



## Biological Nano-Selenium for Eggplant Biofortification under Soil

### Nutrient Deficiency



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**S**OIL nutrient deficiency is a real challenge facing the crop production under sandy soils conditions. This challenge has an increase concern all over the world specially to produce biofortified crops for overcome many human health problems like malnutrition. The current study was carried out to evaluate production of biofortified eggplant enriched in selenium (Se) using biological nano-Se and its bulk form under soil nutrient deficiency stress. In general, the applied 30 ppm of bulk Se-fertilizer achieved many studied attributes, whereas the highest applied dose of biological nano-Se (40 ppm) may need higher doses without recoding toxicity on eggplants. Applied bionano Se recorded an increase up to 100% compared to the control in Se content in eggplant fruits, although this increase in both fruit and leaves were not toxic and the mean values of Se content in fruits were lower than leaves in both seasons and both were in the allowable Se content for human diet. To develop a full picture of eggplant biofortification with Se, additional studies will be needed to answer more open questions regarding producing a safe and healthy eggplant for human nutrition.

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

### 1. Introduction

Globally, about 1.86 million ha cultivated by eggplant or brinjal (*Solanum melongena* L.), with an annual production of 54.08 million ton (Bana et al. 2022a). The main producers of eggplant are China and India with nearly, 84% of the global production including 61 and 23% from China and India, respectively (Bana et al. 2022a). Eggplant, family Solanaceae, is considered an important fruiting vegetable, originated from India, and can be cultivated in open field and in greenhouses (Consentino et al. 2022). This vegetable has a distinguished attributes for human health, which is considered a highly nutritive and functional food due to its high content of flavonoids, phenolics, and other bioactives (Sharma et al. 2022). Furthermore, it has several medicinal and functional properties such as anti-allergic, anti-bacterial, anti-inflammatory, vasodilatory, anti-viral and anti-carcinogenic effects (Sharma et al. 2022). Therefore, many biofortification

programs have been applied on eggplant to be biofortified with many nutrients like iodine (Consentino et al. 2022), phosphorus (Mauro et al. 2022) and micronutrient-embedded NPK (Bana et al. 2022b). An increase concern on the nano-biofortification could be noticed recently as reported in our last publication (El-Ramady et al. 2021a, b; Fawzy et a. 2023a, b).

Climate change and water scarcity are great challenges facing global food security and crop productivity. It is expected that about 2 billion people are suffering from "severe water-scarce" by 2025. In addition, more than 2 million people suffer from malnutrition, which enforce the global scientists to think in deep to find a solution for this issue. Biofortification is a promising tool for improving nutrition of cultivated plants to alleviate human nutrient deficiency (Monika et al. 2023). More than 30 countries have a program for producing

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biofortified crops (around 150 varieties), which enriched by many nutrients (e.g., B, Ca, Cu, Co, I, Fe, Mg, Mn, Ni, S, Se, and Zn), vitamins (Jiang et al. 2021), folate (Viscardi et al. 2020), carotenoids (Morelli and Rodriguez-Concepcion 2023), and others (Dhaliwal et al. 2022). The main approaches of biofortification may include traditional breeding, transgenic, agronomic (Bhardwaj et al. 2022), microbial (Tripathi et al. 2022) and nano-biofortification (Kapoor et al. 2023). Biofortification mainly focuses on the field crops such as cereals (wheat, rice, maize), pulses (common bean, faba bean, lentil, and pea), fodder crops, oilseeds, and horticultural crops including vegetables (tomato, cucumber, potato, and eggplant) and fruits (apple, banana, peach, and raspberries).

Selenium (Se) and its nano-form (nano-Se) have an increase concern due to their distinguished attributes for plant and human health (El-Ramady et al. 2020, 2022a,b). The nano-form of selenium may be existed in red or grey color depending on the kind of synthesis; chemical or biological (El-Ramady et al. 2022a). The biosynthesis of Se is preferable as cost-effective and low toxicity compared to other methods (El-Ramady et al. 2022b). Recently, several studies published on the impact of applied bio-nano-Se on different cultivated plants such as tobacco (Zsiros et al. 2019), *Chrysanthemum morifolium* (Seliem et al. 2020), cucumber (Shalaby et al. 2021), wheat (El-Saadony et al. 2021), tomato (Saffan et al. 2022), *Brassica chinensis* (Zhu et al. 2022), rapeseed (El-Badri et al. (2022), banana (Shalaby et al. 2022a), strawberry (El-Bialy et al. 2023), and radish (Hoang et al. 2023).

