A comparison of the use of different sources of nanoscale iron particles on the concentration of micronutrients and plasma membrane stability in sorghum

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Abstract

The present study aimed to evaluate the method of using iron on the physiologic characteristics of speedfeed sorghum and plasma membrane stability index (PMSI). Therefore, a factorial experiment was conducted in a completely randomized design with three replications. In addition, Zn, Cu, Mn were added to the soils before planting in order to avoid the shortage of micronutrients and find the possible effects of Fe nano materials sources on their sorption. The treatments were done based on two levels of iron including soil application (0.270, 0.405 mg kg⁻¹ of soil) and foliar application (0.25 and 0.5 g of iron per liter with control) from four compost sources including iron oxide nanoparticles, monodisperse iron oxide nanoparticles, polymeric iron oxide nanoparticles, and polymeric monodisperse iron oxide nanoparticles. The results of the interaction among the procedure, level and sources of iron indicated that the concentration of iron in shoots and the concentration of zinc in roots have increased in polymeric iron oxide nanoparticles and monodisperse iron oxide nanoparticles at 0.270 mg kg⁻¹ level while manganese concentration of shoots, and PMSI of roots were significantly increased in the treatments of polymeric monodisperse iron oxide nanoparticles and iron oxide nanoparticles at 0.405 mg kg⁻¹ level, compared to the control group. In foliar application of iron resources, the amount of total chlorophyll in monodisperse iron oxide nanoparticles and the percentage of PMSI of leaf, concentration of Cu, Fe and Mn of roots in polymeric monodisperse iron oxide nanoparticles have significantly increased at the level of 0.25 g L⁻¹ while the concentration of Zn in shoots in iron oxide nanoparticles have significantly increased at level of 0.5 g L⁻¹, in comparison to the control sample. Simple, dual, or triple interaction effects of procedure, source and level of iron were not significant in copper concentration of shoots.

Keywords: Plasma membrane stability, concentration of micro-materials, polymeric monodisperse iron oxide nanoparticles, sorghum.

1. Introduction

Regarding an increase in population, the importance of agriculture for all human societies was emphasized more than any other time. The first and foremost need of any human being is food and food supply which are directly or indirectly associated with agriculture. Although iron is essential for growing plants, which is found everywhere in the Earth crust, the solubility of iron compounds in most soils prevents iron absorption by the plant and expands the signs of iron deficiency in plants (Lucena, 2006). The optimum pH has been reported between 5.5 to 6.5 for proper growth and the optimum absorption of iron. Therefore, a pH greater than 7 can lead to iron deficiency (Korcak, 1987; Bityutskii *et al.*, 2017).

Various methods have been proposed for the removal of iron deficiency in plants such as soil application, foliar application or mixing iron with seeds (Godsey and Johnson, 2001). The use of different types of fertilizers is the main solution for the correction and maintenance of soil fertility and the increase of crop production (Rezaeei *et al.*, 2014; Dong *et al.*, 2016). Borowski and Michalek (2011) reported that foliar application of inorganic iron salts increases chlorophyll a, chlorophyll b and carotenoid of green beans. Sheykhbaglou *et al.*, (2010) showed that the use of nanoscale iron oxide particles increase soybean yields. Ghafari and Razmjoo (2013) reported the application of iron oxide nanoparticles on chlorophyll a, chlorophyll b and total chlorophyll wheat showed a significant increase compared with the control. Ghafariyan *et al.*, (2013) reported that these superparamagnetic iron oxide nanoparticles that inserted into soybean plants and then moved in them increase chlorophyll levels without creating the toxicity of micronutrients. Armin *et al.*, (2014) found that an increase in the concentration of iron leads to an increase in wheat yield and its components.

Few studies have addressed on the herbal toxicity of nanoparticles during a complete plantation course (Rico *et al.*, 2011). In addition, studies conducted on the toxicity of nanoparticles have paid more attention to the cultivation of plants and horticultural crops while they failed to address woody plants (Seeger *et al.*, 2009). The effect of nanoparticles on plants, along with its toxicity has been emphasized for a few generations of plants during a long-term period. However, the determination of long-term effects of nanoparticles on plants is regarded as a neglected area (Wang *et al.*, 2013).

In this study, we have examined the concentration of micronutrients in roots and shoots of the plants as well as the stability of the plasma membrane of the entrance of its elements and plant resistance against stress on the inner membrane of plant including the iron levels. Sullivan (1972) presented a fast and efficient method to determine cell membrane stability in grain sorghum using measuring electrolyte leakage of plant tissues which were exposed to thermal shock. This method is used as a quick, cheap and simple method to evaluate the cell membrane stability in many plants such as sorghum (Sullivan and Ross, 1979). The researches have focused on the effects of nanoscale iron particles fertilizers as well as plant yield and no study has taken into consideration the damages caused by these fertilizers and stress on the plant. This study aimed to compare the use of iron oxide nanoparticles, monodisperse iron oxide nanoparticles, polymeric iron oxide nanoparticles, polymeric monodisperse iron oxide nanoparticles on the concentration of micronutrients and plasma membrane stability of forage sorghum to find the best iron fertilizer and how to use these fertilizers with the least damage to the plasma membrane.

