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The Effect of Different Zinc Application Methods on Yield and Grain Zinc Concentration of Bread Wheat Varieties

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A B S T R A C T

This study was carried out to elucidate the impacts of zinc (Zn) treatments on growth, development, quality and yield of commonly sown bread wheat cultivars under field conditions of Çukurova Region. Three different bread wheat cultivars (Adana-99, Ceyhan-99 and Pandas) were experimented in randomized complete blocks-split plots experimental design with 3 replications. Field experiments were performed by two different Zn application methods; via soil and via soil+foliage. In the both trials, 0, 5, 10, 20, 30, and 40 kg ha^{-1} pure Zn doses were applied to the soil. 0.4% $ZnSO_4.7H_2O$ solution was used for foliar Zn applications. Current findings revealed that Zn treatments had significant effects on grain yield, grain Zn concentration, grain phosphorus (P) concentration and thousand grain weight of bread wheat cultivars, but significant effects were not observed on grain protein concentrations. Soil+foliar Zn treatments were more effective in improving grain Zn concentrations. It was concluded that 10- 20 kg ha^{-1} Zn treatment was quite effective on grain Zn concentrations.

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Introduction

Micronutrient deficiency is a common health issue throughout the world and the problem is common in developing countries effecting almost three billion people (Graham et al., 2001; Welch and Graham 2004; Cakmak et al., 2010b). Among the micronutrients, zinc (Zn) deficiency is a widespread health problem in humans and animals. It is also a quite common case in plants (Cakmak, 2008). It can strictly restrict plant yields. Soil Zn deficiency may be reflected in plants and may create serious risks for human and animal health through plant-originated foods.

Zinc deficiency and other micro element deficiencies have reached to astonishing levels especially in developing countries. Excessive cereal-originated food consumption can be nominated as the primary reason for common Zn deficiency worldwide. Cereals are the primary mineral element and protein source of humans in developing countries (Hotz and Braun, 2004; Cakmak, 2008). In a global study carried out by FAO, Zn deficiency was reported for about 30% of world agricultural lands (Sillanpaa, 1982). Extensive soil analyses carried out recently also indicated Zn deficiency in a significant portion of Turkish agricultural lands (Eyüpoğlu et al., 1995).

Zinc and vitamin-A deficiencies are the most common deficiencies in children with serious impacts on child mortality levels (Black et al., 2008). Zn-deficiency results in deaths of around 500 000 children each year corresponding around 4.4% of total child mortality (Black et al., 2008). There is an urgent need today to enrich Zn-concentration of wheat and other plant grains commonly consumed by humans. It was observed that Zn-enriched cereal grains may significantly reduce child deaths in India (Stein et al., 2007). Development of Zn-rich cultivars or the use of Zn-fertilizers are significant practices to enrich the Zn-contents of grains (Pfeiffer and Mc Clafferty, 2007; Cakmak, 2008).

Very low grain protein content may be the main reason for low amounts of Zn and Fe in cereal grains. Grain Zn and Fe concentrations show a significant positive correlation with grain protein content in a number of wheat collections studied by Cakmak et al. (2010a). Genes affecting accumulations of Zn, Fe and protein in grains are closely linked in Triticum dicoccoides (Cakmak et al., 2004; Uauy et al., 2006). Uptake, translocation, remobilization and grain allocation of Zn and Fe in wheat may be enhanced by improved N nutritional status of this crop due to the enhancesin the abundance of transporter proteins and nitrogenous chelators (Uauy et al., 2006; Cakmak et al., 2010b). High protein and amino acid concentrations in grain can also result with higher bioavailability of micronutrients in the diet (House et al., 1996; Lonnerdal, 2000).

Anti-nutritional components like phytic acid (PA) is also limiting the nutritional level of cereals. Phytic acid binds to metal cations to form insoluble complexes (Raboy, 2002). High phytic acid and low mineral micronutrient contents can cause health problems, related to Fe, Zn, and Mg deficiencies (Welch and Graham, 2002). PA reduces the absorption of dietary Zn and Fe in monogastric animals and humans (Reddy et al., 1989). PA/Zn or PA/Fe molar ratio is an indicator for the bioavailability of Zn and Fe (Ryan et al., 2008; Simic et al., 2009). Also 70-80% of the seed P is found in the form of phytic acid in seeds (Erdal et al., 2002; Raboy et al., 1991).