As far as we know, this is the first report on the producing biofortified eggplant using bio-nano-Se under sandy soil cultivation. Therefore, this work investigated two sources of Se-fertilizers including mineral and nano-form on the productivity of eggplants under nutrient deficiency conditions. Four doses of S-fertilizers were tested in producing of eggplant to know which applied dose of this preferable Se-fertilizer can be used under such studied conditions.

## 2. Materials and Methods

### 2.1 Experimental site and treatments

A field experiment was conducted in a randomized block design in three replicates. This experiment

included nine treatments; the control (using only water), 4 different doses of mineral fertilizer of selenium (i.e., 10, 20, 30 and 40 mg L<sup>-1</sup>), and 4 different doses of biological nano-Se fertilizer (i.e., 10, 20, 30 and 40 mg L<sup>-1</sup>). The treatments were foliar sprayed after 45 and 55 days from planting (**Figure 1**). During the two-summer season of 2017 and 2018, a field experiment was conducted in the Nubaria Experimental Farm (National Research Centre, Egypt). The selected cultivar of eggplant in this study was "Black Classic", which was transplanted after 50 days from seed cultivation, and cultivated in the open field on 5 and 10 April in the first and second season, respectively. Seedlings of eggplant were planted on ridges of 0.8 m width and 30 m length and the plot area was 24 m<sup>2</sup>.

Conventional agricultural practices were followed according to the common ones in the area, where the recommended of NPK were 120, 90, and 80 units, respectively. The 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 (85.5, 11.7, and 2.8% sand, silt and clay, respectively), which is classified as an *Entisol-TypicTorripsamments*. The content of soil organic matter in the topsoil (0-30 cm) was 0.4%, with a high soil alkalinity (pH 8.25), low soil salinity (EC: 0.85 dS m<sup>-1</sup>), and low CaCO<sub>3</sub> (15 g kg<sup>-1</sup>). The soil moisture content at field capacity and the permanent wilting point recorded in average 18 and 8%, respectively. The available soil contents of N, P and K were 12, 4 and 35 mg kg<sup>-1</sup> soil, respectively before the initiation of the experiment, which represent low nutrient contents in studied soil

### 2.2 Selenium fertilizers and their sources

In this study two different forms of Se were investigated during the biofortification process of eggplant including mineral Se-fertilizer as sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>), which dedicated by Nano Food Lab, University of Debrecen, Hungary and nano-form of Se (Se-NPs). The nano-form was prepared biologically at the Lab of Soils, Water and Environment Research Institute (SWERI), Soil Microbiology Department at Sakha station. The size of Se-NPs was 87.7 nm, which was oxidized by using *Bacillus cereus* TAH as reported by Ghazi et al. (2022). The size of Se-NPs was 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.

### 2.3 Plant sampling and analyses

At harvest 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 and branches per plant; fresh and dry weight of leaves (g plant<sup>-1</sup>), Average fruit weight, and the total yield per feddan at harvest stage the mature fruits of eggplant were harvested twice every week along the harvesting season. At harvest, the average fruit weight and fruit yield (kg m<sup>-2</sup>) of each plot were recorded and the total yield (ton fed<sup>-1</sup>) was calculated. The plant samples were divided into leaves, then dried at 65 °C; ground using stainless steel equipment to determine their nutrient contents (i.e., N, P, K and Se) in leaves and fruits of eggplant. From each sample 0.2 g was digested using the mixture of sulfuric and perchloric acids (1:1) for determination of nutrients N, P, K, and Se. Nitrogen was determined using Kjeldahl method, K was determined by flame photometer (NADE LCD Digital Flame photometer FP640, China) and P using the visible spectrophotometer (Single Beam, SP-IV722N, 721N, China) as described by Cottenie et al. (1982). According to Levesque and Vendette (1971), Se content was measured using atomic absorption spectroscopy (SP-IAA1800H, China).

### 2.4 Statistical analyses

Least-significant-differences test (LSD) was used to statistically analyze obtained data of the experiment at the confidence level of 5%. These analyses were 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 Vegetative parameters of eggplant

All selected vegetative parameters and obtained yield of eggplant were tabulated in **Tables (1) and (2)** as a response of these traits to different applied Se-fertilizer doses. In general, the recorded mean values of all parameters were higher for bulk Se-source compared to nano-S form. For each trait, there is a

significant increase in its value with increasing the applied doses of both bulk-Se and nano-form till the level of 30 ppm in general. This increase for the yield was 28.2 and 26.6% for the bulk and nano-Se form, respectively in the second season. A similar increasing rate was recorded for the dry weight of leaves in the second season (28.2 and 21.7 %, respectively) after applying both bulk and nano-Se form. The same trend was observed for the plant length, and fruit weight.

