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Investigation note

Selenium nanoparticles and selenite in lettuce seedling production

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Abstract

The production of quality seedlings in horticultural crops is a fundamental step for their success; to this end, fertilization plays a crucial role. Selenium (Se) has been evaluated for its beneficial effects on plant physiology. Nevertheless, studies at the seedling stage are scarce. Therefore, the present study was conducted at the Autonomous University of San Luis Potosí, Mexico, in 2025 with the aim of evaluating the foliar application of two selenium sources: sodium selenite (Na_2SeO_3) and Se nanoparticles (SeNPs), for the production of lettuce (*Lactuca sativa* L.) seedlings. The treatments were: 1) absolute control, 0 ppm Se (water spraying); 2) 1.5 ppm SeNPs; 3) 3 ppm SeNPs; 4) 4.5 ppm SeNPs; 5) 1.5 ppm Na_2SeO_3; 6) 3 ppm Na_2SeO_3; and 7) 4.5 ppm Na_2SeO_3. After 30 days of germination, dry weight, fresh weight, height, leaf area, shoot/root index, leaf area ratio, chlorophyll index, nitrogen balance index and flavonoid index were determined. The results indicated that foliar application of 3 and 4.5 ppm SeNPs improved the quality of lettuce seedlings, as they generated a better shoot/root balance, higher root dry weight, and a good nitrogen balance. These indicators suggest that seedlings may have more prolonged post-transplant survival and continuous growth, as they can have a greater capacity to absorb water and nutrients. No phytotoxic effects were found on seedling growth.

Keywords:

beneficial elements, nanotechnology, plant stimulation, vegetables.

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In horticultural crops, seedling production represents the beginning of the production process and is a fundamental part of the crop's success or failure. To do this, it is necessary to obtain seedlings with characteristics that allow them to have a high level of field establishment and guarantee continuous growth after transplanting. In their first stage of life, the primary care for seedlings is watering, pest and disease prevention, climate protection, and fertilization.

Recently, several studies have focused on evaluating the effects of some beneficial elements for crops that are not considered essential for plants but, at low doses, can improve growth and development. Among these, selenium (Se) stands out, which is recognized as an essential element in mammals and other organisms, as it participates in various physiological processes (Garza-García *et al*., 2022). In nature, there are different forms of Se: organic ones, consisting of selenoamino acids, and inorganic ones, such as selenite and selenate. Inorganic forms have been used in agricultural systems through biofortification or biostimulation; however, they can be toxic to plants in high concentrations.

In this way, the development of selenium nanoparticles (SeNPs) emerges, which are characterized by sizes of 1 to 100 nm on some of their sides and have greater bioavailability and better biological properties than inorganic sources of Se (Garza-García *et al*., 2022). SeNPs are involved in the stimulation of photosynthetic pigments, the rate of net photosynthesis, gas exchange, the accumulation of osmoprotectants and the synthesis of secondary metabolites, considered to be the primary mechanisms of action in plants (El-Ramady *et al*., 2014). These mechanisms relate to the particle size at the nanometric scale and their physicochemical characteristics, which enable them to enter the plant more efficiently, suggesting that their application can benefit lettuce seedling production and improve quality.

Therefore, the present study aimed to evaluate the foliar application of two selenium sources: sodium selenite (Na_2SeO_3), as an inorganic source and Se nanoparticles (SeNPs) on the production of lettuce seedlings. Seven foliar spray treatments were evaluated on lettuce seedling production: 1) absolute control, 0 ppm Se (water spraying); 2) 1.5 ppm SeNPs; 3) 3 ppm SeNPs; 4) 4.5 ppm SeNPs; 5) 1.5 ppm Na_2SeO_3; 6) 3 ppm Na_2SeO_3; and 7) 4.5 ppm Na_2SeO_3.

Nano selenium was synthesized following the methodology described by Hernández-Díaz *et al*. (2021), at the Center for Research and Assistance in Technology and Design of the State of Jalisco (CIATEJ, by its acronym in Spanish). Three foliar applications of the treatments were made to the lettuce seedlings from the full development of four true leaves, one every week, between 9:30 am and 10 am. The lettuce variety used was iceberg-type Carregón (Enza Zaden, Mexico). The experimental design consisted of randomized blocks with seven treatments and three replications. The experimental units were polystyrene trays with 338 cavities; the experimental plot had 112 seedlings per treatment (1/3 tray). Seedlings were irrigated daily with 1 g of diammonium phosphate (DAP) per liter of water. Seedlings from the center were considered as the useful plot to eliminate the edge factor.

The evaluation of treatments with organic fertilizer and conventional management was made 30 days after germination. Seven seedlings were randomly taken from each treatment from the center of the useful plot. The response variables were as follows: dry weight (DW), fresh weight (FW), seedling height, leaf area, shoot/root index (SRI), leaf area ratio (LAR), chlorophyll index (CI), nitrogen balance index (NBI), and flavonoid index (FI); the latter were measured with DUALEX portable equipment (Optical Leafclip Meter, Pessl Instruments GmbH, Weiz, Austria).

