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Evaluation of Application Methods Efficiency of Zinc and Iron for Canola (*Brassica napus* L.)

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Abstract

In order to evaluation of application method efficiency of zinc and iron microelements in canola, an experiment was conducted in the Agricultural Research Station of Eastern Azerbaijan province in 2008. The experimental design was a RCBD with eight treatments (F_1: control, F_2: iron, F_3: zinc, F_4: iron + zinc in the form of soil utility, F_5: iron, F_6: zinc, F_7: iron+ zinc in the form of solution foliar application, and F_8: iron + zinc in the form of soil utility and foliar application). Analysis of variance showed that there were significant differences among treatments on given traits, antioxidant enzymes activity, fatty acids percentage, plant height, seed weight to capitulum weight ratio, protein percentage, oil percentage, oil yield, 1000 seed weight, seed yield, nitrogen, phosphorous and potassium percentage of leaves, zinc and iron content of leaves and capitulum diameters. The highest seed yield, oil yield, oil percentage, 1000 seed weight, seed weight to capitulum weight ratio and protein percentage were obtained from the soil and foliar application of iron + zinc treatments (F_8). Also, the highest amounts of nitrogen, phosphorous and potassium concentration in leaves were achieved from control treatment which was an indication of non-efficiency of iron and zinc on the absorption rate of these substances in the leaves. The correlation between effective traits on the seed yield, such as, capitalism diameter, number of seed rows in capitulum, seed weight to capitulum weight ratio and 1000 seed weight were positively significant. In general, foliar and soil application of zinc and iron had the highest efficiency in aspect of seed production. The comparison of the various methods of fertilization showed that foliar application was more effective than soil application. Also, micronutrient foliar application increased concentration of elements, especially zinc and iron. Antioxidant enzymes activity was different in response to treatments also the highest palmitoleic, oleic and myristic acid were observed in F_6 and F_7 treatments.

Keywords: foliar application, iron, soil utility, canola, zinc

Introduction

Canola (*Brassica napus* L.) is grown in different agro-climatic zones of the world, differing in soil nutrient status. The use of foliar fertilizing in agriculture has been a popular practice with farmers since the 1950s, when it was learned that foliar fertilization was effective and economic. Recent research has shown that a small amount of nutrients, particularly Zn, Fe and Mn applied by foliar spraying increases significantly the yield of crops (Sarkar *et al.*, 2007; Wissuwa *et al.*, 2008). Also, foliar nutrition is an option when nutrient deficiencies cannot be corrected by applications of nutrients to the soil (Crabtree, 1999; Sarkar *et al.*, 2007; Cakmak, 2008). It is likely therefore, in open-field conditions, where the factors that influence the uptake of the nutrients are very changeable, foliar fertilization can get considerable importance. Among the micronutrients, Zn and Fe nutrition can affect the susceptibility of plants to drought stress (Sultana *et al.*, 2001; Khan *et al.*, 2003; Cakmak, 2008).

Zn is known to have an important role either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a large number of enzymes (Grotz and Guerinot, 2006). Zinc also plays an important role in the production of biomass (Kaya and Higgs, 2002; Cakmak, 2008). Furthermore, zinc may be required for chlorophyll production, pollen function and fertilisation (Kaya and Higgs, 2002; Pandey *et al.*, 2006). Low solubility of Zn in soils rather than low total amount of Zn is the major reason for the widespread occurrence of Zn deficiency problem in crop plants (Cakmak, 2008). High seed-Zn has very important physiological roles during seed germination and early seedling growth (Cakmak, 2008). The review by Cakmak (2008) provides further reasons and relevant research for benefits of high seed-Zn on plant growth. As an activator of CuZn- or Mn-SOD, zinc or manganese is involved in membrane protection against oxidative damage through the detoxification of reactive oxygen species (Marschner, 1995).

