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Effect of foliar application of heavy metals (iron and zinc) on growth, morphological characteristics, yield and quality of flax plant (*Linum usitatissimum* L.)

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Abstract
A field experiment was conducted at Gardarasha Agricultural Farm, College of Agriculture, with global positional system (GPS) reading of 360 0N, 440 01E (03411359 , 03997002 UTM) during growing season 2012-2013 to study the effect of four concentrations of iron (0, 50, 100 and 150 mg Fe L^{-1}) and three levels of zinc (0, 75 and 150 mg Zn L^{-1}) and their interactions on growth, morphological characteristics, yield and quality of flax plant using randomized complete block design with three replicates. The results indicated that the foliar application of heavy metals (Fe and Zn) to a certain level affected significantly at level of significant 1% on oil%, fatty acids content (Palmitic acid, Stearic acid, Oleic acid, Linoleic acid and Linolenic acid, protein content), yield, growth and morphological characteristics of flax plants. On the other hand, the foliar application of high concentration of heavy metals (150 mg L^{-1}) affected negatively on the mentioned characteristics due to their toxic or pollutant effect.

1. Introduction

Flax is an important economic crop which plays an important role in regional policy through its local fabrication as well as exportation [1]. It is one of the most important medicinal plants; therefore, human consumption of the flax is increasing rapidly for food and industrial benefit [2]. Flax is a multipurpose crop that can be harvested for seed to produce linseed oil and for the fiber [3]. Other products substitute for linseed oil in paints. However, the health benefits of flaxseed consumption have helped strengthen markets for the crop. Flaxseed and flaxseed oil are rich in alpha–linolenic acid (ALA), an essential fatty acid that appears to be beneficial for heart disease, inflammatory bowel disease, arthritis and a variety of other health problems. ALA belongs to a group of substances called omega-3 fatty acids that help reduce inflammation [3]. Flax is rich in oil (41%), protein (20%), fatty acids (omeg-3 fatty acid and linoliec acid, which is an omega-6 fatty acid) [4]. In addition, the oil of the plant has special value for human feeding, fabricating paints and different types of varnishes [5]. The Flaxseed production has been declined due to the strong competition between flax and other crops such as wheat, alfalfa ….etc [1]. Thus there is a great gap between the production and consumption especially in seed yield, the gap could be minimized by increasing the yield per unit area through new varieties and improvement of agriculture practices such as fertilization [1]. In calcareous soils the availability of zinc and iron micronutrients is low due to high pH and precipitation of them in carbonic and hydroxyl form, and this causes reduces in ability of micro nutrient uptake by plants and naturally plants requirement increases to these elements [6]. Flax is sensitive to deficiencies of iron and zinc, especially in calcareous soils [3]. Foliar application of micronutrients was successfully used for correcting deficits in crops [1], and increased and improved straw and seed yield [1]. The foliar application of nutrients is more effective as compared to soil applied nutrients because of effective utilization by plant and minimum cost per unit area [7]. Micronutrients play a great role in plant growth as a result of their effects on many physiological processes in plant life, several studies under alluvial soil conditions indicated that the application of some micronutrients as foliar application caused an increase in yield and quality of flax [1]. Zinc is a cofactor of over 300 enzymes and component of a number of dehydrogenases, proteinases and peptidases; thus Zn influences electron transfer reaction including those of Krebs-cycle and hence affecting in plants energy production [3 and 8]. The main factors which are affecting the availability of zinc are pH, carbonate minerals, organic matter, soil texture and interaction between zinc and other microelements, especially Fe. Zinc acts either as metal components of various enzymes or as functional structural or regulatory cofactors. Thus, they are associated with saccharide metabolism, photosynthesis, nucleic acid, lipid metabolism and protein synthesis. Zinc is an essential micronutrient for synthesis of auxin, cell division and the maintenance of membrane structure and function [9]. Zinc is important to membrane integrity and phytochrome activities .In general zinc has a main role in synthesis of proteins, enzyme activating, oxidation and revival reactions and metabolism of carbohydrates [10].

