

Assay on the impact of seed priming with ionic selenium, nanoselenium and micro selenium on early growth, biomolecules and nutrient content in cucumber seedlings

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Abstract

Selenium (Se) is a beneficial nutrient for plants and its application as seed priming is associated with positive effects on their growth. The use of Se occurs in ionic or nanometric form, however, another possible use is in micrometric form, which to our knowledge has not been studied in plants. The objective of the study was to evaluate the seed priming of cucumber (*Cucumis sativus* L.) with sodium selenite (Na_2SeO_3), nanoparticles (SeNPs) and Se microparticles (SeMPs) at concentrations of 0, 0.1, 0.5, 1.0, 1.5 and 3.0 mg L^{-1} of Se, for each of the mentioned forms. Growth, biomass, vigor, biomolecules and nutrients were evaluated in cucumber seedlings grown in a growth chamber for 15 days. The results showed increases in seedling length and biomass for all Se forms, which was reflected in increases in vigor indices from 21.42% to 27.72% for vigor index 1 (length) and from 16.96% to 34.5% for vigor index 2 (biomass), with SeMPs standing out at 1.0 and 1.5 mg L^{-1} . Regarding pigments, variable effects were observed, where some treatments did not modify the concentration of chlorophylls and carotenoids (SeMPs) and others negatively affected (SeNPs and Na_2SeO_3). Reduced glutathione increased from 13.48% to 31.59%, with SeMPs standing out at 1.0 and 1.5 mg L^{-1} . Phenols, flavonoids, proteins, S, K and Mg were also increased with the different Se materials; however, P, Ca, Fe, Zn, Cu and Mn decreased with some Se treatments. The results indicate that it is advisable to apply Na_2SeO_3 , SeNPs and SeMPs, mainly SeMPs at 1.0 and 1.5 mg L^{-1} .

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Introduction

In recent years, the use of plant biostimulants has increased due to the benefits they provide to horticultural crops. A biostimulant is any substance or microorganism applied to plants with the aim of improving nutritional efficiency, stress tolerance and quality characteristics, regardless of nutrient content (du Jardin, 2015). According to this, biostimulation is a biological response triggered by biostimulants, which cause adaptive modification of metabolic processes so that the organism makes adjustments that lead to more efficient use of resources (Juárez-Maldonado *et al.*, 2021).

Within biostimulants, inorganic compounds such as selenium (Se), which is not considered essential for plants, but is considered a beneficial nutrient, stand out (Brown *et al.*, 2022). Se is involved in physiological and metabolic processes of plants, which help them in their growth, quality, absorption and assimilation of nutrients and in the mitigation of stress (Medrano-Macías and Narvaéz-Ortiz, 2022). Se and S in plants share similar properties since they are absorbed, translocated and metabolized via analogous routes (González-Morales *et al.*, 2017). Se has different oxidation states, with selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) being the ways in which plants absorb it due to their high solubility (Foroughbakhch Pournavab *et al.*, 2020). The SeO_4^{2-} is absorbed by the roots of plants through sulfate transporters; in contrast, SeO_3^{2-} is absorbed by means of phosphate transporters and aquaporins (Garduño-Zepeda and Márquez-Quiroz, 2018). Both absorption processes result in the synthesis of organic compounds such as selenomethionine (SeMet), selenocysteine (SeCys) and methylselenocysteine (MSeCys) or in the incorporation of Se into other metabolites (Jiang *et al.*, 2021). The SeO_4^{2-} is absorbed by plants and has to be reduced to SeO_3^{2-} and subsequently to selenide (Se^{2-}), which passes to SeMet, SeCys or MSeCys (El-Ramady *et al.*, 2016). Compared with inorganic forms, organic forms are also absorbed by plants, and the assimilation process is faster (Li *et al.*, 2023).

When SeO_4^{2-} is absorbed by the roots, it does not undergo any chemical modification and is rapidly transported through the xylem to shoots and leaves to later be assimilated into organic forms (Kowalska *et al.*, 2020). In the case of SeO_3^{2-} , when it is absorbed through the roots, it is rapidly assimilated to its organic forms and to other unidentified forms that are mainly retained in the roots, and its translocation to the shoots is low (Wang *et al.*, 2020). Se is biologically active for the growth and development of plants at very low concentrations; however, at high concentrations, it can be toxic (Dawood *et al.*, 2020). Most horticultural crops, including cucumber, are non-accumulators of Se; that is, they are plants where a Se concentration above 20 mg L^{-1} can be toxic (Becvort-Azcurra *et al.*, 2012).

Another way in which Se can be applied to biostimulate crops is in the form of nanoparticles (NPs) and microparticles (MPs). SeNPs and other nutrients have already been evaluated in different crops and under different growing conditions, revealing that positive results can be obtained at low concentrations (Juárez-Maldonado *et al.*, 2021; Sariñana-Navarrete *et al.*, 2023). These materials have been defined as those with at least one dimension $\leq 100 \text{ nm}$, although the biostimulant properties of the materials are observed even with dimensions greater than 100 nm (González-Morales *et al.*, 2022).

The use of MPs in agriculture, whether as nutrients or other compounds, is very rare, with Fe, Zn, B, Cu, Si, chitosan (Cs) and alginate (Alg) being evaluated so far (Wang *et al.*, 2019; Macedo *et al.*, 2021; Read *et al.*, 2021; Jurić *et al.*, 2021a; de Alencar *et al.*, 2024). To date, no studies have been published on SeMPs and the few studies that exist on other compounds in micro form indicate that they have the potential to stimulate processes in plants and improve their yield and quality (Jurić *et al.*, 2021b). MPs have physical dimensions between 1 and $1,000 \mu\text{m}$ (Galogahi *et al.*, 2020). These MPs can have the same composition as NPs and ionic

materials, but because the surface/volume ratio is different from that of NPs and bulk materials, they exhibit different optical, electrical, thermal and magnetic properties than the latter (Joye and McClements, 2014; Galogahi *et al.*, 2020). These findings indicate the potential use of MPs as alternative biostimulants to NPs and bulk materials.

Seed priming is a form of biostimulation that is becoming increasingly important. It consists of a treatment prior to sowing, in which the seeds are soaked in a solution or dispersion with a substance or substances at a specific concentration and for a specified time (Mahakham *et al.*, 2017). This practice can stimulate plants, through what is known as priming memory, associated with the activation of physiological and biochemical processes leading to increased seed germination percentage, vigour, phytochemicals and stress mitigation in plants (Chen and Arora, 2013). Therefore, the objective of the present study was to evaluate seed priming with Se in ionic, nanometric and micrometric forms on initial growth, biomolecules and nutrients in cucumber seedlings, and to demonstrate whether the micrometric form of Se is a recommended option to achieve biostimulation of this crop.

Materials and Methods

Synthesis and preparation of selenium materials

The materials were synthesized and prepared at the Center for Research in Applied Chemistry (Saltillo, Coahuila, Mexico). For the synthesis of the SeNPs, in a glass reactor with mechanical stirring and a nitrogen-vacuum system, selenious acid (H_2SeO_3) and a gum Arabic (GA) solution (2 g L^{-1}) were added as stabilizer, after which the reactor was stirred at 400 rpm at 0°C for 15 min. Next, hydrazine (N_2H_4) was added dropwise to carry out the reduction. The reaction mixture was stirred for 1-2 h in a nitrogen atmosphere at 0°C . Scanning electron microscopy analysis revealed that the SeNPs ranged from 49.8-156.5 nm in size and had a spherical morphology (Figure 1A). The particle size distribution histogram is presented in Figure 1B. The normal curve was broad and showed an average diameter of 98.5 nm for the SeNPs.

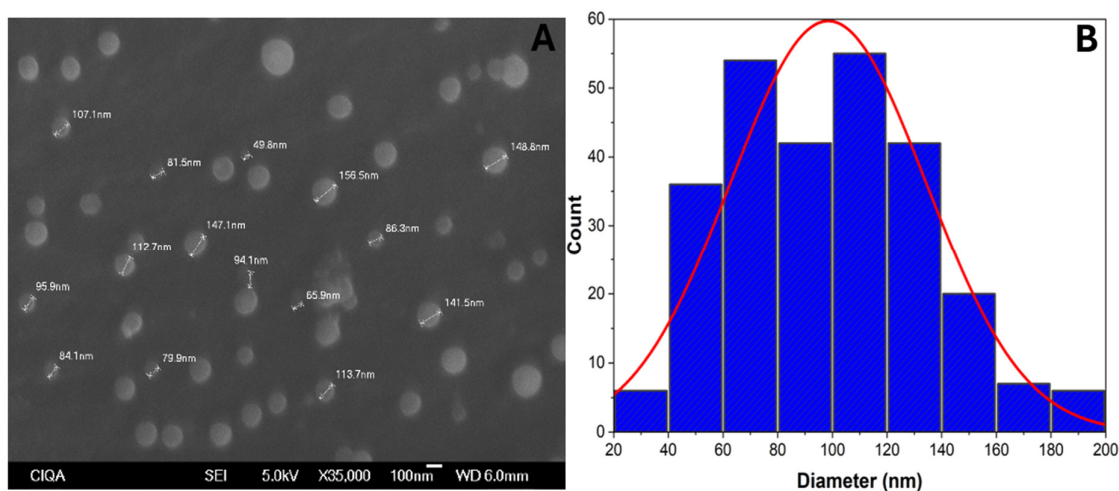


Figure 1. Scanning electron microscopy (A) and histogram of SeNPs size distribution (B)

The GA solutions and the dispersant agrex were added to the SeMPs and the Na_2SeO_3 so that all the Se materials were under the same conditions, since these processes are part of the synthesis and preparation of the SeNPs. GA has excellent stabilizing properties and is widely used to preserve the stability of nano and micromaterials by stimulating the repulsive forces between particles, which limits their tendency to

agglomerate. Furthermore, it is a natural and biodegradable stabilizing agent, which increases its applications (Kong *et al.*, 2014; Mohamed *et al.*, 2025).

A 2 L aqueous dispersion containing 1.1 g of SeNPs was subsequently mixed in the reactor with 4 mL of agricultural dispersant agrex (dioctyl sulfosuccinate 2.3%) and stirred for 30 min at 400 rpm. A stock dispersion was obtained, from which the concentrations to be used were prepared. The preparation of the SeNPs was based on what was reported by Quiterio-Gutiérrez *et al.* (2019) with some modifications. Aqueous samples of Se/GA containing 0.075% and 0.25% Se exhibited zeta potential values of -35.6 mV and -37.1 mV, respectively, suggesting that they can be easily dispersed in aqueous media. Aqueous samples of Se/GA stored for 11 months exhibited very similar values, indicating the colloidal stability of the SeNPs.

