



The Effect of Mycorrhizal fungi inoculation, zinc and iron oxide application on root growth, antioxidant enzyme activities, and mineral elements in sorghum (*Sorghum bicolor*)

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ABSTRACT

Zinc (Zn) and Iron (Fe) are essential micronutrients that play crucial role on crop plants. Adequate amount of these elements is required for metabolic synthesis, enzyme activity and improvement of overall yield. Mycorrhizal also enhance the uptake and facilitate absorption of water and essential elements in crop plants. This study aimed to evaluate the effect of foliar application of Zn and iron Fe oxides in both normal (2 g L⁻¹) and nano form (1.5 g L⁻¹) combined with inoculation mycorrhiza (*Funneliformis mosseae*) on root growth and some physiological characteristics in sorghum. Zn and Fe oxides in both normal and nano forms, increased the root growth. Normal form of Fe and Zn oxides showed the greater effect on this trait. Inoculation with *Funneliformis mosseae*, increased root and shoot dry weight 9.6% and enhanced root length and volume 12.6%. Moreover, Zn and Fe oxides treatments increased the activity of antioxidant enzymes such as guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and catalase (CAT) in leaf tissues. No significant difference was observed between the nano and normal forms regarding these antioxidant enzyme activities. *Funneliformis mosseae* as well as Fe and Zn oxide, increased the concentration of Fe and Zn in the leaves. The highest Fe and Zn concentrations were obtained under foliar application of Zn and Fe oxides in normal form. The results demonstrated that, under symbiosis with the *Funneliformis mosseae*, foliar application of Fe and Zn oxides in their normal form has a greater effect on root growth and enzyme activities in sorghum plants compared to the nano form.

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1. Introduction

Sorghum (*Sorghum bicolor* L) is one of the tropical crops, grown in semi-arid and arid region. It uses as a grain, fodder and industrial crops. Sorghum can be productive under low-input conditions and considered as a drought tolerant (Sarshad *et al.*, 2021). Soils with high sodium adsorption ratio (SAR) and high pH have very low solubility of micronutrients. Therefore, it can affect the absorption of nutrient elements in the soil. Among the micronutrients, Zn is required to maintenance of cell membrane integrity, biosynthesis of proteins and enzymes, hormone regulation, as well as photosynthesis (Yruela *et al.*, 2021). Zn has a major role in protective several chemical compositions such as protein, chlorophyll, membrane lipids, and DNA and enzyme having SH against from ROS (Cakmak, 2002). Studies have demonstrated that grain yield of cereal crops increases significantly with the application of Zn in Zn-deficient soils (Liu *et al.*, 2017).

The solubility of Fe is another challenge to plant growth in soil with high pH, because it can affect on the absorption of Fe. Plant responds to Fe limitation by inducing a series of morphological and physiological changes in the roots to facilitate the mobilization of sparingly soluble Fe compounds in the root media (Cakmak, 2002). Fe is a part of the central cell defense enzyme for peroxidase and catalase (Grillet *et al.*, 2018). About 80% of Fe in plants is concentrated in chloroplasts, Fe also involved in several key chemical processes including synthesis of chlorophyll (a vital pigment required for photosynthesis). Therefore, its deficiency can have a great impact on the reduction of photosynthesis (Brumbarova *et al.*, 2015).

Soil microorganisms such as arbuscular mycorrhizal fungi (AMFs), have been presented as safe and effective biofertilizers in recently years (Wang *et al.*, 2022). Mycorrhiza play an important role to improving carotenoids and modulating the synthesis of antioxidant enzymes, chlorophyll contents and regulation metabolic activities under environmental stress. Colonization by mycorrhiza increases roots and enhance the uptake of nutrients and water (Latef and Chaoping, 2011). AMFs can improve the host's drought resistance by alleviating H₂O₂ and malondialdehyde accumulation, enhancing phosphorus uptake and altering the roots hydraulic (Zhao *et al.*, 2015).

Recently, nanotechnology has been increasingly applied to crop production. Because nano particles (NPs) having high surface area and small in size (<100 nm). Recent studies revealed that nano-fertilizers such as nano Fe and Zn are effective in enhancing absorption and translocation in plants (MonsefAfshar *et al.*, 2013). Nano-fertilizers, unlike conventional fertilizers, reduce the environmental risks associate with chemical fertilizers and soil pollution (Naderi *et al.*, 2011).

