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Biofortification of Vegetables under Stress Conditions Using Biological Nano-Selenium: A Mini-Review

Hassan El-Ramady^{1,4},Shaymaa I. Shedeed², Zakaria F. Fawzy³, Abd El- Mohsin M. El-Bassiony³, Sameh M. El-Sawy³, Sami H. Mahmoud¹, andJózsef Prokisch⁴



¹ Soil and Water Dept., Faculty of Agriculture, Kafrelsheikh University, 33516 Kafr El-Sheikh, Egypt. ²Plant Nutrition Department, Agricultural and Biological Research Institute, National Research Centre, Egypt ³Vegetable Research Dept., Agriculture and Biological Research Institute, National Research Centre, 33 El Behouth St., Dokki, 12622 Giza, Egypt

⁴Institute of Animal Science, Biotechnology and Nature Conservation, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, 138 Böszörményi Street, 4032 Debrecen, Hungary

EGETABLE crops are importantsources of vitamins, and minerals for human, and provides him with several bioactive compounds. Producing safe and healthy vegetables for human diet is of a great global issue. Thisneeds to exploit all available resources, particularly soils understressful conditions such as saline, saline-sodic, waterlogged and low fertile soils), climatic stress (drought, flooding, saltwater intrusion, and heat stress), and along with normal conditions. Nano-biofortification can support the vegetable productivity especially under such conditions by using the biological nanonutrients. Bio-nanonutrients exhibitsmany distinguishableproperties than mineral forms such as higher biological activity, lower toxicity, and better bioavailability. Bio-nanonutrients also promote the vegetable growth, productivity and enhance planttolerance towards different stresses by reinforcing the function of antioxidant enzymes. Thus, production of biofortified vegetables under stressful conditions might be an optimum and sustainable solution particularly by using the biological nanonutrients like selenium. The controlling factors that are needed for a successful nanobiofortification program of vegetables are correlated with growing media, plant species, and method application of nanonutrients. The over dose of nanonutrients maycause a nanotoxicity for cultivated plants, and then human health after consumption. This problem can be managed by following the 4R Nutrient Stewardship concept, which focuses on the right rate, right source, right time, and right place. This program will be discussed in more details in this review article.

Keywords: Soil fertility, Bio-nanofertilizer, Nano-biofortification, Malnutrition, Nanotoxicity.

1. Introduction

Deficiency of nutrients and vitamins (i.e., hidden hunger or malnutrition), are major problems facing several countries all over the world including the Caribbean. sub-Saharan Africa, Mediterranean countries, and Southeast/Western Asia (Morelli et al. 2023). Several approaches have been adapted for and food supplementation biofortification to incorporate required nutrients or vitamins during food processing or crop cultivation (Monika et al. 2023). The main approaches of biofortification include fertilization or agronomic strategies, conventional crop breeding, and genetic engineering or biotechnology methods (Kaur et al. 2020; Lal et al. 2020; Mir et al. 2020). Several materials on biofortification were published which focus on the targeted nutrients such as calcium (Pessoa et al. 2021), copper (Saffan et al. 2022), iodine (Izydorczyk et al. 2021), iron (Buturi et al. 2023), lithium (Naeem e al. 2021), magnesium (Mg), selenium (Cheng et al. 2023), and zinc (Buturi et al. 2023). Some vitamins and bioactive components were also reported in biofortified plants such as vitamins (De Lepeleire et al. 2018; Fitzpatrick and Chapman 2020; Jiang et al. 2021), and carotenoids (Yan et al. 2020; Morelli et al. 2023).

Production of vegetables is a crucial global issue for human health, which can supply the human with many essential minerals, vitamins and bioactives. Vegetables biofortification is a common practice nowadays, which started in many countries several years ago and was applied successfully on production of some vegetables like sweet potato to enrich or supply vitamin A (China and Uganda in 2004), vitamin A on cauliflower (India in 2016), iodine on lettuce (Poland in 2011), iodine on tomato (Italy in 2011), selenium on onion (Norway in 2012), anthocyanin on eggplant (India in 2016), as reported by Kiran (2020). There are other crops already applied in the biofortification program in different countries all over the world like cereals including rice (Barman and Kundu 2023), maize (Msungu et al. 2022), wheat (Chen et al. 2022), and sorghum (Cipriano et al. 2022); oil seeds like soybean (Dai et al. 2020), rapeseed (Praus et al. 2019), and safflower (Rani et al. 2018) and fruit crops like apple (Groth et al. 2021), apricot (Yan et al. 2020), banana (Sperança et al. 2021), and peach (Sun et al. 2020).

