Effects of Adding Fe_2O_3 and Fe_3O_4 Nanoparticles to Soil on Germination and Seedling Characteristics of Oriental Beech

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Effects of Fe-based nanoparticles (Fe_2O_3 and Fe_3O_4) on germination parameters were studied for some seedling characteristics of Oriental beech (*Fagus orientalis*) seeds. Fe_2O_3 and Fe_3O_4 nanoparticle applications were made at concentrations of 400, 800, 1200, 1600, and 2000 mg/L on *Fagus orientalis* seeds collected from 10 different populations, and some germination and seedling characteristics were evaluated. Preliminary results generally indicated that low-dose nanoparticle applications positively affected germination and seedling characteristics, while increases in doses led to decreases in these parameters. Values obtained from high-dose nanoparticle applications were generally lower than those from the control group. The iron nanoparticles affected the parameters to different extents, Fe_2O_3 nanoparticles showed a significant positive effect on germination rate and radicle length, while exhibiting a significant negative effect on germination percentage and plumule length. The populations least affected by high-dose iron nanoparticle applications were Bursa Inegol, Karabuk-Yenice, and Ordu Akkus, while the most affected were the Bartin-Kumluca and Kahramanmaras-Andirin populations.

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INTRODUCTION

Plants are the basis of all living life on Earth. Therefore, understanding and directing plant growth and development is one of the primary study topics for human beings (Ozel *et al*. 2021; Özdikmenli *et al*. 2024). As is known, the growth and development of plants, as in all living organisms, are shaped under the influence of genetic structure (Hrivnak *et al*. 2024) and environmental conditions (Yayla *et al*. 2022; Sevik *et al*. 2024). The main environmental factors affecting plant growth are climatic and edaphic (Key *et al*. 2023; Koc *et al*. 2024). Edaphic factors include many components, such as soil nutrient content, pH, and soil texture and structure (Erdem *et al*. 2023).

The chemical structure of the soil is the most important factor affecting and shaping plant growth. The presence and amount of nutrients required for plant growth are essential. However, metals, essential as nutrients, can become lethal to plant biology at high concentrations. Therefore, it is crucial to provide plants with the optimum amounts of metals so that normal metabolic functions can be maintained without metals deficiency or phytotoxicity (Natasha *et al.* 2022). Moreover, the optimum amount of each metal for plants needs to be determined individually for each plant. The optimum metal concentration and toxicity limit for each plant is different (Pavlovic *et al.* 2021; Kaur *et al.* 2023).

Iron is one of the metals absolutely necessary for plant growth. Iron (Fe) is an essential element for plants and plays many important roles in physiological and metabolic processes. It is a redox active, highly reactive element, and thus higher concentrations of Fe can be toxic to plants (Zuo and Zhang 2011; Kuzmina *et al.* 2023). Although Fe is present in high concentrations in soil, it is not readily taken up by plants. Fe is the third most limiting nutrient for plant growth and metabolism, primarily due to the low solubility of the oxidized ferric form in aerobic environments (Zuo and Zhang 2011). The plant growth depends on the Fe availability that is subjected to several factors including physico-chemical properties of soil and microbial Fe metabolism. Plants secrete protons, phenolics, and metabolites (*e.g.* mugineic acid) to aid conversion of Fe from Fe^{3+} to Fe^{2+} and to increase chelation of Fe for uptake (Ishimaru *et al.* 2011). The optimum amount of Fe and the level of toxicity have been determined in many cases. For example, for *Triticum aestivum*, 10 to 50 mg L^{-1} level is optimum, while 250 mg L^{-1} level is toxic and 1000 mg L^{-1} level is lethal (Kaur *et al.* 2023). In *Oryza sativa*, Fe levels that cause toxicity range from as low as 10 mg Fe L^{-1} to 500 mg Fe L^{-1} or higher (Sahrawat 2005).

In plant development and especially in cultivated plants, the elimination of elemental deficiency in the soil due to long-term production is of great importance in terms of ensuring product continuity. Fertilization for this purpose is vital for plant development (Erdem *et al.* 2023). Soil fertilization with nanoparticles shows promising results for agriculture, especially for crop production (Mielcarz-Skalska *et al.* 2021). In recent years, nanoparticles have been used intensively in many fields, not only for fertilization (Özel *et al.* 2024).

