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Effect of Nano Selenium Seed Priming on Sorghum Seed germination, Seedling Growth and Bacterial Growth

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ABSTRACT: Sorghum is a grain crop that is frequently grown throughout the world's dry and semi-arid regions and provides valuable food, feed, and biofuel. However, biotic and abiotic stressors such as drought, salt, pests, and diseases frequently limit sorghum output. Seed priming is a pre-sowing strategy that improves seed germination and seedling growth in stressful environments. Nano selenium (nano-Se) is a kind of selenium that has been found to improve plant growth and stress tolerance. The effect of nano-Se seed priming on sorghum seed germination, seedling growth, and bacterial growth, on the other hand, has not been thoroughly investigated. The goal of this work was to analyse the influence of nano-Se seed priming on these characteristics and to determine the probable mechanisms involved.

Nano-selenium has been demonstrated to help agricultural crops grow and resist pests and diseases when used as fertilizers. At low concentrations, both selenium and nano-selenium increase plant growth, antioxidant activity, and stress tolerance, which has sparked an increasing interest in evaluating their potential use in crop production and agro-biotechnologies. Therefore, the effect of nanoparticles on plants and microbes must be quantified before their use in soil. This study aimed to examine the toxicity potential of nano-selenium on the germination of sorghum seed, growth of representative soil bacteria *viz*., *Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas koreensis.* Sorghum seeds K-8 and three representative soil bacterial organisms were soaked in different concentrations of nano-selenium (0, 5, 12.5, 25, 50, 100, 200 mg L^{-1}) for 24 h and then seeds were sown in roll towel to quantify germination potential and seedling growth, whereas the growth and survival of representative soil micro-organisms were also assessed.

In this study we came to know that nano-selenium is non-toxic up to 100 mg L^{-1} but above the concentration that is 200 mg L^{-1} decreased the rate of germination potential. However, the growth of bacterial organism grows well in all graded nano-Se, hence it is considered to non-toxic up to 200 mg L^{-1} Hence, this study concluded that nano-selenium is toxic above 100 mg L^{-1} for plant growth but non-toxic for representative soil bacterial growth.

Keywords: Germination rate, toxicity, nano-selenium concentration.

INTRODUCTION

The element selenium is crucial for both plants and animals in which it has a role in a number of physiological functions, including respiration, antioxidant defence, and photosynthesis. Se nanoparticles (Se-NPs) can be made in a variety of ways, although green synthesis utilising plants is more appealing due to a decrease in ecological concerns and an increase in biological activity. Nano-selenium is more active than bulk materials. According to a field study by Qureshi *et al*. (2021), biofortification of selenium (3 mg/L as $SeSO_4$) results in the best growth, quality attributes, and nutrient uptake in the sorghum (*Sorghum bicolor* L.) accessions that are being studied as compared to the control. When compared to other grain crops, sorghum has superior potential to be employed in the supplementation of selenium (Se) to humans and animals due to its high protein content, rustic nature, and exceptional resilience to a variety of stressing situations. It has been demonstrated that the application of Se-NPs promotes the growth of soil bacteria like Pseudomonas aeruginosa (Ni *et al*., 2021).

Selenium has demonstrated a significant protective effect against salt stress in wheat seeds and seedlings. Many biostimulators, including nanoparticles, could be used to increase the germination of grown seeds in challenging conditions (Ghazi *et al*., 2022). Nano-selenium is more active than bulk materials, and several methods of making Se nanoparticles (Se-NPs) have been described, but green synthesis utilising plants is more desirable because it has fewer environmental consequences and boosts biological activity. (Samynathan *et al*., 2023). The synthesis of selenium nanoparticles stabilised by PVP C15 (82 kDa) and ascorbic acid has been shown to have good effects on the length of roots and shoots, the number of roots, and the percentage of seed germination (Siddique *et al*., 2023). Zn seed priming has been demonstrated to improve seed vigour, germination, early seedling growth and biomass production, photosynthetic efficiency, and sugar content, totaling (Adhikary *et al*., 2022). The function of nano selenium (nSe) or selenium nanoparticles (SeNP) on plants' ability to withstand abiotic stress Se NP or nSe has impacts on plant development, strengthens the antioxidant defence system, and increases plant stress tolerance (Khan *et al*., 2023).

