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Effects of iron sources and doses on plant growth criteria in soybean seedlings

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Abstract

In this study, effects of different iron sources and doses on plant growth criteria in soybean (*Glycine max* L.) seedlings were investigated. The experiment was conducted according to factorial experimental design with three replications under controlled conditions. Atakişi variety of soybean (*Glycine max* L.) cultivar was used as a plant material. Three soybean seeds were sown each plastic pot having 1.3 kg soil:sand mixed in 1:1 ratio. Three different Fe sources ($FeSO_4.7H_2O$, Fe-EDDHA and nanoFe) were applied to the pots with three different doses (0-15-30 mg Fe kg^{-1}). The experiment was ended after five weeks of seed sowing. Shoot length, shoot fresh and dry weights, root length, root fresh and dry weights and number of compound leaf in soybean seedlings were determined at the end of the experiment. The highest shoot fresh and dry weights, root fresh and dry weights, compound leaf number were determined in 15 mg kg^{-1} nano Fe applications as 3.56 g, 0.83 g, 2.30 g, 0.33 g and 5, respectively. Increasing the application dose of nano-Fe from 15 to 30 mg kg^{-1} caused to decrease in fresh and dry weights in soybean seedlings. Generally, shoot growth decreased and root length increased in soybean seedlings by increasing Fe application doses. Seedling growth in soybean generally increased depend on the Fe sources in the following order; $FeSO_4.7H_2O$ < Fe-EDDHA < nano-Fe.

Keywords: Soybean, seedling growth, nano Fe, Fe-EDDHA, $FeSO_4.7H_2O$.

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Introduction

Iron is an essential nutrient for all organisms (Zuo and Zhang, 2011). In plants, Fe is also one of the essential micro nutrient and participates in many physiological processes including RNA synthesis, chlorophyll biosynthesis, several enzyme activitions, respiration and redox reactions (Malakouti and Tehrani, 2005; Mimmo et al., 2014; Ye et al., 2015; Zargar et al., 2015). Although, micronutrient elements are needed in rather very small amounts for satisfactory plant growth and production, their deficiency may cause disorder in physiological and metabolic processes involved in the plant.

Iron deficiency is a wide spread agricultural problem in many crops especially in calcareous soils and semi-arid climates. Deficiencies are usually diagnosed by chlorosis in young leaves and are typically found among sensitive crops grown in calcareous soils covering over 30% of the world's surface soil. Excessive iron deficiency may lead to complete crop failure (Lindsay and Schwab, 1982; Chen and Barak, 1982).

Çakmak (2002) reported that even on the world scale, Fe deficiency is wide spread occurring in about 30-50% of cultivated soils. Iron content in soil is generally high, but a large proportion is fixed to soil particles (Mimmo et al., 2014; Bindraban et al., 2015) especially in aerobic soils and high pH levels. Fe is mainly in the insoluble form as ferric iron (Fe^{+3}). Mortvedt et al. (1991) reported that in calcareous soils less than 10 % of the Fe is available to plants. Therefore, these soils are usually deficient in the available form, ferrous iron (Fe^{+2}). (Ye et al., 2015). Because plants usually uptake Fe^{+2} from soil. When the soil-plant-animal-human food

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chain considered, Fe deficiency does not only affect the plant growth and development but also lead to anemia in animals and humans (Li et al., 2014).

The application of Fe fertilizers is still the most effective way to improve Fe efficiency in plants. Inorganic Fe fertilizers chelated Fe fertilizer and organic Fe fertilizer are the common varieties of Fe fertilizers (Laurie et al., 1991). Iron ferrous sulphate ($FeSO_{4}.7H_2O$) and iron chelate products are generally used as inorganic fertilizer (Tisdale et al., 1993). Synthetic chelate iron (Fe-EDDHA) products are mostly applied to prevent or to remove Fe chlorosis in crops grown on calcareous soils. These products consist of a mixture of EDDHA components chelated to Fe.

Recently, the use of nanomaterials in agricultural production has increased (Gogos et al., 2012; Bindraban et al., 2015). Bindraban et al. (2015) and Montalvo et al. (2015) reported that nanomaterials have potential applications as crop fertilizers because of their physical and chemical attributes. The releasing of elements from fertilizers can be controlled and delayed with using of nanoparticles and nano powders. Nano materials consist of nano meter-scale particles with a very small diameter and large specific area (Hochella et al., 2008). Brunner et al. (2006) reported that nano-fertilizers are syhthesized or modified form of traditional fertilizers, fertilizers bulk materials or extracted from different vegetative or reproductive parts of the plant by different chemical, physical, mechanical or biological methods with the help of nanotechnology used to improve soil fertility. Nanoparticles can be made from fully bulk materials.

