

Response of Durum Wheat to Foliar Application of Varied Sources and Rates of Iron Fertilizers

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ABSTRACT

Iron deficiency in soil reduces the quality of durum wheat leading to Fe deficiency in human. Thus, this experiment investigated the effects of foliar application of nano-iron oxide (2 and 4 g L⁻¹), iron chelate (EDTA), (4 and 8 g L⁻¹), iron sulfate (4 and 8 g L⁻¹), and the control on grain yield and quality, yield components, chlorophyll and carotenoids contents, peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX) activities of durum wheat D-85-15-5. Iron application increased activities of all leaf enzymes and chlorophyll of leaf, grain protein, iron and carbohydrate contents, grain carbohydrate, protein, iron yields, and grain yield. Iron source had no effects on enzymes activities, but the highest chlorophyll content, grain yield, grain iron (38%) and protein contents (58%), protein, iron, and carbohydrate yields were produced by application of 2 g L⁻¹ of nano-iron oxide followed by 8 g L⁻¹ iron sulfate. Harvest index, 1,000 grain-weight, and chlorophyll, grain yield, grain iron and protein contents, protein, iron, and carbohydrate yields increased. But, these parameters decreased at the higher rate of nano-iron oxide. Application of 2 g L⁻¹ nano-iron oxide was more effective than the other Fe sources and rates, and is suggested for durum wheat production.

Keywords: Antioxidant enzymes, Chlorophyll content, Iron content, Protein content, Yield.

INTRODUCTION

Micronutrient malnutrition affects over two billion people around the world, especially in the developing countries (McGuire, 1993). Iron deficiency is widespread and of utmost concern to healthcare officials in almost all developing countries (Buyckx, 1993). Iron deficiency has increased from 30% in the 1960s to 40% in the 1990s among the world population (Welch and Graham, 2002).

Durum wheat or pasta wheat (*Triticum turgidum* var durum) has high protein content and good quality for pasta products (macaroni, spaghetti, and other nodules) and making especial bread in the Mediterranean regions (Sissons, 2008). It is the hardest

wheat and durum milling produces coarse particles called semolina, ideal for making pasta and couscous (Matsuo, 1996). Area under cultivation and total production are about 17,000,000 hectares and 26,000,000 tons in the world, respectively (Matsuo, 1996). Durum wheat is better suited than bread wheat under low annual precipitation (300-450 mm). However, such wheat growing areas and growing this wheat in arid and semi-arid regions are affected by Fe deficiency because of high soil pH, free calcium carbonate, low organic matter, drought and salt stresses, imbalanced application of NPK fertilizers, and high bicarbonate content of irrigation water (Narimani *et al.*, 2010; Ali, 2012). The deficiency of iron in the soil causes reduction in wheat grain yield and quality,

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leading to nutrition disorder (Fe deficiency) in human (Ghorbani *et al.*, 2009).

Several approaches were taken to cope with Fe deficiency in the wheat grain. Abbas *et al.* (2009) applied 0, 4, 8, and 12 kg ha⁻¹ in the form of iron sulfate to the soil and showed that iron fertilization increased Fe and protein contents of the wheat grain. With application of 150 g ha⁻¹ iron in the form of Fe₂O₃, Habib (2009) reported that iron and protein contents of the wheat grain were enhanced. Zeidan *et al.* (2010) applied foliar Fe fertilizer (1.0% FeSO₄) and reported that Fe application increased protein and Fe contents of wheat grain. Narimani *et al.* (2010) showed that foliar spray of iron enhanced protein and yield of durum wheat. Welch and Graham (2002) and Cakmak (2008) suggested that Fe deficiency in wheat grain can be alleviated by breeding and selection of cultivars that could absorb more Fe from the soil and accumulate it in the grain, whereas Yip (1997) proposed that Fe deficiency could be overcome by food fortification. However, plant breeding is time consuming and iron fertilizers applied to crops by these methods may reach to target site of crops much below the minimum effective concentration. In addition, the effectiveness of inorganic and chelated forms of Fe fertilizers (FeSO₄, FeEDTA, FeDTPA, FeEDDHA, Fe-citrate) in overcoming Fe deficiency is highly variable depending on their solubility, stability, penetration ability through leaf cuticle, mobility and translocation following diffusion into the leaf tissues (Schonherr *et al.*, 2005; Fernandez *et al.*, 2009).

Reduction of particle size results in increased number of particles per unit of weight and specific surface area of a fertilizer that should increase contact of fertilizer with plant, leading to increase in nutrient uptake (Liscano *et al.*, 2000). Below 100 nm, nano-particles could make plants use fertilizer more efficiently, reduce pollution and be more environmentally friendly, and dissolve in water more effectively, thus, increase their activities (Joseph and Morrison, 2006).

