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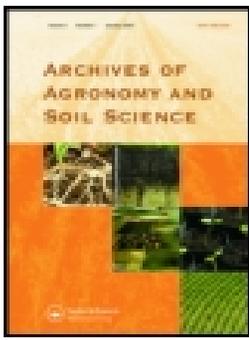
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Effects of nano Fe/SiO₂ fertilizers on germination and growth of barley and maize

Marjan Najafi Disfani^a, Azadeh Mikhak^b, Mohammad Zaman Kassae^c, Amirhossein Maghari^d

^a Faculty of Biosciences & Medical Engineering, University of Teknologi Malaysia(UTM),

Johore Bahru, Malaysia; ^bDepartment of Soil Science, Lorestan University, Khurramabad,

Iran; ^c Department of Chemistry, Tarbiat Modares University, Tehran, Iran; ^d New Hearing

Technologies Research Center, Baqiyatallah University of Medical Science, Tehran, Iran

CONTACT: Azadeh Mikhak, Department of Soil Science, Lorestan University,

Khurramabad, Iran; Email: mikhak_azadeh@yahoo.com

Abstract

Iron nanoparticles, with 30-40 nm diameter, were stabilized on sand. The resulting synthesized Fe/SiO₂ nanoparticles, with different iron contents (0-25 mg kg⁻¹) were employed as fertilizers in probing the mean germination time (MGT), growth, and dry matter of barley and maize and their comparison with common Fe/SiO₂ in a completely randomized design experiment (CRD). The results showed that our fertilizers had significant effects on MGT, with the lowest of 0.58 day for barley and 0.79 day for maize; at 15 and 5 mg kg⁻¹ nano Fe/SiO₂, respectively. Application of 15 mg kg⁻¹ of nano Fe/SiO₂ increased the shoot length: 8.25% and 20.8% for barley and maize, respectively. However, the concentration of 25 mg kg⁻¹ had a negative impact on shoot length in barley. Increasing the concentrations of both

nano and common Fe/SiO₂ particles, increased the root lengths in both plants, however this increase was higher with the application of nano Fe/SiO₂. Likewise, seedling length enlarged with the concentration increase of both Fe/SiO₂ particles and was more pronounced with nano Fe/SiO₂. The application of nano Fe/SiO₂ was more effective compared to the common Fe/SiO₂ in encouraging barley and maize growth. The positive impact was higher in maize than barley.

Keywords: barley, maize, zero-valent iron nanoparticles stabilized on sand (Fe/SiO₂), common Fe/SiO₂, MGT, growth

Introduction

Iron (Fe) is an essential micronutrient for all organisms. It is involved in several vital plant functions, including photosynthesis, respiration and chlorophyll biosynthesis. It is a component in heme, Fe-sulfur cluster, and other Fe binding sites. Regardless of Fe's plentitude in different soil types, it's availability in the form of insoluble Fe (III) makes it a scarce element for plant's up take (Römheld & Marschner 1986). Fe deficiency is observed in calcareous (high pH) and aerobic soils as a result of it's low solubility and availability (Itai et al. 2013; Sánchez-Rodríguez et al. 2015). It is a major nutritional problem often limiting the growth and production in many crops, it induces low CO₂ assimilation rate, reduced yields, chlorosis development (Graham et al. 1997, 2001; Cakmak 2002) increase in the absolute quantity of transporters facing the rhizosphere root exchange surface area (Arahou & Diem 1997; Schmidt et al. 2000; Schmidt & Schikora 2001) decrease in the photosynthetic activity by reducing the light harvesting complexes (LHCs) and electron-transport carriers concentration and drastic decline in carotenoids such as b carotene and neoxanthin (Morales et al. 1990; Donnini et al. 2003, 2009). ENP (engineered nano particles) have aimed to

positively alter the entire agriculture and other associated sectors by preventing disease and molecular treatment, enhancing agricultural productivity *via* genetic improvement of plants and animals (Kuzma 2007; Scott 2007), converting the agricultural and food wastes to applicable energy and other invaluable byproducts by the aid of enzymatic nano bioprocessing, ameliorating the negative effects of pollution level as a result of using pesticides and fertilizers. Plants as a basic component in all ecosystems play a crucial role in the fate and transport of ENPs in the environment through plant uptake and bioaccumulation and they could positively or negatively affect plants (Monica & Cremonini 2009; Ma et al. 2010).

