ARTICLE

EVALUATION OF IRON AND ZINC FOLIAR AND SOIL APPLICATION ON QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF TWO SOYBEAN CULTIVARS

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ABSTRACT

In order to investigate the effects of foliar and soil application of iron and zinc on quantitative and qualitative yield of two soybean cultivars, a field experiment was laid out in factorial arrangement based on completely randomized block design with three replications at Rice Research Institute of Iran (Rasht) in 2014. Treatments include iron foliar application, iron soil application, zinc foliar application, zinc soil application, iron and zinc foliar application, zinc soil application + iron foliar application, iron soil application + zinc foliar application, iron and zinc soil application, iron and zinc foliar and soil application and control was used. Result of showed that iron foliar application + zinc soil application increased stem height and biological yield. Highest rate of seed number per plant,, seed yield, harvest index and seed protein content was observed in zinc soil application treatment (152.4, 16.53 g, 8285.88 kg ha^{-1}, 43.01% and 65.25% respectively). Also results showed that oil and iron content of seed was affected by iron soil application and zinc soil + foliar application. The results of this experiments showed that the consumption of iron and zinc can be improved physiological characteristics, yield and grain quality of soybean.

KEY WORDS
Fe Secostrain, Microelement, Oil, Seed protein, Zinc sulfate.

INTRODUCTION

Soybean (*Glycine max*) is a common legume plant and cultivated for more than 3000 years in Southeastern Asia. Soybean plants first in the world as edible oil and occupies important place in the economy. Climatic and edaphic factors severely affect its production; It has been also well reported that deficiency of micronutrients such as Fe, Mn and Zn affect the soybean production [1]. Furthermore, various researcher reported that the application of essential micronutrients such as zinc, iron and magnesium improve the yield and yield components of crops [2, 3]. Plant sufficient nutrition have an important role in raising level of plants tolerance against a variety of environmental stresses and in this regard, iron and zinc are the most important essential micronutrients in plant nutrition [4]. Micronutrients also play key roles in the release of carbon dioxide, and in optimizing the function of vitamin A and the immune system [5]. Zinc plays an important role as a metal component of enzymes (alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase) or as a functional, structural, or regulator cofactor of a large number of enzymes [6]. Experimental result of [7] also showed that foliar application of micronutrients increased the soybean yield, quality, resistance to pests and diseases and drought stress. They reported that although the need of plants to micronutrients is very little but these nutrients play an important role in growth and development of plants. So that the micronutrients such as iron, copper, boron, zinc and manganese have many contributions in cell wall formation and plant resistance to pests and diseases and environmental stresses.

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Normally fertilization carried out in soil but in this condition very less amount of nutrient reached to the plant system and remaining amount waste through leaching in soil, it also cause land and water pollution. Foliar fertilization is better option to avoid leaching and in this quick translocation of nutrients carried out in different parts of the plant system [8].

The aim of this study was to evaluate the effects of foliar and soil application of iron and zinc, individually and in combination on yield and yield components and grain quality traits of two soybean cultivars.

MATERIALS AND METHODS

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In order to investigate the effects of foliar and soil application of iron and zinc either individually or in combination on yield, yield components and quantitative traits of two soybean cultivars (Williams and Sahar), a field experiment was carry out at rice research institute of Iran, Guilan (37°, 16´ N, 51°, 3´ E and 7 m above sea level) in 2014. The experiment was carried out in factorial arrangement based on completely randomized block design with three replications. Treatments include iron foliar application, iron soil application, zinc foliar application, zinc soil application, iron and zinc foliar application, zinc soil application + iron foliar application, iron soil application + zinc foliar application, iron and zinc soil application, iron and zinc foliar and soil application and control were used.

Table 1: Physical and chemical soil properties

Soil texture	Fe (mg kg^{-1})	P (mg kg^{-1})	(%) N	K (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	Ec (ds m^{-1})
Clay loam	2.2	14	0.03	220	0.22	10.2	2.6

The experimental field received 100 kg ha^{-1} P_2O_5 (in the form of triple superphosphate) and K_2O (in the form of potassium sulfate) before planting. Nitrogen at rate of 100 kg ha^{-1} (in the form of urea) was applied in two stages: before planting and fourth interned stages. Seed were planted on 5 June 2014 in 50 cm row space, 5 cm plant space within row (density of 40 plant m^{-2}) in 3×5 m plots.

