

## Biofortification of *Oryza Sativa* L. using Iron Oxide Nanoparticles for Improved Germination, Growth, and Yield

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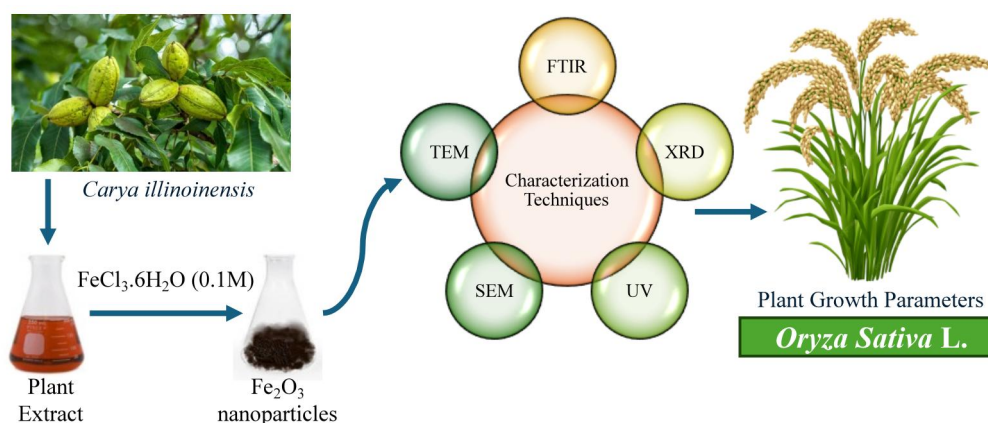
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## ABSTRACT

This study explores the synthesis, characterization, and agricultural applications of iron oxide nanoparticles ( $\text{Fe}_2\text{O}_3$  NPs) for biofortification of *Oryza sativa* L. (rice).  $\text{Fe}_2\text{O}_3$  NPs were synthesized using a plant-based method with *Carya illinoensis* leaf extract and characterized by various techniques. UV-vis spectrophotometry confirmed the successful synthesis of nanoparticles, with an absorption peak around 371 nm, indicating the formation of  $\text{Fe}_2\text{O}_3$  NPs. X-ray diffraction (XRD) analysis revealed the crystalline nature of the nanoparticles, with peaks corresponding to the  $\alpha\text{-Fe}_2\text{O}_3$  phase, confirming high crystallinity and minimal impurity. Fourier Transform Infrared Spectroscopy (FTIR) showed functional groups such as hydroxyl and carbonyl bands, indicating potential interactions between the plant extract and  $\text{Fe}_2\text{O}_3$  NPs. Scanning Electron Microscopy (SEM) images revealed uniform, spherical nanoparticles with a smooth surface morphology, TEM confirmed the presence of iron in the synthesized nanoparticles. In agricultural trials,  $\text{Fe}_2\text{O}_3$  NPs at 100 ppm significantly enhanced rice growth, with increased shoot and root lengths, biomass accumulation, and iron content in seeds. The grain yield and seed iron content were notably higher at 150 ppm, suggesting the potential of  $\text{Fe}_2\text{O}_3$  NPs for biofortification and improved crop productivity.

**Keywords:** Iron oxide nanoparticles; characterization; biofortification; rice growth; improved crop productivity.



## *Figure 1: Schematic Abstract*

### 1. Introduction

Among the various nanomaterials, iron oxide nanoparticles ( $\text{Fe}_2\text{O}_3$  NPs) have garnered interest due to their unique physicochemical properties, including high surface area, stability, biocompatibility, and ability to release essential nutrients in a controlled manner [1]. Iron is an essential micronutrient for plants and plays a crucial role in various physiological processes, including chlorophyll synthesis, photosynthesis, and nitrogen fixation [2]. However, iron deficiency is a common problem in many soils, particularly in regions where rice is grown, leading to poor crop yields and reduced nutritional content in rice grains [3]. Biofortification is a promising strategy for improving the nutritional content of staple crops, and the use of nanoparticles such as  $\text{Fe}_2\text{O}_3$  NPs offers a novel approach to enhancing the iron content in rice seeds while simultaneously improving growth and yield [4]. Iron oxide nanoparticles are known to have a wide range of applications in various fields, including catalysis, environmental remediation, and biomedical sciences, owing to their unique magnetic properties and high surface reactivity [5]. In the agricultural context, these nanoparticles can interact with plant roots and promote nutrient uptake by enhancing the solubility of micronutrients in the soil [6].  $\text{Fe}_2\text{O}_3$  NPs can also stimulate plant growth by improving photosynthetic efficiency and regulating plant hormones, thus promoting better growth and development [7]. Several studies have reported the beneficial effects of  $\text{Fe}_2\text{O}_3$  NPs on plant growth, particularly in crops such as wheat, maize, and soybeans [8]. These studies have shown that  $\text{Fe}_2\text{O}_3$  NPs can enhance seed germination, root and shoot development, biomass accumulation, and overall plant health [8]. Furthermore,  $\text{Fe}_2\text{O}_3$  NPs have been found to improve the bioavailability of essential nutrients, including iron, which is crucial for addressing iron deficiency in plants [9].

Rice (*Oryza sativa* L.) is one of the most important staple crops in the world, providing sustenance to billions of people [10]. Despite its global importance, rice cultivation is often limited by nutrient deficiencies, particularly in soils that are iron-deficient [11]. Iron plays a vital role in rice plant growth and development, and its deficiency can lead to poor seedling growth, reduced grain yield, and lower

