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**Original Research Article**

# Evaluating the Germination Performance of Some Iraqi Barley Cultivars Primed with Iron Nanoparticles

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**Abstract:** Iron nanoparticles exist naturally in the environment, it has distinct properties like reactivity, mobility and toxicity which can have potential harm to the nature when it accumulates in large amounts in the soil. Previous studies used varied levels and materials of iron nanoparticles to evaluate its effect on plants, our study aimed to evaluate the potential effect of Iron Nanoparticles (Fe2O3 NPs) seed priming on some Iraqi barley cultivars at the germination level. The Fe2O3 NPs successfully synthesized using Turmeric extract and examined using Ultraviolet-Visible (UV-vis), Fourier Transform Infrared spectrum (FTIR), Atomic Force Microscope (AFM), Scanning Electron Microscopy (SEM), X-ray diffraction (XRD) and energy dispersive X-ray (EDX). In the experiment, the seed was primed with concentrations of NPs (0,3,6,9 mM) with varied priming periods (6,12, and 24 h) and evaluated the seed germination rate (%) and seed germination speed (h). The experimental data showed statistically insignificant suppression of the seed germination rate compared with control treatment for all cultivars which had 100% germination rate except 9 mM, which was marked significant compared with control treatment, while germination speed delayed and significantly affected by the increase in priming period at the concentrations (6 and 9 mM). The study concluded that Fe2O3 NPs barley seed priming can inhibit the germination and delay germination speed at high concentrations which may pose phytotoxic effect at high levels and long term exposure.

**Keywords:** Barley, Germination Speed, Iron Nanoparticles, Nano-Priming, Seed Germination.

## INTRODUCTION

Barley classified as fourth most important cereal globally, it is essentially used as food, beer source and feeding. It possesses health benefits due to its valuable components, it gained marked interests from agricultural, and food scientists. Barley crops are rich in fibers, which are important to human health compared with other crops, such as rice, wheat, rice, and maize. It is well known that fiber rich diets can provide mitigate diabetes, hypertension, and cardiovascular disease. It has been globally marked to have high potential as a healthy diet (Geng *et al*., 2022; Miralles *et al*., 2021).

Iron (Fe) is a vital micronutrient for all organisms, it considered as the sixth most abundance element globally, and the fourth most prevalent element in the soil. Iron is pivotal element for plant life cycle, as it is essential for metabolic processes in plants (Bhatla & Kathpalia, 2023). Rising applications of iron element grabbed the attention on its environmental inferences, particularly ecotoxicological influences on organisms in diverse ecosystems (Gao *et al*., 2025). In plants Fe is one of the vital micronutrient and involved in many physiological and biochemical processes including chlorophyll biosynthesis, RNA synthesis, enzymatic activation, redox reactions and respiration (Gülser *et al*., 2019). Despite iron nanoparticles formed naturally in the environment, it has definite characteristics such as mobility, reactivity, and toxicity, which can pose real negative effect on human and nature and the use of NPs inevitably makes the environment their end destiny, the particles sized smaller than 100 nm have high penetration potential and pose toxicological risks (Souza *et al*., 2019). The iron oxide particles pose a wide range of essential environmental processes related to plant growth and soil fertility. They often occur as combination of mineral nanoparticles or as nanoscale coatings on other grains in the soil, where they may involve in the transport of nutrients such as phosphate, sulphate, molybdate (Claudio *et al*., 2017).

The eco-toxicity of iron element depends on material features such as surface, dose, plant tolerance, and environmental conditions (Guziałowska-Tic & Tic, 2015). In spite of the fact that plants embody iron elements, which are plant nutritional essential element, it can exert harmful effects (Khan *et al*., 2023). Iron at elevated doses could have potential toxicity and deficiency can supress the physiological and biochemical mechanisms that led to impair growth and development of plants (Rout & Sahoo, 2015). NPs tiny sizes allow them easily to penetrate and pass through cell membrane and consequently distort the cellular functions. Some researchers have concluded in their studies that iron NPs are toxic to some plants at certain doses (Souza *et al*., 2019). In plant tissues, 60 to 300 mg/kg is the appropriate concentration of iron that beneficially effects plants, and exceeds this level considered as phytotoxic (Sun *et al*., 2020). Previous studies outcome has shown varied results either beneficial or phytotoxicity on plant growth and development. Nano-ZVI (nZVI) pose stimulating effects at low doses on cattail (Typha latifolia), peanut (Arachis hypogaea). Arabidopsis thaliana mung bean (Vigna radiate) cucumber (Cucumis sativus) (Sun *et al*., 2020). Due to the inadequate researches on the phytotoxic concentrations of Iron nanoparticles on seed germination, the research intends to assess the effect of varied doses and priming periods on seed germination of Iron nanoparticles on some Iraqi local barley cultivars.