### 3.2 Content of nutrients in leaves and fruits of eggplant

The total content of some selected nutrients including N, P, and K in eggplant leaves in both growing seasons was measured (**Table 3**), whereas this content in eggplant fruits was tabulated in **Table (4)**. Selenium content in the leaves and fruits of eggplants were presented in **Figures (2) and (3)** in both the growing seasons. Both bulk and nano-form of Se were significantly increased by increasing applied Se doses except P in both seasons. The applied dose of 40 ppm of both Se forms recorded the highest mean values of studied nutrients (Tables 3 and 4; Figures 1 and 2). In general, the content values of all studied nutrients were higher in leaves compared to fruits in previous Tables (3 and 4) and Figures (1 and 2) under different applied doses of both Se-forms.

The content of Se in both leaves and fruits were higher after applying bulk Se compared to the nano-form. Although, this increase was 88 and 70% in leaves for both seasons for bulk Se-form, whereas ranged from 94 and 100% for nano-Se form in both two seasons. The mean values of Se content in fruits were lower than leaves in both seasons and both were in the allowable Se content for human diet.

### 3.3 Response of eggplant yield to Se-fertilizers

Applied Se-fertilizers under different doses have a significant impact on eggplant yield (**Fig. 4**). It is observed that a significant increase in the yield of eggplants with increasing applied doses of both bulk and anno-forms of Se in both seasons till the level of 30 ppm and then decreased. The bulk Se-form recorded higher values in eggplant yield (8.5 ton fed<sup>-1</sup>), compared with nano Se-form in both seasons.