The plants sensitive to iron deficiency are apple, avocado, banana, bean, barley, cotton, citrus, grape, oats, peanut, potatoes, pecan, soybean, sorghum and various greenhouse flowers (Chen and Barak, 1982). These chelates increase Fe solubility and function as a transporter through solution to plant (Lucena et al., 1992). Fe-EDDHA (iron ethylenediamine hidroksi pheny acetic acid) is along the most effective synthetic Fe chelates under neutral and alkaline soil conditions (Lucena et al., 1992). Legumes and cereals are two most important foods to humans (Graham and Vance, 2003). Legumes have been used both for their medicinal, cultural as well as nutritional properties providing an important source of protein and oil which can also be converted into biodiesel (Libault et al., 2010). Soybean (*Glycine max* L.) is the highest produced legume crop. According to Food and Agriculture Organization statistic for 2009, about 230 million tons of soybean were produced over the world, ranking the on the world's top commodity production. The objective of this study was to investigate effects of different iron forms on growth criteria in soybean seedlings.

Material and Methods

The experiment was conducted according to factorial experimental design with three replications under controlled conditions in a growth chamber at 25±1ºC and irrigated with distilled water. Atakişi soybean variety was used as a plant material and three soybean seed was sown to each plastic pot having 1.3 kg soil:sand mixed in 1:1 ratio. Three different doses (0, 15, 30 mg kg^{-1}) of $FeSO_{4}.7H_2O$, FeEDDHA and nanoFe were used in the study. The experiment was ended after five weeks after sowing the seeds. Shoot length, shoot fresh and dry weights, root length, root fresh and dry weights and number of compound leaves were determined at the end of five weeks.

Some properties of the growth media were determined using the standard analysis methods (Kacar, 2010). According to the physical and chemical properties of the growth media (Table 1), it had loamy texture class, non-saline, slightly alkaline, low in organic matter, unsufficient in phosphorus and zinc contents, sufficient in calcium, magnesium, manganese and copper contents. Variance analysis of the experimental data was done by SPSS statistical program (SPSS, 2018).

Table 1. Some properties of the growth media used in this study.

Texture	pH	Salinity	Lime	OM	P	K	Ca	Mg	Fe	Mn	Zn	Cu
		dS m^{-1}	%		mg kg^{-1}							
Loamy	7.81	0.361	3.86	1.32	5.50	298	3034	405	5.58	29.84	0.58	0.81

Results and Discussion

The variance analysis of the results and the effects of different iron treatments on seedling growth criteria are given in Table 2 and Table 3, respectively.

Table 2.Variance analysis of the data for plant growth criteria in soybean seedling (F values).

Variation Sources	df	Shoot length	Shoot fresh weight	Shoot dry weight	Root length	Root fresh weight	Root dry weight	Comp. leaf number
Fe source (A)	2	5.43*	2.34	7.85**	0.10ns	5.02*	0.78	1.63ns
Fe dose (B)	2	22.14**	1.39	5.702*	11.55**	11.91**	8.94**	6.13**
AxB	4	2.68ns	3.90*	11.72**	1.49ns	16.21**	8.96**	2.38ns

*significant at 0.05, **significant at 0.01, ns not significant

According to the variance analysis, different iron sources significantly influenced shoot length, shoot dry weight and root fresh weight of soybean seedlings. Similarly Fe application doses had significant effect on all of the plant growth criteria of soybean seedlings, except shoot fresh weight. The effects of interaction between source and dose on shoot length, shoot fresh and dry weights, root fresh and dry weights were found to be significant statistically (Table 2).

According to the Fe application sources, the highest mean values for shoot length, shoot fresh and dry weights and root fresh and dry weights were obtained as 55.16 cm, 2.60 g, 0.54 g, 1.45 g and 0.22 g in nano-Fe application, respectively (Table 3). Increasing iron doses generally caused decreases in the mean values of plant growth criteria, except root length and compound of leaf number. Chakralhoseini et al. (2002) reported that iron at low concentration of 2.5 mg kg-1 in soil increased dry matter weight of soybean, but higher doses of iron decreased soybean growth. In a field experiment, Sheykhbaglou et al. (2010) determined that foliar application of nano-iron oxide at the concentration of 0.75 g L^{-1} was increased leaf + pod dry weight and pod dry weight over the control, but increasing the application dose from 0.75 to 1 g L^{-1} caused reduction in leaf + pod dry weight. They also reported that the highest yield was observed at 0.50 g L^{-1} application rate having 48% increase in grain yield compared with control. In this study, application of 15 mg kg^{-1} nano-Fe increased fresh and dry weights of shoot and root parts in the soybean seedlings over the control, but the highest dose (15 mg kg^{-1}) of nano-Fe application reduced the fresh and dry weights of the plants (Figures 1 and 2).