# MATERIALS AND METHODS

**Turmeric Curcumin Extract Preparation**

The turmeric extract was prepared using 10 grams of commercially available turmeric powder, deposited in a sterile glass beaker capacity for 100 ml of sterile distilled water. Sample was heated using a hot plate at 60°C using a mercuric thermometer with continuous stirring to extract the active compounds of Turmeric Curcumin. It was cooled at room temperature and was filtered using Whatman-1 filter paper kept in a sterilized clean bottle and stored in the refrigerator at 4°C until further use in nanoparticles synthesis (Dikshit *et al.*, 2021).

**Green synthesis of Fe2O3 NPs**

Fe2O3 NPs (0.1 M) were prepared by adding 4.04 g of $Fe(NO_3)_3.9H_2O$ to a glass beaker carries 100 ml of sterile distilled water. The beaker was left on a hot plate until the temperature reached 45-60 °C. Gradually addition of 5 ml of turmeric extract to 0.1 M $Fe(NO_3)_3.9H_2O$ solution with continuous stirring using a magnetic capsule for 5-30 min period until colour change observed. The observed colour changed was from yellow to brown which indicates the formation of iron NPs. The prepared solution was stored in a dark glass bottle at refrigerator until further use (Alshehri *et al*., 2017).

**Characterization of Fe2O3 NPs**

Fe2O3 NPs was prepared using eco-friendly method using turmeric extract as a stabilizing and reducing. Fe2O3 NPs synthesis was examined using multiple analytical and detection techniques to determine structural and morphological features including XRD, SEM, EDX, AFM, UV-Visible spectroscopy, and FTIR. These techniques are providing accurate details about the surface and crystal structure, surface topography, optical and vibrational properties of the NPs sample.

**Seed Priming Experiment**

The seeds were cleaned using running water for 10 min. Afterwards, the seeds of four barley cultivars *Hordeum vulgare* were provided from the Department of Crops, College of agriculture, Tikrit University, Iraq. Healthy and sound seeds were chosen for the nano-priming experiment. Before the germination tests, seeds were sterilized for 10 min in 0.5% NaClO solution then washed 3 times with deionized water to remove all the remnants of disinfectant and dried using filter papers. Suspensions of three different concentrations of Fe2O3 were prepared (3, 6, and 9 mM). Suspensions were added to each petri dish, wherein twelve chosen seeds were placed evenly. Seeds were sown in dark place at 25°C with 70% humidity for (4 days) in climate controlled incubator. The evaporation water loss was compensated every (24 h). Each treatment was performed in three replicates. The control treatments were seeds soaked in distilled water for the same periods of other seeds treated with Fe2O3 NPs. Lined with filter papers, the filter papers kept moist.

**Seed Germination Rate**

The seeds identified germinated when the radicles rise above half of seed length. The germinated seeds were observed every 6h. Germination rate % was measured according to the following equation:

$$Germination\ rate(\%) = \frac{Seeds\ germinated\ normally}{Total\ number\ seeds} \times 100 \qquad Equation\ 1$$

**Data Analysis**

One-way ANOVA analysis of variance have been used analyse the data significance to compare the significance between treatments and control samples using GraphPad prism version (10.6.0).

# RESULTS AND DISCUSSION

The properties analysis of Fe2O3 NPs was done at the Laboratory, University of Kashan/Iran, through the agent BPC company in Tikrit city.

**UV-Visible Spectra Analysis**

UV-vis of the Fe2O3 NPs greenly synthesized, a continuous absorption was observed in the visible range of (200-800) nm due to surface plasma resonance of nano Iron oxide, strong absorption peak recorded at 380 nm that was compatible with the studies that recorded 371nm that illustrated in (Figure 1) (Alshehri *et al*., 2017; Batool *et al*., 2021).

