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Article

Study on phytotoxicity of nano-iron oxide on germination of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method

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Abstract: The present study investigates the effect of nano-Fe_2O_3 particles as a micronutrient on the growth of plant seedlings of Mung (Vigna radiate) and Gram (Cicer arietinum) using the plant agar method. The laboratory experiment was carried out in the test units comprising a petri dish and plant agar medium. The plant agar medium used acts as a false soil that supports the seedlings for germination, prevents precipitation of water-insoluble nanoparticles and eliminates the possibility of any nutrients from external sources other than the nano-Fe_2O_3 particles. Each test unit containing agar media was treated with nano-Fe_2O_3 particle suspension at different concentrations. The effect of nano-Fe_2O_3 particle dose-response on root and shoot growth of the seedlings, as well as on biomass assay, was examined. The results showed that among the various concentrations of nano-Fe_2O_3 particles studied, a good agronomic value over control was observed only for certain optimum concentration i.e., 50 mg/L for mung and 5 mg/L for gram seedlings. Beyond this dose limit, the seedlings showed retardation in their growth characteristics, indicating phytotoxicity.

Keywords: nano-Fe_2O_3; Plant agar; germination; phytotoxicity; biomass; agronomic value

1. Introduction

Advancements in nanotechnology have proven it to be a most diverse and versatile field with promising applications in various branches of science and engineering. In recent years, considerable attention has been directed towards the exploration and utilization of nanomaterials in agriculture, particularly for enhancing plant growth characteristics, disease control, increasing overall crop productivity, optimizing soil health and performance [1-8]. Despite these promising prospects, the question of whether nanomaterials exert predominantly beneficial or potentially harmful effects on plants remains unsolved and continues to be an active area of scientific investigation. To address this, several studies have been undertaken in this direction to understand the effect of different nanomaterials on the seed germination and growth response of a variety of plant species. Existing literature extensively documents research focused on the uptake, accumulation and translocation of nanomaterials within plant systems along with their potential agronomic benefits, physiological impacts and associated phytotoxic effects [9-15].

Among the wide range of engineered nanomaterials, nano-Fe_2O_3 has attracted significant attention due to its extensive use in various industrial sectors, environmental remediation, catalysis and electronics [16]. However, its potential in agriculture, particularly as an agrochemical, is scarcely reported in the literature. Iron is an essential micronutrient that plays a vital role in plant growth and productivity. It is fundamentally involved in key physiological and biochemical processes such as photosynthetic reaction involving chlorophyll production, synthesis of RNA, regulation of hormone biosynthesis, carbohydrates, certain enzyme reactions, growth rate and productivity [17-23]. Several studies have also been reported on the exceptional abilities of nano iron oxide in improving crop growth, enhancing yield attributes and health, micronutrient delivery and contaminant scavenging [24-27]. In recent years, new findings on the potential use of nano-Fe_2O_3 as a micronutrient studied on the germination of a variety of seeds have been reported. For example, nano-iron oxide has been reported to improve seed germination, growth, antioxidant response and nitrogen metabolism of aromatic rice [28]. Green-synthesized nano-Fe_2O_3 has been found to enhance seed germination efficiency of basil (*Ocimum basilicum* L.) cultivars [29]. Hydroponically grown *V. radiata* plants showed enhanced growth and photosynthesis with nano-Fe_3O_4, by boosting chlorophyll and activating antioxidants [30]. In contrast, one study has reported that Fe_2O_3 nanoparticles had little effect on seed germination but impaired root development, chloroplast structure, and photosynthesis in *Kobresia capillifolia* (Decne.) C.B.Clarke seedlings [31]. These nanoparticles induced oxidative stress, reduced biomass, and disrupted nutrient translocation, with roots being more strongly affected than leaves. Collectively, these findings strongly suggest the potential application of nano iron oxide in the agriculture industry.

Although some studies have recently been reported exploring the potential of nano iron oxide as a micronutrient on certain plant systems, to the best of the author's knowledge, very few studies specific to its effect on mung and gram plants have been reported so far. Therefore, the present study aims to investigate the effect of nano-Fe_2O_3 particle suspensions applied as micronutrients on the growth characteristics of mung and gram seedlings using the "plant agar method". Particular emphasis is given on examining the concentration-dependent effects of nano-Fe_2O_3 particles on seed germination behavior and early seedling growth kinetics while addressing potential phytotoxicity associated with the application of this nanomaterial.

2. Materials and methods

2.1. Nano-Fe_2O_3 particles characterization

All the characterizations of nano-iron oxide were carried out at the centralized facility of the Indian Institute of Technology Bombay. The nano-Fe_2O_3 particles used in this study were obtained from I-CanNano^{TM} (Kolkata, India). The reported surface area of the as received sample was 35–40 m^2g^{-1}. Particle size distribution and morphological features of nano-Fe_2O_3 particles were examined using Transmission Electron Microscopy (TEM, Philips CM200 electron microscope, Eindhoven, Netherland, located at Sophisticated Analytical Instrument Facility, IIT Bombay). The crystalline nature of the nanoparticles was verified using X-ray diffraction analysis (XRD, X'Pert Pro Philips, PANalytical, Netherlands, located at Department of Metallurgical Engineering and Materials Science, IIT Bombay). In addition, Fourier transform infrared spectroscopy (FTIR, Nicolet Magna 550 FT-IR spectrometer, Nicolet Instrument Corporation (USA), located at Department of Metallurgical Engineering and Materials Science, IIT Bombay) was employed to confirm the chemical purity of the nanoparticle.