nutritional content in rice grains [12]. Biofortification of rice with iron is therefore a promising approach to addressing iron deficiency in both plants and humans [13]. Traditionally, biofortification has been achieved through agronomic practices such as soil fertilization with iron compounds [14]. However, the use of nanoparticles in agriculture offers a more efficient and controlled method for delivering nutrients to plants, ensuring their optimal uptake and improving crop quality [15]. Nanotechnology-based approaches for biofortification are increasingly being explored as alternatives to conventional methods, as they offer the potential for enhanced nutrient delivery and improved plant performance [16]. In particular, the use of iron oxide nanoparticles for the biofortification of rice has shown promising results [17]. The unique properties of Fe<sub>2</sub>O<sub>3</sub> NPs, such as their ability to be readily absorbed by plant roots and their ability to release iron ions in a controlled manner, make them an ideal candidate for biofortification applications [9]. Moreover, Fe<sub>2</sub>O<sub>3</sub> NPs have been shown to have minimal toxicity to plants, which is essential for their safe application in agriculture [18]. Several studies have highlighted the role of Fe<sub>2</sub>O<sub>3</sub> NPs in improving seed germination, plant growth, and yield in various crops [19], but the impact of Fe<sub>2</sub>O<sub>3</sub> NP treatment on the germination, growth, and yield of rice remains underexplored [18]. Despite the promising potential of Fe<sub>2</sub>O<sub>3</sub> NPs in agricultural applications, there are still several gaps in the current understanding of their effects on plant growth, biomass accumulation, grain yield, and seed nutritional content [20]. While many studies have demonstrated the positive effects of nanoparticles on plant growth, the optimal concentrations, mechanisms of action, and potential toxicity of Fe<sub>2</sub>O<sub>3</sub> NPs in rice cultivation remain unclear [21]. There is also a lack of comprehensive studies that investigate the effects of Fe<sub>2</sub>O<sub>3</sub> NPs on the iron content of rice seeds and their biofortification potential [22]. Furthermore, the long-term effects of nanoparticle application on soil health and plant sustainability are not fully understood, which raises concerns about the environmental impact of nanoparticle use in agriculture [23].

The objective of this study is to investigate the effect of Fe<sub>2</sub>O<sub>3</sub> NPs on the germination, growth, and yield of rice (*Oryza sativa* L.), focusing on their potential for biofortification by increasing the iron content in rice seeds. This research aims to

explore the concentration-dependent effects of Fe<sub>2</sub>O<sub>3</sub> NPs on plant growth, biomass characteristics, grain yield, and seed iron content, providing valuable insights into the potential of Fe<sub>2</sub>O<sub>3</sub> NPs as a biofortification tool for improving crop nutrition and addressing iron deficiency in rice. This study will also evaluate the impact of Fe<sub>2</sub>O<sub>3</sub> NP treatment on seedling health, which is crucial for optimizing crop productivity and ensuring sustainable agricultural practices [21].

## 2. Experimental

### 2.1 Materials and Reagents

Deionized water, pH meter, iron chloride hexahydrate (FeCl<sub>3</sub>·6H<sub>2</sub>O), UV-Vis spectrophotometer, X-ray diffraction (XRD) equipment (Department of Archaeology, Hazara University Mansehra), Fourier Transform Infrared Spectroscopy (FTIR) equipment (CRL, Hazara University Mansehra), Scanning Electron Microscopy (SEM) (CRL University of Peshawar), Energy Dispersive X-ray (EDX) (UK Model INCA 200), Atomic Absorption Spectroscopy (AAS, PerkinElmer S.No 80155070601), nitric acid, perchloric acid. The rice seeds (*Oryza sativa* L.) were obtained from the local market.

### 2.2 Ethical Guidelines for Plant Studies

The *Carya illinoensis* leaves used in this study were purchased from the local herbarium and identified by a Muhammad Asif. All procedures related to the acquisition and use of *Carya illinoensis* plants followed institutional and national ethical guidelines.

### 2.3 Plant Extract Formation and Synthesis of Fe<sub>2</sub>O<sub>3</sub> Nanoparticles

The *Carya illinoensis* leaves were collected, washed with distilled water, dried at room temperature, and then finely powdered. Two grams of the powdered plant material were dissolved in 100 mL of distilled water and boiled at 80°C for 15 minutes to prepare the plant extract. After filtration, the extract was used for the synthesis of Fe<sub>2</sub>O<sub>3</sub> nanoparticles. To synthesize the nanoparticles, 6 g of FeCl<sub>3</sub>·6H<sub>2</sub>O was dissolved in 100 mL of the prepared plant extract. The reaction mixture was heated at 80°C for 2 hours under constant stirring. Following the reaction, the resulting nanoparticle solution was centrifuged at 10,000 rpm for 15 minutes. The pellet obtained was washed with distilled water to remove any residual plant extract,

and the nanoparticles were dried in an oven.

## 2.4 Characterization of $\text{Fe}_2\text{O}_3$ Nanoparticles

The synthesized  $\text{Fe}_2\text{O}_3$  nanoparticles were characterized using several advanced techniques. UV-Vis spectrophotometry was employed to analyze the absorption spectrum of the nanoparticles, with a 1:1 ratio of nanoparticles dissolved in deionized water, and the absorbance was measured within the wavelength range of 350–450 nm using the UV-1700 PharmaSpec. The XRD analysis was conducted at the Department of Archaeology, Hazara University Mansehra to determine the crystalline structure and phase identification of the nanoparticles. FTIR analysis, performed using the FTIR equipment from CRL, Hazara University Mansehra, helped identify the functional groups involved in the synthesis and stabilization of the nanoparticles. The surface morphology and size of the nanoparticles were observed using SEM (JEOL model JSM-IT100) at CRL University of Peshawar, while the elemental composition of the synthesized nanoparticles was analyzed using EDX (UK Model INCA 200).

## 2.5 Plant Growth and Nanoparticle Application

Rice seeds (*Oryza sativa* L.) were surface sterilized using ethanol followed by a distilled water wash. Different concentrations of  $\text{Fe}_2\text{O}_3$  nanoparticle solutions were prepared (Control, 50 ppm, 100 ppm, and 150 ppm). The seeds were soaked in these solutions for 24 hours with constant shaking. Afterward, the seeds were rinsed with distilled water, dried, and sown in pots in the greenhouse. Control seeds were treated with distilled water only. Treatments included: Control, 50 ppm  $\text{Fe}_2\text{O}_3$ , 100 ppm  $\text{Fe}_2\text{O}_3$ , 150 ppm  $\text{Fe}_2\text{O}_3$ . Initially, three seeds were sown in each pot, and after one month, one healthy seedling was maintained per pot. The experiment was performed with three replicates for each treatment in a completely randomized design. Plants were allowed to grow until harvesting (120 days post-sowing). At the time of harvest, root and shoot lengths, as well as fresh and dry weights of the roots and shoots, were measured.