Among the most widely used nanoparticles, Fe_2O_3 and Fe_3O_4 nanoparticles are used for the recovery of contaminated water and soil, offering great promise for use in biomedicine and water treatment due to their superparamagnetic and adsorptive properties. Recent studies suggest that Fe-based nanoparticles may also be beneficial for improving plant growth and Fe nutrient accumulation in agricultural plants. However, the potential adverse effects of nanoparticle applications on other organisms remain a serious concern, despite the fact that it generally has positive effect on agricultural plants (Tombuloglu *et al.* 2022). As a result of the reaction of iron (III) and oxygen, there are about 16 different iron oxide species in nature (Nanography 2024). Iron oxide NPs are often reported in literature using the formula Fe_3O_4, but they also can have different forms such as magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), hematite (α-Fe_2O_3), FeO, ε-Fe_2O_3, and βFe_2O_3. The different forms possess different magnetic behavior. For instance, magnetite and maghemite are ferromagnetic or superparamagnetic; however, hematites are weakly ferromagnetic or antiferromagnetic (Tombuloglu *et al.* 2022). For example, magnetic Fe_3O_4 nanoparticles are superparamagnetic below the size of 20 nm. As the nanoparticle size decreases, this property tends towards paramagnetic or superparamagnetic magnetization (Ajinkya *et al.* 2020). This magnetic property is very valuable for many applications when produced with controlled size and crystal structure.

Nanoparticle compounds can show different properties from those in nature (Nanography 2024). Nanoparticles can be defined as nano-sized powder grains or particles having dimensions in the range of 1 to 100 nm. Compared to their bulk structures, nanoscale materials exhibit different properties due to quantum size effects, size dependence of the electronic structure, and the number of surface atoms. Nanoparticles have enormous surface energies due to their enormous surface area to volume ratio. In this way, while their chemical and physical properties change, their functionality also changes. Therefore, naturally occurring compounds and nanoparticles are compounds of different character (Seyhan 2022).

Iron nanoparticles can have adverse effects, especially on soil organisms, stimulate abundant ROS (reactive oxygen species) production in plants, cause oxidative stress damage, and thus cause inhibition of plant growth. They can cause serious disruptions in photosynthesis, especially in chlorophyll synthesis, leading to adverse effects on plants (Tao *et al.* 2023). It has also been determined that cell density decreases dose-dependently (Ameen *et al.* 2021).

Despite having various studies conducted in agricultural areas, the number of studies on the effects of nanoparticles on forest ecosystems and forest elements is much more limited. In this study, it was aimed to identify the preliminary effects of Fe_2O_3 and Fe_3O_4 nanoparticles on the germination parameters of seeds collected from different locations of oriental beech (*Fagus orientalis*), which is one of the important native tree species in the authors' country, at different doses.

Iron-based nanoparticles can have different effects in soluble ionic forms, and this effect may differ depending on the plant species. Gui *et al.* (2015) observed that inhibition of rice root phytohormones under hydroponic conditions was positively correlated with nFe_2O_3 concentration up to 200 mg/L. However, nFe_2O_3 was reported to cause oxidative stress in maize and roots of Citrus maxima and also reduced leaf chlorophyll content (Li *et al.* 2016; Hu *et al.* 2017). Yang *et al.* (2020) reported that exposure of soybean to nFe_2O_3 did not cause any toxicity stress or physiological disorders, on the contrary, exposure to nFe_2O_3 significantly improved physiological performance by causing increases in chlorophyll content, plant biomass and root growth indices.

However, high amounts of nanoparticles may result in the presence of higher concentrations of iron ions of various types adjacent to the nanoparticles in moist soil due to diffusion mechanism, which may negatively affect plant growth. Therefore, the main hypothesis of the study was "Increasing dosage of Fe-based nanoparticles negatively affects germination and seedling characters in *Fagus orientalis* seeds".

EXPERIMENTAL

Materials and Method

The seeds collected within the scope of the study were obtained from the oriental beech forests of Türkiye. Information regarding the regions and populations, from which the seeds were collected, is given in Table 1.

