International Journal of Environment and Climate Change

12(11): 265-273, 2022; Article no.IJECC.89481
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Toxicity Study of Nanoselenium on Seed Germination, Bacterial Growth and Fish Survivability

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2022/v12i1130971

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/89481

Original Research Article

Received 30 April 2022
Accepted 08 July 2022
Published 11 July 2022

ABSTRACT

Aim: The use of nanoparticles in agriculture has recently increased to sustain crop productivity. Therefore, the effect of nanoparticles on the different ecosystems must be quantified before their use. This study aimed to examine the toxicity potential of nanoselenium on the germination of sorghum seed, growth of soil microorganisms *viz., Bacillus subtilis* and Rhizobium species, and survival of zebrafish. We hypothesize that nanoselenium could be non-toxic to sorghum seed germination, bacillus and rhizobium growth, and zebrafish survival up to 20 mg L^{-1}.

Study Design: Completely randomized design with four or five replications with respect to the experiments.

Place and duration of the Study: Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore

Methodology: Sorghum seeds BTx 623 were soaked in different concentrations (0, 5, 10, 15 and 20 mg L^{-1}) of nanoselenium for 24 h and then sown in Petri-dish to quantify germination potential and seedling growth. The growth medium of microorganisms was challenged from 0 to 20 mg L^{-1} of nanoselenium, and the growth of microorganisms was assessed. Similarly, the survival of zebrafish from 0 to 20 mg L^{-1} of nanoselenium was recorded.

Results: The results showed that nanoselenium up to 20 mg L^{-1} did not cause toxic effects on all organisms representing soil, water and terrestrial ecosystems.

Conclusion: Hence, this study concluded that nanoselenium up to 20 mg L^{-1} is not harmful to organisms representing soil, aquatic and terrestrial ecosystems.

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Keywords: Bacterial growth; ecotoxicity; nanoselenium; seed germination; zebrafish.

1. INTRODUCTION

Currently, the practice of sustainable agriculture is followed to meet the food requirement of the global population. It is projected that the world population will be around 9.8 billion by 2050. To feed this ever-increasing population, global food production must be doubled to meet food, feed and fuel demand. However, crop production is highly impacted by the occurrence of various abiotic stress during the life cycle of the crop [1]. The year-to-year variability in crop yield is largely associated with rainfall and temperature during critical stages of crop development [2]. IPCC [3] as predicted that climate extremes are expected to increase with changes in climate variables, which can significantly limit crop production. Historical observations and model simulations suggested a high risk of drought across the globe [4]. Estimates indicated that across the globe, the yield of cereals, legumes, and oilseeds will be decreased by 10, 50, and 30%, respectively due to drought stress [5]. Future prediction of rainfall indicates a high chance of below-average precipitation in India which can exacerbate the drought stress [6]. Therefore, it is critical to develop crop management technologies to mitigate the drought stress effect to sustain the crop yield.

Studies have indicated that crop management practices like soil management, cultural practices, irrigation management, crop residues and mulching, use of stress-tolerant varieties, and application of nutrients and plant growth regulators can be used to alleviate the drought stress effects in plants [7]. Apart from this, recent studies have indicated that the application of nanomaterials to mitigate abiotic stress is one of the novel approaches [8]. Nanotechnology is a branch of science, that deals with a material having a dimension of 1-100 nm on any one side, which is referred to as a nanoparticle. Through nanomaterials, it is possible to reduce the input cost of agriculture by increasing nutrient use efficiency, controlling pests and diseases with new formulations, and detecting contaminants using nanosensor [9].

Nanoparticles have both positive and negative influences on crops, which are associated with their altered size, shape, and unique physio-chemical and biological properties [10]. Nanomaterials have a significant difference from bulk materials for their surface morphology and large surface area to volume ratio, which makes them more reactive compared to their bulk form [11,12]. The efficiency of nanoparticles is dependent on their chemical composition, shape, surface morphology, and aggregation [13]. Nanomaterials with the same chemical composition and different sizes and shapes and surface properties can create variations in their toxicity potential [14].

Recently studies on assessing the ecotoxicity of nanomaterials are increasing because it is mandatory to quantify the safety of nanomaterials at all trophic levels before their commercial use because there is a direct link between the negative effects of nanoparticles and the survival of organisms [15,16]. Nanoparticles enter the plant system and accumulate in various plant parts and get biomagnified at various trophic levels by consumption [17]. Thus, the use of different types of nanomaterials in agriculture should be regularly examined for their toxicity [18].