Iron plays essential roles in the metabolism of chlorophylls. External application of Fe increased photosynthesis, net assimilation and relative growth in seawater-stressed rice (Sultana *et al.*, 2001). This is especially true for soils of high pH where equilibrium conditions favour the oxida-

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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. Application of Zn or Fe has been reported significant positive effects, in most cases, on growth measurements and chemical composition of safflower (Lewis and McFarlane, 1986), lupine (Brennan *et al*., 2001), cumin (El-Sawi and Mohamed, 2002), soybean (Gadallah, 2000; Heitholt *et al*., 2002), barley (Genc *et al*., 2004; Hebbern *et al*., 2005), wheat (Lu *et al*., 2004), sunflower (Mirzapour and Khoshgoftar, 2006), mustard (Chatterjee and Khurana, 2007), common bean (Fernandes *et al*., 2007) and rice (Wissuwa *et al*., 2008). Even though iron is one of the most abundant elements in soils, the low solubility product of iron minerals makes the inorganic form of iron unavailable to plants and forms the most common widespread nutritional disorder world over (Welch *et al*., 1991). Plants subjected to iron deficiency excess respond in different ways (Abadia, 1992). It is believed that under conditions of iron stress, some plants can increase their absorption capacity for iron. The mechanism affecting the acquisition of this essential microelement is often present in aerobic soils in the form of Fe (Marschner, 1995).

However, to the best of our knowledge, information regarding application method efficiency of zinc and iron on the growth and development of canola is not available. Therefore, the purpose of this study is to understand whether application of micronutrients as foliar application is better or soil application.

Materials and methods

The experiment was conducted on the research farm of Agricultural Research Station of Eastern Azerbaijan province, Azerbaijan, Iran (37°58′ N latitude, 46°3′ E longitude and altitudes of 1320 m) during 2008 growing seasons. Site of study has cold winter and warm summer. The yearly average precipitation (30-years long term period) which is mostly occurred during the spring months is 273.1 mm. The mean annual temperature was 11°C.

Soil sampling and analysis

Prior to the beginning of experiment, soil samples were taken in order to determine the physical and chemical properties. A composite soil samples were collected at a depth of 0-30 cm. It was air dried, crushed, and tested for physical and chemical properties. The research field had a loamy soil. Details of soil properties are shown in Tab. 1.

Field preparation and applying the treatments

After plough in fall and two disks in spring, the land was flatted by leveller and then plots were prepared. The experimental design was laid out in a Randomized Complete Block Design with four replications. The plots had 4 m length and 3 m width consisted of five rows, 0.6 m apart. Between all plots, 1 m distance was kept to eliminate all influence of lateral water movement. According to results of soil analysis 50 k.ha^{-1} super phosphates triple and 300 kg.ha^{-1} urea was used. All of super phosphates triple and one third of urea were distributed in plots and mixed with surface soil before seed sowing. Rest of urea was used when plants have 6 or 8 leaves and at flowering stage as topdress. Different treatments of micronutrient soil application which were including: 25 k.ha^{-1} iron from secostrine and 40 kg.ha^{-1} from zinc sulphate were distributed in plots and mixed with surface soil before seed sowing.

Seed sowing and irrigation

The canola seeds (*Brassica napus* L. C.V. 'SLM$_{046}$') were sown on 15 July. The between row distance and within distance were 60 cm and 25 cm, respectively. The flooding Irrigation was carried out as similar in all of plots during study. Two week after sowing weed control was efficiently performed by hand. Micronutrient foliar application was done at two times, one time when plants had 6 or 8 leaves and another when they have 10 or 12 leaves (early of flowering 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 Tab. 2.

Plant sampling, harvesting and scrutiny

At early flowering, number of leaves and leaf area were counted. Leaf area was estimated by multiplying of length and width of leaves in 0.68 coefficients (Rao and Saran, 1991). Also, fresh leaves samples were taken of similar leaves. The samples were frozen in liquid nitrogen and stored at −80°C until biochemical analysis.

