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Effects of Zn Rates and Application Forms on Protein and Some Micronutrients Accumulation in Common Bean (*Phaseolus vulgaris* L.)

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Abstract: In order to investigation the effects of Zn rates and application forms on protein and element contents (Fe, Cu, Mn, N and Zn) in bean plant, an experiment was conducted as a factorial based on completely randomized design at greenhouse conditions during 2006. Treatments were included zinc rates in 4 levels (10, 20, 30 and 40 mg Zn kg^{-1} $soil^{-1}$ in $ZnSO_4$ source) and 3 application forms (soil application, seed pelleting and foliar spraying). Results showed that Zn rates had significant effect on accumulation of Zn, Cu, Mn and N in bean leaves. Nitrogen accumulation in leaves reduced with increasing of zinc in the soil. Among Zn application forms, spray application had the highest accumulation of Fe, Zn and Mn in leaves (423.17, 282.89 and 88.17 mg kg^{-1}, respectively). The highest Zn content in seed was observed in 20 and 40 mg Zn kg^{-1} $soil^{-1}$ levels (46.39 and 45.62 mg kg^{-1}, respectively). Meanwhile, all treatments of Zn (both rate and application) had not significant effects on Cu and Mn accumulation in bean seeds. According to interaction effects between Zn rates and application forms, the highest Fe content in seed was observed when 40 mg kg^{-1} $soil^{-1}$ of Zn was applied as foliar spraying. The seed protein content nearly was stable while Zn levels was increased from 20 to 40 mg Zn kg^{-1} $soil^{-1}$. Grain yield had significant correlation to zinc and Cu of leaves but, biomass had significant and negative correlation to Zn content of leaves at $p<0.05\%$ probability levels.

Key words: Bean (*Phaseolus vulgaris* L.), protein, micronutrients, zinc, application forms

INTRODUCTION

Zinc is one of the trace elements which are essential for the normal healthy growth and reproduction of crop plants (Alloway, 2004). In plants, zinc does not undergo valency changes and its predominant forms in plant are as: low molecular weight complexes, storage metalloproteins, free ions and in soluble forms associated with the cell walls. Zinc can become inactivated within cells by the formation of complexes with organic legends or by complexes with phosphorus (Brown *et al*., 1993). Zinc has high phloem mobility form leaves to roots, stems and developing grain and form one root to another (Rengel, 2001).

Beans (*Phaseolus vulgaris* L.) are recognized as being susceptible to zinc deficiency. Symptoms of zinc deficiency usually appear on the second set of trifoliate leaves. The leaves become light green and mottled as the deficiency progresses: the area between the leaf veins becomes pale green and then yellow near the tips and outer edges. In cases of severe deficiency, the older leaves may turn grey or brown and die. In the early stages of deficiency the leaves are often deformed, dwarfed and crumpled (Moraghan and Grafton, 1999; Alloway, 2004; Valenciano *et al*., 2007).

Gianquinto *et al*. (2000) reported that in glasshouse and rain shelter conditions, leaf Zn concentration lower than the critical level was reduced by the addition of P to plant grown at low Zn supply. Findings by Haslett *et al.* (2001) showed that foliar treatments with $ZnSO_4$ and chelated Zn forms in wheat (*Triticum aestvium* L.) resulted in shoot Zn concentrations in 7-week-old plants supplied with Zn in the root environment or via foliar spray of ZnO. Study of Page and Feller (2005) showed that dynamics of redistribution Zn_{65}, Mn_{54}, Ni_{63}, Co_{57} and Cd_{109} differed considerably in young wheat. Zn_{65} and

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Cd_{109} were released more slowly form the roots to the leaves and was subsequently redistributed in the phloem to youngest leaves only at trace levels. Hacisalihoglu *et al*. (2004) resulted that Zn efficient genotypes of bean distributed more Zn into young parts of shoot. Also, they reported that the older portions of the shoot for most genotypes had higher dry matter production under Zn deficiency than sufficient Zn supply. Findings of Armour *et al*. (1990) showed that a critical Zn concentration of 10-11 mg kg^{-1} in the youngest fully expanded leaf can be used to diagnose the status of Gallaroy navy beans.