**Figure 2: *Oryza Sativa L.* (a) petri experiment (b) pot experiment**

## 2.6 Measurement of Iron Content in Rice Seeds

The iron concentration in rice seeds was analyzed using AAS (PerkinElmer S.No 80155070601). Approximately 0.5 g of rice powder was digested using a mixture of nitric acid and perchloric acid (2:1 ratio). After 24 hours, the mixture was heated until the appearance of white fumes, filtered, and the iron content was measured using AAS at Alvi Traders, Haripur.

## 2. Results and Discussion

### 3.1 UV-Visible Spectroscopy

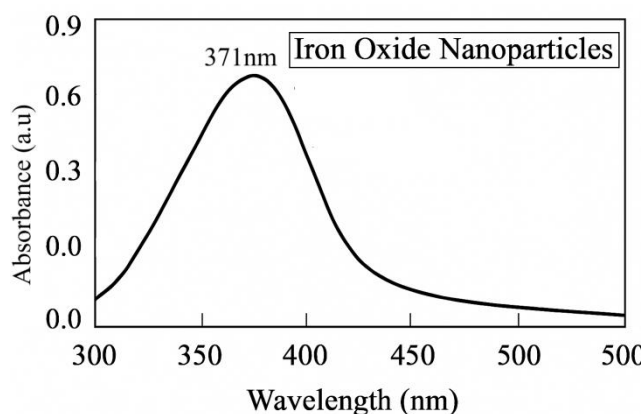


**Figure 3: UV-visible analysis of  $Fe_2O_3$  Nanoparticles**

The synthesis of  $Fe_2O_3$  nanoparticles using *Carya illinoensis* leaf extract was confirmed through UV-Vis spectrophotometry as shown in figure 3, which revealed a distinct absorption peak around 400 nm. This peak is characteristic of iron oxide nanoparticles, indicating successful formation. The presence of this peak suggests that the plant extract played a significant role in reducing iron ions to form  $Fe_2O_3$  NPs, which is typical for green synthesis methods [24]. The sharpness and intensity

of the peak further confirm the crystalline nature and high purity of the nanoparticles. This technique provides a sustainable and eco-friendly approach for nanoparticle synthesis in agricultural applications [25].

### 3.2 Fourier Transform Infrared Analysis

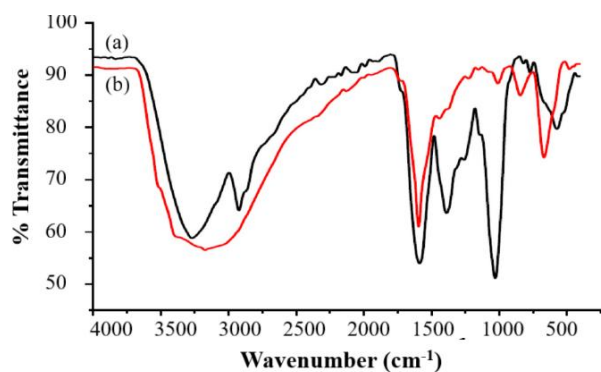


**Figure 4: FTIR Spectrum of (a) *Carya illinoensis* leaves extract, (b)  $\text{Fe}_2\text{O}_3$  Nanoparticles**

Figure 4 shows the FTIR spectra of *Carya illinoensis* leaves extract (a) and  $\text{Fe}_2\text{O}_3$  nanoparticles (b) present valuable insights into the chemical nature and functional groups of these two distinct materials. The FTIR technique is widely used to investigate molecular vibrations, providing detailed information on the bonding environment and functional groups present in the sample [26]. The FTIR spectrum of *Carya illinoensis* leaves extract (a) reveals several prominent bands that suggest the presence of various functional groups. The broad absorption band observed at around  $3400\text{ cm}^{-1}$  is characteristic of the O-H stretching vibration. This broad peak indicates the presence of hydroxyl groups, commonly found in polyphenols, flavonoids, and other bioactive compounds in the plant extract [27]. The intensity of this band suggests a high concentration of hydroxyl-containing functional groups, which is typical for plant extracts rich in phenolic compounds [28]. Next, the C=O stretching vibration appears at around  $1600\text{ cm}^{-1}$ , which is attributed to carbonyl groups (such as in aromatic carbonyls, esters, or acids) present in the plant extract. This band is a clear indication of the presence of conjugated systems or carbonyl-containing compounds like phenolic acids, flavonoids, or other secondary metabolites. Additionally, a band around  $1100\text{ cm}^{-1}$  is observed, corresponding to the C-O stretching vibration. This is indicative of carbohydrates, phenolic acids, or esters,

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further supporting the presence of diverse polyphenolic and carbohydrate-based components in the extract [29]. In contrast, the FTIR spectrum of  $\text{Fe}_2\text{O}_3$  nanoparticles (b) shows some key differences. The broad O-H band around  $3400\text{ cm}^{-1}$  is also present but appears weaker in comparison to the extract. This suggests that the hydroxyl groups are less abundant or more tightly bound in the iron oxide nanoparticles, potentially due to the formation of surface bonds between the extract and the nanoparticles. The band near  $1600\text{ cm}^{-1}$  is less pronounced in the  $\text{Fe}_2\text{O}_3$  spectrum, indicating fewer carbonyl-containing functional groups than in the plant extract. Additionally, the significant band observed around  $600\text{--}700\text{ cm}^{-1}$  is attributed to the Fe-O stretching vibration, a characteristic feature of iron oxide materials such as  $\text{Fe}_2\text{O}_3$ . This band confirms the presence of iron oxide nanoparticles and reflects the vibrational modes associated with the Fe-O bond. The FTIR spectra highlight the distinct chemical nature of the two samples. The leaf extract is rich in hydroxyl, carbonyl, and ester functional groups, indicating its complex polyphenolic and carbohydrate-based composition [30]. In contrast, the  $\text{Fe}_2\text{O}_3$  nanoparticles exhibit characteristic iron oxide-related vibrations, indicating successful synthesis of iron oxide nanoparticles. The differences between the spectra, especially in the hydroxyl and iron oxide bands, suggest potential interactions between the leaf extract and the nanoparticles, which may be involved in the formation and stabilization of the nanoparticles [31].