After that, at flowering stage four upper leaves were taken for determine of N, P, K, Mn, Fe and Zn content of leaves. The samples were washed by distilled water and dried in oven at 70°C during 48 h. Total N, P, K, Mn, Fe and Zn content were determined through atomic absorp-

Tab. 1. Physical and chemical soil properties

	Sand	Silt	Clay	Texture	Calcite	O.M	EC
Depth 0-30 cm	42%	28%	30%	Loamy	18%	0.32%	2.6 d.m^{-1}
	N	P	K	Mn	Fe	Zn	Cu
	0.03%	14 mg.Kg^{-1}	320 mg.Kg^{-1}	2.8 mg.Kg^{-1}	2.2 mg.Kg^{-1}	0.22 mg.Kg^{-1}	1.8 mg.Kg^{-1}

Tab. 2. Different treatments of micronutrient application

F_1	Control	Water
F_2	Iron (soil application)	25 $k.ha^{-1}$ iron from secostrine
F_3	Zinc (soil application)	40 $k.ha^{-1}$ zinc sulphate
F_4	Iron + Zinc (soil application)	25 $k.ha^{-1}$ iron from secostrine + 40 $k.ha^{-1}$ zinc sulphate
F_5	Iron (foliar application)	2 parts per thousand
F_6	Zinc (foliar application)	3 parts per thousand
F_7	Iron + Zinc (foliar application)	2 parts per thousand + 3 parts per thousand
F_8	Iron + Zinc (soil and foliar application)	25 $k.ha^{-1}$ iron from secostrine + 40 $k.ha^{-1}$ zinc sulphate + 2 parts per thousand + 3 parts per thousand

tion method (Perkinelmer 1012, USA). Plants height was measured at end of flowering stage by five sample plant.

At the physiological maturity stage plants were harvested and capitulum diameter, row number in capitulum, number of seed in capitulum, seed weight to capitulum weight ratio, 1000 seed weight, seed yield, total oil percentage and total protein percentage and oil yield were measured.

The fatty acid compositions of the canola seed oils were determined by gas chromatography (GC) (Metcalf *et al.*, 1966). The contents of palmitoleic, linolenic, oleic and myristic acids were determined using a computing integrator. The effects of the independent variables on oil content and palmitoleic, Linolenic, oleic and myristic acid concentrations of the oil were analyzed on a percentage basis.

Oil percentage and protein percentage were measured by soxhlet and Kjeltec method, respectively. Oil yield was calculated via product seed yield in percentage oil.

Antioxidant enzyme activity

Peroxidase

Peroxidase activity was determined by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm (Ghanati *et al.* 2002). The reaction mixture contained 100 µL crude enzyme, 500 µL H_2O_2 5 mm, 500 µL guaiacol 28 mm and 1900 µL phosphate buffer 60 mm (pH 7.0).

Catalase

Catalase activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100 µL crude enzyme extract, 500 µL 10 mm H_2O_2 and 1400 µL 25 mm phosphate buffer. The decrease in the absorbance at 240 nm was recorded for 1 min.

Glutathione reductase

Glutathione reductase activity was determined by the method of Halliwell and Foyer (Halliwell and Foyer, 1978). Its activity was assayed in a 1 mL reaction mixture containing 0.25 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.05 mL of 10 mM oxidized glutathione, 0.12 mL of 1mM NADPH, 0.48 mL of distilled water, and 0.1 mL of enzyme extract. The resultant decrease in NADPH was observed at 340 nm.

Superoxide dismutase

The activity of SOD was measured according to the method of Giannopolities and Ries (1977). The assay medium contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM p-nitro blue tetrazolium chloride, 2 mM riboflavin, 0.1 mM EDTA and 5 mL enzyme extract. Glass test tubes containing the assay medium were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of enzyme activity was determined as the amount of the enzyme to reach an inhibition of 50% nitro blue tetrazolium reduction rate by monitoring the absorbance at 560 nm.

Reduced glutathione

The reduced glutathione content was assayed as described by Griffith and Meister (1979). 200 mg of fresh material was ground with 2ml of 2% metaphosphoric acid and centrifuged at 17,000 rpm for 10 min. Adding 0.6 ml 10% sodium citrate neutralized the supernatant. One milliliter of assay mixture was prepared by adding 100 µl extract, 100 µl distilled water, 100 µl of 6mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) and 700 µl of 0.3mM NADPH. The mixture was stabilized at 25 °C for 3–4 min. Then 10 µl of glutathione reductase was added and read the absorbance at 412 nm in spectrophotometer.