Therefore, nanotechnology such as using nano-scale fertilizer particles may offer new techniques in improving existing crop management. Liu *et al.* (2005) reported that nano-Fe₂O₃ promoted the growth and photosynthesis of peanut. Sheykhbaglou *et al.* (2010) showed that application of nano-iron oxide particles increased soybean yield. Prasad *et al.* (2012) reported that nano-scale zinc oxide particles increased stem and root growth and pod yield of peanut as compared with ZnSO₄ application. Effect of nano-oxide iron alone or with iron chelate and sulfate on wheat production and grain quality, especially Fe content, has not been compared. In addition, there is little information on the accumulation of antioxidant enzymes and their possible role on yield and quality of wheat under nano-oxide iron, iron chelate, and iron sulfate application. Therefore, this experiment was conducted to compare the effects of nano-iron oxide, iron chelate, and iron sulfate rates on antioxidant enzymes accumulation, yield, yield components, and quality of durum wheat.

MATERIALS AND METHODS

Plant Material and Treatment

A field experiment was carried out during two growing seasons (9 November 2010 and 12 November 2011) at the Isfahan University of Technology Agricultural Research Station located at Lavark, Iran (40 km southwest of Isfahan, 32°32'N, 51°23'E, 1630 m asl). The soil was silty clay loam, typic Haplargids, pH= 7.3-7.8, Electrical Conductivity (EC)= 1.2-1.4 dS m⁻¹ and 0.9% organic matter, and contained 4.75 mg kg⁻¹ iron. The mean annual precipitation and mean annual temperature were 159 mm and 15.4°C, respectively. The experiment was laid out as a factorial based on a randomized complete block design (RCBD) with 3 replications. Treatments were the control and three sources of Fe fertilizers including nano-iron oxide, iron chelate, and iron

sulfate at the rates of 2 g L⁻¹ (0.28 kg ha⁻¹) and 4 g L⁻¹ (0.56 kg ha⁻¹); 4 and 8 g L⁻¹ (1.1 kg ha⁻¹); and 4 and 8 g L⁻¹, respectively. Fifty percent of iron fertilizers were foliar sprayed at stem elongation and the rest at flowering stage. The same amount of water was sprayed to the control plots each time. Before plowing, 150 kg ha⁻¹ ammonium phosphate (69 kg ha⁻¹ P₂O₅ and 27 kg ha⁻¹ N) and 150 kg ha⁻¹ potassium sulfate (75 kg ha⁻¹ K₂O and 27 kg ha⁻¹ S) fertilizers were incorporated into the soil by disk and then plots were prepared. Also, 120, 90, and 90 kg ha⁻¹ urea as nitrogen fertilizer were added to the soil before sowing, at six leaves, and at flowering stages, respectively. Seeds of durum wheat cultivar D-85-15-5 were planted in plots 6 m long and 2 m wide consisting of 10 rows with 20 cm spacing. At the maturity stage, 10 plants from each plot were randomly selected and plant height and yield components were measured. At the full maturity stage, plants were harvested (2 m²) and grain and biological yield were determined. Harvest index was calculated as the ratio of grain yield to biological yield.

Protein and Carbohydrate Contents

Protein and carbohydrate contents were measured by Near Infrared Reflectance Spectroscopy. Grains were ground and flour samples were scanned on a NIR systems 6500 scanning spectrophotometer (Pertec 8620-Inframatic Grain Analysis) in reflectance mode as described by Lemons-e-Silva *et al.* (2008).

Grain Iron Content

Two grams of dried sample was placed into a crucible and heated at 550°C for 4 hours. Ten mL of 2N HCl was added to the ashes and then was diluted to 100 volume and iron content was measured by the Atomic Absorption Spectrophotometer (Perkin-Elmer 3030) as described by Davidson and Miller (2005).

Chlorophyll and Carotenoid Contents

Chlorophyll a, chlorophyll b, total chlorophylls and carotenoids contents were extracted from fresh leaves at flowering stage, following the standard method of Lichtenthaler (1994). Fresh leaf samples (0.33 g) were selected randomly from the plants and homogenized in 80% acetone to 10 mL volume.

Enzyme Activities

Catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) activities were determined from the extract prepared according to the methods of Bergmeyer (1970), Nakano and Asada (1981), and Herzog and Fahimi (1973) with some modifications. All steps of the extraction were carried out at 4°C. Fresh leaf samples (0.2 g) were homogenized in a cold mortar in 1 mL of 50 mM Na-phosphate buffer (pH= 7) containing 2 mM α -dithiothreitol, 2 mM EDTA, 0.2% triton X-100, 50 mM Mtris-HCl and 2% polyvinylpyrrolidone and mixed for 15 minutes. The obtained extract was immediately used for determination of enzyme activities. CAT (EC1.11.1.6), the assay of catalase activity was carried out in a total volume of 3 mL of 50 mM Na-phosphate buffer (pH= 7.0) containing 4.51 μ L of H₂O₂ (30%) and 50 μ L of enzyme extract. GPX (EC 1.11.1.7), Guaicol peroxidase activity was determined in 3 mL of 50 mM Na-phosphate buffer (pH 7.8) containing 4.51 μ L of H₂O₂ (30%), 3.35 μ L Guaicol and 50 μ L of enzyme extract. The decrease in absorbance at 240, 270, and 290 nm, respectively, for CAT, GPX and APX, because of degradation of H₂O₂ was monitored every 30 seconds for 2 minutes, using a spectrophotometer U-1800 (Hitachi, Japan). Catalase, Ascorbate peroxidase and Guaicol peroxidase activity was expressed as nanomoles of decomposed per milligram of protein per minute. APX (EC 1.11.1.11), Ascorbate peroxidase activity was



determined in 3 ml of 50 mM Na-phosphate buffer (pH= 7.8) containing 4.51 μ L of H₂O₂ (30%), 100 μ L of 5 mM ascorbate and 50 μ L of enzyme extract. All results correspond to the means of the values obtained with two measurements carried out in three independent experiments.