Commercial SeMPs with a size of 150 μm and a purity of 99.5% (Sigma Aldrich, St. Louis, MO, USA) were used, which were functionalized. In a glass reactor with mechanical stirring, 2 L of distilled water and 4 g of GA were added, and the mixture was stirred (440-500 rpm) for 20 min until the gum arabic was completely dissolved. Then, 1.1 g of commercial SeMPs was added. The dispersion was stirred for 60 min to homogenize the MPs, then 4 mL of agrex was added, and the dispersion was again stirred for 30 min. A stock dispersion was obtained, from which the concentrations to be used were prepared.

Ionic Se was used in the form of sodium selenite (Na_2SeO_3) with a purity of 99% (Sigma Aldrich, St. Louis, MO, USA), which was functionalized. In a glass reactor with mechanical stirring, 0.5 L of distilled water and 3 mL of agrex were added, and the reactor was stirred (440-500 rpm) for 20 min until the mixture was completely dissolved. Next, 0.5 L of an aqueous solution containing 1.5 g of Na_2SeO_3 was added. The mixture was homogenized by stirring for 60 min, 0.5 L of an aqueous mixture containing 3 g of GA was added, and the mixture was stirred again for 30 min. Finally, a stock solution was obtained, from which the concentrations used were prepared.

Plant material and description of treatments

Braga F1 cucumber seeds from Bejo (Waarmenhuizen, Netherlands) were used. The seeds were treated with fludioxonil and had a purity of 99.9% and a germination percentage of 99%. The experiment was carried out at the Plant Physiology Laboratory of the Horticulture Department of Antonio Narro Autonomous Agrarian University (Saltillo, Coahuila, Mexico).

The treatments consisted of a control (distilled water) and five concentrations (0.1, 0.5, 1.0, 1.5 and 3.0 mg L^{-1}) of Na_2SeO_3 , SeNPs and SeMPs. The choice of concentrations was based on the study of Castillo-Godina *et al.* (2016). The solutions and dispersions were prepared in 150 mL beakers containing 50 cucumber seeds in 100 mL of the solutions and dispersions of the different forms of Se. The treatments were subsequently stored in the dark for 24 h at 23-25 °C and stirred every 8 h to prevent the materials from precipitating. All the solutions and dispersions of Se were prepared with distilled water at a pH of 6.

For the assay, after 24 h of seed priming, 10 cucumber seeds were placed in each Petri dish (diameter of 125 mm) that had filter paper that helped retain moisture. Each treatment consisted of five replicates, where one replicate was a Petri dish. The Petri dishes were subsequently placed in a growth chamber with a daytime temperature and relative humidity ranging from 23-25 °C and 35-45%, respectively, and a nighttime temperature and relative humidity ranging from 17-19 °C and 65-75%, respectively. A photoperiod (12 h light per day) was maintained by means of LED lamps with white light, which emitted photosynthetically active radiation (PAR) of 95 $\mu\text{mol m}^{-2}\text{s}^{-1}$. To maintain the moisture of the seeds, distilled water (pH 6) was added when necessary. Finally, after 15 days, germination percentage, radicle and hypocotyl length, fresh and dry biomass of radicle and hypocotyl, vigor, biomolecules and minerals were determined. To determine the dry biomass, the seedlings were dried in a drying oven at 70 °C for 72 h.

Vigor indices

The vigor indices (VIs) of the seedlings were calculated as described in Carballo-Méndez *et al.* (2019) using the equations described below:

$$VI1 = (HL + RL) * \%G \quad (1)$$

$$VI2 = (HDB + RDB) * \%G \quad (2)$$

where

HL: Hypocotyl length; RL: Radicle length; %G: Germination percentage; HDB: Hypocotyl dry biomass; RDB: Radicle dry biomass. The data for VI1 are in cm, and those for VI2 are in mg.

Biochemical determinations

For biochemical determinations, complete seedlings (radicles and hypocotyls) were taken 15 days after the start of the test, placed at -80 °C in a deep freezer and then lyophilized for 72 h in a lyophilizer (Labconco, model FreeZone 2.5 L, Kansas City, MO, USA). The mixed samples were macerated until a fine powder was obtained for analysis.

The pigments were determined as described by Wellburn (1994), where 15 mg of lyophilized mixed tissue was mixed with 1,250 µL of methanol in 2 mL eppendorf tubes. The mixture was subsequently incubated in the dark for 24 h at a temperature of 20-25 °C. After 24 h, the absorbance of the supernatant was read with a UV-Vis spectrophotometer (Genesis 10s UV-Vis, Thermo Scientific, Waltham, MA, USA) at 666, 653 and 470 nm. The concentration of the pigments was expressed in milligrams per gram of dry weight (mg g⁻¹ DW) via the following equations:

$$Chla = [15.65(Abs666) - 7.34(Abs653)] \quad (3)$$

$$Chlb = [27.05(Abs653) - 11.21(Abs666)] \quad (4)$$

$$Carotenoids = \frac{[(1000 * Abs470) - 2.86(Chla) - 129.2(Chlb)]}{221} \quad (5)$$

Chl_a: Chlorophyll *a*; Chl_b: Chlorophyll *b*; Abs: Absorbance. The sum of Chl_a and Chl_b is the total Chl.

The soluble protein concentration was determined via the methodology described by Bradford (1976). A total of 20 mg of lyophilized mixed tissue and 2 mL of phosphate buffer at 100 µM (pH 7-7.2) were mixed in 2 mL tubes. The samples were sonicated for 10 min and centrifuged at 12,500 rpm at 4 °C for 10 min. Subsequently, 0.1 mL of the supernatant and 1 mL of the Bradford reagent were placed in a test tube. The mixture was allowed to stand for 5 min, and its absorbance at 595 nm was evaluated via a UV-Vis spectrophotometer. The data are reported in milligrams per gram of dry weight (mg g⁻¹ DW).

For the quantification of reduced glutathione (GSH), the procedure described by Xue *et al.* (2001) was followed. The extraction was the same as that for the proteins. In test tubes, 0.48 mL of the supernatant, 2.2 mL of Na₂HPO₄ at 0.32 M and 0.32 mL of the 5,5-dithio-bis-2 nitrobenzoic acid dye were added at 1 mM, mixed, allowed to stand for 15 min and read in a UV-Vis spectrophotometer at 412 nm. The data are reported in micromoles per gram of dry weight (µmol g⁻¹ DW).

Total phenols were determined via the methodology described by Singleton *et al.* (1999). They were extracted by placing 20 mg of lyophilized mixed tissue in 2 mL eppendorf tubes, and 2 mL of methanol was added. The mixture was vortexed for 20 s and then sonicated for 5 min. Finally, the samples were centrifuged at 12,000 rpm for 10 min at 4 °C to obtain the supernatant, which was used for quantification, which was carried out in test tubes by adding 50 µL of each sample, 200 µL of the Folin Ciocalteu reagent at 100%, 500 µL of 20% Na₂CO₃ and 5 mL of distilled water. The mixture was allowed to stand for 30 min at 45 °C, after which it was read at 750 nm with a UV-Vis spectrophotometer. The phenol results were expressed as milligram gallic acid equivalents per gram dry weight (mg GAE g⁻¹ DW).

The flavonoids were quantified following the of Zhishen *et al.* (1999). The extraction method was the same as that used for total phenols. For mixing, 250 µL of the sample was placed in a test tube, followed by the addition of 75 µL of 5% NaNO₂ and vortexing. After 5 min, 150 µL of 10% AlCl₃ was added; then, 500 µL of 1 M NaOH was added, plus a final volume of 2,025 µL of distilled water. The absorbance was measured at 510

nm with a UV-Vis spectrophotometer. The results are reported in milligrams equivalents of catechin per gram of dry weight (mg CE g⁻¹ DW). Minerals were determined in the mixed tissue (hypocotyl and radicle). For the digestions, the methodology described by López-Morales *et al.* (2020). Fifty mg of dehydrated sample was weighed into 100 mL beakers, 20 mL of the triacid mixture (1 L of concentrated HNO₃, 100 mL of concentrated HCl, and 25 mL of concentrated H₂SO₄) were added, the mixture was covered with a watch glass, and then it was digested in a heating grill (250 °C) until clarification of the sample. Finally, the digested samples were brought to 25 mL with deionized water and filtered with a 0.45 µm diameter nylon membrane. The quantification of minerals was carried out via the technique of atomic emission spectrometry by plasma (ICP - OES brand Perkin Elmer, model Optima 8300). The results are reported in milligrams per kilogram of dry weight (mg kg⁻¹ DW).

Experimental design and data analysis

A completely randomized design was used, with 16 treatments and five repetitions per treatment. Those five replicates were used to evaluate agronomic and biochemical variables, while only four replicates were used for minerals. A Shapiro-Wilks normality test was performed, followed by analysis of variance and Fisher's LSD test of means ($p \leq 0.05$) for parametric data and the Kruskal-Wallis test ($p \leq 0.05$) for non-parametric data. A Pearson correlation analysis was also performed. All analyses were performed using the statistical softwares Infostat (v2020) and GraphPad Prism 8.

Results

Seed germination and early seedling growth

The different forms of Se did not affect the germination percentage of cucumber seeds or the length of the hypocotyl (Table 1).