2. Material and Methods

A greenhouse experiment was conducted on Speed-feed forage sorghum (*Sorghum Bicolor* (L.) Moench) in a completely randomized factorial design with three replications. The treatments included two levels of iron including soil application (0.270, 0.405 mg kg⁻¹ of soil) and foliar application (0.25 and 0.5 g of iron per liter with control) from four compost sources such as Iron oxide nanoparticles, monodisperse Iron oxide nanoparticles, polymeric iron oxide nanoparticles, polymeric monodisperse Iron oxide nanoparticles. Soil was prepared from 0 to 30 cm depth of training and research greenhouses of Zabol University and after it was air-dried and

passed a 2-mm sieve, some physical and chemical properties were determined including Soil pH and electrical conductivity, soil texture with hydrometer (Bouyoucos, 1997), cation exchange capacity (Rible and Quick, 1960), calcium carbonate equivalent titration method (U.S. Salinity Laboratory Staff, 1954), organic matter by Walkley and Black's method (Walkley and Black, 1934), the Kjeldahl method for total nitrogen (Page *et al.*, 1982), available Phosphorus by Olsen's method (Olsen *et al.*, 1954), the concentration of iron in the soil with extractor DTPA (Lindsay and Norvell, 1978) and was read by atomic absorption spectrometry and potassium extracted by ammonium acetate (Helmke and Sparks, 1996) were determined by flame photometer (Table 1).

Table 1. Some physical and chemical properties of soils in this study

Texture	pH	EC (dS.m ⁻¹)	CEC (Cmol ⁺ kg ⁻¹)	OM (%)	CCE (%)	N (%)	P K Fe mg kg ⁻¹		
Sandy loam	7.77	1.82	9	0.48	20.5	0.12	12.7	30	2.32

Three kilograms of sieved soil were poured in plastic bags. Before planting the seeds, based on soil test and in order to avoid the possible shortage of macro and micro-materials, some materials were added to the vase such as: 75 mg potassium per kg, 75 mg phosphorous per kg, 150 mg of nitrogen per kg in two stages, 2 mg of copper per kg, 5 mg of zinc per kg, and 5 mg of manganese per kg. To use iron fertilizers in soil, the desired amount of fertilizer was mixed with soil before plantation and was added to a three-kilogram vase. Five sorghum seeds were planted at a depth of about 2 to 3 cm in each vase and the vases were irrigated till they reach the filed capacity moisture. After about 10 days of culture, the number of plants in each vase reduced to three. Plants were watered by distilled water during growth period and in weight manner.

Foliar application of iron was performed in two stages, i.e. four-leaf stage and two weeks after the first spraying. Two months after planting, shoots were picked from the crown and then air dried. The roots were washed with distilled water and soil was gently separated. Shoots and roots were dried at 70 °C for 48 hours in the oven, then, the samples were prepared by dry ashing for the analysis of plant and herbal extracts (Motsara and Roy, 2008).

Concentration of micro-materials (iron, manganese, copper and zinc) in plant extracts was measured by atomic absorption spectrometry. Total chlorophyll was determined based on Arnon's method (1949). Plasma membrane stability index (PMSI) was measured by Sairam and Srivastava (2001) method and was calculated according to the following formula:

$$\text{PMSI (\%)} = (1 - (C1/C2)) * 100$$

Formula 1

The experimental data were examined using SAS 9.4 statistical software and the averages were compared using Duncan's Multiple Range Tests at 0.01 and 0.05 significance level.

3. Results

3.1. Characterization of nanomaterials

Figure 1 illustrates FT-IR spectra of four employed nanoparticles in this work. FT-IR spectroscopy is a useful tool for characterization of functional groups.

Figures 1a, b, c, and d indicate iron oxide nanoparticles, monodisperse iron oxide nanoparticles, polymeric iron oxide nanoparticles, and polymeric monodisperse iron oxide nanoparticles, respectively. For "Polymeric Iron oxide nanoparticles" and "Polymeric monodisperse Iron oxide nanoparticles" which their FT-IR spectra are shown in Figure 2c and 2d, respectively, the as-prepared Iron oxide nanoparticles and monodisperse Iron oxide nanoparticles were dispersed in PEG-6000 polymer and then were characterized by FT-IR spectroscopy. Some of the characteristic peaks are representative of attachment of the PEG polymer to the surface of Iron oxide nanoparticles.