Protein Determination

Protein content in the enzymatic extracts was determined by using Bradford method (1976) and Bovine serum albumin (Sigma) was used as standard.

Statistical Analysis

Data of the two years were combined and the combined data were subjected to normal distribution tests and analysis of variance and least significant difference (LSD) for comparison of means were performed using statistical analysis system (SAS 9.1 Institute, 2002).

RESULTS AND DISCUSSION

Chlorophyll and Carotenoid Contents

Nano-iron oxide and iron sulfate application resulted in higher chl a and chl b contents as compared with iron chelate, while nano-iron oxide increased the total chlorophyll content the most, followed by iron sulfate and iron chelate, respectively (Table 2). Carotenoid content and chl a, and b ratio were not affected by iron sources. Iron application increased chlorophyll a, b and total contents as compared with the control and higher rates were more effective. That was perhaps due to the association of Fe with chlorophyll formation (Mazaherinia *et al.*, 2010). In line with our results, Liu *et al.* (2005) reported that nano-Fe₂O₃ application increased chlorophyll content of peanut and Amanullah *et al.* (2012) showed that application of iron sulfate in soil and

foliar spray increased chlorophyll content of maize leaf. Borowski and Michalek (2011) reported that foliar application of iron salt increased chlorophyll a, b and carotenoid contents of French bean. Their results are not in total agreement with ours, since in our results, carotenoid content of the wheat was not affected by iron application. The increase in chlorophyll content of wheat in our experiment could be due to promotion of the absorption and utilization of nutrients such as nitrogen by nano-Fe compound as reported by Liu *et al.* (2005).

Antioxidant Enzymes Activities

On average, the activities of POX, CAT, and APX were not significantly affected by iron sources, but increased by iron application rate (Tables 1 and 2). The increase in the enzymes activities might be due to triggering induction of CAT, POX, and APX genes expression by iron application as reported in *Brassica napus* by Vansuyt *et al.* (1997).

Yield and Yield Components

Plant height, spike length, and number of spike m⁻² were not affected by iron sources, however, iron sulfate application increased biological yield, 1,000-grain weight and grain yield the most, followed by nano-iron oxide and iron chelate, respectively (Table 3).

Iron rate had no marked effect on plant height, spike length, number of grain per spike and biological yield, but number of spike m⁻², 1,000-grain weight, harvest index, and grain yield increased as iron rate increased (Table 4). The increase in grain yield by iron application was perhaps due to increase in chlorophyll content and antioxidant enzymes activities as indicated

Table 1. Analysis of variance for the effect of iron source and rate on chlorophyll, carotenoid contents and CAT, POX, APX enzymes activities.^a

SOV	df	Chl a	Chl b	Chl total	Chl a/b	Carotenoid	CAT	POX	APX
Replication	2	0.0036 ^{ns}	0.0005 ^{ns}	0.0005 ^{ns}	0.027 ^{ns}	0.119 ^{ns}	0.0004 ^{ns}	0.012 ^{ns}	0.0009 ^{ns}
Iron source	2	0.020 ^{**}	0.0029 ^{**}	0.043 ^{**}	0.017 ^{ns}	0.038 ^{ns}	0.003 [*]	0.015 ^{ns}	0.0005 ^{ns}
Iron rate	2	0.187 ^{***}	0.022 ^{***}	0.331 ^{***}	0.168 ^{**}	0.168 [*]	0.002 [*]	0.043 [*]	0.002 ^{**}
Iron source * Iron rate	4	0.046 ^{**}	0.0053 ^{**}	0.097 ^{**}	0.050 ^{ns}	0.051 ^{ns}	0.0007 ^{ns}	0.014 ^{ns}	0.0003
Error	16	0.003	0.0002	0.002	0.055	0.040	0.0004	0.009	0.0003
Total	26								

^a ns, * ** and *** show non-significance and significance at 5, 1 and 0.001% level, respectively.

Table 2. Effects of iron source and rate on chlorophyll, carotenoid contents and CAT, POX and APX enzymes activities of durum wheat.