Selenium (Se) NPs have garnered a lot of attention among the different NPs because of their favourable impact on plant nutrition and health. Selenium is a crucial mineral for both humans and animals. It is also needed for plant metabolism, antioxidant defence, and stress response. However, depending on its availability and quantity, Se is either insufficient or hazardous in soils. Therefore, seed priming with Se NPs can be a workable technique to maximize Se absorption and distribution in plants as well as to increase their resistance to drought stress, which is one of the main risks to global food security. Sorghum (Sorghum bicolor L.) is a significant cereal crop that is widely grown throughout the world in arid and semi-arid climates. It is highly nutritious and remarkably adaptable to adverse environmental factors. However, drought stress continues to have an impact on sorghum production and quality, particularly during the early growth phases. Consequently, seed priming with Se NPs may be a creative way to enhance sorghum performance in drought-prone conditions.

However, the toxicity potential of nano-selenium on different soil organisms was not studied in detail. Hence this study aimed to examine the toxicity potential of nano-selenium on the germination of sorghum seed, growth of representative soil bacteria viz., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas koreensis*.

MATERIALS AND METHODS

Plant materials. The Sorghum variety K-8 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 nano-selenium (0, 5, 12.5, 25, 50, 100, 200 mg L^{-1}) for 12 h as described by Acharya *et al*. (2020. After the expiry of time, the seeds were collected from the soaking solution and sown in petri plate. In each petri plate, 10 seeds were sown

Germination percentage. The percentage of germination was calculated using the formula

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total no. of seeds}} \times 100$$

Seedling vigour index (SVI): Seedling vigour index can be expressed by the following equation

Seedling vigour index = (Average shoot length + average root length) × germination percentage

Root and shoot length measurement. On 14th day after sowing in roll towel, the shoot and root length of 9 seedlings per towel was measured and expressed in cm.

Growth of Soil microorganism. The experiment was designed in a completely randomized design with three replications. Three bacterial organism *Bacillus subtilis* M3T8B1which is a gram- positive rod shaped bacterium commonly found in the soil, *Pseudomonas aeruginosa* PA01 which is a gram-negative rod shaped bacterium that can cause disease in plants and animals, including humans and found widely in the environment, such as in soil, water, and plants, *Pseudomonas koreensis* flhb16 is a species of bacteria that belongs to the Pseudomonas fluorescens complex. Optical density (OD) was recorded in order to determine the effect of nano selenium on the growth of bacterial cells in media consisting of glucose, peptone, beef extract, sodium chloride in the Agilent Cary 60 UV-Vis spectrometry. Control samples (without the addition of nano-Se) and experimental samples (addition of different concentrations of nano selenium) were prepared and the micro cultures were run for 24 h at 37°C with continuous shaking.

Statistical analysis. All the data were statistically analysed using SPSS software (version 16.0). The mean values were compared using Ducan's multiple range test (DMRT) carried out at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of nano-selenium on Sorghum seed germination. According to this study, seed treatment with nano-selenium up to a concentration of 5 to 25 mg L^{-1} had no effect on the germination percentage of sorghum seeds, but at that concentration and above, the germination percentage decreased (Fig. 1c and Table 1). Among nanoparticles, nano-ZnO is determined to be the most phytotoxic, followed by nano-Fe_2O_3 and fullerene. However, loss in root and shoot growth has been proven to be a more sensitive indicator than germination %. The results also show that NPs in low concentrations can be beneficial to seedling growth (Kumar *et al*., 2015). Seed soaking with nanoparticles, specifically TiO_2, ZnO, and chitosan, improved wheat crop germination and seedling growth indices. However, studies have found that nanoparticles have both positive and negative impacts on seed germination, depending on nanoparticle features (metal or carbon-based, size, shape, and surface coating), nanoparticle concentration, growth environment, and plant species.