Silicon (Si) is the second most common element in the earth's crust. It is a useful element for plants. It plays an important role in plant resistance to stresses. However, for various reasons silicon is not a required essential element for plants and as a most abundant element in the earth's crust its deficiency is unlikely therefore, its role has not been considered seriously (Malakouti 2005). Generally, Si impedes the Fe aggregation and due to this feature is mostly used in experiments. The aim of this work is to study the effects of Fe particles on different characteristics of barley and maize and to understand whether SiO₂ is a good stabilizer and could be used in further studies.

Materials and methods

Synthesis of Fe/SiO₂ NPs

Sand was soaked in 0.1 M HCl for 2 h and rinsed three times with deionized (DI) water and dried at 110°C in oven, to remove any impurities. Then, 5.0 g of FeSO₄ · 7 H₂O (98%, Aldrich) was dissolved in 250mL of 30% technical grade methanol and 70% DI water (*v/v*) and 1 g of the dried sand was added. The pH was adjusted to about 7 by 3 M NaOH. Next, 2.0 g of NaBH₄ powder (98%, Aldrich) was dissolved in 15 mL DI water and the solution

was added gradually to the mixture, allowing the foaming to subside between increments which finally resulted in ferric ion (Fe^{3+}) reduction. After the whole addition of the NaBH_4 solution, the black mixture was stirred for 45 min and then centrifuged for 15 min at $5000\times g$. The solid was washed twice with technical grade methanol, effectively substituting methanol for the water in the mixture. The resulting solid was dried for 5 h under the N_2 atmosphere and then broken up with a spatula to form a fine black powder (Valle-Orta et al. 2008; Kassae et al. 2011).

Characterization of the Fe/SiO₂ NPs

The particle size and morphology were investigated by scanning electron microscopy (SEM) using a Holland Philips XL30 microscope with an accelerating voltage of 25 kV. Crystal structures of the samples were examined using a Holland Philips Xpert X-ray powder diffraction (XRD) diffractometer (Cu $K\alpha$, radiation, $\lambda = 0.154056$ nm), at a scanning speed of $2^\circ/\text{min}$ from 10° to 80° (2θ).

Soil characteristics

The calcareous soil was collected from suburbs of Isfahan. The soil was air-dried and sieved through a 2 mm mesh and analyzed according to standard procedures. Soil analysis were measured as follows: pH (Jackson 1967), calcium carbonate (CaCO_3) (Caglar 1949), texture and organic matter (Black 1965), total N (Bremner 1965), extractable P (Olsen & Sommer 1982), extractable potassium (K) (Pratt 1965; Thomas 1982) in addition to extractable iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) (Lindsay and Norvell 1978). The characteristics of the soil in the study are given in Table 1.

Application of the nanoparticles to the soil

Plastic glasses were filled with soil mixture. Then, the Fe/SiO₂ NPs suspensions were added to each one kg pot and were left for overnight stabilization inside a growth chamber. In this experiment, four levels of both Fe/SiO₂ NPs and common Fe/SiO₂ particles, consisting of 0 (control, no NP), 5, 15 and 25 mg kg⁻¹ were used respectively.

Mean germination time

The seeds of maize (cv. Single 704) and barley (cv. Valfajr) which were collected from Isfahan Center for Research of Agricultural Science and Natural Resources were immersed in 10% sodium hypochlorite solution for 30 min, followed by three times rinsing with DI water and finally, soaked in D (Distilled) water for 6 h. 10 seeds per pot were sown at approximately 2.5 cm depth. The experiment was set up in CRD with three replicates and four treatments (0, 5, 15, 25 g kg⁻¹ respectively). Each replicate consisted of one kilogram pots and each pot was filled with 1000 g soil. The pots were placed in the growth chamber with 14 h photoperiod, 65±3% relative humidity, 25/20°C day/night temperature, and 340 mol m⁻²s⁻¹ light intensity. The plants were daily watered and the irrigation of pots was at the field capacity (FC) level.

The mean germination time (MGT)

$$(\text{MGT}) = \sum nt/n \text{ (Matthews \& Khajeh-Hosseini 2007)}$$

n = number of seeds newly germinated (just germinated criterion) at time t.

t = hours from when set to germinate.

