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Foliar Fertilization of Nano-selenium and its Effects on Bioactive Compounds and Enzymatic Activity in Lettuce
Fertilización Foliar de Nanoselenio y sus Efectos sobre Compuestos Bioactivos y Actividad Enzimática en Lechuga

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SUMMARY

Selenium (Se) an oligoelement for mammals is not considered essential in plant fertilization programs. Its deficiency affects almost one million persons, making it a world health problem and nano-biofortification of cultivation is a strategy to attenuate it. Thus, this study determines the foliar selenium nanoparticles (Se-NPs) application effects on yield, enzymatic and non-enzymatic antioxidants, and their accumulation in lettuce. Therefore, six applications of different Se-NPs concentrations: 0, 1, 5, 10, 15, and 20 mg L^{-1} were considered. The results showed that the high doses decrease yield, enzymatic and non-enzymatic antioxidants but increase Se concentration in lettuce leaves. The recommended dosage here is of 1 mg L^{-1} for biomass production and 10 mg L^{-1} for bioactive compounds and enzymatic activity. The recommended daily consumption of lettuce biofortified with 1 mg of Se-NPs (100 g) provides the recommended daily consumption in adults. Thus, the Se-NPs application is an alternative to increase Se concentration and bioactive compounds in lettuce to influence positively, human nutrition after its consumption.

Index words: *antioxidants, biofortification, Lactuca sativa L., nanoparticles, nanotechnology.*

RESUMEN

El Selenio (Se) es oligoelemento indispensable en los mamíferos no se considera esencial en los programas de fertilización de cultivos. Su deficiencia afecta a casi un millón de personas, lo que lo convierte en un problema de salud mundial, y la nanobiofortificación de los cultivos es una estrategia para atenuarla. Así, este estudio determina los efectos de la aplicación foliar de nanopartículas de selenio (Se-NPs) sobre el rendimiento, los antioxidantes enzimáticos y no enzimáticos y su acumulación en lechuga. Por lo tanto, se consideraron seis aplicaciones de diferentes concentraciones de Se-NP: 0, 1, 5, 10, 15 y 20 mg L^{-1}. Los resultados mostraron que las dosis altas disminuyen el rendimiento de antioxidantes enzimáticos y no enzimáticos pero aumentan la concentración de Se en las hojas de lechuga. La dosis recomendada aquí es de 1 mg L^{-1} para producción de biomasa y 10 mg L^{-1} para compuestos bioactivos y actividad enzimática. El consumo diario recomendado de lechuga biofortificada con 1 mg de Se-NPs (100 g), proporciona el consumo diario recomendado en adultos. La aplicación de Se-NPs es una alternativa para aumentar la concentración de Se y compuestos bioactivos en lechuga para influir positivamente en la nutrición humana después de su consumo.

Palabras clave: *antioxidantes, biofortificación, Lactuca sativa L., nanopartículas, nanotecnología.*

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INTRODUCTION

Selenium (Se) is an essential oligoelement for humans; its consumption prevents cellular damage, thyroid alterations, mental confusion, depression, mutations, and cancer, among others (Nayak *et al*., 2021; Rua, Nogales, Carreras, and Ojeda, 2023). The population obtains this nutrient mainly from food of animal origin, such as meat and seafood. According to OMS recommendations, the daily intake of Se by adults should be 40 to 70 μg day^{-1} depending on sex and body condition (weight, pregnancy status in women, etc.) (Bjørklund *et al.,* 2022). However, plants only assimilate a small portion (< 5%) of the applied Se (Zhu, Zhang, Liu, Chen, and Zhang, 2018), so they contain a minimum amount of this micro-element (concentrations between 0.1 and 2.0 mg L^{-1}) due to low availability of Se in soil, which limits its presence in vegetables (Gaucin-Delgado *et al.,* 2020; Guo *et al.,* 2023). An alternative to increase Se in plants is foliar nano-biofortification, which may increase the mineral content in the edible parts of vegetables (Guillén-Enríquez *et al.,* 2022). Although Se is not considered an essential element in plant nutrition, its foliar application increases in *Dracocephalum moldavica* L. (Azimi, Oraei, Gohari, Panahirad, and Farmarzi, 2021), *Solanum lycopersicum* (Dima *et al.,* 2020) and *Cucumis sativus* L. (Li, Li, Li, Zhou, and Wang, 2020), non-enzymatic and enzymatic compounds and antioxidant enzymes, improving the plant defense system by free-radical detoxification coming from oxidative stress generated by biotic and abiotic factors (Sariñana-Navarrete *et al.,* 2023). In addition, performance significantly increases (Shalaby *et al.,* 2021; Wang *et al.,* 2024). Nanoparticles (NPs) applied foliarly and based on trace elements such as Se are an agronomic alternative for nanobiofortification. The nanoparticles (NPs) applied foliarly and based on oligoelements as Se are an agronomical nano-biofortification alternative.