The toxic effect of nanoparticles can be evidenced by genotoxicity, an increase in reactive oxygen species (ROS) production and decreased antioxidant enzyme activity [19]. It is observed that nanoparticles first interact with the cell walls, after entering the cells, the nanoparticle aggravates the alterations in the structure and functions of membranes, molecules, and cell organelles [20]. Initially, the metal radicals produced during oxidative stress, act as a signalling molecule to initiate the antioxidative defence system. If the oxidative damage is higher than antioxidative damage, which will cause toxicity of nanoparticles to the organism [19,20].

Our earlier study has shown that selenium nanoparticles (Se-NPs) possess an antioxidative effect and can improve the yield of sorghum under high-temperature stress [21]. The higher beneficial effect of nanoselenium than bulk selenium is due to their higher surface area, higher bioavailability, size and shape morphology [22]. Bulk selenium particles reduced the stress effect by inducing the accumulation of secondary metabolites and increasing the activity of the antioxidant enzymes [23,24]. Se-NPs mitigate the abiotic stress effects in plants, especially drought, salinity, and heavy metal stress by enhancing the antioxidant enzyme system and reducing the damages caused by ROS [25].

However, the toxicity potential of nanoselenium on different organisms was not studied in detail. Hence, this study aimed to examine the toxicity potential of nanoselenium on the germination of sorghum seed, growth of soil microorganisms *viz*., *Bacillus subtilis* and *Rhizobium* species, and survival of zebrafish. We hypothesize that nanoselenium could be non-toxic to sorghum seed germination, bacillus and rhizobium growth, and zebrafish survival up to 20 mg L^{-1}.

2. MATERIALS AND METHODS

2.1 Synthesis of Nanoselenium

Nanoselenium was synthesised by the chemical reduction method as described in Bisht et al. [26]. Selenious acid was used as the precursor and hydrazine was the reducing agent for the synthesis of nanoselenium. Briefly, 6.4 g of selenious acid was dissolved in water and transferred to a Teflon-lined autoclave to which 5 mL of hydrazine was added. Followed by this the content was transferred to a hot air oven maintained at 120°C for 2 h. After the reaction time, the contents were filtered, and the particles were retained and dried in a hot air oven for 2 h. The contents were washed with water and ethanol five times and used for characterization and toxicity analysis. The synthesised selenium nanoparticle was subjected to particle size analysis and its zeta potential using particle size analyser (Horiba, Nano particle analyser SZ-100).

2.2 Evaluation of Toxicity of Se-NPs

2.2.1 Sorghum seed germination test

The experiment was designed in a completely randomized design with five replications. Sorghum variety BTx 623 was used as seed material. First, the seeds were surface sterilized with 0.1% mercuric chloride for 5 min, followed by washing in deionized water for five times. Sterilized seeds were soaked in different concentrations of nanoselenium (0, 5, 10, 15, and 20 mg L^{-1}) for 12 h as described by Acharya et al. [27]. After the expiry of time, the seeds were collected from the soaking solution and sown in a Petri plate. In each Petri plate, 20 seeds were sown. On 14th day after sowing, the number of seeds germinated per Petri plate was counted. Along with this, the shoot and root length of 10 seedlings per Petri plate was measured and expressed in cm. The emergence of the radicle was considered a criterion for germination. The vigour index is the product of germination percentage and seedling length.

Vigour index = Germination % × Seedling length

2.2.2 Growth of soil microorganism

The experiment was designed in a completely randomized design with five replications. Bacillus (*Bacillus substilis*) is a gram-positive rod-shaped bacterium commonly found in the soil. It is a non-pathogenic bacterium. *Rhizobium* is a gram-negative bacterium that lives in the soil or root nodules of leguminous plants. Bacteria were subcultured in the Luria-Bertani broth in an Erlenmeyer flask under aseptic conditions and incubated for 24 h in a rotary shaker at 30°C and 150 rpm. Luria-Bertani broth consists of 10 g L^{-1} of tryptone, 5 g L^{-1} of yeast extract, and 1 g L^{-1} of sodium chloride. The LB mediums with different nanoselenium concentrations (0, 5, 10, 15, and 20 mg L^{-1}) were prepared by sonication and autoclaved at 121°C for 20 min and 15 lbs sq inch^{-1} pressure. After sterilization, the LB medium was poured into a sterile Petri plate under an aseptic condition and kept undisturbed. The subculture of bacillus and rhizobium having 0.1 optical density was used for inoculation. The subculture was inoculated into the plates by spot inoculation method. After inoculation, the plates were wrapped with kiln film and incubated for overnight. After the incubation period, the plates were taken, and the growth of microorganism colonies were measured and expressed in cm.