While the lowest mean values for shoot length (44.02 cm), shoot fresh (2.12 g) and dry (0.38 g) weights, root fresh (0.97 g) and dry (0.16 g) weights were found in 30 mg kg^{-1} Fe application dose, the highest mean values for root length (25.36 cm) was obtained in the highest Fe application dose (Table 3). According to the interactions between source and Fe dose, the highest values of shoot fresh (3.56 g) and dry (0.83 g) weights, root fresh (2.30 g) and dry (0.33 g) weights were determined in 15 mg kg^{-1} dose of nano-Fe application (Figure 1 and 2). Rui et al. (2016) found that application of iron oxide nanoparticles (Fe_2O_3NPs) as a fertilizer increased root length, plant height and biomass of peanut plants over the control and EDTA-Fe treatments. They reported that the root dry biomass did not display the statistical difference between Fe_2O_3NPs and EDTA-Fe treatments, and also no impact on biomass increases was found in the shoot dry weight. Alidoust and Isoda (2013) found that root length of soybean seedlings increased by 6, 8, 19, 27, 37, and 40% at 50, 100, 250, 500, 1000, and 2000 mg L^{-1} of nanoparticle Fe (IONPs) applications, respectively as compared to the controls. In another study, Liu (2016) determined that Fe nanoparticles (FeOxNPs) significantly increased root length of lettuce seedlings by 12–26 % as a Fe fertilizer at low application rates (5–20 ppm). Similarly in this study, at 15 and 30 mg kg^{-1} application doses of Fe, the mean values of shoot length decreased 25.54% and 27.28%, and the mean values of root length increased 24.64% and 31.53% over the control treatment, respectively.

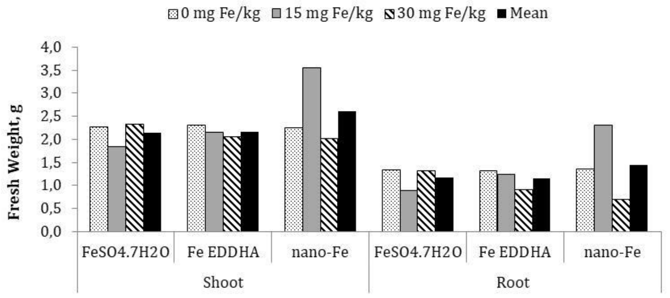

Figure 1. The effects of iron sources and doses on fresh weight in soybean seedlings

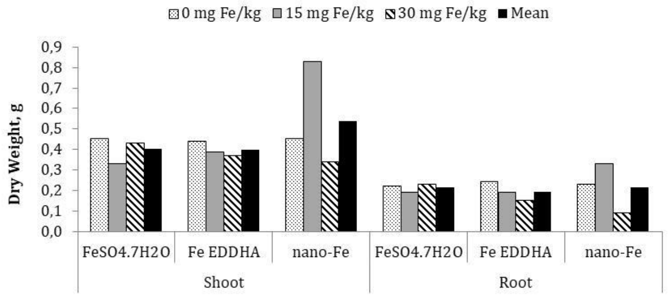

Figure 2. The effects of iron sources and doses on dry weight in soybean seedlings

In this study although the highest mean values of plant growth criteria were obtained in nano-Fe applications compare to the other Fe sources, 30 mg kg^{-1} Fe dose generally decreased plant growth. According to the results, it can be concluded that Fe application dose more than 15 mg kg^{-1} adversely affected seedling growth criteria in soybean.

According to the Fe sources, the highest mean values of plant growth criteria were generally found in 15 mg kg^{-1} iron doses of Fe-EDDHA and nano-Fe while the lowest means of these parameters were obtained in 15 mg kg^{-1} dose of $FeSO_4.7H_2O$. It was reported that soluble inorganic Fe fertilizer has little ameliorative effect to improve the available Fe content in alkaline calcareous soils (Cesco et al., 2000; Lucena et al., 2010). In this study, the mean values of plant growth criteria obtained in different iron sources showed the following decreasing trend: nano-Fe>Fe-EDDHA> $FeSO_4.7H_2O$.

Table 3. Effects of different iron sources and doses on growth criteria in soybean seedlings.