Figure 1. FT-IR spectrum of (a) Iron oxide nanoparticles; (b) Monodisperse iron oxide nanoparticles; (c) Polymeric iron oxide nanoparticles; (d) Polymeric monodisperse iron oxide nanoparticles

In order to characterize the phase of the prepared nanoparticles, XRD patterns of iron oxide and monodisperse iron oxide nanoparticles were recorded and were shown in Figure 2a and b, respectively.

Figure 2. XRD patterns of (a) Iron oxide nanoparticles; (b) Monodisperse iron oxide nanoparticles

Figure 3 illustrates the morphology and elemental composition of the prepared nanoparticles. SEM images of iron oxide nanoparticles and monodisperse iron oxide nanoparticles are displayed in Figure 3a and b, respectively. Energy-dispersive X-ray (EDX)

analysis was used to specify the type and quantitative amounts of the elements in each sample. Figure 3c and d represent the EDX analyses of iron oxide nanoparticles and monodisperse iron oxide nanoparticles, respectively.

Figure 3. SEM images and EDX spectra of (a) and (c) iron oxide nanoparticles; (b) and (d) monodisperse iron oxide nanoparticles

2.2. Characterization of plant

Results of ANOVA in Table 2 indicated that simple effects (except level) and dual interactions of level and sources of iron and triple interactions among procedure, levels and sources of iron were significantly important at significance level of 0.05 and PMSI of leaf was significant at the level of 0.05 under the simple

effects of iron sources and the effects of dual interactions (except procedure and levels) and the triple interactions of procedure, source and level of iron. Further, simple effects (except level) and the effects of dual interactions (except procedure and levels) and the effects of triple interactions of procedure, sources and level showed that total chlorophyll of leaves was significant at the 0.05 level of significance (Table 2).

Table 2. Results of ANOVA for procedure, sources and level of iron on the quality characteristics and the concentration of micro-materials in plant roots and shoots

S.O.V	df	Mean square		
		PMSI of root	PMSI of leaf	Total chlorophyll
Procedure	1	278.9001*	21.5880ns	24.9228*
Iron sources	4	248.2164*	359.2417*	11.1844*
Level	1	0.4752ns	12.4852ns	0.0183ns
Procedure*Level	1	17.7561ns	1.2702ns	0.5282ns
Procedure*Iron sources	4	73.5357ns	198.3990*	8.4408*
Level*Iron sources	4	252.2203*	106.4792*	2.3761*
Procedure*Level*Iron sources	4	946.2491*	79.4955*	7.1568*
Error	40	15.1639	1.8747	0.1190
C.V. (%)	-	12.6311	9.3112	7.6412

S.O.V	df	Mean square			
		Cu content in shoots	Fe content in shoots	Mn content in shoots	Zn content in shoots
Procedure	1	0.0365ns	754.6597*	12.9828*	47.4904*
Iron sources	4	0.5334ns	3390.8192*	13.3382*	39.3494*
Level	1	0.6448ns	1746.6852*	4.0093*	8.8627*
Procedure*Level	1	0.8401ns	2780.5233*	5.8593*	174.2169*
Procedure*Iron sources	4	0.1294ns	2369.4607*	6.8951*	132.5607*
Level*Iron sources	4	0.6209ns	2133.6880*	5.5170*	120.9008*
Procedure*Level*Iron sources	4	0.3793ns	2433.2811*	0.8246*	65.8066*
Error	40	0.2410	0.3732	0.0184	0.2465
C.V. (%)	-	13.2001	1.6134	2.9140	4.3988

S.O.V	df	Mean square			
		Cu content in roots	Fe content in roots	Mn content in roots	Zn content in roots
Procedure	1	13.2634*	16887.7282*	150.8603*	24.2316*
Iron sources	4	1.2963*	143325.7411*	403.4397*	585.0356*
Level	1	7.3290*	127161.5599*	309.9917*	598.1883*
Procedure*Level	1	0.0686ns	10701.0944*	159.2184*	926.7726*
Procedure*Iron sources	4	1.6021ns	58616.4719*	442.2122*	955.0887*
Level*Iron sources	4	2.6732*	126076.1516*	596.2099*	783.0423*
Procedure*Level*Iron sources	4	0.5422*	68174.5962*	353.3202*	802.6822*
Error	40	0.2495	0.340	0.2525	0.2462
C.V. (%)	-	13.9477	0.0856	2.0925	3.7450

(*and ** denoted significance at the 5 and 1 percent level, respectively; ns is not significant).