After being subjected to health tests and showing no issues in their embryo and endosperm parts, and demonstrating healthy and normal development performance, the collected seeds were used in germination tests. One of the main objectives of the research was to identify the effects of iron nanoparticles on the germination parameters of oriental beech seeds as well as to establish toxic threshold values. For this purpose, under sterile and hygienic conditions in the laboratory, five different nanoparticle concentrations for both Fe_2O_3 (average diameter: <30 nm, purity: >99.5%) and Fe_3O_4 (average diameter: <20 nm, purity: >99.5%) nanoparticles were prepared in the amounts of 400, 800, 1200, 1600, and 2000 mg/L, thus a total of ten concentrations were then stored in sterile concentration bottles to be applied to the seeds.

Table 1. Populations from Which the Seeds Were Collected

	Region	Coordinates		Altitude (m)	Exposure	Stand Type
1	Adapazari-Karasu	41° 01' 13"	30° 44' 45"	300 to 500	Northwest	Knd$_2$
		41° 00' 38"	30° 45' 01"			
2	Balikesir-Dursunbey	39° 27' 35"	28° 33' 54"	800 to 1000	North	Knd$_1$
		39° 27' 30"	28° 32' 44"			
3	Bartin-Kumluca	41° 29' 43"	32° 26' 32"	450 to 700	North	Knd$_3$
		41° 29' 55"	32° 27' 54"			
4	Bursa-Inegol	39° 58' 44"	29° 29' 04"	700 to 1200	Northwest	Knd$_3$
		39° 58' 02"	29° 28' 46"			
5	Canakkale-Kalkim	39° 46' 56"	27° 09' 46"	400 to 600	North	Knd$_2$
		39° 46' 11"	27° 09' 10"			
6	Duzce-Yigilca	31° 25' 35"	31° 25' 35"	700 to 1100	Northwest	Knd$_3$
		31° 25' 35"	31° 25' 35"			
7	Kahramanmaras-Andirin	37° 46' 12"	36° 22' 10"	1400 to 1800	North	Knd$_3$
		37° 44' 52"	36° 23' 34"			
8	Karabuk-Yenice	41° 09' 08"	32° 16' 37"	600 to 900	North	Knd$_1$
		41° 08' 52"	32° 15' 53"			
9	Ordu-Akkus	40° 47' 25"	36° 58' 50"	1100 to 1400	North	Knd$_2$
		40° 46' 56"	36° 59' 05"			
10	Zonguldak-Devrek	41° 12' 27"	31° 53' 15"	500 to 700	Northwest	Knd$_1$
		41° 16' 13"	32° 12' 52"			

The beech seeds treated with prepared concentrations in five different doses were placed in single-use sterile petri dishes prepared with quantitative filter papers to be used in germination tests. Five repetitions were performed for each dose, and nanoparticle treatment was applied to 150 seeds for each dose, with 30 healthy seeds per repetition. A total of 900 seeds, including the control group, were used in the germination tests. Germination tests were conducted in a 3M Climacell brand germination cabinet. The temperature of the germination medium in the cabinet was set to 20 °C, relative humidity to 70%, and the lighting duration to 12 h.

Oriental beech seeds, placed in 100-mL petri dishes in a way not to touch each other, were monitored for germination by exposing them to 10 mL of nanoparticle solution daily. On the 7th day of application, the number of germinated seeds was counted to calculate the germination rate (GR). The applications continued for 35 days, and at the end of the 35th day, the seedling height (SH), root collar diameter (RCD), plumule length (PL), radicle length (RL), and radicle thickness (RT) were measured using a digital micro-meter compass. Non-germinated seeds were cut to check if they were healthy, and the germination percentage (GP) was calculated by comparing the total number of germinated seeds to the total number of healthy seeds. Similarly, the germination rate was calculated as the ratio of the number of germinated seeds on the 7th day to the number of healthy seeds. The data obtained were analysed using SPSS 22.0 software package, and variance analysis and Duncan test were applied to the data.

RESULTS

Data showing the changes in germination rate is presented in Table 2.