Different treatments of micronutrient soil application which were including: 20 kg ha^{-1} iron from secostrine and 30 kg ha^{-1} from zinc sulphate were distributed in plots and mixed with surface soil before seed sowing. Micronutrient foliar application was done at three times, one time when plants had two nodes, second time in 50% flowering and another in 50% podding stage. The iron and zinc were sprayed on plants with concentrate of 2 parts per thousand and 3 part per thousand, respectively. In control treatments plants were sprayed by water. Description of all treatments is shown in [Table 2].

Table 2: Different treatments of micronutrient application

F_1	Iron (foliar application)	2 parts per thousand
F_2	Iron (soil application)	20 kg ha^{-1} iron from secostrine
F_3	Zinc (foliar application)	3 parts per thousand
F_4	Zinc (soil application)	30 kg ha^{-1} zinc sulphate
F_5	Iron + Zinc (foliar application)	2 parts per thousand + 3 parts per thousand
F_6	Zinc (soil application)+ Iron (foliar application)	30 kg ha^{-1} zinc sulphate+2 parts per thousand
F_7	Iron (soil application)+ Zinc (foliar application)	20 kg ha^{-1} iron from secostrine+3 parts per thousand
F_8	Iron + Zinc (soil application)	20 kg ha^{-1} iron from secostrine + 30 kg ha^{-1} zinc sulphate
F_9	Iron + Zinc (soil and foliar application)	20 kg ha^{-1} iron from secostrine + 30 kg ha^{-1} zinc sulphate + 2 parts per thousand + 3 parts per thousand
F_{10}	Control	Water

At harvest, ten plants were taken at random from the central ridge to estimate: plant height, number of seed/plant, seed yield, biological yield and harvest index, straw and seed yields were determined from the three central ridges. Moreover, samples of soybean seeds were oven dried at 60-70°C for 48 hours ground to pass through a 0.5 mm sieve and sub samples of 0.2 g portions were wet digested using a mixture of sulphuric (H_2SO_4) and perchloric (HClO) 4acids. The digest was analyzed for N. Total nitrogen percentage (N %) in seeds was determined to the modified micro Kjeldahl method. Crude protein content in seeds was estimated by multiplying nitrogen percentage X 6.25.

Total oil content of soybean seeds was determined by using the soxhlet device; the pure seeds of each treatment were dried and weighted before insert into the device. The chloroform was used as solvent, it is a popular solvent seed oil extraction, particularly for lipids of intermediate polarity and when mixed with methanol it becomes a general extraction solvent. So the dried and powdered seed samples were inserted into the soxhlet device and the extraction was completed by evaporating the solvent.
After that, for determine of Fe and Zn content of seeds, the samples were washed by distilled water and dried in oven at 70°C during 48 h. Total Fe and Zn content were determined through atomic absorption method (Elmer Perkin 3030). Data analysis performed by using Statistical Analysis System (SAS) and the mean comparisons were evaluated based on Least Significant Differences (LSD).

RESULTS AND DISCUSSION

Seed Yield: The results showed that, the interaction effect of cultivar and treatment on the grain yield was significant [Data not shown]. Mean comparison showed that the maximum amount of seed yield was observed in Sahar × zn soil application treatment (F_4) (8774.8 kg ha^{-1}). There was no significant difference between F_4 and F_3. Minimum amount of seed yield was observed in Williams × Iron + zinc soil and foliar application (4107.1 kg ha^{-1}) [Table 3]. The interaction of various micro-nutrients (Fe, Zn) showed a positive and significant response to growth and yield parameters of soybean. Heitholt *et al* [9] reported that seed yield of soybean increased while Cu, Mn, Zn, and Fe applied individually.

Rose *et al* [10] reported that two time's foliar applications of zinc (40 kg ha^{-1}) increased soybean grain yield from 58 to 208 percent. Considering the results of this study 25 kg ha^{-1} Fe and 40 kg ha^{-1} Zn produced the highest seed yield and biomass. In contrast, Kobraee *et al* [11] also claimed that zinc and iron application at the same time could be lead to higher dry matter and seed yield as compared to using them separately.

Shiraiwa *et al* [12] declared that pod number and seed number are two important factors which affect soybean seed yield. Variation in dry matter production in seed filling period is a prime factor for different seed yield in soybean genotypes. In soybean, as a result of increasing the ratio of source-sink during flowering stage, seed yield was increased by the seed number enhancement [13].

One of the most important factors which are determiner in soybean seed yield is pod number per plant. The rate of pod set in soybean raised with an enhancement in source vigor at the time at which sink/source ratio was altered by flower thinning and defoliation. Furthermore, the ratio of dry matter enhancement in soybean seeds to its shoot is a crucial factor which affects the rate of podding in soybean [13].