Nano selenium (nano-Se) is well known with distinguished characteristics as antistressor, biostimulant, nano-insecticide, and nanofertilizer (El-Ramady et al. 2020, 2022a, b; Kang et al. 2022; Medrano-Macías et al. 2022). This product can promote cultivated plants under different stresses such as heat stress (Seliem et al. 2020), drought and heat stress (El-Saadony et al. 2021), drought and heat stress (Shalaby et al. 2021), salinity (El-Badri et al. 2022), organic pollutants (Liu et al. 2022), heavy metals stress (Zhu et al. 2022), and nano-toxicity (Kamali-Andani et al. 2023). Many benefits were reported for nano-Se on several agro-cultivation practices such as improving the rate of germination under salinity stress (Ghazi et al. 2022), reducing the hazards of irrigation with low water quality (Saffan et al. 2022), acclimatization of banana Seedlings (Shalaby et al. 2022a), and enhance rooting of strawberry seedlings (El-Bialy et al. 2023). Nano-Se can also improve lettuce growth, nutrient availability, and photosynthesis (Cheng et al. 2023), protect melon plants from insect infestations and pathogens by enhancing their resistance to biotic stress (Kang et al. 2022), enhance cowpea photosynthesis process and/or photosynthetic pigments, and antioxidant capacity (Lanza et al. 2021), and improve tomato by promoting plant enzyme activities especially phenylalanine ammonia-lyase, β -1,3-glucanase, superoxide dismutase (Joshi et al. 2021).

Therefore, this review was designed to highlight the impacts of bio fortification for alleviating stress conditions of vegetables using biological nanoselenium. Biofortification and malnutrition, vegetables and human health, and the biofortification of vegetables under stress are the main issues in this study.

2. Biofortification and malnutrition

The global agriculture has a great challenge that needs to follow the mission of "Farm to Fork to Gut". The humanity already encountered a series of huge challenges starting from the global COVID-19 pandemic (coronavirus disease) in 2020, droughts, and floods in Europe, North America, and Asia extreme snow disasters, and unprecedented locust attacks in Africa (Jiang et al. 2021). These series ofchallenges have had devastating impacts on food security, human health, and the entire environment (Jiang et al. 2021). Due to the previous global crises, human health suffers from many troubles especially the malnutrition and / or hidden hunger because of micronutrient deficiencies. Malnutrition is considered a global challenge facing the world in order to develop a think-tank to alleviate and provide the right access to food globally and also secure them nutritionally (Shukla et al. 2022). Several nutrients have been confirmed their deficient in the human diet in many developing countries (mainly African and Asian Continent) such as calcium (Ca), iron (Fe), iodine (I), magnesium (Mg), selenium (Se), and zinc (Zn). Although, the proper solution for the malnutrition is the biofortification, there are many options to improve dietary foods with essential micronutrients including food supplementation, fortification, and dietary diversification (Shukla et al. 2022). Biofortification is "a revolutionary technique for improving plant nutrition and alleviating human micronutrient deficiency" (Monika et al. 2023). The main approaches of biofortification, the targeted crops, and nutrients that can be applied to the program of biofortification are presented in Figure 1. Among, biofortification with essential micronutrients in the targeted crop could be achieved through agronomic practices, conventional breeding, genetic engineering, and microorganism approaches. These approaches can be employed in the pulse crops (e.g., chickpea, pigeon pea, and lentils), which showed great potential to overcome micronutrient deficiencies prevalent among the vulnerable group (Shukla et al. 2022).