Plant growth and biomass measurement

From each plot, 7 plants were randomly sampled and plant growth including, shoot length, root length and seedling length (sum of root and shoot (cm)), was measured. Then, shoot and root of the plants were excised. The culture conditions of both germination and growth were

similar. The roots and shoots dry weight (mg) was measured at different concentrations of NPs.

Leaf iron concentration

The leaf Fe concentration was measured in samples collected after terminated fertilization by inductively coupled plasma (ICP) (GBC Scientific Equipment, Braeside, VIC, Australia). The tissue samples (0.5 g) were well grounded and placed in the oven with a temperature gradient of $5^{\circ}\text{C min}^{-1}$ until the temperature reached 500°C . The samples were taken out of the oven, and 10 ml of hydrochloric acid (HCl) 20% (v/v) was added. The samples were then placed in a boiling water bath and filtered through Watman 42 filter paper (GE Healthcare, Cleveland, Ohio, USA).

Statistical analysis

For combined analysis of three variables, plant type (barley and maize), applied substance type (common Fe/SiO₂ and nano Fe/SiO₂) and the applied concentration (0-control, 5, 15 and 25 mg kg⁻¹) on each of the measured (MGT, shoot length, root length, seedling length and biomass). For data analysis three-way ANOVA was used. In order to univariate analysis One-Way ANOVA and independent Sample t-test were employed. The significance level was 0.05. All statistical analyses were performed using SPSS software (version 17.0).

Results

Characterization of Fe/SiO₂ NPs

Synthesized Fe/SiO₂ NPs were characterized using X-ray Diffraction (XRD) and SEM (Figure 1a, Figure 1b). The structural properties of Fe/Sand NPs were analyzed by XRD, where silicon oxide (Quartz-low) showed a very sharp diffraction peak at 26.61° that

corresponds to a d-spacing of 3.34 Å with an index of (1 0 1). This was along with the diffraction peaks at 20.83°, 50.16°, 60.05° and 68.34° were indexed to (1 0 0), (1 1 2), (2 1 1), and (3 0 1) planes of α -SiO₂. In addition, two diffraction peaks at $2\theta = 44.68^\circ$ and 65.01° with index of (1 0 0) and (2 0 0) indicated the formation of FeNPs (Figure 1a). The SEM image of Fe/SiO₂ NPs portrayed rather dispersed spherical NPs (Figure 1b).

Mean germination time (MGT)

The effect of different concentrations of common Fe/SiO₂ and nano Fe/SiO₂ (0, 5, 15, 25 mg kg⁻¹) on seed germination of barley and maize are presented in (Figure 2). In our analyses, P group value shows the significance level between common and nano Fe/SiO₂, P plant value shows the significance level between barley and maize and P treatment value shows the significance level between different concentrations of common and nano Fe/SiO₂. Combined analysis results show that all three variables simultaneously affect the MGT (mean germination time) index ($P < 0.05$).

In general, nano Fe/SiO₂ had a greater impact on germination time reduction compared to the common Fe/SiO₂ in both plants. This impact was higher in maize compared to barley since the time was only 68.6% with using 5 mg kg⁻¹ nano Fe/SiO₂ compared to 100% in the control. The most effective concentration in barley was 15 mg kg⁻¹ nano Fe/SiO₂ which time reduction was reported 51.7%. Generally, by increasing concentration of Fe particles, the seeds absorb less water and the germination is slightly delayed.

Plant growth

No growth inhibition of the treated plants was evident. Combined analysis results showed that all three variables simultaneously affect shoot length and seedling length index ($P < 0.05$). According to the results the application of both Fe/SiO₂ types had a positive effect on the shoot length in maize (Table 2) which the highest increase (26.25%) was observed at the

concentration of 15 mg kg⁻¹ nano Fe/SiO₂. However, further increase to 25mg kg⁻¹ had a very little impact on shoot elongation. In contrast, the application of common Fe/SiO₂ increased shoot length gradually ranging from 5.87% to 18.75%. At the same time in barley, although shoot length increased with the application of both particles and reached its highest amount at the concentration of 15 mg kg⁻¹, however further increase had an adverse effect and in turn had an unfavorable effect for shoot length. Generally, high concentrations of Fe/SiO₂ in both plants weren't effective for increasing the seedling length which may be due to no absorption in high concentrations by the plants.