The Se-NPs have shown high bioactivity and antioxidant capacity besides a positive effect on the plant's primary and secondary metabolism (Lanza and Dos Reis, 2021) improving harvest quality (Narváez-Ortiz *et al.,* 2018; Sayed *et al.,* 2024). Concerning inorganic Se, the Se-NPs have shown the advantages of having greater bioactivity, bioavailability, and lesser toxicity (Azimi *et al.,* 2021).

Lettuce (*Lactuca sativa* L.) is one of the most important vegetables because of its fresh consumption or mixed in salads (Sularz, Smoleń, Koronowicz, Kowalska, and Leszczyńska, 2020; Javaid *et al.,* 2024). In 2023, approximately 27.83 million tons of lechugas will be produced worldwide (FAO, 2023) and in Mexico, lechuga production will be 569 810 tons (SIAP, 2023). The interest of consumers in lettuce has been increasing due to its composition low in calories, and minimal microbial charge of beneficial phytochemicals for human health, such as phenolic acids, and flavonoids, among others (El-Nakhel *et al.,* 2020). These bioactive compounds are important since people are in search of consuming food with greater nutritional quality (Garza-García *et al.,* 2022); thus, bioactive components increase through nano-biofortification, which is of great interest (Hernández-Hernández *et al.,* 2019; Nazir, Anwar, Mahmood, and Shafiq, 2024). Therefore, the objective of the present research is to determine the effects of Se-NPs on yield, bioactive compounds, enzymatic activity, and Se accumulation in lettuce by foliar sprays.

MATERIALS AND METHODS

Plant Material and Growing Conditions

The study was performed in a greenhouse located at Instituto Tecnológico de Torreon, Coahuila, México. Lettuce (*Lactuca sativa* L.) cv. Isla Parris (Huertas®, México) seeds germinated in agricultural foam (Peat Foam, Growing Medium). Twenty days after sowing, the seedlings were transplanted to 5 kg black plastic polyethylene pots containing river sand as substrate and perlite (80:20, v/v) previously sterilized with 5% NaClO. Inside the greenhouse, the pots were set in a double line with a population density of four plants m^{-2}; the drip irrigation system was used, applying Steiner nutritional solution (Steiner, 1984), providing irrigation thrice a day. Each irrigation consisted of applying 0.1 L of water from transplant to plant stage and 1 to 1.5 L from plant stage to harvest. The pH and electrical conductivity of the nutritional solution were 5.5 and 2.0 dS m^{-1}, respectively. The experiment took place during the winter months (January - March), minimum and maximum temperature fluctuated from 12 to 20 °C, respectively, and the minimum and maximum humidity from 20 to 30 percent.

Treatments, Experimental Design, and Samplings

The Se-NPs were synthesized by the Centro de Investigación en Química Aplicada according to the methodology of Quiterio -Gutiérrez *et al*. (2019). The size was from 2-20 nm in spherical form and was applied with adherence ADH (adhesive acrylic resin) on leaves every 15 days with a total of six applications during the first hectare in the morning. The experiment was set up in a complete randomized design, each treatment with seven repetitions.

At 60 days after transplant, 10 plants were collected per treatment and their composition was determined: fresh (FW) and dry (DW) weight (g); photosynthetic pigments (mg g^{-1} FW); total phenols content (mg GAE 100 g^{-1} FW); flavonoids (mg QE 100 g^{-1} FW); antioxidant capacity (µM equivalent in Trolox 100 g^{-1} DW); glutathione (mg 100 g^{-1} DW); enzymatic activity: GXP (UTP^{-1}); catalase -CAT- (UTP^{-1}), superoxide dismutase -SOD- (U mL^{-1}) and Se content (µg kg^{-1}).

Se-NPs Application

The evaluated treatments consisted of foliar application of Se-NPs using the following concentrations: 0, 1, 5, 10, 15, and 20 mg L^{-1} (Gaucin-Delgado *et al*., 2020). To prepare the different doses a stock solution of Se-NPs was used. Subsequently, the five doses of nanoparticles were prepared in a one-liter volumetric flask, and each of the nanoparticles' concentrations was poured separately and completed with distilled water. The solutions were placed in manual sprayers with a capacity of 100 mL^{-1}, and the different nanoparticle treatments were applied via foliar spraying during the first hours of the morning, spraying was carried out every fifteen days after transplantation (DAT).

Biomass Production and Dry Weight

For yield quantification, lettuce plants of each treatment were collected and weighed in an analytical balance (Ohaus Corporation, Pine Brook, NJ, USA) expressing the result in grams. The lettuce leaves were placed in paper bags and deposited in a drying oven (Novatech S.A. de C.V., Ohaus® 547A) Ohaus Mexico at 72 °C for 24 h. The sampling was weighed in an analytical balance (Ohaus Corporation, Pine Brook, NJ, USA) and the results were expressed in grams.