Treatments	Chl a (mg g ⁻¹)	Chl b (mg g ⁻¹)	Chl total (mg g ⁻¹)	Chl a/b (mg g ⁻¹)	Carotenoid (mg g ⁻¹)	CAT (unit ml ⁻¹)	POX (unit ml ⁻¹)	APX (unit ml ⁻¹)
Iron source								
Nano-iron oxide	0.88 ^a	0.304 ^a	1.18 ^a	2.89 ^a	12.98 ^a	0.177 ^a	0.78 ^a	0.177 ^a
Iron chelate	0.79 ^b	0.268 ^b	1.06 ^c	2.96 ^a	13.07 ^a	0.176 ^a	0.70 ^a	0.166 ^a
Iron sulfate	0.87 ^a	0.298 ^a	1.16 ^b	2.92 ^a	12.95 ^a	0.186 ^a	0.73 ^a	0.181 ^a
LSD	0.057	0.014	0.043	0.235	0.201	0.020	0.098	0.018
Iron rate								
Control	0.69 ^c	0.231 ^b	0.92 ^c	2.98 ^{ab}	13.04 ^{ab}	0.162 ^b	0.68 ^b	0.158 ^b
Level 1	0.90 ^b	0.315 ^a	1.21 ^b	2.86 ^b	13.11 ^a	0.189 ^a	0.71 ^b	0.179 ^a
Level 2	0.96 ^a	0.317 ^a	1.28 ^a	3.03 ^a	12.85 ^b	0.188 ^a	0.81 ^a	0.188 ^a
LSD	0.057	0.014	0.043	0.235	0.201	0.020	0.098	0.018

^a Values within the column and each experimental factor, followed by the different letters are significantly different according to the Least Significant Difference test (LSD) at 0.05 probabilities.



in Table 2, and increase in some yield components as indicated in Table 4. In addition, Sheykhbaglou *et al.* (2010) reported that nano-iron oxide increased pod and leaf dry weight and yield of soybean, but had no effects on plant height and other growth and yield parameters. Also, Ghodsi *et al.* (2012) reported that nano-iron oxide increased plant height and yield of sunflower. Their results were in general agreement with ours. In line also with our results, Habib (2009) reported that application of 150 g ha^{-1} Fe as Fe_2O_3 increased wheat grain yield. Narimani *et al.* (2010) showed that foliar application of Fe fertilizer increased yield and yield components of durum wheat and Zeidan *et al.* (2010) reported that application of 1% FeSO_4 increased yield and yield components of wheat. Furthermore, Ali (2012) applied 0.2 to 0.6 mg L^{-1} Iron sulphate fertilization ($\text{FeSO}_4 \cdot \text{H}_2\text{O}$) and reported that yield and components increased as the rate of Fe increased. In line with our results, Liu *et al.* (2005) reported that nano- Fe_2O_3 promoted the growth and photosynthesis of peanut and Sheykhbaglou *et al.* (2010) showed that the application of nano-iron oxide particles increased soybean yield.

Grain Quality

Grain carbohydrates, protein and iron contents also carbohydrate, protein and iron yield were significantly affected by iron source, iron rate and their interactions (Table 5). Nano-iron oxide produced the highest grain iron content and yield and grain protein content and yield, followed by iron sulfate and iron chelate, respectively (Table 6). Whereas, iron sulfate and nano-iron oxide applications produced higher grain carbohydrate contents and yield as compared with iron chelate (Table 6). In general, with increasing iron rate, grain carbohydrates, protein and iron contents also carbohydrate, protein and iron yield increased. In agreement with our results, Habib (2009) reported that application of

150 g ha^{-1} Fe as Fe_2O_3 increased wheat grain yield and Zeidan *et al.* (2010) reported that application of 1% FeSO_4 increased wheat grain protein and Fe contents. In accordance with our results, Ali (2012) also reported that Fe application increased grain protein content and yield of durum wheat and Monsef Afshar *et al.* (2012) reported that application of 1 per 1,000 nano-iron chelate increased Fe and protein content of cowpea seed. The increase in grain quality of wheat in our experiment by application of iron fertilizers may be due to the role of Fe in enhancing accumulation of assimilate in the grain as concluded by Zeidan *et al.* (2010).

Interaction

There was interaction between iron rate and iron source on 1,000-grain weight, harvest index, chlorophyll a, b, and total contents (Table 7). Highest 1,000-grain weight and harvest index were produced by application of 2 g L^{-1} of nano-iron oxide or 8 g L^{-1} iron sulfate, while the greatest chl a, b, and total chlorophyll contents were obtained by application of 2 g L^{-1} of nano-iron oxide followed by 8 g L^{-1} iron sulfate (Table 7). Harvest index, 1,000-grain weight, and chlorophyll a, b, and total contents increased as the rate of iron sulfate and iron chelate increased, but these parameters decreased at the higher rate of nano-iron oxide.

Grain protein and iron contents and yield of grain, protein, iron and carbohydrate were affected by iron rate and source (Table 8). Application of 2 g L^{-1} of nano-iron oxide increased grain protein and iron contents, total grain yield, and total grain yield of protein, iron, and carbohydrate, followed by application of 8 g L^{-1} iron sulfate. In general, application of higher rates of iron sulfate or iron chelate increased these measured parameters, but higher rate of nano-iron oxide reduced them. These results suggested that Fe fertilizer application rate in nano form need to be carefully regulated. Kampfenkle *et al.* (1995) concluded that excess Fe in plant cell may cause oxidative

Table 3. Analysis of variance for the effect of iron source and rate on yield and yield components of durum wheat.