In terms of seedling length in maize while both Fe/SiO₂ particles had a positive effect, however, it was observed that common Fe/SiO₂ was more effective than nano Fe/SiO₂ and the maximum percentage of seedling length (43.0) was at the concentration of 25 mg kg⁻¹ of common Fe/SiO₂ (Table 3). On contrary, nano Fe/SiO₂ was more effective on seedling length in barley compared to common Fe/SiO₂. Among the four different concentrations of nano Fe/SiO₂, the highest seedling length (33 cm) was obtained with the application of 15 mg kg⁻¹ of nano Fe/SiO₂ followed by the length of (28 cm) for 25 mg kg⁻¹ of nano Fe/SiO₂ in barley. However, no significant difference was observed in this index with the application of common Fe/SiO₂.

The influence of Fe/SiO₂ particles on the root elongation in barley and maize at four concentrations ranging from to 25 mg kg⁻¹ is shown in Table 4. It was observed that root elongation was enhanced by the exposure to both Fe/SiO₂ particles at any concentration in maize and barley. In the presence of common Fe/SiO₂, root length increased by 2.5, 20.0 and 11.9% in barley and 6.4, 11.7 and 26.7% in maize at the concentration of 5, 15 and 25mg kg⁻¹, respectively, as compared to the control group and 22.5, 27.5 and 38.8% in barley and 13.6, 19.1 and 47.9 % in maize at the concentration of 5, 15, 25 mg kg⁻¹, respectively in the presence of nano Fe/SiO₂. Compared to the control roots, the nano Fe/SiO₂ tended to enhance

the root elongation in barley and maize. The results also indicate that nano Fe/SiO₂ was more effective than common Fe/SiO₂.

Biomass

The effect of common Fe/SiO₂ and nano Fe/SiO₂ on root dry matter of maize and barley are shown in (Table 5). Combined analysis result showed that each three variables simultaneously didn't affect the root dry matter ($P > 0.05$). However, the dual effects plant type (barley and maize) and substance type (common Fe/SiO₂ and nano Fe/SiO₂) ($P = 0.019$), plant type and substance concentration ($P < 0.001$) and substance concentration and plant type ($P < 0.001$) were significant (Table 5). Both Fe/SiO₂ particles were favorable for the root dry matter in barley and maize, in particular maize. The results also indicate that nano Fe/SiO₂ was more effective than common Fe/SiO₂. Root dry weight increased gradually in both maize and barley. In such condition, the greatest increase in root dry weight in barley was 15.9% and 14.3% at 25 mg kg⁻¹ nano Fe/SiO₂ and common Fe/SiO₂. The greatest increase in root dry weight was 79.5% for nano Fe/SiO₂ and 54.7% for common Fe/SiO₂ compared to the control in maize.

Fe concentration in leaves

Combined analysis results showed that all three variables simultaneously affected the leaf Fe concentration ($P < 0.05$) (Table 6). Compared to control, the application of both Fe/SiO₂ particles improved leaf's Fe concentration in all tested plants, mostly in barley. This increase had a direct relationship with the concentration increase in both forms. The maximum value of it was observed with the application of common and nano Fe/SiO₂ at 25 mg kg⁻¹ (40.3%, 122.2% in barley and 6.3%, 70.3 % in maize, respectively) (Table 6).