Singh (2007) indicated that seed coating treatments significantly increased the fertilizer-Zn use efficiency and its physiological efficiency compared to soil application and improved the Zn content in seeds similar to that of soil application. Hardiman *et al*. (1984) investigated factors affecting the distribution of cadmium, copper and lead and their effect upon yield and zinc content in bush bean and reported that uptake of heavy metals are associated with a decrease in zinc content and yield in plants. Study of Madani *et al*. (2007) in Iran showed that an application of 40 kg Zn sulfate ha^{-1} soil followed by two foliar applications carried out before flowering and during grain formation of soybean significantly improved quantitative parameters of soybean yield. Researchers at International Center for Tropical Agriculture evaluated a core collection of more than 1000 accessions of common beans and found Fe concentrations from 34 to 89 μg g^{-1} (average of 55 μg g^{-1} Fe). Also, Zinc concentrations in these accessions ranged from 21 to 54 μg g^{-1}(average of 35 μg g^{-1} Zn). Gregoria (2002) examined some bean accessions from Peru were recently found that some genotypes had very high levels of Fe, averaging up to 100 μg g^{-1} Fe.

Many studies reported that zinc is important for high yielding and high quality crops such as beans (Alloway, 2004; Teixeira *et al*., 2004; Gianquinto *et al*., 2000; Beebe *et al*., 1999; Brown *et al*., 1993). Gabal *et al*. (1985) findings showed that foliar application of 50 to 100 ppm Zn significantly increased total dry matter, seed yield in Giza-3 bean cultivar. The zinc status of soils and crops are easily assessed by soil or plant analysis and it is important that farmers should investigate land where poor yields are obtained without other obvious explanations such as drought or disease, especially in areas with highly susceptible soils (Alloway, 2004). Generally, the aims of this study was to investigate different Zn application forms and rates effects on accumulation of Zn, Fe, Cu, Mn, N and protein content in bean in greenhouse conditions.

MATERIALS AND METHODS

A greenhouse experiment was conducted as a factorial based on Completely Randomized Design (CRD) with 4 replications. Experiment was carried out at the greenhouse of the Sari Agriculture Science and Natural Resources University in north of Iran during 2006. Treatments were included zinc rates in 4 levels (10, 20, 30 and 40 mg Zn kg^{-1} $soil^{-1}$ from $ZnSO_4$ source) and 3 application forms (soil application, seed pelleting and foliar spraying). In foliar application treatments, plants were supplied with zinc sulphat ($ZnSO_4$) as foliar spray at the 10 days after flowering. Seeds of bean plants (*Phaseolus vulgaris* L.) cv. Contender were grown in pots. Bean seeds were pre-germinated for 2 day, after which 10 seeds of bean were sown in pots filled with clay loam soil. One week after sowing, the seedlings were thinned to five per pot. The daily air temperature ranged from 37°C (maximum at days) to 10°C (minimum at night), which the daily average temperature being about 20°C. For each sampling, one plant was selected from each pot in maturity stage and grain dried to 14% moisture and then elements contents in each sample were determined.

The amounts of grain protein content (using Kjeldhal method) in seeds, Zn, Fe, Cu, Mn and N accumulated in seeds and total leaves have been determined. To determine Zn concentration in leaf and grain, after drying at 60°C and grinding, seed and leaves samples were changed to ash at 550°C for 8 h and then the ashes were dissolved in 3.3% HCl (v/v) to determine Zn and Fe by an atomic absorption spectrometer (Varian SpectrAA-10). Analysis of variance was preformed by SAS and Duncan's Multiple Range Test (DMRT) was used at $p = 0.05$ level.

RESULTS AND DISCUSSION

Elements content in leaves: Application form of Zn had significant effect on Zn and Cu content in leaf. Results showed that Zinc application had effect on Zn, Cu, Mn and N in leaf. Interaction between application form and Zn rates were significant for Zn and Cu accumulation in leaves (Table 1). Among application methods, spraying caused the highest concentration of Fe, Zn and Mn in leaves, while, soil application had the highest amount of Cu in leaves (Table 2). Haslett *et al*. (2001) reported that foliar application of Zn in wheat compared to other applications had about two fold Zn concentration in shoot. Also, findings by Hacisalihoglu *et al*. (2004) are in agreement to these results.