### 3.3 X-ray Diffraction (XRD) Analysis

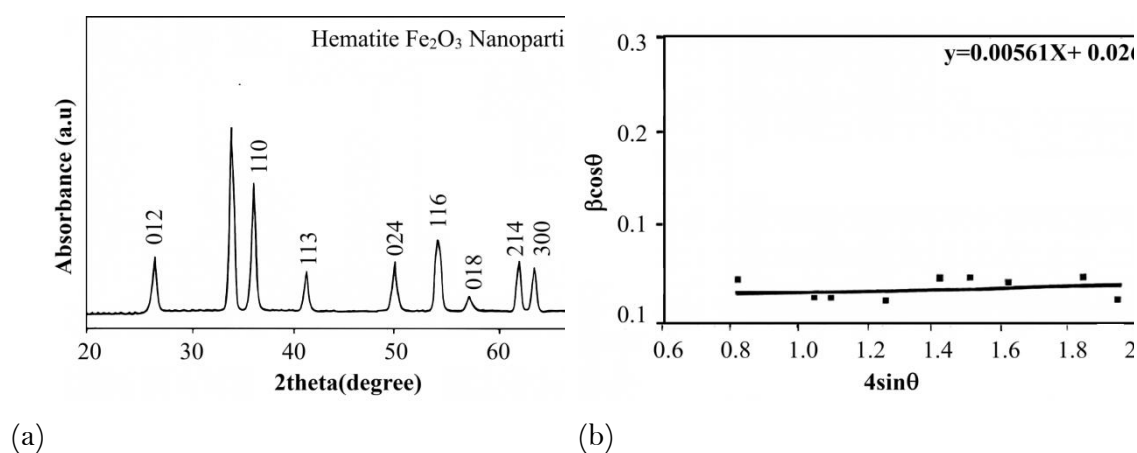


**Figure 5: (a) XRD analysis of  $\text{Fe}_2\text{O}_3$  Nanoparticles, (b) Williamson-Hall Plot of  $\text{Fe}_2\text{O}_3$  Nanoparticles**

The X-ray diffraction (XRD) analysis of hematite ( $\text{Fe}_2\text{O}_3$ ) nanoparticles displayed in Figure 5a shows distinct peaks at specific  $2\theta$  values. These peaks are characteristic of the crystalline nature of hematite, and their intensities provide insights into the material's crystallinity and phase composition [32]. The prominent peaks at around  $30^\circ$ ,  $35^\circ$ ,  $40^\circ$ ,  $50^\circ$ ,  $55^\circ$ ,  $60^\circ$ , and  $65^\circ$  correspond to the Miller indices 012, 110, 113, 024, 116, 018, and 214, respectively. These positions align well with the JCPDS (Joint Committee on Powder Diffraction Standards) card for hematite, confirming the presence of the  $\alpha\text{-Fe}_2\text{O}_3$  phase. The sharp and well-defined peaks suggest that the nanoparticles possess high crystallinity, and the relatively strong peak at  $35^\circ$  (110) indicates the dominant crystallographic plane. The diffraction pattern further supports the expected hexagonal close-packed (hcp) structure of hematite, as seen in the JCPDS reference (card no. 33-0664). The consistency of the observed peaks with the JCPDS data validates the synthesis of hematite nanoparticles with the desired structural and phase characteristics. In addition, the absence of significant peaks other than those associated with hematite suggests minimal impurities, which is crucial for applications such as catalysis and sensors where high purity is essential [33].

The Williamson-Hall plot presented in Figure 5b provides a method for analyzing the crystallite size and microstrain in the  $\text{Fe}_2\text{O}_3$  nanoparticles. Based on the linear relationship of  $\beta\cos\theta$  versus  $4\sin\theta$ , the crystallite size for the nanoparticles was calculated to be approximately 5.56 nm using the intercept from the plot. This value is consistent with the nanoparticle size observed in other methods, such as X-ray diffraction (XRD), confirming the small scale of the synthesized  $\text{Fe}_2\text{O}_3$  nanoparticles. The grain size, which is obtained through traditional methods, is much larger at around 79 nm. This difference between the crystallite size and the grain size suggests that the nanoparticles consist of small crystallites, but they are aggregated into larger grains [34]. The discrepancy between crystallite and grain size highlights the importance of distinguishing between the two when analyzing nanomaterials, as the crystallite size reflects the fundamental building blocks of the material, while the grain size encompasses the overall physical structure [8]. The crystallite size of 5.56 nm obtained from the Williamson-Hall plot further supports