Statistical analysis of data

All data were analyzed from analysis of variance (ANOVA) using the MSTAT-C. Least significant difference test was used to measure statistical differences between treatment methods and controls. Also the charts were drawn by Excel, Microsoft Office 2003.

Results and discussion

The results of analysis of variance showed that, effect of micronutrients was significant on all traits except number of leaves in plant, leaf area, number of seed row in capitulum, seed number in capitulum, kernel to seed percentage and Mn content (Tab. 3 and 4).

Tab. 3. Effect of micronutrient application on some agronomical traits of canola

S.O.V	d.f	PH	LN	LA	CD	SW	PP	OP
Block	3	110.78	18.58	0.53	1.40	65.56	0.04	2.54
Treatment	7	97.19**	7.30	0.11	2.17*	59.19**	3.37**	13.69**
error	21	20.53	3.88	0.15	1.06	6.33	0.005	0.49
C.V		3.51	9.20	23.54	5.45	3.71	0.34	1.62
S.O.V	d.f	KS	OY	SY	LN	LZ	LM	
Block	3	3.03	12664.27	42443.65	0.11	28.53	261.69	
Treatment	7	3.89	155428.34**	489700.60**	0.16**	256.56**	9.81	
error	21	4.59	9975.70	53768.74	0.01	16.22	14.69	
C.V		2.80	4.60	4.64	4.35	14.30	5.45	

*, ** significant at the 0.05 and 0.01 probability levels, respectively

PH: plant height; LN: leaf number; LA: leaf area; CD: capitulum diameter; SW: seed weight; PP: protein percentage; OP: oil percentage; Kernel to seed percentage; OY: oil yield; SY: seed yield; LN: leaf nitrogen; LP: leaf phosphorous; LK: leaf potassium; LI: leaf iron; LZ: leaf zinc; LM: leaf manganese

Antioxidant enzyme activity

The highest POD activity was observed in F$_6$ and F$_8$ treatment while the lowest activity was related to F$_2$ treatment (Tab. 5). There was not significant difference between F$_1$ and F$_3$ and between F$_5$ and F$_7$ treatments (Tab. 5).

It seems that Zn foliar application causes increase of POD activity because soil application had not additive effect on activity of this enzyme in contrast Fe application decreased POD activity. Our findings were in agreement with the results reported by Jiang and Huang (2001) and Habibi *et al.* (2004). The simultaneous increase in the activity of these enzymes contributes to a decrease of the deleterious effects of H_2O_2 under stress.

Control treatment had the highest CAT activity (Tab. 5). The lowest CAT activity was achieved from F$_4$, F$_6$ and F$_8$ treatments, as among these treatments were not significant difference (Tab. 5). Similarly, there was no difference between F$_3$ and F$_5$ application method. Fe and Zn application decreased CAT activity, due to scavenging of reactive oxygen species by other antioxidant enzymes. These results were agreement with the results reported by Bailly *et al.* (2000).

Glutathione reductase was affected by fertilizers too. As F$_2$ and F$_3$ treatments had the highest enzyme activity while the lowest was observed from F$_8$ treatment (Tab. 5). There was not significant difference between F$_4$ and F$_6$ treatments too (Tab. 5). The highest and the lowest GSH activity were related to F$_1$, F$_2$, F$_3$ and F$_6$, F$_7$, F$_8$ treatments, respectively (Tab. 5).

The results showed that, F$_7$ and F$_8$ treatments increased significantly SOD activity. The lowest activity was observed in F$_1$, F$_3$ and F$_5$ treatments (Tab. 5). Cakmak (2000) reported that Zn deficiency may inhibit the activities of a number of antioxidant enzyme.

It has been demonstrated that environmental stress induces oxidative stress in plant tissues. Exposes chloroplasts to excessive excitation energy, may increase generation of reactive oxygen species and induce oxidative stress. To overcome the effects of oxidative stress, plants make use of a complex antioxidant system. Relatively higher activities of reactive oxygen species scavenger enzymes have been reported in many stressed plants, which suggest that the antioxidant system plays an important role in plants against environmental stresses (Habibi *et al.,* 2004).

