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Selenium Seed Priming and Biostimulation Influence the Seed Germination and Seedling Morphology of Jalapeño (*Capsicum annuum* L.)

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Abstract: The priming of seeds is shown as a viable technique to improve germination, the growth of the radicle and plumule, and the seedling vigor index, which gives rise to seedlings with higher quality and tolerance to environmental growing conditions. Sodium selenite (Na_2SeO_3) and selenium nanoparticles (nSe) were used as priming media and postgermination biostimulation in seeds of jalapeño pepper, in concentrations of 1, 5, 10, 15, 20, 25, 30, 35, 40, and 45 mg L^{-1} for the two Se species, and control treatment. This research aimed to determine the priming response of jalapeño pepper regarding the germination percent, germination speed index, radicle length, plumule length, fresh weight, and seedling vigor index. The stimulation and phytotoxicity thresholds were also computed. The results showed a percentage of germination greater than 80% in all concentrations evaluated. Most variables of jalapeño pepper presented stimulation responses at Na_2SeO_3 doses lower than 5 mg L^{-1} and nSe doses lower than 15 mg L^{-1}. The higher daily germination was favored by nSe on the fifth day compared to the sixth day of Na_2SeO_3; in addition, the higher cumulative germination occurred on the sixth day with nSe and on the eighth day with Na_2SeO_3. The use of low Na_2SeO_3 concentrations positively favors germination and the morphological traits of the shoots. Likewise, the use of Se in nanometric form was friendlier, that is, the degree of tolerance to Se was higher.

Keywords: priming; biostimulation; phytotoxicity; nanomaterials; sodium selenite

1. Introduction

Seed priming is a technique used with the purpose of increasing the rate and uniformity of emergence in many plant species of economic importance, since this influences the quality and yield of crops [1]. Former seed priming techniques were defined as hydropriming (seeds pretreated with water), halopriming (seeds immersed into inorganic salt solutions such as NaCl, KNO_3, $CaCl_2$, etc.), osmopriming (solutions with sugar, polyethylene glycol, glycerol, sorbitol, or mannitol), and hormonal priming (seeds pretreated with hormones that promote the growth and development of seedlings) [2]. In recent years, the priming technique has been modified and other resources and procedures have emerged,

such as high and low temperatures, solutions based on bioactive molecules or microorganisms (bio-priming), and nanomaterial solutions (nano-priming) [3].

Seed priming techniques have been widely reported, mainly those related to plant species of agricultural interest. In cereals, this practice has been implemented in *Zea mays* L. [4], where the use of Cu nanoparticles encapsulated with chitosan increased the leaf area, shoot dry weight, and seedling root length; in *Triticum aestivum* L. [5], seeds osmoprimed with polyethylene glycol (PEG 8000) and KNO_3 at −0.3, −0.6 and −0.9 MPa increased the germination rate and root and shoot length, and improved the seedling growth; in *Oriza sativa*, ZnO nanoparticles [6] did not affect the germination rate; however, it induced changes in the physiological and biochemical attributes of seedlings. In horticultural species, seed imbibition has been used in *Solanum lycopersicum* L. with C nanomaterials [7], potassium nitrate [8], salicylic acid, hydrogen peroxide, and ascorbic acid [9]; in *Citrullus lanatus* L. [10] using Ag nanoparticles; and in *Capsicum annuum* L. with beneficial microorganisms [11,12], sodium chloride [13], and inorganic salts such as K, Mg, and Ca [1].

Se is a non-essential element for plants; however, it is considered beneficial in amounts much lower than those required by an essential element, due to its inducing a large number of changes in plant metabolism. Its chemical similarity to S facilitates its absorption and it can play the same role in the biochemical system [14]. Seed priming based on selenium in different crops has been widely studied, that is, in rice with Na_2SeO_3 between 0.8 and 1.0 mg L^{-1} [15], between 30 and 60 µmol L^{-1} [16,17]; Na_2SeO_4 between 0.5 and 6.0 mg L^{-1} [18], between 15 and 105 µmol L^{-1} [16,19]; and nSe between 0.5 and 10 mg L^{-1} [18,20]; maize with Na_2SeO_3 between 2 and 5 mg L^{-1} [21]; sorghum with Na_2SeO_3 at 25 µmol L^{-1} [22]; alfalfa with Na_2SeO_3 between 0.5 and 8 µmol L^{-1} [23,24]; quinoa with Na_2SeO_4 between 3 and 9 mg L^{-1} [25] and with nSe at 1 mg L^{-1} [26]; marigold with Na_2SeO_4 between 0.5 and 4 mg L^{-1} [27]; *Brassica rapa* L. with Na_2SeO_3 between 75 and 125 µmol L^{-1} [28]; *Setaria italica* with nSe at 1 mg L^{-1} [29]; lentil with Se between 250 and 750 mM [30]; and tomato with Na_2SeO_3 between 0.5 and 3 mg L^{-1} [31] and nSe between 25 and 100 mg L^{-1} [32].

On the other hand, fertilizer salts also have been applied to crops in the germination media, that is, by moistening the filter paper with treatment solutions [33–36]. In relation to Se solutions applied to the germination media of pepper seed, Leon-Morales et al. [37] moistened filter paper containing pepper seeds with 8 mL of Na_2SeO_3 and Na_2SeO_4 solutions ranging from 1.25 to 5 µM and distilled water for the control, and Hassan et al. [38] added Se between 10 and 50 mg L^{-1} in the soil per potted pepper plant. By combining seed priming with moistening of the filter paper with the same treatment solutions, Aloui et al. [39,40] primed seeds of Anaheim Chili cultivar with 50 mM of NaCl solution for a 24 h imbibition time and moistened the filter paper with 5 mL of NaCl solutions (0, 2, 4, 6, 8, 10, 12 g L^{-1}) in the germination phase for 14 days [39], and irrigated the potted plants with 250 mL of NaCl solution at the same doses for four months of crop cycle [40]. Smith and Cobb [41] studied the accelerated germination of pepper seeds; they performed seed priming with KNO_3, KCl, NaCl, K_2SO_4, Na_2SO_4, NaCl/$CaCl_2$, $Ca(NO_3)_2$, $CaCl_2$, Na_2HPO_4, and K_2HPO_4 solutions ranging from 10 to 300 mM, and moistened the filter paper with the same salt solutions every two days during the germination.