Discussion

In this study, the effect of chemically synthesized Fe/SiO₂ NPs on some characteristics of barley and maize as strategically important crops, including MGT, plant growth, biomass and leaf Fe concentration were evaluated. In order to minimize nano iron particles aggregation sand (SiO₂) as a neutral stabilizer was used due to its abundance, low cost and plant resistance increase to environmental stresses. Also, its consumption with iron reduced iron toxicity and improved soil texture quality for better water and nutrients penetration. According to the obtained results, SiO₂ was a good stabilizer since it was observed that plant grew better and absorbed more Fe. Because of nano Fe's well documented toxicity to plants, the negative effect of Fe exposure on seed germination in barley and maize was expected. Also according to the provided results, the effect of nano Fe/SiO₂ on MGT was more significant. Our results contradicts the findings of El-Temsah et al. (2012) who showed that nZVI had an inhibitory effect on seed germination in all tested plant in soil and aqueous suspension. Toxic effects of three NPs types, Au, Ag, and Fe₃O₄ were evaluated in cucumber and lettuce using germination tests, revealing no or only low toxicity in all concentrations (62, 100, and 116 mg L⁻¹) (Barrena et al. 2009). Moreover, in terms of plant growth, our observation on root elongation was in line with Wang et al. (2011) who observed no inhibited root elongation in soybean at any concentration of Fe₂O₃ NPs (IONPs) and in fact a positive effect on root elongation was reported in comparison to bulk Fe₂O₃ (IOBKS). Moreover, Fe₃O₄ and BPs treatment tended to enhance the root elongation in rye grass and pumpkin plants. This enhancement effect may be due to dissolved iron ions from Fe₃O₄ NPs or BPS serving as a nutrient. However, Lee et al. (2010) showed that the root elongation was negatively influenced at all exposure concentrations (400, 2000, and 4000 mg L⁻¹ Fe₃O₄). Also, the increase of nZVI had an inhibitory effect on shoot length in all tested plants and complete inhibition was observed at (El-Temsah et al. 2012). They could positively or negatively affect plants (Monica & Cremonini 2009; Ma et al. 2010). Mushtaq (2011)

demonstrated less root growth compared to controls in cucumber for Fe₃O₄ ENPs within 100–5000 mg L⁻¹ treatments as a result of stressing conditions. In an experiment by Sadeghi et al. (2011) the highest grain yield was observed using 0.5 g L⁻¹ nano Fe oxide that showed 48% increase in comparison with the control. Studying four rice cultivars, Pereira et al. (2013) analyzed changes in multiple variables including root and shoot lengths, number of lateral roots, photosynthetic pigments, and internal CO₂ concentration with the increase of Fe ENP from 4 to 9 mM in the growth medium. To assess ZnO iron doped (Fe at ZnO) ENPs toxicity in *Pisum sativum* (L.) four indicators (seed germination, uptake, chlorophyll and H₂O₂ content and enzymatic activity) were studied. No signs of necrosis, stunting, chlorosis or wilting were found, while various physiological and biochemical responses in terms of plant growth, chlorophyll content and induction of reactive oxygen species (ROS) were observed (Mukherjee et al. 2014). Libralato et al. (2015) analyzed the phytotoxicity of three types of NPs: ionic (FeCl₃), micro and nano sized zerovalent iron (nZVI) on the seed germination, seedling elongation, germination index and biomass in three species of plants: *Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum*. No significant phytotoxicity effects were detected for both micro and nano sized (nZVI), and bio stimulation effects such as an increased seedling length and biomass production were detected at the highest exposure concentrations. Our observation on barley seedling length dosed with nano Fe/SiO₂ was in accordance with the observations made by Ma et al. (2013). These researchers reported that *Typha latifolia* dosed with high doses of nZVI demonstrated some signs of toxic effects. Compared to the controls they were shorter, while plants exposed to lower levels of nZVI (<50 mg) grow better. Their results also suggested that *Typha latifolia* treated with 25 and 50 mg L⁻¹ of nZVI was positive on biomass, while concentrations over 200 mg L⁻¹ had many dried leaves and thus lower biomass compared to the initial plants. Comparably, poplar fresh biomass was also reduced at 1000 mg L⁻¹ as a result of these dried leaves. In this study also,

low dosages of nano Fe/SiO₂ resulted in increased biomass in barley and maize. The application of Fe₃O₄ ENPs in *Arabidopsis thaliana* did not encourage seed germination and leaf production (Lee et al. 2010). Using magnetite (Fe₃O₄) as nanoparticles, Zhu et al. (2008) detected magnetic signals ranging from <0.1 to 0.4–1.2 mT g⁻¹ manifesting NPs accumulation in plant tissues. These findings confirm our results which the application of Fe/SiO₂ particles caused Fe accumulation in leaves. In addition, Si role in the strength of plant cell walls, SiO₂ NPs have served as a proper dispersing agent for Fe-NPs so that it causes better Fe movement in plant and with the application of nano SiO₂, Fe concentration becomes more in leaves.