Photosynthetic Pigments

The photosynthetic pigments were determined by the method described by Wellburn (1994). Fresh leaf weight was 0.3 g and 10 mL of pure methanol (CH_3OH) was added and incubated at room temperature in darkness for 24 h. Subsequently, absorption was quantified at 653 nm (chlorophyll b: Chl b) and 666 nm (chlorophyll a: Chl a). The pigment concentration calculus was performed with the following formula:

$$\text{Chl a: } [15.65(A666) - 7.34(A653)] / \text{Chl a} * (V1 * p1) / p2 * 2^2 * n \quad (1)$$

$$\text{Chl b: } [27.05(A653) - 11021(A666)] / \text{Chl b} * (V1 * p1) / p2 * 2^2) * n \quad (2)$$

Extract Preparation for Phytochemical Compounds

To obtain the extracts, 2 g of fresh sampling were mixed in 10 mL of ethanol at 80% in screw-cap test tubes and placed in an orbital shaker (AAH3D1265U, OS-3000 Shaker) at 20 rpm in darkness at room temperature for 24 h. The supernatant was used for the analytical assays.

The total phenolic content was determined by modifying the Folin-Ciocalteu method; 50 µL of ethanolic extract (Singleton, Orthofer, and Lamuela, 1999) were diluted in 3 mL of Mill-Q (Merck, NJ, USA) water; 250 µL of Folin-Ciocalteu (1 N) were added, shaken, and let stand for 3 min for the reaction. Subsequently, 750 µL of Na_2CO_3 (20%) and 950 µL of Mill-Q (mQ) water were added; the solution was left to stand for 2 h and the samples were quantified in an ultra-violet visible (UV-Vis) at 760 nm. The standard solution was prepared with gallic acid. The results are expressed in mg GAE 100 g^{-1} FW.

The total flavonoids were determined by spectrophotometry (Souza *et al.,* 2014); 250 µL of the ethanolic extract were taken, mixed with 1.25 mL of mQ water and 75 µL of $NaNO_2$ (5%), let stand for 5 min and 150 µL of $AlCl_3$ (10%) were added. Subsequently, 500 µL of NaOH (1 M) was added and 275 µL of mQ water, was vigorously shaken and the samples were quantified in a UV-Vis spectrophotometry at 510 nm. The standard was prepared with quercetin dissolved in absolute ethanol (y = 0.0122x-0.0067; r2 = 0.965). The results are expressed in mg QE 100 g^{-1} FW.

The total antioxidant capacity was measured by the DPPH+ *in vitro* method (Moretti *et al.*, 2013). A $DPPH^+$ (Sigma-Aldrich, U.S.A.) solution was prepared in ethanol at a concentration of 0.025 mg mL^{-1}. Then, 50 µL of the ethanolic extract was mixed with 1.950 µL of DPPH+ solution; after 30 min, the samples were quantified in a UV-Vis spectrophotometer at 517 nm. The results were expressed in µM equivalent to 100 g^{-1} Trolox DW.

Glutathione (mg 100 g^{-1} DW) was determined following the method of Xue, Hartikainen, and Piironen (2001) by 5,5-ditio-bis-2 nitrobenzoic acid (DTNB) reaction. Then, a mixture of the extract of 0.480 mL, 2.2 mL of sodium dibasic phosphate (Na_2HPO_4 at 0.32 M) buffer and 0.32 mL of DTNB (1 mM) dye were placed in a test tube. The mixture was shaken in a vortex and read in a spectrophotometer UV-Vis (Thermo Fisher Scientific, model G10S, Waltham, MA, USA) at 412 nanometre.

Enzymatic Activity

Glutathione peroxidase (GPX) was determined (EC 1.11.1.9) [U g^{-1} of total proteins (UTP^{-1}), where U is equal to reduced glutathione mM (GSH) per milliliter per minute] by Flohé and Günzler (1984). Then, 200 µL of the extract, 400 µL of GSH (0.1 mM), and 200 µL of Na_2HPO_4 (0.067 M) were placed in a test tube. The mixture was preheated in bain-marie at 25 °C for 5 min; after that, 200 µL of H_2O_2 to start the catalytic reaction at 26 °C for 10 min. The reaction was stopped by adding 1 mL of trichloroacetic acid at 1%. The mixture was placed in an ice bath for 30 min and centrifuged at 1000 × g at 4 °C for 10 min. To evaluate GPX, 480 µL of supernatant, 2.2 mL of Na2HPO4 (0.32 M) and 320 µL of acid dye 5.5-ditio-bis-2-nitrobenzoic (DTNB) of 1 mM in a test tube. Absorption was measured by a UV-Vis spectrophotometer (Thermo Fisher Scientific, model G10S, Waltham, MA, U.S.A.) at 412 nanometre.