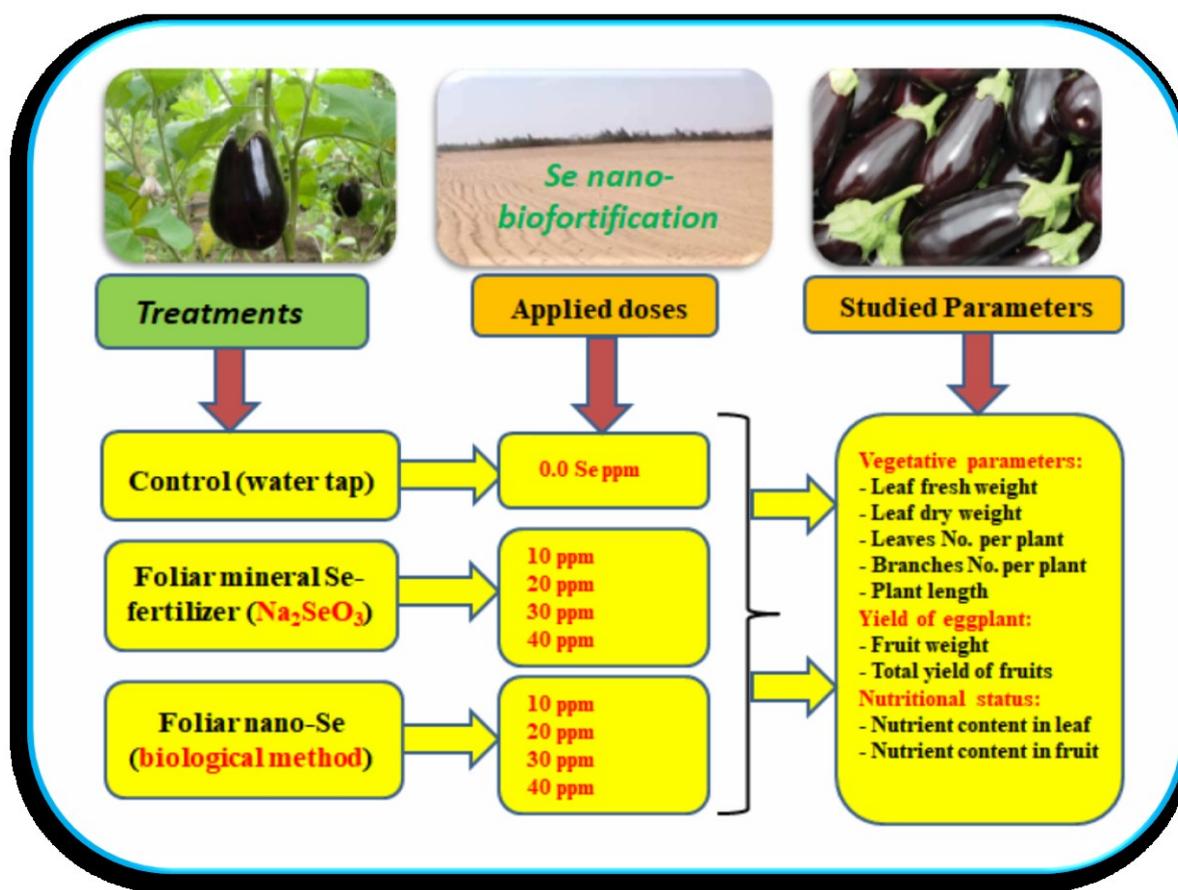


Fig. 1. An overview on the main applied treatments in the study including different applied doses and the studied parameters.

Table 1. Response of vegetative growth parameters of eggplant to applied Se-fertilizers.

Treatments (Applied doses)	Fresh weight of leaves (g)		Dry weight of leaves (g)		Number of leaves per plant		Number of branches per plant	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season
<b>Mineral Se-fertilizer</b>								
Control	66.73c	68.15d	8.58c	9.18c	42.17d	42.56c	9.33c	9.33d
10 ppm	69.11bc	71.79c	9.29b	10.26bc	45.39c	45.61b	9.56bc	9.67c
20 ppm	71.15b	74.91b	9.97b	11.30b	46.56c	48.28b	10.28b	10.78b
30 ppm	77.42a	78.50a	10.95a	12.80a	55.22a	57.72a	12.06a	12.50a
40 ppm	75.84a	77.09a	10.46ab	12.05ab	52.50b	55.33a	11.39a	12.44a
<b>Nano Se-fertilizer</b>								
Control	66.36b	67.58b	8.56b	9.15b	42.11b	42.50c	8.67b	8.83b
10 ppm	67.17b	68.82b	8.79b	9.50b	43.28b	44.28bc	9.22b	9.17b
20 ppm	68.65b	71.08a	9.18ab	10.10b	44.56b	46.44b	9.50b	9.56b
30 ppm	72.37a	73.78a	10.22a	11.69a	48.28a	48.94a	11.78a	12.06a
40 ppm	70.73ab	73.27a	9.85a	11.12ab	48.17a	48.83a	11.44a	11.89a

