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## Broccoli Biofortification Using Biological Nano- and Mineral Fertilizers of Selenium: A Comparative Study under Soil Nutrient Deficiency Stress

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NUTRIENT deficiency on cultivated plants may lead to human malnutrition when consumes these plants. So, the biofortification is a promising approach in increasing several crops in their contents of essentials nutrients for human health like selenium (Se). The current study focused on using two different sources of Se-fertilizers for biofortifying broccoli under nutrient deficiency stress in sandy soil. The applied doses of selenium in each form were 10, 20, 30, and 40 ppm in the form of mineral and biological nano Se-fertilizers. The vegetative parameters and yield of broccoli were determined. In general, the highest growth and yield components were attained for applying the dose of 30 mg Si $L^{-1}$ for both the soluble and nano Se-forms. The studied parameters included fresh and dry weight of leaves and broccoli head, head diameter and its length gave the highest values when the dose of 30 ppm from both Se-fertilizers applied. The content of N, P, K, and Se nutrients significantly increased by increasing applied Se-doses till 40 ppm in both Se-fertilizers except P. The harvested yield of head broccoli was obtained after applied dose of 30 ppm in case of both Se-fertilizers, with an increase rate for mineral Se-fertilizer 60.1 and 57.2%, whereas were 51.8 and 47.4% for nano-Se fertilizer for both seasons, respectively. The biological Se-nanofertilizer may be is preferable for a safe biofortification. The most distinguished findings in the current study that producing biofortified broccoli rich in Se for human health can be achieved under grown in sandy soils or nutrient deficiency stress. Further studies are needed to answer more open questions regarding producing a safe and healthy food for human nutrition.

**Keywords:** Soil fertility, Bio-nanofertilizer, Potassium deficiency, Nitrogen deficiency.

### 1. Introduction

Broccoli (*Brassica oleracea* L.) is considered one of the most common and important cruciferous vegetables all the over the world. Its economic value increases every year (Francisco et al. 2017). Its florets and sprouts are the consumed parts, while stalks and leaves are wasted after harvesting (Li et al. 2022a). Broccoli crop exerts several human health benefits due to its high content of bioactive compounds (e.g., minerals, vitamins, fiber, glucosinolates and phenolic compounds), and has also exhibits anti-cancer effect (Li et al. 2022a). Several cultivars of broccoli are commonly grown in the Mediterranean region yet, it is characterized by itslong production period (Lopez-Zaplana et al. 2021). Many factors affect its productivity including biotic (Zhang et al. 2022), and abiotic stresses such as salinity (Ali et al. 2022), wounding stress (Guan et al. 2021), pH of growing media (Li et al. 2022b), cold stress (Lopez-Zaplana et al. 2021), and applied nanomaterials like nanocarbon (Li et al. 2022c). The resistance of broccoli against plant diseases can be increased by induction of secondary

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metabolites synthesis, accumulation of sugar alcohols, and unsaturated fatty acids (Zhang et al. 2022).

Selenium (Se) is an essential nutrient for human and animal life, but for higher plants its importance is still not confirmed. However, its venifical role in broccoli biofortification, and production of crop and/or sprouts has been confirmed by many researchers such as Bañuelos et al. (2016), Tian et al. (2018), El-Ramady et al. (2020), and Mao et al. (2020). Selenium biofortification can enhance crop tolerant to heavy metals stress (Ghorai et al. 2022). Nano-selenium and selenium have an increase concern especially for managing cultivated plants under environmental stress and improving quality of crops (Hossain et al. 2022). Several studies on selenium and nano-Selenium were recently published which focus on sources in soil (El-Ramady et al. 2022a), role in plant nutrition or as a biostimulant (Medrano-Macías and Narvaéz-Ortiz 2022) for improving crop quality (El-Ramady et al. 2022a), as, even under undesirable conditions such as cold (Chongping et al. 2022). This significance was detected on the yield and quality traits of pomegranate (Zahedi et al. 2019), tomato (Liu et al. 2022), radish (Huang et al. 2023). Apart from mineral and chemical nano-Se, biological nano-Se has distinguished attributes due to its non-toxic, and eco-safety use as confirmed by some recent publications on cucumber (*Cucumis sativus* L.) (Shalaby et al. 2021), wheat (T*riticum asetivum* L.) (El-Saadony et al. 2021), pakchoi (*Brassica chinensis*) (Zhu et al. 2022), rapeseed (*Brassica napus* L.) (El-Badri et al. (2022), banana (Shalaby et al. 2022), and radish (Hoang et al. 2023).

