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Effect of Bio-fabricated Selenium Nanoparticles in Morphological Growth Parameters of Rice (*Oryza sativa* L.) Seedlings at Early Growth Stage

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Authors' contributions

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

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ABSTRACT

The present study highlights the effect of Bio-fabricated Selenium Nanoparticles in Morphological Growth Parameters of Rice (*Oryza sativa* L.) Seedlings at Early Growth Stage. On plant growth, selenium (Se) exhibits both positive and negative effects. Lower sodium selenite concentrations promoted the growth of rice (*Oryza sativa* L.) seedlings, while greater sodium selenite concentrations inhibited seedling growth. Physiological studies indicated that Se (sodium selenite) at a concentration between 2 and 6mg/L can promote rice seedling growth; however higher concentration (>6mg/L) became inhibitory. Se treatments activated antioxidative system to enhance

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stress resistance in plants. Besides, proteins involved in photosynthesis in shoot, as well as carbohydrate and protein metabolism in root were up-regulated under lower Se treatment. When taken as a whole, these facts support the growth of seedlings. Higher Se treatment, however, may result in the production of excessive reactive oxygen species (ROS) and selenoproteins, which suppress photosynthesis and primary metabolism and impede seedling growth. In order to analyze the Se response pathways in higher plants and create Se-enriched rice cultivars in the future, our study offered new insights into the Se response in rice at the morphological level.

Keywords: Selenium; rice; nanoparticles; seedling vigour index; growth rate.

1. INTRODUCTION

Selenium (Se) in low doses is reported to exert an array of beneficial effects on various plants. It is reported to improve the overall crop growth and vigour by enhancing the uptake of nutrients. All these beneficial effects are observed only when Se is supplied in very low concentration. Conversely, at higher concentrations Se creates nutrient imbalance and causes decrease in growth of plant (Ran et al., 2024). While Se acts as antioxidant at low concentration, the role is reversed at high dose where it acts as pro-oxidant and causes significant increase in production of ROS and RNS like NO and peroxynitrite (Lehotai *et al.*, 2016). Hence to achieve the positive effect of Se phytofortification, balance between these beneficial and toxic effects needs to be maintained and the chemical form, method of application needs to be outlined. Many studies have shown that Se in very low concentration enhances plant growth and development like other toxic elements which is generally termed as “Hormesis” (Poschenrieder *et al*., 2013). The increased growth can be attributed to the Se mediated uptake and redistribution of essential nutrients (Feng *et al*., 2009; Gupta and Gupta, 2017).

The essentiality of Se for animals is well established and Se as a constituent of various selenoproteins is involved in free radical scavenging thus preventing cancer, immunological functions like pathogen resistance especially against the viral pathogen, thyroid function and spermatogenesis (Adhikary et al., 2022). In the case of higher plants, the essentiality of Se is still debated but in low concentration, selenium is found to be beneficial for many plants (Pilon-Smits and Quinn, 2010). The beneficial effect can be attributed to the upregulation of various pathways involved in plant stress tolerance. The beneficial effect of Se in plants includes maintenance of cellular structure and function, enhanced photosynthesis by protecting photosynthetic machinery from oxidative stress, growth and development, distribution of essential elements, reduced metal toxicity and regulation of ROS (Gupta and Gupta, 2017). All these functions are interrelated and they contribute to the overall growth and development of plants under stressed and non-stressed conditions. Selenium in optimal concentration is found to exert an array of beneficial effects in various crop plants though the essentiality of the nutrient in the case of plants is not well established (Bala et al., 2024). A large number of experimental evidence suggests that Se in low concentration improves crop yield, impart tolerance towards a wide range of abiotic stresses. At higher concentrations, Se disrupts the physiological functions of plants which are generally attributed to the generation of ROS which causes oxidative stress (Ran et al., 2024).

As the essentiality of Se is well established for animals especially humans and other mammals, bio-fortification of cultivated crops and fodders with Se is of great importance for the improvement of nutritional quality of grains and fodder (Setty et al., 2023). Hence finding the optimum dose of Se for specific plant species, stage of plant growth, Se species, Growth substrate, application method and accumulated tissue Se concentration is of utmost importance.

2. MATERIALS AND METHODS

The experiment was conducted at Banaras Hindu University, Varanasi, India during the June month of year 2022, where SeNPs of optimal size were synthesized using varying sodium selenite concentrations and raisin extract. The green-synthesized SeNPs were then characterized using advanced methods (Setty et al., 2023). Green-synthesized SeNPs were prepared in concentrations of 10µM, 20µM, 25µM, 30µM, and 35µM, labeled T1 to T6, with T1 as the control. Rice seeds (HUR-105) from

Banaras Hindu University were soaked, sterilized, and tested for seedling vigour index and growth analysis parameters like Absolute Growth Rate (AGR) and Relative Growth Rate (RGR). SVI is one of the important indicators of seedling growth was calculated by the formula given by Abdul-Baki and Anderson (1973). Growth analyses are used to estimate growth in terms of having functional and structural significance. From the recorded morpho-physiological data, various growth parameters are estimated. Absolute Growth Rate (AGR) (Hunt, 1990) is the absolute value of dry matter accumulated between two intervals. Relative Growth Rate (RGR) expresses the total plant dry weight increase in a time interval in relation to the initial weight (Williams, 1946).