Conclusion

According to our results, the application of Fe/SiO₂ NPs were more effective in improving plant characteristics compared to the common Fe/SiO₂ due to their higher absorption by plants. This impact was more noticed in maize with the application of nano Fe/SiO₂ compared to barley which may be due to physiological and structural differences in plants.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 1. Properties of the calcareous soil

Cu	Fe	Mn	Zn	Total N	K ava.
mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	%	mg kg ⁻¹
0.97	2.33	8.14	0.54	0.103	238
P ava.	T.N.V	O.C	pH	E.C.	Depth
mg kg ⁻¹	mg kg ⁻¹	%		dS m ⁻¹	cm
14.4	34.5	1.365	7.88	2.19	30

T.N.V.: Total volatile nitrogen; O.C. organic carbon; E.C.: Electrical conductivity

Table 2. Effect of common and nano Fe/SiO₂ on shoot length in barley and maize (cm)

Plant	Group	Treatment	Mean ±SE	<i>P</i> (Treatment)	<i>P</i> (Group)
Barley	Common	Control	13.00 ±0.19	0.091 ^{ns}	0.484 ^{ns}
		5	13.10 ±0.01		
		15	13.76 ±0.48		
		25	12.60 ±0.86		
	Nano	Control	13.00 ±1.16	0.528 ^{ns}	
		5	13.65 ±1.72		
		15	14.17 ±1.26		
		25	12.75 ±0.65		
Maize	Common	Control	16.00 ±0.26	0.0001*	0.195 ^{ns}
		5	16.94 ±0.50		
		15	18.10 ±0.10		
		25	19.00 ±0.22		
	Nano	Control	16.00 ±0.23	0.0001*	
		5	18.40 ±1.11		
		15	20.20 ±0.81		
		25	16.27 ±1.04		

P values: Plant: *P*=0.0001*; Plant×Group: *P*=0.0001*; Plant×Treatment: *P*=0.0001*;

Group×Treatment: *P*=0.0001*; Plant×Group×Treatment: *P*=0.0001*

*shows significant figures while ns shows nonsignificant figures

Table 3. Effect of different concentrations of common and nano Fe/SiO₂ on seedling length in barley and maize (cm)

Plant	Group	Treatment	Mean±SE	<i>P</i> (Treatment)	<i>P</i> (Group)
Barley	Common Fe/SiO ₂	Control	25.00 ±0.13	0.0001*	0.083 ^{ns}
		5	26.00 ±0.67		
		15	27.00 ±0.23		
		25	23.00 ±0.63		
	Nano Fe/SiO ₂	Control	25.00 ±0.31	0.0001*	
		5	28.00 ±1.45		
		15	33.00 ±0.44		
		25	28.00 ±1.28		
Maize	Common Fe/SiO ₂	Control	30.00±1.30	0.001*	0.171 ^{ns}
		5	37.00±2.66		
		15	42.00±1.46		
		25	43.00±1.80		
	Nano Fe/SiO ₂	Control	30.00±0.13	0.001*	
		5	30.33±0.42		
		15	31.00±0.90		
		25	34.76±1.00		

P values: Plant: *P*=0.0001*; Plant×Group: *P*=0.596^{ns}; Plant×Treatment: *P*=0.0001*; Group×Treatment: *P*=0.0002*; Plant×Group×Treatment: *P*=0.138^{ns}

*shows significant figures while ns shows nonsignificant figures

1 **Table 4.** Effect of different concentrations of common and nano Fe/SiO₂ on root elongation
 2 in barley and maize (cm)

Plant	Group	Treatment	Mean±SE	<i>P</i> (Treatment)	<i>P</i> (Group)
Barley	Common Fe/SiO ₂	Control	8.00 ±0.19	0.001*	0.083 ^{ns}
		5	8.20 ±0.16		
		15	9.60 ±0.14		
	Nano Fe/SiO ₂	25	8.95 ±0.91	0.001*	
		Control	8.00 ±0.40		
		5	9.80 ±0.31		
Maize	Common Fe/SiO ₂	15	10.20 ±0.41	0.0001*	0.171 ^{ns}
		25	11.10 ±1.00		
		Control	11.00 ±0.48		
	Nano Fe/SiO ₂	5	11.70 ±0.26	0.0001*	
		15	12.29 ±0.57		
		25	13.94 ±0.38		
Nano Fe/SiO ₂	Control	11.00 ±0.63	0.0001*		
	5	12.50 ±0.55			
	15	13.10 ±0.44			
		25	16.27 ±1.04		