**Table 2. Response of plant length and yield of eggplant to applied doses of Se-fertilizers.**

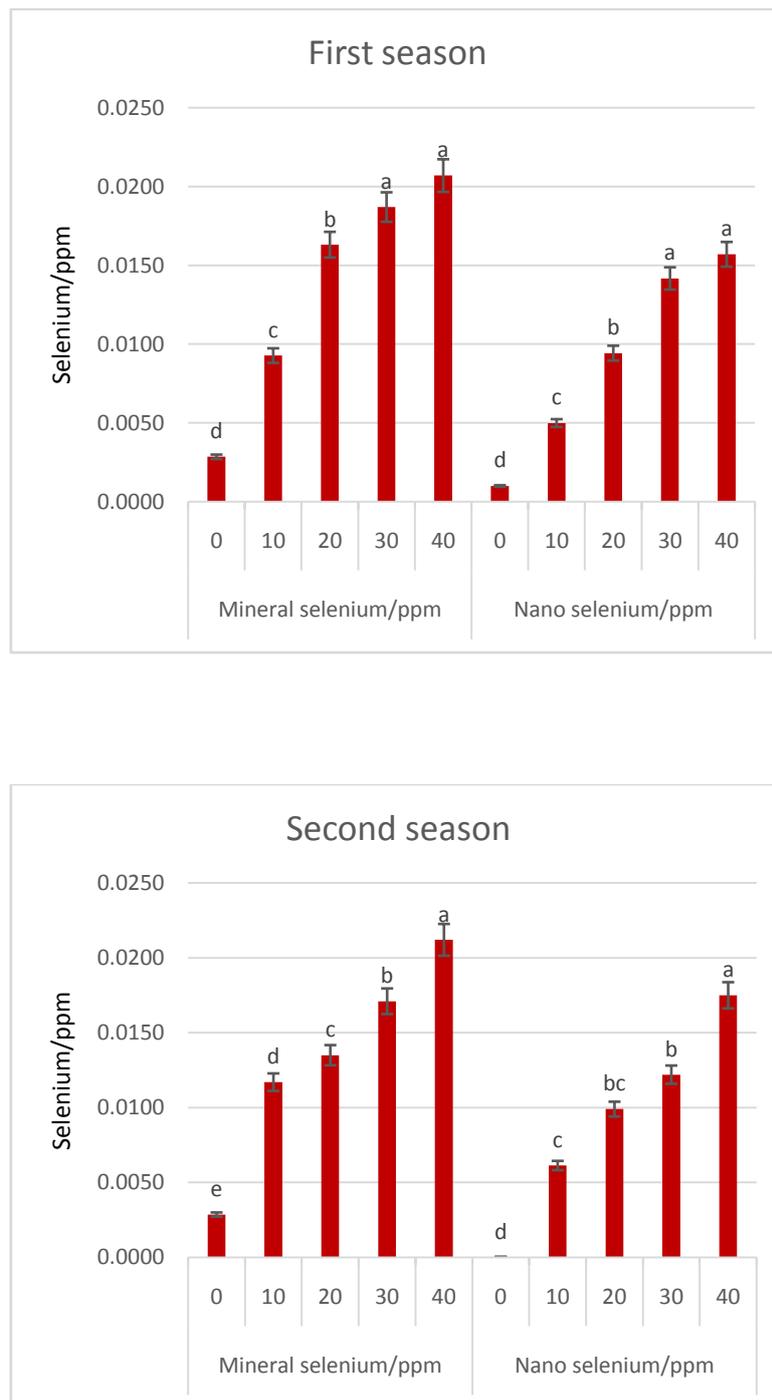
Treatments (Applied doses)	Plant length (cm)		Average fruit weight (g)		Total yield (ton/fed.)	
	First season	Second season	First season	Second season	First season	Second season
<b>Mineral Se-fertilizer</b>						
Control	46.88b	47.78c	53.14d	53.35d	6.55c	6.07c
10 ppm	48.45b	50.18b	55.18c	54.48c	7.18b	7.04b
20 ppm	49.17b	51.28b	56.92b	57.14b	7.47b	7.48b
30 ppm	53.58a	56.03a	58.64a	59.77a	8.11a	8.46a
40 ppm	51.70ab	55.15a	58.66a	59.80a	7.92ab	8.17a
<b>Nano Se-fertilizer</b>						
Control	45.40c	45.51c	52.11c	49.78c	6.48b	5.96b
10 ppm	46.98b	47.93bc	53.67b	52.17b	6.93b	6.65b
20 ppm	48.58b	50.38b	55.32a	54.69a	7.48ab	7.49a
30 ppm	51.49a	54.83a	55.83a	55.47a	7.89a	8.12a
40 ppm	51.66a	54.75a	55.72a	55.30a	7.58a	7.65a