Therefore, this is work involved two different fertilizers of selenium including mineral and biological nanofertilizer and their impacts on broccoli production under soil nutrient deficiency (i.e., sandy soil). The main questions in this manuscript are which Se-fertilizer is better for prompting broccoli production under such nutrient deficiency? Which applied dose of this preferable Se-fertilizer can be used under such studied conditions?

## 2. Materials and Methods

### 2.1 Experimental location

A field experiment was conducted in the Nubaria Experimental Farm of the National Research Centre during the two winter seasons of 2016-2017 and 2017-2018. This field is situated in an arid climate region at an altitude of 27 m above mean sea level and is intersected by latitude of 30° 30N and longitude of 30° 20E. The soil of the experimental site was deep, well-drained sandy texture, which is classified as an Entisol-Typic Torripsamments, composing of 85.5% sand, 11.7% silt and 2.8% clay. Soil organic matter in the topsoil (0-80 cm depth) was 0.4%, with an alkaline pH of 8.25, low soil salinity (EC: 0.85 dS $m^{-1}$), and lime ($CaCO_3$: 1.5%). The average soil water content at field capacity and the permanent wilting point was 18 and 8%, respectively. Available N, P and K contents within the surface soil layer were 12, 4 and 35 mg $kg^{-1}$ soil, respectively prior to cultivation, which represents a low fertile soil.

### 2.2 Experimental design and treatments

A field experiment was conducted in a Randomized Block Design in three replicates. This experiment included nine treatments: control treatment using only water, 4 different doses of selenium (i.e., 10, 20, 30 and 40 mg $L^{-1}$), and 4 different doses of biological nano-Se fertilizer (i.e., 10, 20, 30 and 40 mg $L^{-1}$). These doses were sprayed on plants at 45 and 55 days from planting.

### 2.3 Source of selenium fertilizers

Mineral selenium fertilizer was prepared using sodium selenite ($Na_2SeO_3$), which was dedicated by Nano Food Lab, University of Debrecen, Hungary. Nanofertilizer of selenium was prepared biologically at the Soils, Water and Environment Research Institute (SWERI), Soil Microbiology Department. The size of selenium nanoparticles was 87.7 nm using Bacillus cereus TAH as reported by Ghazi et al. (2022). The nanoparticle sizes were measured using high resolution transmission electron microscope (HR-TEM, Tecnai G20, FEI, The Netherlands), by Nanotechnology and Advanced Material

Central Lab, Agriculture Research Center (ARC). and other properties were measured using TEM and X-ray in Nanotechnology Lab in Agricultural Research Center, in Giza.

### 2.4 Plant analyses

At harvesting stage, a random sample of five plants were chosen from each plot and subjected for analysis. The following plant growth parameters were measured at harvest; plant length (cm); number of leaves per plant; head fresh and dry weight (g plant$^{-1}$), head length and its diameter, as well as the yield per plant and the total yield per fed. The plant samples were divided into leaves and head, then dried at 65 °C; ground using stainless steel equipment's to determine their nutrient contents i.e., N, P, K and Se in leaves and head of broccoli. From each sample 0.2 g was digested using the mixture of sulfuric ($H_2SO_4$) and perchloric ($HClO_4$) acids (1:1) for N, P, K, and Se determination. Nitrogen was determined using Kjeldahl method, K was determined by flame photometer (NADE LCD Digital Flame photometer FP640, China) and P with the visible Spectro-photometer (Single Beam, SP-IV722N, 721N, China) as described by Cottenie et al. (1982). While, Se content was determined using atomic absorption spectroscopy (SP-IAA1800H, China) as described by Levesque and Vendette (1971).

### 2.5 Statistical analyses

Data of the experiment were subjected to statistical analyses of Dunken's test at the confidence level of 5% conducted on means of treatments to measure the considered significantly different according to the procedures of the procedure outlined by Gomez and Gomez (1984).