Superoxide dismutase may function as a reactive oxygen species scavenger, by converting O_2^- to H_2O_2 (Baily *et al.* 2000). Hydrogen peroxide is converted to oxygen and water by CAT and POX . Even though high SOD activity protects plants against superoxide radicals, it can not be considered solely responsible for membrane protection against peroxidation. In general, micronutrient application had different effects on antioxidant enzymes activity, in some cases increase and in some cases decrease was observed.

Tab. 4. Effect of micronutrient application on antioxidant enzyme activity and fatty acid percentage of canola

S.O.V	df	POD	CAT	GR	GSH	SOD	Palmitoleic acid	Linolenic acid	Oleic acid	Myristic acid
Block	2	2.16	2.16	0.00	0.00	3.51	1.29	0.64	0.37	0.16
Treatment	7	30.76**	13.94**	0.01**	0.01**	24.49**	22.47**	9.84**	18.19**	3.47**
Error	14	0.97	0.50	0.00	0.00	0.24	4.38	1.34	1.42	0.43
C.V		3.10	3.58	2.63	3.46	3.53	9.89	17.36	1.76	16.66

*, ** significant at the 0.05 and 0.01 probability levels, respectively

POD: peroxidase, CAT: Catalase; GR: glutathione reductase; SOD: superoxide dismutase

Tab. 5. Comparison of means on antioxidant enzyme activity and fatty acid percentage of canola

	Treatments	POD	CAT	GR	GSH	SOD	palmitoleic acid	Linolenic acid	Oleic acid	Myristic acid
F_1	control	30.66cd	24.00a	0.46b	0.73a	10.50d	17.66c	3.7e	61.66b	2.60c
F_2	Iron (soil application)	26.33e	21.33b	0.52a	0.75a	12.33c	20.00bc	6.00cd	67.33a	2.93bc
F_3	Zinc (soil application)	29.00d	20.00c	0.51a	0.71a	11.00d	22.33ab	8.00abc	68.66a	3.40bc
F_4	Iron + Zinc (soil application)	31.00c	18.00d	0.40d	0.65b	13.00c	17.00c	8.66ab	68.66a	3.66bc
F_5	Iron (foliar application)	33.00b	19.33c	0.43c	0.64b	15.33b	22.00ab	9.00a	68.00a	4.20b
F_6	Zinc (foliar application)	36.00a	17.66d	0.40d	0.59c	15.00b	25.00a	5.00de	69.66a	5.50a
F_7	Iron + Zinc (foliar application)	34.00b	19.66c	0.36e	0.58c	18.00a	22.33ab	6.66bcd	68.33a	5.50a
F_8	Iron + Zinc (soil and foliar application)	34.66ab	17.66d	0.32f	0.62bc	17.66a	23.00ab	6.33cd	67.66a	4.03b

For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

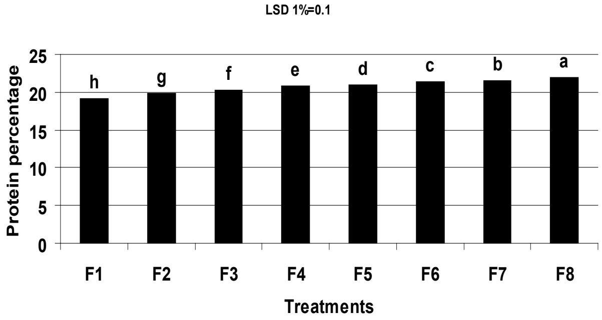

Fig. 1. Changes of protein percentage due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

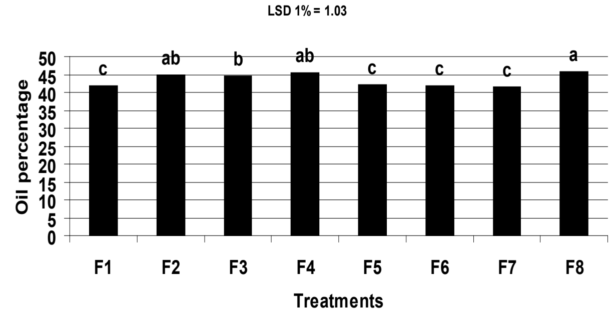

Fig. 2. Changes of oil percentage due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