Agricultural approaches have come into prominence as an alternative solution for micro element deficiency problems. Breeding of new cereal genotypes rich in Zn and/or widespread use of micro element-containing fertilizers are considered among the significant strategies to overcome such deficiencies (Cakmak, 2008).

Among micro elements, Zn has a special significance since it both restricts plant yields and reduces product quality. The present study was carried out to determine the impacts of soil and soil+foliar zinc treatments on thousand grain weight, yield, grain Zn and P concentrations and protein concentration of bread wheat cultivars commonly sown in Çukurova region.

Materials and Methods

This study was carried out in the experimental fields of Eastern Mediterranean Agricultural Research Institute during 2005-2006 and 2006-2007 wheat growing seasons. Total annual precipitation in the first and second seasons were 414 mm and 657 mm, respectively. Differences were observed in distribution of the precipitations in years throughout growing seasons. There have been significant differences in the distribution of rainfall over the years during the trials. There was no rainfall during 13 November-20 January in the second wheat season of trials (Table 1). Drought conditions effected emergence and tillering in this season.

The research area is composed of clay loamy soil, slightly alkaline reaction, slightly salted, high lime content, low organic matter, low P content, high potassium (K) content and low Zn concentration (Table 2).

Soil texture was analyzed by using hydrometer method (Bouyoucus, 1952), Scheibler calcimeter was used to measure soil lime contents (Cağlar, 1949), Walkey-Black method was used to determine soil organic matter content (Jackson, 1959), Wheatstone bridge method was used to measure soil salinity from saturation paste extracts (U. S. Salinity Laboratory Staff, 1954). A pH meter was used to measure soil pH from the same extract (Jackson, 1959). Available P and K levels were measured according to principles specified in Olsen et al. (1954) and Jackson (2005) respectively. Soil extractable Zn was determined according to method of Lindsay and Norvell (1978) by exraction with DTPA (diethylenetriamine pentaacetic acid) using a soil:solution ratio of 1:2 and shaking time of two hours.

In the study, two different field experiments were conducted where one involved Zn application to soil and other to soil+leaf. Zinc doses and varieties were research subjects in both trials. Trials were set up in 3 replicates, according to the split parcel trial design in randomized blocks. According to this; Zinc doses were randomly distributed to the main plots and varieties were randomly distributed to the sub-plots. Adana-99, Ceyhan-99 and Pandas bread wheat varieties were used in the research. Sub-plots had 5 rows and were 5 m long. All cultivars were sown at a sowing rate of 400 grain m^{-2}. Zn doses were 0, 5, 10, 20, 30, and 40 kg Zn ha^{-1} and treatments were application through soil and soil+foliar. For soil zinc treatments, zinc was sprayed on the soil surface before sowing and then incorporated with a disk harrow. 22% zinc sulphate ($ZnSO_4.7H_2O$) was used as Zn source for this aim. 0.4% $ZnSO_4$ solution (based on 500 gr Zn ha^{-1}) was used for foliar zinc treatments. Treatments of foliar solutions were done twice during tillering and bolting periods in 15 day intervals. 60 kg N ha^{-1} in ammonium sulphate form was applied at sowing time for base fertilization and additional 100 kg N ha^{-1} in urea form was applied at bolting stage for topdressing. Phosphorus (P) was applied in form of TSP (42-44%) in dose of 60 kg P ha^{-1} and incorporated into soil before sowing.

Table 1 Climatic datas related to growing seasons of the trials.

Months	Temperature (°C)			Precipitation (mm)			Relative Humidity (%)		
	Long term	I. Year	II. Year	Long term	I. Year	II. Year	Long term	I. Year	II. Year
October	21.6	19.8	21.0	43.5	37.9	156.3	60	61	71
November	15.3	13.9	13.2	73.9	64.6	91.5	63	67	65
December	11.1	12.1	9.3	124.4	64.1	0.0	67	70	58
January	9.7	8.8	8.7	109.4	36.3	34.1	65	63	63
February	10.4	10.6	11.2	88.9	131.6	127.0	65	73	72
March	13.3	14.1	14.2	65.8	46.2	75.7	65	76	70
April	17.5	18.5	16.6	52.5	9.3	115.4	67	71	64
May	21.7	22.4	23.5	47.0	19.8	32.0	66	69	70
June	25.6	26.0	26.0	20.6	4.5	25.0	67	73	69
Total				626	414.3	657			

Table 2 Soil physical and chemical characteristics of the field experiment location.