Catalase -CAT- (UTP^{-1}, where U is equal to the equivalent mM of H_2O_2 consumed per milliliter per minute) was quantified by Dhindsa and Matowe (1981). The measurement was performed in two steps [at time 0 (T0) and time 1 (T1)]. In T0, 100 µL of extract, 400 µL of H_2SO_4 (5%) and 1 mL of H_2O_2 (100 mM) were added to an Eppendorf tube (Hamburg, Germany) and shaken for 30 s. After that, absorption was measured in a UV-Vis spectrophotometer (Thermo Fisher Scientific, model G10S, Waltham, MA, USA) at 270 nm. Catalase determination was based on quantifying the oxidation rate of H_2O_2 by absorption difference (T0-T1).

Superoxide (SOD) determination (EC 1.15.1.1) (U mL^{-1}, where U is defined as the necessary enzyme amount to exhibit a dismutation of 50% of the SOD radical) was performed by using the kit SOD Cayman 706002®. A mixture of 20 µL extract, 200 µL of radical detectors (tetrazolium salt), and 20 µL oxidase xanthine solution was placed in a microplate. The microplate was covered with a transparent cover (kit), shaken for 10 s, and then incubated at 26 °C for 30 min. Next, absorbance was measured at a length of 450 nm using a plate reader (BioTek, model ELx808, Winooski, VT, USA). The principle of the trial is based on using tetrazolium salt to detect the superoxide radicals generated by xanthine oxidase and hypoxanthine.

Selenium Content

Dry samples of lettuce were ground in a porcelain mortar and digested in nitric acid-perchloric acid (3:1) using a plate and heated at 100 °C. The solution was filtered and boiled up to 100 mL with deionized water. The selenium concentration in lettuce was determined by an inductively coupled plasma-optical emission spectrometry (ICP-OES) series 700 Agilent Technologies, USA). The results are expressed in micrograms per kilogram (µg kg^{-1}) of dry matter (DM).

Statistical Analyses

The normality and homogeneity of the variation of the data obtained will be verified using Kolmogorov-Smirnov and Bartlett tests, respectively to prove the normal variation of the variable. Subsequently, the simple and multiple comparisons of mean analyses of variance (ANOVA) were performed through Fisher's least significant difference (LSD) test with a probability of 5% ($P \leq 0.05$) and the help of the statistical analysis system package SAS v 9.0 (SAS Institute, 2002).

RESULTS AND DISCUSSION

Biomass Production

Foliar spraying with Se-NPs affected biomass production; the dosage of 1 mg L^{-1} of Se-NPs increased by 35.8% of lettuce fresh weight compared to the control (Figure 1). The application of low Se-NPs doses may increase biomass production of the cultivation since selenium nanoparticles may prevent cellular oxidation through several mechanisms. For example, they may improve the plant antioxidation system, stimulate chlorophyll biosynthesis, increase stomatal opening, and improve the CO_2 assimilation rate (Hasanuzzaman *et al.,* 2020; Li *et al.,* 2020). The biomass produced by a conventional lechuga weighs between 200 and 500 g. Moreover, photosynthesis is promoted as well as water and nutrient absorption that stimulate plant growth and biomass (Sindireva *et al.,* 2023; Zulfiqar and Ashraf, 2021).

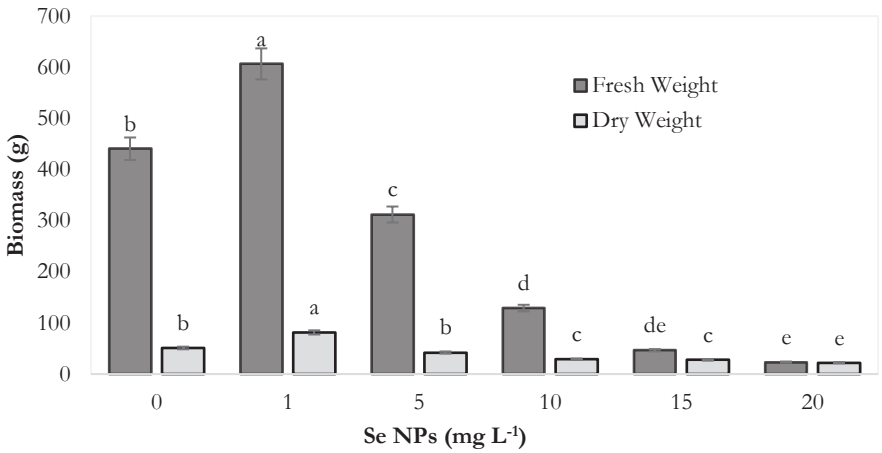

Figure 1. Biomass production in lettuce plant subjected to different concentration of nanoselenium particles (Se-NPs).