Maximum accumulation of Zn, Cu and Mn in leaves were obtained in 40 mg Zn kg^{-1} $soil^{-1}$ but Fe

Table 1: The analysis of variance for Zn application forms and rates effects on protein and some micronutrients accumulation in common bean

		Means of squares										
		Fe		Zn		Cu		Mn		Protein	N	
Source of variance	df	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf	Yield (g plant)
A	2	**	NS	**	**	NS	**	NS	NS	**	NS	NS
Zn R	3	NS	NS	**	**	NS	*	NS	**	**	**	*
A* Zn R	6	*	NS	NS	**	**	*	NS	NS	**	NS	NS
CV%	-	19.82	24.81	15.51	18.23	17.36	16.87	11.13	17.09	5.15	13.16	23.35

*,**Significant at the 5 and 1% levels of probability, respectively, NS = Non Significant, A = Application forms, Zn R = Zn Rates

Table 2: Results of mean comparison of Zn application forms and rates effect on protein and some micronutrients accumulation in common bean

	Fe (mg kg $^{-1}$)		Zn (mg kg $^{-1}$)		Cu (mg kg $^{-1}$)		Mn (mg kg $^{-1}$)				
Treatments	Seed (Trait)	Leaf (Trait)	Seed (Trait)	Leaf (Trait)	Seed (Trait)	Leaf (Trait)	Seed (Trait)	Leaf (Trait)	Protein (%) seed	N (%) leaf	Yield (g plant)
Application forms											
SA$^{\Delta}$	81.62^{ab}	335.93^{b}	40.25^{b}	63.81^{b}	7.37^{a}	14.99^{a}	15.81^{a}	76.28^{b}	26.27^{a}	3.31^{a}	6.56^{a}
SP	74.07^{b}	397.94^{ab}	37.36^{c}	73.14^{b}	8.36^{a}	11.75^{b}	15.66^{a}	81.54^{ab}	24.68^{ab}	3.00^{a}	7.04^{a}
FS	92.90^{a}	423.17^{a}	47.42^{a}	282.89^{a}	8.09^{a}	12.25^{b}	15.83^{a}	88.17^{a}	25.56^{b}	3028^{a}	6.39^{a}
Zn rates (mg kg^{-1} soil)											
10	77.53^{a}	434.50^{a}	38.91^{b}	165.87^{a}	8.45^{a}	11.77^{b}	16.01^{a}	80.87^{b}	24.61^{b}	3.27^{b}	6.61^{ab}
20	87.47^{a}	356.12^{a}	45.62^{a}	157.03^{a}	8.06^{ab}	12.93^{b}	16.63^{a}	70.82^{b}	26.93^{a}	3.63^{a}	5.45^{b}
30	78.18^{a}	362.07^{a}	36.65^{b}	173.58^{a}	7.22^{b}	12.50^{b}	15.06^{a}	76.49^{b}	25.44^{b}	3.00^{b}	7.47^{a}
40	88.27^{a}	390.02^{a}	46.39^{a}	163.31^{b}	7.94^{ab}	14.77^{a}	15.36^{a}	99.80^{a}	25.04^{b}	2.90^{b}	7.12^{a}

Means within the same column followed by the same superscript letter(s) were not significantly different according to DMRT (p<0.05), $^{\Delta}$SA: Soil Application, SP: Seed Pelleting and FS: Foliar Spray

Table 3: Interaction effect of Zn application forms and rates on protein and some micronutrients accumulation in common bean