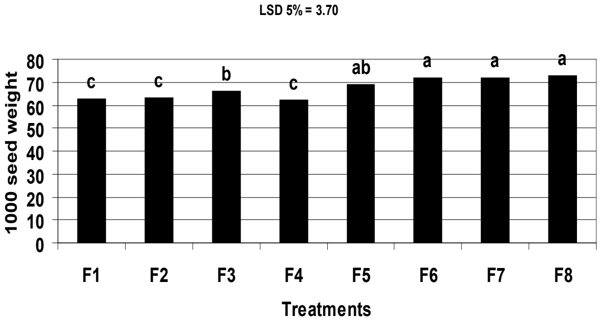

Fig.3. Changes of 1000 seed weight due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

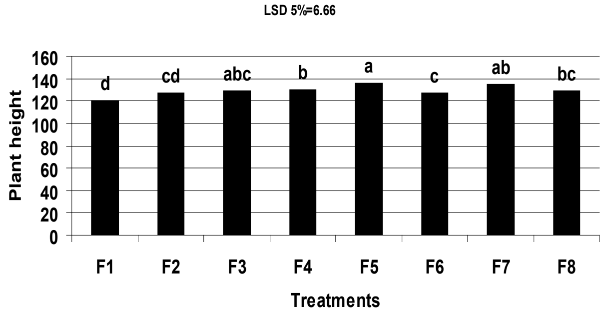

Fig. 4. Changes of plant height due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

Fatty acids

Analysis of fatty acids by GC showed that, the application of Zn and Fe increased the fatty acids compared to the control (Tab. 5). In case of palmitic, palmitoleic and myristic acid foliar application was better than soil application while regarding linolenic acid soil application had better effect. Between foliar application and soil application was not difference on oleic acid content but applica-

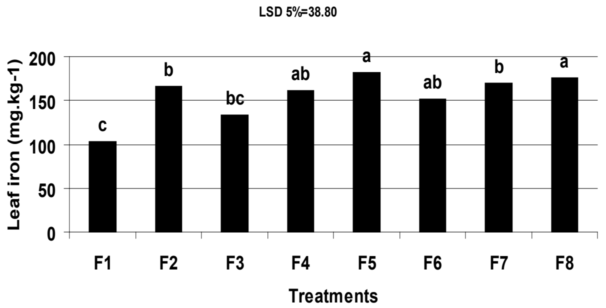

Fig. 5. Changes of leaf iron due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

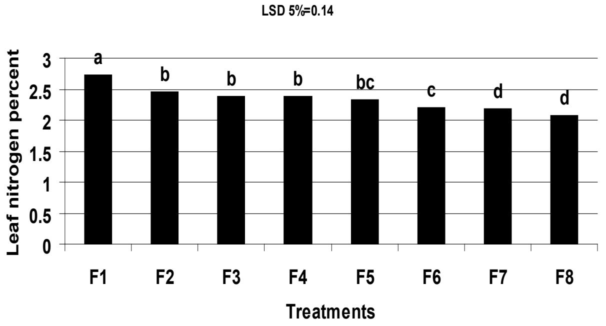

Fig. 7. Changes of leaf nitrogen due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

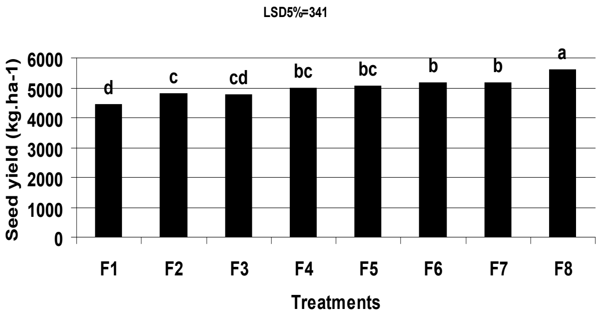

Fig. 9. Changes of seed yield due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