2.2. Preparation of nano-Fe_2O_3 particle suspension

A nano-Fe_2O_3 particle suspension of varying weight-based concentration was prepared by dispersing the required amounts of nanoparticles in deionized water. The dispersion process involved initial mixing with a mechanical stirrer, followed by ultrasonication for 30 min using a probe-type ultrasonicator (Misonix Incorporated, New York, located at IIT Bombay Nanofabrication Facility (IITBNF)) with a power of 100 W operating at a frequency of 40 kHz. The resulting well-dispersed nanoparticle suspensions were subsequently used for all experimental investigations.

2.3. Germination of seeds

Seeds of mung (*Vigna radiata*) and gram (*Cicer arietinum*) used in the present investigation were obtained from a local source and stored under dry, dark conditions at room temperature until experimentation. Prior to use, the seeds were surface sterilized to eliminate microbial contamination by immersing them in 5% sodium hypochlorite solution for 10 min. This was followed by repeated washing with deionized water to ensure complete removal of any residual sterilizing agent. The germination of these sterile mung and gram seeds was then initiated by placing them on moistened cotton in petri dishes and incubating them in the dark at a controlled temperature of 25 ± 1 °C for a period of 24 h. Germination of the seeds was assessed, and the sprouted seeds were taken for further study [32].

2.4. Plant agar method

The experiment was performed using sterile Petri dishes (87 mm × 18 mm) containing 30 mL of dual-layer agar culture media. Defined concentrations of nano-Fe_2O_3 particle suspensions were incorporated into the dishes, with treatments for mung seeds including a control and 10, 20, 50, 100, 500, 1000, and 2000 mg/L, while gram seeds received a control and 5, 10, 20, 50, 100, 500, 1000, and 2000 mg/L. Initially, 20 mL of a 2.5% agar solution was poured into each Petri dish, followed by the appropriate nano-Fe_2O_3 concentration. To minimize nanoparticle agglomeration, the media were rapidly solidified in a freezer. Subsequently, a second uniform agar layer was created by gently adding 10 mL of 1% agar solution over the solidified base layer [32].

Ten seedlings were carefully positioned on the surface of the agar in each prepared Petri dish. The dishes were then incubated in darkness at 25 ± 1 °C. Three replicates were established for each nano-Fe_2O_3 concentration, and seedlings were allowed to grow for 60 h. The exposure duration for seed germination of mung bean (*Vigna radiata*) and gram (*Cicer arietinum*) was determined based on our preliminary experiments conducted using the Petri dish method. Further, short-term germination assays conducted during the first week of seedling development (typically within 3–7 days) widely accepted method for assessing nanoparticle phytotoxicity, as early germination and seedling growth are highly sensitive indicators of toxic effects [32]. At the end of the exposure period, seedlings were removed and their growth was measured using a ruler. Control treatments consisted of agar media without nano-Fe_2O_3. Representative Petri dish setups for mung and gram seedlings are shown in **Figures 1 and 2**, respectively. For biomass analysis, treatments exhibiting the maximum and minimum seedling growth relative to the control were selected.

2.5. Biomass assay

After the designated growth period, seedlings were collected and thoroughly rinsed with deionized water. The roots and shoots were carefully separated, and their fresh weights were recorded immediately. To determine dry biomass, the separated plant parts were oven-dried at 70 °C for 24 h and weighed repeatedly until a stable mass was achieved. All treatments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical evaluation was carried out using Student's *t*-test, with differences considered statistically significant at $p < 0.05$.

(**a**) Control (**b**) 50 mg/L (**c**) 2000 mg/L

Figure 1. Petri Dish Test Units Showing Mung Seedlings Placed on Dual Agar Culture. (**a**) Control and with added Doses of (**b**) 50 mg/L and (**c**) 2000 mg/L nano-Fe_2O_3 Particle Suspension.

(**a**) Control (**b**) 50 mg/L (**c**) 2000 mg/L

Figure 2. Petri Dish Test Units Showing Gram Seedlings Placed on Dual Agar Culture. (**a**) Control and with added Doses of (**b**) 5 mg/L and (**c**) 2000 mg/L nano-Fe_2O_3 Particle Suspension.

3. Results and discussion

3.1. Characterization of nano-Fe_2O_3 particles

Figure 3 presents a transmission electron microscopy (TEM) image of nano-Fe_2O_3 powder illustrating its nanoscale morphology and revealing that the particles have an average size range of approximately 10–30 nm. **Figure 4** shows the X-ray diffraction pattern of nano-Fe_2O_3 powder. The diffraction peaks correspond to specific crystal planes of Fe_2O_3, indicating the high structural integrity of the nanoparticles. Furthermore, **Figure 5** shows the Fourier-transform infrared (FTIR) spectra of the nano-Fe_2O_3 powder, highlighting the chemical functional groups present on the particle surface. The spectra exhibit distinct absorption bands at around 561 cm^{-1} and 472 cm^{-1}, which can be attributed to the characteristic Fe–O vibrational modes. In addition, a broad absorption band observed near 3400 cm^{-1} indicates the existence of hydroxyl (–OH) groups on the surface of the nanoparticles, suggesting surface-adsorbed water [33]. Collectively, the TEM, XRD, and FTIR analyses provide a comprehensive characterization of nano-Fe_2O_3, confirming both its nanoscale morphology and its crystalline and chemical properties.