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

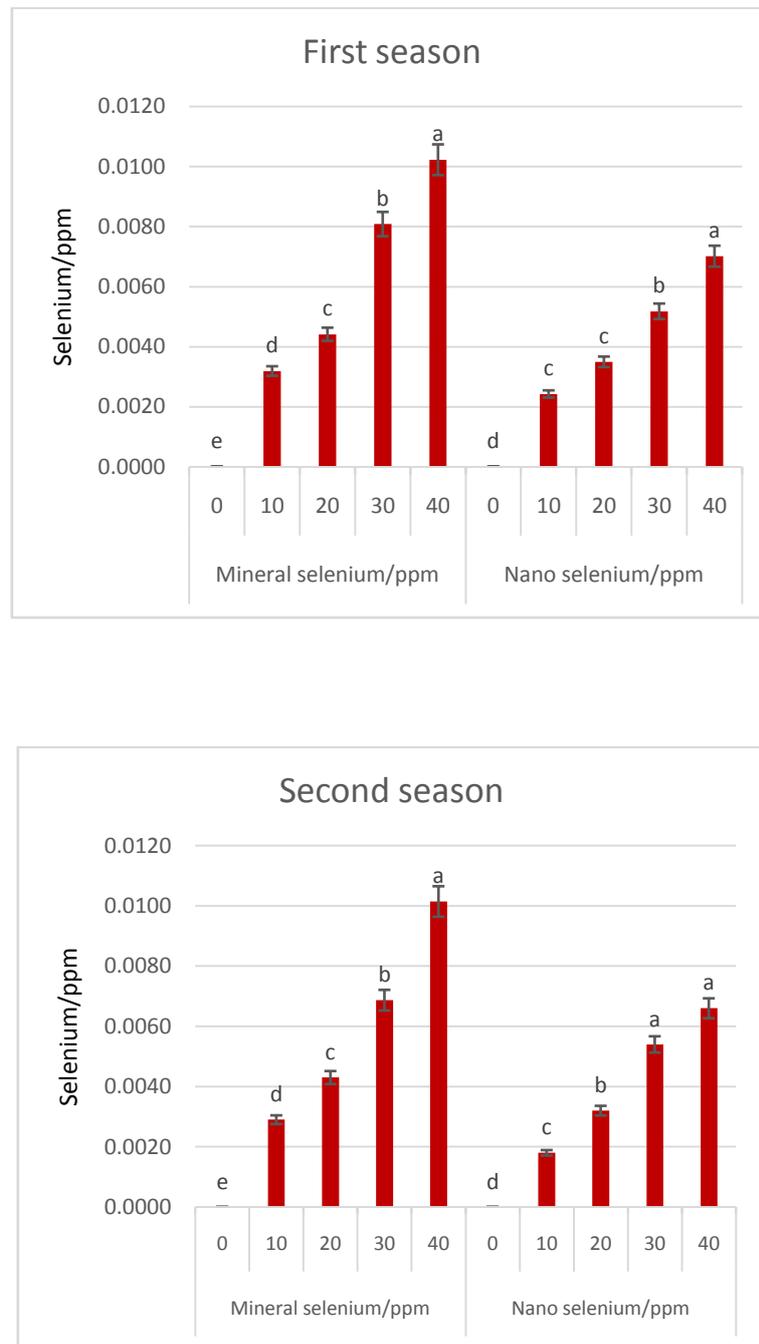
Treatments (Applied doses)	N content in leaf (%)		P content in leaf (%)		K content in leaf (%)	
	First season	Second season	First season	Second season	First season	Second season
<b>Mineral Se-fertilizer</b>						
Control	1.38d	1.28d	0.25	0.23	1.54d	1.51e
10 ppm	1.61c	1.63c	0.28	0.28	1.62d	1.63d
20 ppm	1.73bc	1.82b	0.33	0.35	1.76c	1.84c
30 ppm	1.78b	1.89ab	0.35	0.39	1.92b	1.99b
40 ppm	1.95a	1.97a	0.37	0.41	2.11a	2.15a
<b>Nano Se-fertilizer</b>						
Control	1.37c	1.27b	0.23	0.20	1.44c	1.35b
10 ppm	1.46b	1.40b	0.25	0.24	1.50c	1.45b
20 ppm	1.68a	1.71a	0.26	0.25	1.70b	1.75a
30 ppm	1.72a	1.80a	0.32	0.34	1.75ab	1.83a
40 ppm	1.77a	1.88a	0.34	0.35	1.83a	1.86a