Table 2. Changes in Germination Percentage (%)

Pop.	Fe_3O_4						Fe_2O_3					
	Cont.	400	800	1200	1600	2000	Cont.	400	800	1200	1600	2000
1	5.9	5.6	5.5	5.0	5.0	4.3	5.9	7.0	6.7	6.7	6.3	5.5
2	5.4	5.7	5.6	5.3	5.1	4.7	5.4	7.1	6.7	6.3	6.6	5.6
3	5.6	5.2	5.1	4.9	4.6	4.5	5.6	6.9	6.6	5.9	6.0	5.1
4	5.2	5.9	5.8	5.5	5.3	5.0	5.2	7.1	6.8	6.7	6.6	6.0
5	5.8	5.4	5.4	4.9	4.6	4.6	5.8	7.0	6.4	6.1	6.1	5.4
6	5.3	5.6	5.4	4.9	4.9	4.8	5.3	6.6	6.7	6.4	6.2	6.0
7	5.4	5.0	5.1	4.7	4.6	4.3	5.4	6.4	6.2	6.1	6.1	5.2
8	5.2	5.8	5.5	5.2	5.1	5.1	5.2	6.9	6.8	6.6	6.3	5.9
9	5.6	6.1	5.8	5.5	5.3	5.2	5.6	7.4	6.9	6.6	6.5	6.3
10	5.3	5.4	5.3	5.1	4.7	4.5	5.3	6.7	6.7	6.4	6.0	5.1
Av.	5.5	5.6	5.5	5.1	4.9	4.7	5.5	6.9	6.7	6.4	6.3	5.6
StD.	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.4

Pop.; Population number, Cont.; Control, Av.; Average, StD.; Standard deviation

When the changes in germination percentage were examined, it was observed that in both nanoparticle applications, the germination percentage significantly increased at low doses and began to decrease with increasing doses. The germination at a dose of 400 mg/L increased by approximately 50% in the Fe_3O_4 nanoparticle application, while it increased more than twice in the Fe_2O_3 nanoparticle application. Afterwards, the germination started to decrease with increasing doses, but even at the highest dose application of 2000 mg/L, it did not fall below the values in the control group.

Table 3. Changes in Germination Percentage (%)

Pop	Fe_3O_4						Fe_2O_3					
	Control	400	800	1200	1600	2000	Control	400	800	1200	1600	2000
1	68.4	84.0	80.7	78.9	77.2	76.3	68.4	71.1	71.9	67.1	64.2	63.1
2	71.6	84.7	85.5	79.6	77.1	77.4	71.6	75.1	75.4	73.0	70.4	69.5
3	59.3	78.9	77.3	74.9	73.9	73.5	59.3	66.5	64.6	60.8	59.5	59.4
4	75.2	85.5	84.1	80.9	79.5	79.0	75.2	80.9	79.7	74.9	73.6	72.8
5	66.0	80.9	79.2	77.0	75.4	75.8	66.0	70.6	70.4	65.1	64.0	63.4
6	69.9	85.0	83.5	79.0	76.3	77.0	69.9	74.1	72.5	68.7	65.6	65.7
7	57.7	77.6	77.0	74.8	73.0	72.9	57.7	65.5	64.8	58.8	56.7	55.1
8	74.6	83.5	85.6	80.8	79.7	78.9	74.6	78.2	79.9	74.3	71.6	71.1
9	78.8	87.4	86.4	81.4	78.9	77.0	78.8	82.3	81.9	77.0	72.5	72.0
10	59.8	82.0	80.1	78.8	75.9	74.2	59.8	69.7	68.7	63.9	62.4	62.6
Av.	68.1	83.0	81.9	78.6	76.7	76.2	68.1	73.4	73.0	68.4	66.1	65.5
StD.	7.3	3.1	3.5	2.4	2.3	2.1	7.3	5.8	6.2	6.3	5.8	5.8

When the changes in germination percentage was examined based on population, it was observed that the highest germination rates were obtained in the P4 and P9 populations, while the lowest germination rates were obtained in the P7 population. The trend showing the changes in germination percentage is presented in Table 3.