## 3. Results

### 3.1 Response of vegetative parameters of broccoli to applied Se-fertilizers

Fresh and dry weight of leaves and head, besides head diameter and its length, number of leaves per plant, and plant length are presented in Tables 1 and 2. The studied vegetative parameters including dry weight of leaves and head increased significantly with increasing the dose of Si application up to 30 ppm for both types of Se-fertilizers (**Table 1**). The recorded values were higher in case of mineral Se-fertilizer compared to Se nano fertilizer. The same trend was observed for number of leaves per plant, head length and its diameter, which recorded the highest values when treated with 30 ppm with both Se-fertilizers (**Table 2**), except for the number of leaves per plant (at 20 ppm mineral Si-form). Comparing with getting values of previous studied parameters, mineral Se-fertilizer still give the highest values compared to nano-Se-fertilizer. The increase in head dry weight (%) for the nanofertilizer in both seasons were 46.0 and 50.8, whereas were 56.3 and 59.9% for mineral Se-fertilizer comparing with control.

**Table 1. Response of the fresh and dry weight of leaves and head to applied Se-fertilizers.**

| Treatments (Applied doses) | Fresh weight of leaves (g) | | Dry weight of leaves (g) | | Fresh weight of head (g) | | Dry weight of head (g) | |
|---|---|---|---|---|---|---|---|---|
| | First season | Second season | First season | Second season | First season | Second season | First season | Second season |
| **Mineral Se-fertilizer** | | | | | | | | |
| Control | 98.23 c | 99.34 e | 12.43 d | 11.06 c | 116.8 e | 117.9 d | 13.98 d | 13.44 c |
| 10 ppm | 120.12 a | 122.8 b | 15.60 c | 15.91 b | 141.1 c | 144.9 c | 14.51 d | 14.25 c |
| 20 ppm | 125.51 a | 131.0 a | 17.78 b | 17.26 b | 167.4 b | 165.2 b | 20.16 a | 20.89 ab |
| 30 ppm | 109.54 b | 116.6 c | 20.45 a | 19.38 a | 187.1 a | 185.3 a | 21.86 a | 21.50 a |
| 40 ppm | 105.96 b | 111.1 d | 15.73 c | 16.11 b | 128.5 d | 135.6 c | 18.13 b | 18.80 b |
| **Nano Se-fertilizer** | | | | | | | | |
| Control | 87.14 b | 88.37 b | 14.06 d | 11.51 c | 117.5 e | 118.9 c | 14.06 d | 13.56 c |
| 10 ppm | 91.19 b | 88.57 b | 16.12 c | 15.16 b | 136.8 d | 138.4 b | 16.12 c | 16.72 b |
| 20 ppm | 100.62 a | 103.0 a | 18.69 b | 17.06 ab | 165.2 b | 171.9 a | 18.69 b | 19.05 a |
| 30 ppm | 98.96 a | 100.4 a | 20.53 a | 18.92 a | 178.5 a | 175.2 a | 20.53 a | 20.46 a |
| 40 ppm | 87.70 b | 88.23 b | 18.85 b | 15.58 b | 149.8 c | 148.3 b | 18.85 b | 19.40 a |

Means within column having the same letters are not significantly different according to the Duncan's Multiple Range Test (at p <0.05)

**Table 2. Response of head and leaves to applied different doses of Se-fertilizers.**

| Treatments (Applied doses) | Number of leaves per plant | | Plant length (cm) | | Head length (cm) | | Head diameter (cm) | |
|---|---|---|---|---|---|---|---|---|
| | First season | Second season | First season | Second season | First season | Second season | First season | Second season |
| **Mineral Se-fertilizer** | | | | | | | | |
| Control | 17.61 c | 16.22 c | 35.62 d | 33.56 c | 10.78 | 10.55 d | 10.74 c | 10.48 c |
| 10 ppm | 20.83 a | 21.06 a | 43.08 ab | 44.96 a | 11.47 | 11.60 c | 14.48 b | 14.21 b |
| 20 ppm | 21.50 a | 21.56 a | 42.18 b | 43.59 a | 13.92 | 14.35 ab | 18.27 a | 20.00 a |
| 30 ppm | 20.78 a | 20.7 ab | 44.50 a | 45.15 a | 15.20 | 15.31 a | 18.96 a | 21.05 a |
| 40 ppm | 20.06 b | 19.67 b | 38.65 c | 38.18 b | 12.97 | 13.89 b | 15.04 b | 15.06 b |
| **Nano Se-fertilizer** | | | | | | | | |
| Control | 17.44 c | 16.72 c | 37.30 bc | 36.11 b | 10.61 | 10.28 d | 11.56 c | 11.74 c |
| 10 ppm | 18.72 bc | 18.78 b | 38.97 b | 38.67 b | 12.56 | 13.27 b | 13.92 b | 13.35 c |
| 20 ppm | 19.83 b | 19.39 b | 41.61 a | 41.71 a | 14.11 | 14.65 ab | 17.43 a | 18.72 b |
| 30 ppm | 21.11 a | 21.33 a | 40.29 ab | 40.69 ab | 14.80 | 15.70 a | 18.72 a | 20.69 a |
| 40 ppm | 18.72 bc | 18.67 b | 37.96 bc | 37.12 b | 14.47 | 14.68 ab | 17.50 a | 17.83 b |