Although the use of nano-fertilizers can positively effect on the growth and physiology of crop plants. But, physiological aspects of nano Zn and Fe oxide compared to Zn and Fe oxide in normal form along with the use of mycorrhizal fungi, on root growth and the physiological changes in the root of sorghum are partially unknown. Hence, the present study was carried out to investigate the effects of foliar application of Zn and Fe oxide in both nano and normal form with the use of mycorrhizal on root characters, root growth, antioxidant enzymes activities and concentration of mineral nutrients in sorghum.

2. Materials and Methods

2.1. Plant Culture

To study Fe and Zn oxide in normal and nano form with inoculation of mycorrhizal fungi on antioxidant enzyme activities, root growth and concentration of mineral elements in leaves tissues in sorghum variety Sepideh, a pot experiment was conducted as a factorial design (completely randomized block) 2*3*3 with three replicates at the Yazd University, Iran in 2021. 54 pots (35×35 and height 33cm) were filled with sandy loam soil, EC=1.8 ds m⁻¹ and pH= 7.26. Before use, soil was sterilized by autoclave. Treatments included inoculation of plants with mycorrhiza at two levels: without mycorrhiza and inoculation with mycorrhiza (*Funneliformis mosseae*), as first factor, foliar application of Fe oxide at three levels: 0 (without foliar application of Fe oxide), 2 gl⁻¹ Fe oxide, and 1.5gl⁻¹ Fe oxide in nano form, as the second factor and foliar application of Zn oxide at three levels: 0 (without foliar application of Zn oxide), 2gl⁻¹ Zn oxide, and 1.5gl⁻¹ Zn oxide in nano form, as the third factor.

Five seeds in each pot, were planted. After germination, the plants were thinned to two plants. With the same amount of water, pots were irrigated every four days. To apply mycorrhizal in pot, 70 spores of *Funneliformis mosseae* were placed in the soil of the pot for each seed. Foliar application of two elements, Fe and Zn oxide, was done at the beginning of the stem elongation and the beginning of flowering stages. To measure antioxidant enzyme activities, physiological traits and root growth, at the milking the seeds stage, root of plants was removed from the soil and washed and root traits and aerial parts were measured. Data collected at the milking the seeds stage, included the shoot dry weight, root volume, root length and root dry weight.

2.2. Determination of antioxidant enzyme activities and ion concentration in the leaves

The concentrations of Zn and Fe in the in leaves were measured by using atomic absorption (Shimadzu AA-6300 Double Beam Atomic Absorption). To measured antioxidant enzyme activities in the leaves, superoxide

dismutase (SOD) activity measured according to the Dhindsa *et al.* (1981), CAT according to Taranishi *et al.* (1974), APX according to Nakano & Asada (1981) and GPX was determined as described by Urbanek *et al.* (1991).

2.3. Statistical analyses

All data were analyzed with SAS (9.1). Statistical analyses were carried out using ANOVA ($P=0.05$). The significant differences between individual means, were determined by using the *Least Significant Difference (LSD)*.

3. Results

3.1. Root Traits

Analysis of data showed that mycorrhiza and foliar application of Fe and Zn had significant effects on shoot dry weight, root length, root volume, and root dry weight (Table 1). The results showed that using mycorrhiza increased these traits in sorghum, so the highest dry weight of the shoot (243.7 g/plant), root length (49.1 cm), root volume (122.7 cm³), and root dry weight (40.6 g/plant) were related to the conditions of using mycorrhiza. This increase for the shoot and root dry weight was 9.6%. For root length and volume was 12.6% (Table 2).

The results showed that the application of Fe and Zn oxides in both normal and nano forms increased root traits. But the effects of Fe and Zn oxides in normal form were greater than Fe and Zn oxides in nano form. The highest shoot dry weight (253.6 and 254.1 g), root length (49.9 and 48.5 cm), root volume (124.8 and 121.2 cm³) and root dry weight (42.2 and 42.3g) were related to 2 g l⁻¹ Fe and Zn oxide respectively. In this study, the amount of increase in the root and shoot dry weight (8.3, the length and volume of roots were 8.3 and 6.1% during the consumption of Fe oxide in normal form compared to nano Fe oxide. This increase for Zn oxide compared to nano Zn oxide was equal to 10.1 and 1.2% (Table 2).