Iron is one of the essential micronutrients which plays a vital role in growth and development of plants and occupy an important portion by virtue of its essentiality in increasing crop yields [11]. It is well known, that the productivity of flax plants could be sustained through nutrition and fertilization. Iron is essential for the formation of chlorophyll, the green pigments that capture light to produce food for the plant. Iron is also necessary for the proper functioning of many plant enzyme systems that influence respiration and plant metabolism and helps oxidize sugar for energy. The foliar feeding of iron causes increase in flax production [10].

Since there is little or no studies about the interaction effect of Zn and Fe on yield and quality of flax, for above reasons this investigation was selected to study the effect of foliar application of zinc, iron and their interactions on growth characteristics, yield, yield components, oil%, protein%, fatty acids content and anatomical characteristics of flax.

2. Material and Methods

A field experiment was conducted at Gardarasha Agricultural Farm, College of Agriculture, University of Salahaddin/ Erbil, with global positional system (GPS) reading of 360 0N, 440 01 E (03411359 ,03997002 UTM), during the growing season of 2012-2013, to investigate the effect of foliar feeding of four concentrations of iron (0, 50 ,100 and 150 mg L^{-1}) (using Fe-EDDHA which contain 13% Fe) and three concentrations of Zn (0, 75 and 150 mg Zn L^{-1}) using liquid $ZnSO_4$ (10% Zn) and their interaction on morphological characteristics, yield and quality of flax using randomized complete block design with 3 replicates .

The seeds were sown on the first of December, 2012 using 58 Kg ha^{-1} of Thorshansity 72 cultivar (Poland cultivar). The constant level of nitrogen (15 Kg N/donum) in the form of urea 46% N was applied before seeding. The area of each experimental unit was 1.5 m^2. The experiment was done under rain-feed condition with annual rainfall of (605.6 mm /growing season); weed control was done 3 times using hand method. The spraying treatments were done at 50% of flowering on 4th of April 2013.

Random representative samples of ten plants were used at full maturity stage from every experimental unit to estimate the following characteristics:

1 Stem length (cm).
2 Technical stem length (cm).
3 Fruiting zone length (cm).
4 Stem diameter (mm).
5 Number of fruiting branches /plant.
6 Number of capsules /plant.
7 Number of seeds/capsule.
8 Seed index

Plants were harvested from the whole plot to determine:

1 Biological yield (kg ha^{-1}).
2 Seed yield (kg ha^{-1}).
3 Harvest Index (%).

Seed oil was determined by soxhlet extraction apparatus using hexane according to the methods described by [12]. Oil yield was calculated by multiplying seed yield kg/ha by seed oil percentage. Total Nitrogen was determined using Micro-Kjeldahl method then protein% was determined as follow: Protein% = N% × 6.25

Fatty acid was determined according to methods described by [13]. Physical and chemical analysis for soil of experimental sites represent in (table 2) [14].

Table 1. *Symbols for the concentrations of iron and zinc applied in foliar feeding (treatments)*

Symbols	Fe_0	Fe_1	Fe_2	Fe_3	Zn_0	Zn_1	Zn_2
Amount of applied heavy metal (mg $L^{-1)}$	0	50	100	150	0	75	150

Table 2. *Some chemical and physical properties of the studied soil.*

PSD g kg^{-1}			Textural name		Water content (Kpa)				
Clay	Silt	Sand			S.P		F.C		W.P
350.7	521.3	128.0	Silty clay loam		50.52		26.81		14.98
Soluble ions $mmol_cL^{-1}$									
Ca^{2+}	Mg^{2+}	Na^{1+}	K^{1+}	HCO_3^{1-}	CO_3^{2-}	Cl^{1-}	SO_4^{2-}	pH	EC dS m^{-1}
2.63	0.72	0.43	0.20	2.32	0.0	0.54	1.32	7.51	0.42
OMg kg^{-1}	$CaCO_3$ g kg^{-1}	Active $CaCO_3$ g kg^{-1}		Available phosphorus (mg kg^{-1}soil)					
10.1	24.31	4.52		2.47					

For the anatomical study paraffin methods was applied [15]. Statistical analysis were done using SPSS program version 20 for comparing between means using Duncan's multiple rang test at probability ($p \leq 0.95$) as mentioned by Cochran and Cox [16] .