It was observed that the application of Fe nanoparticles significantly affected the germination percentage. In both nanoparticle applications, the germination percentage increased significantly at low doses, with this increase being higher in the Fe_3O_4 nanoparticle application. However, in both applications, the germination percentage decreased significantly at the application of 1200 mg/L, and it continued to decrease with increasing doses. While the germination percentage in Fe_3O_4 nanoparticles was generally higher than that in the control group even at high doses, the germination percentages obtained at 2000 mg/L application in Fe_2O_3 nanoparticles were generally lower than those in the control group.

When the changes in germination percentage were examined based on population, it was observed that the highest germination percentages were obtained in the P4, P8, and P9 populations. The lowest germination percentages were obtained in the P3 and P7 populations. The graph showing the changes in seedling height is presented in Table 4.

Table 4. Changes in Seedling Height (cm)

Pop	Fe_3O_4							Fe_2O_3				
	Cont.	400	800	1200	1600	2000	Cont.	400	800	1200	1600	2000
1	7.1	7.4	7.4	7.2	6.9	6.8	7.1	7.1	7.0	6.8	6.5	6.1
2	7.2	7.7	7.6	7.3	7.1	7.1	7.2	7.4	7.3	7.0	6.6	6.4
3	6.4	7.2	7.2	6.9	6.8	6.5	6.4	6.9	6.7	6.3	6.2	5.9
4	7.2	7.9	7.9	7.6	7.3	7.2	7.2	7.7	7.5	7.2	7.0	6.7
5	7.0	7.5	7.3	7	6.8	6.6	7.0	7.1	6.9	6.7	6.4	6.1
6	7.1	7.6	7.6	7.3	7.1	7.0	7.1	7.4	7.3	6.9	6.5	6.2
7	6.4	7.1	7.0	6.7	6.6	6.4	6.4	6.8	6.6	6.2	6.1	5.9
8	7.2	7.9	7.8	7.3	7.2	7.1	7.2	7.6	7.4	7.3	6.8	6.7
9	7.3	8.2	8.1	7.8	7.5	7.2	7.3	8.3	7.6	7.4	6.8	6.4
10	6.6	7.3	7.2	6.9	6.7	6.5	6.6	7.0	6.8	6.6	6.4	6.0
Av.	7.0	7.6	7.5	7.2	7.0	6.8	7.0	7.0	7.3	7.1	6.8	6.5
StD.	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.5	0.4	0.4	0.3

Similar to germination values, it was determined that Fe nanoparticle applications significantly affected the seedling height as well. In both nanoparticle applications, seedling height increased significantly at low doses, with this increase being higher in the Fe_3O_4 nanoparticle application. However, seedling height decreased with increasing nanoparticle levels. In the Fe_2O_3 nanoparticle application, it was determined that the decrease in seedling height with increasing doses was at higher levels.

Seedling height was also observed to be varying significantly on a population basis. The highest seedling heights were observed in the P4, P8, and P9 populations, while the lowest seedling heights were observed in the P3, P7, and P10 populations. The graph showing the changes in root collar diameter is presented in Table 5.

Table 5. Changes in Root Collar Diameter (mm)

Pop	Fe_3O_4						Fe_2O_3					
	Cont.	400	800	1200	1600	2000	Cont.	400	800	1200	1600	2000
1	0.8	0.9	0.8	0.9	0.9	0.8	0.8	0.9	0.8	0.9	0.8	0.8
2	0.9	0.9	1.0	0.8	0.9	0.9	0.9	1.0	1.0	0.9	0.9	0.8
3	0.8	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.9	0.9	0.9	0.8
4	0.9	0.9	0.9	1.0	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9
5	0.8	0.9	0.9	0.8	0.9	0.8	0.8	0.9	0.9	0.9	0.8	0.8
6	0.8	0.9	0.9	0.9	0.9	0.8	0.8	1.0	0.9	0.9	0.8	0.8
7	0.8	0.9	0.8	0.9	0.8	0.7	0.8	0.9	0.9	0.9	0.8	0.8
8	0.9	1.0	1.0	0.8	0.9	0.9	0.9	1.0	0.9	0.9	0.9	1.0
9	0.9	1.0	0.9	1.0	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.9
10	0.8	0.9	0.8	0.9	0.8	0.8	0.8	0.9	0.8	0.8	0.8	0.8
Av.	0.8	0.9	0.9	0.9	0.9	0.8	0.8	0.9	0.9	0.9	0.9	0.8
StD.	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1

As shown in Table 5, the values of root collar diameter generally ranged between 0.8 and 1.0 mm, and it was not possible to say that there was a significant change depending on both the population and the nanoparticles applied. According to these results, it can be said that the root collar diameter did not change noticeably depending on the nanoparticle applications. The graph showing the changes in plumule length is presented in Table 6.