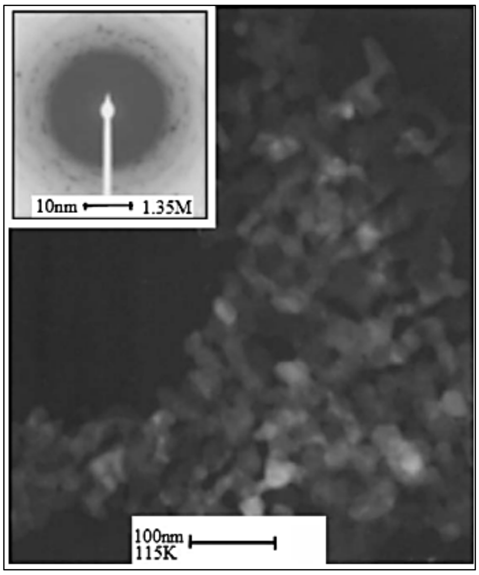


Figure 3. TEM Photograph of nano-Fe_2O_3 Particles with its Diffraction Pattern.

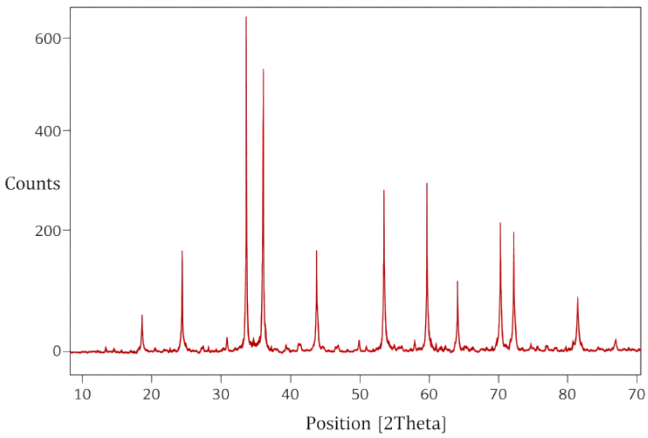


Figure 4. XRD Pattern of nano-Fe_2O_3 Particles.

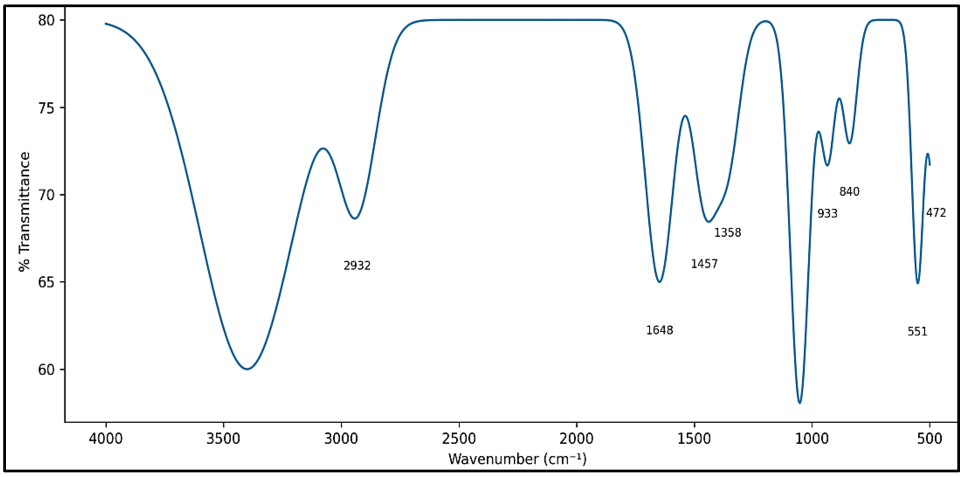


Figure 5. FTIR Spectra of nano-Fe_2O_3 Powder.