3.2. Antioxidant enzyme activities in leaf tissues

Interaction between mycorrhiza, Fe and Zn oxide treatments had a significant effect on the CAT, APX, GPX and SOD in leaves tissues (Table 1). The results showed that antioxidant enzymes activity in the condition of using mycorrhiza was higher than in the case of not using mycorrhiza. About of three enzymes, CAT, APX and GPX, there was no difference between the foliar application of Fe and Zn oxides in nano form and Fe and Zn oxides in normal form. The activity of these three enzymes was the same and they were statistically at the same level. But about of the SOD, application of Zn and Fe oxide in the normal form caused a greater increase in the activity of this enzyme compared to their nano form. The increase in the activity of SOD at Fe and Zn oxide in normal form compared to its in nano form was equal to 25.7% (Table 3).

3.3. Concentration of Zinc and Iron in leaf

The analysis of variance showed that, mycorrhiza, Fe and Zn oxide treatments had significant effect on the Fe in leaves tissues. About the concentration of Zn in leaves, data showed that only mycorrhiza and Zn treatments had a significant effect on that (Table 1). The results of mean square of mycorrhizal on concentration of Fe and Zn showed that the highest concentration of Fe (44.7) and Zn (48.3 mg/kg of dry weight) in leaves was related to the treatment using mycorrhizal. The increase in Fe concentration compared to the condition of not using mycorrhiza was 27.9%. This increase for Zn, was 24.1% (Table 2).

The results showed that the concentration of Fe and Zn increased more during the application of Fe and Zn oxide in normal form than nano form. Therefore, the highest Zn and Fe in leaves was obtained during foliar application of Zn and Fe oxide in normal form. The increase in the concentration of Fe and Zn during foliar application of Fe oxide (13.1 and 6.7%) and Zn oxide (9.6 and 12.1%) compared to the nano form.

Table 1. Analysis of variance (ANOVA) of mycorrhiza, Fe and Zn oxide in normal and nano form on root traits, antioxidant enzyme activities in leaves and Concentration nutrient element in leaves in sorghum

Source of Variation (S.O.V)	df	Concentration nutrient element in leaves		Antioxidant enzyme activities in leaves				Root traits			
		Fe	Zn	SOD	CAT	APX	GPX	Root dry weight	Root length	Root volume	Shoot dry weight
Block	2	0.12 ^{ns}	0.02 ^{ns}	60.5 ^{ns}	235.1 ^{ns}	2673.4 ^{ns}	143.1 ^{ns}	5.7 ^{ns}	0.06 ^{ns}	43.6 ^{ns}	143.4 ^{ns}
Mycorrhiza (M)	1	1.71 ^{**}	0.56 ^{**}	2455.7 ^{**}	5611.1 ^{**}	87900.2 ^{**}	3888.1 ^{**}	16.1 ^{**}	32.6 ^{**}	2345.6 ^{**}	2314.7 ^{**}
Iron oxide (Fe)	2	3.44 ^{**}	0.89 ^{ns}	2134.3 ^{**}	2333.7 ^{**}	78999.4 ^{**}	3188.2 ^{**}	18.2 ^{**}	34.5 ^{**}	2653.5 ^{**}	2543.8 ^{**}
Zinc oxide (Zn)	2	4.39 ^{**}	2.77 ^{**}	1122.2 ^{**}	2567.8 ^{**}	65688.5 ^{**}	1909.6 ^{**}	44.9 ^{**}	56.1 ^{**}	6543.4 ^{**}	4365.6 ^{**}
Fe*M	2	0.1 ^{ns}	0.002 ^{ns}	860.9 ^{**}	167.7 ^{ns}	4005.6 ^{ns}	1899.7 ^{**}	7.8 ^{ns}	5.8 ^{ns}	234.2 ^{ns}	234.6 ^{ns}
Zn*M	2	0.06 ^{ns}	0.006 ^{ns}	398.6 ^{**}	187.6 ^{ns}	4577.7 ^{ns}	1111.9 ^{**}	5.4 ^{ns}	3.6 ^{ns}	12.1 ^{ns}	182.5 ^{ns}
Fe*Zn	4	0.06 ^{ns}	0.006 ^{ns}	288.9 ^{**}	112.4 ^{ns}	16900.6 ^{**}	1187.9 ^{**}	3.8 ^{ns}	2.3 ^{ns}	6.1 ^{ns}	195.1 ^{ns}
Fe*Zn*M	4	0.01 ^{ns}	0.01 ^{ns}	220.1 ^{**}	990.1 ^{**}	8056.4 [*]	600.2 [*]	1.6 ^{ns}	0.76 ^{ns}	5.6 ^{ns}	5.62 ^{ns}
Error	19	0.02	0.01	40.1	123.5	2567.8	137.7	7.5	6.8	313.5	453.6
CV%		12.3	7.8	11.7	7.8	11.4	4.7	13.4	21.7	14.6	13.9