3 *P* values: Plant: *P*=0.0001*; Plant×Group: *P*=0.596^{ns}; Plant×Treatment: *P*=0.0001*;

4 Group×Treatment: *P*=0.0002*; Plant×Group×Treatment: *P*=0.138^{ns}

5 *shows significant figures while ns shows nonsignificant figures

Table 5. Influence of different concentrations of common and nano Fe/SiO₂ on root dry matter (mg) of barley and maize

Plant	Group	Treatment	Mean±SE	<i>P</i> (Treatment)	<i>P</i> (Group)
Barley	Common Fe/SiO ₂	Control	5.59 ±0.40	0.0001*	0.0001*
		5	5.70 ±0.35		
		15	6.11 ±0.11		
		25	6.39 ±0.21		
	Nano Fe/SiO ₂	Control	5.09 ±0.82	0.0004*	
		5	4.99 ±0.71		
		15	5.23 ±0.54		
		25	5.90 ±1.20		
Maize	Common Fe/SiO ₂	Control	20.00 ±2.40	0.0001*	0.276 ^{ns}
		5	24.00 ±1.60		
		15	29.10 ±1.77		
		25	30.94 ±0.24		
	Nano Fe/SiO ₂	Control	20.00 ±1.17	0.0001*	
		5	28.00 ±1.54		
		15	30.00 ±0.63		
		25	35.90 ±0.26		

P values: Plant: *P*=0.0001*; Plant×Group: *P*=0.0001*; Plant×Treatment: *P*=0.0001*; Group×Treatment: *P*=0.0019*; Plant×Group×Treatment: *P*=0.138^{ns}

*shows significant figures while ns shows nonsignificant level

Table 6. Influence of different concentrations of common and nano Fe/SiO₂ on Fe concentration (mg kg⁻¹) in barley and maize

Plant	Group	Treatment	Mean±SE	<i>P</i> (Treatment)	<i>P</i> (Group)
Barley	Common Fe/SiO ₂	Control	81.00 ±1.50	0.0001*	0.006*
		5	87.00 ±2.73		
		15	100.00 ±0.39		
		25	113.67 ±1.53		
	Nano Fe/SiO ₂	Control	81.00 ±7.00	0.0001*	
		5	127.00±2.81		
		15	133.33±5.31		
		25	180.00±9.25		
Maize	Common Fe/SiO ₂	Control	37.00 ±0.18	0.062 ^{ns}	0.0001*
		5	37.00 ±0.50		
		15	38.32 ±0.88		
		25	39.33 ±1.76		
	Nano Fe/SiO ₂	Control	37.00 ±0.30	0.0001*	
		5	49.00 ±0.62		
		15	58.00 ±0.41		
		25	63.00 ±1.92		

P values: Plant: *P*=0.0001*; Plant×Group: *P*=0.0001*; Plant×Treatment: *P*=0.0001*; Group×Treatment: *P*=0.0001*; Plant×Group×Treatment: *P*=0.0001*

*shows significant figures while ns shows nonsignificant level

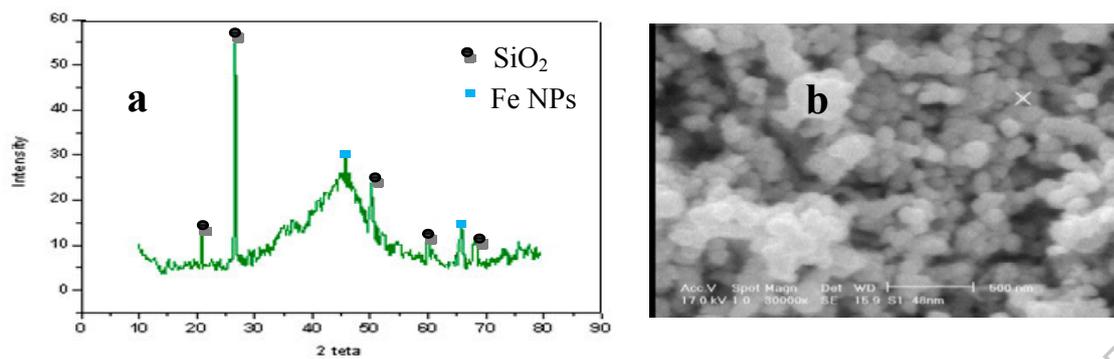


Figure 1. Diffraction (XRD) scanning pattern of the Fe/SiO₂ NPs (a), Electron microscope (SEM) images of the Fe/SiO₂ NPs (b)

