

## ENHANCING RICE SEEDLING GROWTH WITH FE<sub>3</sub>O<sub>4</sub> NANOPARTICLES: MITIGATING ARSENIC STRESS FOR SUSTAINABLE AGRICULTURE

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### Abstract

Arsenic contamination of soil presents a significant challenge to rice cultivation and food security globally. This study evaluates the effects of Fe<sub>3</sub>O<sub>4</sub> nanoparticles on rice seedling growth kinetics under arsenic stress. Over a 7-day period, germination rates, shoot lengths, and root lengths were measured. The control group showed a 75% germination rate, with average shoot and root lengths of 1.06 cm and 41 cm, respectively. Treatment groups exposed to 10 ppm, 20 ppm, and 50 ppm Fe<sub>3</sub>O<sub>4</sub> nanoparticles yielded germination rates of 41%, 66%, and 79%, and corresponding shoot lengths of 2.06 cm, 2.97 cm, and 3 cm, respectively. Root lengths were enhanced to 66 cm, 75 cm, and 79 cm at these respective concentrations. The findings indicate that Fe<sub>3</sub>O<sub>4</sub> nanoparticles, particularly between 20 ppm to 50 ppm, promote the growth of rice seedlings without adverse effects even under arsenic stress. This advancement holds promise for improving sustainable agriculture and addressing arsenic-related food security concerns in affected regions, potentially leading to increased crop yields.

### INTRODUCTION

Rice (*Oryza sativa*) is a cornerstone of global food security, providing sustenance to a significant portion of the world's population (Chaudhry et al., 2023; R. K. Gupta, Naresh, Hobbs, Jiaguo, & Ladha, 2003). However, rice cultivation faces an increasingly daunting challenge: the contamination of agricultural soils with arsenic (Faisal et al., 2023; Sharma, Tjell, Sloth, & Holm, 2014). Arsenic, a naturally occurring metalloid, poses grave risks to human health and ecosystems due to its inherent toxicity and widespread

distribution. As arsenic accumulates in rice plants, it threatens crop yields and soil quality and ultimately jeopardises food security. In this context, the role of iron oxide nanoparticles as potential mitigators of arsenic toxicity in rice plants emerges as a promising avenue of research (Rai et al., 2018; Ur Rahim et al., 2021).

Arsenic poisoning in plants, particularly rice, is a complex issue that can result in a range of diverse consequences (Finnegan & Chen, 2012). Arsenic exists in various forms, but the inorganic forms of

the element, namely arsenite and arsenate, are commonly present in soils (Nriagu et al., 2007). Plants efficiently assimilate these forms, leading to their accumulation in various plant tissues, including rice grains (Bakhat et al., 2017). Therefore, rice serves as a major pathway through which arsenic can be introduced into the human diet. Prolonged exposure to arsenic-rich rice poses notable health risks, such as the onset of developmental issues, skin irritations, and cancer (Biswas et al., 2021).

Addressing arsenic toxicity in rice cultivation necessitates innovative approaches that transcend traditional methodologies. Iron oxide nanoparticles, particularly Fe<sub>3</sub>O<sub>4</sub> NPs, have garnered considerable attention due to their unique physicochemical properties and potential applications in environmental remediation (Ahmad et al., 2024). These nanoparticles possess a high surface area and reactivity, enabling them to adsorb a wide range of contaminants, including heavy metals and metalloids like arsenic (K. Gupta, Joshi, Gusain, & Khatri, 2021). Their small size and tailored surface chemistry provide a platform for efficient interaction with arsenic species in the soil, reducing their bioavailability to plants (Riaz et al., 2020; Yang et al., 2020).