Many published articles reported about the strong relationship between biofortification and malnutrition with focus on different topics such as:

1- The suggested interactions among malnutrition and possible strategies for vitamin biofortification (Jiang et al. 2021),

2- Studying recent strategies for pulse biofortification to combat malnutrition (Shukla et al. 2022),

3- Carotenoids biofortification in plant with focus on their post-harvest stability and assessing bioaccessibility of the biofortified products (Morelli et al. 2023),

4- The strongrelation between both biofortification and phytoremediation with focus on sulphur amendments for long-term field studies using different S-doses to maximize the food safety and ecosystem health (Cao et al. 2023),

5- Studying biofortification as a long-term solution to improve global health (Monika et al. 2023),

6- Benefits of biofortification strategies in the field using chelated forms of both Fe and Zn minerals as preferable toots for biofortification programs of carrots (Buturi et al. 2023),

7- Field-scale studies quantify limitations for wheat grain zinc biofortification in dryland areas with reducing P-fertilizer use (Li et al. 2023), and

8- Exploring the complementarity of dietary diversification and fortification to combat micronutrient deficiencies to establish evidence of effectiveness of combined strategies to foster policy adoption (Bechoff et al. 2023).

3. Vegetables and human health

Vegetables are important edible plants of high nutritional value for human health. More than 1000 vegetable crops are recognized, whereas globally at least 402 vegetables are cultivated and commercialized (Dias 2019). The main classification of vegetables is based on either being leafy and stalk vegetable (53% of the total), fruit and flower vegetables (15%) and belowground or root, bulb, and tuber vegetables (17%), as reported by Dias (2019). Concerning the group of leafy and stalk vegetables, it includes lettuce, head cabbages, chicory, coriander, kales, collards, Chinese cabbage, Swiss chard, Brussels sprouts, pak-choi, mustards, rocket, watercress, spinach, purslane, spinach, celery, asparagus, chives, fennel, and parsley (Figure 1). Regarding the group of fruit and flower vegetables, it includes cauliflower, cucumber, tomato, eggplant, pepper, watermelon, melon, pumpkin, squash, zucchini, bitter gourd, green pea, beans, lentil, okra, sweet maize, broccoli, artichoke, etc. The third group (i.e., root, bulb, and tuber vegetables) includes carrot, garden beet, turnip, radish, rutabaga, parsnip potato, sweet potato, cassava, onion, celeriac, garlic, leek, shallot, (Dias 2019).

There are several human health benefits of vegetables including the antioxidants, natural colorants (e.g., carotenoids), vitamins, and minerals,

as confirmed by several researchers (e.g., Ramya and Patel 2019; Dias 2019; Kumar et al. 2020). As essential source for human nutrition, vegetables should include the adequate and proper nutrients and vitamins for human diets, and production of biofortified vegetables sometimes is needed (Figure 2). Vegetables may have several phytochemicals, which are important for human health such as vitamin A, vitamin C (ascorbic acid), vitamin E (tocopherols), vitamin K, vitamin B9 (folate or folic acid), α -carotene, β -carotene, lycopene, flavonoids xanthophylls, dietary fiber, (anthocyanidin), and phenolic acids (Table 1).

4. Nano-biofortification of vegetables under stress

Nano-biofortification is a new biotechnological approach that has been used in enriching different crops with many essential nutrients in a nano-form to supply human diet with balanced diet (El-Ramady et al. 2021a). Nano biofortification can be achieved by applying essential nutrients (mainly Cu, Fe, Se and Zn) as nano-fertilizers using foliar or soil or hydroponics application (El-Ramady et al. 2021a, b). Vegetable crops are well-known with their short duration period of growth, which sometimes needs for 2-3 months. This very short growing period makes the biofortification process is more critical than other crops, which their growth may extend to one year or more. Therefore, the biofortification programs of vegetables are very critical and special case due to the short growth periods, the expected toxicity after applying higher doses of nutrient candidates, and growing under stress conditions (El-Ramady et al. 2021a). the following sub-sections will include some stress conditions like nutrient deficiency stress, and other stresses.

4.1 Under soil nutrient deficiency conditions

During the last years, some selected vegetable crops (i.e., broccoli, onion, pepper and eggplant) were grown successfullyunder the alkaline sandy soil conditions, which suffer from deficiency inavailable soil nutrient. At the same time, biological nano Se and its bulk form under different doses were foliar applied on these selected previous vegetable crops for biofortification program (Tables 2-4). The positive outcomes of these nano-applications ondifferent vegetables were identifiedi.e., broccoli (Fawzy et al. 2023a), onion (Fawzy et al. 2023b), eggplant (Mahmoud et al. 2023), and pepper (unpublished data). The production of these vegetables significantly increased by increasing the applied doses of both bulk and nano-Se, with priority to bulk

Se (sodium selenate). The reason may back to the lower applied doses of nano-Se, whereas the higher doses (up to 100 ppm or more) were the best as reported by other crops such as banana (Shalaby et al. 2022a), tomato (Saffan et al. 2022), strawberry (El-Baily et al. 2023).