2.2.3 Zebrafish survivability

The experiment was designed in a completely randomized design with three replications. Zebrafish (*Danio rerio*) is one of the small tropical freshwater fish, and it is the best model to study the toxicity of nanomaterial on aquatic ecosystems because of its small size, very high reproducibility, and easy handling. Different concentrations of nanoselenium solution (0, 5, 10, 15, and 20 mg L^{-1}) were prepared and sonicated and to which seven adult zebrafish were left in the tank to test their survivability. Fishes were given their natural environment other than nanoselenium treatment. On the next day the survival percentage of fish in the tank of each concentration of nanoselenium was examined.

2.3 Statistical Analyses

The data from each experiment were analyzed using SAS program. Observations were analyzed using the PROC GLM procedure of SAS. The standard error was shown as an estimate of variability, and means of various variables were separated for significance by the LSD test at a probability level of 0.05.

3. RESULTS AND DISCUSSION

It is mandatory to increase food production to feed the ever-growing population by adopting new technologies like nanotechnology. However, the safety of the nanomaterial to the ecosystem is of high priority. In the present study, the toxicity of nanoselenium was studied. The major finding of this study was seed germination, microbial growth, and the survival percentage of zebrafish were not affected at 5, 10, 15, and 20 mg L^{-1} of nanoselenium.

3.1 Nanoparticle Characterization

The analysis of synthesized nanoselenium in the particle size analyzer (Horiba, Nano particle analyser SZ-100) indicated that the particle size of the synthesized nanoselenium was 580.2 nm and the zeta potential of the synthesized nanoselenium falls between -40 mV to 0 mV (Fig. 1a-b).

3.2 Impact of Nanoselenium on Sorghum Seed Germination

Uniform seed germination is important for better crop establishment [28]. The seed germination process involves the activation of enzymes namely amylases, proteases, and lipases, and these enzymes are involved in the breaking down of macromolecules for the growth and development of the embryo [29]. The present study indicated that seed treatment with nanoselenium from 5 to 20 mg L^{-1} did not affect the sorghum seed germination percentage (Fig. 2a). This study showed that hydrolytic enzymes were not affected by treatment with nanoselenium. However, studies have indicated seed germination was positively influenced by chitosan or zinc oxide nanoparticles treatment [30]. In contrast, silver nanoparticle influences seed germination in both positive and negative way [31]. Application of high concentration of copper nanoparticle of 800 mg L^{-1} in cowpea reduced the germination index, chlorophyll content, carotenoids and increased the lipid peroxidation and total sugars [32]. Similar to seed germination, the effect of nanoselenium on root length, shoot length and vigour index was not obvious compared to control (Fig. 2b-e).

