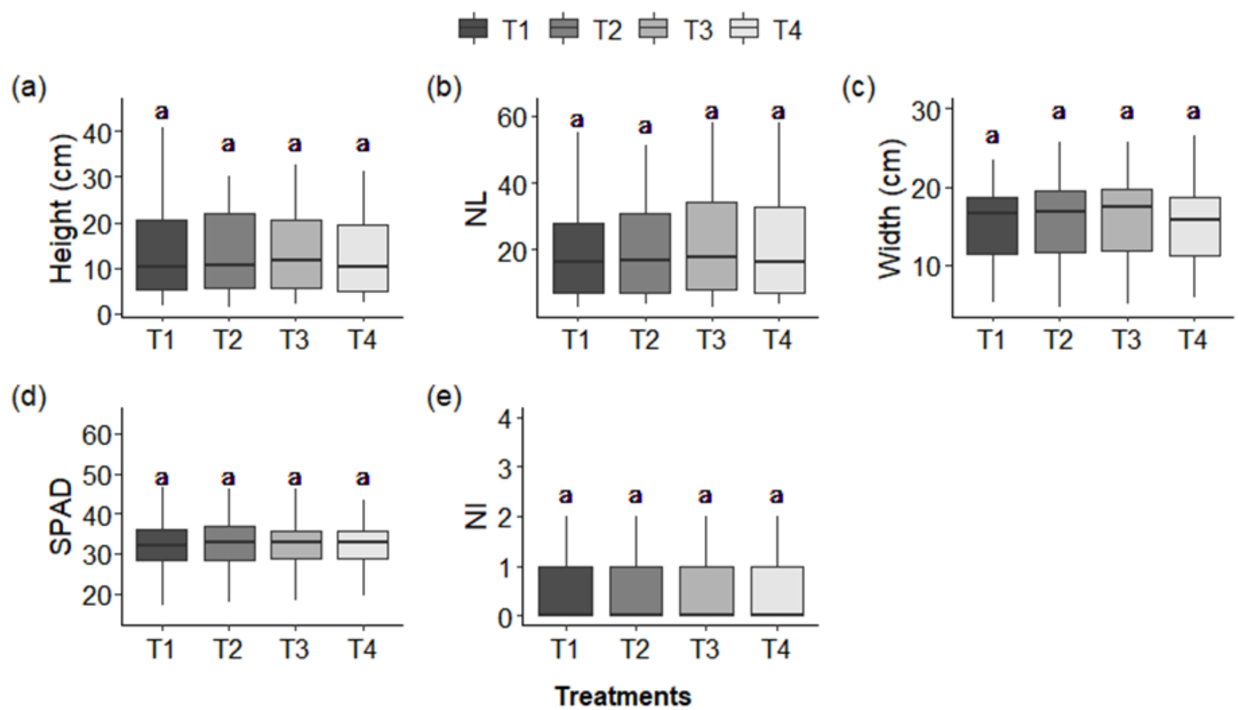


Note. T1, control group without application of chitosan; T2, application of a chitosan solution of low molecular weight (2 kDa); T3, application of a chitosan solution of medium molecular weight (50 kDa); and T4, application of a chitosan solution of high molecular weight (970 kDa). Means with the same letters are not significantly different according to Tukey's Studentized range honestly significant difference separation test at $P \leq \alpha = 0.05$ ($n = 12$)

The EC represents the total dissolved salts in the root substrate (medium). Measuring this parameter provides a general indication of nutrient deficiency or excess. Lower or higher values generally result from an imbalance in the relationship between the amount of fertilizer applied and the plant's needs. The EC of the leachate for all treatments varied mainly between 0.5 and 2 dS/m. Although the EC of the leachate typically results in a lower EC than the substrate solution, and although all plants were fertilized in the same way and rate, the EC value for T1 and T2 was lower compared to T3 and T4. However, this did not influence plant growth (Figure 4).