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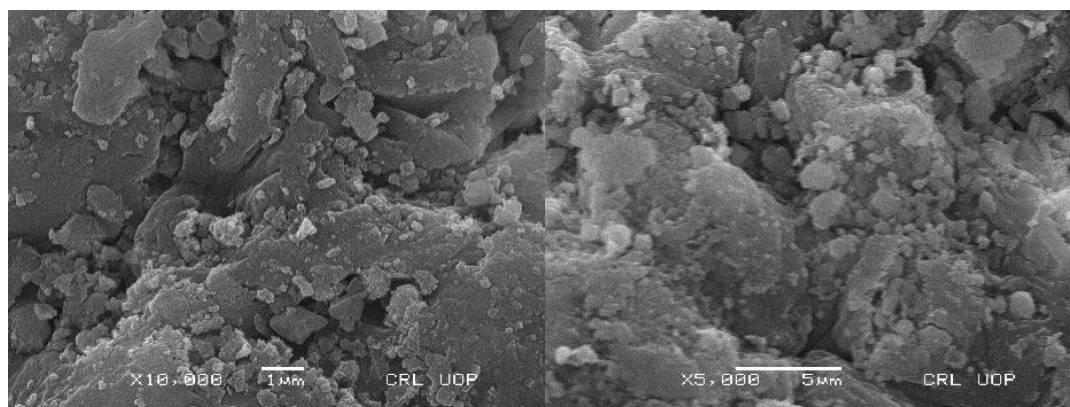
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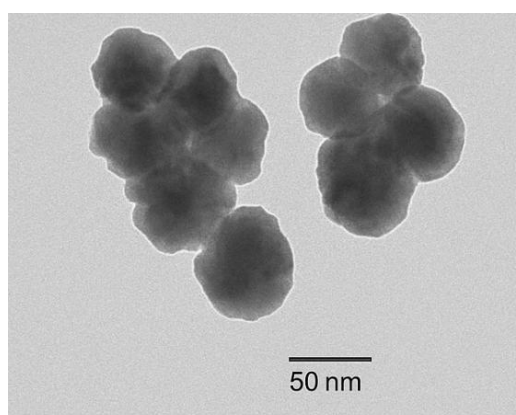
the nanocrystalline nature of the  $\text{Fe}_2\text{O}_3$  nanoparticles. Such small crystallites typically offer enhanced properties such as high surface area and increased catalytic activity, making these nanoparticles suitable for applications in fields such as catalysis, sensing, and energy storage [35]. The low microstrain observed indicates minimal structural defects, which contributes to the overall stability of the nanoparticles.

| Sample   | a (Å) | c (Å)  | Crystallite Size (nm) | Grain Size (nm) | Crystallite Size (nm) (W-H Plot) |
|----------|-------|--------|-----------------------|-----------------|----------------------------------|
| Hematite | 5.032 | 13.538 | 6.2                   | 79              | 5.56                             |

### 3.4 Scanning electron microscopy (SEM) micrographs & Transmission electron microscopy (TEM)



(a)



(b)

**Figure 6: (a) SEM micrographs of  $\text{Fe}_2\text{O}_3$  Nanoparticles (b) TEM micrograph of  $\text{Fe}_2\text{O}_3$  Nanoparticles**

Figure 6a displays the scanning electron microscopy (SEM) micrographs of  $\text{Fe}_2\text{O}_3$  nanoparticles, offering a comprehensive view of their surface morphology and particle distribution. The SEM images reveal that the nanoparticles are relatively uniform in size, with well-defined spherical shapes [36]. The particles appear to be loosely aggregated, with some degree of clustering, but without significant large-scale agglomeration. This indicates that the nanoparticles have good dispersibility and stability in their synthesized form. The high magnification images highlight the surface texture of the nanoparticles, showing a rough, granular appearance typical of metal oxide nanoparticles like  $\text{Fe}_2\text{O}_3$ . This surface roughness can contribute to the material's high surface area, which is beneficial for applications such as catalysis and adsorption [37]. Additionally, the clarity and sharpness of the SEM images confirm the crystallinity of the nanoparticles. Overall, the SEM analysis provides valuable insights into the structural properties of  $\text{Fe}_2\text{O}_3$  nanoparticles, reinforcing their suitability for various advanced applications [38].

Figure 6b presents the transmission electron microscopy (TEM) micrographs of  $\text{Fe}_2\text{O}_3$  nanoparticles, providing a detailed view of their morphology and size distribution at high magnification. The images reveal well-defined, spherical nanoparticles with a relatively uniform size, which is characteristic of controlled synthesis processes. The particles are dispersed, with no significant agglomeration, indicating good dispersion and stability of the nanoparticles. The TEM analysis confirms the crystalline nature of the  $\text{Fe}_2\text{O}_3$  nanoparticles, as observed by their sharp, distinct boundaries [39]. The nanoparticle size observed in the TEM images is consistent with the values calculated from the XRD and Williamson-Hall plot, reinforcing the accuracy of the characterization methods. Additionally, the absence of significant defects or irregularities in the particle shape suggests a high-quality synthesis process. These nanoparticles, due to their small size and uniformity, hold great promise for various applications, including catalysis, magnetic materials, and environmental remediation, where consistent size and structure are crucial for performance [40].

### 3.5 Plant Growth Parameters

The utilization of nanoparticles (NPs) in agricultural practices has gained significant

attention in recent years due to their potential in enhancing plant growth, development, and resistance to environmental stressors [41]. Among the various metal oxide nanoparticles, iron oxide ( $\text{Fe}_2\text{O}_3$ ) nanoparticles have shown promising results in improving the physiological processes of plants [18]. The results presented in Figures 7 and 8, and Tables 1 and 2, to understand the effects of different  $\text{Fe}_2\text{O}_3$  NP concentrations on plant growth, biomass characteristics, grain yield, and seed characteristics.

**Table 1: *Effect of  $\text{Fe}_2\text{O}_3$  NP concentration on plant growth and biomass characteristics (mean  $\pm$  SD).***

| $\text{Fe}_2\text{O}_3$<br>Concentration<br>(ppm) | NP | Shoot<br>Length<br>(cm) | Root<br>Length<br>(cm) | Shoot<br>Fresh<br>Weight<br>(g) | Root<br>Fresh<br>Weight<br>(g) | Shoot<br>Dry<br>Weight<br>(g) | Root<br>Dry<br>Weight<br>(g) |
|---------------------------------------------------|----|-------------------------|------------------------|---------------------------------|--------------------------------|-------------------------------|------------------------------|
| Control                                           |    | $80 \pm 5$              | $8 \pm 1$              | $40 \pm 3$                      | $25 \pm 2$                     | $30 \pm 2$                    | $20 \pm 1$                   |
| 50ppm                                             |    | $85 \pm 4$              | $11 \pm 1$             | $50 \pm 4$                      | $45 \pm 3$                     | $42 \pm 3$                    | $40 \pm 2$                   |
| 100ppm                                            |    | $100 \pm 3$             | $15 \pm 2$             | $62 \pm 5$                      | $60 \pm 4$                     | $55 \pm 4$                    | $55 \pm 3$                   |
| 150ppm                                            |    | $75 \pm 5$              | $10 \pm 1$             | $95 \pm 6$                      | $75 \pm 4$                     | $85 \pm 5$                    | $65 \pm 3$                   |