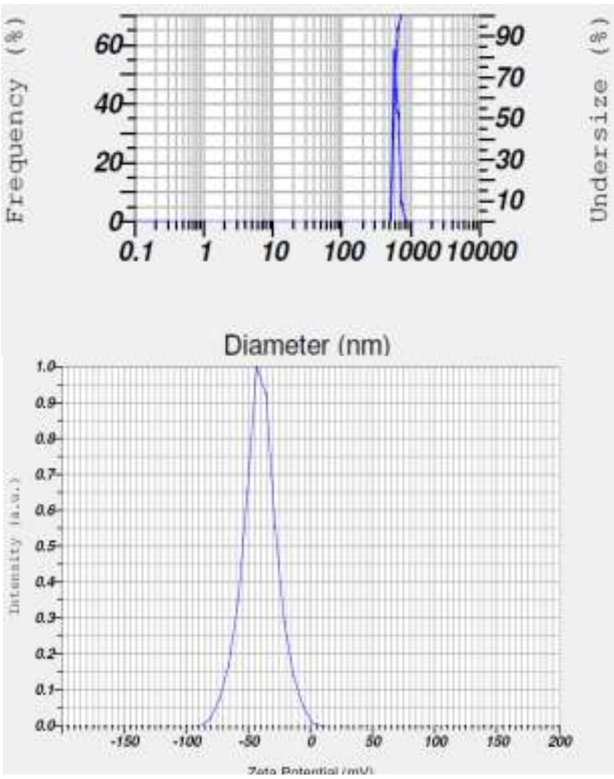

Fig. 1. Showing the (a) Particle size distribution and (b) zeta potential of synthesized nanoselenium using particle size analyser (Horiba, Nano particle analyser SZ-100)

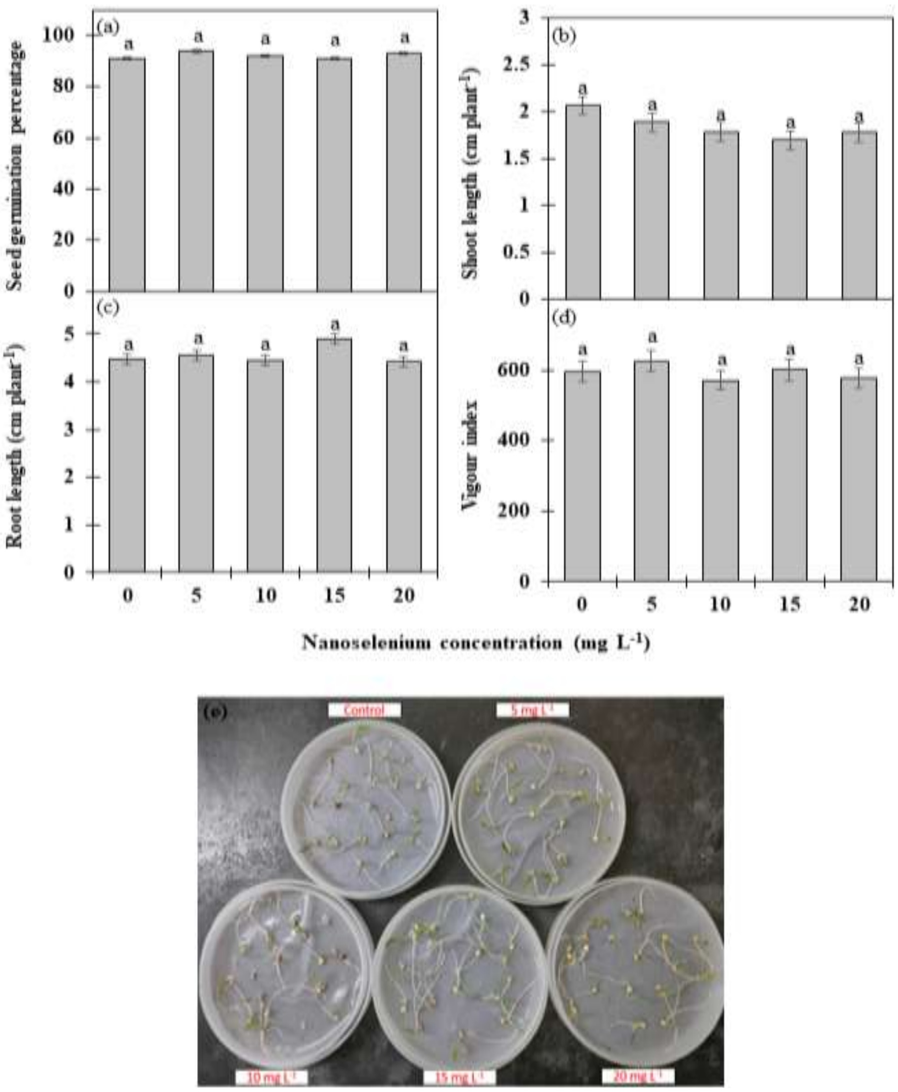

Fig. 2. Effect of different concentrations of nanoselenium on (a) sorghum seed germination percentage, (b) shoot length (cm plant^{-1}), (c) root length (cm plant^{-1}), (d) vigour index, and (e) an overview of the experimental setup. Results indicated that the seed germination, seedling growth, and vigour index were not affected at all concentrations of nanoselenium