3. Results and Discussion

Data in table (3) indicates that the levels of applied Fe not affected significantly on the studied characteristics. Although the Fe didn't affect significantly on biological yield, seed yield and harvest index (HI) but the highest value of them were recorded from Fe_3, Fe_2, Fe_2 respectively. The similar results were obtained by [1]; they suggested that Fe foliar application can improve the seed and straw yields of flax varieties.

Table 3. *Effect of Fe foliar application on some growth characteristics and yield of flax*

	Stem length (cm)	Technical stem length (cm)	Fruiting zone length (cm)	Stem diameter (mm)	No. of fruiting branches / plant	No. of capsules / plant	No. of seeds /capsule	Seed index	Biological yield (Kg ha^{-1})	Seed yield (Kg ha^{-1})	HI
Fe_0	65.15 a	51.87 a	13.28 a	0.72a	4.3 a	10.6 a	7.17a	7.17 a	5307.41 a	1356.78 a	0.26 a
Fe_1	69.70 a	55.57 a	14.13 a	0.71a	4.69 a	13.03 a	6.88a	6.88 a	5533.33 a	1402.84 a	0.26 a
Fe_2	66.62 a	53.70 a	12.92 a	0.66a	4.32 a	10.66 a	7.33a	7.33 a	5785.19 a	1418.24 a	0.27 a
Fe_3	65.10 a	51.94 a	13.16 a	0.62a	4.31 a	11.32 a	7.72a	7.19 a	6048.15 a	1382.13 a	0.23 a

Table 4. *Effect of Zn foliar application on some growth characteristics and yield of flax*

	Stem length (cm)	Technical stem length (cm)	Fruiting zone length (cm)	Stem diameter (mm)	No. of fruiting branches / plant	No. of capsules/plant	No. of seeds /capsule	Seed index	Biological yield (Kg ha^{-1})	Seed yield (Kg ha^{-1})	HI
Zn_0	69.48 a	56.25a	13.23 a	0.67a	4.53a	11.05a	7.73a	7.28 a	6138.89 a	1541.66 a	0.26ab
Zn_1	62.49 b	50.20b	12.29 a	0.63a	4.13 a	10.56a	7.20a	7.2 a	4775.00 a	1310.77 a	0.28a
Zn_2	67.96 b	53.37ab	14.59 a	0.73a	4.55 a	12.60a	6.94a	6.9a	6091.67 a	1317.57 a	0.22b

Table 5. *The interaction effect of Fe and Zn foliar application on some growth characteristics and yield of flax.*

Treat.	Stem length (cm)	Technical stem length (cm)	Fruiting zone length (cm)	Stem diameter (mm)	No. of fruiting branches/ plant	No. of capsules/ plant	No. of seeds /capsule	Seed index	Biological yield (Kg ha^{-1})	Seed yield (Kg ha^{-1})	HI
Fe_0Zn_0	64.98 ab	52.98 b	12.00 a	0.64 a	4.37 a	9.07 a	6.87 ab	6.17 a	5311.11 ab	1425.87 a	0.27 ab
Fe_0Zn_1	59.93 b	48.70 b	11.23 a	0.63 a	3.83 a	9.07 a	7.63 ab	6.17 a	4800.00 ab	1190.64 a	0.25 ab
Fe_0 Zn_2	70.53 ab	53.93 ab	16.60 a	0.87 a	4.70 a	13.67 a	7.00 ab	6.27 a	5811.11 ab	1453.84 a	0.25 ab
Fe_1 Zn_0	75.97 a	62.10 a	13.87 a	0.71 a	4.80 a	12.30 a	6.73 b	6.21 a	5377.78 ab	1598.44 a	0.31 ab
Fe_1 Zn_1	64.56 ab	50.33 b	14.23 a	0.71 a	4.63 a	12.57 a	7.03 ab	6.03 a	5111.11 ab	1300.27 a	0.25 ab