At the lowest concentration of 50 ppm, the shoot length increased to  $85 \pm 4$  cm compared to the control group ( $80 \pm 5$  cm), and the root length showed a notable increase of  $11 \pm 1$  cm compared to  $8 \pm 1$  cm in the control. At 100 ppm, the shoot length reached its peak at  $100 \pm 3$  cm, and the root length increased further to  $15 \pm 2$  cm. However, at 150 ppm, a decrease in shoot length was observed, with the length reduced to  $75 \pm 5$  cm. This suggests that  $\text{Fe}_2\text{O}_3$  NPs have a positive effect on shoot and root elongation up to a certain concentration (100 ppm), beyond which their effect is diminished, possibly due to the toxicity or imbalance caused by higher nanoparticle concentrations. Similar results have been observed in other studies where nanoparticles were found to stimulate growth at low concentrations but inhibit growth at higher levels due to potential toxicity (Bano et al., 2017). The fresh and dry weight of both shoot and root increased progressively with increasing  $\text{Fe}_2\text{O}_3$  NP concentration. At 50 ppm, shoot fresh weight was  $50 \pm 4$  g, and root fresh weight was  $45 \pm 3$  g. At 100 ppm, the values increased significantly, with shoot fresh

weight reaching  $62 \pm 5$  g and root fresh weight  $60 \pm 4$  g. Furthermore, the dry weight of both shoot and root followed the same trend. Shoot dry weight increased from  $30 \pm 2$  g in the control to  $55 \pm 4$  g at 100 ppm, and root dry weight increased from  $20 \pm 1$  g to  $55 \pm 3$  g. At 150 ppm, the shoot dry weight reached its maximum at  $85 \pm 5$  g, but the root dry weight only increased to  $65 \pm 3$  g, suggesting that higher concentrations may lead to disproportionate growth between the shoot and root systems. This is consistent with findings in other studies, where the growth stimulation effect of NPs is attributed to their interaction with plant hormones and improvement in nutrient uptake.

**Table 2: Effect of  $\text{Fe}_2\text{O}_3$  NP concentration on grain yield and seed characteristics (mean  $\pm$  SD).**

| $\text{Fe}_2\text{O}_3$<br>Concentration<br>(ppm) | NP | Grain<br>Yield (g) | 1000<br>Grain<br>Weight<br>(g) | Number<br>of<br>Spikes | Spike<br>Length<br>(cm) | Number<br>of<br>Tillers | Seed<br>Iron<br>Content<br>( $\mu\text{g/g}$ ) |
|---------------------------------------------------|----|--------------------|--------------------------------|------------------------|-------------------------|-------------------------|------------------------------------------------|
| Control                                           |    | $8 \pm 0.5$        | $25 \pm 1$                     | $10 \pm 1$             | $15 \pm 1$              | $13 \pm 2$              | $20 \pm 2$                                     |
| 50ppm                                             |    | $10 \pm 0.8$       | $40 \pm 2$                     | $12 \pm 1$             | $21 \pm 2$              | $16 \pm 2$              | $35 \pm 3$                                     |
| 100ppm                                            |    | $15 \pm 1.2$       | $45 \pm 3$                     | $14 \pm 1$             | $29 \pm 3$              | $20 \pm 3$              | $44 \pm 3$                                     |
| 150ppm                                            |    | $16 \pm 1.5$       | $50 \pm 3$                     | $11 \pm 1$             | $20 \pm 2$              | $18 \pm 2$              | $58 \pm 4$                                     |

From Table 1, it is evident that  $\text{Fe}_2\text{O}_3$  NPs have a notable impact on plant growth and biomass accumulation, as shown by the significant differences in shoot length, root length, fresh weight, and dry weight of both shoot and root at varying concentrations of  $\text{Fe}_2\text{O}_3$  NPs. In Table 2, the effect of  $\text{Fe}_2\text{O}_3$  NP concentration on grain yield and seed characteristics is outlined. The concentration of  $\text{Fe}_2\text{O}_3$  NPs significantly affected grain yield, 1000 grain weight, number of spikes, spike length, number of tillers, and seed iron content. At 50 ppm, the grain yield increased to  $10 \pm 0.8$  g from  $8 \pm 0.5$  g in control. This increase became more pronounced at 100 ppm, with the grain yield reaching  $15 \pm 1.2$  g. The highest concentration of 150 ppm further boosted grain yield to  $16 \pm 1.5$  g. These results indicate that  $\text{Fe}_2\text{O}_3$  NPs have a stimulatory effect on grain yield, which could be attributed to enhanced nutrient uptake, increased photosynthesis, and improved root function (Siddiqui et al., 2015).