SOV	df	Plant height	Spike length	Number of spikes m ⁻²	number of grains per spike	1000-grain weight	Biological Yield	Grain yield	Harvest index
Replication	2	14.9 ^{ns}	0.49 ^{ns}	8493 ^{ns}	2.21 ^{ns}	7.62 ^{ns}	109744 ^{ns}	16959 ^{ns}	20.1 ^{ns}
Iron source	2	13.2 ^{ns}	0.12 ^{ns}	23104 ^{**}	0.94 ^{ns}	71.3 ^{**}	343652 [*]	1422959 [*]	65.1 [*]
Iron rate	2	199.9 [*]	0.12 ^{ns}	28133 ^{***}	12.4 ^{ns}	261.3 ^{***}	114614 ^{ns}	2704731 ^{**}	282.6 ^{***}
Iron source*				13557 [*]					
Iron rate	4	48.7 ^{ns}	0.16 ^{ns}		23.7 ^{ns}	85.9 ^{**}	144635 ^{ns}	2343625 ^{**}	159.7 ^{**}
Error total	16	150.6	0.712	3578.4	14.49	3.61	92799	24283	18.4
	26								

^{ns} non-significance; *, ** and *** show and significance at 5, 1 and 0.001% level, respectively.

Table 4. Effects of iron source and rate on yield and yield components of durum wheat.

Treatments	Plant height (cm)	Spike length (cm)	Number of spikes m ⁻²	number of grains per spike	1000-grain weight (g)	Biological Yield (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	Harvest index (%)
Iron source								
Nano-iron Oxide	93.88 ^a	7.22 ^a	545.8 ^a	33.32 ^a	49.23 ^b	15880 ^{ab}	8914.4 ^a	56.13 ^a
Iron chelate	95.08 ^a	6.99 ^a	520.7 ^b	33.18 ^a	48.62 ^b	15250 ^b	8300 ^b	54.42 ^b
Iron sulfate	92.66 ^a	7.07 ^a	533.1 ^a	33.37 ^a	51.76 ^a	16115.8 ^a	9044.4 ^a	56.13 ^a
LSD	12.3	0.84	59.8	3.80	1.90	962.7	492.5	4.29
Iron rate								
Control	90.22 ^a	7.01 ^a	496.6 ^b	33.91 ^a	47.62 ^c	15584.4 ^a	8000 ^b	51.38 ^b
Level 1	92.22 ^a	7.04 ^a	513.3 ^b	33.94 ^a	52.07 ^b	15760 ^a	9070 ^a	57.55 ^a
Level 2	99.20 ^a	7.22 ^a	525.7 ^a	34.03 ^a	55.08 ^a	16271.3 ^a	9688.9 ^a	59.54 ^a
LSD	12.3	0.84	59.8	3.80	1.90	962.7	492.5	4.29

^a Values within the column and each experimental factor, followed by different letters are significantly different according to the Least significant difference test (LSD) at 0.05 probabilities.

Table 5. Analysis of variance for the effect of iron source and rate on grain carbohydrate, protein, iron contents and grain carbohydrate, protein, iron yield.^a

SOV	df	Carbohydrate content	Grain protein content	Iron contents	Carbohydrate yield	Grain protein yield	Iron yield
Replication	2	11.6 ^{ns}	0.18 ^{ns}	12.7 ^{ns}	58508073 ^{ns}	4559770 ^{ns}	10153 ^{ns}
Iron source	2	27.8 ^{**}	3.9 [*]	306.5 ^{**}	174355232 ^{**}	15168413 ^{**}	86958 ^{**}
Iron rate	2	32.5 ^{**}	35.4 ^{***}	829.1 ^{**}	875877908 ^{**}	67658225 ^{**}	366203 ^{**}
Iron source*			15.5 ^{**}	486.9 ^{**}	233652830 ^{**}	37693504 ^{**}	152301 ^{**}
Iron rate	4	24.4 ^{**}					
Error total	16	3.34	1.09	12.04	10873129	1151481	2249
	26						

^{ns} non-significance; *, ** and *** show and significance at 5, 1 and 0.001 % level, respectively.



Table 6. Effects of iron source and rates on grain carbohydrate, protein, iron contents and grain carbohydrate, protein, iron yield.

Treatments	Carbohydrate content (g kg ⁻¹)	Grain protein content (g kg ⁻¹)	Iron contents (mg kg ⁻¹)	Carbohydrate yield (kg h ⁻¹)	Grain protein yield (kg h ⁻¹)	Iron yield (kg h ⁻¹)
Iron source						
Nano-iron oxide	764.5 ^a	147.5 ^a	115.0 ^a	6815.5 ^a	1314.8 ^a	1.03 ^a
Iron chelate	741.5 ^b	134.4 ^b	103.3 ^c	6154.4 ^b	1115.5 ^b	0.86 ^c
Iron sulfate	776.0 ^a	139.1 ^{ab}	109.4 ^b	7018.4 ^a	1258.1 ^a	0.99 ^b
LSD	1.83	1.04	3.47	329	107	0.047
Iron rate						
Control	741.4 ^b	117.6 ^b	98.3 ^b	5931.2 ^b	940.8 ^b	0.79 ^b
Level 1	761.2 ^a	149.2 ^a	113.1 ^a	6904.0 ^{ab}	1353.2 ^{ab}	1.03 ^{ab}
Level 2	779.4 ^a	154.2 ^a	116.3 ^a	7551.5 ^a	1494.0 ^a	1.12 ^a
LSD	1.83	1.04	3.47	329	107	0.047

^a Values within the column of each experimental factor, followed by different letters are significantly different according to the Least significant difference test (LSD) at 0.05 probabilities.