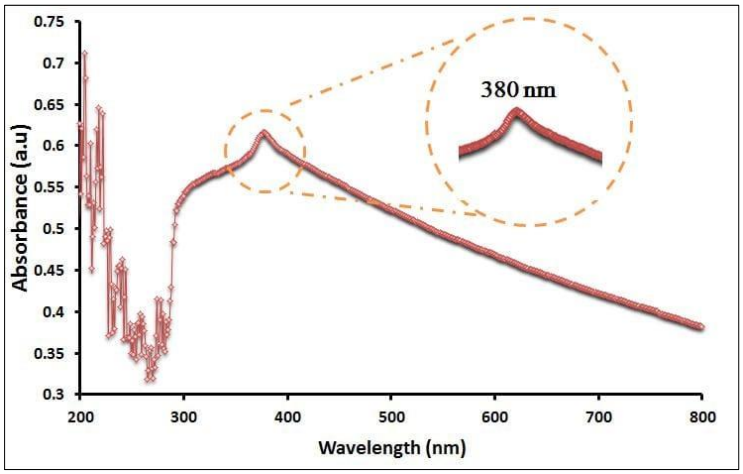


**Figure 1: UV-Spectroscopy of *Fe2O3 NPs***

**FTIR Spectroscopy Analysis**

Figure (2) represents the FTIR spectrum of Fe2O3 NPs prepared using the green synthesis method by turmeric extract. It shows distinct peaks confirming the success of the preparation process. A broad peak appears between 3444 and 3391 $cm^{-1}$, which is attributed to O–H vibrations resulting from the hydroxyl groups in the phenolic compounds of the turmeric extract, indicating its role as a stabilizing and reducing agent. A peak at 1635 $cm^{-1}$ also appears, which is attributed to C=O or C=C vibrations in aromatic compounds, and a peak at 1383 $cm^{-1}$ is owing to C–H vibrations or residues of $NO_3^-$ ions from iron nitrate. More importantly, the peaks appear in the range 614–420 $cm^{-1}$, which clearly indicate Fe–O bonds, thus confirming the formation of iron oxide nanoparticles. It is inferred that turmeric extract acted as a reducing agent and successfully facilitated the biosynthesis process to produce Fe2O3 NPs. These results suggest the formation of monodentate and bidentate that was consistent with the results of (Tuutijärvi *et al*., 2010; Zhang *et al*., 2014).

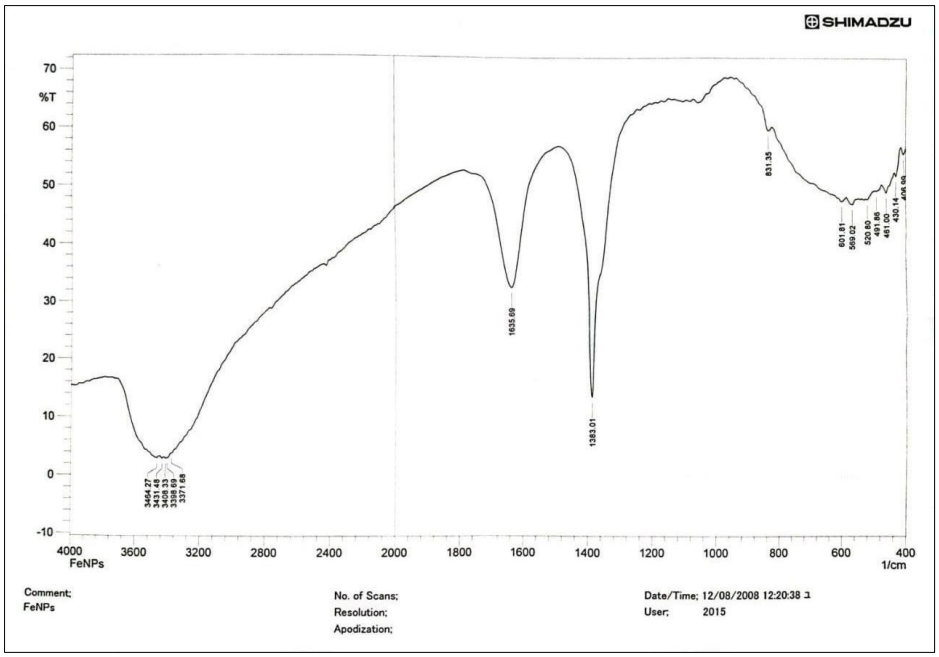


**Figure 2: FTIR analysis of Fe2O3 NPs**

**AFM Analysis**

AFM is crucial for measuring the size distribution range in nanoparticles, the particle surface composed of diameters heights ranging from 9.164-95.15) nm. According to the AFM image, the surface is considered as rough with varied dimensions that give the surface roughness which increases the efficiency of nanoparticles. The colorimetric description presented in the diagram revealed that all the diameters less than 100 nm were consisted with SEM analysis.