The use of iron oxide nanoparticles to mitigate arsenic toxicity aligns with the principles of green nanotechnology. By harnessing the potential of nanomaterials, researchers can design environmentally friendly solutions to pressing challenges (Imran & Alsayeqh, 2022). Biosynthesis, involving the use of biological entities like plant extracts and microorganisms, further enhances the eco-friendly nature of these nanoparticles' production (Bouafia & Laouini, 2021). Different type of phytochemicals are present in plants including flavonoids, terpenoids and phenols (Rahman et al., 2022). Biosynthesized Fe<sub>3</sub>O<sub>4</sub> NPs can be optimized for specific applications, offering a tailored approach to combating arsenic toxicity in rice plants (Bouafia & Laouini, 2021).

This work aims to investigate the capacity of iron oxide nanoparticles, specifically Fe<sub>3</sub>O<sub>4</sub> NPs, to alleviate arsenic toxicity in rice plants. This research intends to further our comprehension of

the intricate relationship between nanotechnology and plant biology by examining how nanomaterials interact with arsenic and influence plant physiological responses. Moreover, it aims to elucidate the mechanisms by which iron oxide nanoparticles can decrease the absorption and buildup of arsenic in rice plants, thereby offering potential for improving crop productivity and protecting human well-being.

The following sections of this work will explore the production of Fe<sub>3</sub>O<sub>4</sub> nanoparticles and their impact on rice plants. This paper analyzes the advantages and difficulties of incorporating nanotechnology into agriculture.

## Materials and Methods

### Synthesis of iron oxide nanoparticles

The synthesis of iron oxide nanoparticles was conducted using an adapted methodology from the study conducted by (Kanagasubbulakshmi & Kadirvelu, 2017). Aqueous solution of FeCl<sub>3</sub>·6H<sub>2</sub>O at a concentration of 0.01 M was mixed with plant extract (Moringa) in equal volumes. Following 60 minutes of stirring, the liquid was subsequently allowed to rest at ambient temperature for an additional 30 minutes, leading to the creation of a colloidal suspension. The suspension was subjected to centrifugation many times. The Fe<sub>3</sub>O<sub>4</sub>-NPs were dried at a temperature of 40 °C to obtain the final product.

### Experimental Design

Fe<sub>3</sub>O<sub>4</sub> nanoparticle solutions with varying concentrations (10, 20, and 50 ppm) and As<sub>2</sub>O<sub>3</sub> (10 ppm) were administered to seedlings that were positioned on petri plates (Khan et al., 2020). The seeds were let to undergo germination in a dark environment. The percentages of seed germination were meticulously documented over a span of 7 days. Additionally, the length of both the root and shoot were measured after a period of 7 days. The vigor index was computed using a specific formula.

vigor index (I) = germination percentage / seedling length (Barpete et al., 2015)

**Statistical Analysis**

The data underwent a one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test ( $p \leq 0.05$ ). The data was analyzed using the Statistix8 software.

**Results and Discussion**

**Seed Germination**

A controlled experimental setting was used to expose rice seedlings to different concentrations of

Fe<sub>3</sub>O<sub>4</sub> nanoparticles in order to assess their effect on shoot growth. The average germination percentages were as control (41%), 10 ppm (66%), 20 ppm (75%), and 50 ppm (79%). Seedlings subjected to 20 ppm and 50 ppm Fe<sub>3</sub>O<sub>4</sub> nanoparticles showed a substantial increase in germination % as compared to the control, with increments of 83% and 93% respectively. In contrast, the 10 ppm treatment resulted in a lower increase of 66%.