Table 7. The interaction between fertilizer source and rate on 1000-grain weight, harvest index and Chlorophyll contents of durum wheat.

Iron source	Iron rate	1000-grain weight (g)	Harvest index (%)	Chl a (mg g ⁻¹)	Chl b (mg g ⁻¹)	Chl total (mg g ⁻¹)
Nano-iron oxide	Control	44.81 ^d ^a	47.37 ^c	0.69 ^{fg}	0.23 ^e	0.93 ^f
	2 g L ⁻¹	57.50 ^d	65.03 ^a	1.09 ^a	0.38 ^a	1.48 ^a
	4 g L ⁻¹	48.12 ^c	52.90 ^{bc}	0.87 ^{cd}	0.29 ^{cd}	1.18 ^d
Iron chelate	Control	44.81 ^d	47.37 ^c	0.69 ^{fg}	0.23 ^e	0.93 ^f
	4 g L ⁻¹	46.40 ^{ed}	49.08 ^{bc}	0.76 ^{ef}	0.27 ^d	1.03 ^e
	8 g L ⁻¹	54.46 ^{bc}	55.09 ^b	0.96 ^{bc}	0.30 ^c	1.27 ^c
Iron sulfate	Control	44.81 ^d	47.37 ^c	0.69 ^{fg}	0.23 ^e	0.93 ^f
	4 g L ⁻¹	52.60 ^c	51.65 ^{bc}	0.86 ^{de}	0.29 ^{cd}	1.15 ^d
	8 g L ⁻¹	62.30 ^a	66.67 ^a	1.05 ^{ab}	0.35 ^b	1.40 ^b
LSD		3.28	7.42	0.098	0.023	0.073

^a Values within the column of each experimental factor, followed by different letters are significantly different according to the least significant difference test (LSD) at 0.05 probabilities.

Table 8. The interaction between fertilizer source and rate on yield and grain quality of durum wheat.^a

Iron source	Iron rate	Grain yield (kg ha ⁻¹)	Grain protein content (g kg ⁻¹)	Iron contents (mg kg ⁻¹)	Carbohydrate yield (kg h ⁻¹)	Grain protein yield (kg h ⁻¹)	Iron yield (kg h ⁻¹)
Nano-iron oxide	Control	7500 ^d ^a	117.6 ^c	96.33 ^f	5490.4 ^e	882.8 ^e	0.72 ^f
	2 g L ⁻¹	10633.3 ^a	185.3 ^a	133.3 ^a	8461.3 ^a	1970.4 ^a	1.41 ^a
	4 g L ⁻¹	9000 ^{bc}	139.6 ^{cd}	108.0 ^{cd}	6887.8 ^b	1256.4 ^d	0.97 ^{cd}
Iron chelate	Control	7500 ^d	117.6 ^c	96.33 ^f	5490.4 ^e	882.8 ^e	0.72 ^f
	4 g L ⁻¹	8266.7 ^{cd}	129.0 ^{de}	103.6 ^{de}	6129.3 ^{cd}	1066.8 ^d	0.86 ^e
	8 g L ⁻¹	9133.3 ^b	156.6 ^{bc}	114.0 ^c	7067.5 ^b	1430.3 ^c	1.04 ^c
Iron sulfate	Control	7500 ^d	117.6 ^c	96.33 ^f	5490.4 ^e	882.8 ^e	0.72 ^f
	4 g L ⁻¹	8910 ^{bc}	133.3 ^{de}	105.0 ^{de}	6648.2 ^{bc}	1187.7 ^{de}	0.94 ^d
	8 g L ⁻¹	10333.3 ^a	166.3 ^b	127.0 ^b	8269.8 ^a	1718.4 ^b	1.31 ^b
LSD		852.9	1.80	6.00	57.07	18.57	0.82

^a Values within the column of each experimental factor, followed by different letters are significantly different according to the least significant difference test (LSD) at 0.05 probabilities.

stress suggesting cellular Fe concentration must be finely regulated to avoid possible cellular damage. The results suggested that application of 2 g L⁻¹ nano-iron oxide was more effective than other Fe sources and rates, because nano-iron oxide had more particles per unit of weight and specific surface area that increased contact of fertilizer with plant, leading to increase in Fe and other nutrients uptake (Liscano *et al.*, 2000; Liu *et al.*, 2005). In addition, nano-oxide iron particles below 100 nm perhaps made Fe more efficient and dissolved in water more effectively, thus, increased their activities (Joseph and Morrison, 2006).

CONCLUSIONS

The foliar application of iron increased leaf activities of CAT, POX and APX, chlorophyll a, b, total contents, grain protein, iron and carbohydrate contents, grain carbohydrate, protein and iron yields and grain yield. Application of 2 g L⁻¹ nano-iron oxide was more effective than the other sources and rates of iron fertilizers, therefore, application of 2 g L⁻¹ nano-iron oxide is suggested for wheat production in this and similar areas.