Results of ANOVA in Table 2 indicated that simple effects, the effects of dual interactions and the effects of triple interactions of procedure, sources and level on the concentrations of iron, manganese and zinc were significant at the level of 0.05 in shoots but it was not meaningful for copper concentration of shoots. As shown in Table 2, ANOVA results indicated that simple effects, the effects of dual interactions and

those of triple interactions among procedure, sources and level on the concentrations of iron, manganese and zinc were significant at level of 0.05 in roots. Further, results of ANOVA for the simple effect, dual interaction of iron level and sources, and triple interactions of iron procedure, sources and level on the concentration of copper were significant ($P < 0.05$) (Table 2).

Table 3. Results of the comparison of the mean effects of triple interaction of procedure, source and level of iron on PMSI of leaf and root and total chlorophyll

Procedure	Level	Treatment	PMSI of root (%)	PMSI of leaf (%)	Total chlorophyll (mg g ⁻¹ fresh weight)	
Soil application	0	Control	15.19e	5.87j	3.94ef	
	0.270 (mg kg ⁻¹)	Iron oxide nanoparticles	14.32e	21.81c	3.02ghi	
		Monodisperse iron oxide nanoparticles	31.53c	25.13b	3.49fg	
		Polymeric Iron oxide nanoparticles	17.56e	15.20de	3.09gh	
		Polymeric monodisperse Iron oxide nanoparticles	40.59a	11.63ghi	6.34c	
	0.405 (mg kg ⁻¹)	Iron oxide nanoparticles	41.26a	17.61d	4.32de	
		Monodisperse iron oxide nanoparticles	24.23d	24.05bc	4.61d	
		Polymeric Iron oxide nanoparticles	33.68bc	16.18de	3.88ef	
		Polymeric monodisperse Iron oxide nanoparticles	11.54e	9.18i	2.40i	
	Spraying	0	Control	15.19e	5.87j	3.94ef
		0.25 (g L ⁻¹)	Iron oxide nanoparticles	15.00e	12.54fgh	6.32c
			Monodisperse iron oxide nanoparticles	30.37cd	10.93hi	7.92a
Polymeric Iron oxide nanoparticles			30.72cd	11.78fghi	2.80hi	
Polymeric monodisperse Iron oxide nanoparticles			10.91e	30.95a	4.25de	
0.5 (g L ⁻¹)		Iron oxide nanoparticles	16.29e	10.10hi	7.30b	
		Monodisperse iron oxide nanoparticles	14.32e	24.37b	6.19c	
		Polymeric Iron oxide nanoparticles	13.87e	14.34ef	3.23gh	
		Polymeric monodisperse Iron oxide nanoparticles	38.85ab	14.05efg	5.83c	

(means with the same letters in each column are not significantly different (p<0.01)).

A comparison of the mean effects of triple interactions of procedure, sources and level of iron showed that the highest percentage of PMSI of root (the lowest membrane damage) was related to the concentration of iron oxide nanoparticles 0.405 mg kg⁻¹ (41.26%) in the soil application of fertilizers which increased 2.71 times compared to the control (15.19%) (Table 3). In spraying, in 0.5 g L⁻¹ concentration, the highest percentage of PMSI of root was related to the polymeric mono-

disperse iron oxide nanoparticles (38.85%) which significantly increased, compared to the control, but no significant difference was observed, compared to the soil application of fertilizers (Table 3). The lowest amount of PMSI of root (10.91%) was observed in application of spraying and in the concentration of polymeric monodisperse iron oxide nanoparticles at 0.25 g L⁻¹ concentration of, decreased to 39.23%, compared to the control (Table 3).

A comparison of the results of mean effects of triple interactions of procedure, sources and level of iron in Table 3 showed that the highest percentage of PMSI of leaf (the lowest membrane damage) was related to the polymeric monodisperse iron oxide nanoparticles (30.95%) in the application of spraying fertilizers at the concentration of 0.25 g L^{-1} , increased 5.27 times with the highest membrane damage (the lowest percentage of PMSI of leaf), compared to the control (5.87%) (Table 3). In soil application of fertilizers, the highest percent of PMSI of leaves (25.13%) was related to monodisperse iron oxide nanoparticles at the concentration of 0.270 mg kg^{-1} which showed a significant increase, compared to the control group as well as a the use of fertilizers (Table 3). Based on the comparison of mean effects of triple interactions of procedure, sources and level of iron (Table 3), the highest amount of total chlorophyll in the use of spraying of $0.25 \text{ gram per liter}$ was observed in monodisperse iron oxide nanoparticles (7.92 mg g^{-1}), which was significantly increased, compared to the control (3.94 mg g^{-1}). Also, the highest amount of total chlorophyll was observed in iron oxide nanoparticles at the use of spraying 0.5 g L^{-1} . The lowest amount of total chlorophyll was observed in polymeric monodisperse iron oxide nanoparticles in the soil application of 0.405 mg kg^{-1} of iron and was significantly different compared to control (Table 3).