Means within column having the same letters are not significantly different according to the Duncan's Multiple Range Test (at p <0.05).

### 3.2 Content of nutrients in leaf and head of broccoli

The total content of N, P, K, and Se in both head and leaves of broccoli are presented in **Table 3, Figs. 1and 2**. These contents increased in both leaves and head of broccoli plants by increasing the applied dose of Se till 40 ppm except in case for P, which exhibited non-significant variations. The leaves of broccoli contained lower contents of N, P, K, and Se than the heads. The increase rates (%) in these nutrients in broccoli head were 42.9, 250, and 63.9%, after applying 40 ppm of mineral, respectively comparing with control in the second season, whereas for and nano-Se fertilizer were 49.1, 120, and 57.1% for NPK, respectively. In case of Se content in leaves, it was higher than in head of broccoli for each applied dose in each Se-fertilizer (**Figs. 1and 2**).

**Table 3. Content of nutrients in broccoli leaves as a response to applied Se-fertilizers.**

| Treatments (Applied doses) | N content in leaf (%) | | P content in leaf (%) | | K content in leaf (%) | |
|---|---|---|---|---|---|---|
| | First season | Second season | First season | Second season | First season | Second season |
| **Mineral Se-fertilizer** | | | | | | |
| Control | 2.12 d | 2.19 e | 0.11a | 0.12a | 1.68 c | 1.72 d |
| 10 ppm | 2.22 d | 2.35 d | 0.21a | 0.22a | 1.80 b | 1.86 c |
| 20 ppm | 2.40 c | 2.62 c | 0.21a | 0.27a | 1.80 b | 1.90 c |
| 30 ppm | 2.73 b | 2.93 b | 0.25a | 0.33a | 2.28 a | 2.64 b |
| 40 ppm | 2.94 a | 3.13 a | 0.31a | 0.42a | 2.40 a | 2.82 a |
| **Nano Se-fertilizer** | | | | | | |
| Control | 2.15 c | 2.24 d | 0.13a | 0.15a | 1.56 d | 1.54 e |
| 10 ppm | 2.60 b | 2.73 c | 0.20a | 0.26a | 1.80 c | 1.76 d |
| 20 ppm | 2.75 ab | 3.16 b | 0.21a | 0.29a | 1.86 c | 2.00 c |
| 30 ppm | 2.84 a | 3.30 a | 0.24a | 0.32a | 2.04 b | 2.27 b |
| 40 ppm | 2.87 a | 3.34 a | 0.29a | 0.33a | 2.40 a | 2.42 a |

Means within column having the same letters are not significantly different according to the Duncan's Multiple Range Test (at p <0.05).

**Table 4. Content of nutrients in broccoli head as a response to applied Se-fertilizers.**

| Treatments (Applied doses) | N content in leaf (%) | | P content in leaf (%) | | K content in leaf (%) | |
|---|---|---|---|---|---|---|
| | First season | Second season | First season | Second season | First season | Second season |
| **Mineral Se-fertilizer** | | | | | | |
| Control | 3.71 e | 3.23 e | 0.43 d | 0.41 c | 2.06 d | 2.00 d |
| 10 ppm | 3.89 d | 3.50 d | 0.56 c | 0.61 b | 2.16 d | 2.18 cd |
| 20 ppm | 4.11 c | 3.84 c | 0.64 b | 0.73 a | 2.28 c | 2.34 c |
| 30 ppm | 4.49 b | 4.42 b | 0.65 b | 0.74 a | 2.64 b | 2.54 b |
| 40 ppm | 4.88 a | 5.02 a | 0.69 a | 0.81 a | 2.76 a | 2.77 a |
| **Nano Se-fertilizer** | | | | | | |
| Control | 3.63 e | 3.10 d | 0.41 d | 0.38 c | 2.00 c | 1.91 c |
| 10 ppm | 3.91 d | 3.53 c | 0.52 c | 0.55 b | 2.16 b | 2.15 b |
| 20 ppm | 4.07 c | 3.78 b | 0.57 b | 0.62 b | 2.22 b | 2.25 b |
| 30 ppm | 4.21 b | 3.99 b | 0.63 a | 0.71 a | 2.40 a | 2.42 ab |
| 40 ppm | 4.37 a | 4.24 a | 0.65 a | 0.75 a | 2.49 a | 2.47 a |