Fig. 1. A brief of the most common vegetables including the commercial and cultivated number, and the main classification of vegetable crops with some photos.



Fig. 2. The main targeted nutrients for biofortification, which could be applied in the biofortified crops (sources: Kaur et al. 2020; Lal et al. 2020; Mir et al. 2020).

| Phytochemicals | Function in human body | Sources of vegetables | Impacted human diseases |
|---------------------------------------|--|--|--|
| Vitamin A | Promotes growth, essential for vision, skin and human immune system | Pumpkin, carrot, sweet potato, pepper, and spinach | Heart diseases, cancer, and stroke |
| Vitamin C | Improveswound and bone healing, | Broccoli, cabbage, pepper, | Cardiovascular disease, healthy |
| (ascorbic acid) | strengthens blood vessels, and increases the absorption of iron | pineapples, potato, strawberry, tomato, watermelon | immune system, scurvy prevention, and wound healing |
| Vitamin E (tocopherols) | Acts as antioxidant to protect cells from the damage caused by free | Lentil, chickpea, green leafy vegetables | Heartdisease, cancer, diabetes, and healthy immune system |
| Vitamin K | Helpsto make 4 of 13 proteins needed for blood clotting to stop wounds from continuously bleeding | Broccoli, Brussels sprouts, cabbage, green onions, lentil, and leafy greens | Osteoporosis, blood coagulation problem |
| VitaminB9 (folateor folic acid) | Importantin red blood cell formation and for healthy cell growth and function | Spinach, mustard greens, lettuce, okra, broccoli, Brussels sprouts | Birth defects, cancer, heart disease |
| α-Carotene | Acts as antioxidants, and enhances immune function | Broccoli, cabbage, carrot, green bean, kale, lettuce, spinach, squash, sweet potato | Cardiovasculardiseases and cancers, ischemic, stroke, tumor growth |
| β-Carotene | Acts as an antioxidantto protect the body from damaging free radicals; Beta carotene can convert into vitamin A | Broccoli, spinach, turnip greens; orange vegetables such as carrots, pumpkins, sweet potato | Cancer, cataracts, coronary artery disease, heart disease, night blindness prevention, provitamin A activity, psoriasis |
| Lycopene | Antioxidant can improve heart health and a lower risk of certain types of cancer | Tomato, watermelon, carrot, strawberry | Atherosclerosis, breast andprostate cancer, heart disease,male infertility |
| Xanthophylls | Protects human against phototoxic damage | Okra, spinach, summer squash, turnip greens, sweet corn | Atherosclerosis, cancer, macular degeneration |
| Dietary fiber | Important to maintain digestive health, as well as reduce blood cholesterol | Pea, Brusselssprouts, parsnips, broccoli | Diabetes, heart disease, colorectal cancer |
| Flavonoids (anthocyanidin) | Anticancer, antioxidant, anti- inflammatory, antiviral properties, neuroprotective and cardio- protective effects | Parsley, strawberry, red onion, celery, hot pepper, lettuce, garlic | Heart disease, cancer initiation, diabetes, cataracts, blood pressure, allergies |
| Phenolic acids | Antioxidants can avert the damage of cells resulted from free-radicals | Carrot, green chicory, mushrooms, eggplant, strawberry, potato | Endothelial dysfunction, cancer, anti-microbial, anti-inflammatory |

| Fable 1. The main constituents of | f vegetables that have a | a positive impact on | ı human health | and their sour | ces (adapted |
|--|--------------------------|----------------------|----------------|----------------|--------------|
| from Ramya and Patel 2 | 2019; Yahia et al. 2019; | ; Kumar et al. 2020) |). | | |

Table 2. Response of the yield (ton fed⁻¹) of some vegetables to applied doses of Se-fertilizers cultivated in sandy soil (pH=8.25, EC= 0.85 dS m⁻¹, available N, P and K were 12, 4 and 35 mg kg⁻¹ soil, respectively).