Year	Texture (%)				pH	Salt	$CaCO_3$	Org. M.	P_2O_5	K_2O	Zn
	Sand	Silt	Clay	Class	(1:2.5)	(mmhos/cm)	(%)	(%)	(kg da^{-1})		(mg kg^{-1})
I. Year	43	29	27	CL	7.85	0.15	15.2	1.4	30.2	128	0.23
II. Year	26	39	36	CL	7.87	0.28	14.2	1.9	30.0	93	0.25

Table 3 Results of zinc treatments on grain yields (kg ha^{-1}) of bread wheat cultivars.

Grain yields (kg ha^{-1})		Soil Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	4620^{e-j}	4530^{f-j}	5650^{a-d}	5540^{a-e}	5790^{abc}	5390^{a-g}	5250
	Ceyhan-99	4530^{f-j}	5080^{a-h}	5170^{a-h}	5820^{ab}	5080^{a-h}	4840^{c-j}	5090
	Pandas	4030^{j-k}	5230^{a-h}	5250^{a-h}	4760^{d-j}	4430^{g-j}	4760^{d-j}	4740
	Avr.	4390	4940	5360	5370	5100	5000	5030^{ARC}
2nd Year	Adana-99	4530^{f-j}	5650^{a-d}	5170^{a-h}	5080^{a-h}	5010^{a-h}	4370^{hij}	4970
	Ceyhan-99	4060^{ijk}	4880^{b-j}	5070^{a-h}	5570^{a-e}	4940^{a-j}	4800^{d-j}	4890
	Pandas	3310^{k}	4810^{d-j}	5450^{a-f}	5210^{a-h}	5870^{a}	4020^{jk}	4780
	Avr.	3970	5110	5230	5290	5270	4400	4880^{ARC}
General Mean.	Adana-99	4580	5090	5410	5310	5400	4880	5110
	Ceyhan-99	4300	4980	5120	5700	5010	4820	4990
	Pandas	3670	5020	5350	4980	5150	4390	4760
	Avr.	4180^{c}	5030^{a}	5290^{a}	5330^{a}	5190^{a}	4700^{b}	
CV (%)	11.94							
LSD	year: (ns), * cultivar: (ns), cultivar x year int.: (ns), **zinc: 311.1, zinc x year int.: (ns), zinc x cultivar int.: (ns), *zinc x cultivar x year int.: 971.2, ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							
Grain yields (kg ha^{-1})		Soil + Foliar Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	4940	5650	6010	6930	6010	5700	5870^{a}
	Ceyhan-99	4860	6110	5500	6420	4810	5310	5500^{b}
	Pandas	4390	5450	5330	5440	5250	4240	5020^{c}
	Avr.	4730	5740	5620	6260	5360	5080	5460^{aARC}
2nd Year	Adana-99	3890	4860	4410	5120	4670	4400	4560^{d}
	Ceyhan-99	4060	4270	4910	4860	4920	4950	4660^{d}
	Pandas	3700	4630	4700	5160	5610	3800	4600^{d}
	Avr.	3880	4580	4670	5050	5070	4380	4610^{bARC}
General Mean.	Adana-99	4420	5250	5210	6020	5340	5050	5220^{a}
	Ceyhan-99	4460	5190	5200	5640	4860	5130	5080^{a}
	Pandas	4050	5040	5020	5300	5430	4020	4810^{b}
	Avr.	4310^{d}	5160^{b}	5140^{b}	5650^{a}	5210^{b}	4730^{c}	
CV (%)	10.26							
LSD	**year: 179.0, **cultivar:245.0, **cultivar x year int.: 346.0, ** zinc: 310.0, zinc x year int. (ns), zinc x cultivar int. (ns), zinc x cultivar x year int. (ns), ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							

For the analysis of mineral nutrients, ground grain samples were subjected to acid-digestion (ca. 0.2 g sample in 2 ml 30% H_2O_2 and 5 ml 65% HNO_3) in a closed vessel microwave system (MarsExpress; CEM Corp., Matthews, NC, USA). After digestion, the total sample volume was finalized to 20 ml by adding double-deionized water. Concentrations of mineral nutrients Zn were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista-Pro Axial, Varian Pty Ltd, Mulgrave, Australia). Measurements were checked by using certified standard reference materials obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Grain P levels were analyzed spectrophotometrically in accordance with Barton (1948) method. Total N content was determined by using the Dumas combustion method (Leco FP-428 analyzer). Grain protein was determined by multiplying the total N by 5.7 as a conversion factor (Anon, 2000; Elgün et al., 2002).