* P<0.05 and ** P<0.01: Numbers represent F values at 5% level, ns: not significant

Table 2. Effect of different Fe and Zn oxide in normal and nano form with inoculation of mycorrhiza on root traits and concentration of zinc and iron concentration in leaves of sorghum.

Treatments	Root dry weight (g per plant)	Root length (cm)	Root volume (cm ⁻³)	Shoot dry weight (-g per plant)	Zn (mg kg ⁻¹ dry weight)	Fe (mg kg ⁻¹ dry weight)
Mycorrhiza						
No use of mycorrhiza	36.7b	107.3b	42.9b	220.3b	36.7b	32.2b
Use of mycorrhiza	40.6a	122.7a	49.1a	243.7a	48.3a	44.7a
Fe oxide						
0	24.9c	103.2c	41.3c	209.9c	33.1b	35.3c
2 g l ⁻¹ Fe oxide normal form	38.7b	117.2b	46.9b	232.5b	35.8b	38.6b
1.5 g l ⁻¹ Fe oxide nano form	42.2a	124.8a	49.9a	253.6a	41.2a	41.4a
Zn oxide						
0	53.6c	104.3b	4107b	214c	35.1c	33.1b
2g l ⁻¹ Zn oxide normal form	38b	119.7ab	47.9a	228.1b	39.4b	34.6b
1.5 g l ⁻¹ Zn oxide nano form	42.3a	121.2a	48.5a	254.1a	43.6a	38.8a

The values represent means of three replications and the means with different letters. Letter is statistically significant at p<0.05

Table 3. Effect of different Fe and Zn oxide in normal and nano form with inoculation of mycorrhiza on antioxidant enzyme activities in leaves of sorghum.

Treatments	CAT	Antioxidant enzyme activities					
		APX	GPX	SOD			
(unit/mg protein. Min)							
No use of mycorrhiza	Fe oxide =0	Zn oxide= 0	0.38f	0.25c	0.33e	21.2f	
		Zn oxide nano= 1.5	0.39f	0.26c	0.34e	22.3ef	
		Zn oxide =2	0.42d	0.27c	0.34e	23.6def	
	Fe oxide nano=1.5	Zn oxide= 0	0.41de	0.28bc	0.35e	25.2cde	
		Zn oxide nano= 1.5	0.42de	0.27c	0.42d	25.3cde	
		Zn oxide =2	0.43cd	0.28bc	0.42d	26.3bcd	
	Fe oxide=2	Zn oxide= 0	0.41def	0.26c	0.34e	24.2de	
		Zn oxide nano= 1.5	0.52b	0.27c	0.44d	25.2cde	
		Zn oxide =2	0.52b	0.27c	0.44d	26.2bcd	
	Use of mycorrhiza	Fe oxide =0	Zn oxide= 0	0.41def	0.27c	0.45cd	25.4cd
			Zn oxide nano= 1.5	0.52b	0.27c	0.45cd	25.4cd
			Zn oxide =2	0.54a	0.28bc	0.46bcd	26.4bcd
Fe oxide nano=1.5		Zn oxide= 0	0.45c	0.31b	0.48b	28.3b	
		Zn oxide nano= 1.5	0.57a	0.39a	0.52a	28.3b	
		Zn oxide =2	0.58a	0.41a	0.52a	38.1a	
Fe oxide=2		Zn oxide= 0	0.50b	0.31b	0.47bc	27.1bc	
		Zn oxide nano= 1.5	0.56a	0.39a	0.52a	29.3b	
		Zn oxide =2	0.59a	0.42a	0.52a	29.5a	