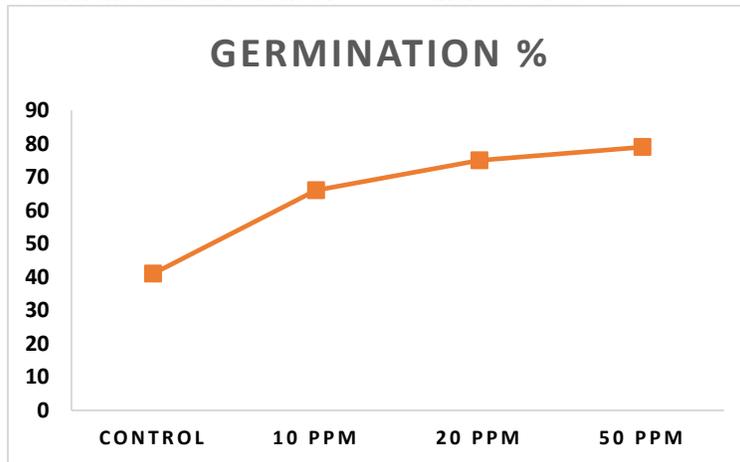


Figure 1: Seed germination % of rice seeds treated with various concentrations of nanoparticles and arsenic

**Shoot and Root Lengths**

The study assessed the influence of Fe<sub>3</sub>O<sub>4</sub> nanoparticles on the development of rice seedlings. The measured mean lengths of the shoots and roots were 1.06 cm and 2.57 cm, respectively, in the control group. Similarly, at a concentration of 10 ppm. The shoot length has

been extended to 2.06 cm, while the root length has been elongated to 3.3 cm. An increase in shoot length to 2.97 cm and root length to 3.4 cm was found at a concentration of 20 ppm. The highest dose, 50 ppm, resulted in a shoot length of 3 cm and root length of 3.73 cm.

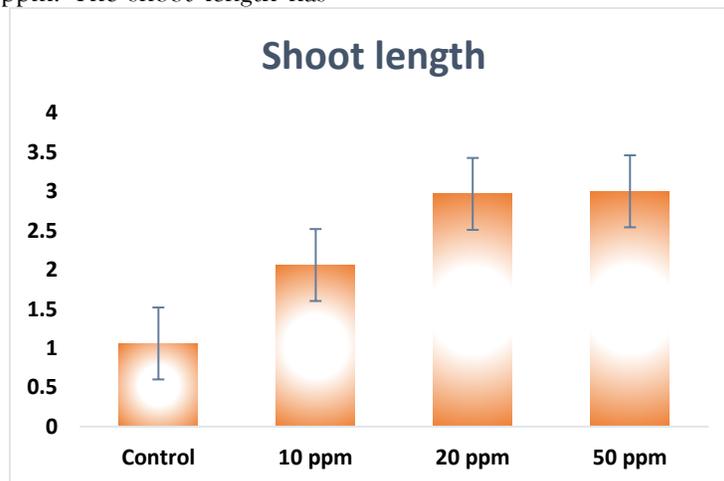


Figure 2: Shoot length of rice seedlings treated with various concentrations of nanoparticles and arsenic

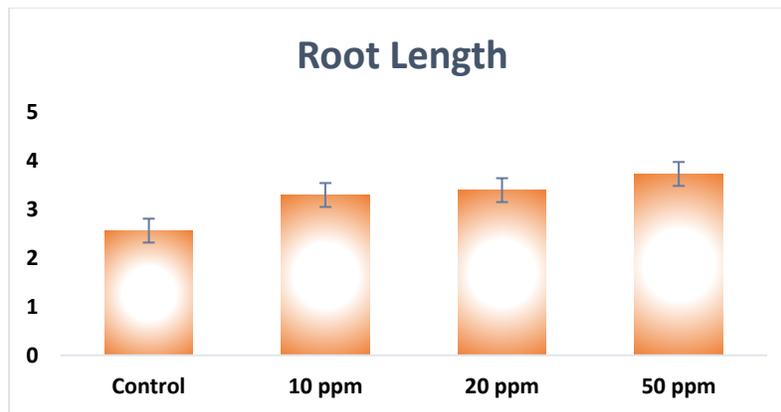


Figure 3: Root length of rice seedlings treated with various concentrations of nanoparticles and arsenic

**Seedling Biomass**

The experiment demonstrated that the use of Fe<sub>3</sub>O<sub>4</sub> nanoparticles at different concentrations resulted in a proportional increase in seedling biomass compared to the control group. The seedlings in the control group exhibited an average biomass of 0.07067 g. Seedlings subjected to a concentration of 10 parts per million (ppm) of

nanoparticles exhibited a marginal enhancement in biomass, reaching a value of 0.07333 grammes. At a concentration of 20 ppm, a more significant rise was noted, with an average biomass of 0.088 g, indicating improved growth. At a concentration of 50 ppm, the average biomass was 0.08967 g, suggesting that the upward tendency levels off at higher doses.