3.3 Impact of Nanoselenium on the Growth of Soil Microbes

Nanoparticles can be classified into four types based on toxicity, namely (i) carbon-based nanomaterials, (ii) metal and metal-based nanomaterials, (iii) nanomaterials based on metallodendrimers and (iv) metal composites [33]. The microbial interactions of nanoparticles would lead to the persistent entry of nanoparticles into the food chain. Studies indicated that bacteria are more sensitive to nanoparticles compared to human fibroblast [34,35]. The present study showed that the growth of bacillus and rhizobium was not affected by the presence of nanoselenium up to 20 mg L^{-1} (Fig. 3a-d). Like the present study, the growth of *E. coli* was not affected by the application of iron composite nanoparticles and there was no toxicity [36]. However, iron nanoparticle influences bacterial growth in both positive and negative way [37]. In contrast, microbe treated with 500 mg L^{-1} SeNPs get destructed within 12 hours because SeNPs change the membrane permeability and it caused the leakage of proteins and polysaccharides [38]. The decreased effect of selenium nanoparticles on microbial growth may be associated with efflux systems, redox state of the metal ions, extracellular complexation of metals, and the changes in membrane composition [39,40].

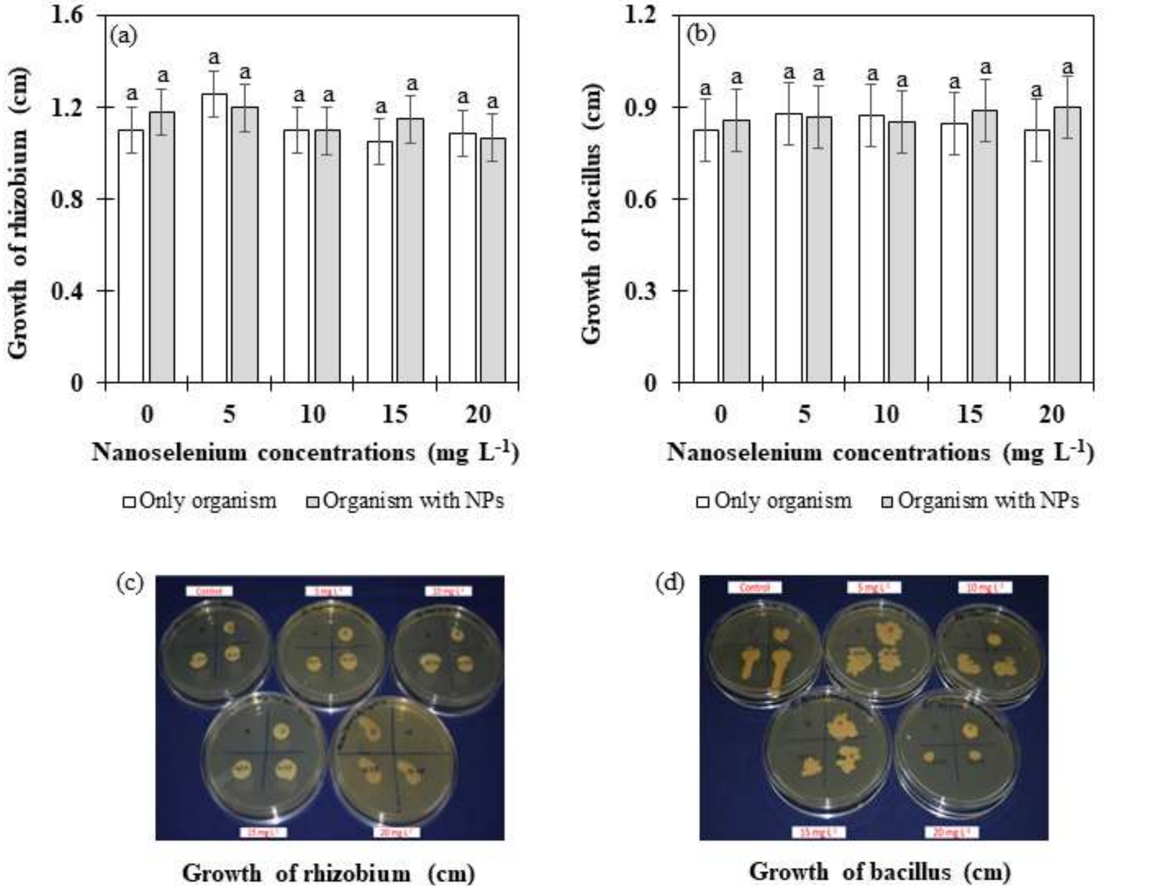

Fig. 3. Effect of different concentrations of nanoselenium on the growth of (a) rhizobium (cm), (b) bacillus (cm), (c) a view of rhizobium growth, and (d) a view of bacillus growth. The result indicated that the growth of rhizobium or bacillus were not affected at all concentrations of nanoselenium