The values represent means of three replications and the means with different letters/ letter is statistically significant at $p < 0.05$

4. Discussion

To completing their life cycle and carry out physiological and biochemical function, plants need various micro and macronutrients. Among micronutrients, Zn and Fe is considered to be the most required nutrients in plant metabolism (Samreen *et al.*, 2017). Although Zn and Fe plays an important role in the physiological processes in plants, it is possible to use as nano form. Plants can rapidly uptake nano-fertilizers. Nano- fertilizers can save fertilizer consumption and minimize environmental pollution (Derosa *et al.*, 2010). In this study, the results showed that although the use of Fe and Zn oxide in normal and nano forms increased the growth, length and volume of roots in sorghum variety Sepideh, but the effect of both Fe and Zn oxide in the normal form was better than its nano form in root growth (Table 2). According to the results of this study, the researchers reported that the use of nano-fertilizers such as nano-Fe can improve the growth of plants. But in this study, Fe and Zn oxide in nono form had less effect compared to their normal form on root growth (Table 2). Moghadam *et al.* (2012) studied the effect of Fe nano-fertilizer on spinach and reported that application of 4 kg ha⁻¹ Fe nano-fertilizer increased leaf area index by 47% and leaf weight 58% compared to control.

Mycorrhiza also had a positive effect on root growth in sorghum and this effect was greater during the foliar application of Fe and Zn oxide. If arbuscular mycorrhiza can be successfully established, the fungal mutualist will provide nutrients such as nitrogen and phosphorous and water to the plant (Bitterlich *et al.*, 2018; Jayne and Quigley, 2014). Mycorrhiza caused more absorption and increased concentration of Zn and Fe elements and also increased the activity of antioxidant enzymes in leaves of sorghum. In a study of three enzymes, SOD, CAT and POD in the leaves of the wheat plant, conducted by Al-Masoudi and Al-Semmak (2021), it was shown that the addition of nano-Zn at a concentration (0, 1, and 2 g l⁻¹) led to a significant increase in the enzyme SOD and CAT at 2 g l⁻¹, while it led to a significant decrease in the enzyme POD compared to the control on a plant. The results in this study about activity of CAT, APX and GPX, showed that, application of Fe and Zn oxide in both normal and nano forms increased the activity of them in sorghum, and this increase was largely the same for the nano and normal form of Fe and Zn oxide and there was no difference between the foliar application of Fe and Zn oxides in nano form and Fe and Zn oxides in normal form (Table 3). Heidari *et al.* (2018) reported that the maximum grain yield, GPX and APX enzymes activity in leaves of maize were obtained at foliar application of nano Fe chelate. The positive effect of spraying Fe nano-fertilizer on basil plants was also observed in the experiments of Peyvandi *et al.* (2011). They indicated that Fe in nano form increased stem length, root length, chlorophyll content and shoot dry weight compared with the common Fe fertilizers.

5. Conclusion

Fe and Zn play an important role in the physiological and biochemical processes in plants, and their deficiency occurs in most soils with a pH higher than 7. Different combinations of these two elements are recommended for plants. Form oxide is one of those examples. Today, nano forms of Fe and Zn oxide have been prepared and provided to farmers. The results showed that foliar application of Fe and Zn oxides in normal form had a greater effect on the root growth and root characteristics in sorghum than nano form. In the meantime, the use of mycorrhizal improved root growth and caused better absorption of Zn and Fe in the soil and increased the activity of antioxidant enzymes in leaves of sorghum. In general, the results showed that the foliar application of Fe and Zn oxides in the normal form had a greater and better effect than nano Fe and Zn oxides in sorghum.

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