In this context, this research aimed to evaluate the influence of Se priming of jalapeño pepper seeds (*Capsicum annuum* L.) and postgermination biostimulation with sodium selenite (Na_2SeO_3) and selenium nanoparticles (nSe) on the germination percent, germination speed index, radicle length, plumule length, fresh weight, and seedling vigor index, as well as compute the stimulation and phytotoxicity thresholds to find the limiting doses for stimulation from Se treatments and the limiting time for stimulation from the time after sowing.

2. Materials and Methods

2.1. Plant Material and Experimental Site

Seeds of Durango-F1 hybrid jalapeño pepper (*Capsicum annuum* L.) with determined growth habits (Starseeds International Inc., Puebla, Mexico) were used. The experiment was carried out in the Plant Physiology laboratory of the Horticulture Department and Seeds laboratory in the Plant Breeding Department at the Agricultural University "Antonio Narro" in Saltillo, Mexico (25°21′ N, 101°01′ W, altitude 1743 m).

2.2. Treatments and Experimental Design

Se treatments were prepared in distilled water at 1, 5, 10, 15, 20, 25, 30, 35, 40, and 45 mg mL^{-1} [42]. Sodium selenite (Na_2SeO_3 99%, Sigma Aldrich, St. Louis, MO, USA) and 20 nm diameter spherical selenium nanoparticles (nSe) dispersed in Chitosan-PVA [43] were used; the nSe were synthesized in the Research Center of Applied Chemistry (Saltillo, Mexico) according to Kong et al.'s [44] methodology by using a glass reactor equipped with mechanical stirring, temperature control, and an inert atmosphere system, where an aqueous solution of selenious acid (H_2SeO_3) and a Chitosan-PVA solution were mixed at 400 rpm at a temperature of 0 °C. Subsequently, N_2H_4 was added to perform the reduction. A completely randomized design was used.

2.3. Seed Priming

The seed priming process was carried out according to Portuguez-Garcia et al. [45]. Treatments were prepared with 30 mL of Se concentrations in plastic containers; 60 seeds were submerged in each container, shaken for 5 min, and left to rest for a 24 h imbibition time at a temperature of 25 °C and dark conditions. Control treatment consisted of a distilled water solution and the same imbibition procedure. Containers were covered with PM996 parafilm paper (Merck KGaA, Darmstadt, Germany). After the imbibition time, the seeds were rinsed with distilled water and dried at room temperature for 30 min in order to be germinated.

2.4. Germination

Four replications of 15 seeds of each treatment were germinated in transparent plastic trays (15 × 10 × 5 cm) with Whatman No. 1 filter paper (CTR Scientific, Monterrey, Mexico) adjusted to the base and moistened with 10 mL of the Se treatment solutions [33,46] and distilled water for control treatment. The germination medium consisted of a top of paper [47,48], the 15 seeds were equidistantly placed, and the trays were kept for 13 days in an EGCS 3S, 301 3SHR germination chamber (Equitec, Madrid, Spain) at a temperature of 25 °C, 75% relative humidity, and 500 µmol photons m^{-2} s^{-1} white light with a 12/12 h photoperiod [48,49].

2.5. Measurements

The counts of seed germination and normal seedlings were carried out at 5, 6, 8, 11, and 13 days after sowing (DAS). Neither germination nor normal seedlings were detected visually before 5 DAS, from the criterion for the beginning of radicle emergence that implies a radicle length from 1 to 2 mm [49], so that the beginning of germination was considered 4 DAS. Germination as a function of Se treatments was calculated with Equation (1) [50]:

$$Germination = \frac{Germinated\ seeds}{Sown\ seeds} \times 100 \quad (1)$$

Daily and cumulative germination in function of time were calculated with Equation (2) from the germinated seeds at the *i*-day after sowing and the total number of sown seeds, deduced from Romano and Stevanato [51], McNair et al. [52], and Ranal and de Santana [53].

$$Germination = \frac{\sum(Germinated\ seeds\ at\ the\ i\text{-}day)}{Sown\ seeds} \times 100 \quad (2)$$

Normal seedlings were considered those seedlings that were healthy and presented uniform development in the radicle and well-defined primary root, ending in a fine tip, with numerous root hairs, a well-developed plumule, long and narrow cotyledons, increased green coloration from the base of the plumule, and length ratio (radicle/plumule) is greater than the unity [47].

Daily and cumulative germination speed index (GSI) were computed with Equation (3) from the germination percent (GP) of normal seedlings at the *i*-day after sowing [34,50,54,55].