Table 6. Changes in Plumule Length (cm)

Pop	Fe_3O_4						Fe_2O_3					
	Cont.	400	800	1200	1600	2000	Cont.	400	800	1200	1600	2000
1	1.1	1.0	0.9	1.0	1.0	0.9	1.1	0.9	1.0	0.8	0.8	0.8
2	1.1	1.0	1.0	1.0	1.0	1.0	1.1	1.0	0.9	0.9	0.9	0.9
3	0.9	1.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8
4	1.1	1.0	1.0	1.0	1.0	1.0	1.1	0.9	1.0	1.0	0.9	0.8
5	1.0	1.0	0.9	1.0	0.9	0.9	1.0	0.9	0.9	0.8	0.8	0.8
6	1.1	1.0	1.0	1.0	1.0	0.9	1.1	0.9	0.9	0.9	0.9	0.8
7	0.9	0.9	1.0	0.9	0.9	0.8	0.9	0.8	0.9	0.9	0.8	0.8
8	1.1	1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	0.9	0.8
9	1.1	1.1	1.1	1.0	1.1	1.0	1.1	1.0	1.0	1.0	0.9	0.9
10	1.0	1.0	0.9	0.9	0.9	0.9	1.0	0.9	0.8	0.9	0.8	0.8
Av.	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.9	0.9	0.9	0.9	0.8
StD.	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0

It can be said that the changes in plumule length were not significant in the Fe_3O_4 application, while they tended to decrease with increasing doses in the Fe_2O_3 application. It was observed that the plumule length, which was in the range of 0.9 to 1 mm in the control group for the Fe_2O_3 application, dropped to the level of 0.8 mm in most populations with the application of Fe_2O_3. The graph showing the changes in radicle thickness is presented in Table 7.

Table 7. Changes in Radicle Thickness (mm)

Pop	Fe_3O_4						Fe_2O_3					
	Cont.	400	800	1200	1600	2000	Cont.	400	800	1200	1600	2000
1	1.2	1.0	1.0	1.0	0.9	0.9	1.2	1.0	0.9	0.9	0.8	0.9
2	1.3	1.1	1.0	0.9	1.0	0.9	1.3	1.2	1.0	1.0	0.9	0.9
3	1.1	1.0	1.0	1.0	0.8	0.8	1.1	1.1	1.0	0.9	0.9	0.9
4	1.3	1.1	1.0	1.0	1.0	0.9	1.3	1.0	0.9	0.9	1.0	0.9
5	1.1	1.1	1.0	1.0	1.0	0.9	1.1	1.1	1.0	0.9	0.9	0.9
6	1.2	1.0	1.1	1.0	0.9	0.9	1.2	1.0	0.9	0.9	1.0	0.9
7	1.0	0.9	1.0	0.9	0.9	0.8	1.0	0.9	1.0	0.8	0.8	0.8
8	1.2	1.0	1.0	1.0	1.1	0.9	1.2	1.1	1.0	0.9	1.0	0.9
9	1.3	1.1	1.1	1.1	1.0	0.9	1.3	1.1	1.0	0.9	1.0	0.9
10	1.1	1.0	1.0	0.9	0.9	0.9	1.1	0.9	1.0	0.8	0.9	0.9
Av.	1.2	1.0	1.0	1.0	1.0	0.9	1.2	1.0	1.0	0.9	0.9	0.9
StD.	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0

As shown in Table 7, the radicle thickness significantly decreased after the application of Fe nanoparticles, when it was 1 mm or more in the control group, and it continued to decrease with increasing doses. This decrease was much faster in the Fe_2O_3 application. In the Fe_2O_3 application, from the dose of 1200 mg/L onwards, the average radicle length in most populations is observed to be around 0.8 mm. The graph showing the changes in radicle length is presented in Table 8.