Effect of nano-selenium on Sorghum shoot length. The results showed that graded nano-Se up to 100 mg L^{-1} has no effect on the length it grows at all mg L^{-1} compared to the control, but it reduces at 200 mg L^{-1} (Fig. 1a). This study demonstrated maize root and shoot length and biomass increased up to 100 mgL^{-1}treated plants but only at 200 mg L^{-1} did greater levels cause a decrease in growth metrics, with root and shoot length being shorter than the control (Srivastav *et al.*, 2021). According to a study, treatment with 100 mg L^{-1} nano-selenium under high Ec values of 14 ds m^{-1} improved the vegetative growth parameters of shoot length by 22.8% over untreated controls (Ghazi *et al.*, 2022).

Effect of nano-selenium on Sorghum root length. The findings showed that graded nano-Se up to 50 mg L^{-1} lengthens the roots, whereas 200 mg L^{-1} decreases root length in comparison to control (Fig. 1b). According to studies, seed germination as well as the early growth of shoots, roots, and other plant parts are all affected differently by nanoparticles at different concentrations. While some nanoparticles are harmful to plants and prevent their growth when present in higher concentrations, low levels of them can lengthen roots and shoots (Talebi, 2018).

Effect of nano-selenium on Sorghum seed vigour index. The results showed that, except for 200 mg L^{-1} concentration, all graded concentrations from 5 to 100 mg L^{-1} increased the vigour index in comparison to control(Fig 1d).A study on the impacts of nano-TiO_2 on Chinese fir seed germination, seedling growth, and physiology revealed that nano-TiO_2 had a substantial impact on seed germination rate, germination energy, germination index, and vigour index. Low concentrations of this impact were positively associated, whereas high concentrations reduced seedling development and germination rates (Li *et al.*, 2017). Another study found that the percentage of germination, germination rate index, root and shoot length, fresh weight, vigour index, and chlorophyll content of seedlings all increased significantly as nano-anatase concentration was raised (Dehkourdi and Mosavi 2013).

Effect of nano-selenium on soil microorganism. As a result, it has no effect on the growth of graded nano-Se from 5-200 mg L^{-1}, according to the data, which reveal that the growth of bacillus and Pseudomonas sp rises at both lag and log phases compared to control (Fig. 2). It has been demonstrated, for instance, that 5 nm diameter anionic and cationic gold nanoparticles are not naturally antibacterial and do not inhibit bacterial growth (Fuller *et al.*, 2020). A panel of bacteria that both drug-resistant and drug-susceptible have been demonstrated to be rendered inactive by silver nanoparticles, but bacterial growth remains unaffected (Lara *et al.*, 2010).

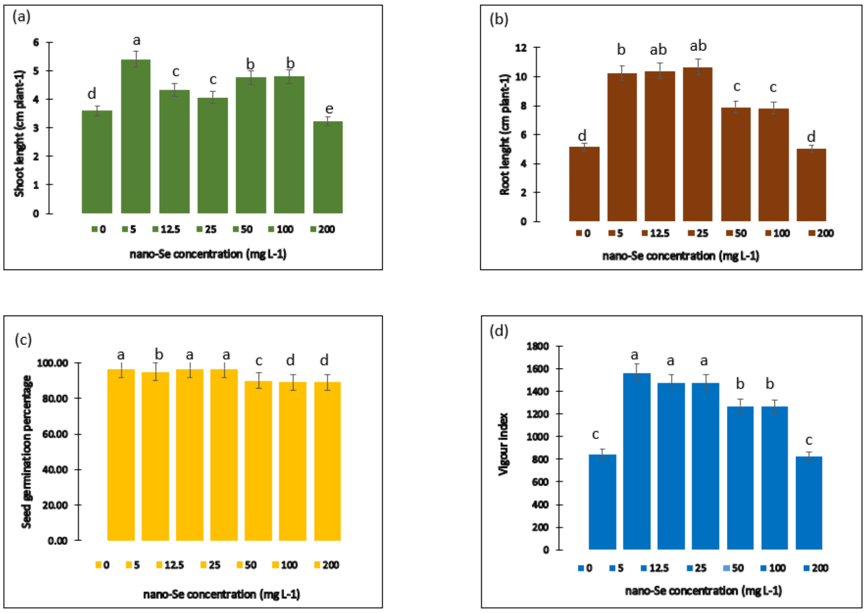

Fig. 1. Effect of graded nano-selenium concentrations on Sorghum (a) shoot length (cm plant^{-1}), (b) Root length (cm plant^{-1}), (c) seed germination percentage, (d) Vigour index.