The statistical analysis consisted of an ANOVA; in case of significant differences ($p \leq 0.05$), a Tukey mean test was performed ($p \leq 0.05$). A principal component analysis (PCA) was performed to visualize the differences of the different concentrations of SeNPs (treatments) in lettuce seedling production, using the FactoMineR (V. 3.5.3) and Factoextra (V. 3.5.3) packages. All analyses and the figure were obtained using R software (V 4.1.0.) in RStudio (V 1.4.1717).

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The results of the foliar application of Se nanoparticles (SeNPs) and sodium selenite (Na_2SeO_3), as a source of inorganic Se, are presented in Tables 1 and 2.

Table 1. Effect of foliar application of inorganic Se (Na_2SeO_3) and Se nanoparticles (SeNPs) on the biomass and height of lettuce seedlings.

Source of Se	Dose (ppm)	Dry weight (g)			Fresh weight (g)			Plant height (cm)
		Total	Shoot	Root	Total	Shoot	Root	
Control	0	0.2 ±0.07 a	0.2 ±0.02 a	0.06 ±0.01 b	2.8 ±0.4 a	2.1 ±0.2 a	0.7 ±0.1 a	9.3 ±1 a
SeNPs	1.5	0.2 ±0.03 a	0.2 ±0.03 a	0.07 ±0.01 ab	2.9 ±0.3 a	2.4 ±0.6 a	0.6 ±0.1 a	10.1 ±0.8 a
SeNPs	3	0.3 ±0.05 a	0.2 ±0.03 a	0.08 ±0.02 a	3.2 ±0.6 a	2.6 ±0.4 a	0.7 ±0.1 a	9.9 ±0.6 a
SeNPs	4.5	0.3 ±0.04 a	0.2 ±0.03 a	0.08 ±0.01 a	3.1 ±0.4 a	2.6 ±0.4 a	0.7 ±0.2 a	10.4 ±0.5 a
Na_2SeO_3	1.5	0.3 ±0.02 a	0.2 ±0.02 a	0.07 ±0.01 ab	2.9 ±0.3 a	2.2 ±0.3 a	0.6 ±0.1 a	9.6 ±0.5 a
Na_2SeO_3	3	0.3 ±0.06 a	0.2 ±0.05 a	0.06 ±0.01 ab	2.8 ±0.3 a	2.3 ±0.4 a	0.5 ±0.2 a	9.9 ±0.4 a
Na_2SeO_3	4.5	0.2 ±0.03 a	0.2 ±0.02 a	0.07 ±0.01 ab	2.9 ±0.4 a	2.3 ±0.5 a	0.6 ±0.1 a	9.2 ±0.9 a

Mean values with different letters in the same column are statistically different ($p < 0.05$).

Table 2. Effect of foliar application of inorganic Se (Na_2SeO_3) and Se nanoparticles (SeNPs) on the growth and physiology of lettuce seedlings.

Source of Se	Dose (ppm)	Leaf area (cm^2)	Leaf area ratio (LAR)	Shoot/root index (SRI)	Chlorophyll index (CI)	Nitrogen balance index (NBI)	Flavonoid index
Control	0	30.9 ±2.1 e	182 ±19 c	2.4 ±0.3 b	11.8 ±2.5 c	22.5 ±1.1 b	0.5 ±0.08 abc
SeNPs	1.5	32.2 ±1.9 de	165.4 ±15 cd	2 ±0.3 d	13.2 ±2.6 a	24.5 ±1.2 ab	0.6 ±0.07 a
SeNPs	3	27.2 ±1.8 f	127.9 ±13 d	2.3 ±0.2 c	12.5 ±2.1 b	23.1 ±2.6 ab	0.5 ±0.07 abc
SeNPs	4.5	34.4 ±2.1 d	164 ±17 cd	2.8 ±0.7 a	11.1 ±2.2 c	26.3 ±2.6 a	0.4 ±0.08 c
Na_2SeO_3	1.5	40.2 ±2.9 c	211.7 ±19 b	2.5 ±0.3 b	12.1 ±2.1 bc	21.6 ±2.5 b	0.6 ±0.07 ab
Na_2SeO_3	3	44.0 ±3.1 b	222.3 ±18 b	2.5 ±0.5 b	13.3 ±1.2 a	22.3 ±1.2 b	0.6 ±0.08 a
Na_2SeO_3	4.5	46.9 ±2.7 a	281.1 ±21 a	2.5 ±0.4 b	11.8 ±1.7 c	23.8 ±2.2 ab	0.4 ±0.03 bc

Mean values with different letters in the same column are statistically different ($p < 0.05$).

There were no significant differences in the variables of dry and fresh weight (total and shoot); however, differences in root dry weight were observed between the control and the treatments with 3 and 4.5 ppm of SeNPs. Significant differences were not observed in seedling height either ($p > 0.05$) (Table 1), which could indicate that the treatments evaluated did not cause a phytotoxic effect. Significant differences were found in the variables of leaf area, leaf area ratio (LAR), shoot/root index (SRI), chlorophyll, nitrogen balance index (NBI), and flavonoids ($p < 0.05$) (Table 2).