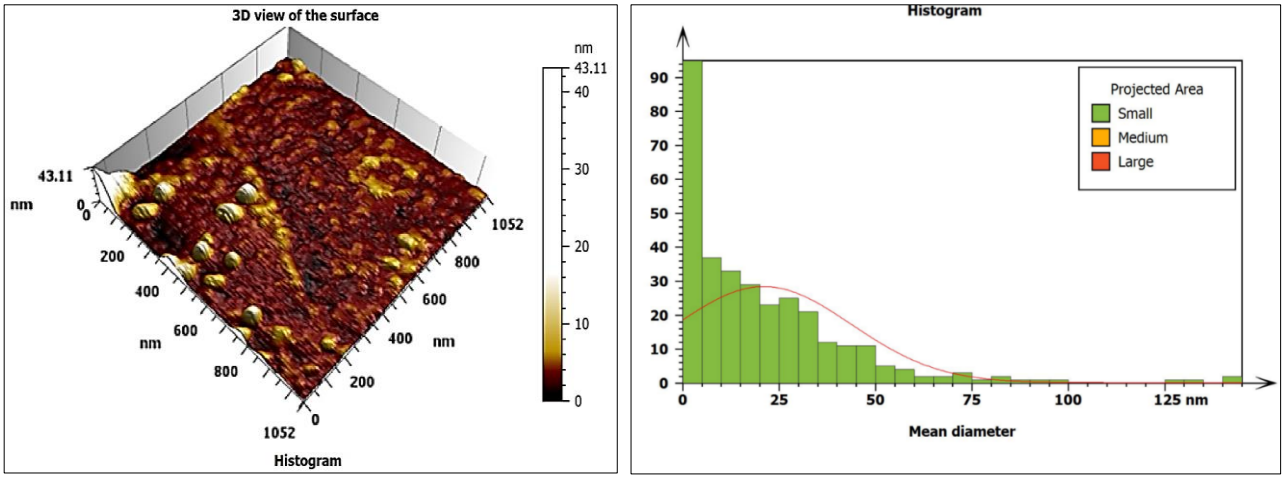


**Figure 3: AFM Results of Fe2O3 NPs**

**SEM Analysis**

The shape and sizes of Fe2O3 NPs that prepared using turmeric aqueous extract evaluated using SEM that described in (Figure 4). The Fe2O3 NPs particles were spherical shape and the size average about 60nm. The SEM analysis revealed that the particles within the sample located within the range of nanoparticles (1-100) nm.