		Fe (mg kg $^{-1}$)		Zn (mg kg $^{-1}$)		Cu (mg kg $^{-1}$)		Mn (mg kg $^{-1}$)				
Treatments		Seed (Trait)	Leaf (Trait)	Seed (Trait)	Leaf (Trait)	Seed (Trait)	Leaf (Trait)	Seed (Trait)	Leaf (Trait)	Protein (%) seed	N (%) leaf	Yield (g plant)
SA$^{\Delta}$	10*†	72.07^{bc}	359.99^{bc}	38.79^{bcd}	82.16^{b}	8.89^{b}	12.57^{b-d}	16.72^{ab}	81.96^{b-e}	24.61^{cd}	3.22^{bc}	7.51^{a}
	20	90.40^{bc}	378.66^{bc}	48.05^{b}	328.18^{a}	8.18^{b}	11.67^{b-d}	16.01^{ab}	70.39^{de}	26.45^{a-c}	3.50^{ab}	5.80^{a}
	30	63.18^{c}	391.27^{a-c}	30.61^{d}	71.03^{b}	6.76^{bc}	9.83^{d}	14.23^{b}	76.86^{c-e}	23.41^{b}	2.76^{c}	8.08^{a}
	40	75.45^{bc}	301.13^{c}	43.07^{bc}	65.67^{b}	8.54^{b}	18.08^{a}	15.12^{ab}	89.47^{a-d}	27.50^{a}	3.08^{bc}	6.36^{a}
SP	10	84.71^{bc}	407.37^{a-c}	38.08^{b-d}	79.19^{b}	8.18^{b}	10.38^{cd}	14.95^{ab}	65.59^{e}	24.87^{b-d}	3.22^{bc}	6.19^{a}
	20	92.99b	287.90^{c}	43.95^{bc}	41.89^{b}	4.89^{c}	15.12^{ab}	16.46^{ab}	67.03^{de}	27.54^{a}	4.05^{a}	4.48^{a}
	30	85.42^{bc}	297.29^{c}	44.13^{bc}	384.18^{a}	7.82^{b}	13.47^{bc}	16.01^{ab}	85.97^{b-e}	27.48^{a}	3.32^{bc}	6.81^{a}
	40	69.40^{bc}	391.34^{a-c}	38.44^{b-d}	41.92b	7.47^{b}	14.76^{b}	16.01^{ab}	108.86^{a}	23.65^{d}	2.69^{c}	8.18^{a}
FS	10	75.81^{bc}	539.13^{a}	39.86^{b-d}	336.26^{a}	8.54^{b}	12.38^{b-d}	16.37^{ab}	95.07^{a-c}	24.35^{d}	3.37^{bc}	6.13^{a}
	20	79.01^{bc}	401.80^{a-c}	44.85^{bc}	100.41^{b}	11.03^{a}	12.01^{b-d}	17.44^{a}	75.04^{c-e}	26.79^{ab}	3.34^{bc}	5.69^{a}
	30	85.96^{bc}	397.67^{a-c}	35.21^{cd}	65.50^{b}	7.09^{b}	14.20^{b}	14.94^{ab}	66.66^{de}	25.43^{a-d}	2.91^{bc}	7.54^{a}
	40	119.95^{a}	477.59^{ab}	57.66^{a}	82.33^{b}	7.83^{b}	11.47^{b-d}	14.95^{ab}	101.27^{ab}	23.96^{d}	2.94^{bc}	6.81^{a}

Means within the same column followed by the same superscript letter(s) were not significantly different according to DMRT (p<0.05), †: mg kg^{-1} soil, $^{\Delta}$SA: Soil Application, SP: Seed Pelleting and FS: Foliar Spray

accumulation were not significantly affected by Zn rates (Table 2). Interaction effects of Zn rates and application forms showed that the highest amount of Fe in leaves was in 10 mg Zn kg^{-1} soil^{-1} (foliar application), Zn in leaves was in 30 mg Zn kg^{-1} soil^{-1}, (seed pelleting), Cu in leaves was in 40 mg Zn kg^{-1} soil^{-1} (soil application), Mn and N in 40 and 20 mg Zn kg^{-1} soil^{-1} (seed pelleting), respectively (Table 3). Grain yield had significant correlation to Zn and Cu content in leaves. By contrast biological yield had significant and negative correlation to Zn content in leaves at p<0.05% probability levels (Table 4). Carsky and Reid (1990) showed the Zn concentration of whole young corn plants was significantly correlated with relative corn yield.

Elements content in seed: Results of ANOVA showed that Zn content of seed was effected by Zn rate in p≤0.01% probability levels. The application forms of Zn had significant effect on Fe and Zn content in seed in p≤ 0.01% probability levels (Table 1). In foliar application the concentration of Fe and Zn in seed was more than other application forms, However, Cu and Mn content in seed were not differed in any application forms (Table 2). The maximum Zn content in seed was observed in 40 and 20 mg kg^{-1} soil^{-1} (46.39 and 45.62 mg kg^{-1}), respectively (Table 2). Cichy *et al.* (2005) reported that reduce the incidence of Zn deficiency may be through the development of high Zn dry beans.