Photosynthetic Pigments and Phytochemical Compounds

The Se-NPs induced an increase in total chlorophyll content, and foliar application of 10 mg L^{-1} increased by 47% (Figure 2a). Using increasing doses of Se in albacar the biomass production decreases as Se concentration increases. The increase in photosynthetic pigment content may be attributed to the capacity of Se to protect chloroplast enzymes and increase the biosynthesis of the photosynthetic pigments (Thakur, Bhattacharya, Khosla, and Puri, 2019). High Se-NPs dosage exerts phytotoxic effects inducing cyto-toxicity, genotoxicity, and chloroplast damage when oxidative stress conditions are induced, which obstructs the photosynthesis process by disturbing the activity of photosystem I and causes low cultivation yield (Da Cruz Ferreira *et al.,* 2020; Puccinelli, Malorgio, Rosellini, and Pezzarossa, 2019). Selenium toxicity in plants can occur by forming too many selenoproteins and inducing oxidative stress. The toxicity of selenium depends on the age of the plant and the chemical form of this element. At a Se concentration ≥ 50 mg L^{-1} dry weight (DW) in tomato it is toxic and causes a 25% reduction in biomass without visible symptoms (Gaucin-Delgado *et al.,* 2020). Similarly, applying Na_2SeO_3 and Na_2OH_4 a (5 µM) reduced germination growth in pepper and altered reproductive development (Hernández-Díaz *et al.,* 2021). Selenium at high levels was also found to interact with other toxic metals/metalloids and accelerates toxic effects (Raghda'a Ali Al-Khafajy, AL-Taey, AND AL-Mohammed, 2020).

In the results of the present study, the plant response to Se-NPs depends on the dose used since a low dosage promotes lettuce weight, causing a phytotoxic effect, and damaging the plant metabolic processes (Hawrylak-Nowak *et al.,* 2015). Additionally, high Se dosage can maintain a close correlation with the net photosynthesis inhibition and growth reduction in plants (Zulfiqar and Ashraf, 2021). The toxic effects of Se in plant cells are related to the interference of selenium metabolism (Malerba and Cerana, 2018; Qu *et al.,* 2023), which results in foliar chlorosis and high doses cause a decrease in protein synthesis and low production of dry matter (Liu *et al.,* 2022b). Selenium toxicity generally occurs in high foliar concentrations (Terry, Zayed, De Souza, and Tarun, 2000; Silva *et al.,* 2023).

Foliar spraying Se-NPs affected the phytochemical compounds positively (total phenolic, flavonoids, antioxidant activity and glutathione (Figure 2b, e), obtaining greater accumulation of these compounds with 10 mg L^{-1}, sur-passing control in 12, 19, 31 and 11%, respectively. The NPs have demonstrated to improve the plants cultivated under stress by many mechanisms, such as improving the antioxidant defense system, promoting photosynthesis, and increasing water, nutrients and phytohormones (Zhong and Cheng, 2017). This type of response depends on the dose used, since the Se-NPs may induce negative responses to the determined dosage, while in others, they may induce the opposite effect or simply not have any effect (Joudeh and Linke, 2022). The previous result may be explained because plants experience oxidative stress caused by the foliar application of Se-NPs, and the plants need to adapt metabolically to overcome the stress, which induces secondary metabolite synthesis (Mosa *et al.,* 2018). This adaptation improves the functional properties in many plant species including lettuce, when greater secondary metabolite biosynthesis is induced (Zahedi, Hosseini, Meybodi, and da Silva, 2019). These secondary metabolites include phenolic compounds, flavonoids, alkaloids, glycosides, and tannins among others which help to increase the antioxidant capacity in the fruit (Sindireva *et al.,* 2023).