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REFERENCES

1. Abbas, G., Khan, M. Q., Khan, M. J., Hussain, F. and Hussain, I. 2009. Effect of Iron on the Growth and Yield Contributing Parameters of Wheat (*Triticum aestivum* L.). *J. Anim. Plant Sci.*, **19**: 135-139.
2. Ali, E.A. 2012. Effect of Iron Nutrient Care Sprayed on Foliage at Different Physiological Growth Stages on Yield and Quality of some Durum Wheat (*Triticum durum* L.) Varieties in Sandy Soil. *Asian. J. Crop Sci.*, **4**: 139-149.
3. Amanullah, M. M., Archana, J., Manoharan, S. and Subramanian, K. S. 2012. Influence of Iron and AM Inoculation on Metabolically Active Iron, Chlorophyll Content and Yield of Hybrid Maize in Calcareous Soil. *J. Agron.*, **11(1)**: 27-30.
4. Bergmeyer, N. 1970. Method of Enzymatic Analyses. *Acad. Verlag. Berl.*, **1**: 636-647.
5. Borowski, E. and Michalek, S. 2011. The Effect of Foliar Fertilization of French Bean with Iron Salts and Urea on some Physiological Processes in Plants Relative to Iron Uptake and Translocation in Leaves. *Acta Sci. Pol. Hortorum. Cultus.*, **10(2)**: 183-193.
6. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. *Anal. Biochem.*, **72**: 248-254.
7. Buyckx, M. 1993. The International Community's Commitment to Combating Micronutrient Deficiencies. *Food Nutr. Agar.*, **7**: 2-7.
8. Cakmak, I. 2008. Enrichment of Cereal Grains with Zinc: Agronomic or Genetic Biofortification? *Plant Soil*, **302**: 1-17.
9. Fernandez, V., Orera, I., Abadia, J. and Abadia, A. 2009. Foliar Iron-fertilization of Fruit Trees: Present Knowledge and Future Perspectives. *J. Hort. Sci. Bio.*, **84**: 1-6.
10. Ghodsi, A., Astaraei, A. L., Emami, H. and Mirzapour, M. H. 2012. Effect of Nano-iron Oxide Powder and Urban Solid Waste Compost Coated Sulfur on Sunflower Yield and Yield Components in Saline-sodic Soil. *Environ. Sci.*, **9(3)**: 111-118.
11. Ghorbani, S., Khiabani, B. N., Amini, I., Ardakani, M. R., Pirdashti, H. and Moakhar, S. R. 2009. Effect of Iron and Zinc on Yield and Components of Mutant Line, Wheat. *Asian. J. Bio. Sci.*, **2**: 74-80.
12. Habib, M., 2009. Effect of Foliar Application of Zn and Fe on Wheat Yield and Quality. *Afr. J. Biotechnol.*, **8**: 6795-6798.
13. Herzog, V. and Fahimi, H. 1973. Determination of Activity of Proxidase. *Anal. Biochem.*, **55**: 554-562.
14. Joseph, T. and Morrison, M. 2006. Nanotechnology in Agriculture and Food. <http://nanoforum.org>
15. Kampfenkle, K., Montagu, M. V. and Inze, D. 1995. Effect of Iron Excess on *Nicotiana glumbaginifolia* Plants. *Plant Physiol.*, **107**: 725-735.