The analysis was performed using analysis of variance (ANOVA).Experimental results were subjected to LSD test using JUMP statistical software.

Results and Discussion

Effects of Treatments on Grain Yield

Grain yields of bread wheat cultivars in two trials (soil and soil+foliar zinc treatment trials) are given in Table 3. In soil Zn treatments trial, treatments had positive significant effects (1%) on yields. The greatest yield was obtained with 20 kg Zn ha^{-1} where 27.5% yield increase occured over control treatment where 5, 10 and 30 kg Zn ha^{-1} was also located in the same group. Yields decreases were observed when 40 kg ha^{-1} Zn applied. The differences in grain yields of the cultivars were significant at 5% level. General means of grain yields of cultivars in soil application trial were 5110, 4990 and 4760 kg ha^{-1} for Adana-99, Ceyhan-99 and Pandas, respectively. Zinc x cultivar x year interaction was also found significant (5%) with regard to grain yield (Table 3).

In soil+foliar Zn trial, grain yields were changed significantly (1%) positive (Table 3). However, yield decreases were observed with 40 kg ha^{-1} Zn treatment. The greatest yield was obtained from 20 kg Zn ha^{-1} soil+foliar treatment with 31.1% increase compared to control treatment. The differences in grain yields based on years were found significant (1%). Differences in yields of the cultivars were also significant (5%). Adana-99 cultivar had a grain yield of 5220 kg ha^{-1} and it was followed by Ceyhan-99 with 5080 kg ha^{-1} and Pandas cultivar with 4810 kg ha^{-1}. Differences in climatic conditions at different years interacted significantly with Zn doses (Table 3). High but irregular precipitation during second growing season might be the reason for low yield results occurred in second year compared to first year both in two trials. There were no rainfall during 13 November-20 January in the second wheat season of trials. Drought conditions effected emergence and tillering in this season (Table 1).

It was reported that Zn treatments provided 5-550% increases in grain yields of wheat in different regions of Central Anatolia Region of Turkey (Cakmak et al., 1995). Besides, Kalaycı et al. (1993) reported 50-60% increases in wheat and barley grain yields with soil Zn treatments. In a study of Gomez-Coronado et al. (2016) foliar application was not significantly effected grain yield, but increased yield about 10% in soil and 7% in soil+foliar applications. In another study (Kalaycı et al., 1996; Yılmaz et al., 1996), where soil, seed, foliar and combined treatments compared, soil and seed treatments were found more effective than foliar treatments. There exists researches with various positive effects of Zn treatments on yields of other crops (Togay et al., 2005; Daghan et al., 2013; Cakmak et al., 2010a; Zou et al., 2012).

Table 4 Effects of zinc treatments on grain zinc concentrations (mg kg^{-1}) of cultivars.