In proceed to the previous studies [14] all methods of Zn application for plants significantly increased grain yield. Micronutrients increases photosynthesis rate and improves leaf area duration thus seed yield will be increased. Zinc plays important role in tryptophan biosynthesis, later is precursor of auxine also zinc is founded in phosphoenolpyruvate carboxylase structure. Another element that is iron is necessary to chlorophyll synthesis and its critical element in electron transport chain in photo systems. Iron deficiency leads to many disorders in chloroplasts. Ferredoxin is an important iron-containing protein involved in electron-transfer.

Biological Yield: The results showed that, the interaction of cultivar and fertilizer treatment on biological yield was significant [Data not shown]. Mean comparison showed that the maximum amount of biological yield was observed in

Sahar × zinc (soil application) + Iron (foliar application) (F_6) (25785.9 kg ha^{-1}). There was no significant difference between F_6 and F_4 (24880.7 kg ha^{-1}). Minimum amount of biological yield was observed in Williams× iron + zinc soil and foliar application (13674.3 kg ha^{-1}) [Table 3]. Such effects of micronutrients (Zn) application might be due to their critical role in crop growth, involving in photosynthesis processes, respiration and other biochemical and physiological activates and thus their importance in achieving higher yields [15]. Also, Cakmak [16] reported that zinc plays an important role in the biomass production. Zn and Fe applications separately have better effect than their combination. Decrease of biological yield in F_9 (Fe+ Zn. soil and foliar application) can be attributed to antagonistic effect among Fe and Zn in their combination.

Iron plays essential roles in the metabolism of chlorophylls. External application of Fe increased photosynthesis, net assimilation and relative growth in seawater-stressed rice [17]. This is especially true for soils of high pH where equilibrium conditions favour the oxida tion of plant-available Fe^{+2} to unavailable Fe^{+3}. Plant yield on many soils is, therefore, limited by poor Fe availability, rather than a low Fe content in the soil. Also Fe leaching is the main pathway for Fe loss in coarse-textured soil with high pH, while excessive Fe uptake was the main pathway for Fe loss in clay-textured and acid soil [4].

Table 3: The mean comparison of the interaction of cultivars and treatments on measured traits in experiment

Cultivar	treatments	Mean Squares				
		Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)	Plant height (cm)	Seed/plant
	F_1	6646.6 bcdef	14684.8 h	43.80 bc	143.90 b	18.96 gh
	F_2	5866.9 efg	14685.4 h	39.91 de	143.20 b	116.08 hij
	F_3	7519.4 abcdef	18351.3 d	42.07 cd	144.36 b	105.13 k
	F_4	8263.5 abc	17868.3 ef	46.46 ab	13740 bc	147.40 bc
	F_5	6637.3 bcdef	17200.8 fg	39.48 de	138.16 bc	108.73 jk
Williams	F_6	8074.9 abcd	17971.8 ef	47.88 a	168.73 a	133.30 def
	F_7	5459.5 fg	16181.6 g	33.86 f	140.30 bc	101.60 k
	F_8	8043.6 abcd	16997.9 fg	45.24 abc	131.43 cd	140.26 cde
	F_9	4107.1 g	19268.0 d	26.30 i	126.90 de	86.73 l
	F_{10}	6079.6 def	13674.3 h	41.59 cde	136.06 bcd	126.13 fg
	F_1	8774.8 a	25785.9 a	34.46 f	130.73 cd	131.13 ef
	F_2	7618.8 abcde	18386.9 de	41.66 cde	101.80 ij	141.50 cd
	F_3	6330.8 cdef	23492.9 b	26.95 hi	110.80 ghi	119.00 ghi
	F_4	8251.6 abc	21561.7 e	38.38 de	117.80 efgh	157.40 a

Sahar	F_5	6304.4 cdef	19352.0 d	32.23 fg	99.40 j	110.13 ij
	F_6	6640.9 bcdef	17505.1 ef	38.15 e	108.60 hi	146.76 bc
	F_7	5651.3 efg	18331.2 de	30.84 fg	119.80 efg	154.83 ab
	F_8	8639.3 ab	24880.7 a	34.26 f	115.93 fgh	148.93 abc
	F_9	6258.6 cdef	17320.8 ef	30.00 gh	126.90 de	152.86 ab
	F_{10}	6050.4 def	21405.7 c	39.32 ed	120.85 ef	126.29 fg

In each column, the means that have at least the same letters are not significantly different by L.S. Means test.

Harvest Index: Mean comparison showed that the highest harvest index was recorded in Williams × zinc soil application (F_4) (47.88%). Lowest harvest index also recorded in Williams× iron + zinc soil and foliar application (26.3%) [Table 3]. Bameri *et al* [18] demonstrated that foliar application with micronutrient either separately or in mixture significantly increased harvest index.