Table 8. Changes in Radicle Length (cm)

Pop	Fe_3O_4						Fe_2O_3					
	Cont.	400	800	1200	1600	2000	Cont.	400	800	1200	1600	2000
1	5.9	5.6	5.5	5.0	5.0	4.3	5.9	7.0	6.7	6.7	6.3	5.5
2	5.4	5.7	5.6	5.3	5.1	4.7	5.4	7.1	6.7	6.3	6.6	5.6
3	5.6	5.2	5.1	4.9	4.6	4.5	5.6	6.9	6.6	5.9	6.0	5.1
4	5.2	5.9	5.8	5.5	5.3	5.0	5.2	7.1	6.8	6.7	6.6	6.0
5	5.8	5.4	5.4	4.9	4.6	4.6	5.8	7.0	6.4	6.1	6.1	5.4
6	5.3	5.6	5.4	4.9	4.9	4.8	5.3	6.6	6.7	6.4	6.2	6.0
7	5.4	5.0	5.1	4.7	4.6	4.3	5.4	6.4	6.2	6.1	6.1	5.2
8	5.2	5.8	5.5	5.2	5.1	5.1	5.2	6.9	6.8	6.6	6.3	5.9
9	5.6	6.1	5.8	5.5	5.3	5.2	5.6	7.4	6.9	6.6	6.5	6.3
10	5.3	5.4	5.3	5.1	4.7	4.5	5.3	6.7	6.7	6.4	6.0	5.1
Av.	5.5	5.6	5.5	5.1	4.9	4.7	5.5	6.9	6.7	6.4	6.3	5.6
StD.	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.4

The Fe nanoparticle applications were observed to significantly affect radicle length. Particularly, in the low-dose Fe_2O_3 application, radicle length increased significantly; however, in both applications, radicle length decreased significantly with increasing doses. Especially, in the Fe_2O_3 nanoparticle application at 2000 mg/L, radicle length showed a significant decrease.

When the changes in radicle length were examined the basis of population, it was observed that the highest values were obtained in the P4, P6, P8, and P9 populations. The lowest germination percentages were obtained in the P3 and P7 populations.

DISCUSSION

The study results showed that iron nanoparticle applications affected almost all of the measured characteristics. Generally, germination and seedling characteristics were positively affected at low nanoparticle doses, but decreases began in these parameters with increasing doses. Values obtained from high-dose nanoparticle applications were generally lower than those in the control group. Therefore, it was found that low nanoparticle applications had a positive effect on germination and seedling characteristics, but they had a negative effect as the dose increases. This result means that the main hypothesis of the study is accepted. This finding is in compliance with the information obtained in the literature.

Recent studies suggest that Fe-based nanoparticles may also be beneficial for improving plant growth and Fe accumulation in plants. For instance, it is stated that the addition of 2 mg kg^{-1} Fe_2O_3 nanoparticles can increase chlorophyll content, regulate phytohormones, and increase Fe content. In contrast, the negative effects of Fe-based nanoparticles have also been reported in many studies, and their use in such applications in the future has been discussed. For example, Fe_3O_4 nanoparticles have been reported to cause oxidative stress in ryegrass and pumpkin grown hydroponically. Additionally, it is stated that Fe_2O_3 nanoparticles at 50 mg L^{-1} concentration can disrupt photosynthesis by reducing chlorophyll content, while nanoparticles at a concentration of 500 mg L^{-1} can cause excessive accumulation of ROS in cells, leading to DNA damage and a decrease in mitotic index in plant cells (Li *et al.* 2021). Low concentrations of nZVI nanoparticles were found to have positive effects on *Oryza sativa* seeds and seedlings, such as increased root and shoot length, biomass and photosynthetic pigment content. However, seedlings prepared with 160 mgL^{-1} nZVI were subjected to oxidative stress and SEM micrographs also revealed root tissue damage at this concentration (Guha *et al.* 2018).