Means within column having the same letters are not significantly different according to the Duncan's Multiple Range Test (at $p < 0.05$)

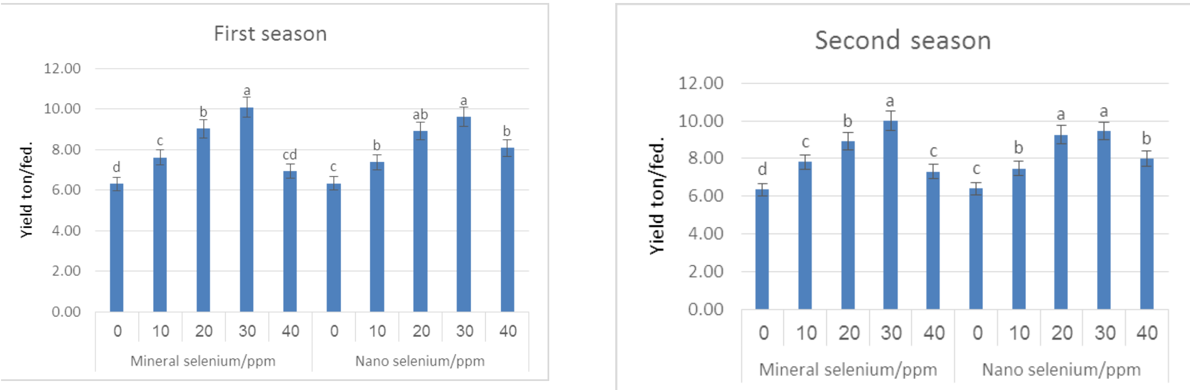


**Fig. 1. Effect of selenium source and concentration on selenium (ppm) in leaves of broccoli plants during both seasons.**

Means within column having the same letters are not significantly different according to the Duncan's Multiple Range Test (at $p < 0.05$).

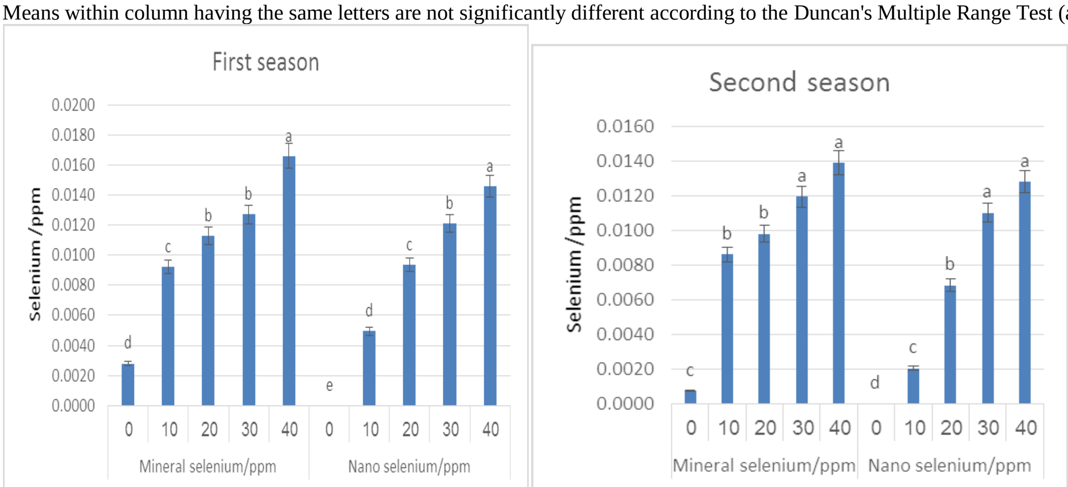


**Fig. 2. Effect of selenium source and concentration on selenium (ppm) in head of broccoli plants during both seasons Means within column having the same letters are not significantly different according to the Duncan's Multiple Range Test (at $p < 0.05$).**