The maximum of Fe content in seed was obtained in spray and soil application forms of Zn fertilizer

Table 4: Correlation coefficients between studied traits

Trait		Leaf					Seed						
		Fe	Zn	Cu	Mn	N	Fe	Zn	Cu	Mn	Protein	Yield	Biomass
Leaf	Fe	1											
	Zn	0.53ns	1										
	Cu	-0.17ns	-0.35ns	1									
	Mn	0.01ns	0.88*	0.23ns	1								
	N	-0.28ns	0.37ns	0.38ns	-0.14ns	1							
Seed	Fe	0.81*	0.75*	-0.38ns	0.26ns	0.360ns	1						
	Zn	0.71ns	0.22ns	-0.31ns	0.28ns	0.130ns	0.00ns	1					
	Cu	0.06ns	0.31ns	-0.16ns	0.08ns	-0.590ns	0.90*	0.02ns	1				
	Mn	-0.61ns	0.38ns	-0.24ns	0.23ns	0.640ns	0.009ns	0.56ns	0.03ns	1			
	Protein	-0.02ns	0.09ns	0.04ns	-0.32ns	0.001ns	0.32ns	0.21ns	-0.89*	0.03ns	1		
	Yield	0.59ns	0.78*	0.84*	0.22ns	-0.001ns	-0.06ns	-0.08ns	-0.91*	-0.40ns	0.00ns	1	
	Biomass	0.65ns	-0.92ns	0.69ns	0.26ns	-0.040ns	-0.34ns	0.93*	0.91*	-0.70ns	-0.30ns	0.00ns	1

*Significant at the 5 levels of probability, ns: non significant

(92.90 and 81.62 mg kg^{-1}) (Table 2). Also, the maximum amount of Zn content in seed was belonged to spray application form of Zn (47.24 mg kg^{-1}). In these cases, Haslett *et al*. (2001) showed that foliar application of Zn in inorganic or organic forms is equally suitable for providing adequate Zn nutrition to wheat. Interaction effects between Zn rates and application forms showed that the highest concentration of Fe in seed was observed in 40 mg Zn kg^{-1} $soil^{-1}$ in foliar application. But, the highest content of Cu was belonged to 40 mg Zn kg^{-1} $soil^{-1}$ with seed pelleting treatment (Table 3). Also, Fe content of seed had significant and positive correlation with Fe content in leaves (r = 0.81*) (Table 4). In wheat, grain Zn were correlated with Fe, P, Mg and S and positive correlations between grain Fe and Zn have been reported in which it may reflect common transport mechanisms to grains (McDolanld *et al*., 2007; Garvin *et al*., 2006). Grain yield had a significant negative correlation to Zn amount of seed (Table 4).

Protein content of seed: Variance analysis (Table 1) showed that all treatments had signification effects on protein content at 1% levels of probability, in which the highest protein content of seed was obtained in 20 mg Zn kg^{-1} $soil^{-1}$. Bakhash Kelarestaghi *et al*. (2007) also reported that using Zn fertilizer increased grain yield and protein percentage up to 25 and 40%, respectively. Meanwhile, the highest and lowest protein was observed in soil application and seed pelleting form of Zn (Table 2).

The protein content was almost stable when Zn levels increased from 20 to 40 mg kg^{-1} $soil^{-1}$ (Table 2). Interaction effects between rate and application forms of Zn were significant ($p \leq 0.01\%$). The lowest protein content in bean seed was obtained in spray application of Zn fertilizer and different levels of Zn (Table 3). The most fundamental effect of zinc on protein metabolism is through its involvement in the stability and function of genetic material (Brown *et al*., 1993).

CONCLUSION

Zinc is an element required for growth and reproduction of plants. When zinc fertilizer applied to soils, the balance of other micronutrients and macronutrients could be affected. In this experiment, Zn application forms and rates had significant effect on protein content and accumulation of some elements in seed and leaves of bean. The different plant response observed upon these three different ways might be related to different Zn-uptake strategies in leaf and root cells. The highest Zn content in seed was observed in 20 and 40 mg Zn kg^{-1} $soil^{-1}$ (46.39 and 45.62 mg kg^{-1}), respectively. The highest Cu and Mn contents were obtained when 40 mg Zn kg^{-1} $soil^{-1}$ was applied. All treatment of Zn had not significant effects on Cu and Mn content in bean seed. The highest protein was observed in soil application and in seed pelleting the lowest protein content produced. Grain yield had significant correlation to zinc and Cu of leaves but, biomass had significant and negative correlation to Zn leaves in $p<5\%$ probability levels.

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