Whereas, F_4 treatment produced maximum grain and biological yield, so increase harvest index in this treatment is absolutely. The increase in the studied characters due to micronutrients may be attributed to its influences in enhancing the photosynthesis process and translocation of photosynthetic products to the seed as a result of increase enzymatic activity and other biological activities.

Plant Height: Results showed that the highest plant height was recorded in Williams × zinc soil application + iron foliar application (F_6) (168.73 cm) and lowest plant height recorded in Sahar × iron + zinc soil and foliar application (99.40 cm) [Table 3]. Micronutrients have a structural role in chlorophyll. These elements can be easily sprayed on leaf, thus leaves chlorophyll concentration increased by micronutrient foliar application, which in turn, lead to an increase in plant height and yield. Also zinc, increased plant height via increasing internodes distances. Plant height was significantly affected by foliar application of Fe, Mn and Zn individually and combined [18].

Seed Number of Plant: Maximum amount of seed number per plant was observed in Sahar × zinc soil application (F_4) (157.4). There was no significant difference between F_4 and F_3, F_2, F_1. Minimum amount of seed number per plant was observed in Williams× iron + zinc soil and foliar application (86.73) [Table 3]. Maximum 100 grain weight was found in Williams × zn soil application treatment (F_4) (20.17 g). There was no significant difference between F_4 and F_3. Minimum 100-seed weight was found in Sahar× Iron + Zinc soil and foliar application (10.87 g) [Table 3].

Number of pods per plant in soybean was enhanced by zinc application. Zinc application enhanced soybean yield by influencing the number of seeds per plant and seed weight. Zeidan *et al* [15] also reported that yield components in lentil are enhanced by foliar application of micronutrients. Due to the enzymatic activity enhancement, microelements effectively increased photosynthesis and translocation of assimilates to the seed. Previous researches showed that highest pods per plant was produced by foliar spraying at flowering and poding stage and increase of number of pods per plant due to foliar application could be attributed to significant effect of microelements on reproductive organs, such as stamens and pollens. These authors revealed that since soybean is a self-pollinated crop, stamen activity enhances the number of flowers that can fertile well and as a result, larger number of pods per plant will be produced [19]. Probably zinc may increase the amount of carbon hydrates and led to increase rates of seed.

Seed Protein Content: The results of this study showed that there were significant interaction effects of cultivar and treatment on seed protein content [Data not shown]. The lowest seed protein content (34 %) was recorded in Sahar × iron + zinc soil and foliar application treatment (38.84%) while the highest seed protein content (67.83%) was noted in Williams × iron foliar application + zinc soil application (F_6) [Table 4].
Fe+Zn foliar application compared to the control treatment could increase the grain protein content about 16% [Table 4]. Baybordy and Mamedov [4] explained that Iron and zinc are two important elements in enzymes structure involved in amino acid biosynthesis and because amino acids are the base of protein synthesis, protein content increases in the case of using these micronutrients. The results obtained by Thalooth *et al* [20] showed that using of zinc sulfate increases grain protein content of mungbean. In addition, iron is involved in the metabolism of nitrogen and increases leaf area and has a direct impact on the process of protein production. So it can be expected that iron foliar application, increased the plant protein production.

Seed Oil Content: The highest oil percentage was obtained from Sahar× iron soil application (12.22%).The lowest oil percentage was obtained from Sahar× iron + zinc soil and foliar application (7.7%) [Table 4]. It seems that, soil application of micronutrients is more benefit to oil biosynthesis. Singh and Sinha [21] reported the decrease in oil concentration may be due to oxidation of some polyunsaturated fatty acids. In general, there was not significant different between micronutrient application method.

Seed Iron Content: Mean comparisons showed that the interaction between Sahar × iron soil application could be increasing the concentration of iron in the grain about 9% compared to the control treatment and

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32% compared to the iron foliar application treatment in Williams cultivar, that showed minimum seed iron content (72.51%) [Table 4]. Baybordy and Mamedov [4] by foliar application with iron in canola increased the amount of iron in grain that confirmed the results of this experiment.