## 3.3 Response of broccoli yield to applied Se-fertilizers

The role of applied Se-fertilizers and their different doses on the yield of broccoli is presented in Fig. 3. For both Se-fertilizers, the yield of broccoli was significantly higher by increasing the applied dose of each fertilizer up to 30 mg $L^{-1}$ thereafter decreased considerably. The recorded yield in case of mineral Se-fertilizer in both seasons (10.1 and 10.0 ton fed-1) was higher compared to nano-Se-fertilizer (9.63 and 9.45 ton $fed^{-1}$). An increase rate in the yield was obtained after applying 30 ppm mineral Se-fertilizer (60.1 and 57.2% for both seasons, respectively), whereas increase rate values were 51.8 and 47.4% for nano-Se fertilizer after applying the same dose and for both seasons, respectively.

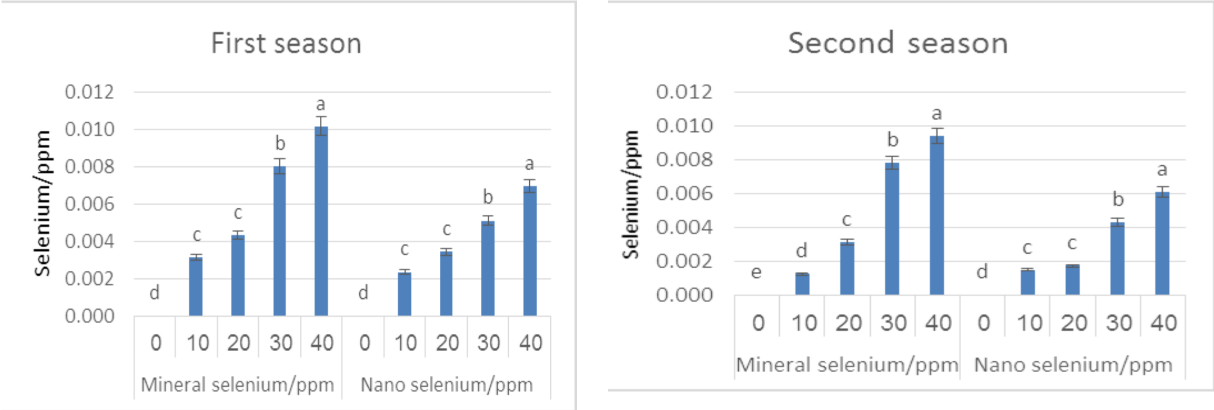


**Fig. 3. Effect of selenium source and concentration on yield (ton/fed.) of broccoli plants during booth seasons Means within column having the same letters are not significantly different according to the Duncan's Multiple Range Test (at p <0.05).**

## 4. Discussion

Great challenges face our world including climate change, desertification, malnutrition, poverty, pollution, global food security, etc. How to feed the entire population all over the world is a serious problem, which needs different programs like the Sustainable Development Goals (SDGs) in 2015 by the United Nations (El-Ramady et al., 2022c and d). Biofortification is a crucial approachto overcome many of the previous problems while improve human health (El-Ramady et al. 2020). This health start from the sustainable plant nutrition (El-Ramady et al. 2022d), sustaining soil fertility (El-Ramady et al. 2022c), with focus on soil and human health (El-Ramady et al. 2022e), and the nexus soil-water-plant-human (Brevik et al. 2022). The current study focuses on the production of biofortifying broccoli enriched in Se using nano Se-fertilizer and its bulk mineral form.

Selenium and nano-selenium biofortification for human health was reported by several researchers such as El-Ramadyet al. (2020, 2022a, b). This biofortification approach was carried out using mineral and/or nanofertilizers. For establishing a successful program of biofortification, it is essential to know the proper crop (important for human diet), the target nutrient and applied dose (which reflects its essentiality for human nutrition), the suitable application method (foliar or soil or in vitro application), etc. In the present investigation, the main questions were which applied dose is effective for Se-biofortification program? Which one is better biological nano-Se fertilizer or its bulk mineral one? To what extent this Se-biofortification program of broccoli can be achieved under nutrient deficiency stress condition? The answer of all previous questions would be presented in the next section.