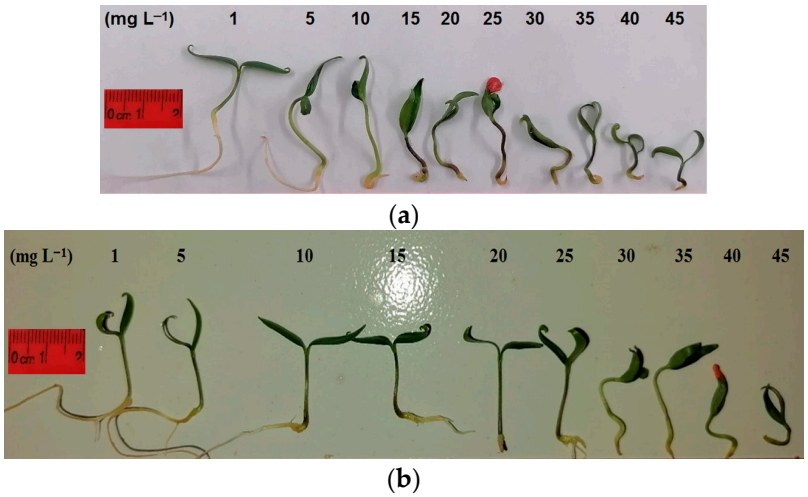
$$GSI = \frac{\sum(GP\ of\ normal\ seedlingss\ at\ the\ i\text{-}day)}{i\text{-}day} \times 100 \quad (3)$$

Seedling vigor index was calculated as a function of mass with the seedling fresh weight (Equation (4)) and a function of size with the seedling length (Equation (5)), where the germination was expressed in decimal to simplify the whole number, and the seedling length was the sum of the radicle and plumule lengths [46,56–58].

$$\text{Seedling vigor index (on weight)} = \text{Germination (decimal)} \times \text{Seedling fresh weight} \quad (4)$$

$$\text{Seedling vigor index (on length)} = \text{Germination (decimal)} \times \text{Seedling length} \quad (5)$$

Radicle and plumule lengths were measured with a 500-197-30 digital caliper (Mitutoyo Co., Ltd., Kanagawa, Japan), the radicle length from the hypocotyl base to the radicle apex and the plumule length from the radicle–hypocotyl intersection to the cotyledon base (Figure 1) [35]. Fresh weight of normal seedlings was measured using a PR224/E analytical balance (Ohaus Co., Parsippany, NJ, USA). Seedling size and biomass were measured on the 15th day after sowing.



(**a**)

(**b**)

Figure 1. Seedlings of jalapeño pepper from seed priming with selenium: (**a**) sodium selenite (Na_2SeO_3); (**b**) selenium nanoparticles (nSe).

The biostimulant or phytotoxic response of the crop influenced by a chemical reagent can be expressed as the relative phytotoxicity index [59] or tolerance index to the chemical reagent [60], and is calculated as the rate: chemical reagent/control. In this research, the biostimulation and phytotoxicity thresholds were computed as a function of the Se and control treatments, from a defined transition value ($y_t > 0$) stated according to the measured variables (y_i), where the biostimulation threshold uses the measured values greater than the transition value (Equation (6)), and the phytotoxicity threshold uses the measured values lower than the transition value (Equation (7)).

$$y_i > y_t \quad Biostimulation = \frac{y_i - y_t}{y_{max} - y_t} \quad (6)$$

$$y_i < y_t \quad Phytotoxicity = \frac{y_i - y_t}{y_t - y_{min}} \tag{7}$$

Also, the biostimulation and phytotoxicity thresholds were computed as a function of the time where the control treatment (*Co*) was stated as the transition value ($y_t = Co - Co = 0$); however, these were expressed as benefit and non-benefit thresholds, respectively, (Equations (8) and (9)), because phytotoxicity does not imply intoxication but rather something undesirable (Figure S1).

$$(y_i - Co) > y_t \qquad Benefit = \frac{(y_i - Co) - y_t}{y_{max} - y_t} = \frac{y_i - Co}{y_{max}} \tag{8}$$

$$(y_i - Co) < y_t \qquad Non{-}benefit = \frac{(y_i - Co) - y_t}{y_t - y_{min}} = \frac{y_i - Co}{-y_{min}} \tag{9}$$

where y_{max} is the maximum observed value that will tend to 1 in the biostimulation/benefit threshold, and y_{min} is the minimum observed value that will tend to −1 in the phytotoxicity/non-benefit threshold.

Sinkkonen [61] simulated the phytochemical effects of phytotoxic compounds present in the soil on seed germination depending on plant density, and fixed a germination probability according to the crop to describe the stimulation and phytotoxicity thresholds in the hormesis curve. Belz and Duke [62] simulated the hormesis curves of the root length of lettuce exposed to phytotoxic compounds and temperature with initial values of 1 to 2 cm of root length.

Since the radicle is a very important organ of the seedling, it is an indicator of germination performance [43]. A radicle length of jalapeño pepper from which germination is achieved (stimulation) or inhibited (phytotoxicity) by the exogenous addition of selenium has not been reported. In this research, the stimulation and phytotoxicity of the parameters of jalapeño pepper influenced by selenium were calculated from a transition value (y_t) of the radicle length stated as y_t = 1.5 cm from the average of the initial values of radicle length in lettuce used by Belz and Duque [62]; the Se dose of transition of the stimulation and phytotoxicity was obtained (Na_2SeO_3 at 5.5 mg L^{-1}). From the Na_2SeO_3 and nSe observed values at the 5 mg L^{-1}, the lower values of the plumule length, seedling length, fresh weight, seedling speed index on length, and seedling speed index on weight were assigned at the 5 mg L^{-1} rounded dose to obtain the corresponding transition values (y_t) (Figure 2).

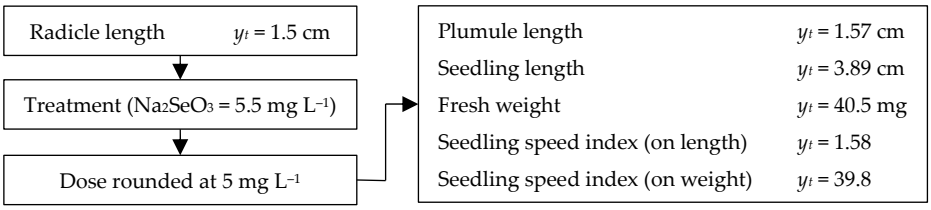


Figure 2. Scheme to calculate the transition values (y_t) for stimulation and phytotoxicity.