Grain zinc concentrations (mg kg^{-1})		Soil Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	23.2	26.1	24.0	26.7	31.0	23.5	25.7^{de}
	Ceyhan-99	22.5	24.4	24.1	24.3	27.1	29.0	25.2^{e}
	Pandas	24.6	25.2	26.7	29.0	29.4	28.1	27.2^{cd}
	Avr.	23.4^{f}	25.2^{ef}	24.9^{ef}	26.7^{de}	29.2^{bcd}	26.9^{de}	26.1^{bARC}
2nd Year	Adana-99	27.2	27.3	31.8	29.0	32.2	31.6	29.9^{ab}
	Ceyhan-99	29.9	27.0	36.5	28.4	29.4	32.1	30.6^{a}
	Pandas	23.4	27.4	33.0	31.1	29.9	26.9	28.6^{bc}
	Avr.	26.8^{de}	27.2^{cde}	33.8^{a}	29.5^{bcd}	30.5^{b}	30.2^{bc}	29.7^{aARC}
General Mean.	Adana-99	25.2^{fg}	26.7^{efg}	27.9^{b-f}	27.8^{b-f}	31.6^{a}	27.6^{c-f}	27.8
	Ceyhan-99	26.2^{efg}	25.7^{efg}	30.3^{abc}	26.4^{efg}	28.3^{b-e}	30.6^{ab}	27.9
	Pandas	24.0^{g}	26.3^{efg}	29.9^{a-d}	30.1^{a-d}	29.7^{a-d}	27.5^{def}	27.9
	Avr.	25.1^{c}	26.2^{bc}	29.4^{a}	28.1^{ab}	29.8^{a}	28.5^{a}	
CV (%)	8.62							
LSD	**year: 1.28, cultivar (ns), **cultivar x year int. : 1.61, **zinc: 2.22, *zinc x year int. : 3.13, *zinc x cultivar int. : 2.78, zinc x cultivar x year int. (ns), ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							

Grain zinc concentrations (mg kg^{-1})		Soil + Foliar Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	22.6^{o}	30.4^{j-m}	31.0^{j-m}	38.2^{gh}	35.2^{g-j}	30.8^{j-m}	31.4^{d}
	Ceyhan-99	24.7^{n-o}	27.7^{l-o}	32.0^{j-m}	29.2^{k-n}	32.8$^{\text{ı-l}}$	33.7^{h-k}	30.0^{d}
	Pandas	24.1^{n-o}	28.8^{k-n}	31.1^{j-m}	31.4^{j-m}	32.8$^{\text{ı-l}}$	31.8^{j-m}	30.0^{d}
	Avr.	23.8^{f}	29.0^{de}	31.4^{cd}	32.9^{c}	33.6^{c}	32.1^{cd}	30.5^{bARC}
2nd Year	Adana-99	27.1^{mno}	42.4^{cf}	44.1^{cde}	50.3^{ab}	51.3^{a}	50.6^{a}	44.3^{a}
	Ceyhan-99	30.6^{j-m}	40.1^{d-g}	50.6^{a}	45.0^{bcd}	43.8^{de}	38.0$^{\text{f-ı}}$	41.3^{b}
	Pandas	23.0^{o}	38.0$^{\text{f-ı}}$	41.1^{c-f}	39.0^{e-h}	41.8^{c-f}	46.3^{abc}	38.2^{c}
	Avr.	26.9^{ef}	40.2^{b}	45.3^{a}	44.8^{a}	45.7^{a}	45.0^{a}	41.3^{aARC}
General Mean.	Adana-99	24.8$^{\text{hı}}$	36.4^{d-g}	37.6^{b-f}	44.3^{a}	43.3^{a}	40.7^{abc}	37.8^{a}
	Ceyhan-99	27.7^{h}	33.9^{fg}	41.3^{ab}	37.1^{c-g}	38.3^{be}	35.8^{d-g}	35.7^{b}
	Pandas	23.5$^{\text{ı}}$	33.4^{g}	36.1^{d-g}	35.2^{d-g}	37.3^{c-f}	39.0^{bcd}	34.1^{c}
	Avr.	25.3^{c}	34.6^{b}	38.3^{a}	38.8^{a}	39.6^{a}	38.5^{a}	
CV (%)	9.07							
LSD	**year: 1.55, **cultivar: 1.54, * cultivar x year int. : 2.18 , **zinc: 2.68, **zinc x year int. : 3.79, ** zinc x cultivar int.: 3.78, **zinc x cultivar x year int.: 5.34, ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							

Table 5 Effects of zinc treatments on grain grain phosphorus concentrations (%) of cultivars.