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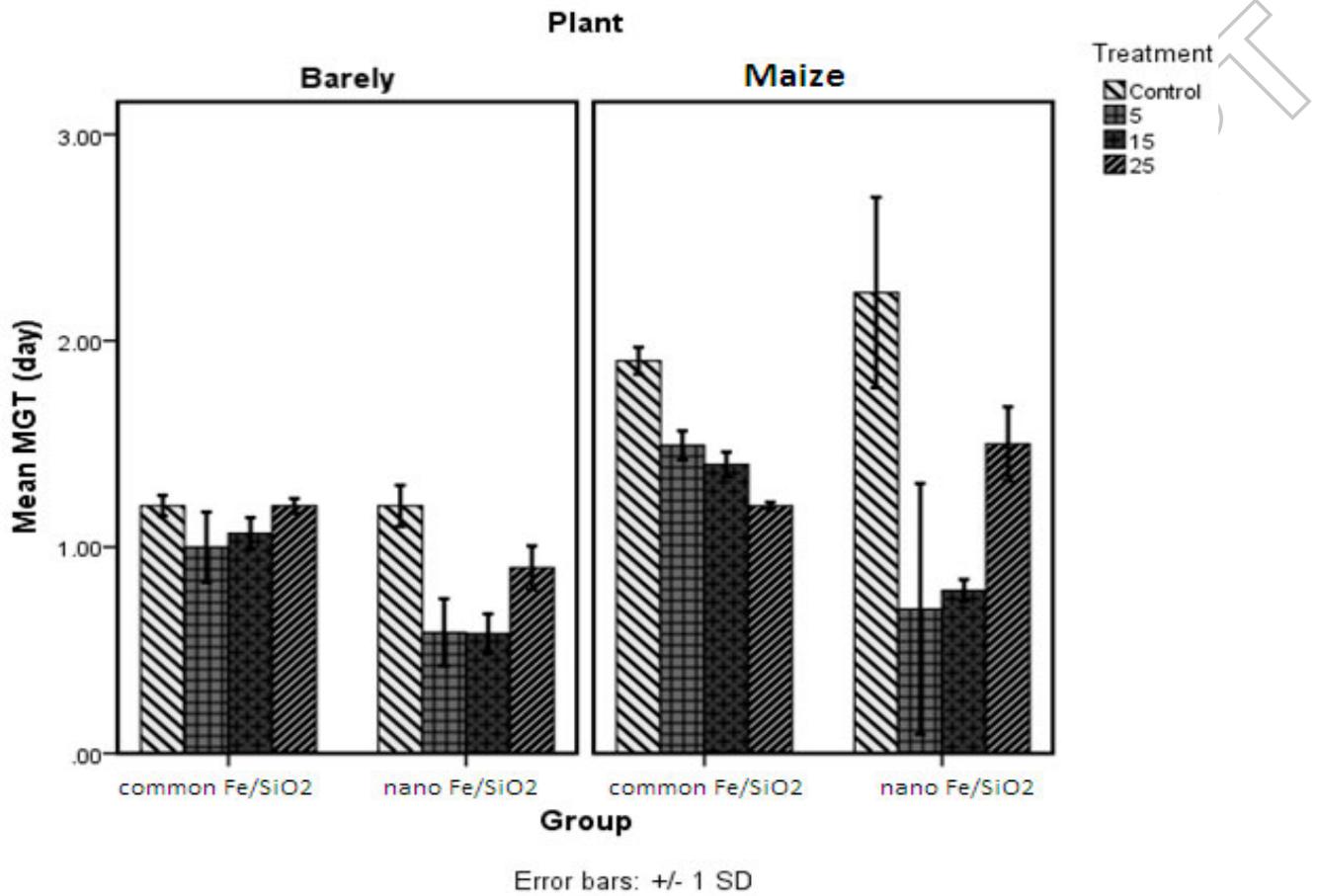


Figure 2. Mean germination time in barley and maize

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