Based on the results of comparing the mean triple interaction of procedure, sources and level (Table 4), the highest concentration of iron in shoots was observed in polymeric iron oxide nanoparticles (56.10 mg kg^{-1}) at the level of 0.270 mg kg^{-1} , increased 1.5 times, compared to the control (28.79 mg kg^{-1}). Further, polymeric

monodisperse iron oxide nanoparticles (44.79 mg kg^{-1}) had the highest increase at the 0.25 g L^{-1} , indicating the most significant concentration of iron in shoots (Table 4). The results of comparison of mean effects of triple interactions of procedure, sources and level of iron (Table 4) indicated that the lowest concentration of manganese in the soil application of iron sources was observed in monodisperse iron oxide nanoparticles (3.21 mg g^{-1}), which significantly reduced, compared to the control (44.23%).

The highest concentration of Manganese in the soil application of shoot is polymeric monodisperse iron oxide nanoparticles (9.68 mg kg^{-1}) at the level of 0.405 mg kg^{-1} , which significantly increased 2.09 times, compared to the control (4.63 mg kg^{-1}).

According to the results of comparing the mean triple interaction of procedure, sources and level of iron (Table 4), the highest concentration of zinc in shoots was observed in iron oxide nanoparticles (25.83 mg kg^{-1}) at the level of 0.5 g L^{-1} , which increased 2.87 times, compared to the control (8.98 mg kg^{-1}). Among the sources of iron that were used to soil application, the highest amount of zinc on shoot was observed in polymeric monodisperse iron oxide nanoparticles (21.63 mg kg^{-1}) at level of iron of 0.270 mg kg^{-1} (Table 4).

The lowest concentration of zinc (7.22 mg kg^{-1}) in plant shoot in the use of spraying was observed in the treatment of polymeric iron oxide nanoparticles at the level of 0.5 g L^{-1} and was significantly reduced, compared to the control (Table 4). Simple, dual and triple interactions effects of procedure, source and level of iron were not significant in copper concentration of shoots of sorghum (Table 2).

Table 4. The results of the comparison of mean effects of triple interaction of procedure, source and level of iron on the concentration of iron, manganese and zinc in shoot

Procedure	Level	Treatment	Fe	Mn	Zn
			mg kg ⁻¹		
Soil application	0	Control	28.79j	4.63g	8.98e
		Iron oxide nanoparticles	29.21j	3.94i	9.55de
		Monodisperse iron oxide nanoparticles	29.36j	3.21i	8.60ef
		Polymeric Iron oxide nanoparticles	56.10a	5.05e	9.52de
	0.270 (mg kg ⁻¹)	Polymeric monodisperse Iron oxide nanoparticles	33.10h	6.37b	21.63b
		Iron oxide nanoparticles	28.72j	3.63j	8.79e
		Monodisperse iron oxide nanoparticles	33.58h	4.73f	9.59de
		Polymeric Iron oxide nanoparticles	34.42g	6.26c	7.73fg
	0.405 (mg kg ⁻¹)	Polymeric monodisperse Iron oxide nanoparticles	39.68d	9.68a	10.35d
	0	Control	28.79j	4.63g	8.98e
		Iron oxide nanoparticles	31.42i	4.16h	9.55de
		Monodisperse iron oxide nanoparticles	31.00i	4.73f	9.25e
Spraying		Polymeric Iron oxide nanoparticles	35.52f	4.10h	15.15c
	0.25 (g L ⁻¹)	Polymeric monodisperse Iron oxide nanoparticles	44.79b	3.63j	7.73fg
		Iron oxide nanoparticles	35.73f	3.37k	25.83a
		Monodisperse iron oxide nanoparticles	37.15e	3.42k	21.89b
		Polymeric Iron oxide nanoparticles	40.89c	3.58j	7.22g
	0.5 (g L ⁻¹)	Polymeric monodisperse Iron oxide nanoparticles	36.63e	6.05d	7.36g

(means with the same letters in each column are not significantly different ($p < 0.01$)).

With regard to the comparison of the mean for triple interaction of procedure, sources and level of iron (Table 5), the results showed that the highest concentration of copper in roots (5.68 mg kg⁻¹) was

observed in polymeric monodisperse iron oxide nanoparticles at the level of 0.25 g L⁻¹, and it has significantly increased, compared to the control (2.63 mg kg⁻¹).