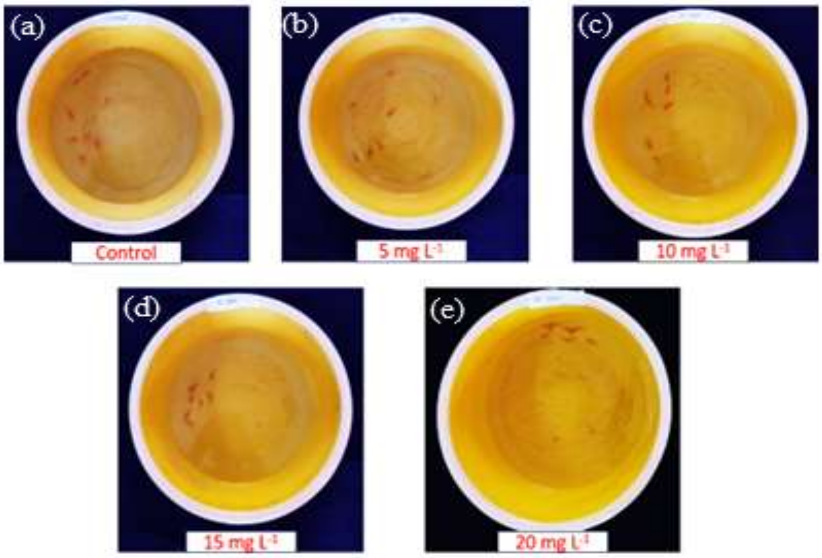

Fig. 4. Effect of different concentrations of nanoselenium on survival of zebrafish. In all the treatments, the fishes survived indicating no significant differences among the treatments

3.4 Impact of Nanoselenium on the Survival Percentage of Zebrafish

In general, the toxicity of nanomaterial to aquatic ecosystems is very important because it is the main point of nanomaterial entry into the various ecosystem. The zebrafish is an established vertebrate model for studying the development and disease [41]. The zebrafish and human genomes share ~70% similarity [42,43]. Therefore, the study was conducted with zebrafish to evaluate the toxicity potential of nanoselenium. The result indicated that nanoselenium did not affect the survivability of zebrafish (Fig. 4). Similar to the present study, gold, magnesium, copper and carbon nanotubes did not cause any toxicity to zebrafish as evidenced by 100% survivability [44]. In contrast, 100 mg L^{-1} of TiO_2NP caused oxidative damage in zebrafish [45].