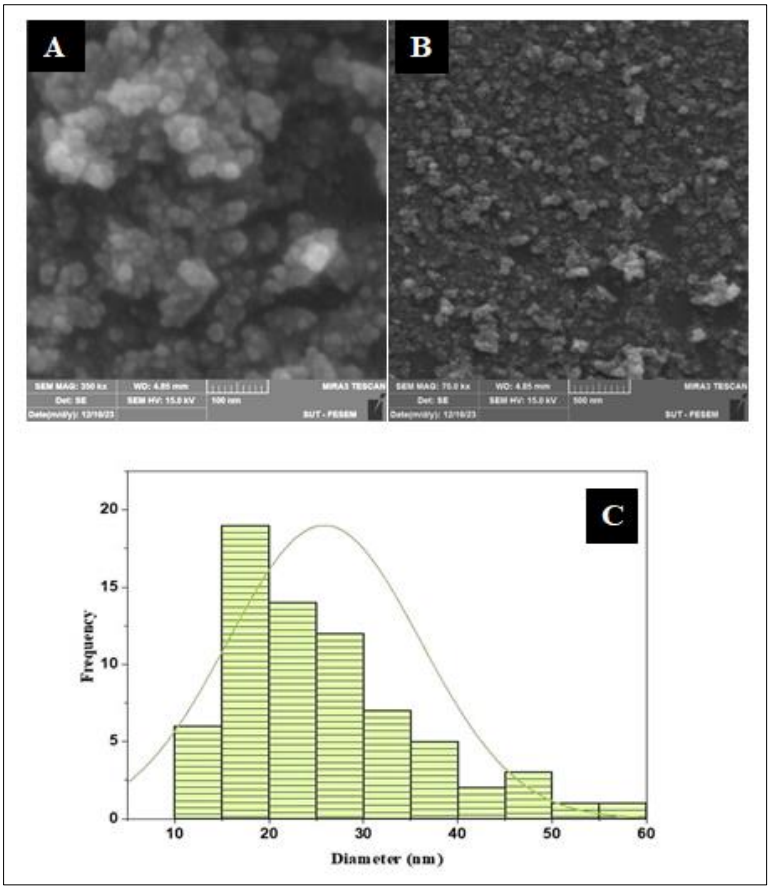


**Figure 4: SEM results of Fe2O3 NPs prepared by green synthesis method of turmeric extract under magnification (a) 500 nm, (b) 100 nm (c) Size distribution**

**EDX Analysis**

The results of EDX that illustrated in the (Figure 5) proved the successful of green synthesis of Fe2O3 NPs. The relative abundance of iron was 80%, disclose that iron was the prevalent element, representing 80% of detected elements. Conversely, oxygen was 20%, revealing that the tested compound was an iron oxide, which is consistent with the XRD results (Althomali *et al*., 2021).

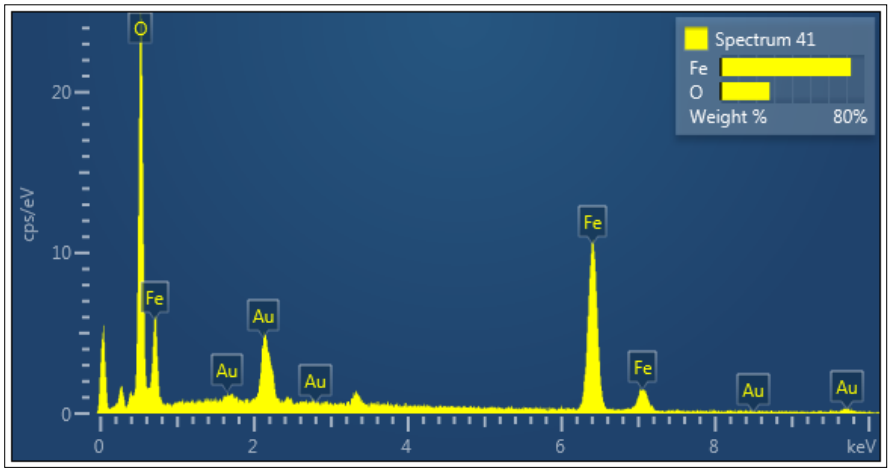


**Figure 5: EDX analysis of Fe2O3 NPs**

**XRD Analysis**

The results of XRD test showed the presence of a bunch of evident peaks at an angle 20 for X-ray diffraction reached 30.241°، 35.631 ° ، 43.285° ،57.273° ،62.927° ،71.378° the peaks values matched the peaks values information in the file (JCPDS 00-039-1346) of $Fe_2O_3$ that was a cubic features depending on peak areas (Bhosale *et al.*, 2015).