Plant measurements and dry matter

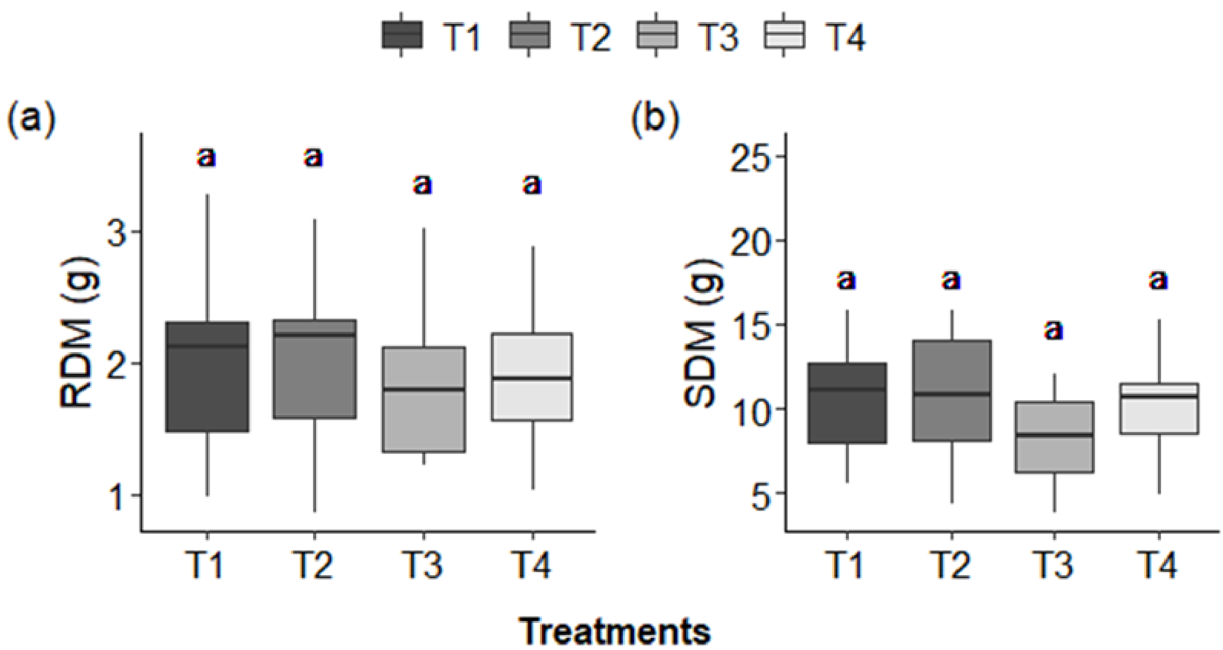
Figure 4. (a) Plant height (cm), (b) the number of leaves – NL, (c) width (cm), (d) SPAD index, and (e) number of inflorescences – NI of Geranium pot plants growth under chitosan treatments.



Note. T1, control group without application of chitosan; T2, application of a chitosan solution of low molecular weight (2 kDa); T3, application of a chitosan solution of medium molecular weight (50 kDa); and T4, application of a chitosan solution of high molecular weight (970 kDa). Means with the same letters are not significantly different according to Tukey's Studentized range honestly significant difference separation test at $P \leq \alpha = 0.05$ ($n = 12$)

<u>Figure 4 shows the results obtained for the plant growth variables. There were no significant statistical differences between treatments (p < 0.05) for any variable. This result indicates that regardless of the molecular weight of the chitosan solution used, the plants did not show different growth responses.</u>

Figure 5. (a) Root Dry Matter – RDM (g), and (b) shoot dry matter – SDM (g) of Geranium pot plants growth under chitosan treatments.



Note. T1, control group without application of chitosan; T2, application of a chitosan solution of low molecular weight (2 kDa); T3, application of a chitosan solution of medium molecular weight (50 kDa); and T4, application of a chitosan solution of high molecular weight (970 kDa). Means with the same letters are not significantly different according to Tukey's Studentized range honestly significant difference separation test at $P \leq \alpha = 0.05$ (n = 12)

<u>Figure 5 shows the results obtained for the roots and shoots dry matter. Following the same trend of the growth variables, there were no significant statistical differences between the</u>

treatments ($p < 0.05$) for any variable. This result indicates that regardless of the molecular weight of the chitosan solution used, the plants did not show different dry matter accumulation responses.

Discussion/Implications

There is a worldwide trend to explore new alternatives to increase the yield of agricultural products while decreasing the usage of resources and time without chemical treatments that degrade the soil quality and aquatic environments. Among these methods is the use of biostimulants such as chitosan. However, the effectiveness of chitosan is dependent on many other factors such as dosage, application method, and the environmental conditions of the plant. From the data collected, the applied water volume, leachate, pH, and EC of the medium molecular weight chitosan had a significant impact in comparison with the rest of the treatments. The other variables such as plant height, number of leaves, SPAD, number of inflorescence, and nitrate didn't have much difference between all of the four treatment groups. Based on the data collected, it seems like chitosan with a molecular weight of 50 kDa is effective in decreasing the amount of water required to hit container capacity which directly decreases the amount of leachate. Moreover, from the data, medium molecular weight chitosan also seems to decrease the pH and increase EC in comparison to the other groups. However, because there was no significant difference for the other variables (height, number of leaves, SPAD, number of inflorescence, and nitrate) between the four treatments, the decrease usage of water in treatment does not impact the growth rate of geraniums nor the leachate's nutrient composition. Because we only experimented with one application method, results regarding chitosan's effectiveness at promoting plant growth may change with other application methods such as drenching, other concentrations of the product and different timing.

Conclusion and future perspectives

From the experiment conducted, data has shown that medium molecular weight chitosan (50 kDa) is effective at decreasing the amount of water required to hit container capacity and leachate from the pots. However, given that no differences were found concerning the growth and accumulation of dry matter in the plants, future research can point to the efficiency of using irrigation water. Having a plant of the same commercial size as the control group with less water would be both an environmental and economic achievement due to the reduced volume of water used, the cost associated with that water, and the operation of irrigation systems. The lower volume of water would be associated with the possibility of using a proportion less than 100% of container capacity, and the lower volume of leachate collected would be associated with lower fertilizer losses. In this context, as a future perspective, using chitosan in combination with different container capacities can be investigated.

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