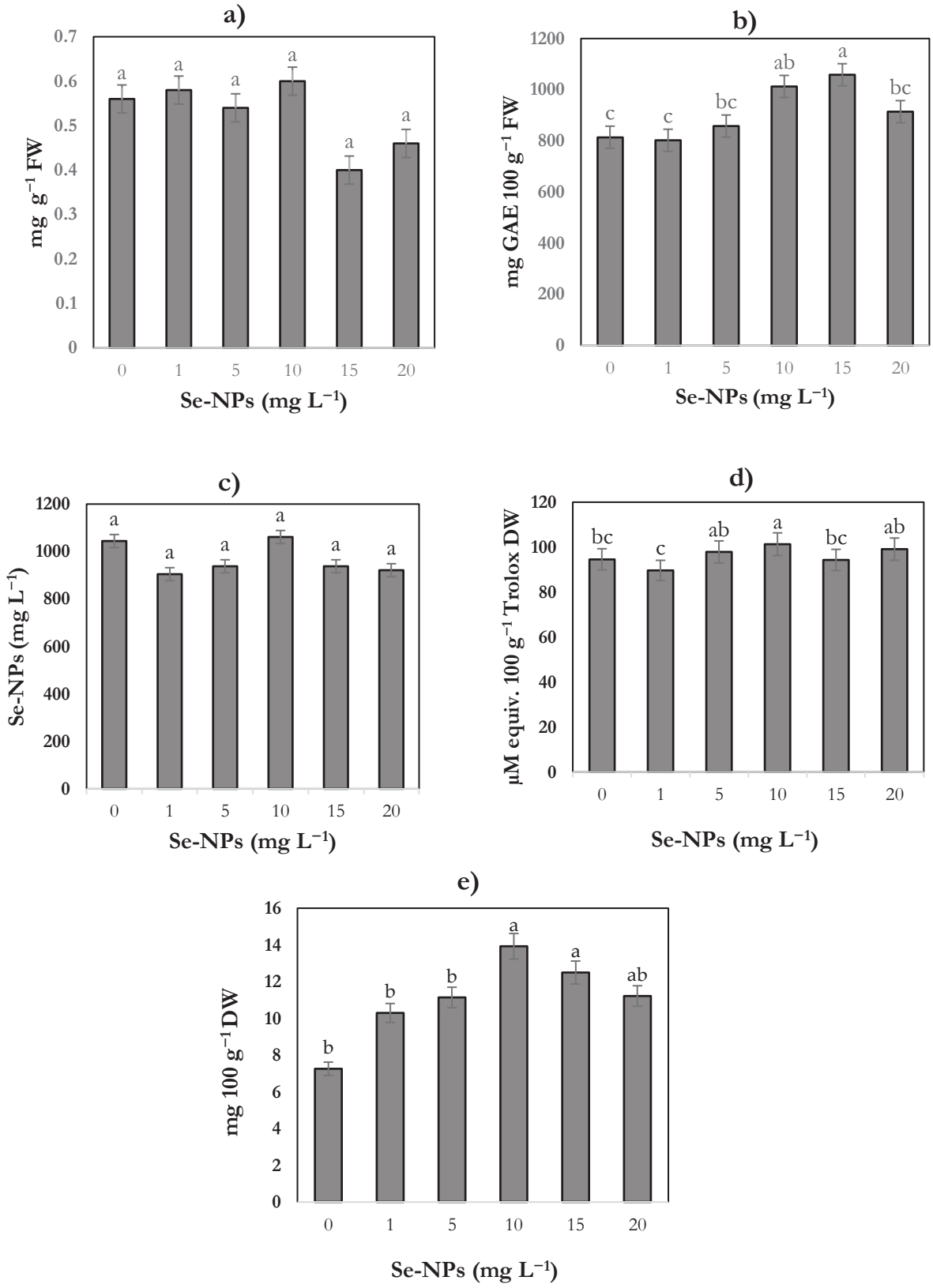

Figure 2. Effect of nano-Se particles on (a) total chlorophyll, (b) total phenolic content, (c) flavonoids, (d) total antioxidant and (e) glutathione in lettuce.

Selenium is an essential component of the selenoenzymes selenoproteins, which show antioxidant functions that improve nutrient quality (Zhu *et al*., 2018). Moreover, the secondary metabolites in plants may fluctuate inversely according to the type and level of the stress applied, affecting plant metabolism (Luo *et al.,* 2019). In extreme cases, the plants cannot overcome stress and their survival decreases drastically (Bocchini *et al.,* 2018). The toxicological effects of the Se-NPs are determined not only by the physicochemical characteristics, time of exposure on the plant, and development stage in which the nano product contacts the plant (Chomchan, Siripongvutikorn, Puttarak, and Rattanapon, 2017). Thus, the metabolic profile and general response of the nanoelements depend on the type of plant, concentration, and stimulation time (Garza-García *et al.,* 2022). Improving the secondary metabolites through nano-biofortification has opened a new investigation area that may have important economic benefits for the agri-food industry. Since these compounds may delay oxidation and increment the nutritional food quality (El-Ramady *et al.,* 2021), their consumption is beneficial for human health due to their antioxidant and anticarcinogenic characteristics (Sarwar *et al.,* 2020).

The previous results can be explained because glutathione synthesizes starting from constituent amino acids, such as cysteine in different cellular compartments and may move through the apoplast or symplast as selenocysteine for its incorporation in the glutathione ascorbate cycle to remove H_2O_2 (Sindireva *et al.,* 2023). The multifunctionality of glutathione allows diverse functions that cannot be performed by the antioxidants since it interacts with various proteins through sulfurdoped titanium dioxide (TiO_2) exchange, which allows interaction in biosynthetic routes, detoxification, antioxidant biochemistry and homeostasis redox (Dos Santos *et al.,* 2020). On the other hand, the reduction in glutathione activity is probably due to the stress caused by the high Se-NPs dosage, which causes toxicity in the plant. The presence of this enzyme in the control treatment is because under normal conditions the plants produce reactive oxygen species (ROS), which perform a key function in plant growth and development (Tan, Mo, Lau, and Xu, 2019), thus, generating specialized compounds in the plant that modify primary metabolic routes. With these compounds, plant organisms may adapt easily to new conditions, recognizing several threat signals by their receptors and sensors that activate defense responses to stabilize themselves against stress (Golubkina *et al.,* 2023).