| Applied doses of Se- | Broccoli | Onion | Pepper | Eggplant | | | |
|-------------------------------|--|--------|--------------------------|----------|--|--|--|
| fertilizers | | | (ton fed ⁻¹) | | | | |
| Mineral Se-fertilizer (sodium | selenite: Na ₂ SeO ₃) | | | | | | |
| Control | 6.36 d | 16.77c | 7.42c | 6.07c | | | |
| 10 ppm | 7.82 c | 28.04b | 8.18b | 7.04b | | | |
| 20 ppm | 8.91 b | 28.97b | 8.99a | 7.48b | | | |
| 30 ppm | 10.00 a | 34.62a | 8.46b | 8.46a | | | |
| 40 ppm | 7.32 c | 35.57a | 8.17b | 8.17a | | | |
| Biological Nano Se-fertilizer | | | | | | | |
| Control | 6.41 c | 17.89c | 5.92c | 5.96b | | | |
| 10 ppm | 7.47 b | 26.85b | 7.28b | 6.65b | | | |
| 20 ppm | 9.27 a | 26.50b | 8.09a | 7.49a | | | |
| 30 ppm | 9.45 a | 33.01a | 8.12a | 8.12a | | | |
| 40 ppm | 8.00 b | 32.98a | 7.49b | 7.65a | | | |

The size of selenium nanoparticles was 87.7 nm using *Bacillus cereus* TAH as reported by Ghazi et al. (2022); fed = 4200 m^2 = acre.



Fig. 3. List of some published studied on nano-biofortification on vegetable crops, where the list of refs. [1] Hossain and Bezbaruah (2021), [2] Li et al. (2013), [3] Zhu et al. (2008), [4] Cifuentes et al. (2010), [5] Gonzalez-García et al. (2021), [6] Deng et al. (2020), [7] Wang et al. (2020), [8] Hernandez-Hernandez et al. (2019), [9] Wang et al. (2019), [10] Fortis-Hernández et al. (2022), [11] Shalaby et al. (2021), [12] Abedi et al. (2021), [13] RajaeeBehbahani et al. (2020), [14] Huang et al. (2023), [15] Quiterio-Gutierrez et al. (2019), [16] El-Bialy et al. (2023), [17] Mahmoud et al. (2023), [18] Fawzy et al. (2023a), [19] Fawzy et al. (2023b), [20] Li et al. (2021), [21] Semida et al. (2021), [22] Skiba et al. (2020), [23] Raza et al. (2022), [24] Salama et al. (2019), [25] Obrador et al. (2022).

Table 3. Content of nutrients in edible parts of vegetables as response to applied Se-fertilizers cultivated in sandy soil (pH=8.25, EC= 0.85 dS m⁻¹, available N, P and K were 12, 4 and 35 mg kg⁻¹ soil, respectively).

| Applied doses of Se- | Broccoli (heads) | | | Onion (bulbs) | | | | |
|--|------------------|--------|---------|---------------|-------|-------|--------|----------|
| fertilizers | N (%) | P (%) | K (%) | Se (ppm) | N (%) | P (%) | K (%) | Se (ppm) |
| Mineral Se-fertilizer (sodium selenite: Na ₂ SeO ₃) | | | | | | | | |
| Control | 3.23 e | 0.41 c | 2.00 d | 0.0000 e | 0.67d | 0.12 | 1.35d | 0.0000d |
| 10 ppm | 3.50 d | 0.61 b | 2.18 cd | 0.0013 d | 0.71d | 0.18 | 1.72c | 0.0021c |
| 20 ppm | 3.84 c | 0.73 a | 2.34 c | 0.0031 c | 0.79c | 0.26 | 1.78c | 0.0040b |
| 30 ppm | 4.42 b | 0.74 a | 2.54 b | 0.0078 b | 1.05b | 0.29 | 2.03b | 0.0096a |
| 40 ppm | 5.02 a | 0.81 a | 2.77 a | 0.0094 a | 1.45a | 0.36 | 2.29a | 0.0109a |
| Biological nano Se-fertilizer | | | | | | | | |
| Control | 3.10 d | 0.38 c | 1.91 c | 0.0000 d | 0.56e | 0.10 | 1.17c | 0.0000d |
| 10 ppm | 3.53 c | 0.55 b | 2.15 b | 0.0015 c | 0.70d | 0.15 | 1.38b | 0.0022c |
| 20 ppm | 3.78 b | 0.62 b | 2.25 b | 0.0017 c | 0.79c | 0.23 | 1.58b | 0.0036c |
| 30 ppm | 3.99 b | 0.71 a | 2.42 ab | 0.0043 b | 0.87b | 0.27 | 1.78ab | 0.0052b |
| 40 ppm | 4.24 a | 0.75 a | 2.47 a | 0.0061 a | 1.05a | 0.32 | 1.92a | 0.0080a |