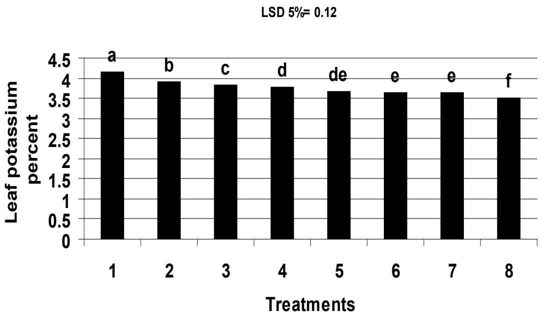

Fig. 6. Changes of leaf potassium due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

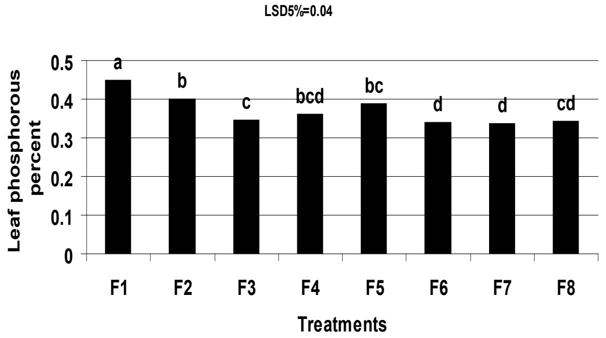

Fig. 8. Changes of leaf phosphorous due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (soil and foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

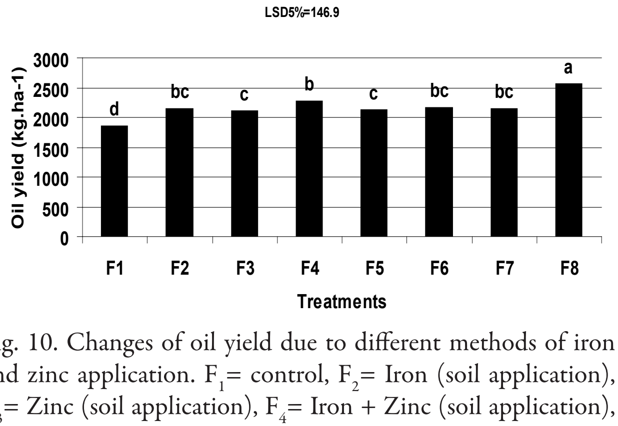

Fig. 10. Changes of oil yield due to different methods of iron and zinc application. F_1= control, F_2= Iron (soil application), F_3= Zinc (soil application), F_4= Iron + Zinc (soil application), F_5= Iron (foliar application), F_6= Zinc (foliar application), F_7= Iron + Zinc (foliar application), F_8= Iron + Zinc (soil and foliar application). For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

tion of these elements increased significantly oleic content than control treatment (Tab. 5). Zakaria *et al.* (2001) concluded that Zn foliar application significantly increased the total unsaturated fatty acids in cotton.

The highest palmitoleic acid was related to F_3, F_5, F_6, F_7 and F_8 treatments also, the highest and the lowest linolenic acid percentage were achieved from F_3, F_4, F_5 and control treatment, respectively (Tab. 5).

The highest myristic percentage was achieved from F_6 and F_7 treatments (Tab. 5). It was observed that control treatment had the lowest myristic percentage (Tab. 5).

Canola oil consists of different types of saturated and unsaturated fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid etc.). The palmatic acid and stearic acid are the major saturated fatty acids, whereas oleic and linoleic acids are unsaturated. Fatty acid composition of canola in particular and other oil seed crops in general, are influenced by fertilizing management. The findings of present study are shows that fatty acid composition is affected by iron and zinc application, for example, Kheir *et al.* (1991) found that the higher N-rate increased the percentage of unsaturated fatty acids and decreased saturated fatty acids of flax oil.

Protein percentage

The highest and the lowest protein percentage were observed in F_8 treatment and control, respectively. Kaya and Higgs (2002) pointed that the application of Zn fertilisers significantly increased the protein content of tomato. But it was in consistent with results of Singh, S. (2000) which suggest that protein content does not affected by fertilizers treatment and is related to genotypes. Cakmak *et al.* (1989) stated that under condition of zinc deficiency protein content decreases. Iron and zinc are two important elements in enzymes structure involved in amino acid biosynthesis and thus amino acids are the base of protein synthesis, protein content increases in use of these micronutrients. Our results are agreed with results of Zakaria et al (2001) on cotton.