The application of Fe through soil as well as foliar application caused a marked increase in the total content of Fe in the soybean plants. Whereas in foliar application method iron absorption is faster and easier that soil application the highest iron content was observed in these treatments. In soil application due to organic matters banding with chemical fertilizers that have high ability to absorb and hold nutrients, and positioning these substances near hairy roots, results in better availability to plant and thus causing in higher iron content. Studying previous researches show that best results achieved in using iron sulphate [22]. And may be the use of zinc sulphate causes better absorption in iron. Iron deficiency leads to chlorophyll degradation and chlorosis. It's reported that, iron is an essential element in protein synthesis and iron deficit decreases plant growth. Also, iron is involved electron transport in photosystems. Already decrease of chlorophyll content due to iron deficit was reported.The results of this study indicate that due to high humidity in the weather conditions of Gilan province, iron soil application can be more effective than Iron foliar application to increase the concentration of this element in grain. Because iron would be oxide in the air and the plants would not be able to absorb it.

Seed Zinc Content: The highest zinc content was obtained from Williams × zinc soil application (60.42 mg kg^{-1}), there was no significant difference between zinc soil application and zinc foliar application treatment (59.27 $mg.kg^{-1}$). The lowest zinc content was obtained from Sahar × iron soil application (33.54 mg kg^{-1}) [Table 4]. Grain micronutrients concentration depends on their uptake by root during the seed development stage and remobilization from plant tissues to grain through phloem. Amount of remobilization from this way has largely depended on each element moves in the phloem and zinc has good remobilization from leaves to the grain. Kazemi Poshtmasari *et al* [23] also increased concentration of this element in bean grain by using of zinc foliar application, which confirmed the results of this experiment. Zinc is essential element in enzymatic system such as superoxide dismutase enzyme. Zinc plays important role in auxine and protein synthesis and it is essential for seed setting. Abdili *et al* [24] observed that consumption of sulfate zinc in 40 kg per hectare increase grain yield of soybean by 19%, zinc content of grain by15%, the concentration of the plant by 46% and total uptake of Zn by grain 37% rather than control treatment.

Table 4: The mean comparison of the interaction of cultivars and treatments on measured traits in experiment

Cultivar	Treatments	Mean Squares			
		Protein content (%)	Oil content (%)	Fe content (mg kg^{-1})	Zn content (mg kg^{-1})
Williams	F_1	54.80 de	11.54 abc	87.49 j	36.76 j
	F_2	53.08 e	9.40 ef	88.44 hi	38.40 i
	F_3	54.43 de	11.34 bc	72.51 o	58.50 b
	F_4	65.92 a	10.58 d	90.26 gh	44.71 ef
	F_5	56.45 de	10.62 d	74.44 n	38.80 i
	F_6	46.52 f	9.78 e	79.31 l	41.40 h
	F_7	55.49 de	8.44 g	73.57 no	60.42 a
	F_8	54.93 de	11.06 cd	79.79 l	45.90 e
	F_9	67.83 a	8.45 g	93.60 de	53.32 c
	F_{10}	34.89 h	8.90 fg	94.35 d	44.17 fg
Sahar	F_1	47.99 f	10.62 d	92.19 ef	33.54 l
	F_2	62.23 abc	9.18 efg	106.22 a	35.50 jk
	F_3	38.84 gh	12.22 a	98.27 c	53.51 c
	F_4	43.16 fg	7.72 h	77.46 m	34.43 k
	F_5	54.03 e	11.84 ab	90.23 gh	47.61 d
	F_6	59.81 bcd	9.36 ef	89.38 hi	34.73 kl

F_7	63.33 abc	8.98 fg	101.15 b	42.77 gh
F_8	58.52 cde	9.41 ef	91.34 fg	39.68 i
F_9	62.67 abc	7.70 h	83.42 k	59.27 ab
F_{10}	44.21 fg	8.82 fg	97.66 c	34.25 k

In each column, the means that have at least the same letters are not significantly different by L.S. Means test.

CONCLUSION

Uses of micro nutritious elements especially zinc and iron alone or combination had positive effect on yield and yield components. Iron and zinc uptake are controlled by the two major factors, availability of these elements in the soil and the ability of plants to acquire them. Application methods of micronutrients are very important to attain the best absorption. Sometimes response of the plants is different to application methods of fertilizers, for example in calcareous soil Fe and Zn are not available for plants, in this times, foliar application is a useful method for nourish of the plants. The results of this study demonstrated that, Fe and Zn had positive effect on yield and quality of soybean oil, protein, zinc and content. Overall, when plants like soybean are not supplied with an optimum amount of Fe and Mo due to environmental limitation, growth inhibition and physiological changes will be appear more quickly, depending on the strength and duration of the imposed stress. Negative response to two combination (Fe+Zn) foliar application on quantitative and qualitative characteristics of soybean may be attributed to micronutrient uptake problems and antagonistic effect among Fe and Zn in their combination.

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
None

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