2.6. Statistical Analysis

Analysis of variance and mean tests by Fisher's least significant difference (LSD) ($p \leq 0.05$) were performed in the statistical software Infostat® 2016 (Cordoba, Argentina) [63].

3. Results

3.1. Germination Percent

Seed germination percent significantly decreased by 6.7% with Na_2SeO_3 at 15 mg L^{-1} in relation to the control, while the nSe treatments did not statistically modify the seed germination (Figure 3). Germination ranged from 93.33 to 100% in all Se and control treatments.

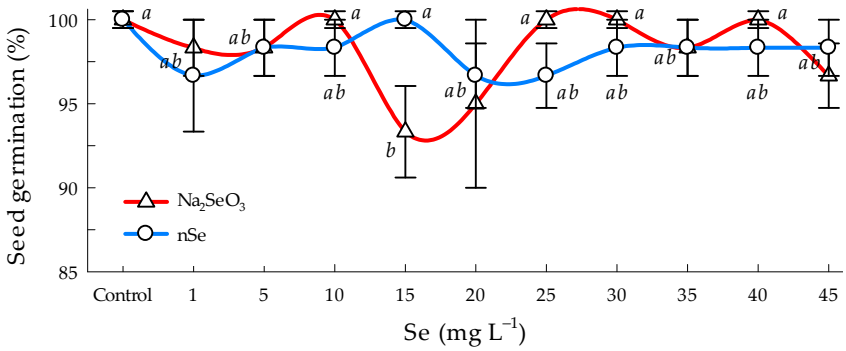


Figure 3. Germination percent of jalapeño pepper (*Capsicum annuum* L.) from seed priming with selenium. Different letters indicate significant differences between treatments (LSD Fisher's test $p \leq 0.05$). Mean values ± standard error. n = 4, eu = 15 seeds.

Regarding germination over time, the higher daily germination was 62 and 65% with nSe and Na_2SeO_3 imbibition on the fifth and sixth days after sowing (DAS), respectively (Figure 4a). According to Se doses of 1 to 45 mg L^{-1}, the limiting day for stimulation (Figure S2) ranged from 6 to 8 DAS for both the nSe and Na_2SeO_3 imbibition (Figure 4c). Meanwhile, the mean cumulative germination reached 88% at 6 and 8 DAS with nSe and Na_2SeO_3 imbibition, respectively (Figure 4b). Also, according to Se doses, the limiting day for stimulation in the cumulative germination ranged from 8 to 11 DAS for both the Na_2SeO_3 and nSe imbibitions (Figure 4d).

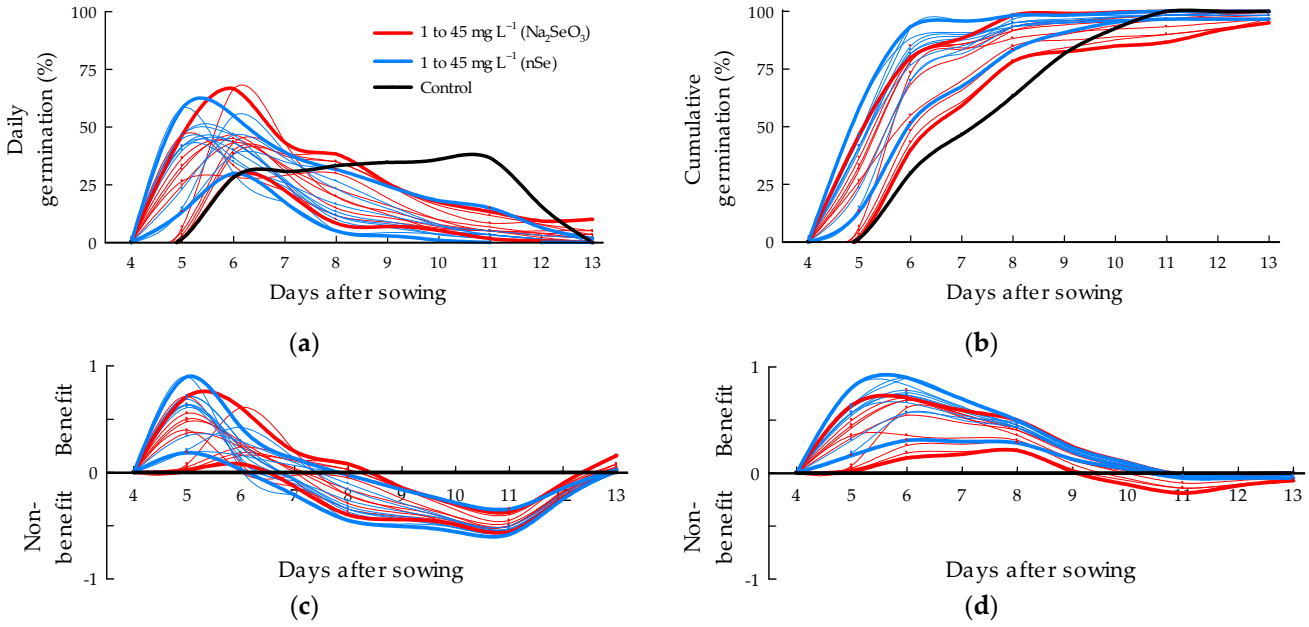


Figure 4. Germination as a function of time of jalapeño pepper (*Capsicum annuum* L.) from seed priming with selenium: (**a**) Daily germination; (**b**) Cumulative germination; (**c**) Biostimulation and phytotoxicity of daily germination; (**d**) Biostimulation and phytotoxicity of cumulative germination.