Seedling Vigour Index I = Seedling Length (cm) × Germination %

Seedling Vigour Index II = Seedling Dry Weight (g or mg) × Germination %

$$Absolute\ Growth\ Rate\ (g\ day-1) = \frac{W2 - W1}{t2 - t1}$$

$$Relative\ Growth\ Rate\ (gg^{-1}day^{-1}) = \frac{In\ W2 - In\ W1}{t2 - t1}$$

2.1 Statistical Analysis

All the data collected were analyzed by R-studios software. The significant, the means were compared at 1% significant level (α = 0.01). Design used was Completely Randomized Design. Graphs were plotted using the Origin pro 2024 software with error bars depicting the standard error of mean.

3. RESULTS

Seedling Vigour Index I and II were calculated at 15, and 30 days after sowing and the observations are reported in Fig. 1. Treatment T4 (20 µM) performed better concerning both the indices and in all days at which the data is recorded. It showed 43%, 60%, and 35% increase over control regarding SVI I and 90%, 80%, and 100% increase over control regarding SVI II at 10, 20, and 30 days after sowing respectively. Toxic concentrations of SeNP decreased the seedling growth and vigour. Various growth parameters viz. Absolute growth rate (AGR) and Relative growth rate (RGR), were calculated based on the various observations recorded at 15 and 30 days of crop growth and similarly like SVI, treatment T4 was found to be superior regarding all the growth analysis parameters concerning AGR and RGR. T5 and T6 showed a 24% and 25% decrease over control in both AGR and RGR respectively (Fig. 1 and Table. 1) and both treatments were toxic and found at par. At concentrations of 30 and 35 µM toxic effects were visible in roots and leaves. The withering and drying of leaves from the tip were observed at concentrations starting from 30 µM and the extent of drying increased with increase in concentration. Similarly in roots subjected to 30 and 35 µM of Se, showed thickening of primary roots with pinkish colouration and produced few to no lateral roots.

Table 1. Effect of SeNP on Absolute growth rate (AGR) and Relative growth rate (RGR) in rice genotype HUR-105 at 10, 20 and 30 days after germination

Treatment	AGR I	AGR II	RGR I	RGR II
	10 DAS	20 DAS	30 DAS	30 DAS
T1 (Control)	0.12±0.002[d]	0.08±0.0002[d]	0.20±0.001[a]	0.04±0.001[bc]
T2 (10 µM)	0.14±0.001[c]	0.08±0.0017[c]	0.20±0.002[a]	0.04±0.001[b]
T3 (20 µM)	0.20±0.004[b]	0.17±0.0033[b]	0.19±0.004[a]	0.05±0.001[a]
T4 (25 µM)	0.22±0.001[a]	0.19±0.0023[a]	0.19±0.002[a]	0.05±0.001[a]
T5 (30 µM)	0.11±0.001[e]	0.06±0.0003[e]	0.20±0.001[b]	0.04±0.000[c]
T6 (35 µM)	0.09±0.01[f]	0.06±0.0003[e]	0.20±0.002[b]	0.05±0.001[d]
±SE(d)	0.003	0.003	0.003	0.001
CD at 5%	0.006	0.005	0.007	0.003
CD at 1%	0.009	0.008	0.010	0.004