Table 1. Percentage of seed germination and length of hypocotyls and radicles of cucumber seedlings

Treatments (mg L ⁻¹)	Germination (%)	Hypocotyl length (cm)	Radicle length (cm)	Total length (cm)
AC	100 ± 0.00 ^{a*}	1.67 ± 0.16 ^{a*}	3.27 ± 0.47 ^{cd}	4.94 ± 0.45 ^{dc}
Na ₂ SeO ₃ -0.1	100 ± 0.00 ^a	1.47 ± 0.07 ^a	4.05 ± 0.55 ^{a-d}	5.52 ± 0.55 ^{a-c}
Na ₂ SeO ₃ -0.5	100 ± 0.00 ^a	1.53 ± 0.08 ^a	4.30 ± 0.46 ^{ab}	5.83 ± 0.41 ^{a-d}
Na ₂ SeO ₃ -1.0	100 ± 0.00 ^a	1.50 ± 0.08 ^a	4.10 ± 0.86 ^{a-c}	5.60 ± 0.91 ^{a-c}
Na ₂ SeO ₃ -1.5	98 ± 4.47 ^a	1.57 ± 0.05 ^a	4.11 ± 0.38 ^{a-c}	5.68 ± 0.41 ^{a-c}
Na ₂ SeO ₃ -3.0	100 ± 0.00 ^a	1.49 ± 0.17 ^a	4.03 ± 0.78 ^{a-d}	5.52 ± 0.77 ^{a-c}
SeNPs-0.1	100 ± 0.00 ^a	1.51 ± 0.08 ^a	3.95 ± 0.47 ^{a-d}	5.46 ± 0.52 ^{a-c}
SeNPs-0.5	100 ± 0.00 ^a	1.58 ± 0.06 ^a	3.15 ± 0.71 ^d	4.73 ± 0.67 ^c
SeNPs-1.0	100 ± 0.00 ^a	1.48 ± 0.04 ^a	3.83 ± 1.08 ^{b-d}	5.31 ± 1.07 ^{b-c}
SeNPs-1.5	100 ± 0.00 ^a	1.58 ± 0.34 ^a	3.15 ± 0.91 ^d	4.73 ± 1.00 ^c
SeNPs-3.0	100 ± 0.00 ^a	1.67 ± 0.13 ^a	3.68 ± 0.97 ^{b-d}	5.35 ± 0.96 ^{b-c}
SeMPs-0.1	100 ± 0.00 ^a	1.50 ± 0.09 ^a	3.60 ± 0.88 ^{b-d}	5.10 ± 0.93 ^{a-c}
SeMPs-0.5	100 ± 0.00 ^a	1.55 ± 0.07 ^a	4.45 ± 0.73 ^{ab}	6.01 ± 0.79 ^{a-c}
SeMPs-1.0	100 ± 0.00 ^a	1.54 ± 0.14 ^a	4.52 ± 0.82 ^{ab}	6.06 ± 0.77 ^{ab}
SeMPs-1.5	96 ± 5.48 ^a	1.43 ± 0.06 ^a	4.88 ± 0.85 ^a	6.31 ± 0.89 ^a
SeMPs-3.0	100 ± 0.00 ^a	1.52 ± 0.08 ^a	3.87 ± 0.35 ^{b-d}	5.39 ± 0.37 ^{a-c}
CV (%)	1.77	8.28	18.75	13.77

*Notes: AC: Control; SeNPs: Selenium nanoparticles; SeMPs: Selenium microparticles; CV: Coefficient of variation. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$ and *Kruskal-Wallis, $p \leq 0.05$); $n = 5$; ± standard deviation (SD)

In terms of radicle length, the opposite occurred, as the SeMPs treatments at 1.5, 1.0 and 0.5 mg L⁻¹ and the Na₂SeO₃ treatment at 0.5 mg L⁻¹ promoted increases of 49.23, 38.22, 36.08 and 31.49%, respectively, in comparison with those of the control (Table 1). Compared with those of the control, the total length of the seedlings treated with SeMPs at 1.5, 1.0 and 0.5 mg L⁻¹ increased by 27.67, 22.42 and 21.41%, respectively (Table 1).

On the other hand, for the fresh biomass of the hypocotyl, all the levels of SeMPs were greater than those of the control, with increases of 12.74, 19.93, 19.6, 17.89 and 11.98% for 0.1, 0.5, 1.0, 1.5 and 3.0 mg L⁻¹ of SeMPs, respectively (Table 2). For SeNPs, the level of 0.5 mg L⁻¹ increased by 14.62%, and for 3.0 mg L⁻¹ of Na₂SeO₃, it increased by 15.77% (Table 2). Regarding fresh radicle biomass, all treatments with Se were superior to the control, being the most outstanding SeMPs at 0.5, 1.0 and 1.5 mg L⁻¹ and SeNPs at 0.1 mg L⁻¹, increasing 81.38, 74.53, 92.65 and 69.2%, respectively (Table 2). The total fresh biomass presented a similar trend, where the control presented the lowest value; however, it was statistically equal to that of Na₂SeO₃ at 0.1 mg L⁻¹ and that of SeNPs at 1.5 mg L⁻¹. The best treatments were SeMPs at 0.5 and 1.5 mg L⁻¹, with increases of 28.67 and 28.52%, respectively, compared with those of the control (Table 2).

Table 2. Fresh and dry biomass of cucumber seedlings

Treatments (mg L ⁻¹)	HFB (mg)	RFB (mg)	TFB (mg)	HDB (mg)	RDB (mg)	TDB (mg)
AC	206.90 ± 20.83 ^a	34.32 ± 3.22 ^f	241.22 ± 22.94 ^f	12.52 ± 2.10 ^{c*}	1.36 ± 0.26 ^{ef}	13.88 ± 2.32 ^f
Na ₂ SeO ₃ -0.1	214.13 ± 15.31 ^{de}	49.04 ± 8.98 ^{de}	263.17 ± 22.71 ^{d-f}	16.18 ± 1.26 ^a	1.91 ± 0.41 ^{a-c}	18.09 ± 1.62 ^{a-c}
Na ₂ SeO ₃ -0.5	224.60 ± 9.62 ^{b-c}	54.26 ± 3.60 ^{b-c}	278.86 ± 13.13 ^{b-c}	15.32 ± 1.08 ^{a-c}	2.05 ± 0.38 ^{ab}	17.37 ± 1.38 ^{a-d}
Na ₂ SeO ₃ -1.0	219.6 ± 25.48 ^{c-e}	52.23 ± 8.83 ^{b-e}	271.83 ± 33.52 ^{c-e}	14.92 ± 1.39 ^{a-c}	1.70 ± 0.32 ^{a-e}	16.62 ± 1.65 ^{c-e}
Na ₂ SeO ₃ -1.5	224.82 ± 17.44 ^{b-e}	47.56 ± 5.46 ^c	272.38 ± 21.59 ^{c-e}	15.11 ± 0.87 ^{a-c}	1.45 ± 0.25 ^{c-f}	16.56 ± 1.07 ^{c-e}
Na ₂ SeO ₃ -3.0	239.53 ± 12.54 ^{a-c}	54.49 ± 10.14 ^{b-c}	294.02 ± 19.34 ^{a-c}	15.63 ± 1.23 ^{ab}	1.38 ± 0.67 ^{d-f}	17.01 ± 1.83 ^{a-e}
SeNPs-0.1	227.86 ± 11.75 ^{a-e}	58.07 ± 5.94 ^{a-d}	285.93 ± 15.64 ^{a-e}	15.12 ± 1.13 ^{a-c}	1.63 ± 0.44 ^{b-e}	16.75 ± 1.40 ^{b-e}
SeNPs-0.5	237.15 ± 16.16 ^{a-c}	52.66 ± 5.94 ^{b-c}	289.81 ± 21.39 ^{a-d}	15.22 ± 0.94 ^{a-c}	1.02 ± 0.19 ^f	16.24 ± 0.88 ^{de}
SeNPs-1.0	228.27 ± 14.32 ^{a-c}	48.33 ± 4.92 ^{de}	276.60 ± 16.34 ^{c-e}	15.78 ± 0.73 ^a	1.70 ± 0.31 ^{a-c}	17.48 ± 0.74 ^{a-d}
SeNPs-1.5	211.64 ± 24.63 ^{de}	46.73 ± 10.28 ^c	258.37 ± 33.89 ^{ef}	14.03 ± 1.41 ^{bc}	1.41 ± 0.39 ^{d-f}	15.44 ± 1.66 ^{ef}
SeNPs-3.0	224.64 ± 14.33 ^{b-e}	47.83 ± 5.07 ^c	272.47 ± 17.68 ^{c-e}	15.43 ± 0.29 ^{ab}	1.66 ± 0.29 ^{b-e}	17.09 ± 0.51 ^{a-e}
SeMPs-0.1	233.26 ± 15.59 ^{a-d}	52.99 ± 12.23 ^{b-c}	286.25 ± 27.25 ^{a-e}	16.20 ± 0.26 ^a	1.85 ± 0.53 ^{a-d}	18.05 ± 0.74 ^{a-c}
SeMPs-0.5	248.15 ± 15.37 ^a	62.25 ± 9.89 ^{ab}	310.40 ± 24.48 ^a	15.82 ± 0.85 ^a	1.66 ± 0.42 ^{b-e}	17.48 ± 1.06 ^{a-d}
SeMPs-1.0	247.48 ± 20.63 ^a	59.90 ± 4.95 ^{a-c}	307.38 ± 24.57 ^{ab}	16.53 ± 1.30 ^a	2.14 ± 0.10 ^a	18.67 ± 1.33 ^a
SeMPs-1.5	243.92 ± 17.75 ^{ab}	66.12 ± 9.55 ^a	310.04 ± 26.43 ^a	16.21 ± 1.34 ^a	2.17 ± 0.40 ^a	18.38 ± 1.67 ^{ab}
SeMPs-3.0	231.7 ± 15.54 ^{a-d}	51.16 ± 11.13 ^{c-e}	282.86 ± 26.03 ^{a-c}	15.58 ± 1.04 ^{ab}	1.79 ± 0.27 ^{a-e}	17.37 ± 1.18 ^{a-d}
CV (%)	7.52	15.30	8.40	7.55	22.39	8.18

*Notes: AC: Control; SeNPs: Selenium nanoparticles; SeMPs: Selenium microparticles; CV: Coefficient of variation; HFB: Hypocotyl fresh biomass; RFB: Radicle fresh biomass; TFB: Total fresh biomass; HDB: Hypocotyl dry biomass; RDB: Radicle dry biomass; TDB: Total dry biomass. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$ and *Kruskal-Wallis, $p \leq 0.05$); $n = 5$; \pm SD

For the dry biomass of the hypocotyl, the control showed the lowest values, with the best results being Na₂SeO₃ at 0.1 mg L⁻¹ and SeMPs at 0.1, 0.5, 1.0 and 1.5 mg L⁻¹, with increases of 29.23, 29.39, 26.35, 32.02 and 29.47%, respectively (Table 2). Compared with the control, the treatments with 0.1 and 0.5 mg L⁻¹ of Na₂SeO₃ and 0.1, 1.0 and 1.5 mg L⁻¹ of SeMPs increased the dry biomass of the radicle by 40.44, 50.73, 36.02, 57.35 and 59.55%, respectively (Table 2). However, for the total dry biomass, the control had the lowest value statistically, and only the SeNPs concentration of 1.5 mg L⁻¹ was equal, with the SeMPs concentration of 1.0 mg L⁻¹ being the highest accumulation of total dry biomass, with 34.51% more total dry biomass than the control (Table 2).