3.2. Effect on root length and shoot length of seedlings

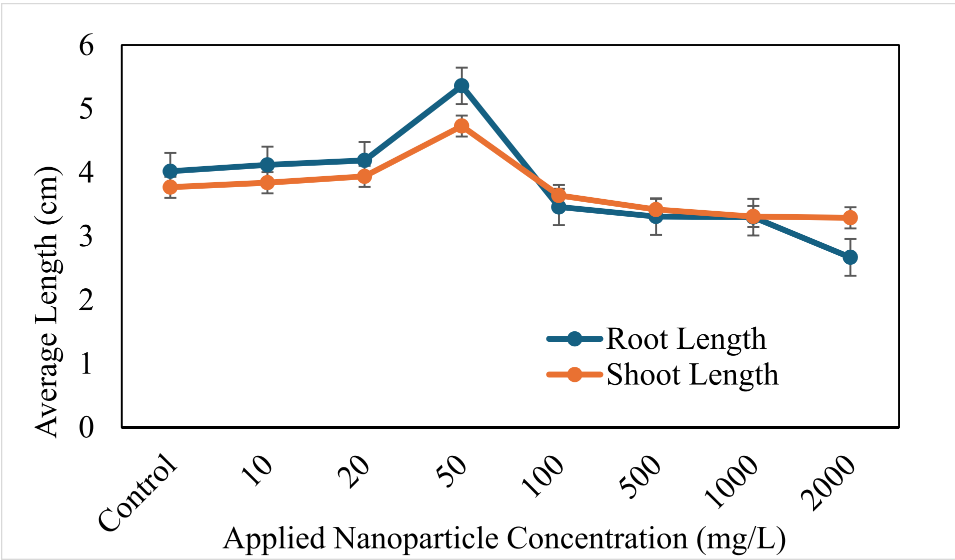
The root and shoot growth responses of mung and gram seedlings to varying concentrations of nano-Fe_2O_3 particle suspensions, along with the corresponding dose-response curves, are presented in **Figures 6 and 7**, respectively. For the construction of these dose-response curves, the reported values represent the mean ± standard deviation (SD) calculated from three replicates, with each replicate containing ten seeds. In the case of mung seedlings, the treatment with 50 mg/L nano-Fe_2O_3 exhibited the most favorable growth performance (**Figure 6a**). At this optimal concentration, hypocotyl length increased by 25.46%, while root length was enhanced by 33.33% relative to the control seedlings. However, when the concentration exceeded this level, seedling growth was markedly inhibited. For instance, treatment with 2000 mg/L nano-Fe_2O_3 resulted in a decrease of 12.73% in hypocotyl length and 33.58% in root length compared to the control, indicating that excessively high nanoparticle concentrations can exert toxic effects on mung seedling development (**Figure 7a**).

A similar trend was observed for gram seedlings, although the optimal concentration differed. The best growth response was recorded at 5 mg/L nano-Fe_2O_3, where shoot length increased by 3.60% and root length by 46.51% compared to the control seedlings (**Figure 6b**). Increasing the nanoparticle concentration beyond 5 mg/L led to substantial growth inhibition. Specifically, treatment with 2000 mg/L nano-Fe_2O_3 caused reductions of 54.05% and 58.95% in shoot and root lengths, respectively, relative to untreated seedlings. These findings clearly demonstrate that both mung and gram seedlings exhibit a concentration-dependent response to nano-Fe_2O_3, with growth promotion occurring only within a specific dose range. Beyond this optimal range, higher nanoparticle concentrations induce toxicity, resulting in significant suppression of root and shoot development (**Figure 7b**). This pattern highlights the importance of careful optimization of nanoparticle dosage for promoting early seedling growth without triggering detrimental effects.

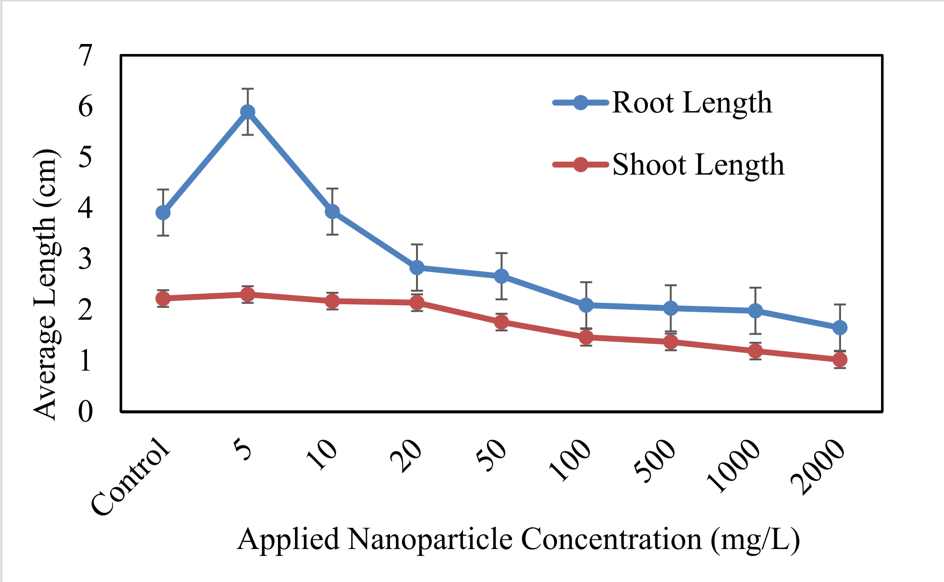
(**a**) Mung Seedlings (Control ⟶ 2000 mg/L)

(**b**) Gram Seedlings (Control ⟶ 2000 mg/L)

Figure 6. Root and Shoot Growth of (**a**) Mung Seedlings and (**b**) Gram Seedlings as a Function of Applied Concentration of nano-Fe_2O_3 Particle Suspension.



(**a**)



(**b**)

Figure 7. Dose-response Curves of nano-Fe_2O_3 Particle on Root and Shoot of (**a**) Mung Seedlings and (**b**) Gram Seedlings.