The 1000 grain weight showed a similar trend, increasing from  $25 \pm 1$  g in control to  $50 \pm 3$  g at 150 ppm. This suggests that  $\text{Fe}_2\text{O}_3$  NPs contribute not only to increasing the number of grains but also to improving their size. The number of spikes increased from  $10 \pm 1$  in the control to  $14 \pm 1$  at 100 ppm, indicating that  $\text{Fe}_2\text{O}_3$  NPs enhance the reproductive capacity of the plants. However, at 150 ppm, the number of spikes decreased to  $11 \pm 1$ , possibly due to the inhibitory effects of high nanoparticle concentrations. Similarly, the spike length increased with increasing  $\text{Fe}_2\text{O}_3$  NP concentration, from  $15 \pm 1$  cm in the control to  $29 \pm 3$  cm at 100 ppm. This improvement in spike length may contribute to the overall increase in grain yield, suggesting that  $\text{Fe}_2\text{O}_3$  NPs enhance the development of reproductive structures. The number of tillers, which is directly related to the plant's ability to produce secondary shoots and increase its biomass, increased with  $\text{Fe}_2\text{O}_3$  NP concentration. At 50 ppm, the number of tillers increased to  $16 \pm 2$  from  $13 \pm 2$  in the control, and at 100 ppm, it reached  $20 \pm 3$ . This trend is indicative of the positive role of  $\text{Fe}_2\text{O}_3$  NPs in enhancing branching and overall plant structure, which is consistent with other reports where nanoparticles have been shown to stimulate vegetative growth and improve the number of tillers. The seed iron content, an important parameter for nutritional quality, increased significantly with increasing  $\text{Fe}_2\text{O}_3$  NP concentration. The iron content in the seeds increased from  $20 \pm 2$   $\mu\text{g/g}$  in the control to  $58 \pm 4$   $\mu\text{g/g}$  at 150 ppm. This increase is especially significant as iron is a crucial micronutrient for both human nutrition and plant metabolism. The presence of  $\text{Fe}_2\text{O}_3$  NPs likely enhanced the bioavailability of iron in the soil, thereby increasing its uptake by the plants and enriching the seed iron content. This has important implications for agricultural practices, especially in regions where iron deficiency is prevalent. Despite the overall beneficial effects observed at lower concentrations, it is important to note that at the highest concentration of 150 ppm, some parameters, particularly shoot length and number of spikes, showed a slight decrease compared to the 100 ppm concentration. This suggests that while  $\text{Fe}_2\text{O}_3$  NPs have beneficial effects at lower to moderate concentrations, excessive concentrations may cause toxicity or stress to the plants [19]. Similar findings have been observed in other studies where nanoparticles caused growth inhibition at high

concentrations due to their potential to disrupt cellular processes or induce oxidative stress.

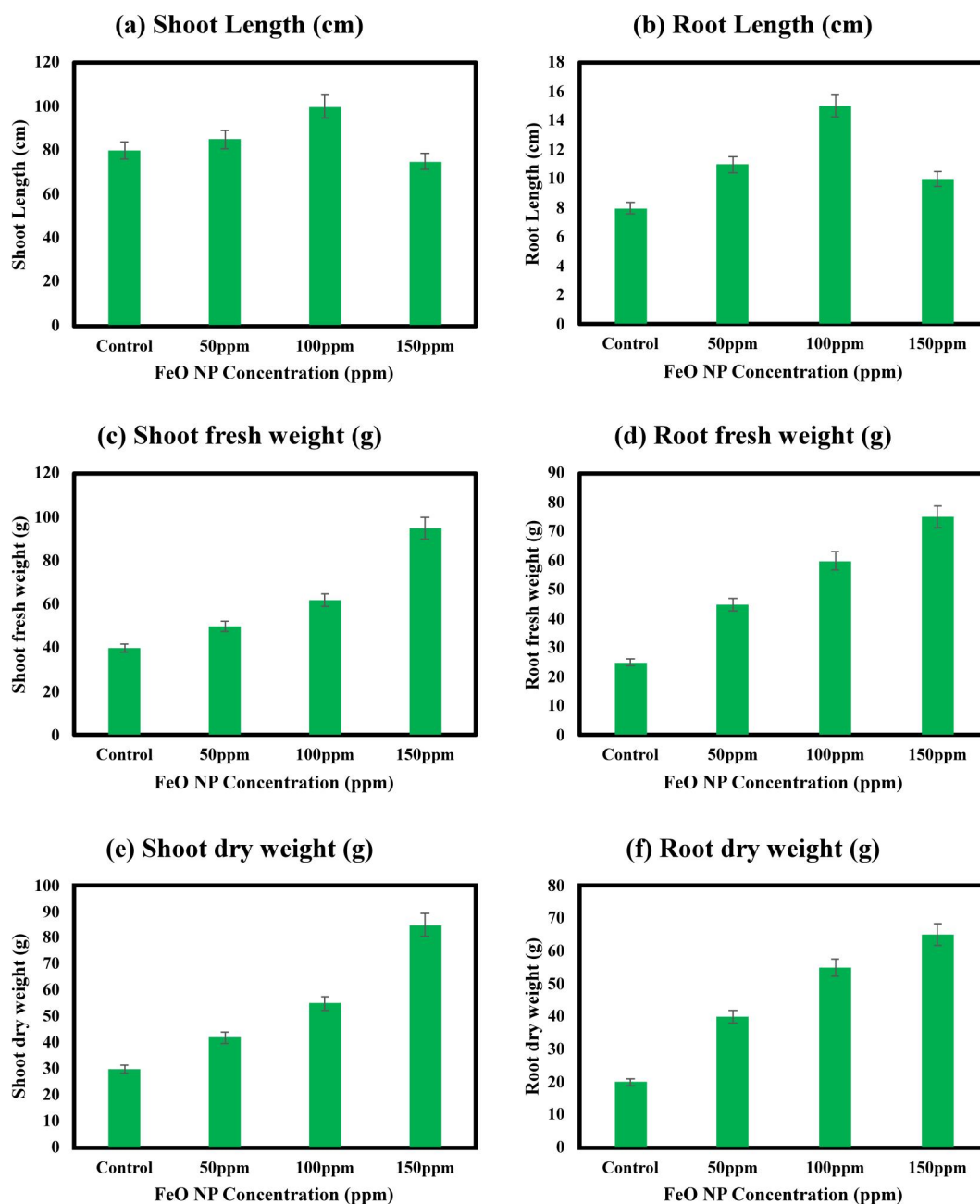


**Figure 7:** Effect of  $\text{Fe}_2\text{O}_3$  NP concentration on plant growth and biomass characteristics (mean  $\pm$  SD): (a) Shoot Length (cm), (b) Root Length (cm), (c) Shoot Fresh Weight (g), (d) Root Fresh Weight (g), (e) Shoot Dry Weight (g), (f) Root Dry Weight (g)