Enzymatic Activity and Selenium Content in Lettuce

The Se-NPs foliar application modified the enzymatic activity (GPX, CAT, SOD, and Se) significantly, in lettuce plants (Figure 3a-3c). The best enzymatic activity was observed with 10 mg L^{-1} of NPs surpassing 8.56 and 47% of the control treatment. These results evidence that with this dose a positive stress is produced in the plant when its defence mechanism activates. Nanobiofortification is a simple manner to improve vegetable food quality and attenuate the oligoelement deficiencies in the population. In the present study, the concentration of Se in lettuce leaves increased proportionally to the dose sprayed (Figure 3d).

The previous result is probably due to Se interacting in the cellular metabolism of the plant as the catalytic center of proteins regulating the enzymatic activities (Chauhan *et al.,* 2019). For example, GXP, CAT, and SOD are important eliminators of free radicals and form part of the first line of defense against oxidative stress (Złotek, Świeca, and Jakubczyk, 2014: Garza-Alonso *et al.,* 2023). Consequently, plants have a wide range of cellular protection mechanisms designed to neutralize or control the excess of ROS before they may cause damage to the metabolism or cellular structure (Dima *et al.,* 2020). This system of defense includes antioxidant and enzymatic components, such as CAT, GPX, and SOD, among others (Drobek, Frąc, and Cybulska, 2019). Studies have demonstrated that Se foliar application increases SOD, CAT, and GPX enzymatic activities on leaves of *Spinacia oleracea* (Golubkina *et al*., 2023) and *Solanum lycopersicum* (Durmuş, Çolak, and Karaköse, 2019; Liu *et al.,* 2022a) Likewise, Huang *et al*. (2023), confirmed that the NPs within subcellular organelles may induce cascades of oxidative stress signaling in the cells. However, to counteract the increase in ROS levels, the organisms activate antioxidant defense mechanisms, which allow them to increase or decrease lipid peroxidation and the antioxidant capacity, according to the dosage used (Montaño-Herrera *et al.,* 2022).

Moreover, high Se-NP dosages (15 and 20 mg L^{-1}) cause physical changes and biochemical alterations in plants, expressing phytotoxicity caused by their application. Likely, the cells may experiment with apoptosis a self-destruction process regulated and highly organized inherent to their development and survival, which may be unchained in response to the stress situations (Morales-Espinoza *et al.,* 2019). In contrast, cellular death results from exposure to toxic compounds and severe stress (Khurana, Tekula, Saifi, Venkatesh, and Godugu, 2019). Furthermore, it is difficult to know the effect the nanoparticles may cause when they interact with a living system (Li, Wu, Li, Yang, and Yang, 2017), which mainly depends on the physicochemical characteristics of the NPs that are the main cause of the effects generated (López *et al.,* 2021). Thus, morphology, superficial charge, concentration, and size distribution are properties that may provoke different results in the same system if they are individually modified (Andrade *et al.,* 2018).