With respect to vigor index 1, the levels of SeMPs at 1.5, 1.0 and 0.5 mg L⁻¹ showed an increase resulted in increases of 27.72, 22.63 and 21.42%, respectively, compared with those of the control, whereas those of the other treatments were statistically equal to those of the control (Figure 2). In terms of vigor index 2, the control presented the lowest values; however, the best treatments were SeMPs at 1.0 and 1.5 mg L⁻¹, with increases of

34.5 and 32.4%, respectively (Figure 2). Figure 3 shows images of the development of the seedlings during the experiment.

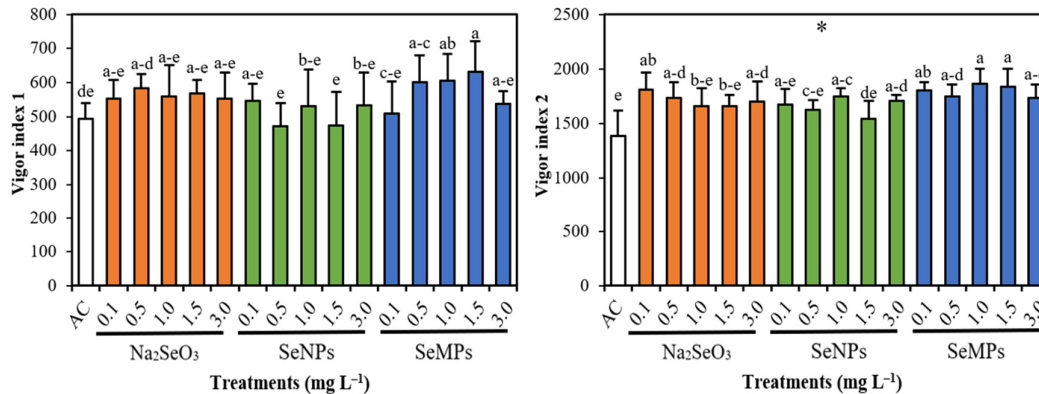


Figure 2. Vigor indices of cucumber seedlings

AC: Control; SeNPs: Selenium nanoparticles; SeMPs: Selenium microparticles. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$ and *Kruskal-Wallis, $p \leq 0.05$). $n = 5$; the ranges of the bars represent the SD.

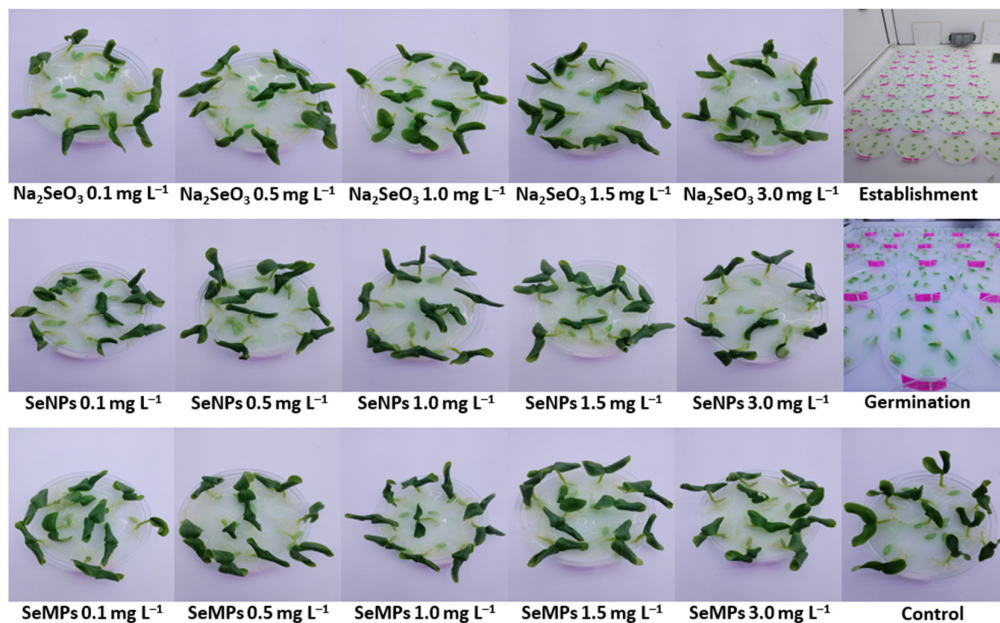


Figure 3. Development of the seedlings at the end of the experiment

SeNPs: Selenium nanoparticles; SeMPs: Selenium microparticles. The diameter of the Petri dishes is 125 mm

Photosynthetic pigments

The concentration of photosynthetic pigments varied among the treatments. Compared with the control, the 0.1 mg L⁻¹ SeNPs treatment decreased the concentration of Chl a by 18.91% (Table 3). Regarding Chl b , the treatments of Na₂SeO₃ at 1.0, 1.5 and 3.0 mg L⁻¹ and SeNPs at 0.1 and 1.0 mg L⁻¹, showed decreasing trends of 10.68, 17.94, 19.23, 24.35 and 14.10%, respectively, compared to the control (Table 3). For total Chl, decreasing trends were also found for Na₂SeO₃ at 1.5 mg L⁻¹ (15.09%) and SeNPs at 0.1 mg L⁻¹ (20.42%) (Table 3). For carotenoids there were no significant differences between Se treatments and the control (Table 3). The Chl a /Chl b ratio increased in all the Se treatments, with the control resulting in the lowest value (Table 3). The

treatments of Na₂SeO₃ at 0.5 mg L⁻¹ and SeNPs at 0.5 mg L⁻¹ increased the ratio to 15.08 and 13.68%, respectively, compared to the control.

Table 3. Photosynthetic pigments of cucumber seedlings

Treatments (mg L ⁻¹)	Chl _a (mg g ⁻¹ DW)	Chl _b (mg g ⁻¹ DW)	Chl _T (mg g ⁻¹ DW)	Caro (mg g ⁻¹ DW)	Chl _a /Chl _b Ratio
AC	6.66 ± 0.30 ^{a-d}	2.34 ± 0.10 ^{ab}	9.01 ± 0.39 ^{a-d}	1.00 ± 0.06 ^{a-c*}	2.85 ± 0.06 ^g
Na ₂ SeO ₃ -0.1	6.66 ± 0.57 ^{a-d}	2.12 ± 0.13 ^{b-f}	8.78 ± 0.70 ^{a-c}	1.15 ± 0.12 ^{ab}	3.14 ± 0.09 ^{bc}
Na ₂ SeO ₃ -0.5	7.14 ± 0.53 ^{ab}	2.18 ± 0.14 ^{b-d}	9.32 ± 0.67 ^{ab}	1.21 ± 0.11 ^a	3.28 ± 0.04 ^a
Na ₂ SeO ₃ -1.0	6.53 ± 0.90 ^{a-d}	2.09 ± 0.23 ^{c-f}	8.62 ± 1.13 ^{a-c}	1.06 ± 0.20 ^{a-d}	3.12 ± 0.10 ^{b-d}
Na ₂ SeO ₃ -1.5	5.73 ± 0.39 ^{de}	1.92 ± 0.08 ^{c-g}	7.65 ± 0.47 ^{cf}	0.80 ± 0.09 ^c	2.98 ± 0.09 ^{d-g}
Na ₂ SeO ₃ -3.0	5.88 ± 1.01 ^{c-c}	1.89 ± 0.25 ^{fg}	7.77 ± 1.27 ^{d-f}	0.86 ± 0.19 ^{de}	3.10 ± 0.12 ^{c-c}
SeNPs-0.1	5.40 ± 0.84 ^e	1.77 ± 0.18 ^g	7.17 ± 1.02 ^f	0.80 ± 0.12 ^c	3.05 ± 0.18 ^{c-f}
SeNPs-0.5	7.01 ± 1.17 ^{ab}	2.15 ± 0.27 ^{b-c}	9.16 ± 1.44 ^{a-c}	1.15 ± 0.23 ^{a-c}	3.26 ± 0.16 ^{ab}
SeNPs-1.0	5.90 ± 0.86 ^{c-c}	2.01 ± 0.23 ^{d-g}	7.91 ± 1.09 ^{c-f}	0.90 ± 0.17 ^{c-c}	2.94 ± 0.10 ^{fg}
SeNPs-1.5	6.50 ± 0.76 ^{a-d}	2.15 ± 0.20 ^{b-c}	8.65 ± 0.95 ^{a-c}	0.99 ± 0.17 ^{a-c}	3.02 ± 0.09 ^{c-f}
SeNPs-3.0	6.86 ± 0.63 ^{a-c}	2.29 ± 0.13 ^{a-c}	9.15 ± 0.75 ^{a-c}	1.05 ± 0.12 ^{a-d}	3.00 ± 0.14 ^{c-f}
SeMPs-0.1	6.29 ± 0.89 ^{b-c}	2.10 ± 0.25 ^{b-f}	8.39 ± 1.14 ^{b-f}	0.87 ± 0.17 ^{de}	3.00 ± 0.07 ^{c-f}
SeMPs-0.5	6.81 ± 1.43 ^{a-c}	2.21 ± 0.34 ^{d-d}	9.02 ± 1.78 ^{a-d}	0.97 ± 0.29 ^{b-c}	3.08 ± 0.17 ^{c-f}
SeMPs-1.0	7.45 ± 0.70 ^a	2.44 ± 0.17 ^a	9.89 ± 0.87 ^a	1.10 ± 0.12 ^{a-c}	3.05 ± 0.09 ^{c-f}
SeMPs-1.5	6.88 ± 0.56 ^{a-c}	2.22 ± 0.14 ^{d-d}	9.10 ± 0.70 ^{a-c}	0.98 ± 0.10 ^{a-c}	3.10 ± 0.07 ^{c-e}
SeMPs-3.0	6.41 ± 0.57 ^{b-c}	2.16 ± 0.13 ^{b-c}	8.57 ± 0.70 ^{b-c}	0.83 ± 0.11 ^{de}	2.97 ± 0.10 ^{c-g}
CV (%)	12.43	9.31	11.63	16.00	3.66

*Notes: AC: Control; SeNPs: Selenium nanoparticles; SeMPs: Selenium microparticles; CV: Coefficient of variation; Chl_a: Chlorophyll a; Chl_b: Chlorophyll b; Chl_T: Total chlorophyll; Caro: Carotenoids; DW: Dry weight. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$ and *Kruskal-Wallis, $p \leq 0.05$). $n = 5$; \pm SD.