3.3. Biomass analysis

3.3.1. Wet biomass

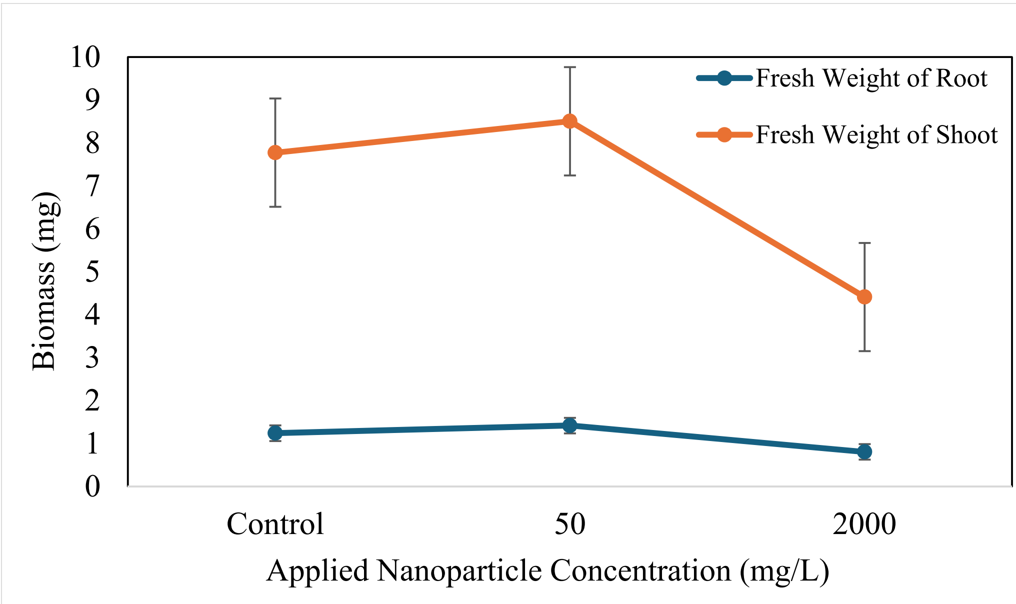
Mung seedlings exposed to 50 mg/L of nano-Fe_2O_3 exhibited a notable increase in biomass. At this concentration, root length and hypocotyl length increased by 33.33% and 25.46%, respectively. Biomass measurements from the same treatment group showed increases of 14.24% in root biomass and 9.39% in shoot biomass compared with the control seedlings, indicating a positive stimulatory effect of nanoparticles at the optimal concentration. However, when the concentration of nano-Fe_2O_3 was raised to 2000 mg/L, the root length and hpocotyl length decreased by 33.58% and 12.73%, respectively and a significant inhibitory effect on biomass was observed, with root biomass declining by 34.98% and shoot biomass decreasing by 43.23% relative to the control, demonstrating the onset of toxicity at higher nanoparticle doses (**Figure 8a**).

Similarly, gram seedlings displayed a dose-dependent response to nano-Fe_2O_3 treatment. The lower concentration of 5 mg/L showed the most favorable growth response, with shoot length and root length increasing by 3.60% and 46.51%, respectively, compared with the control seedlings. Biomass measurements from the same treatment group showed corresponding increases of 26.08% in shoot biomass and 17.99% in root biomass, indicating a stimulatory effect of nano-Fe_2O_3 at this optimal concentration. However, when the nanoparticle concentration was increased to 2000 mg/L, substantial growth inhibition was observed, with shoot and root lengths decreasing by 54.05% and 58.95%, respectively, and shoots and root biomass declining by 53.80% and 36.07% relative to the control seedlings, demonstrating toxicity at higher nanoparticle doses (**Figure 9a**). These results collectively indicate that both mung and gram seedlings respond positively to nano-Fe_2O_3 only within a narrow, low-dose range, while higher concentrations exert toxic effects that markedly suppress the accumulation of both root and shoot biomass. This clearly emphasizes the critical importance of optimizing nanoparticle concentrations for promoting seedling growth without causing detrimental impacts.