3.2. Germination Speed Index

The germination speed index (GSI) over time was computed from the normal seedlings which were fewer than the total germinated seeds, so that the daily GSI was reduced with a high variation at 5 and 6 days after sowing (DAS) with Na_2SeO_3 imbibition (Figure 5a); consequently, the cumulative GSI was also reduced with a high variation from 5 DAS (Figure 5b). According to Se doses of 1 to 45 mg L^{-1}, the limiting day for stimulation (Figure S2) in the daily GSI ranged from 5 to 8 DAS for Na_2SeO_3 imbibition and from

6 to 8 DAS for nSe imbibition (Figure 5c), while the limiting day for stimulation in the cumulative GSI ranged from 5 to 11 DAS for Na_2SeO_3, and from 8 to 13 DAS for nSe imbibition (Figure 5d).

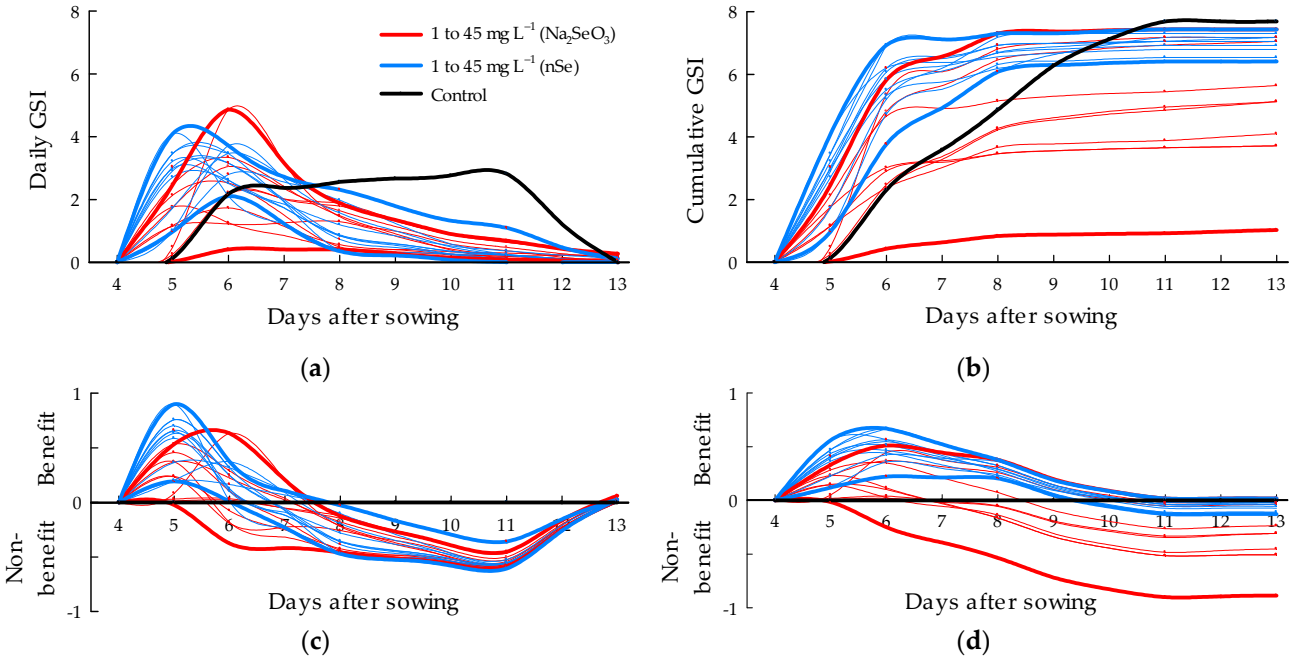


Figure 5. Germination speed index (GSI) as a function of time of jalapeño pepper (*Capsicum annuum* L.) from seed priming with selenium: (**a**) Daily GSI; (**b**) Cumulative GSI; (**c**) Biostimulation and phytotoxicity of daily GSI; (**d**) Biostimulation and phytotoxicity of cumulative GSI.

3.3. Radicle Length

Seed priming of *Capsicum annuum* L. with Se influenced the radicle length of the seedlings. By increasing the nSe dose of seed imbibition, the radicle length of the seedlings increased by 12.5% with 1 mg L^{-1} and was greatly reduced from 10 mg L^{-1}, compared to the control treatment, until reaching a radicle growth inhibition of 95.5% at the higher Na_2SeO_3 dose (Figure 6a). By using a transition value (y_t) for the radicle length of y_t = 1.5 cm, the limiting doses for stimulation (Figures 2 and S2) are located between 5 and 15 mg L^{-1} for Na_2SeO_3 and nSe imbibition, respectively (Figure 6b). According to observed data, by increasing the initial radicle length the limiting Se doses for stimulation are reduced.

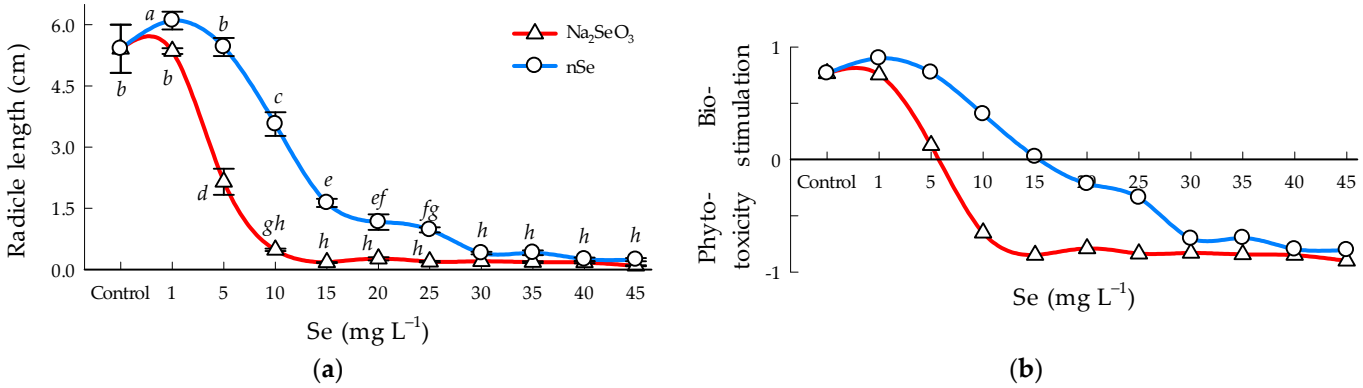


Figure 6. Radicle length of jalapeño pepper (*Capsicum annuum* L.) seedlings from seed priming with selenium: (**a**) Observed values; (**b**) Biostimulation and phytotoxicity. Different letters indicate significant differences between treatments (LSD Fisher's test $p \leq 0.05$). Mean values ± standard error. n = 4, eu = 15 seeds.