Plant Growth Criteria	Fe doses (mg kg^{-1})	Fe sources			Mean
		$FeSO_4.7H_2O$	Fe-EDDHA	Nano-Fe	
Shoot length (cm)	0	60.55 a*	60.58 a	60.46 a	60.53 A**
	15	42.27 cd	44.20 cd	48.75 bc	45.07 B
	30	39.10 cd	36.70 d	56.27 ab	44.02 B
Mean		47.31 B*	47.16 B	55.16 A	
Shoot fresh weight (g)	0	2.26 b*	2.29 b	2.24 b	2.27
	15	1.84 b	2.15 b	3.56 a	2.52
	30	2.32 b	2.04 b	2.00 b	2.12
Mean		2.14	2.16	2.60	
Shoot dry weight (g)	0	0.45 b**	0.44 b	0.45 b	0.45 AB*
	15	0.33 b	0.39 b	0.83 a	0.52 A
	30	0.43 b	0.37 b	0.34 b	0.38 B
Mean		0.40 B**	0.40 B	0.54 A	
Root length (cm)	0	19.25	19.32	19.28	19.28 B*
	15	22.63	23.77	25.70	24.03 A
	30	27.80	25.29	23.00	25.36 A
Mean		23.23	22.79	22.66	
Root fresh weight (g)	0	1.33 bc**	1.31 bc	1.34 b	1.33 A**
	15	0.89 de	1.24 bcd	2.30 a	1.48 A
	30	1.31 bcd	0.90 cde	0.69 e	0.97 B
Mean		1.18 B*	1.14 B	1.45 A	
Root dry weight (g)	0	0.22 bc**	0.24 b	0.23 bc	0.23 A**
	15	0.19 bc	0.19 bc	0.33 a	0.24 A
	30	0.23 b	0.15 cd	0.09 d	0.16 B
Mean		0.21	0.19	0.22	
Compound leaf number	0	4.00	4.00	4.00	4.00 B**
	15	4.67	5.00	5.00	4.89 A
	30	5.00	5.00	3.67	4.56 A
Mean		4.5 6	4.67	4.22	

*significant at 0.05 level, ** significant at 0.01 level.

Our results were corresponding with the results of similar researches. Mortvedt et al. (1972) reported that FeEDDHA application was more effective than ferrous sulphate at the same rate iron. Hassani et al. (2015) applied Fe fertilizer with chemical and nano form as their combination and separately. They determined that nano Fe had more effect on plant growth criteria than the chemical fertilizer in mint plant. Rui et al. (2016) determined that iron oxide nanoparticles increased root length, plant height, biomass values compare to chelated Fe application in the peanut plant. They also reported that iron oxide nanoparticles can replace traditional Fe-fertilizers. Similiarly, Sheykhbaglou et al. (2010), Nadi et al. (2013) and Singh et al. (2017) reported that nano Fe particles have beneficial effects on plant growth criteria and grain yield in soybean and fababean, respectively.

Previous researches showed that nano fertilizers lead an increase in the use efficiency of plant nutrients, reduce soil toxicity, decrease the potential adverse effects of excessive chemical fertilizer use and fertilizer application frequency (Brunner et al., 2006; Liu et al. 2016; Khadka et al., 2017). Nano fertilizers have large surface area and particle size less than the pore size of root and leaves of the plant which can increase penetration into the plant from applied surface and improve uptake and nutrient use efficiency of the nano fertilizer (Liscano et al., 2000). They can be applied frequently in small amounts and are environmentally friendly compare to traditional chemical fertilizers (Liu and Lal, 2016). On the other hand, Rameshaiah et al. (2015) reported that excessive doses of nano materials used in agricultural lands may be cause hazardous effects on living organisms and human health. The selection and cultivation of iron toxicity tolerant genotype may be another suitable option for its toxicity management (Khadka et al., 2017).

Conclusion

The different iron sources and application doses significantly influenced plant growth criteria in soybean seedlings. The highest shoot fresh and dry weights, root fresh and dry weights, compound leaf number were determined in 15 mg kg^{-1} nano Fe applications compared with the $FeSO_4.7H_2O$ and Fe-EDDHA treatments. The dose of 30 mg kg^{-1} nano-Fe application decreased fresh and dry weights in soybean seedlings, but increased the root length. Increasing the dose of Fe application from the different Fe sources generally decreased shoot growth and increased root length. Effect of nano-Fe on the seedling growth was more effective than Fe-EDDHa and $FeSO_4.7H_2O$. As a result, it can be suggested that using of nano-Fe fertilizer will be beneficial on soybean seedling growth with an application dose lower than 30 mg kg^{-1}.

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