Grain phosphorus concentrations (%)		Soil Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	0.307^{c-j}	0.270^{k-l}	0.293^{e-l}	0.267^{l}	0.280^{h-l}	0.270^{jkl}	0.282^{c}
	Ceyhan-99	0.373^{a}	0.287^{f-l}	0.283^{g-l}	0.220^{m}	0.280^{h-l}	0.280^{h-l}	0.287^{c}
	Pandas	0.347^{ab}	0.270^{kl}	0.280^{h-l}	0.277$^{ı-l}$	0.280^{h-l}	0.320^{b-f}	0.296^{bc}
	Avr.	0.342^{a}	0.276^{e-f}	0.286^{de}	0.254^{f}	0.280^{e}	0.291^{cde}	0.288^{bARC}
2nd Year	Adana-99	0.333^{bcd}	0.340^{abc}	0.313^{b-h}	0.313^{b-h}	0.323^{b-e}	0.317^{b-g}	0.323^{a}
	Ceyhan-99	0.340^{abc}	0.283^{g-l}	0.320^{b-f}	0.320^{b-f}	0.303^{d-k}	0.333^{bcd}	0.317^{a}
	Pandas	0.337^{bcd}	0.310$^{c-ı}$	0.337^{bcd}	0.293$^{e-ı}$	0.310$^{c-ı}$	0.267^{l}	0.309^{ab}
	Avr.	0.337^{a}	0.311^{bc}	0.323^{ab}	0.309^{bcd}	0.312^{bc}	0.306^{bcd}	0.316^{aARC}
General Mean.	Adana-99	0.320	0.305	0.303	0.290	0.302	0.295	0.303
	Ceyhan-99	0.357	0.295	0.302	0.270	0.292	0.307	0.302
	Pandas	0.342	0.290	0.308	0.285	0.295	0.293	0.302
	Avr.	0.339^{a}	0.293^{bc}	0.304^{b}	0.282^{c}	0.296^{bc}	0.298^{bc}	
CV (%)	7.33							
LSD	**year: 0.010, cultivar (ns), *cultivar x year int.: 0.015 , **zinc : 0.017, *zinc x year int.: 0.024, zinc x cultivar int. (ns), **zinc x cultivar x year int.: 0.036, ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							

Grain phosphorus concentrations (%)		Soil + Foliar Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	0.300	0.283	0.293	0.293	0.283	0.280	0.288^{c}
	Ceyhan-99	0.386	0.263	0.306	0.290	0.296	0.276	0.303^{bc}
	Pandas	0.336	0.256	0.270	0.286	0.276	0.323	0.291^{c}
	Avr.	0.341^{a}	0.267^{f}	0.290^{de}	0.290^{de}	0.285^{ef}	0.293^{ef}	0.294^{bARC}
2nd Year	Adana-99	0.363	0.343	0.343	0.336	0.340	0.293	0.339^{a}
	Ceyhan-99	0.330	0.300	0.330	0.310	0.310	0.313	0.315^{b}
	Pandas	0.323	0.306	0.306	0.313	0.300	0.296	0.307^{b}
	Avr.	0.338^{a}	0.316^{bc}	0.326^{ab}	0.320^{bc}	0.316^{cd}	0.306^{cd}	0.320^{aARC}
General Mean.	Adana-99	0.332^{b}	0.313^{bcd}	0.318^{bc}	0.315^{bc}	0.311^{bcd}	0.295^{cde}	0.314^{a}
	Ceyhan-99	0.358^{a}	0.281^{e}	0.318^{bc}	0.300^{cde}	0.303^{cde}	0.295^{cde}	0.309^{ab}
	Pandas	0.330^{b}	0.281^{e}	0.288^{de}	0.300^{cde}	0.288^{de}	0.310^{cd}	0.299^{b}
	Avr.	0.340^{a}	0.292^{c}	0.308^{b}	0.305^{bc}	0.301^{bc}	0.300^{bc}	
CV (%)	7.17							
LSD	**Year: 0.008, *cultivar: 0.010, **cultivar x year int.: 0.015, **zinc: 0.013, **zinc x year int.: 0.019, *zinc x cultivar int.: 0.026, zinc x cultivar x year int.: ns, ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							

Wheat response to Zn treatments was investigated in 3 wheat cultivars under Eskişehir conditions between the years 1993-1996. Different Zn doses were applied through soils, leaves and seeds. Results revealed that soil Zn treatments provided significant increases in yield and yield components and it was followed respectively by seed and foliar Zn treatments (Özbek and Özgümüş, 1998). In a parallel study carried out at Anadolu Agricultural Research Institute and Konya Bahri Dağdaş International Winter Cereals Research Center, respectively 35 and 69% increases were obtained in wheat yield with soil Zn treatments (Cakmak, 1994). Quite similar outcomes were also indicated by other researchers (Ceylan et al., 1998; Özbek and Özgümüş, 1998; Yılmaz et al., 1998; Taban et al., 1998; Mungan and Doran, 2003). Previous reserachers also indicated various positive effects of Zn treatments on yield levels of several crops (Cakmak et al., 1998; Kalayci et al., 1999; Togay et al., 2005; Daghan et al., 2013).