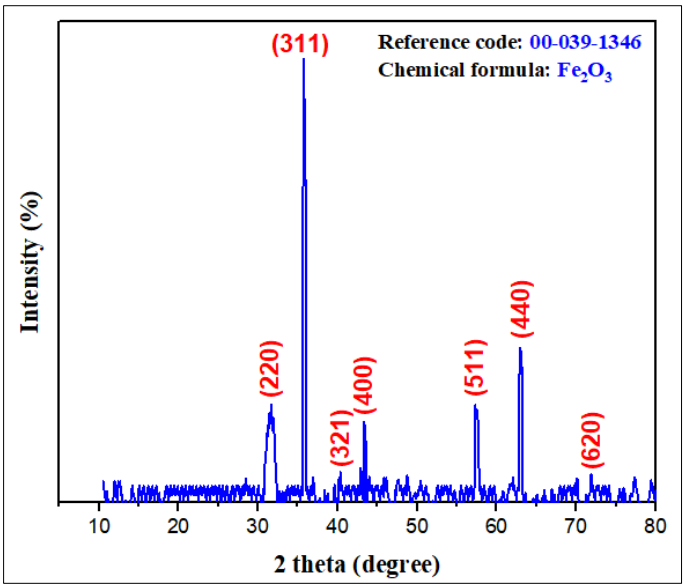


**Figure 6: XRD analysis of Fe2O3 NPs**

**Germination Percentage %**

The barley cultivars were primed with three different Fe2O3 NPs concentrations and the control sample was primed with water. As illustrated in Table 1, the seeds that not treated with Fe2O3 NPs had 100% germination rate after two days of sowing. In most treatments, exposure to Fe2O3 NPs had slight impact on total seed germination rate after (3 days), but in some cases delayed germination. After 3 days of sowing, the germination rate recorded was higher than 90%. There are differences in germination rate between all the treatments were insignificant except the concentration of 9mM which was significant compared to control treatments for (Hadhar, IPA99 and Zarqaa) cultivars. The results obtained disclosed that the germination rate and Fe2O3 NPs concentration inversely correlated, the germination rate decreased with concentration increase. At 3 and 6 mM concentration, the Fe2O3 NPs had no inhibition effect on barley cultivars germination.

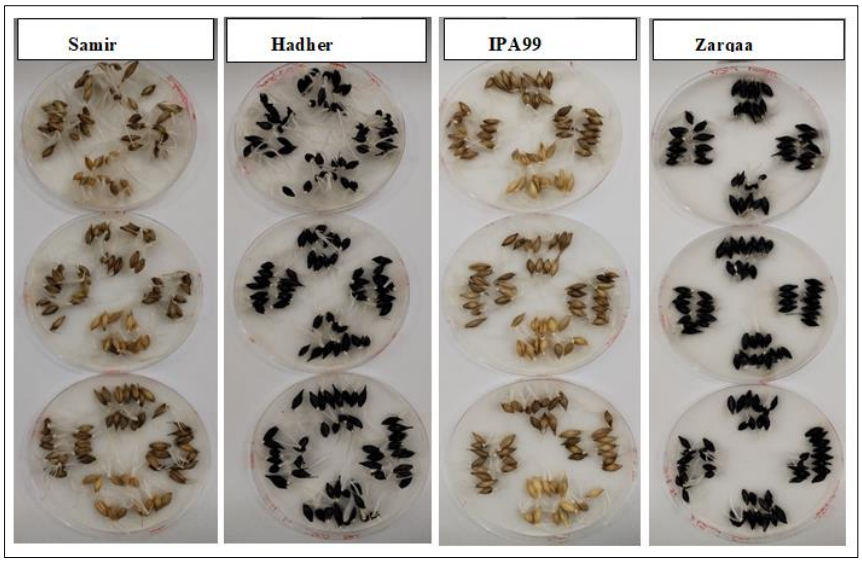


**Figure 7: Four barley cultivars sown in petri dishes treated with three different concentrations of Fe2O3 NPs**

**Table 1: Germination rate % of barley cultivars exposed to varied concentrations of Fe2O3 NPs (mean±SD)**

| Cultivar | Dose (mM) | Germination rate % | | |
|---|---|---|---|---|
| | | 6h | 12h | 24h |
| **Samir** | **Control** | 100±0$^{a}$ | 100±0$^{a}$ | 100±0$^{a}$ |
| | **3** | 100±0$^{a}$ | 99±12$^{a}$ | 97±10$^{a}$ |
| | **6** | 97±0$^{a}$ | 96±6$^{a}$ | 92±6$^{a}$ |
| | **9** | 97±6$^{a}$ | 93±2$^{a}$ | 90±6$^{a}$ |
| **Hadhar** | **Control** | 100±0$^{a}$ | 100±0$^{a}$ | 100±0$^{a}$ |
| | **3** | 100±0$^{a}$ | 100±0$^{a}$ | 99±1$^{a}$ |
| | **6** | 99±0$^{a}$ | 100±0$^{a}$ | 93±12$^{a}$ |
| | **9** | 99±0$^{a}$ | 94±6$^{a}$ | 85±10$^{b}$ |
| **IPA99** | **Control** | 100±0$^{a}$ | 100±0$^{a}$ | 100±0$^{a}$ |
| | **3** | 100±0$^{a}$ | 99±6$^{a}$ | 97±6$^{a}$ |
| | **6** | 99±6$^{a}$ | 97±3$^{a}$ | 97±6$^{a}$ |
| | **9** | 97±4$^{a}$ | 90±6$^{a}$ | 86±4$^{b}$ |
| **Zarqaa** | **Control** | 100±0$^{a}$ | 100±0$^{a}$ | 100±0$^{a}$ |
| | **3** | 100±0$^{a}$ | 98±10$^{a}$ | 98±2$^{a}$ |
| | **6** | 99±0$^{a}$ | 97±12$^{a}$ | 90±0$^{a}$ |
| | **9** | 97±6$^{a}$ | 90±3$^{a}$ | 87±10$^{b}$ |