The size of selenium nanoparticles was 87.7 nm using *Bacillus cereus* TAH as reported by Ghazi et al. (2022); fed = 4200 m² = acre.

| Applied doses of Se- | Pepper (fruits) | | | | Eggplant (fruits) | | | |
|--|-----------------|-------|--------|----------|-------------------|-------|-------|----------|
| fertilizers | N (%) | P (%) | K (%) | Se (ppm) | N (%) | P (%) | K (%) | Se (ppm) |
| Mineral Se-fertilizer (sodium selenite: Na ₂ SeO ₃) | | | | | | | | |
| Control | 1.56d | 0.34 | 1.25c | 0.0000e | 1.32d | 0.41 | 0.94d | 0.0000e |
| 10 ppm | 1.69c | 0.40 | 1.52b | 0.0039d | 1.42c | 0.51 | 1.09c | 0.0029d |
| 20 ppm | 1.73c | 0.45 | 1.58b | 0.0049c | 1.48c | 0.59 | 1.14c | 0.0043c |
| 30 ppm | 2.04b | 0.51 | 1.75a | 0.0066b | 1.61b | 0.63 | 1.39b | 0.0069b |
| 40 ppm | 2.25a | 0.71 | 1.85a | 0.0098a | 1.73a | 0.74 | 1.70a | 0.0101a |
| Biological nano Se-fertilizer | | | | | | | | |
| Control | 1.47c | 0.29 | 1.19c | 0.0000d | 1.12c | 0.38 | 0.72d | 0.0000d |
| 10 ppm | 1.58c | 0.37 | 1.28c | 0.0021c | 1.19bc | 0.47 | 0.91c | 0.0018c |
| 20 ppm | 1.65bc | 0.39 | 1.48b | 0.0044b | 1.25b | 0.59 | 1.03c | 0.0032b |
| 30 ppm | 1.75b | 0.43 | 1.55ab | 0.0051b | 1.38a | 0.63 | 1.20b | 0.0054a |
| 40 ppm | 1.92a | 0.48 | 1.59a | 0.0073a | 1.39a | 0.68 | 1.39a | 0.0066a |

Table 4. Content of nutrients in edible parts of vegetables as response to applied Se-fertilizers cultivated in sandy soil (pH=8.25, EC= 0.85 dS m⁻¹, available N, P and K were 12, 4 and 35 mg kg⁻¹ soil, respectively).

The size of selenium nanoparticles was 87.7 nm using *Bacillus cereus* TAH as reported by Ghazi et al. (2022); fed = 4200 m^2 = acre.

4.2 Under salinity stress

In general, the main factors controlling the effectiveness of any biofortification program may include all information related to plant species (genotypes, and phenotypes), soil characteristics (mainly pH, salinity or EC, fertility or CEC, and texture), types of application (foliar, soil, hydroponics, seed priming), and forms/doses of applied nutrients and climatic conditions (Szerement et al. 2022). Salinity stress is considered one of the major environmental stresses, which decrease theproductivity of cultivate by more than 50%.

Applying nano-Se, mitigates and enhances the production under suchstressful conditions (**Table 5**). The role of applied nano-Se for producing biofortified crops under salinity stress may depend on its applied dose, method of preparation, and plant species. It is found that, applied nano-Se can alleviate salt stress in plants by improving the plant growth, and photosynthesis, as well as reducing the oxidative stress in plants under salt stress. This effect mainly depends on the nano-size, doses, types, exposure time, and plants species (Etesami et al. 2021).