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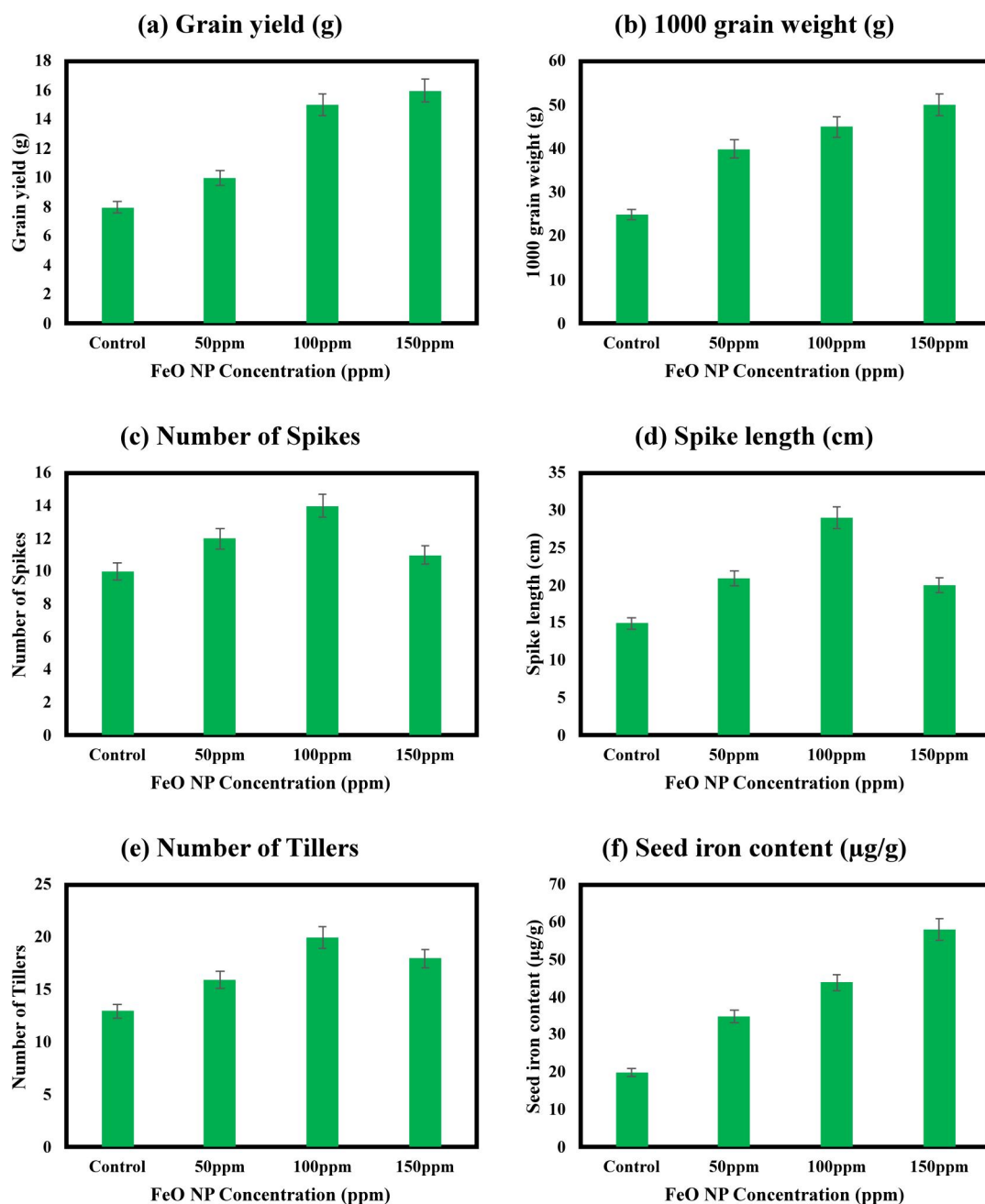
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**Figure 8:** Effect of  $\text{Fe}_2\text{O}_3$  NP concentration on grain yield and seed characteristics (mean  $\pm$  SD): (a) Grain Yield (g), (b) 1000 Grain Weight (g), (c) Number of Spikes, (d) Spike Length (cm), (e) Number of Tillers and (f) Seed Iron Content ( $\mu\text{g/g}$ )

### Conclusion

In this study, iron oxide nanoparticles ( $\text{Fe}_2\text{O}_3$  NPs) were successfully synthesized using a plant-based method with *Carya illinoensis* leaf extract, offering an eco-

friendly and sustainable approach to nanoparticle production. The synthesized nanoparticles were characterized using several advanced techniques to confirm their formation and properties. UV-Vis spectrophotometry revealed an absorption peak around 400 nm, confirming the successful synthesis of Fe<sub>2</sub>O<sub>3</sub> NPs. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the nanoparticles, with well-defined peaks corresponding to the  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> phase, suggesting high crystallinity and minimal impurities. Fourier Transform Infrared Spectroscopy (FTIR) further revealed the presence of functional groups such as hydroxyl and carbonyl, indicating the involvement of plant extract components in the stabilization of Fe<sub>2</sub>O<sub>3</sub> NPs. Scanning Electron Microscopy (SEM) showed that the nanoparticles had a uniform spherical morphology, and Energy Dispersive X-ray (EDX) analysis confirmed the presence of iron in the synthesized nanoparticles. In agricultural trials, Fe<sub>2</sub>O<sub>3</sub> NPs at concentrations of 50, 100, and 150 ppm were applied to *Oryza sativa* L. (rice) seeds. Results showed that Fe<sub>2</sub>O<sub>3</sub> NPs significantly enhanced plant growth, with increased shoot and root lengths, biomass accumulation, and iron content in seeds. The concentration of 100 ppm produced the most significant growth and yield improvements, while 150 ppm further increased grain yield but caused a slight decline in some growth parameters, suggesting potential toxicity at higher concentrations. The seed iron content increased progressively with Fe<sub>2</sub>O<sub>3</sub> NP concentration, with 150 ppm showing the highest value, demonstrating the potential of Fe<sub>2</sub>O<sub>3</sub> NPs for biofortification. In conclusion, Fe<sub>2</sub>O<sub>3</sub> NPs have proven to be a promising tool for enhancing plant growth, improving yield, and biofortifying crops, particularly with increased iron content. However, optimizing nanoparticle concentrations for maximal benefit without toxicity is crucial for future agricultural applications.

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