Table 5. The results of the comparison of mean effects for triple interaction of procedure, source and level of iron on the concentration of copper, iron, manganese and zinc in root

Procedure	Level	Treatment	Cu	Fe	Mn	Zn
			Mg kg ⁻¹			
Soil application	0	Control	2.63cdefgh	677.15j	19.94h	6.93ij
		Iron oxide nanoparticles	2.47defgh	339.15q	24.52f	8.66gh
		Monodisperse iron oxide nanoparticles	2.42efgh	527.68l	17.58ij	75.20a
		Polymeric Iron oxide nanoparticles	2.79bcdefg	875.42c	30.42c	7.86hi
		Polymeric monodisperse Iron oxide nanoparticles	3.47bcd	744.47h	22.94g	5.90k
		Iron oxide nanoparticles	2.00fgh	791.15g	13.15l	7.82hi
	0.405 (mg.kg ⁻¹)	Monodisperse iron oxide nanoparticles	1.89gh	709.89i	28.73d	3.49l
		Polymeric Iron oxide nanoparticles	1.73h	892.84b	33.63b	6.31jk
		Polymeric monodisperse Iron oxide nanoparticles	1.84gh	402.31p	13.36l	9.46fg
Spraying	0	Control	2.63cdefgh	677.15j	19.94h	6.93ij
		Iron oxide nanoparticles	3.52bc	662.00k	18.36i	21.48c
		Monodisperse iron oxide nanoparticles	2.97bcdef	460.42o	14.94k	7.59i
		Polymeric Iron oxide nanoparticles	3.79b	841.42d	28.21d	9.65f
		Polymeric monodisperse Iron oxide nanoparticles	5.68a	1007.94a	66.05a	13.60d
		Iron oxide nanoparticles	3.63bc	793.10f	26.68e	29.16b
	0.25 (g L ⁻¹)	Monodisperse iron oxide nanoparticles	2.94bcdef	517.79n	14.00l	9.12fg
		Polymeric Iron oxide nanoparticles	3.36bcde	810.47e	30.94c	12.31e
		Polymeric monodisperse Iron oxide nanoparticles	2.68cdefgh	524.63m	16.94j	9.30fg

(means with the same letters in each column are not significantly different (p<0.01)).

The lowest concentration of copper in roots was observed in polymeric iron oxide nanoparticles (1.73 mg kg^{-1}) at the level of 0.405 mg kg^{-1} , which failed to have a significant difference, compared to that of the control (Table 5).

As shown in Table 5, the results indicated that the highest concentration of iron in roots ($1007.94 \text{ mg kg}^{-1}$) was observed in polymeric monodisperse iron oxide nanoparticles at the level of 0.25 g L^{-1} , and it has significantly increased compared to control ($677.15 \text{ mg kg}^{-1}$).

The lowest concentration of iron and manganese in the roots in the treatment of iron oxide nanoparticles (339.15 and 13.15 mg kg^{-1} , respectively) in the soil application was observed in the soil application of iron at the level of 0.270 mg kg^{-1} and 0.405 mg kg^{-1} , which have significantly reduced, compared to that of the control (99.66% and 51.63% , respectively) (Table 5). Based on the mean comparison of triple interaction of procedure, sources and level of iron (Table 5), the results showed that the highest concentration of manganese in roots (66.5 mg kg^{-1}) was observed in polymeric monodisperse iron oxide nanoparticles at the level of 0.25 g L^{-1} , and it has significantly increased, compared to the control (19.94 mg kg^{-1}). Although in the treatment of polymeric monodisperse iron oxide nanoparticles, one can observe the highest concentration of copper, iron and manganese in the root (Table 5), the most damage to membrane can be observed at the root of plant (Table 3).

As it is evident from Table 5, the highest concentration of zinc in roots was observed in soil application of monodisperse iron oxide nanoparticles (75.20 mg kg^{-1}) at the level of 0.270 mg kg^{-1} , and it was found it had increased 10.85 times compared to that of the control (6.93 mg kg^{-1}). The lowest concentration of zinc in the root was observed in monodisperse iron oxide nanoparticles (3.49 mg kg^{-1}) in soil application

in 0.405 mg kg^{-1} , which was significantly reduced, compared to the control.

4. Discussions

4.1. Characterization of nanomaterials

As shown in Figure 2, the peaks appeared at $550\text{--}650 \text{ cm}^{-1}$ are assigned to Fe-O bonds of Fe_3O_4 lattice in all the spectra for the FT-IR characterization. The peaks at around $3100\text{--}3400 \text{ cm}^{-1}$ as well as the appeared peaks at 1652 cm^{-1} are due to the stretching and bending vibration of either water molecules present in KBr or the OH groups of PEG polymer around Iron oxide nanoparticles, respectively. The difference between “iron oxide nanoparticles” and “Monodisperse iron oxide nanoparticles” samples is in their synthetic procedure in which for the former no additive except that iron salts and NaOH is added and for the latter, in addition to the mentioned materials, PEG 8000 is used in the synthetic procedure. Therefore, it is expected that some characteristic peaks attributing to PEG-8000 is appeared in the FT-IR spectrum of “Monodisperse iron oxide nanoparticles” (Figure 2b). As can be seen in Figure 2b, there are vibrations around 2800 and 2900 cm^{-1} which can be attributed to the C-H stretching bands of the polymer. In a study, Dang *et al.*, (2011) reported the interaction between the PEG 6000 and Cu nanoparticles. They observed that two absorption peaks appear at 1690 and 1760 cm^{-1} after attachment of the polymer to the copper nanoparticles. Here, these two characteristic peaks are appeared at 1645 and 1715 cm^{-1} . Therefore, attachment of PEG-6000 polymer to Iron oxide nanoparticles is confirmed for both samples (Figure 2c and d).