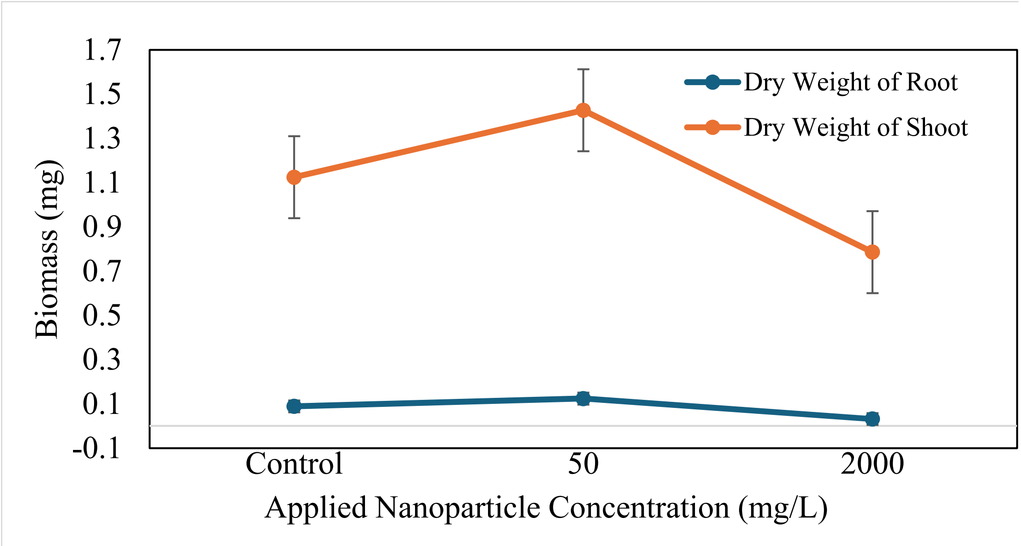
3.3.2. Dry biomass

Dry matter yield serves as a critical indicator for assessing the overall nutrient content and its total uptake by plants. Therefore, the dry matter yield of roots and shoots was recorded separately. The result showed that for Mung seedlings treated with 50 mg/L of nano-Fe_2O_3, there was a substantial enhancement of biomass, with shoot dry matter increasing by 26.91% and root dry matter showing an even greater improvement of 49.81% compared to the control seedlings. This indicates that moderate levels of nano-Fe_2O_3 can significantly stimulate growth and biomass accumulation in mung seedlings. However, exposure to a much higher concentration of 2000 mg/L led to a pronounced reduction in dry matter, with shoot mass declining by 30.09% and root mass decreasing by 61.39% relative to the reference seedlings, demonstrating that excessive nanoparticle concentrations can exert toxic effects and markedly inhibit plant growth (**Figure 8b**).

A similar trend was observed in gram seedlings, although the optimal and inhibitory concentrations differed slightly. At a low concentration of 5 mg/L nano-Fe_2O_3, gram seedlings exhibited considerable improvements in dry matter accumulation, with shoot dry matter increasing by 38.79% and root dry matter by 34.39% over the untreated control. This enhancement indicates that a relatively small dose of nano-Fe_2O_3 is sufficient to promote nutrient uptake and stimulate growth in gram seedlings. Conversely, treatment with the highest tested concentration of 2000 mg/L resulted in a significant reduction in biomass, with shoot mass decreasing by 31.54% and root mass declining by 52.89% relative to the control seedlings (**Figure 9b**).

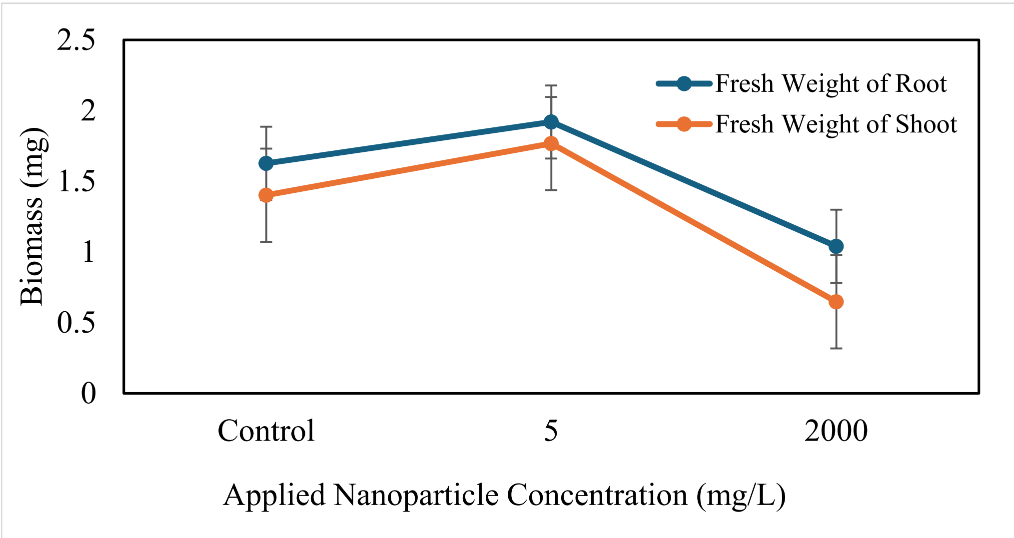


(a)