Oil yield and percentage

The F2, F4 and F8 treatments produced the highest oil percentage .The lowest oil percentage was obtained from foliar application treatment and control treatment. It seems that, soil application of micronutrients is more benefit to oil biosynthesis. Singh and Sinha (2005) reported the decrease in oil concentration may be due to oxidation of some polyunsaturated fatty acids. The highest and the lowest oil yield were achieved from F8 and control treatments. In general, there was not significant different between micronutrient application method. Because of direct relation between seed yield and oil yield, increase in seed yield causes in higher oil yield (Singh, S. 2000).

1000 seed weight

The highest 1000 seed weight was observed in F_8 treatment and other foliar application treatments. Zinc is required for the biosynthesis of the plant growth regulator such as IAA and for carbohydrate and N metabolism which leads to high yield and yield components. This may be due to provision of macro and micro nutrients at latter stages which might have enhanced accumulation of assimilate in seeds and thus resulting in heavier seeds.

Seed yield

The highest seed yield was produced from F_8 treatment. Masoni *et al.* (1996) showed that, zinc increases seed yield. Heitholt *et al.* (2002) reported that seed yield of soybean increased while Cu, Mn, Zn, and Fe applied individually. The increase in yield with soil applied Fe might have been the result of increase in the amount of available Fe in soil. The increase in seed yield with foliar application of Fe could be attributed to the direct absorption of the element by the foliar sprayed with Fe.

In proceed to the previous studies (Cakmak, 1999), 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 photosystems. Iron deficiency leads to many disorders in chloroplasts. Ferredoxin is an important iron-containing protein involved in electron-transfer. Singh *et al*. (1975) observed that, zinc increases canola significantly seed yield. The lowest seed yield was obtained from control treatment.

Leaf nitrogen content

The results showed that, micronutrient application decreased leaf nitrogen content. The highest nitrogen content was related to control treatment while the lowest content was obtained from F_8 treatment. Foliar application had more effect on nitrogen content reduction. It seems that, decrease in nitrogen content is due to competitive effect between elements such as zinc and nitrogen.

Leaf phosphorous content

Similar upon results, micronutrient fertilizing decreased phosphorous content in leaves. These results were inconsistent with that obtained from Movchan and Sobornikova (1972). The highest phosphorous content was related to control treatment. Zinc decreases phosphorous content because there is competitive effect between phosphorous and zinc in uptake of ions. Sahota and Arora (1981) reported that there was no significant effect of Zn on P content of leaves.

Leaf potassium content

There was significant decrease in potassium content of plants which were treated with iron or zinc than control plants. The lowest potassium content was observed in F_8 treatment. It shows that, iron and zinc decreases potassium content in leaves of canola. Stoyanova and Doncheva (2002) remarked that the K content of the stems and leaves was not significantly affected by different concentrations of Zn.

Leaf iron content

The highest iron contents were observed in four treatments, F_8, F_6, F_5 and F_4, respectively . The application of Fe through soil as well as foliar application caused a marked increase in the total content of Fe in the fenugreek 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 (Kalbasi *et al.* 1998). 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 (Agarwala *et al.*, 1965). Also, iron is involved electron transport in photosystems. Already decrease of chlorophyll content due to iron deficit was reported by Masoni *et al.* (1996).

Leaf zinc content

The highest zinc content was observed in F_6, F_7 and F_8 treatments, respectively. Foliar application of zinc increased zinc content more than soil application. Zinc uptake is easier form leaves in compare soil application. Zinc uptake is lower in lime soils. Grawel and Graham (1999) have been reported that, zinc application increases zinc concentration in seed, root and leaves. Increase in Zn concentration reported in soil application of S and Fe. These results were in agreement with those reported by El-Gazzar *et al.* (1979) and Foregoni *et al.* (1974). 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.

Conclusions

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 canola oil. In addition to this, we also suggest that Ca and Fe should be sprayed on plants to reach the best quality and quantity in canola production.

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