Table 5. Production of vegetables under salinity stress and applied nano-Se.

| Biofortified vegetable | Applied nano-Se | Method of preparing | Salinity level | Growth media | Recommended applied dose | Reference |
|------------------------|--|--------------------------|------------------------------------|--|-----------------------------|---------------------------------------|
| Cucumber | 25 mg L^{-1} | Biological (41-87 nm) | Soil EC 4.49 dS m^{-1} | Saline soil | 25 ppm | Shalaby et al. (2021) |
| Bell pepper | 10 and 50 mg L^{-1} | Chemical (2–20 nm) | Solution 25 and 50 mM NaCl | Peat and perlite (1:1) | 25 ppm | Gonzalez-García et al. (2021) |
| Tomato | From 1 to 20 mg L^{-1} | Chemical (2–20 nm) | Solution 50 mM NaCl | Peat and perlite (1:1) | 20 ppm | Morales- Espinoza et al. (2019) |
| Strawberry | $10 \text{ and } 20 \text{ mg } \text{L}^{-1}$ | Chemical (10–45 nm) | Saline soil (25, 50, 75 mM NaCl | Perlite, coco peat and sand (5:7:23) | 20 ppm | Zahedi et al. (2019) |

4.3 Nanotoxicity and biofortification

The program of biofortification should be very critical and precise because it may increasenutrient accumulationin cultivated plants when applied at higherdoses than the allowable onesof these nutrients. These phenomena are noticed after applying intensive nanomaterials or nanoparticles which induce phytotoxicity *via* producing excess reactive oxygen species and oxidative stress, and this in turnlead to imbalanced metabolic and biological processes in plants (Sharma et al. 2022). Therefore, there is an urgent need for the understanding of various biochemical and physiological responses of plants to nanotoxicity, evaluating phytotoxicity, and

developing mitigation strategies for cultivated vegetable crops. Recently, a great concern on nanotoxicity can be noticed on different crops and nanomaterials/nanoparticles such as nanotoxicity of nano-applications in agriculture (Ali et al. 2021; Muthukrishnan 2022), graphite-derived nanomaterials (Wu et al. 2023), and the biomagnification and bioaccumulation of NPs in vegetables and their phytotoxicity, which may cause serious effects on human health (Sharma et al. 2022). The successful biofortification should depend on the following items: (1) The applied recommended doses of nanonutrients can achieve positive impacts on plant growth of biofortified and edible plants (Gil-Díaz et al. 2022),

(2) The successful biofortification program could be also applied to reduce the uptake of toxic heavy metals (e.g., Cd, Pb, Hg) by cultivated plants when some nutrients applied like selenium (Sardar R, et al. 2022a, b; Shalaby et al. 2022b),

(3) Under higher applied doses of nanonutrients, toxic impacts on cultivated plants are expected and the toxic effects on plants may include different physiological, morphological, and genotoxic changes (Kumar et al. 2023). The toxic level differs from plant to other and may stimulate the regeneration of the oxidative stress (i.e., excess of free radicals or ROS), which disturbs the functions and stability of plant cells by affecting biomolecules including DNA, carbohydrates, protein, and membrane lipids (Sharma et al. 2022),

(4) The growing media are very important in this program whose characterization are crucial such as pH, salinity, available nutrients and application method (Almendros et al. 2022), as well as the forms, types of nanonutrients and the methods of their transmission into cultivated plants (Salama et al. 2021), and

(5) The main strategies to mitigate the nano-toxicity may represent in effective antioxidants, which are considered the better means to minimize this oxidative stress and reduce free radicals induced nanotoxicity (Sharma et al. 2022). Before this approach, the **4R Nutrient Stewardship** can be considered as a sustainable solution, which means "apply the right source of nutrients at the right rate, at the right time, and in the right place".

5. Conclusions

Biofortification is a promising tool for producing biofortified vegetable crops, especially nano selenium, which is essential for human health. Unlikely, not all essential nutrients for human nutrition can be biofortified in the nano-form using all edible plants but there are several obstacles that prevent this approach. Production of biofortified vegetables is a great mission, which is needed in different regions all over the world. This production can depend on the nano-forms of many nutrients, but the acceptable recommendation for different nanonutrients and plant species still is an unattainable dream. The reason is simply back to the unknown recommendation applied dose of many needed nanonutrients for vegetable production. This may cause application of higher doses, which can lead to nanotoxicity on cultivated plants, and their consumption humans is a serious for human health. On the other hand, nano-biofortification program for vegetable production can be applied under stressful conditions, but with a more concern on the production requirements. Therefore, mitigation

strategies should be kept in mind to avoid the nanophytotoxicity and associated negative impacts on human health. Several studies are needed concerning the nano-biofortification of vegetables under both stressful and non-stressful conditions.

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6. References

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