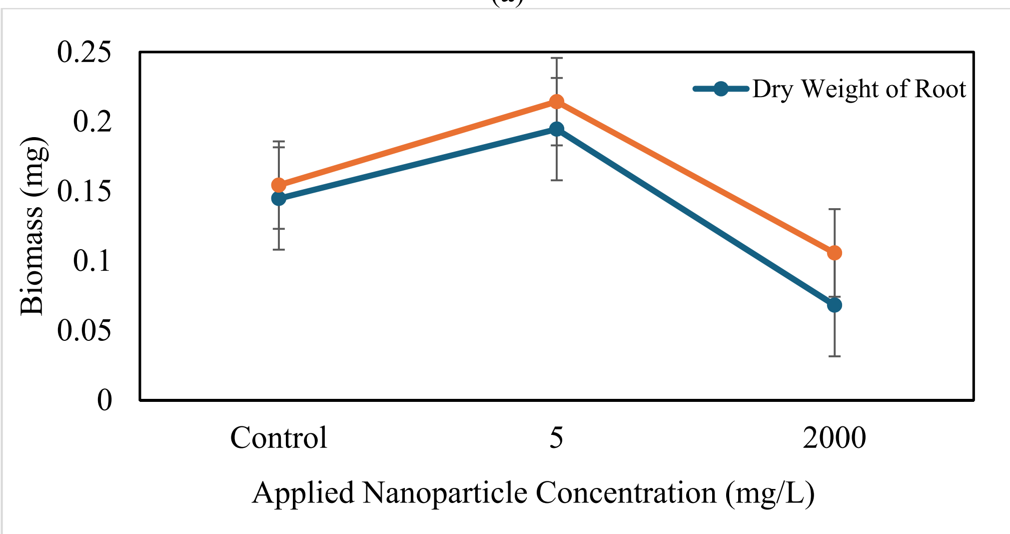


(**b**)

Figure 8. Biomass Assay of Mung Seedlings (**a**) Wet Biomass (**b**) Dry Biomass.



(**a**)



(**b**)

Figure 9. Biomass Assay of Gram Seedlings (**a**) Wet Biomass (**b**) Dry Biomass.

4. Discussion

Based on a comprehensive evaluation of growth response parameters and biomass accumulation assays conducted across a range of nano-Fe_2O_3 concentrations, the present findings clearly indicate that the physiological and developmental effects of iron oxide nanoparticles on plant systems are highly concentration-dependent and are further modulated by particle size and the mode of application. The differential response observed among test species suggests that nano-Fe_2O_3 does not exert a uniform effect but instead interacts dynamically with plant metabolic and cellular processes. In the current investigation, the most pronounced stimulatory effects were recorded at a concentration of 50 mg/L for mung (*Vigna radiata*) seedlings and at 5 mg/L for gram (*Cicer arietinum*) seedlings. This enhanced performance at lower to moderate concentrations can be largely attributed to the nanoscale dimensions of the particles employed, which substantially increase their surface area–to–volume ratio, chemical reactivity, and bioavailability, thereby facilitating more efficient interaction with plant tissues.

A substantial body of literature corroborates these observations, reporting improvements in agronomic traits as a direct function of nanoparticle size and dose-dependent responses of nano-Fe_2O_3 particles across multiple plant species. These beneficial effects have been documented from the earliest stages of plant development, including seed germination, root initiation, nutrient uptake, and subsequent translocation within plant tissues. One of the common observations supporting the positive effect of nano-Fe_2O_3 in various plants is attributed to its enzymatic peroxidase-like activity responsible for various growth, photosynthesis, antioxidant activity, reduced heavy metal toxicity and improving tolerance to abiotic stresses such as drought and nutrient limitation [34-40].

Furthermore, previous investigations have identified an optimal particle size range of approximately 20–40 nm for nano-Fe_2O_3 to exert maximum positive effects on seed germination rate, root elongation, shoot development, chlorophyll biosynthesis, and overall biomass production. This size range closely corresponds to the particle dimensions utilized in the present study, thereby providing strong experimental support for the observed enhancement in mung and gram seedling growth [41,42]. A recent study on pea, mung bean, wheat, and barley seeds demonstrated that Fe_3O_4 nanoparticles significantly affect germination, early seedling growth, and fungal infection in a species-specific manner under microwave-sterilised conditions. The findings suggest that optimally applied nano-Fe_3O_4 has strong potential as an effective seed-priming agent to improve early crop performance in controlled environments [43]. Another study confirms that α-Fe_2O_3 nanoparticles with a size range of 30–67 nm are effective in promoting okra seed germination and early seedling growth. An application concentration of 0.5 mM was found to be optimal, resulting in significantly higher germination, enhanced root and shoot elongation, and increased fresh biomass compared to untreated controls. These results indicate that moderately sized nano-Fe_2O_3 applied at low concentrations provides the most beneficial growth response [44]. Specifically, one of the studies exploring the effect of nano-Fe_2O_3 on sunflower seed showed that smaller Fe_2O_3 nanoparticles (4.5 nm) significantly inhibited germination speed, indicating a negative impact at very small dimensions, likely due to increased reactivity and stress induction. In contrast, relatively larger Fe_2O_3 nanoparticles (16.7 nm) exhibited a comparatively less inhibitory effect, demonstrating that nanoparticle size plays a critical role in determining the biological response of seeds to iron oxide exposure [45].

In addition to particle size, dose level is a critical determinant of biological response. Several reports have demonstrated that appropriate concentrations of nano-Fe_2O_3 significantly improve germination efficiency, early vegetative growth, and root biomass relative to untreated controls in crops such as wheat [24,26], *Quercus macdougallii* [36], *Carum copticum* L. [46] and maize [47]. Consistent with the findings of the present study, a nano-Fe_3O_4 concentration of 150 mg/L was identified as the optimal dose for mung seedlings, resulting in enhanced shoot elongation and the highest fresh and dry biomass accumulation [48]. These findings collectively reinforce the positive influence of nano-Fe_2O_3 on mung and gram seedlings as observed in the present investigation. The reported enhancement in growth and biomass accumulation is also indicative of effective uptake and internal translocation of nano-Fe_2O_3 particles through plant root systems, most likely via the apoplastic pathway, allowing their movement into cortical and vascular tissues [49-53]. This also supports and explains the observed increase in biomass accumulation by the mung and gram seedlings in the present study.