The observed diffraction peaks in XRD pattern of both samples are in good agreement with that of pure cubic spinel structure for magnetite (JCPDS Card No.: 19-629) (Figure 2a and b). For monodisperse

iron oxide nanoparticles (Figure 2b), in addition to the pure Iron oxide nanoparticles, some other peaks are also observed which are representative of some impurities which mainly correspond to γ -FeOOH phase. Also a peak at $2\theta=19$ is observed which could be attributed to PEG-8000 which was used during the synthesis of Iron oxide nanoparticles (Figure 2b). These results show that the main phase of the employed nanoparticles is cubic Iron oxide nanoparticles.

As illustrated in Figure 3a and b, based on the results of SEM images, the iron oxide nanoparticles (Figure 3a) prepared without adding any surfactant or polymer are more agglomerated than monodisperse iron oxide nanoparticles (Figure 3b) prepared in the presence of PEG-8000.

Figure 3c and 3d show EDX analyses of iron oxide nanoparticles and monodisperse iron oxide nanoparticles, respectively. It can be seen that for monodisperse iron oxide nanoparticles, in addition to iron and oxygen, carbon is also found which confirms the existence of the residue of the applied polymer during the synthesis.

4.2. Characterization of plant

4.2.1. The effect of iron sources on the PMSI of root, leaf, and total chlorophyll

Based on the results in Table 3, using two different levels of iron sources and procedure can play a positive effect on root PMSI. In other words, an increase in the root PMSI leads to less damage in plant root. In the present study, an increase of root PMSI was observed in addition to increasing the root PMSI at 0.405 mg kg^{-1} of iron oxide nanoparticles although no significant difference was observed, compared to the control sample. This amount of decrease in PMSI of root caused a high damage to the plasma membrane of roots and it can be reason for the observed high concentration of copper, iron and manga-

nese in the root which will be further discussed (Table 5). It was found that under stress, Plasmalma is of the first organ that is damaged (Levitt, 1980). As a result of damage to the cell membrane, the permeability has increased; therefore, electrolyte leakage from the cell caused plant wilting (Blume and Ebercon, 1981).

Table 3 displays the positive effect of the iron sources on the leaf PMSI. Based on the results, the leaf PMSI can increase by 5.27 times, compared to the control sample which indicates a lower level of damage in the leaf plasma membrane. The highest PMSI value of leaf is observed in polymeric monodispersed iron oxide nanoparticles at 0.25 g L^{-1} level while the lowest value is observed in the control sample. In a study conducted by Delfani *et al.*, (2014), the damage of plasma membrane was low for iron and nanoscale iron particles in the presence of magnesium, and the lowest amount of damage with 27.9% was observed for nanoscale iron particles with magnesium at 0.25 g L^{-1} .

As shown in Table 3, the use of iron sources can changes the total chlorophyll content of the plant. In addition, the foliar use of iron sources considerably influenced on increasing the chlorophyll content. The highest value of chlorophyll was observed for the foliar use of monodisperse iron oxide nanoparticles at 0.25 g L^{-1} . The results of Ghafari and Razmjoo (2013)' study is in line with the results of the present study as they reported the application of iron oxide nanoparticles on chlorophyll a, chlorophyll b and total chlorophyll in wheat plant showed a significant increase, compared to the control sample.

4.2.2. The effect of iron sources on plant micronutrients

Table 4 indicates 0.270 mg kg^{-1} for Fe concentration in plant shoots when fertilization is performed with polymeric iron oxide nanoparticles. Based on the results, an increase occurred in the Fe concentration in plant shoot in soil use of iron sources including mono-

dispersed iron oxide and polymeric monodisperse iron oxide nanoparticles. In addition, the same result was observed in foliar application when monodisperse iron oxide nanoparticles, polymeric iron oxide nanoparticles, and iron oxide nanoparticles were used. The results are consistent with the results of the previous studies (Márquez-Quiroz *et al.*, 2015; Hernández-Castro *et al.*, 2015) in which fertilizing with iron sources could significantly improve the Fe concentration in cowpea plant. In the greenhouse experiment, it was reported that some of polymeric hydrated sources provides small environment within the root zone which keeps Iron sulfate available to plants compared with its non- polymeric use (Mikkelsen, 1994). The use of polymers to supply nutrients may provide more exact conditions for the needs of the plant, while one should regard the protection of the quality of the environment and the preservation of natural resources (Mikkelsen, 1994).