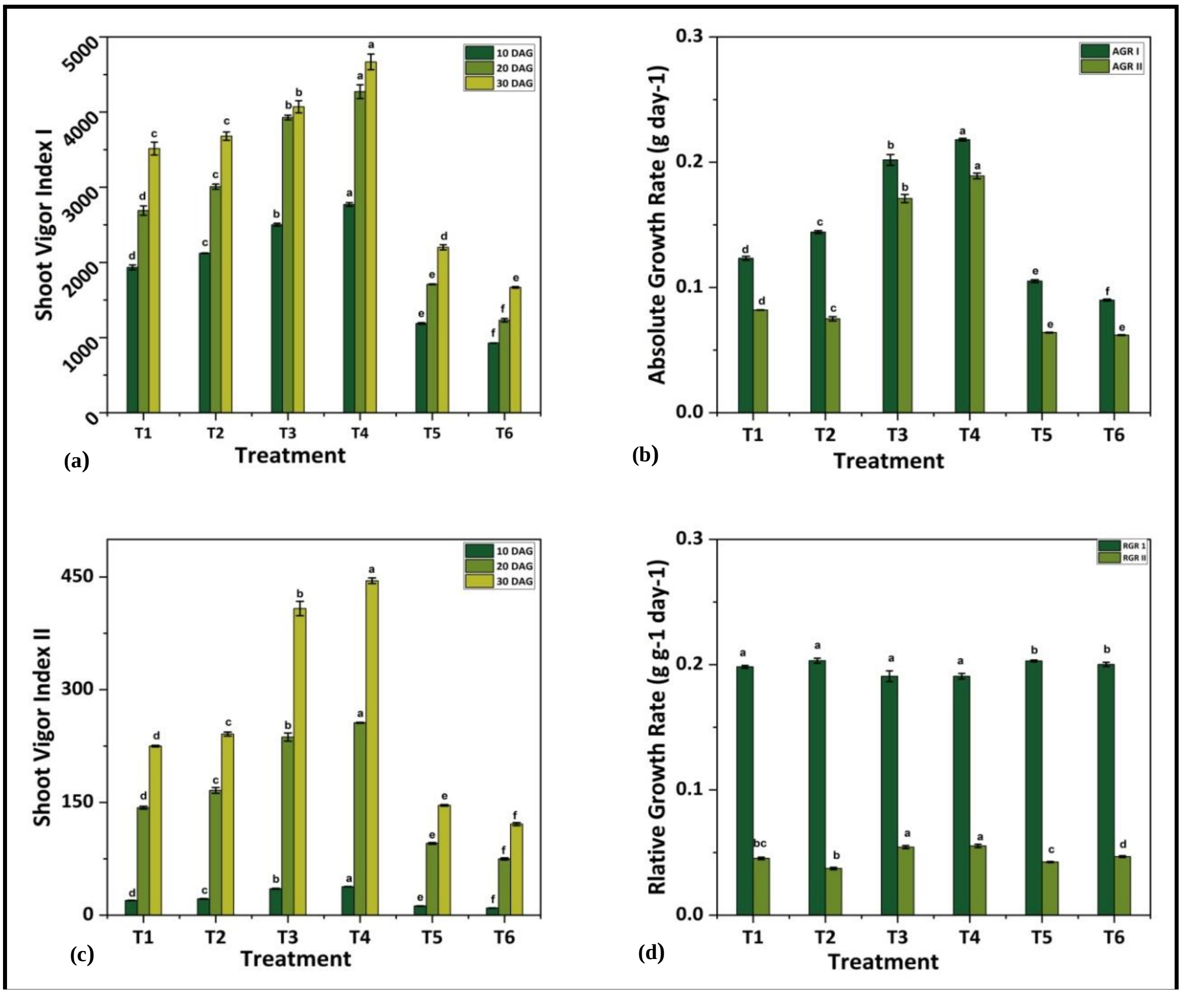

Fig. 1. Effect of SeNP on (a) Seedling Vigour Index I, (b) Seedling Vigour Index II, (c) Absolute growth rate (AGR) and (d) Relative growth rate (RGR) in rice genotype HUR-105 at 10, 20 and 30 days after germination

4. DISCUSSION

Seedling Vigour Indices show the robustness of seedling growth. In the present study enhancement of seedling vigour at lower concentrations of Se is a result of enhanced growth at those concentrations. Recent studies conducted concerning the effect of SeNP on seedling vigour showed a similar increase in SVI in horse gram (Antony *et al.*, 2021), pea (Li *et al.*, 2021), and maize (Nawaz *et al.*, 2021). In rice, a 35 - 90% increase in vigour index (SVI I, II) was observed in response to SeNP treatment in current experiment. At higher concentrations, seedling vigour was reduced significantly. This reduction in vigour resulted from the decreased root and shoot growth and decreased dry matter accumulation.

In the current investigation, calculated growth kinetics AGR and RGR showed improvement at 10-25 µM of SeNP. This increase at the lower concentration can be attributed to the greater uptake of essential nutrients and consequent higher dry matter production. AGR and RGR showed significant reduction only at very high concentrations and at moderate concentrations, these are found at par with control. Pezzarossa *et al.* (1999) reported similar observations in tomato supplied with selenium and sulphate.

5. CONCLUSION

SeNP in low doses improve the growth and physiology of crop plants by imparting beneficial effect on nutrient uptake and redistribution, improved photosynthesis, and increased metabolism. Conversely, at higher doses, it significantly reduces the growth and vigour of the plant by inducing nutrient imbalance, decreasing dry matter accumulation, increased production of

ROS. As per the current study, 10 to 25 μM of SeNP supplied is found to be optimum for rice growth under hydroponic culture while 30 μM was found to be the threshold from which the toxicity was evident. 35 μM was found to be very toxic and showed many adverse effects on rice growth. Further study in the direction of SeNP accumulation in various plant tissue from such culture, suitable method and form of application can be undertaken. Further research in these parameters can help us in SeNP fortified crop production for nutritional security.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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