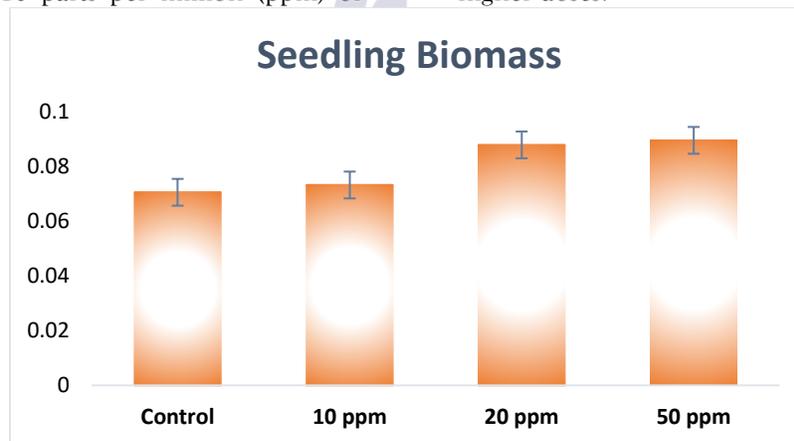


Figure 4: Seedling biomass of rice seedlings treated with various concentrations of nanoparticles and arsenic

**Seedling vigour**

Compared to the control group, seedlings exposed to a concentration of 10 ppm exhibited a decrease in vigour index, indicating a potentially harmful impact of this nanoparticle concentration on the initial growth of the seedlings. On the other hand,

seedlings that were exposed to 20 and 50 ppm showed increased vigour indices compared to the control group. This suggests that these concentrations have a beneficial effect on the growth and development of the seedlings.

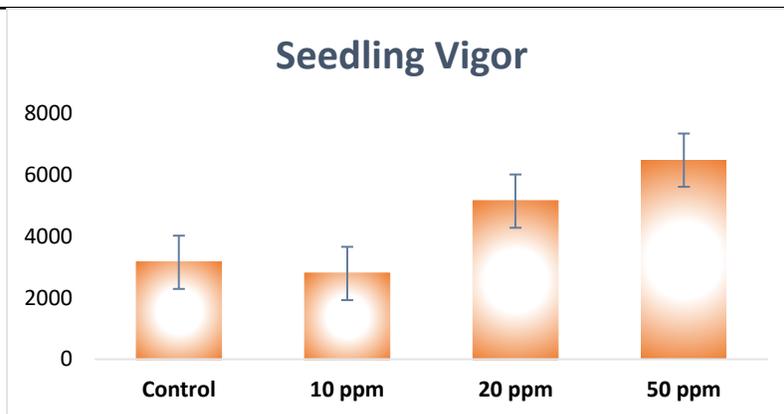


Figure 5: Seedling vigour of rice seedlings treated with various concentrations of nanoparticles and arsenic

### Discussion

This study aimed to investigate the effects of Fe<sub>3</sub>O<sub>4</sub> nanoparticles on rice seedling germination across varying concentrations. Our results revealed that increasing the concentration of Fe<sub>3</sub>O<sub>4</sub> nanoparticles positively influenced germination rates, with percentages rising from 41% in the control group to 66%, 75%, and 79% at 10 ppm, 20 ppm, and 50 ppm, respectively. These findings underscore the potential of Fe<sub>3</sub>O<sub>4</sub> nanoparticles to enhance seedling growth, with more pronounced effects observed at higher concentrations. Our results are in line with results of (Tovar, Briceño, Suarez, Flores, & González, 2020).