Treat.	Stem length (cm)	Technical stem length (cm)	Fruiting zone length (cm)	Stem diameter (mm)	No. of fruiting branches/ plant	No. of capsules/ plant	No. of seeds /capsule	Seed index	Biological yield (Kg ha$^{-1)}$	Seed yield (Kg ha$^{-1)}$	HI
Fe_1 Zn_2	68.57 ab	54.28 ab	14.28 a	0.72 a	4.63 a	14.23 a	6.87 ab	6.21 a	6111.11 ab	1309.80 a	0.21 b
Fe_2 Zn_0	71.74 ab	57.40 ab	14.33 a	0.68 a	4.67 a	12.63 a	7.93 a	6.02 a	7833.33 a	1778.95 a	0.23 b
Fe_2 Zn_1	62.40 b	51.30 b	11.10 a	0.63 a	4.00 a	9.30 a	7.33 ab	5.92 a	3966.67 b	1315.69 a	0.36 a
Fe_2 Zn_2	65.73 ab	52.40 b	13.33 a	0.68 a	4.30 a	10.03 a	6.73 b	6.20 a	5555.56 ab	1160.09 a	0.21 b
Fe_3Zn_0	65.23 ab	52.50 b	12.73 a	0.66 a	4.30 a	10.20 a	7.60 ab	6.37 a	6033.33 ab	1363.38 a	0.23 b
Fe_3 Zn_1	63.07 ab	50.47 b	12.60 a	0.56 a	4.07 a	11.30 a	6.80 ab	6.11 a	5222.22 ab	1436.47 a	0.28 ab
Fe_3 Zn_2	67.00 ab	52.87 b	14.13 a	0.64 a	4.57 a	12.47 a	7.17 ab	6.05 a	6888.89 ab	1346.56 a	0.20 b

Table 6. *Effect of Fe foliar application on oil percentage, oil yield and fatty acids of flax*

	Oil (%)	Oil yield (Kg ha^{-1})	Protein	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
			(%)					
Fe_0	20.82 b	28220.01 a	25.47b	6.33a	5.75a	19.28a	16.61a	51.43d
Fe_1	20.37 b	28709.43 a	29.10a	6.23b	5.47ab	19.28a	14.00b	54.62a
Fe_2	21.64a	30634.69 a	27.65a	5.98c	5.15b	18.23b	16.43a	53.90c
Fe_3	18.88 c	25992.33 a	22.43c	5.94d	5.16b	17.75c	16.45a	54.47b

Table 7. *Effect of Zn foliar application on oil percentage, oil yield and fatty acids of flax.*

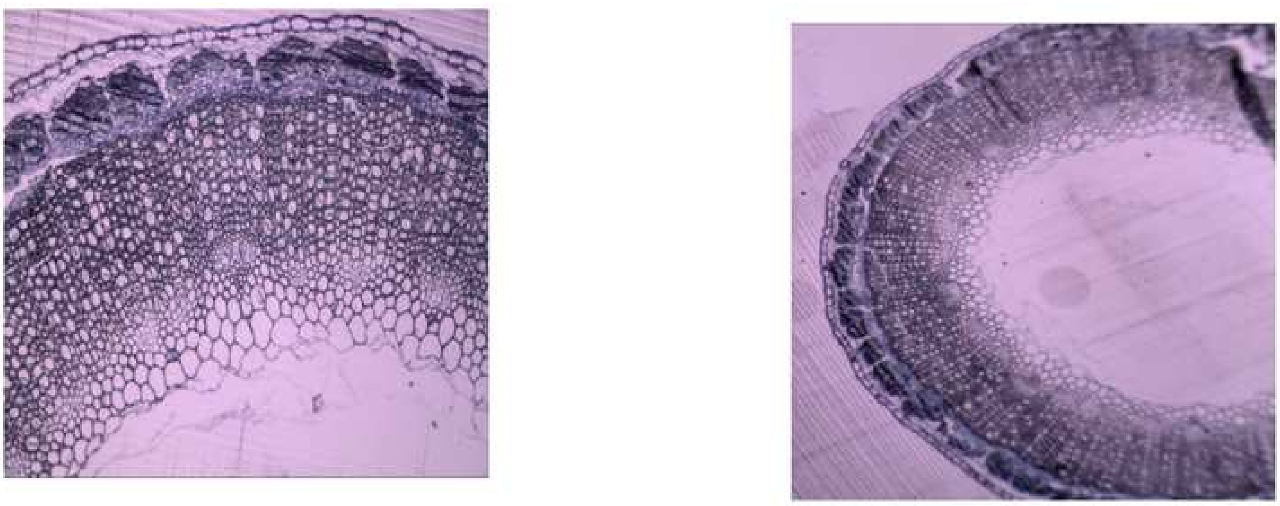
	Oil (%)	Oil yield (Kg ha^{-1})	Protein	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
			(%)					
Zn_0	19.35c	30195.52 a	25.99ab	6.03c	5.24a	17.99 c	15.86a	53.86b
Zn_1	20.02b	26135.92 a	25.00b	6.28a	5.40a	18.37b	15.24a	54.98a
Zn_2	21.92a	28835.90 a	27.50a	6.05b	5.51a	19.54a	16.51a	51.98c