Effects of Treatments on Grain Zinc Concentrations

Zinc concentrations of bread wheat cultivar grains at soil and soil+foliar Zn treatment trials are given in Table 4. Effects of soil treatments on grain Zn concentrations were significant (1%). Grain Zn concentration was 25.1 mg kg^{-1} in control treatment while, increased to 29.4 mg kg^{-1} with 10 kg ha^{-1} Zn treatment. Further increments did not increase concentration significantly. Zinc concentration of the first and second year was measured as 26.1 and 29.7 mg kg^{-1}, respectively. Differences in zinc concentrations of the cultivars were not significant (Table 4).

Effects of soil+foliar Zn treatments on grain Zn concentrations were found significant (1%) (Table 4). While grain Zn concentration was found as 25.3 mg kg^{-1} in control treatment, it increased with soil+foliar Zn treatments and the values varied between 34.6-39.6 mg kg^{-1}. Zn concentrations of grains were increased 51.0-56.5% by Zn application compared to control. Significant differences were observed in grain Zn concentrations of the years (1%) where first and second year was measured as 30.5 and 41.3 mg kg^{-1}, respectively. The differences in Zn concentrations of the cultivars were significant (1%). Grain zinc concentration was measured as 37.8 mg kg^{-1} in Adana-99 cultivar, 35.7 mg kg^{-1} in Ceyhan-99 cultivar and 34.1 mg kg^{-1} in Pandas cultivar (Table 4).

Cakmak et al. (2010a) reported that soil and foliar combined treatment are the most efficient method to improve grain Zn concentrations. Yılmaz et al. (1998) reported significant increases in grain yield, plant and grain Zn concentrations with soil, foliar, seed and combined Zn treatments varied based on genotypes and species. Foliar Zn treatments may increase Zn levels of both the whole grain and the endosperm (Jiang et al., 2007; Cakmak et al., 2010a, Phattarakul et al., 2012, Zou et al., 2012; Zhang et al., 2012; Xue et al., 2012).

Effects of treatments on grain phosphorus concentrations

Effects of soil and soil+foliar Zn treatments on grain P concentrations (%) of bread wheat cultivars are provided in Table 5. According to combined analysis results (2005-2006 and 2006-2007), the effects of soil Zn treatments on grain P concentrations were found to be significant (1%). Zn treatments decreased grain P concentrations. While grain P concentration was 0.339% in control treatment, grain P concentrations decreased with soil Zn treatments. The lowest value was observed in 20 kg ha^{-1} soil Zn treatment (0.282%). Significant differences were observed between the years (1%). While grain P concentration was measured as 0.288% in the first year, the value was measured as 0.316% in the second year. Significant differences were not observed among grain P concentrations of the cultivars (Table 5).

Table 6 Effects of zinc treatments on grain weights (g) of cultivars.