3.4. Plumule Length

By increasing the dose of seed imbibition with nSe, the plumule length significantly increased by 13.7 and 16.4% at 20 and 25 mg L^{-1} and decreased significantly between 14.4 and 22.6% (40 and 45 mg L^{-1}, respectively), while the Na_2SeO_3 priming significantly increased this parameter by 19.2% at 5 mg L^{-1}, and decreased significantly between 17.1 and 41.8% (15 and 45 mg L^{-1}, respectively), in relation to the control (Figure 7a). According to the 5 mg L^{-1} dose, the transition value of plumule length was (y_t = 1.57 cm). The plumule length from the seed priming with the two Se species had tri-phasic inverse hormesis curves due to the fact that the control treatment was placed below the transition values (Figure S1); the best response occurred at 5 and 25 mg L^{-1} for Na_2SeO_3 and nSe, respectively, with limiting doses for stimulation from 1 to 10 mg L^{-1} for Na_2SeO_3, and from 5 to 35 mg L^{-1} for nSe (Figure S2). After the second inflection point, the Se become toxic for the growth of the plumule (Figure 7b).

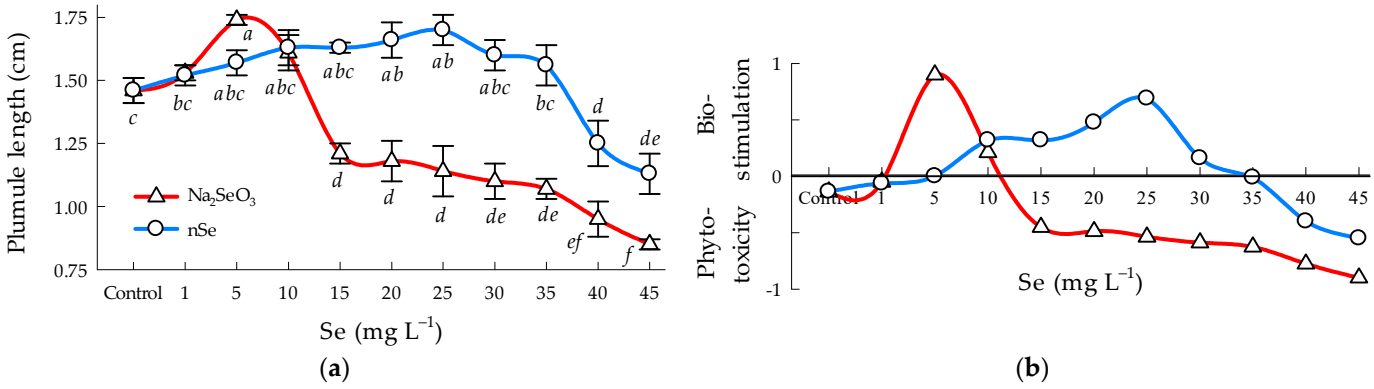


Figure 7. Plumule length of jalapeño pepper (*Capsicum annuum* L.) seedlings from seed priming with selenium: (**a**) Observed values; (**b**) Biostimulation and phytotoxicity. Different letters indicate significant differences between treatments (LSD Fisher's test $p \leq 0.05$). Mean values ± standard error. n = 4, eu = 15 seeds.

3.5. Fresh Weight and Seedling Length

Seed priming of *Capsicum annuum* L. with the two Se species negatively influenced the fresh weight and the seedling length. By increasing the Se concentration, the fresh weight of the seedlings significantly decreased by between 12.1 and 56.6% (10 and 45 mg L^{-1}, respectively) with nSe imbibition, and between 20.5 and 52.2% (5 and 45 mg L^{-1}) with Na_2SeO_3, in relation to the control. The seedling length significantly increased by 10.9%, and significantly decreased by between 52.5 and 80.1% (15 and 45 mg L^{-1}) with nSe, and between 40.4 and 86.3% (5 and 45 mg L^{-1}) with Na_2SeO_3, in relation to the control (Figure 8a). According to the 5 mg L^{-1} dose, the transition values were: fresh weight (y_t = 40.5 mg) and seedling length (y_t = 3.89 cm). The fresh weight and seedling length presented typical hormesis curves (Figure S1) which implies that it is feasible to extend the discretization of Se doses from 0 to 5 mg L^{-1}. The limiting doses for stimulation (Figure S2) ranged between 5 and 15 mg L^{-1} for Na_2SeO_3 and nSe, respectively (Figure 8b).

3.6. Seedling Vigor Index

The seedling vigor indexes related to the fresh weight and to the seedling length reduced as the Se doses increased from 0 to 45 mg L^{-1}. The seedling vigor index (fresh weight) significantly decreased by between 13.6 and 57.3% (10 and 45 mg L^{-1}, respectively) with nSe, and between 21.8 and 53.8% (5 and 45 mg L^{-1}, respectively) with Na_2SeO_3, in relation to the control (Figure 9a). Meanwhile, the seedling vigor index (length) significantly decreased by between 65.2 and 91.3% (15 and 45 mg L^{-1}, respectively) with nSe, and between 55 and 93.5% (5 and 45 mg L^{-1}) with Na_2SeO_3, in relation to the control (Figure 9a). According to the 5 mg L^{-1} dose, the transition values were (y_t = 39.8) of seedling vigor

index (fresh weight) and (y_t = 1.58) of seedling vigor index (length). Both the seedling vigor indexes presented typical hormesis curves (Figure S1) which implies that it is feasible to extend the discretization of Se doses from 0 to 5 mg L^{-1}. The limiting doses for stimulation (Figure S2) ranged between 5 and 15 mg L^{-1} for Na_2SeO_3 and nSe, respectively (Figure 9b).