The shoot data demonstrate a positive correlation between the concentration of Fe<sub>3</sub>O<sub>4</sub> nanoparticles and the growth of both shoot and root lengths. The highest nanoparticle treatment (50 ppm) led to an almost threefold increase in shoot length and a 45% increase in root length compared to the control. The treatment with a concentration of 20 ppm exhibited the greatest shoot length, indicating a potential ideal concentration for promoting shoot growth. In contrast, the length of the roots continued to rise as the concentration increased.

Studies have demonstrated that Fe<sub>3</sub>O<sub>4</sub> nanoparticles have the ability to improve plant development. This effect was observed in wheat, where the application of a high concentration of nanoparticles resulted in higher plant growth, photosynthesis, and respiration. Additionally, it

led to an increase in the concentration of photosynthetic pigments in the leaves (Ahmad et al., 2024)(Feng et al., 2022). The observed elongation of shoot lengths in our experiment at 10 ppm and 50 ppm, as opposed to the control, can be attributed to this phenomenon.

The effect of Fe<sub>3</sub>O<sub>4</sub> nanoparticles also varies depending on their concentration. The research conducted on tomato plants shown that the application of 20 mg/L nano-Fe<sub>3</sub>O<sub>4</sub> resulted in a reduction of cadmium buildup and an enhancement of nutrient intake, thereby indicating a positive impact at certain concentrations (Rahmatizadeh et al., 2019). Our data demonstrate that shoot length was significantly increased in the 10 ppm and 50 ppm concentrations compared to both the control and the 20 ppm treatment. The observed correlation between greater concentrations of nanoparticles and increased root length is consistent with the results reported by Li et al. (2020), suggesting that Fe<sub>3</sub>O<sub>4</sub> nanoparticles have the ability to improve both shoot and root growth in plants.

Our findings indicate that Fe<sub>3</sub>O<sub>4</sub> nanoparticles have the potential to enhance the growth of seedlings, particularly at a concentration of 20 ppm. However, the growth benefits seem to plateau beyond this concentration. The observed increase in seedling biomass at low concentrations of Fe<sub>3</sub>O<sub>4</sub> nanoparticles is consistent with previous research indicating that these particles can promote plant growth through enhanced nutrient uptake (Rajput et al., 2018). Nevertheless, the

occurrence of the plateau effect at elevated concentrations aligns with the notion that an excess of nanoparticles could result in saturation or toxicity, hence restricting any additional growth advantages (Rico et al., 2011).

According to the literature, it has been found that moderate levels of nanoparticles can improve the process of seed germination and the growth of seedlings by increasing the absorption of water and the utilization of nutrients (Rico et al., 2011; Raliya et al., 2015). Nevertheless, lesser doses may not yield a satisfactory level of stimulation for enhancement, or could potentially disrupt regular physiological processes, resulting in diminished vitality (Rajput et al., 2018). Thus, the improved vigor indices at 20 and 50 ppm correspond with the idea of an optimal concentration window where Fe<sub>3</sub>O<sub>4</sub> nanoparticles stimulate growth more efficiently without phytotoxicity.

## Conclusions

Fe<sub>3</sub>O<sub>4</sub> nanoparticles at concentrations of 20 ppm and 50 ppm had a substantial positive impact on the growth of rice seedlings. This improvement was observed in various aspects such as germination, shoot and root length, and biomass. The significance of this phenomenon is particularly pronounced when considering the impact of arsenic stress, since these nanoparticles have the ability to alleviate its detrimental consequences. This study highlights the capacity of Fe<sub>3</sub>O<sub>4</sub> nanoparticles to improve the growth of rice seedlings in the presence of arsenic stress. However, additional research is required to determine the most effective dosages. Further investigation is warranted to examine the exact processes by which nanoparticles interact and determine the most effective dosage for promoting sustainable growth of rice seedlings under arsenic-induced stress.

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