Antioxidants and proteins

The GSH increased with Se treatments in all its forms, with the control having the lowest value. The levels of 1.0 and 1.5 mg L⁻¹ of SeMPs showed the highest values with increases of 29.05 and 31.59%, respectively, compared to the control (Figure 4). For total phenols, the level of 1.5 mg L⁻¹ of SeMPs was the one that increased them to the greatest extent (122.32%), followed by Na₂SeO₃ at 0.5 mg L⁻¹ (69.95%), compared to control (Figure 4). The flavonoids only showed increases of 14.73, 14.36 and 14.36% with the levels of Na₂SeO₃ at 0.1 and 0.5 mg L⁻¹ and SeNPs at 3.0 mg L⁻¹, respectively, compared to the control (Figure 4). The rest of the treatments were equal to or inferior to the control. Regarding soluble proteins, it is observed that SeNPs at levels of 0.5, 1.0, 1.5 and 3.0 mg L⁻¹ showed the best results, with increases of 7.98, 5.23, 6.05 and 5.87%, respectively, over the control (Figure 4). The Na₂SeO₃ concentrations of 0.1, 1.0 and 1.5 mg L⁻¹ also increased by 2.93, 3.76 and 3.21%, respectively.

Mineral concentration

The concentration of macronutrients (Table 4) was modified with the different forms and concentrations of Se. For P, statistically, some Se treatments were equal to or lower than the control. With respect to K, Na₂SeO₃ at 0.1 and 1.5 mg L⁻¹ were superior to the control by 6.88 and 8.21%, respectively. The rest of the treatments were equal to or lower than the control, with respect to K. For Ca, the Na₂SeO₃ treatments at 1.5 and 3.0 mg L⁻¹, SeNPs at 0.1 mg L⁻¹ and SeMPs at 0.5, 1.0, 1.5 and 3.0 mg L⁻¹ were equal to the control, the rest were inferior. For Mg, some improvements were observed with the treatments SeNPs at 1.0 mg L⁻¹ (17.22%) and SeMPs at 0.5 (5.01%), 1.5 (3.98%) and 3.0 mg L⁻¹ (5.13%). The S data indicate that the levels of all the forms of Se significantly surpassed those of the control. The levels of Na₂SeO₃ (1.0 mg L⁻¹), SeNPs (1.5 and 3.0 mg L⁻¹) and SeMPs (1.5 and 3.0 mg L⁻¹) presented the highest values of S, with increases of 4.0, 4.21, 3.44, 4.28 and 3.98%, respectively.

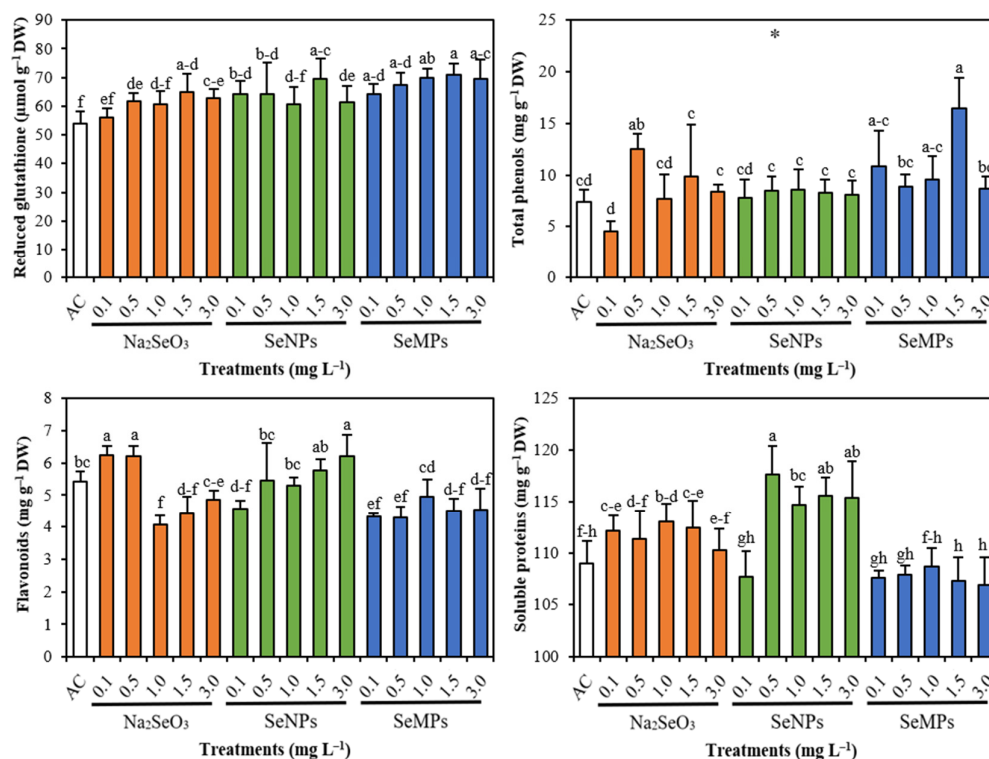


Figure 4. GSH, total phenols, flavonoids and soluble proteins of cucumber seedlings

AC: Control; SeNPs: Selenium nanoparticles; SeMPs: Selenium microparticles; DW: Dry weight. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$ and *Kruskal-Wallis, $p \leq 0.05$). $n = 5$; the ranges of the bars represent the SD.

Table 4. Macronutrients of cucumber seedlings

Treatments (mg L ⁻¹)	P (mg kg ⁻¹ DW)	K (mg kg ⁻¹ DW)	Ca (mg kg ⁻¹ DW)	Mg (mg kg ⁻¹ DW)	S (mg kg ⁻¹ DW)
AC	9298 ± 200.55 ^{a*}	5345 ± 48.59 ^{c-c*}	22811 ± 301.95 ^{a*}	9119 ± 38.14 ^{d-g*}	1071.71 ± 4.47 ^g
Na ₂ SeO ₃ -0.1	8443 ± 29.10 ^{c-f}	5713 ± 32.88 ^{ab}	19457 ± 155.34 ^{cf}	9201 ± 22.95 ^{b-c}	1104.19 ± 3.97 ^{c-c}
Na ₂ SeO ₃ -0.5	8233 ± 59.99 ^f	5637 ± 51.47 ^{a-c}	20088 ± 249.16 ^{c-f}	9005 ± 54.10 ^{c-g}	1096.68 ± 6.49 ^{cf}
Na ₂ SeO ₃ -1.0	8776 ± 62.05 ^{a-c}	5589 ± 50.32 ^{a-c}	20267 ± 247.58 ^{b-f}	8266 ± 39.93 ^g	1114.62 ± 7.95 ^{ab}
Na ₂ SeO ₃ -1.5	8918 ± 67.76 ^{ab}	5784 ± 31.50 ^a	20911 ± 168.96 ^{a-c}	8470 ± 25.82 ^g	1098.74 ± 2.20 ^{d-f}
Na ₂ SeO ₃ -3.0	8986.93 ± 60.68 ^{ab}	5309.02 ± 29.45 ^{c-f}	21039.59 ± 278.81 ^{a-d}	9143.66 ± 53.33 ^{c-g}	1093.28 ± 8.60 ^f
SeNPs-0.1	8426.22 ± 154.41 ^{c-f}	5431.50 ± 89.29 ^{a-c}	21373.08 ± 175.35 ^{a-c}	8883.18 ± 51.22 ^{fg}	1099.69 ± 11.85 ^{c-f}
SeNPs-0.5	9525 ± 100.79 ^a	5479 ± 21.76 ^{c-d}	20237 ± 40.84 ^{b-f}	9127 ± 64.61 ^{c-g}	1102.25 ± 2.22 ^{c-f}
SeNPs-1.0	8517 ± 79.89 ^{b-f}	5349 ± 20.75 ^{b-c}	20171 ± 99.19 ^{c-f}	10690 ± 57.46 ^a	1096.19 ± 3.37 ^{cf}
SeNPs-1.5	8568.29 ± 100.20 ^{b-c}	5188.28 ± 42.60 ^{cf}	17472.91 ± 188.74 ^f	9129.44 ± 93.06 ^{c-g}	1116.85 ± 13.56 ^{ab}
SeNPs-3.0	8871 ± 65.43 ^{a-c}	5659 ± 27.20 ^{a-c}	18128 ± 138.68 ^f	9248 ± 85.42 ^{a-c}	1108.68 ± 7.22 ^{a-c}
SeMPs-0.1	8429 ± 77.04 ^{c-f}	5226 ± 20.78 ^{d-f}	19957 ± 141.32 ^{d-c}	9187 ± 20.75 ^{b-f}	1097.16 ± 7.36 ^{cf}
SeMPs-0.5	8267 ± 138.04 ^{cf}	5216 ± 62.57 ^{d-f}	22971 ± 28.54 ^a	9576 ± 48.99 ^{ab}	1107.71 ± 3.13 ^{b-d}
SeMPs-1.0	8383 ± 59.36 ^{d-f}	5019 ± 54.88 ^f	21593 ± 320.86 ^{a-c}	9347 ± 26.00 ^{a-d}	1097.89 ± 8.52 ^{d-f}
SeMPs-1.5	8244 ± 94.83 ^{cf}	4987 ± 16.77 ^f	21949 ± 87.41 ^{ab}	9482 ± 39.93 ^{a-c}	1117.65 ± 3.78 ^a
SeMPs-3.0	8623 ± 69.00 ^{a-d}	5204 ± 54.94 ^{d-f}	22947 ± 64.36 ^a	9587 ± 69.54 ^{ab}	1114.38 ± 0.83 ^{ab}
CV (%)	1.13	0.84	0.92	0.58	0.63

*Notes: AC: Control; SeNPs: Selenium nanoparticles; SeMPs: Selenium microparticles; CV: Coefficient of variation; DW: Dry weight. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$ and *Kruskal-Wallis, $p \leq 0.05$). $n = 4$; ± SD.

For micronutrients (Fe, Zn, Cu and Mn) (Table 5), no positive effects were observed, however, most Se treatments for all four micronutrients showed decreasing trends.