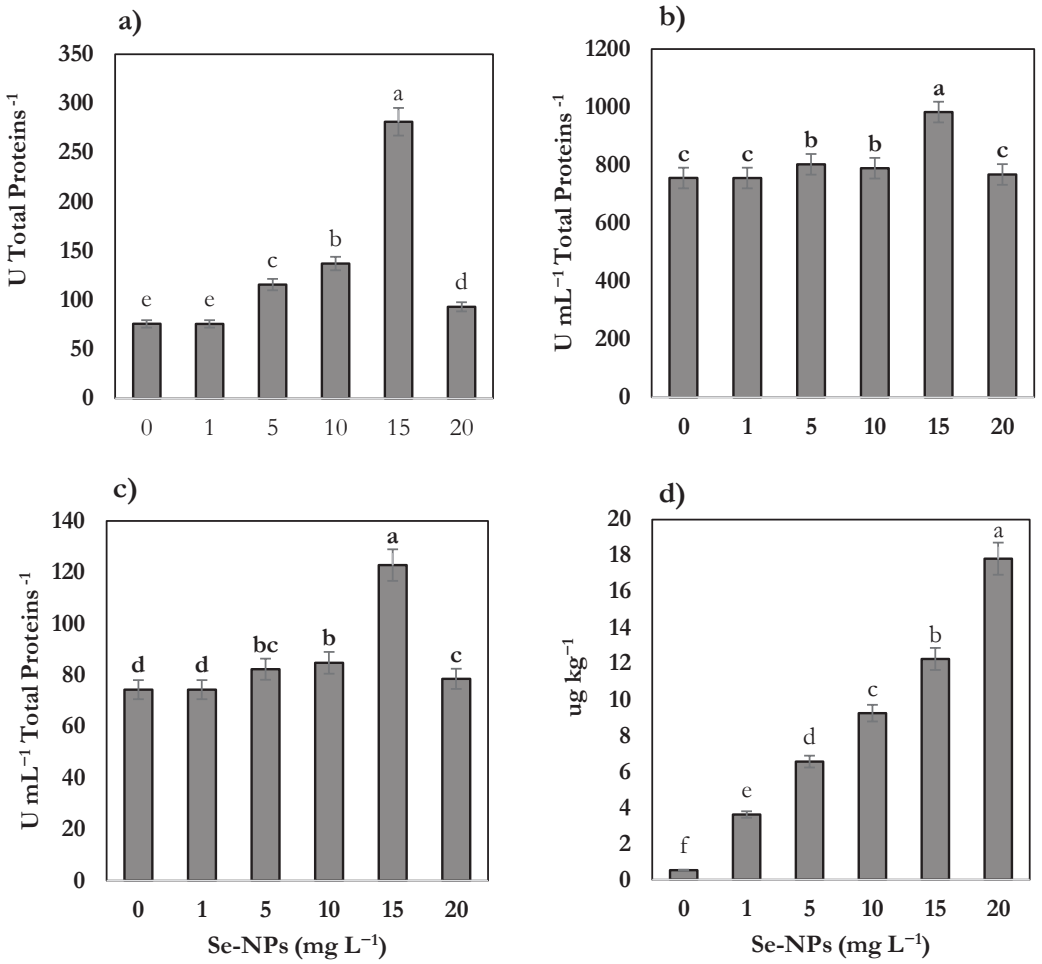

Figure 3. Doses effect of selenium nanoparticles on the content of (a) glutathione peroxidase, (b) catalase, (c) superoxide dismutase SOD, and (d) selenium in lettuce.

As pointed out (Rajput *et al.,* 2018), Se accumulation in the plant depends on many factors, mainly on the concentration of the element used. The Se-NPs foliar applications in high dosage caused phytotoxicity due to high bioaccumulation, damaging the plant metabolism and structure. Therefore, future studies should determine the optimum dose and application frequency to avoid negative effects and know the toxicity mechanisms, which are closely related to dose, composition, chemical structure, particle size, and superficial area.

In this sense, our results suggest that the daily consumption of lettuce biofortified with 1 mg L^{-1} of Se-NPs (100 g) provides the recommended daily consumption in adults of 55 mg day^{-1} (Stefani, Halim, Andayani, and Witjaksono, 2020; Paramo, Feregrino, Guevara, Mendoza, and Esquivel, 2020). With the above, foliar spraying with selenium in short-term and fast-growing crops such as lettuce, emerges as an alternative to improve nutritional quality and increase the capacity to accumulate Se in their tissues, with the alternative of reducing Se deficiency. in humans. In this sense, foliar spraying with 1 mg L^{-1} of Se-NPs on lettuce plants may contribute easily and simply to the daily intake recommended in adults of 55 mg day^{-1} (Stefani, Halim, Andayani, and Witjaksono, 2020; Páramo *et al.,* 2020).

CONCLUSIONS

The Se-NPs foliar application in high dosage reduces yield, nutraceutical quality, and enzymatic activities (GPX, CAT, and SOD) but increases Se concentration in lettuce leaves. The recommended dose is 10 mg L^{-1} maximizing the bioactive compounds and enzymatic activity. Therefore, the Se-NPs application may modulate biomass production and the accumulation of bioactive compounds, contributing to improving lettuce's nutritional quality.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Data sets used or analyzed during the current study are available from the corresponding author upon reasonable request.

COMPETING OF INTERESTS

The authors declare that they have no competing interests.

FINANCING

Not applicable.

AUTHORS' CONTRIBUTIONS

Conceptualization: J.M.G.D., P.P.R, and L.G.H.M. Formal analysis: M.C.H.A., S.C.R.R., and M.G.R.A. Investigation: J.M.G.D., S.C.R.R., and L.G.H.M. Methodology: M.G.R.A., and S.C.R.R. Project administration: P.P.R., and R.L.S. Supervision: P.P.R., and R.L.S. Validation: M.C.H.A., P.P.R., and L.G.H.M.; Visualization: P.P.R. Writing - original draft: J.M.G.D., P.P.R., and L.G.H.M. Writing - review and editing: P.P.R., and L.G.H.M. authors read and approved the manuscript.

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