**Table 4: Content of nutrients in eggplant fruits as a response to applied Se-fertilizers**

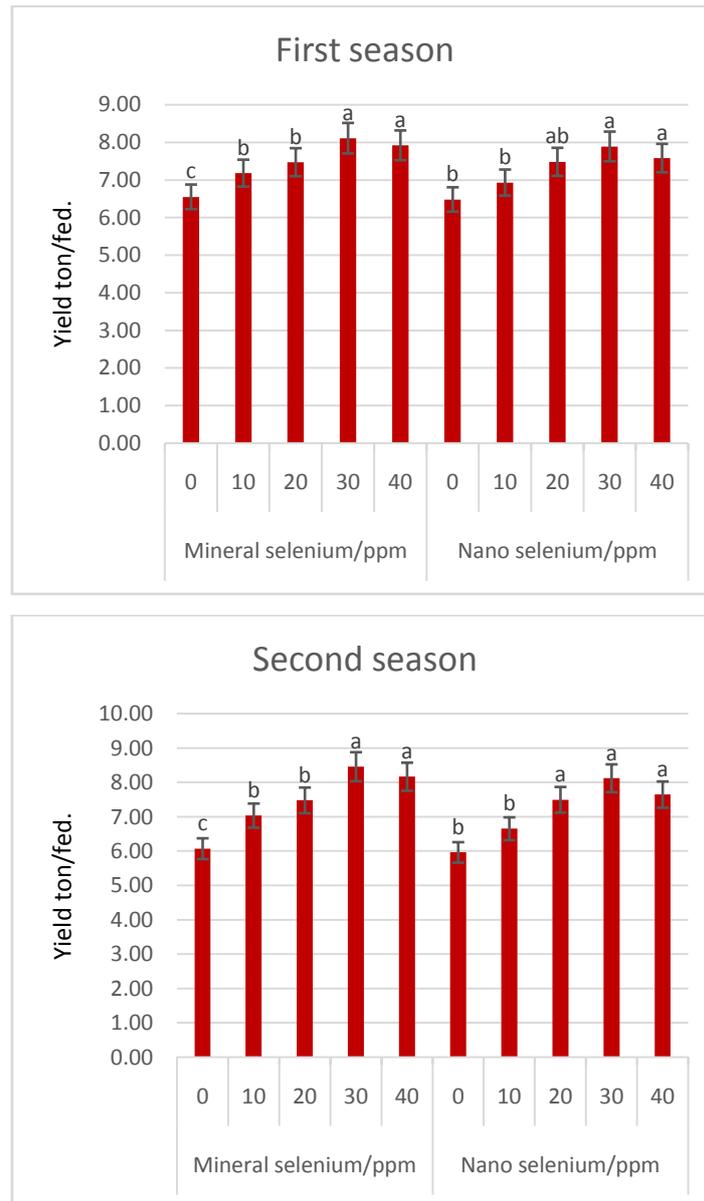
Treatments (Applied doses)	N content in fruit (%)		P content in fruit (%)		K content in fruit (%)	
	First season	Second season	First season	Second season	First season	Second season
<b>Mineral Se-fertilizer</b>						
Control	1.35d	1.32d	0.43	0.41	0.96d	0.94d
10 ppm	1.42cd	1.42c	0.52	0.51	1.06cd	1.09c
20 ppm	1.46c	1.48c	0.62	0.59	1.16c	1.14c
30 ppm	1.54b	1.61b	0.66	0.63	1.32b	1.39b
40 ppm	1.67a	1.73a	0.71	0.74	1.72a	1.70a
<b>Nano Se-fertilizer</b>						
Control	1.22c	1.12c	0.41	0.38	0.82d	0.72d
10 ppm	1.27bc	1.19bc	0.47	0.47	0.94c	0.91c
20 ppm	1.31b	1.25b	0.55	0.59	1.00c	1.03c
30 ppm	1.39a	1.38a	0.61	0.63	1.21b	1.20b
40 ppm	1.40a	1.39a	0.65	0.68	1.35a	1.39a



**Fig. 2. Effect of selenium source and concentration on selenium (ppm) in leaves of eggplant plants during studied seasons.**



**Fig. 3. Effect of Se sources and doses on Se (ppm) in eggplant fruits of during the studied seasons.**



**Fig. 4. Effect of selenium sources and doses on yield (ton/fed.) of eggplant plants during the studied seasons.**

#### 4. Discussion

Biofortification is a promising approach for enriching the harvested plants with needed nutrients to face the malnutrition. Malnutrition is a crucial problem that billions of people suffer from all over the world. Several crops have been used in the biofortification program, whereas a few studies involved eggplants such as Bana et al. (2022a, b), and Consentino et al. (2022). As far as we know, there is no published articles on the biofortification of eggplants with nano Se-form. Therefore, this study is considered a first report on this case study

under soil nutrient deficiency stress. The current study is an attempt to answer the main question: which source of applied Se-fertilizer and which applied dose was the most effective in biofortifying the eggplant fruits? It is worthing to mention that toxic level of Se for plants may up to 10 mg Se kg<sup>-1</sup>, whereas this level for nano-Se can be reached up to 200 ppm depending on the plant species (El-Ramady et al. 2020). Concerning the recommended daily intake of Se is about 55 µg day<sup>-1</sup>, toxic to human up to 400 µg day<sup>-1</sup>, whereas these levels of

nano-Se did not yet establish (El-Ramady et al. 2020).