The parameters under study were determined to be affected by iron nanoparticles at different levels. While Fe_2O_3 nanoparticles showed a significant positive effect on germination rate and radicle length, a significant negative effect was observed on germination percentage and plumule length. The studies conducted on the subject indicate that nanoparticles of different sizes and concentrations affect germination and seedling characteristics at different levels. In a study, the effects of Fe_3O_4 nanoparticles were investigated on seed germination in tobacco plant (*Nicotiana tabacum*), and it was found that the radicle lengths of seeds treated with 5 nm (30 mg/L concentration) and 10 nm (10 and 30 mg/L concentration) were significantly shorter than those of control seeds. In contrast, the radicle lengths of seeds treated with 10 nm (3 mg/L concentration) and 20 nm (10 mg/L concentration) were found to be significantly longer than those of control seeds.

Most of the seeds treated with nanoparticles show significantly higher seed germination percentages (Alkhatib *et al.* 2021).

In a study conducted by Asadi-Kavan *et al.* (2020), the effects of iron oxide nanoparticles (Fe_2O_3) on the germination and seedling growth of *Oenothera biennis* were determined, and it was found that Fe_2O_3 increased the germination percentage 89.2%, the germination tolerance index 53.4%, and the root tolerance index 82.2%. The study concluded that the use of Fe_2O_3 at low and medium concentrations did not have toxic effects on this plant. Gupta *et al.* (2022) reported that Fe_3O_4 nanoparticle application in *Cucumis sativus* increased seed yield 52.2%, total chlorophyll content 17.3%, germination percentage 17.0%, and seedling vigor 72.6%. A significant increase also was observed in starch, soluble proteins, soluble sugars, and fat content in seeds obtained from plants treated with Fe_3O_4 nanoparticles. It was determined that Fe_2O_3 and Fe_3O_4 nanoparticles significantly increased the germination rate (approximately 37% for Fe_2O_3; approximately 63% for Fe_3O_4), plant biomass and pigmentation in *Hordeum vulgare* (Tombuloglu *et al.* 2022).

In *Nicotiana tabacum*, it is found that Fe_3O_4 nanoparticles accumulate only in clusters around the root cell wall, indicating that Fe_3O_4 nanoparticles move through the apoplastic pathway in roots (Yuan *et al.* 2018; Alkhatib *et al.* 2021). It is stated that Fe_3O_4 nanoparticles are efficiently taken up by roots and transported to leaves regardless of their size, but small-sized Fe_3O_4 nanoparticles can be more reactive due to their size properties, which then lead to cell stress and membrane damage (Tombuloglu *et al.* 2024). In seeds treated with Fe_3O_4 nanoparticles, it is indicated that chlorophyll is completely consumed and albinism occurs, potentially causing a lethal effect on chlorophyll synthase (Alkhatib *et al.* 2021). Germination is found to be decreasing in *Helianthus annuus* seeds treated with Fe nanoparticles, indicating a decrease in the content of elements associated with this process (Ca, Mg, K, P, and Na), and it is suggested that these elements are adsorbed by Fe nanoparticles (Kornarzyński *et al.* 2020). As can be seen, the effects of nanoparticles on plants vary depending on size and dose, but generally, nanoparticle applications that increase efficiency at low doses are found to be harmful with increasing doses.

As a result of the study, the effects of iron nanoparticle applications on germination and seedling characteristics of beech seeds were evaluated at the population level, and it was determined that populations P4, P8, and P9 were the least affected by high-dose iron nanoparticle applications, while populations P3 and P7 were the most affected. When these populations were examined, it was observed that the least affected populations were mostly located at moderate altitudes, while the most affected populations were located at low or high altitudes, *i.e.*, at extreme altitudes.

CONCLUSIONS

1. The results of the study revealed that high doses of Fe_2O_3 and Fe_3O_4 nanoparticles negatively affected germination and seedling development. However, it was determined that 400 mg/L nanoparticle application positively affected almost all characteristics. For this reason, 400 mg/L Fe_2O_3 and Fe_3O_4 nanoparticles are recommended, especially in seedling production studies.
2. The study results indicate that some populations are less affected by iron-based nanoparticles. These populations can be used as a priority seed source for seedling

production. However, it is recommended that studies on this subject to focus on more advanced stages, such as the seedling stage, before this stage.

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