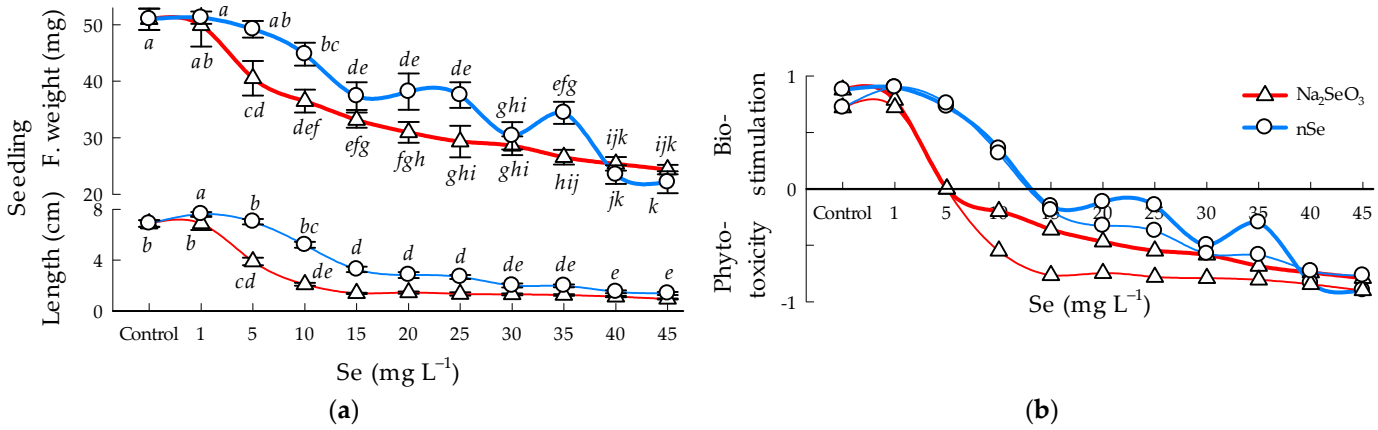


Figure 8. Fresh weight and seedling length of jalapeño pepper (*Capsicum annuum* L.) from seed priming with selenium: (**a**) Observed values; (**b**) Biostimulation and phytotoxicity. Different letters indicate significant differences between treatments (LSD Fisher's test $p \leq 0.05$). Mean values ± standard error. n = 4, eu = 15 seeds.

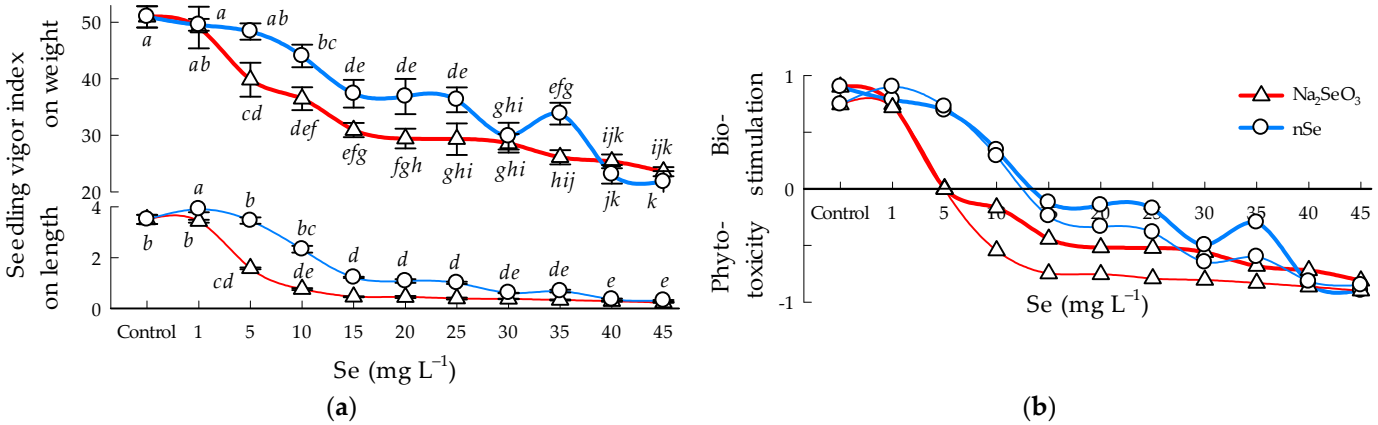


Figure 9. Seedling vigor indexes of jalapeño pepper (*Capsicum annuum* L.) from seed priming with selenium: (**a**) Observed values; (**b**) Biostimulation and phytotoxicity. Different letters indicate significant differences between treatments (LSD Fisher's test $p \leq 0.05$). Mean values ± standard error. n = 4, eu = 15 seeds.