4. CONCLUSIONS

This study showed that nanoselenium from 5 to 20 mg L^{-1} did not affect the seed germination process, bacillus and rhizobium growth and zebrafish survivability. Overall, nanoselenium, up to 20 mg L^{-1} did not have any toxic effect on terrestrial, soil and aquatic ecosystem representing sorghum seed germination, soil microbes and zebrafish respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Godoy F, Olivos-Hernández K, Stange C, Handford M. Abiotic stress in crop species: Improving tolerance by applying plant metabolites. Plants. 2021;10(2):186.
2. Kukal MS, Irmak S. Climate-driven crop yield and yield variability and climate change impacts on the US Great Plains agricultural production. Scientific reports. 2018;8(1):1-8.
3. IPCC, 2021: Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. 2021;3−32.
4. Mishra A, Liu SC. Changes in precipitation pattern and risk of drought over India in the context of global warming. Journal of Geophysical Research: Atmospheres. 2014;119(13):7833-41.
5. Farooq M, Gogoi N, Barthakur S, Baroowa B, Bharadwaj N, Alghamdi SS, Siddique KH. Drought stress in grain legumes during reproduction and grain filling. Journal of Agronomy and Crop Science. 2017;203(2):81-102.
6. Kulkarni A, Gadgil S, Patwardhan S. Monsoon variability, the 2015 Marathwada drought and rainfed agriculture. Current Science. 2016;111(7):1182-93.
7. Rao CS, Gopinath KA. Resilient rainfed technologies for drought mitigation and sustainable food security. Mausam. 2016;67(1):169-82.
8. Zhao L, Lu L, Wang A, Zhang H, Huang M, Wu H, Xing B, Wang Z, Ji R. Nano-biotechnology in agriculture: use of nanomaterials to promote plant growth and stress tolerance. Journal of agricultural and food chemistry. 2020;68(7):1935-47.
9. Mittal D, Kaur G, Singh P, Yadav K, Ali SA. Nanoparticle-based sustainable agriculture and food science: Recent advances and future outlook. Frontiers in Nanotechnology. 2020 Dec 4;2:10.
10. Wang RH, Xin JH, Tao XM. UV-blocking property of dumbbell-shaped ZnO crystallites on cotton fabrics. Inorganic chemistry. 2005;44(11):3926-30.
11. Roduner E. Size matters: Why nanomaterials are different. Chemical Society Reviews. 2006;35(7):583-92.
12. Riaz S, Ashraf M, Hussain T, Hussain MT, Rehman A, Javid A, Iqbal K, Basit A, Aziz H. Functional finishing and coloration of textiles with nanomaterials. Coloration Technology. 2018;134(5):327-46.
13. Ion I, Culetu A, Gherase D, Sirbu F, Ion AC. Environmental applications of carbon-based nanomaterials. Acetylcholinesterase biosensors for organophosphate pesticide analysis. New Applications of Nanomaterials. Bucharest: Academiei Române. 2014;33-51.
14. Patra JK, Baek KH. Antibacterial activity and synergistic antibacterial potential of

biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects. Frontiers in microbiology. 2017;8:167.

15. Tripathi DK, Singh S, Singh S, Pandey R, Singh VP, Sharma NC, Prasad SM, Dubey NK, Chauhan DK. An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and phytotoxicity. Plant Physiology and Biochemistry. 2017;110:2-12.
16. Singh S, Vishwakarma K, Singh S, Sharma S, Dubey NK, Singh VK, Liu S, Tripathi DK, Chauhan DK. Understanding the plant and nanoparticle interface at transcriptomic and proteomic level: a concentric overview. Plant Gene. 2017;11:265-72.
17. Tripathi DK, Singh S, Singh VP, Prasad SM, Chauhan DK, Dubey NK. Silicon nanoparticles more efficiently alleviate arsenate toxicity than silicon in maize cultiver and hybrid differing in arsenate tolerance. Frontiers in Environmental Science. 2016;4:46.
18. Prasad R, Bhattacharyya A, Nguyen QD. Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. Frontiers in microbiology. 2017;8:1014.
19. Zia-ur-Rehman M, Qayyum MF, Akmal F, Maqsood MA, Rizwan M, Waqar M, Azhar M. Recent progress of nanotoxicology in plants. Nanomaterials in Plants, Algae, and Microorganisms. 2018;143-74.
20. do Amaral DF, Guerra V, Motta AG, e Silva DD, Rocha TL. Ecotoxicity of nano-materials in amphibians: a critical review. Science of the total environment. 2019;686:332-44.
21. Djanaguiraman M, Belliraj N, Bossmann SH, Prasad PV. High-temperature stress alleviation by selenium nanoparticle treatment in grain sorghum. ACS omega. 2018;3(3):2479-91.
22. Menon S, Shanmugam VK. Chemopreventive mechanism of action by oxidative stress and toxicity induced surface decorated selenium nanoparticles. Journal of Trace Elements in Medicine and Biology. 2020;62:126549.
23. Schiavon M, Pilon-Smits EA. The fascinating facets of plant selenium accumulation–biochemistry, physiology, evolution and ecology. New Phytologist. 2017;213(4):1582-96.
24. Zohra E, Ikram M, Omar AA, Hussain M, Satti SH, Raja NI, Ehsan M. Potential applications of biogenic selenium nanoparticles in alleviating biotic and abiotic stresses in plants: A comprehensive insight on the mechanistic approach and future perspectives. Green Processing and Synthesis. 2021;10(1): 456-75.
25. El-Ramady H, Abdalla N, Taha HS, Alshaal T, El-Henawy A, Faizy SE, Shams MS, Youssef SM, Shalaby T, Bayoumi Y, Elhawat N. Selenium and nano-selenium in plant nutrition. Environmental Chemistry Letters. 2016;14(1):123-47.
26. Bisht, Neha, Priyanka Phalswal, Pawan K. Khanna. Selenium nanoparticles: A review on synthesis and biomedical applications. Materials Advances. 2022;3(3):1415-31.
27. Acharya P, Jayaprakasha GK, Crosby KM, Jifon JL, Patil BS. Nanoparticle-mediated seed priming improves germination, growth, yield and quality of watermelons (*Citrullus lanatus*) at multi-locations in Texas. Scientific reports. 2020;10(1):1-6.
28. Chen K, Arora R. Priming memory invokes seed stress-tolerance. Environmental and Experimental Botany. 2013;94:33-45.
29. Ashraf M, Foolad MR. Pre-sowing seed treatment—A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Advances in agronomy. 2005;88:223-71.
30. Afrayeem SM, Chaurasia AK. Effect of zinc oxide nanoparticles on seed germination and seed vigour in chilli (*Capsicum annuum* L.). J. Pharmacogn. Phytochem. 2017;6(5):1564-6.
31. Almutairi ZM, Alharbi A. Effect of silver nanoparticles on seed germination of crop plants. International Journal of Nuclear and Quantum Engineering. 2015;9(6):689-93.
32. Lapaz AD, Santos LF, Yoshida CH, Heinrichs R, Campos M, Reis AR. Physiological and toxic effects of selenium on seed germination of cowpea seedlings. Bragantia. 2019;78:498-508.
33. Sardoiwala MN, Kaundal B, Choudhury SR. Toxic impact of nanomaterials on microbes, plants and animals. Environmental Chemistry Letters. 2018;16(1):147-60.
34. Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, Bruinink A, Stark WJ. *In vitro* cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility.