In addition, Table 4 lists the results related to the effect of using different iron sources on the concentrations of other micronutrients. In spraying iron sources, the least amount of concentration in manganese in shoot (3.37 mg kg^{-1}) was achieved after using iron oxide nanoparticles at the iron level of 0.5 g L^{-1} . The findings of the present study are congruent with the results of previous studies. Ghasemi-Fasaei *et al.*, (2005) demonstrated that foliar or soil applications of iron reduce the manganese of shoots, due to the antagonistic relationship of iron on absorption and transmission of manganese from root to the shoots.

Further, based on the results in Table 4, the soil use of polymeric monodisperse iron oxide nanoparticles leads to a significant increase in Mn concentration in plant shoot. Roosta *et al.* (2015) reported that the highest amount of manganese was observed in shoots of lettuce and in a variety of iceberg and iron nano-chelates treatment and this amount of manganese in leaves was

significantly affected by plant varieties and their interaction with iron resources.

Zn content could increase by 2.87 times when 0.5 g L^{-1} iron oxide nanoparticles was used as iron source, compared to the control sample including the highest value of Zn in plant shoot among all the samples. In addition, monodisperse iron oxide nanoparticles caused an increase in Zn content of the plant shoot at the same level of iron fertilization. Further, polymeric iron oxide nanoparticles source resulted in decreasing the Zn content of the plant shoot (Table 4). Roosta *et al.*, (2015) concluded that the amount of zinc and iron of leaves were significantly affected by plants varieties and their interactions with sources of iron and the highest amount of zinc was found in shoot of lettuce in iceberg varieties and iron nano chelate treatment. The findings are consistent with the results of the present research.

As shown in Table 5, regarding the related data for Cu micronutrient, the highest amount of Cu was obtained after using of 0.25 g L^{-1} of polymeric monodispers iron oxide nanoparticles. However, no significant difference in Cu content was observed in other samples, compared to the control sample. These results are congruent with Broschat and Moore (2004)'s study who showed that FeEDTA and FeEDDHA have increased the amount of copper of African marigolds leaves, but other sources failed to have consistent effect on the amount of copper of leaves.

As displayed in Table 5, the use of polymeric monodispers iron oxide nanoparticles at 0.25 g L^{-1} Fe content in the plant root could significantly increased by 48.85% (Table 5). The results were congruent with the study of Armin *et al.* (2014) and Márquez-Quiroz *et al.* (2015). In addition, the foliar use of this iron source resulted in increasing iron absorption of root from soil. Further, based on the results in Table 3, plasma membrane damage was observed in the root PMSI in this sample which is regarded as the reason

for improving the permeability of cellular membrane (Blume and Ebercon, 1981).

As shown in Table 5, the soil use of iron oxide nanoparticles at 0.405 and 0.270 mg kg⁻¹ can decrease the amount of Fe and Mn of roots by 99.66% and 51.63%, respectively. Regarding the main reason, we can refer to quick growth in plant, i.e. dry weight gain from 0.56 in control group to 2.53 and 2.19 g pot⁻¹ for the iron and manganese in iron oxide nanoparticles, respectively, which is justified by the dilution effect at the root (Feil *et al.*, 2005). In addition, the use of iron sources leads to an increase in the Mn content in roots (Table 5). The results were consistent with the results reported by Roosta *et al.* (2015).

As displayed in Table 5, the Zn content in roots is influenced by nanostructured fertilizers. The soil application of 0.270 mg kg⁻¹ in monodisperse iron oxide nanoparticles caused an increase of 10.85 times in the Zn content of the root plant, compared to the control sample. The least concentration of Zn was observed for 0.405 mg kg⁻¹ in monodisperse iron oxide nanoparticles. The decrease occurred by an increase in the iron fertilizer concentration is related to dilution since dry weight increase from 0.56 in the control sample to 2.27 gram in pot in monodisperse iron oxide nanoparticles usually leads to a decrease due to the effect of dilution (Feil *et al.*, 2005).

5. Conclusion

The present study aimed to evaluate the effect of iron oxide nanoparticles embedded in polymeric matrix on the physiologic characteristics of speedfeed sorghum and plasma membrane stability index (PMSI). To this aim, some iron oxidesources including iron oxide nanoparticles, monodisperse iron oxide nanoparticles, polymeric iron oxide nanoparticles and polymeric monodisperse iron oxide nanoparticles were examined. Based on the results, polymeric monodisperse iron

oxide nanoparticles prepared by using polymer leads to a significant increase in total chlorophyll, the PMSI of roots and shoots, Zn and Fe contents of shoots, as well as Cu and Fe contents in roots. The result of the present study can pave the way for further investigation on novel nanostetured nutrients in order to improve growth parameters of plants.

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