4. Discussion

4.1. Germination Percent

Seed germination is a three-phase process [64], that is, the absorption of water by the imbibed seed, the restart of metabolic processes, and the emergence of the radicle. The treatment of seeds with mineral salts and nanomaterials prior to the start of the germination process has been documented; however, the results in hybrids, species, minerals, and nanomaterials used are very varied [35,65]. Seed imbibition with nSe and nZnO in *Brassica napus* modulated the expression levels of genes related to the ABA and GA pathways, parameters not evaluated in this research, which influenced seed germination and early seedling development [66]. Seed priming has demonstrated its effect by stimulating yield in seeds with low germination parameters [19,64]. The type of raw material used to treat seeds for priming processes can influence the results. Different materials have been used for tests,

such as selenium, zinc, titanium, silicon, silver, iron, carbon nanomaterials, and inorganic salts [3]. Seed pretreatment with nanomaterials to accelerate germination rate and speed, vigor index, and other germination parameters has been widely documented [3,19,33], and is consistent with results obtained with Se nanoparticles where germination was not inhibited; the maximum reduction (3.3%) of this parameter at the 1, 20, and 25 mg L^{-1} doses of seed priming with nSe relative to the control treatment was statistically maintained at the non-significant threshold. Seed pretreatment of *Zea mays* L. with Na_2SeO_4 and $ZnSO_4.H_2O$ maintained the seedling vigor index [58] under normal conditions, and decreased this parameter by almost 50% under water stress conditions [67]. Seed pretreatment of *Oriza sativa* L. with Se and salicylic acid influenced the metabolism of starch, improved the integrity of the membrane, and increased the synthesis of metabolites, which led to better germination and seedling development, even under stress conditions [68].

4.2. Response of Seedlings to Seed Priming with Minerals—Salts and Nanomaterials

Simultaneous evaluation of mineral salts and nanomaterials with the same component on the structures of developing seedlings under controlled conditions occasionally exhibits differences in the same concentration within a variable. In seed pretreatment, the use of nanoparticles and other materials results in growth stimulation and a noticeable improvement in morphological and metabolic characteristics [69]. However, it has been reported that high doses used can cause toxicity and development of abnormal morphological structures [42]. Seeds of *Foeniculim vulgare* Mill. pretreated with TiO_2 and TiO_2 nanoparticles in the same concentration showed significant differences in seedling weight, and shoot and plumule dry weight, while the root and plumule lengths did not change significantly [33]. Seeds of *Capsicum annuum* L. separately pretreated with ZnO nanoparticles at 100, 200, and 500 ppm showed reductions higher than 50% on radicle length, while the plumule length and dry weight of the seedlings were not significantly modified, taking the control treatment as reference [35]. Another study of *Capsicum annuum* L. treated with Se and Se nanoparticles presented toxicity symptoms in seedlings with doses higher than 10 mg L^{-1} for both Se species, namely, a drastic decrease in radicle length and biomass of leaves and roots, as well as the rupture of apical meristems, stopping or slowing down the seedling growth [42]. Also, the root weight significantly increased in the treatments of nSe with 0.5 and 1 mg L^{-1}, compared to the control. According to current research, the seed priming of *Capsicum annuum* L. with selenium influenced growth stimulation in the radicle, fresh weight and length of the seedling, and the seedling vigor index with Na_2SeO_3 doses lower than 5 mg L^{-1}, and with nSe doses lower than 15 mg L^{-1}, and influenced the phytotoxicity or inhibition in the growth of these parameters at doses higher than the mentioned Se doses. The aforementioned agreed with what was mentioned about low doses conferring a stimulating response and high doses inducing toxicity or inhibition [70,71]. The growth stimulation threshold of the plumule was extended to 10 and 35 mg L^{-1} with Na_2SeO_3 and nSe, respectively.

4.3. Bioestimulation and Phitotoxicity

Regarding the germination percentage from Se seed priming over time, seedling attributes greater than the control represented a benefit, and consequently the lower ones were not beneficial (Figure S1). The hormesis is a biphasic dose–response relationship where low doses induce stimulation and high doses induce inhibition, with multiple applications in toxicology and biological disciplines [70,71]. The simultaneous plotting of biostimulation and phytotoxicity can be implemented in the cost–benefit analysis of nanofertilizers [72] and other fertilizers used on agricultural crops [73,74]. The limiting doses for stimulation (Figure S2) for the radicle length, fresh weight, and seedling vigor index were 5 and 15 mg L^{-1} for Na_2SeO_3 and nSe, respectively, whilst in the plumule length these limits extend to 10 and 35 mg L^{-1} in the same order. These results are consistent with the findings of García Márquez et al. [75], who stated that applications of Na_2SeO_3 doses less than 5 mg L^{-1} favored the Se biofortification and antioxidant contents in *Lactuca sativa*

L., *Solanum lycopersicon* L., *Fragaria x ananassa* L., and *Ocinum basilicum* L. Sotoodehnia-Korani et al. [42] mentioned that nSe doses higher than 10 mg L^{-1} are associated with severe toxicity and abnormality in leaf and root development.

5. Conclusions

Simultaneous evaluation of two or more materials containing the same reagent or active substance offers a broad threshold of scenarios and responses, which allows the expansion of knowledge about the active substance compared to individual evaluation. Seed priming of *Capsicum annuum* L. with Na_2SeO_3 and nSe in the same concentrations exhibits seedling tolerance to the evaluated materials and the changes in morphology that may occur. The use of both inorganic selenium salts (Na_2SeO_3) and the nanoparticulate form (nSe) shows them as raw materials with potential to improve germination attributes, mainly as a growth stimulant in low concentrations for radicle growth. Both Se species used for seed priming influenced growth stimulation in the radicle, fresh weight and seedling length, and seedling vigor index with Na_2SeO_3 and nSe doses lower than 5 and 15 mg L^{-1}, respectively, and influenced phytotoxicity or inhibition in the growth of these parameters at the corresponding higher doses. Seed priming and post-emergence biostimulation is a technique in which seed priming reagents can be used to moisten the germination media that is, the filter paper used in the seed germination chamber, and the soil or substrate that's supports plants for their growth and production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10020119/s1, Figure S1: Schematic representation of dose–response or hormesis curves; Figure S2: Components of the dose–response curve.

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