Table 8. *The interaction effect of Fe and Zn foliar application and their on oil percentage, oil yield and fatty acids of flax.*

	Oil (%)	Oil yield (Kg ha^{-1})	Protein (%)	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
Fe_0Zn_0	17.60 f	251.30b	22.04 d	6.55ab	5.68 c	18.90 d	16.53a	51.59h
Fe_0Zn_1	21.75 c	257.64 b	24.36 cd	6.35bc	5.69 c	18.18 f	16.62a	52.5 f
Fe_0 Zn_2	23.10 b	337.65b	30.00 a	6.04d	6.18 a	20.75 b	16.38a	50.19 k
Fe_1 Zn_0	21.65 c	345.91a	29.13 ab	5.98d	4.78 g	17.3 1 g	13.94ab	54.69c
Fe_1 Zn_1	19.00e	247.56 b	28.29 ab	6.61a	5.94 b	21.04 a	11.53b	57.35 a
Fe_1 Zn_2	20.45d	267.79b	29.88 a	6.16cd	5.64 c	19.49 c	16.83a	51.83g
Fe_2 Zn_0	22.13 c	393.66a	28.61 ab	5.92d	4.90 fg	17.99e f	16.32a	54.49c
Fe_2 Zn_1	18.00 f	237.33 b	26.11bc	6.06d	5.28 d	17.80 f	16.39a	54.24d
Fe_2 Zn_2	24.81 a	288.05 b	28.25 ab	5.96d	5.20 de	18.90 e	16.58a	52.98e
Fe_3Zn_0	16.02 g	216.94 c	24.17 cd	5.61e	5.14 def	17.79 f	16.36a	54.65c
Fe_3 Zn_1	21.32 cd	302.90 b	21.25 d	6.16cd	4.97 efg	16.44 k	16.41a	55.83b
Fe_3 Zn_2	19.31 e	259.94 b	21.88 d	6.04d	5.32 d	19.01 e	16.51a	52.93e

Table 9. *Effect of Fe and Zn foliar application and their interaction on the thickness of fibrous region.*

Treatments	Fibrous diameter (µ)	Treatments	Fibrous Diameter (µ)
Fe_0	20.67 b	Fe_0Zn_0	20.00 bc
Fe_1	24.67 a	Fe_0Zn_1	18.00 c
Fe_2	22.00 b	Fe_0Zn_2	24.00 abc
Fe_3	25.33 a	Fe_1Zn_0	26.00 abc
Treatments		Fe_1Zn_1	30.00 a
Zn_0	22.50 a	Fe_1Zn_2	18.00 c
Zn_1	23.00 a	Fe_2Zn_0	20.00 bc
Zn_2	24.00 a	Fe_2Zn_1	18.00 c
		Fe_2Zn_2	28.00 ab
		Fe_3Zn_0	24.00 abc
		Fe_3Zn_1	26.00 abc
		Fe_3Zn_2	26.00 ab



A: The treatment combination (Fe_1 Zn_1) with high thickness.

B: The treatment combination (Fe_0Zn_1) with lowest thickness.

Figure 1. *Anatomical features of fiber thickness: A. (Fe_1 Zn_1) with high thickness, B. (Fe_0Zn_1) with lowest thickness*

Table (4) shows the significant effect of foliar application of Zn on stem length(cm), technical length (cm) and HI, the highest values were (67.96, 56.25 cm and 0.28 cm) obtained from Zn_2, Zn_0, and Zn_1 respectively, while the lowest value were (69.48, 50.20 and 0.22 cm) were obtained from Zn_0 , Zn_1 and Zn_2 respectively.