*Different letters refer to significant differences among the means in different treatments within the column using one-way ANOVA (P≤0.05).*

**Germination Speed**

**Table 2: Germination speed of barley cultivars exposed to different concentrations of Fe2O3 for different time periods**

| Cultivar | Dose (mM) | Germination Speed (h) | | |
|---|---|---|---|---|
| | | 6h | 12h | 24h |
| **Samir** | **Control** | 48$^{a}$ | 48$^{a}$ | 50$^{a}$ |
| | **3** | 48$^{a}$ | 48$^{a}$ | 50$^{a}$ |
| | **6** | 48$^{a}$ | 52$^{b}$ | 60$^{b}$ |
| | **9** | 72$^{b}$ | 72$^{c}$ | 80$^{c}$ |
| **Hadhar** | **Control** | 48$^{a}$ | 48$^{a}$ | 50$^{a}$ |
| | **3** | 48$^{a}$ | 48$^{a}$ | 53$^{b}$ |
| | **6** | 48$^{a}$ | 51$^{b}$ | 57$^{c}$ |
| | **9** | 72$^{b}$ | 72$^{c}$ | 86$^{d}$ |
| **IPA99** | **Control** | 44$^{a}$ | 48$^{a}$ | 48$^{a}$ |
| | **3** | 48$^{a}$ | 49$^{a}$ | 54$^{b}$ |
| | **6** | 48$^{a}$ | 59$^{b}$ | 62$^{c}$ |
| | **9** | 72$^{b}$ | 72$^{c}$ | 82$^{d}$ |

| Cultivar | Dose (mM) | Germination Speed (h) | | |
|---|---|---|---|---|
| | | 6h | 12h | 24h |
| Zarqaa | Control | 46[a] | 48[a] | 48[a] |
| | 3 | 48[a] | 56[a] | 58[b] |
| | 6 | 48[a] | 68[b] | 72[c] |
| | 9 | 72[b] | 72[c] | 86[d] |

*Different letters refer to significant differences among the means in different treatments within the column using one-way ANOVA (P≤0.05).*

In the past decade, the iron materials effects on environment have been studied, but no consistent outcomes have been reached (Saif *et al.*, 2016). The effect of iron materials essentially depends on their properties (type, , size, surface properties, and synthesis method), test plant tolerance, and environmental conditions (Zahra *et al.*, 2021). Smaller particles have more tendency to penetrate into the cells, and pose potential harmful consequences. Several researches have revealed iron NPs with smaller size pose higher toxic effects on organisms (Malhotra *et al*., 2020; Soenen & De Cuyper, 2010; Verma & Pandey, 2017). Taking the two germination indicators (seed germination rate and germination speed) together, we found all the Fe2O3 NPs caused insignificant inhibition or toxicity to barley cultivars at the tested levels. Our results compatible with varied previous studies concluded similar findings to ours (Libralato *et al*., 2016; Malek *et al*., 2023; Sun *et al*., 2020; Waqas Mazhar *et al*., 2022). Particularly, we conclude that concentrations at (6 and 9 mM) Fe2O3 NPs did not pose more significant toxicity. Suggesting that these materials have been used are safe at tested doses. The dose-dependent effect was not obvious for seed germination rate and germination speed within doses we used. Results from all treatments evidenced that Fe2O3 NPs at concentrations used was not phytotoxic. Germination percentage didn't show significant toxic effects compared to control samples and germination speed significantly affected and dose dependent.

## CONCLUSIONS

The results of Fe2O3 nanoparticle characterization tests demonstrated the success and efficiency of the green synthesis method using turmeric extract. Seed priming performance was evaluated using germination tests. Our study results showed that Fe2O3 NPs generally had insignificant inhibition effect at low doses and short-time exposure of seeds to Fe2O3 NPs. It showed that the Fe2O3 NPs materials had insignificant phytotoxicity to all barley cultivars at the tested low doses. In conclusion, the tested Fe2O3 NPs pose no obvious phytotoxic effect within the dose range (3-6 mM), while, generally delay the germination. High dose and long-time exposure have inhibition effect on seed germination rate % that seen significant at 9 mM for 24h priming period.

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