To answer this question, the impacts of applied Se-fertilizers on growth and productivity of eggplant will be the main area for answer. We can add the impacts of Se fertilizers on the vegetative parameters, yield and nutrient contents in both leaves and fruits of eggplants, from one side and kind of Se-fertilizers and applied doses, from the other hand. In the present study, all studied vegetative attributes increased significantly with increasing applied both Se-fertilizers with priority to mineral fertilizer. The role of Se-fertilizers (including nano-form) in enhancing growth of cultivated plants under stress was confirmed by many studies (Shalaby et al. 2022b), such as phytopathogen on wheat (El-Saadony et al. 2021), soil salinity and heat stress on cucumber (Shalaby et al. 2021), salinity on rapeseed (El-Badri et al. (2022), salinity on wheat (Ghazi et al. 2022), heavy metals stress on pak choi (Zhu et al. 2022), water salinity on tomato (Saffan et al. 2022), organic pollutant on tomato (Liu et al. 2022), cadmium stress on rice (Barman and Kundu 2023), and nano-stress on mung bean (Kamali-Andani et al. 2023). From previous studies, it was reported that applied nano-Se can mitigate growth of stressful plants by different pathways like protects plant organs from oxidative stress like chlorophyll, increases root and leaf activity, alleviates the toxicity of heavy metals, and regulate metabolism of C, N and oxidative stress (Wang et al. 2023). In our study, Se and its nanoform enhanced the growth and production of eggplants under soil nutrient deficiency stress due to their role in supporting plants under this stress. These results are in agreements with results of many other crops as mentioned for tomato, cucumber, wheat, rice, and mung bean.

These are surprising finding in the current study concerning the bulk Se-form, which achieved

higher values in almost studied parameters compared to nano-Se form. The reason may back to low dose of applied nano-Se (up to 40 ppm), whereas applying up to 100 ppm of biological nano-Se was recorded distinguished results for our previous studies on the cultivated banana, tomato, wheat, and strawberry, and may be up to 200 ppm for *Chrysanthemum morifolium*. Further studies are required to better understand the extent to which applied doses of Se-fertilizers can promote the eggplant production under nutrient deficiency stress. These data also must be interpreted with caution because the trend of the study may depend on the kind of stress, plant species, and type of experiment (field, pots or *in vitro*, or hydroponics).

Why applied Se-fertilizers can promote growth and production of eggplants? Under sandy soil conditions, the soil suffers from the nutrient deficiency, which might impact on growing and developing cultivated plants. Applied selenium in both forms can support eggplant under this stress by promoting uptake of macro- or micro-nutrients from the soil, improving the nutritional status of eggplant leaves, and finally could increase the yield and productivity (Cheng et al. 2023). The other benefits of applied nano-Se may include up-regulating 42 metabolites (mainly carbohydrates, amino acids), and metabolic pathways related to C and N metabolism, which may improve various physiological attributes under stress (Wang et al. 2023). The foliar applied doses of Se-fertilizers in our study did not record any toxicity symptoms on cultivated eggplants, which may support their opportunity for eggplant biofortification to overcome hidden-hunger and the malnutrition, as reported by Bana et al. (2022a).

There was no significant difference between applied dose of 30 and 40 ppm Se on the yield and vegetative growth of eggplants with propriety to apply 30 ppm Se-fertilizer. The optimum applied

dose of Se-fertilizer for biofortification mainly depends on the cultivated plants, Se-form and method of application. In case of foliar application, the applied bionano-Se may reach up to 20 ppm on pak choi (Zhu et al. 2022), 25 on cucumber (Shalaby et al. 2021), or up to 100 ppm on tomato (Saffan et al. 2022), strawberry (El-Baily et al. 2023), and banana (Shalaby et al. 2022a). The method of Se synthesis is very important that can control the impact and toxicity of applied dose of nano-Se on cultivated plants. It is preferable the biological synthesis of nano-Se compared to the chemical and physical methods due to its low toxicity, its high bioavailability to plants, and eco-friendly tool (Kang et al. 2022). The soil application of nano-Se (produced by laser ablation process) promoted growth of eggplant up to 25  $\mu\text{g kg}^{-1}$  (Gudkov et al. 2020), whereas foliar application of bio-nano-Se on eggplant in our study was up to 40 ppm or improved nutritional quality of radish (Huang et al. 2023). Hence, it could conceivably be hypothesized that this topic needs more investigations.

## 5. Conclusions

Biofortification has become an urgent approach in the last decade for enriching the producing plants for overcome the malnutrition. The recent study was designated to highlight producing biofortified eggplants, as a first report, using biological nano-selenium and its bulk form as well. The present results are significant in at least two major respects; confirming on the biological for of anno-Se, and not so high applied dose is needed. However, these results were very encouraging to continue investigation using higher doses of bionano-Se and under different kind of stresses. Finally, the combination of findings provides some support for the conceptual premise that biological nano-Se is a promising fertilizer for producing biofortified eggplants.

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