Data present in table (5) shows that the interaction treatments affected significantly on stem length (cm), technical length (cm), seed/capsule, biological yield (kg ha^{-1}) and HI. The highest values were (75.97, 62.10, 7.93, 7833.33 and 0.36) obtained from the combination treatments (Fe_1Zn_0, Fe_1Zn_0, Fe_2Zn_0, Fe_2Zn_0 and Fe_2Zn_1) respectively, this could be attributed to Fe acts as catalylst formation [10] and improves the photosynthesis processes, leading to more dry matter production. lower levels of Fe was transported from roots to shoot in plants that grown in nutrient solutions with Zinc low activity, compared with the same plants that grown in nutrients with more activity zinc [9] . While [3] mentioned that there is no affect of Fe and Zn on flax yield.

Table (6) shows that the increases in Fe foliar application caused significant increase in oil %, the highest value (21.64 %) was recorded from Fe_2 treatment; this may due to that the micronutrients requirements under undesirable soil conditions, such as pH, low organic matter content, high $CaCO_3$ content of the soil [5].

Table (7) pointed out that Zn application caused a significant increase in protein%, since Zn is the main composition of ribosome and essential for their development, amino acids accumulated in plant tissues and protein synthesis decline by zinc deficient [17] and fatty acids such as saturated (Palmitic acid) and unsaturated fatty acids (Oleic acid, Linoleic acid and Linolenic acid). But there is no significant effect on stearic acid. It seems that Zn has a beneficial effect on the formation of fatty acids, the improvement of physiological performance in tested plants due to more nutrients uptake by treated flax plants from soil, these results are in harmony with those obtained by [5]. This refers to the role of Zn foliar application in nutrients balance of flax under undesirable soil conditions.

Table (8) refers to significant combination effect of levels of applied Fe and Zn on oil % and oil yield the highest values were (24.81% and 393.66 kg ha^{-1}) obtained from the treatment combinations (Fe_2Zn_2 and Fe_2Zn_o) respectively, this may be due creating the best condition for plant growth and yield under the mentioned interaction treatments. The same table refers to significant combination effect of levels of applied (Fe and Zn) in protein and palmitic acid, stearic acid, oleic acid linoleic acid and linolenic acid, the highest values were (29.88, 6.61, 6.18, 21.04, 16.83 and 57.35%) obtained from the treatment combinations (Fe_1 Zn_2, Fe_1 Zn_1, Fe_0 Zn_2, Fe_1 Zn_1, Fe_1 Zn_2 and Fe_1 Zn_1) respectively this may be due to positive relation between Zn and Fe, [6] mentioned that zinc deficiency is lead to iron deficiency, due to prevent of transfer of Fe from root to shoot in Zinc deficiency conditions. lower levels of Fe was transported from roots to shoot in plants that grown in nutrient solutions with Zinc low activity, compared with the same plants that grown in nutrients with more activity zinc [6].

Table (9) shows that the increase in Fe foliar application caused significant increase in the fibrous diameter (μ), the highest value (25.33) micron was recorded from Fe_3 treatment; otherwise, there is no significant effect of applied Zn foliar application on fiber thickness although the thickness of fibrous region increase with increasing Zn treatments.

The same table shows that the interaction treatments affected significantly on the fibrous diameter, the highest value (30) micron was recorded from the treatment combination (Fe_1Zn_1) (Application of 50 mg L^{-1}Fe +75 mg L^{-1}Zn), anatomical changes in stem anatomy recorded due to the effect of Fe and Zn foliar application and their interactions (Fig. 1)

4. Conclusion

The results indicated to significant increase in most of the studied parameters like stem length, technical stem length, oil%, oil yield, protein content, fatty acids and thickness of fiber with increase in concentration of Fe and Zn foliar application to a certain level, then decrease in value of some parameters in case of higher concentration (100 and 150 mg L^{-1}) of F e and Zn respectively. For the mentioned results and conclusion it is necessary to apply higher concentrations of Fe and Zn in the future studies for limiting the toxic level or lethal dosage of them.

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