Tab 1: Effect of nano-selenium at graded concentrations of seed germination percentage, shoot length, root length and vigour index are represented in mean ± SD.

Nano-selenium (mg L^{-1})	Seed germination percentage	Shoot length	Root length	Vigour index
0 mg L^{-1}	96.66 ± 0.12^{a}	3.60 ± 0.06^{d}	5.13 ± 0.09^{d}	843.84 ± 16.3^{c}
5 mg L^{-1}	95.00 ± 0.06^{b}	5.40 ± 0.06^{a}	10.23 ± 0.12^{b}	1563 ± 32^{a}
12.5 mg L^{-1}	96.66 ± 0.13^{a}	4.33 ± 0.09^{c}	10.40 ± 0.12 ^{ab}	1473 ± 26.6^{a}
25 mg L^{-1}	96.66 ± 0.07^{a}	4.07 ± 0.09^{c}	10.67 ± 0.09^{ab}	1474 ± 25.7^{a}
50 mg L^{-1}	90.00 ± 0.13^{c}	4.77 ± 0.15^{b}	7.90 ± 0.06^{c}	1267 ± 64.9^{b}
100 mg L^{-1}	89.00 ± 0.17^{d}	4.80 ± 0.06^{b}	7.83 ± 0.09^{c}	1263 ± 67.3^{b}
200 mg L^{-1}	89.00 ± 0.14^{d}	3.23 ± 0.15^{e}	5.03 ± 0.07^{d}	826 ± 44.3^{c}

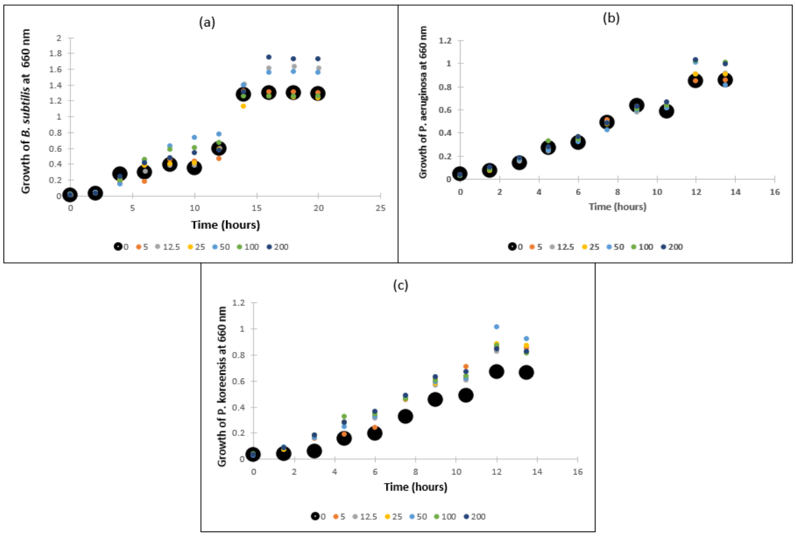

Fig. 2. Effect of graded nano-selenium concentrations on the growth of bacterial organism (a) *Bacillus subtilis* (b) *Pseudomonas aeruginosa* (c) *Pseudomonas koreensis.*

CONCLUSIONS

This study demonstrated that nano-selenium at concentrations between 5 and 100 mg L^{-1} had no effect on bacterial growth or seed germination. Overall nano-selenium activity shows that concentrations between 5 and 100 mg L^{-1} did not cause any toxicity, but concentrations over 200 mg L^{-1} had a hazardous effect on the germination of sorghum seeds. Meanwhile nano-selenium up to a concentration of 200 mg L^{-1} has no effect on representative soil bacterial growth.

FUTURE SCOPE

Future research on this topic might look into how nano-selenium affects different types of plants and crops. Investigating how nano-selenium affects various bacterial and microorganism species in soil can potentially be of interest. This could aid in our comprehension of the advantages and disadvantages of employing nano-selenium in agriculture and other sectors.

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Conflict of Interest. None.

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