Environmental science & technology. 2006;40(14):4374-81.
35. Limbach LK, Li Y, Grass RN, Brunner TJ, Hintermann MA, Muller M, Gunther D, Stark WJ. Oxide nanoparticle uptake in human lung fibroblasts: Effects of particle size, agglomeration, and diffusion at low concentrations. Environmental science & technology. 2005;39(23):9370-6.
36. Williams DN, Ehrman SH, Pulliam Holoman TR. Evaluation of the microbial growth response to inorganic nano-particles. Journal of nanobiotechnology. 2006;4(1):1-8.
37. Wu D, Shen Y, Ding A, Mahmood Q, Liu S, Tu Q. Effects of nanoscale zero-valent iron particles on biological nitrogen and phosphorus removal and microorganisms in activated sludge. Journal of hazardous materials. 2013;262:649-55.
38. Zhang H, Li Z, Dai C, Wang P, Fan S, Yu B, Qu Y. Antibacterial properties and mechanism of selenium nanoparticles synthesized by *Providencia* sp. DCX. Environmental Research. 2021;194: 110630.
39. Fang J, Lyon DY, Wiesner MR, Dong J, Alvarez PJ. Effect of a fullerene water suspension on bacterial phospholipids and membrane phase behavior. Environmental Science & Technology. 2007;41(7):2636-42.
40. Silver S. Bacterial resistances to toxic metal ions-a review. Gene. 1996;179(1):9-19.
41. Dooley K, Zon LI. Zebrafish: A model system for the study of human disease. Current opinion in genetics & development. 2000;10(3):252-6.
42. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L, McLaren S. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013; 496(7446):498-503.
43. Kettleborough RN, Busch-Nentwich EM, Harvey SA, Dooley CM, De Bruijn E, Van Eeden F, Sealy I, White RJ, Herd C, Nijman IJ, Fényes F. A systematic genome-wide analysis of zebrafish protein-coding gene function. Nature. 2013;496(7446):494-7.
Tang T, Zhang Z, Zhu X. Toxic effects of TiO2 NPs on zebrafish. International Journal of Environmental Research and Public Health. 2019;16(4):523.
44. Haque E, Ward AC. Zebrafish as a model to evaluate nanoparticle toxicity. Nanomaterials. 2018;8(7):561.
45. Tang T, Zhang Z, Zhu X. Toxic effects of TiO2 NPs on zebrafish. International Journal of Environmental Research and Public Health. 2019;16(4):523.

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