Table 5. Micronutrients of cucumber seedlings

Treatments (mg L ⁻¹)	Fe (mg kg ⁻¹ DW)	Zn (mg kg ⁻¹ DW)	Cu (mg kg ⁻¹ DW)	Mn (mg kg ⁻¹ DW)
AC	187.40 ± 2.70 ^{ab*}	96.06 ± 1.29 ^{a*}	52.00 ± 0.65 ^{a*}	42.50 ± 0.38 ^{a*}
Na ₂ SeO ₃ -0.1	153.10 ± 0.68 ^{a-d}	71.22 ± 0.86 ^{a-f}	36.10 ± 0.50 ^{d-g}	39.20 ± 0.00 ^{a-d}
Na ₂ SeO ₃ -0.5	157.10 ± 0.76 ^{a-c}	86.34 ± 0.91 ^{ab}	36.00 ± 0.00 ^{d-h}	42.20 ± 0.23 ^{ab}
Na ₂ SeO ₃ -1.0	149.50 ± 1.05 ^{a-d}	74.76 ± 0.57 ^{a-d}	37.00 ± 0.23 ^{a-d}	38.30 ± 0.20 ^{a-f}
Na ₂ SeO ₃ -1.5	140.70 ± 0.60 ^{d-g}	75.30 ± 0.23 ^{a-c}	36.10 ± 0.38 ^{d-g}	38.50 ± 0.20 ^{a-c}
Na ₂ SeO ₃ -3.0	144.30 ± 2.75 ^{b-f}	60.44 ± 1.92 ^b	34.21 ± 0.73 ^{gh}	32.49 ± 0.44 ^{fj}
SeNPs-0.1	140.96 ± 2.76 ^{c-g}	73.56 ± 1.21 ^{a-c}	39.17 ± 0.82 ^{ab}	40.70 ± 0.39 ^{a-c}
SeNPs-0.5	141.20 ± 2.65 ^{c-g}	68.16 ± 0.85 ^{c-g}	32.10 ± 0.20 ^h	37.50 ± 0.20 ^{a-g}
SeNPs-1.0	136.60 ± 0.23 ^{c-g}	70.56 ± 0.48 ^{b-f}	38.20 ± 0.23 ^{a-d}	35.60 ± 0.00 ^{b-h}
SeNPs-1.5	148.38 ± 6.14 ^{a-c}	56.33 ± 1.52 ^b	36.33 ± 0.93 ^{c-g}	31.29 ± 0.42 ^j
SeNPs-3.0	140.50 ± 1.65 ^{c-g}	65.76 ± 0.59 ^{fg}	35.50 ± 0.20 ^{e-h}	33.90 ± 0.20 ^{d-j}
SeMPs-0.1	233.30 ± 4.84 ^a	65.88 ± 0.75 ^{fg}	35.10 ± 0.20 ^{f-h}	31.60 ± 0.33 ^{ij}
SeMPs-0.5	118.70 ± 2.36 ^g	67.62 ± 1.01 ^{c-g}	36.90 ± 0.82 ^{a-c}	32.00 ± 0.00 ^{b-j}
SeMPs-1.0	120.50 ± 2.36 ^g	68.88 ± 1.45 ^{d-g}	36.00 ± 0.33 ^{d-h}	32.70 ± 0.38 ^{a-j}
SeMPs-1.5	132.80 ± 1.35 ^{fg}	68.88 ± 0.59 ^{c-g}	36.30 ± 0.20 ^{b-f}	32.30 ± 0.20 ^{g-j}
SeMPs-3.0	165.40 ± 0.23 ^{ab}	73.92 ± 0.44 ^{a-c}	38.90 ± 0.20 ^{a-c}	35.30 ± 0.20 ^{e-i}
CV (%)	1.73	1.42	1.34	0.76

*Notes: AC: Control; SeNPs: Selenium nanoparticles; SeMPs: Selenium microparticles; CV: Coefficient of variation; DW: Dry weight. Different letters indicate significant differences between treatments (*Kruskal-Wallis, $p \leq 0.05$). $n = 4$; \pm SD.

Correlation analysis

Correlation analysis revealed positive and negative relationships between the growth variables, biomass, bioactive compounds and minerals (Figure 5). For example, GSH was positively related to the fresh biomass of the hypocotyl (0.35), radicle (0.38) and total (0.38). Likewise, phenols were positively related to the fresh and dry biomass of the radicle and to GSH, with coefficients of 0.34, 0.35 and 0.36, respectively. The concentration of flavonoids was positively related to that of proteins, with a coefficient of 0.48. The macronutrients and micronutrients presented positive relationships, with coefficients ranging between 0.91 and 0.99. Regarding the variables that showed negative relationships, there are proteins with the variables of fresh and dry biomass and lengths, with coefficients that ranged between -0.42 and -0.18. Negative relationships of all nutrients with dry biomass of the radicle and total length are also observed.

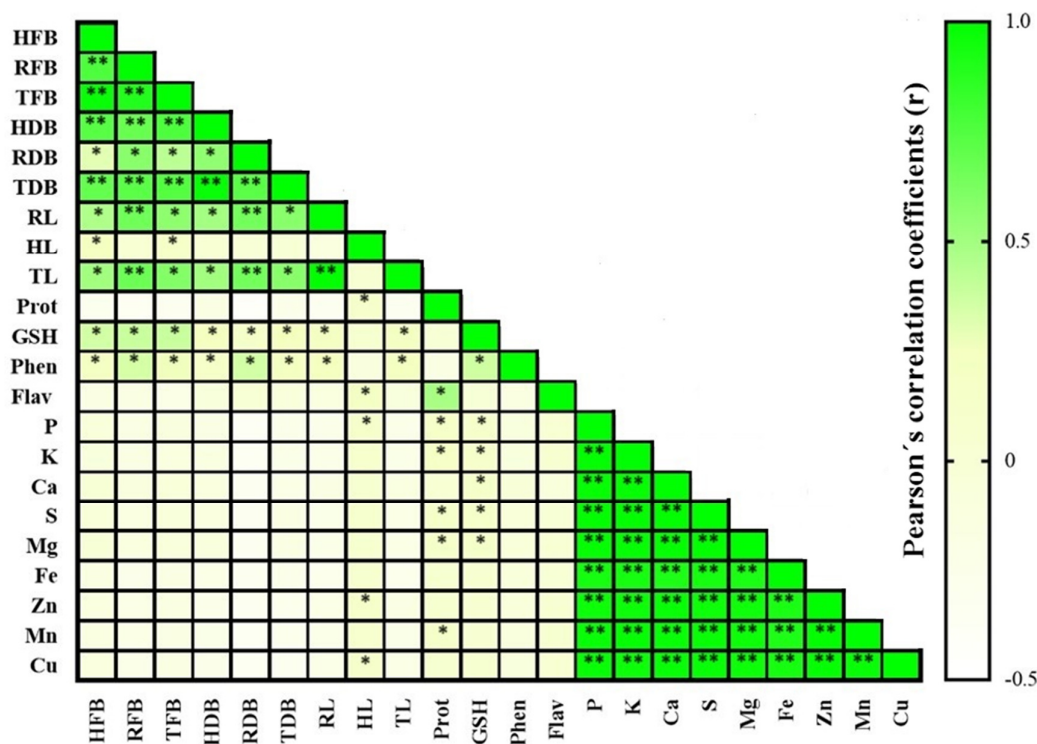


Figure 5. Correlation matrix between growth variables, biomass, bioactive compounds and minerals of cucumber seedlings

HFB: Hypocotyl fresh biomass; RFB: Radicle fresh biomass; TFB: Total fresh biomass; HDB: Hypocotyl dry biomass; RDB: Radicle dry biomass; TDB: Total dry biomass; RL: Radicle length; HL: Hypocotyl length; TL: Total length; Prot: Proteins; GSH: Reduced glutathione; Phen: Phenols; Flav: Flavonoids. Coefficients $> +0.1$ and $< +0.6$ indicate significant correlations (*), and coefficients $> +0.6$ indicate highly significant correlations (**), according to a previously described scale (Schober *et al.*, 2018). The rest were not significant or presented negative correlations

Discussion

Seed germination and early seedling growth

Seed priming with this type of material can improve germination and stimulate initial seedling growth, but the results can also be null or negative. In this regard, the results of this experiment indicate that the germination percentage did not change, mainly because the control treatment percentage was 100%. Different results are reported by Hussain *et al.* (2023) by demonstrating that concentrations of $75 \mu\text{mol L}^{-1}$ of Na_2SeO_3 decreased the germination percentage in *Brassica rapa* L. seeds, however, concentrations of 100 and $125 \mu\text{mol L}^{-1}$ did not modify it, with respect to the control. Vera-Reyes *et al.* (2023) reported that the germination percentage of tomato seeds was not affected using graphite-MPs. In contrast, García-Locascio *et al.* (2024) reported that SeNPs at 10 mg L^{-1} improved the percentage of germination in tomato seeds.

The NPs, owing to their electrical, magnetic, and optical properties and the composition of their corona, can enter more easily through the seed coat and create a greater number of pores to speed up the absorption of water, which increases the speed of germination and therefore the early growth of seedlings (Khodakovskaya *et al.*, 2009). This effect can also be provoked with the use of MPs, since they also have physicochemical properties that allow them to enter the cell interior more easily and trigger different responses (Weiss *et al.*, 2019; Vera-Reyes *et al.*, 2023). However, ionic materials, NPs and MPs do not necessarily enter the cell interior, since it is possible that they adsorb on the seed coat due to its ion exchange zones or bind to receptors found in the seed

coat and the signalling process is carried out, which can give rise to different responses in terms of germination and development of the seedlings (Yu *et al.*, 2019). As for the types of receptors, there is no knowledge, but it is believed that they may be those of sulfates and phosphates for ionic forms and, for NPs and MPs, if they do not enter the cell interior, it is possible that they accumulate in the seed coats, mainly in the cell walls (Schwab *et al.*, 2016; Schiavon and Pilon-Smits, 2017).

Importantly, when NPs and MPs come into contact with water, soil, the epidermis or internal fluids of plants, they adsorb components of organic matter or biomolecules (proteins and peptides) from the environment, forming a corona; therefore, the first interaction likely occurs between the corona of NPs and MPs and the receptors and surface components of plant cell walls and membranes (Juárez-Maldonado *et al.*, 2019; Weiss *et al.*, 2019). For this reason, NPs and MPs of the same composition have different impacts on plants, both due to the differences in the physical properties and the composition of the corona (Weiss *et al.*, 2019; Juárez-Maldonado *et al.*, 2021).