The phytotoxicity observed at a concentration above 50 mg/L for mung seedlings, and 5 mg/L for gram seedlings is consistent with the data reported in literature, where phytotoxicity of nano-Fe_2O_3 above 100 mg/L for different plant systems was observed [53,54]. Another recent study showed that exposure to nano-zero valent iron (nZVI) severely inhibited mung bean germination and growth, with nZVI showing the strongest phytotoxic effects. The toxicity is linked to excessive iron accumulation, oxidative stress, and impaired photosynthesis, highlighting the need for careful modification and dosage control [55]. Further, the results obtained in the present study aligns with the similar results presenting beneficial effects on seed germination, root elongation at certain dose levels and associated phytotoxicity at higher dose levels for mung (*Vigna radiata*) [56,57] and gram (*Cicer arietinum*) [58,59] seedlings has been observed in separate studies validating the potential use of nano-Fe_2O_3 particles as an efficient fertilizer supplement or micronutrient source in sustainable agricultural practices, provided that optimal dose thresholds are carefully defined and adhered to for specific crop systems.

The growth-promoting or inhibitory effects of nano-Fe_2O_3 on mung and gram seedlings can be explained through several interconnected physicochemical and biochemical mechanisms reported in the literature. Due to their nanoscale dimensions and high surface reactivity, Fe_2O_3 nanoparticles can adhere to the seed coat and root epidermis, facilitating entry via apoplastic and symplastic pathways [49,60]. During seed imbibition, nano-Fe_2O_3 has been shown to enhance water uptake and accelerate germination by modifying seed coat permeability and stimulating early metabolic activity [61]. Once internalized, nanoparticles may remain localized in root cortical tissues or undergo partial dissolution, releasing Fe^{3+}/Fe^{2+} ions that are transported through the xylem to aerial tissues [62]. The released iron improves micronutrient availability, supporting chlorophyll biosynthesis, photosynthetic electron transport, and enzymatic reactions involved in respiration and nitrogen assimilation [63,64]. At optimal concentrations, nano-Fe_2O_3 can enhance root surface area and permeability, facilitating improved nutrient uptake and stimulating root elongation [20,60]. Additionally, optimal concentration of nano-Fe_2O_3 induces controlled production of reactive oxygen species (ROS), which function as signaling molecules, activating antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidases, thereby promoting stress tolerance and growth [65,66]. However, at higher concentrations, excessive ROS generation through Fenton-type reactions may disrupt membrane integrity, impair mitochondrial function, and inhibit cell division, leading to growth suppression [67,68]. Additionally, nanoparticle exposure may influence phytohormonal balance, including gibberellins and auxins, promoting root elongation and cell division [69]. However, these responses are concentration dependent; excessive nanoparticle accumulation can result in oxidative stress, lipid peroxidation, membrane damage, and inhibition of mitotic activity, ultimately suppressing seedling growth [1]. Studies on nano-zero valent iron (nZVI) exposure in mung bean further confirm significant reductions in germination indices and seedling growth at high doses, accompanied by strong upregulation of antioxidant defense systems indicative of oxidative stress toxicity [55]. Collectively, these findings support that nano-Fe_2O_3-seedling interactions are governed by a balance between enhanced micronutrient availability and oxidative stress-mediated toxicity, resulting in improved germination and growth at optimal concentrations but phytotoxicity at higher levels.

It is noteworthy that the optimal nano-Fe_2O_3 dose above which phytotoxic effect was observed in gram seedlings was nearly ten times lower than for Mung seedlings. The lower optimal concentration observed for Gram (*Cicer arietinum*) compared with Mung bean (*Vigna radiata*) may arise from species-specific differences reported in nanomaterial–plant interaction studies. Responses of leguminous plants to metal-oxide nanoparticles vary widely among species due to differences in seed size, seed coat permeability, root architecture, rhizosphere chemistry, iron uptake mechanism and oxidative stress tolerance [70,43]. In legumes such as Gram, iron nanoparticles have been reported to enhance nodulation and nitrogen fixation at lower concentrations because of higher uptake efficiency. This may explain the observed species–specific dose differences, where Mung requires higher nanoparticle concentrations to achieve comparable physiological responses [71]. However, further mechanistic studies are needed to confirm these differences.

5. Conclusion

The exposure of nano-Fe_2O_3 particles at varying concentrations has been found to affect the root/shoot growth and biomass assay of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings. Based on the additive concentration, a positive or negative effect was observed. A beneficial effect was found at 50 mg/L for mung and 5 mg/L for gram seedlings, beyond which the seed germination and growth were inhibited. It is noteworthy that accumulation and uptake of nano-Fe_2O_3 particles by the root's dependents on the exposure concentrations. Therefore, nano-Fe_2O_3 used at a certain optimal concentration might be ideal as an agrochemical. However, extensive field studies are to be conducted to thoroughly assess and understand the translocation of nano-iron oxide into plant tissues, its effect on crop yield and the potential threat to the environment and food security.

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