The PCA showed that the treatments had different effects on the variables evaluated in the seedlings (Figure 1). Two dimensions (dimensions 1 and 2) explained 54.58% of the total variation in the data; dimension 1 showed a different effect of treatments with 3 ppm and 4.5 ppm of SeNPs compared to the rest of the treatments (Figure 1).

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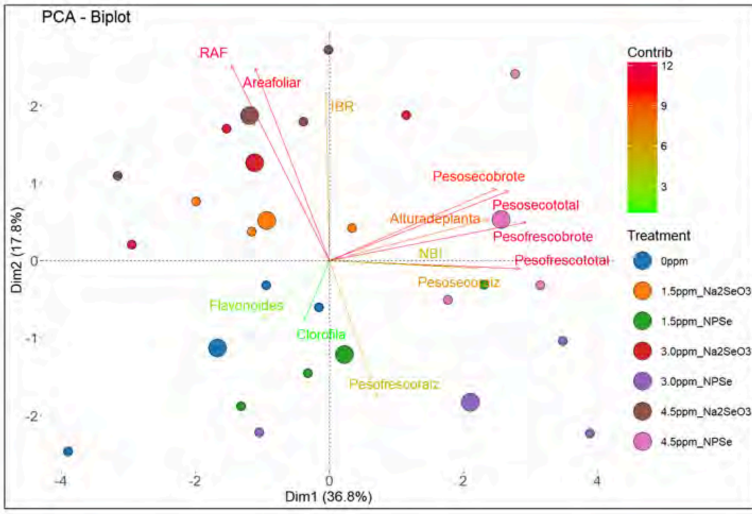
Figure 1. Principal component analysis (PCA) using the seven treatments of the different selenium sources. The colored arrows indicate the contribution of each variable. The table contains the contribution (%) of each variable to each dimension.



Eigenvalue	4.78	2.31
Variabilidad (%)	36.77	17.8
Variable	**Dim 1**	**Dim 2**
Peso fresco brote	17.61	1.01
Peso fresco total	16.55	0.05
Peso seco total	14.78	3.42
Peso seco brote	12.73	3.61
Altura de planta	11.72	1.22
Peso seco raíz	9.90	0.04
Índice de balance de nitrógeno (NBI)	6.52	0.02
Relación área foliar (RAF)	4.39	26.79
Área foliar	2.51	26.05
Flavonoides	1.94	2.31
Peso fresco raíz	1.05	13.03
Clorofila	0.29	2.51
Índice brote:raíz (IBR)	0.01	19.93

SeNP treatments stood out for lettuce seedling production because they led to better SRI and NBI, two critical indicators of seedling quality. Treatments with 3 and 4.5 ppm of Na_2SeO_3 had greater leaf area. The SRI indicates the balance between the shoot and the root of the seedlings; a good balance can guarantee better transplant survival, since transpiration is prevented from exceeding the soil's water absorption capacity. Likewise, a low LAR value is also an indicator of greater resistance to post-transplant stress. Among the SeNP doses, the most outstanding doses were 1.5 and 4.5 ppm, which, in addition to the aforementioned quality indices, could lead to the best values of chlorophyll and NBI, two important indicators of the nitrogen status of plants, which may help their nutrition and rapid growth after transplantation.

The results obtained are relevant due to the lack of information on the use of SeNPs in the production of vegetables at their first phenological stage. Most studies have focused on the post-transplant stages and the evaluation of final production variables (Gaucin-Delgado *et al*., 2024; Golubkina *et al*., 2024). A study by Huang *et al*. (2023) on Purple Rome crisp lettuce found that Na_2SeO_3 doses lower than 8 µM significantly promoted seedling growth. The authors also found that the enzyme activity and gene expression of antioxidant enzymes increased significantly with exogenous application of Se. Another important effect they report is an increase in nutrient accumulation in lettuce in the seedling stage.

Other authors have demonstrated a positive effect of SeNPs on seedling ontogenesis (Malagoli *et al*., 2015) and on organogenesis and physiology, which promotes their growth and protects them against abiotic stress (Mozafariyan *et al*., 2014; Tarrahi *et al*., 2021). The mechanism of action of SeNPs on root growth has been related to the regulation of phytohormone biosynthesis, such as auxins, gibberellins, and cytokinins (Khai *et al*., 2024). Increased glycerate accumulation and enrichment in the citrate cycle (Krebs cycle) have also been reported in chili seedling roots (Campos-García *et al*., 2025). On the other hand, higher doses of Na_2SeO_3 (16 µM) have been reported to inhibit seedling growth and induce the production of reactive oxygen species, causing oxidative damage to membrane lipids and cell death (Bano *et al*., 2021; Huang *et al*., 2023).

Conclusions

The foliar application of selenium nanoparticles (SeNPs) can improve the quality of lettuce seedlings, specifically, with doses of 3 and 4.5 ppm of SeNPs, since they generated a better shoot/root balance (SRI), due to a higher root dry weight and a good nitrogen balance (NBI), indicators

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that give a high post-transplant survival capacity, better establishment and continuous growth of the crop. No phytotoxic effects of the treatments with both Se sources were identified on lettuce seedling growth.

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