On the other hand, the length and fresh and dry biomass of the seedlings increased with the different forms of Se, with the greatest increase occurring with the SeMPs. The germination process of the seeds and the initial growth of the seedlings are accompanied by different changes in metabolism, and among these metabolites, those that play crucial roles are phytohormones, which can be regulated by supplementation with Se (Skrypnik *et al.*, 2022), as mentioned by Lehotai *et al.* (2012) indicating that 40 μM of Na_2SeO_3 in *Arabidopsis thaliana* L. increased the expression of the *ARR5::GUS* gene, responsible for encoding cytokinins. Similarly, El-Badri *et al.* (2021) mention that the seed priming of *Brassica napus* L. with SeNPs modulated the levels of expression of the *ABA* and *GA* genes, which subsequently promoted the germination of the seeds and the early growth of the seedlings under conditions of salinity stress. Colman *et al.* (2019) reported that tomato seed priming with CsMPs at 0.1 mg mL^{-1} increased the expression of the *DR5::GUS* gene, which is responsible for the encoding of auxins and is associated with an increase in the percentage of seed germination and dry biomass of seedlings.

The increases in length and biomass are also explained by the fact that the NPs and MPs can induce rapid mobilization of the reserves contained in the seeds, mainly carbohydrates (Acharya *et al.*, 2020). Additionally, the Se can stimulate nitrogen metabolism, which is directly related to plant growth and biomass (Zhang *et al.*, 2023). Domokos-Szabolcsy *et al.* (2012) indicate that Se in low concentrations can directly stimulate organogenesis, which can also explain the growth and accumulation of biomass in this study.

Vigor indicates the ability of seeds to germinate and develop under different conditions and is directly related to the quality of the seedlings in different phenological stages (Carballo-Mendez *et al.*, 2019). In this study, the vigor index used depended directly on the length and biomass of the hypocotyl and radicle, which are variables for which there were more significant differences. Sariñana-Navarrete *et al.* (2024) indicate that, in jalapeño pepper seeds, the use of 1 mg L^{-1} of SeNPs increased the vigor index with respect to the length of the seedling, and increasing the SeNPs concentration (5 to 45 mg L^{-1}) decreased the vigor index. The same authors evaluated the application of Na_2SeO_3 , which decreased the vigor as the concentration increased (5 to 45 mg L^{-1}). Vera-Reyes *et al.* (2023) reported that tomato seed priming with graphite-MPs at 50 mg L^{-1} improved the vigor index.

Se in plants can be assimilated into SeMet, SeCys, or MSeCys or can be incorporated into other sulfur metabolites, such as glucosinolates and GSH, which can stimulate development and growth (García Márquez *et al.*, 2020; Danso *et al.*, 2023). SeMet and SeCys are predominant in cereal grains and legumes, whereas MSeCys and selenoglucosinolates are the main forms of Se in species of the *Brassicaceae* family and *Allium* genus, which is directly associated with growth, biomass and yield of these crops (Erbersdobler *et al.*, 2017; Hu *et al.*, 2021; Dobrzyńska *et al.*, 2023). In nonaccumulating vegetables such as cucumber, tomato, and lettuce, the SeMet is the form of Se that is present at the highest concentration and is apparently the main regulator of the accumulation of biomass (Hu *et al.*, 2022).

The toxicity caused by high concentrations is well documented and is associated mainly with the inadvertent incorporation of selenoamino acids into proteins and the replacement of cysteine and methionine, which leads to protein misfolding and the subsequent overproduction of reactive oxygen species (ROS) that cause imbalances at all omic levels in plants (Gupta and Gupta 2017). This toxicity response is more common in nonaccumulating crops (Dall'Acqua *et al.*, 2019).

On the other hand, when NPs or MPs are applied and enter the interior of a cell, they have various effects depending on the shape, size and composition of the material; however, they can release the ions or substances that compose them and can be incorporated into different metabolic routes (Juárez-Maldonado *et al.*, 2021; Jurić *et al.*, 2021a). Therefore, NPs and MPs normally have a wider variety of biological effects than ionic materials (Juárez-Maldonado *et al.*, 2019; Weiss *et al.*, 2019).

Photosynthetic pigments

Se is a beneficial nutrient that has been evaluated in different crops and has shown varied effects in terms of its photosynthetic pigment content. The different forms of Se did not improve the content of chlorophylls; however, in some treatments, some of the contents decreased with respect to those in the control. This effect is possibly because Se in any of its forms can act as a prooxidant and induce the synthesis of ROS, which cause peroxidation of the chloroplast membrane, decreasing the synthesis of pigments (Hussein *et al.*, 2019). Another possible reason why the photosynthetic process could be affected is due to the substitution of cysteines for SeCys in ferredoxin, which is a protein composed of Fe-S and cysteine, a sulfur amino acid; if it occurs, this substitution would affect the correct folding of the protein and it would cease to be functional, thus affecting electron transport (Guignardi and Schiavon, 2017). In addition, Zsiros *et al.* (2019) mention that the synthesis of pigments can be decreased to a certain extent without affecting the structure and function of the photosynthetic machinery, which can be related to the results of this study, where there was a decrease in pigments in some treatments; however, this decrease did not affect the growth and biomass variables, which are dependent on photosynthesis.

Se treatments did not modify carotenoids concentrations compared to the control. This response likely depends on the association between chlorophylls and carotenoids in the photosystems or as a response to the ROS synthesized by the action of Se (Simkin *et al.*, 2022; Keshari *et al.*, 2023). In this case, García-Locascio *et al.* (2024) reported that tomato seed priming with SeNPs at 1.0 mg L⁻¹ decreased the concentration of chlorophylls in the leaves; however, 10 mg L⁻¹ of SeNPs increased it. Ishtiaq *et al.* (2023) reported that tomato seed priming with SeNPs increased the concentration of carotenoids in plant leaves under standard growth conditions and water deficit. Hussain *et al.* (2023) mention that with the use of Na₂SeO₃ in seeds of *B. rapa*, it was possible to increase the concentrations of chlorophylls and carotenoids with certain treatments; however, other treatments decreased the concentrations of these pigments. Wang *et al.* (2019) indicate that applications of CuONPs and CuOMPs at 200 and 400 mg kg⁻¹ did not affect the concentrations of chlorophyll *a* and *b* in lettuce.

On the other hand, the increase in the value of the Chl*a*/Chl*b* ratio can be explained by the decrease in these pigments due to the Se materials. The Chl*b* is more abundant in the PSII, and its main function is as an accessory pigment in the collection of light; for this reason, under stress conditions, it can oxidize faster than can Chl*a* (Caffarri *et al.*, 2014). This selective oxidation leads to a relatively higher concentration of Chl*a* than of Chl*b*, thus increasing the Chl*a*/Chl*b* ratio (Zhao *et al.*, 2020). In this way, plants can optimize their photosynthetic efficiency under stress conditions, ensuring maximum energy capture with reduced light harvesting capacity (Caffarri *et al.*, 2014; Zhao *et al.*, 2020).

Antioxidants and proteins

The increases in bioactive compounds can be attributed to the different effects of different forms of Se. In the first instance, ionic Se acts as a prooxidant on its own or by forming selenoamino acids and replacing cysteine and methionine in proteins are generated ROS as a result of imbalances in metabolism; ROS act as signalers and induces the synthesis of bioactive compounds such as GSH, ascorbic acid, phenols and carotenoids, to neutralize these oxidant species (Gupta and Gupta 2017; Khalofah *et al.*, 2021). When in contact with cellular components, NPs and MPs can act as damage-associated molecular patterns (DAMPs) as a result of misfolded proteins arranged on the corona, which leads to the generation of ROS (Iglesias *et al.*, 2019; Juárez-Maldonado *et al.*, 2019). Additionally, Se can bind to receptors on cell walls or membranes, including receptors present in the seed coat, which triggers signaling that can increase the synthesis of bioactive compounds, as mentioned by Khan *et al.* (2023) by indicating that the seed coat has a great capacity to store Se, since several forms of this mineral have been detected (organic and inorganic, adsorbed or bound to receptors). These findings indicate that seed priming with Se is a promising option for biostimulating the antioxidant system of crops and improving their agronomic and functional characteristics.

Similar results are shown by Ishtiaq *et al.* (2023) who indicate that tomato seed priming with SeNPs increased the α -techoferol, flavonoids, anthocyanins, ascorbic acid and GSH contents in plants under standard growth conditions and water deficit. Similarly, Jurić *et al.* (2021b) reported that the application of Zn/Alg/Cs MPs in strawberry improved the accumulation of total phenols, flavonoids and anthocyanins in fruits. Adhikary *et al.* (2022) in their study, they reported that the priming of rice seeds with Na₂SeO₃ individually or in combination with Na₂SeO₄ at a concentration of 50 $\mu\text{mol L}^{-1}$ increased the content of phenols and soluble proteins. Importantly, all the treatments with Se increased the concentration of GSH, possibly due to the similar characteristics of Se and S, considering that GSH is a sulfur metabolite and that Se at relatively low doses can stimulate its metabolism, which increases its concentration (González-Morales *et al.*, 2017).

In general, SeNPs and SeMPs, unlike Na₂SeO₃, can penetrate the seed testa and be internalized in different cellular organelles, either by endocytosis or pore formation, which stimulates the synthesis of ROS by the interaction of the surface charges of the corona of the nano and micromaterials with the different cellular organelles, which generates a cellular response that ranges from biostimulation to cell death depending on the level of oxidative stress generated by the increase in ROS (López-Vargas *et al.*, 2020).

Mineral concentration

It is important to mention that no Se was detected in the seedling tissue. This could be due to different phenomena, such as the low Se concentrations used in the experiment, and the fact that some of the Se could have remained adsorbed in the seed testa, which can retain this mineral thanks to the ion exchange zones. In addition, the cell walls and membranes of the seed testa contain different receptors that can bind to Se (Khan *et al.*, 2023). These scenarios can prevent the entry of Se at the cellular level and its subsequent translocation to developing organs. In addition, it has been proven that Se in its different forms (mainly SeNPs and SeMPs due to their properties) not only acts at the receptor level, but also a certain concentration enters the cellular level and evokes other responses (Zhou *et al.*, 2020; Juárez-Maldonado *et al.*, 2021).