16. Lemonse-Silva, C. F., Kothe Milach, S. C., Anjos-e Silva, S. D. and Montero, C. R. 2008. Near Infrared Reflectance Spectroscopy (NIRS) to Assess Protein and Lipid Contents in (*Avena sativa* L.). *Crop Breed. Appl. Bio.*, **8**: 127-133.
17. Lichtenthaler, H. K., 1994. Chlorophylls and Carotenoids Pigments of Photosynthetic. *Methods Enzymol.*, **148**: 350-382.
18. Liscano, J. F., Wilson, C. E., Norman-Jr, R. J. and Slaton, N. A. 2000. Zinc Availability to Rice from Seven Granular Fertilizers. *AAES Res. Bull.*, **963**: 1-31.
19. Liu, X. M., Zhang, F. D., Zhang, S. Q., He, X. S., Fang, R., Feng, Z. and Wang, Y. 2005. Effects of Nano-ferric Oxide on the Growth and Nutrients Absorption of Peanut. *Plant Nutr. Fert. Sci.*, **11**: 14-18.
20. Matsuo, R. R., Dexter, J. E. and MacGregor, A. W. 1982. Effect of Sprout Damage Durum Wheat and Spaghetti Quality. *Cereal Chem.*, **59**: 468-472.
21. Matsuo, R. R. 1996. Durum Wheat: Its Unique Pasta-making Properties. In: "*Wheat production, properties and quality*" (Eds): Bushuk, W. and Rasper, V. F., Chapman and Hall. London. PP. 169-178.
22. Mazaherinia, S., Astarai, A. R., Fotovat, A. and Monshi, A. 2010. Nano-iron Oxide Particles Efficiency on Fe, Mn, Zn and Cu Concentrations in Wheat Plant. *World Appl. Sci. J.*, **7**: 36-40.
23. McGuire, J. 1993. Addressing Micronutrient Malnutrition. *SCN News*, **9**: 1-10.
24. Monsef Afshar, R., Hadi, H. and Pirzad, A. 2012. Effect of Nano-iron Foliar Application on Qualitative and Quantitative Characteristics of Cowpea, under end Season Drought Stress. *Intl. Res. J. Appl. Basic Sci.*, **3(8)**: 1709-1717.
25. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.*, **22**: 867-880.
26. Narimani, H., Rahimi, M. M., Ahmadikhan, A. and Vaezi, B. 2010. Study on the Effects of Foliar Spray of Micronutrient on Yield and Yield Components of Durum Wheat. *Appl. Sci. Res.*, **2(6)**: 168-176.
27. Nestel, P. and Nalubola, R. 2000. Manual for wheat flour fortification with iron (Part 1) Guidelines for the development, implementation, monitoring, and evaluation of a program for wheat flour fortification with iron. <http://www.mostproject.org>.
28. Prasad, T. N. V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Raja-Reddy, K., Sreepasad, T. S., Sajanalal, P. R. and Pradeep, T. 2012. Effect of Nanoscale Zinc Oxide Particles on the Germination, Growth and Yield of Peanut. *J. Plant Nutr.*, **35**: 905-927.
29. SAS Institute., 2002. *The SAS System for Windows: Released 9.1*. SAS Inst. Cary, NC.
30. Schonherr, J., Fernández, V. and Schreiber, L. 2005. Rates of Cuticular Penetration of Chelated Fe(III): Role of Humidity, Concentration, Adjuvants, Temperature and Type of Chelate. *J. Agri. Food Chem.*, **53**: 4484-4492.
31. Sheykhbaglou, R., Sedghi, M., Tajbakhsh Shishevan, M. and Sharifi, S. R. 2010. Effects of Nano-iron Oxide Particles on Agronomic Traits of Soybean. *NotulaeSci. Bio.*, **2**: 112-113.
32. Sissons, M. 2008. Role of Durum Wheat Composition on the Quality of Pasta and Bread. *Food Global Sci. Book*, **2(2)**: 75-90.
33. Vansuyt, G., Lopez, F., UGent, D. I. and Briat, J. F. 1997. Iron Triggers a Rapid Induction of Ascorbate Peroxidase Gene Expression in *Brassica napus*. *Febs Lett.*, **410**: 195-200.
34. Welch, R. M. and Graham, R. D. 2002. Breeding Crops for Enhanced Micronutrient Content. *Plant Soil*, **245**: 205-214.
35. Yip, P. 1997. Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Degree of Master of Arts. MSc. Thesis, Calgary, Alberta, Canada.
36. Zeidan, M. S., Manal, F. and Hamouda, H. A. 2010. Effect of Foliar Fertilization of Fe, Mn and Zn on Wheat Yield and Quality in Low Sandy Soils Fertility. *World J. Agric. Sci.*, **6**: 696-699.

واکنش گندم دوروم به برگپاشی با منابع و مقادیر مختلف کود آهن

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چکیده

کمبود آهن با کاهش کیفیت گندم دوروم باعث کمبود آهن در انسان می‌شود. بنابراین این پژوهش با هدف ارزیابی تاثیر محلول‌پاشی نانوآکسید آهن (۲ و ۴ گرم در لیتر)، کلات آهن (۴ و ۸ گرم در لیتر) و سولفات آهن (۴ و ۸ گرم در لیتر) و شاهد (عدم محلول‌پاشی) بر عملکرد دانه و اجزای عملکرد، محتوای کلروفیل و کارتنوئید و فعالیت آنزیم‌های آنتی‌اکسیدانی گندم دوروم لاین D-85-15-5 صورت گرفت. کاربرد آهن باعث افزایش فعالیت آنزیم‌های و محتوای کلروفیل برگ، پروتئین، آهن و کربوهیدرات دانه، عملکرد پروتئین، آهن و کربوهیدرات و عملکرد دانه شد. منابع آهن تاثیر چندانی بر فعالیت آنزیم‌ها نداشتند، اما بالاترین محتوای کلروفیل، عملکرد دانه، محتوای پروتئین دانه (افزایش ۵۸٪) و آهن دانه (افزایش ۳۸٪)، عملکرد پروتئین، آهن و کربوهیدرات مربوط به کاربرد ۲ گرم در لیتر نانوآکسید آهن و پس از آن ۸ گرم در لیتر سولفات آهن بود. شاخص سطح برگ، وزن هزار دانه، محتوای کلروفیل، عملکرد دانه، محتوای پروتئین و آهن دانه و عملکرد پروتئین، آهن و کربوهیدرات در سطح ۸ گرم در لیتر سولفات آهن و کلات آهن افزایش یافت. در حالیکه این پارامترها در سطح بالاتر نانوآکسید آهن کاهش یافتند. کاربرد ۲ گرم در لیتر نانوآکسید آهن بیشترین تاثیر را نسبت به منابع و سطوح کودی دیگر داشت و برای تولید گندم دوروم پیشنهاد شده است.