Previous published articles reported that broccoli could be biofortified with Se such as Bachiega et al. (2016), McKenzie et al. (2017), Tian e al. (2018), and Rao et al. (2021), but so far there are published articles on biofortifying broccoli with Se in poor fertile soils of low nutrient content which could not satisfy plant needs. In the present study, we need to answer many questions such as: is there any difference between applied sources of Se-fertilizers in the biofortification program of broccoli? The answer can be clarified through the presented results. Firstly, several previous studies confirmed that many vegetative attributes of crops including broccoli were improved with increasing applied dose of both forms (mineral and nanofertilizers) of Si such as Hossain et al. (2019), Zsiros et al. (2019), and Li et al. (2022d). Secondly, the dry weight of both leaves and heads as well as studied vegetative parameters of broccoli increased with increasing the dose of foliar application of Se. Additionally, Se has distinguishable and potential physiological functions in Se plant physiology, because its stimulates some enzymatic antioxidants (Wen et al. 2021). Thirdly, these studied parameters recorded highest values in case of mineral form of Si compared to nano Se-fertilizer. This result may back to the easily translocate of selenate from roots to shoots via xylem and assimilation of it in the leaf chloroplasts (El-Ramady et al. 2015). These results are in agreement with results of Zsiros et al. (2019), who reported that applied selenate dose of 10 ppm obtained higher fresh weight and chlorophyll content in tobacco compared to nano Se-fertilizer (100 ppm). Fourthly, the applied Se-dose for biofortification mainly depends on used crop, where in this study acceptable dose was 30 ppm (from both Se-forms), although this dose recorded to be only 20 ppm nano-Se for groundnut (Hussein et al. 2019).

Why broccoli crop was selected in this study to be biofortified with Se? because this crop belongs the family of Brassicaceae, which is very well-known to be rich in bioactive components, mainly glucosinolates, and antioxidants (Li et al. 2022a). One reason more for preferring broccoli in this study is due to the ability of broccoli to accumulate selenium compared to other crops, as a secondary accumulator of Se. Another important fact is that biofortifying Se as sodium selenate is more effective in promoting the accumulation of Se than using sodium selenite, and it leads to more rapid absorption and distribution (Bachiega et al. 2016).

Which one is better biological nano-Se fertilizer or its bulk mineral one? To what extent this Se-biofortification program of broccoli can be achieved under nutrient deficiency stress condition? Although, the mineral Se fertilizer recorded the highest yield of broccoli compared to nano Se-fertilizer, the nano-form is preferable due to its low toxicity, and a sustainable solution. This trend was confirmed by Huang et al. (2023), who reported that foliar biofortification of radish with biological nano-Se can promote the growth and yield. One reason more to prefer biological nano-Se fertilizer is the lower Se-content in head (which will be consumed by humans), compared to leaves of broccoli confirming that bio- nano -Se fertilizer can produce a safe head of broccoli for human nutrition.

Finally, are Se-fertilizers supporting growth and producing broccoli under sandy soils (nutrient deficiency stress)? Findings of the current study confirmed that both of Se-fertilizers enhanced the growth and broccoli productivity under such stress conditions due to its effective role of Se under stress conditions. These results are in the same trend of results obtained by Hussein et al. (2019), who reported about the supportive role of nano-Se under sandy soil conditions by enhancing the antioxidant defense systems in groundnut and improvement of plant tolerance. The promising role biological nano-Se in supporting crop productivity under different stresses such as salinity stress on rapeseed (Brassica napus L.) (El-Badri et al. (2022), toxicity stress of heavy metals on Brassica chinensis (Zhu et al. 2022), salt stress on wheat (Ghazi et al. 2022), and biotic stress on sugar beet (Abou-Salem et al. 2022).

## 5. Conclusions

Biofortification programs have been become effective approaches to overcome malnutrition in several countries all over the world. The present study is an example on producing broccoli biofortified with Se by investigating 2 different sources of Se-fertilizers (i.e., mineral and biological nano-fertilizer). The key results support using the mineral Se-form, which recorded the highest values in vegetative growth parameters and yield. The most important finding in the present study is containing the broccoli head lower content of Se compared to mineral form, which supports the safe consumption of such head of broccoli. One more important finding from this study is the ability of biological nano-Se fertilizer in increasing broccoli growth and its productivity under nutrient deficiency stress by increasing the tolerance cultivated plants to such stress and enhancing nutritional status of plants.

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