According to this, the lack of detection of Se may also be due to the technique used by ICP-OES with a detection limit of 0.020 mg L⁻¹, so it would be advisable to quantify it in subsequent experiments using ICP-MS, which offers lower detection limits. Now, after plants absorb Se, they can convert it into volatile compounds that are released into the atmosphere. This process is carried out through the action of specialized enzymes (selenocysteine lyase and methyltransferases) that convert organic Se compounds (SeCys and SeMet) into dimethylselenide (DMSe) and dimethyldiselenide (DMDSe), which are the volatile compounds (Lanza and dos Reis, 2021).

Among the nutrients determined, some very interesting phenomena were observed, mainly with P and S. One of the forms of Se used was SeO₃²⁻, which is absorbed by plants through phosphate and aquaporin

transporters. In this study, a decrease in the accumulation of P in all forms of Se was observed. Jafari and Moghaddam (2022) reported that concentrations of 8 and 12 mg L⁻¹ of Na₂SeO₃ in mint caused a decrease of P.

With respect to S, all concentrations and forms of Se increased its accumulation, which is very interesting since SeO₄²⁻ is the form of Se that is absorbed by sulfate transporters (González-Morales *et al.*, 2017). In this sense, and in accordance with the data of this experiment, we can say that SeO₃²⁻ can improve S uptake, possibly by inducing the expression of genes that code for sulfate transporter proteins, which is mentioned by Inostroza-Blancheteau *et al.* (2013) who applied Na₂SeO₃ to different wheat genotypes and demonstrated that the expression of the *TaeSultr1.1a* and *TaeSultr4.1* genes, which encode sulfate transporter proteins, was increased. SeNPs can also improve S uptake, as shown by Kang *et al.* (2024) by indicating, the use of SeNPs at 30 μM increased the accumulation of Se and S in tomato roots and shoots, a phenomenon caused by the increase in the expression of the *Sultr1:3* gene, which encodes a sulfate transporter. This effect can also be attributed to the SeMPs, since similar trends were observed between the NPs and MPs.

Regarding Ca, a decrease was observed in most of the treatments with Se. Some studies have shown that the accumulation of Ca can decrease in *Pteris vittata* with the addition of Na₂SeO₃ (Feng *et al.*, 2009) but increase in corn (Hawrylak-Nowak, 2008). Other authors mention that the application of anionic forms of Se (SeO₃²⁻ and SeO₄²⁻) can increase the concentration of a cation such as Ca (Longchamp *et al.*, 2016).

Mg showed increases with Se treatments, since this nutrient is essential in some metabolic pathways where Se intervenes, since it is a cofactor of the GSH-synthase enzyme (Peng *et al.*, 2015). Another possible mechanism to explain the increase in some nutrients (K, Mg and S) in cucumber seedlings is that NPs induce the overexpression of genes encoding aquaporins (*TIP1:1*, *PIP2* and *PIP1:1*), which increases the entry of nutrients at the cellular level (Akdemir, 2021), a mechanism that the MPs possibly share. In addition, this type of materials can create pores in cell walls and membranes, which facilitate the entry of water and nutrients. Additionally, they are inducers of the synthesis of phytohormones and the subsequent formation of root hairs, which improves the absorption of nutrients (Khodakovskaya *et al.*, 2009; Colman *et al.*, 2019; Khai *et al.*, 2022). Soliman *et al.* (2023) showed that the use of SeNPs in wheat increased the concentrations of N, P, K, and Mg and the expression of the *TIP1* and *PIP1* genes.

Most concentrations and forms of Se decreased the accumulation of micronutrients compared to the control. In the first instance, one of the forms of Fe that plants absorb is Fe³⁺, which requires the union of siderophores; however, these compounds have L-methionine as a precursor, an amino acid that can be replaced by SeMet, inhibiting thus the formation of the compound and therefore the absorption of Fe (Zagrodzki *et al.*, 2020). Different results were shown by Alves *et al.* (2020) reporting that Na₂SeO₃ at 1.0, 5.0 and 10 μmol L⁻¹ increased the concentration of Fe in tomato roots and shoots.

Regarding Zn, several investigations have focused on its interactions with Se, but the results are highly variable, showing positive and negative effects on its accumulation in plants. Feng *et al.* (2009) indicate that in *P. vittata*, the application of Na₂SeO₃ caused a decrease in the accumulation of Zn; however, Xue *et al.* (2020) mention that, in *Brassica chinensis* L., the concentration increased. This finding indicates that the plant species will be decisive in the expected results, since *B. chinensis* is an accumulating culture of Se (Abdalla *et al.*, 2020). For their part, Söderlund *et al.* (2016) and Huang *et al.* (2018) mention that the additions of SeO₃²⁻ and SeO₄²⁻ increases the pH of the medium, which can greatly inhibit the mobilization of Zn²⁺. Neysanian *et al.* (2020) showed that SeNPs in tomato plants at a concentration of 3.0 mg L⁻¹ increased the contents of Fe and Zn and that 10 mg L⁻¹ decreased them.

Research has indicated that Se has an antagonistic relationship with Cu (Liu *et al.*, 2021), which was evidenced in this study, where all concentrations and forms of Se caused a decrease in the accumulation of Cu. Mn is another micronutrient that is negatively affected by supplementation with Se; in this sense, a possible explanation for this phenomenon is that it can repress the expression of the transporter *Nramp5*, a fundamental

transporter that regulates the absorption of Mn (Cui *et al.*, 2018; Li *et al.*, 2019). Wu *et al.* (2020) reported that with the use of Na₂SeO₃ in *C. violifolia*, the concentration of Cu was not modified and that of Mn decreased, in addition, with the use of Na₂SeO₄, both micronutrients decreased compared with those in the control. Selim *et al.* (2022) in their study, they reported that the seed priming of *Medicago interexta* with 25 mg L⁻¹ of SeNPs did not affect the concentrations of Cu and Mn; however, it increased those of Fe, Zn, Ca, P and K.

The results of the accumulation of P, Ca and micronutrients for most of the treatments with Se indicate that there was a decrease compared with that of the control; however, the concentrations of these nutrients were within the appropriate levels for this species of plant, according to Campbell (2000). Only the K values were below those recommended by the same author (3-4%), which can be explained by the fact that the seedlings were hydrated with distilled water and depended on the reserves of the seeds; in addition, as they are seedlings, concentrations lower than those recommended for adult plants (Anwar *et al.*, 2020) are normal.

Finding modifications in nutrient concentrations in this type of experiments where seedlings were only hydrated with distilled water is very complex, since they only depend on the reserves of the seeds, but it is possible, since Se under these conditions can modify mineral transport patterns and even cause nutrient losses through the radicle (exudates) and cotyledons (in the form of volatile or soluble compounds). García-Locascio *et al.* (2024) show that priming tomato seeds with SeNPs modified the nutrient concentration in 7-day-old seedlings hydrated with distilled water.

In general, Se materials influence nutrient absorption by improving the expression of genes that code for aquaporins, sulfate and phosphate transporters, and by stimulating the synthesis of phytohormones that improve root growth, which in turn improves nutrient absorption and transport (Garduño-Zepeda and Márquez-Quiroz, 2018; Zahedi *et al.*, 2019). However, SeNPs and SeMPs, thanks to their size and properties, are more efficient in nutrient absorption, as they can create pores in membranes, facilitating their entry and transport (Mahakham *et al.*, 2017). In addition, Na₂SeO₃, SeNPs, and SeMPs increase the activity of antioxidant enzymes (SOD, CAT, APX, GPX, etc.) and the concentration of secondary metabolites, neutralizing ROS, which preserves the integrity of membranes and the nutrient transport system (Gupta and Gupta 2017; Khalofah *et al.*, 2021). However, Se in any of its forms can also compete with the absorption of certain nutrients such as P, Ca, Fe, Zn, Cu and Mn, which inhibits their uptake through the root system, as observed in the present study.

Correlation analysis

Correlation analysis showed positive and negative relationships between the different variables, confirming certain patterns observed in the results. Antioxidants such as GSH, phenols, and flavonoids were positively related to fresh and dry biomass and proteins. In this sense, by accumulating more antioxidants (due to the effect of Se), plants avoid cellular damage caused by ROS and can use more energy to continue growing, rather than repairing damage (Fujita and Hasanuzzaman, 2022). Minerals presented negative relationships with dry root biomass and total length, which has a logical explanation, since most nutrients showed a decrease. However, under these situations, plants can show improved growth through compensatory mechanisms, such as the redistribution of nutrients from less essential parts to those most crucial for growth (Zhao, 2024). Now, Se plays an important role in the efficient use of water and N and in the stimulation of phytohormones that promote plant growth (Zahedi *et al.*, 2019).

Limitations and future prospects

The study presents relevant data mainly due to the use of SeMPs, which have not been evaluated in agricultural systems. However, the study had some limitations such as the non-detection of Se, which is a variable that, if detected, would have given more relevance to the research. Regarding the growth chamber where the experiment was carried out, there were some details regarding the temperature control, since it was

not completely automated. In this sense, a 100% automated chamber would possibly give us data with less variation. In this sense, the presented research shows preliminary data on the different forms of Se under in vitro conditions, so it would be interesting and advisable to test these materials in cucumber plants and other crops of economic importance under field or greenhouse conditions and evaluate growth parameters, yield, nutraceutical and nutritional quality, antioxidant enzyme activity, phytohormones, expression of sulfate, phosphate and aquaporin transporter proteins and the level of Se biofortification in edible organs.

Conclusions

The results indicate that the applications of Na₂SeO₃, SeNPs and SeMPs were effective in improving initial growth, biomass, bioactive compounds and the accumulation of some nutrients such as S, K and Mg in cucumber seedlings, with SeMPs standing out at concentrations of 1.0 and 1.5 mg L⁻¹. However, some treatments decreased variables such as photosynthetic pigments and nutrient concentrations (Ca, P, Fe, Zn, Cu, and Mn). However, these reductions did not affect seedling growth. While some treatments with Na₂SeO₃ and SeNPs decreased pigments, SeMPs showed similar results to the control. Regarding micronutrients, although they decreased with the three forms of Se, they still present values within the sufficiency ranges, which motivates future research to conduct studies at more advanced plant stages involving nutritional plans and thus elucidate their effects on nutrient dynamics in these scenarios.

Authors' Contributions

Conceptualization: OSA, ABM; Data curation: OSA; Formal analysis: OSA; Funding acquisition: ABM; Investigation: OSA; Methodology: ABM; Project administration: SGM; Resources: GCP, MPA, ABMD; Software: OSA; Supervision: JMM, DLOB; Validation: ABM, GCP; Visualization: JMM, SGM; Roles/Writing - original draft: OSA; and Writing - review & editing: OSA, ABM.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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