Grain weights (g)		Soil Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	40.4	46.1	42.9	42.9	45.4	43.2	43.5^{a}
	Ceyhan-99	39.8	45.5	44.8	47.7	45.1	44.6	44.6^{a}
	Pandas	41.0	46.5	47.1	45.2	44.9	42.2	44.5^{a}
	Avr.	40.4^{c}	46.0^{a}	44.9^{ab}	45.3^{a}	45.1^{a}	43.3^{b}	44.2^{aARC}
2nd Year	Adana-99	35.6	35.8	38.1	37.2	37.7	37.3	37.0^{c}
	Ceyhan-99	33.7	33.8	36.9	34.6	35.8	37.3	35.3^{d}
	Pandas	38.5	39.9	38,0	40.1	40.8	39.5	39.5^{b}
	Avr.	35.9^{e}	36.5^{de}	37.7^{d}	37.3^{de}	38.1^{d}	38.0^{d}	37.3^{bARC}
General Mean.	Adana-99	38.0	41.0	40.5	40.0	41.6	40.2	40.2^{b}
	Ceyhan-99	36.7	39.7	40.9	41.1	40.5	40.9	40.0^{b}
	Pandas	39.7	43.2	42.6	42.6	42.9	40.9	42.0^{a}
	Avr.	38.2^{b}	41.3^{a}	41.3^{a}	41.3^{a}	41.6^{a}	40.7^{a}	
CV (%)	4.5							
LSD	**year: 0.69, ** cultivar: 0.87, **cultivar x year int. : 1.22, ** zinc:1.20, **zinc x year int. :1.70, zinc x cultivar int. (ns), zinc x cultivar x year int. (ns), ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							
Grain weights (g)		Soil + Foliar Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	41.0	43.3	45.9	44.4	42.3	42.9	43.3^{b}
	Ceyhan-99	41.2	46.7	47.3	47.3	44.2	42.9	44.9^{a}
	Pandas	42.6	46.2	44.8	45.5	44.3	41.9	44.2^{ab}
	Avr.	41.6^{d}	45.4^{ab}	46.0^{a}	45.7^{a}	43.6^{bc}	42.6^{cd}	44.1^{aARC}
2nd Year	Adana-99	35.7	36,0	36.4	37,0	37.5	36.8	36.6^{d}
	Ceyhan-99	34.8	38.2	35.1	35,0	35.3	38.1	36.1^{d}
	Pandas	39.0	43.0	40.3	40.7	41.5	38.9	40.6^{c}
	Avr.	36.5^{f}	39.0^{e}	37.3^{ef}	37.6^{ef}	38.1^{ef}	37.9^{ef}	37.7^{bARC}
General Mean.	Adana-99	38.4	39.6	41.2	40.7	39.9	39.9	39.9^{c}
	Ceyhan-99	38.0	42.4	41.2	41.2	39.7	40.5	40.5^{b}
	Pandas	40.8	44.6	42.5	43.1	42.9	40.4	42.4^{a}
	Avr.	39.1^{c}	42.2^{a}	41.6^{ab}	41.6^{ab}	40.8^{ab}	40.3^{bc}	
CV (%)	5.1							
LSD	**year: 0.81, **cultivar: 0.99, **cultivar x year int. : 1.40, **zinc: 1.40, *zinc x year int.: 1.98, zinc x cultivar int. (ns), zinc x cultivar x year int. (ns), ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							

Table 7 Effects of zinc treatments on protein contents (%) of cultivars.

Grain protein contents (%)		Soil Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	12.5	11.8	12.4	12.5	12.4	11.8	12.2
	Ceyhan-99	12.9	11.5	12.6	11.6	12.8	12.1	12.2
	Pandas	12.8	12.3	12.2	12.1	13.3	13.6	12.7
	Avr.	12.7	11.9	12.4	12.0	12.8	12.5	12.4^{ARC}
2nd Year	Adana-99	11.7	11.9	12.1	12.2	11.6	12.2	11.9
	Ceyhan-99	12.1	11.7	11.9	12.4	11.6	12.9	12.1
	Pandas	11.8	12.9	12.6	12.7	12.8	13.4	12.7
	Avr.	11.9	12.2	12.2	12.4	12.0	12.9	12.2^{ARC}
General Mean.	Adana-99	12.1	11.8	12.2	12.3	12.0	12.0	12.1^{b}
	Ceyhan-99	12.5	11.6	12.2	12.0	12.2	12.5	12.2^{b}
	Pandas	12.3	12.6	12.4	12.4	13.0	13.5	12.7^{a}
	Avr.	12.3	12.0	12.3	12.2	12.4	12.7	
CV (%)	6.9							
LSD	year (ns), **cultivar: 0.41, cultivar x year int. (ns), zinc (ns), zinc x year int. (ns), zinc x cultivar int. (ns), zinc x cultivar x year int. (ns), ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							

Grain protein contents (%)		Soil + Foliar Treatment						
		Zinc Level (kg ha^{-1})						
		0	5	10	20	30	40	Avr
1st Year	Adana-99	13.4	14.4	13.0	13.0	13.4	13.4	13.4^{a}
	Ceyhan-99	12.5	13.0	12.6	12.6	11.9	12.3	12.5^{bc}
	Pandas	12.1	13.2	12.6	12.0	12.9	12.6	12.6^{b}
	Avr.	12.7	13.5	12.7	12.5	12.7	12.7	12.8^{aARC}
2nd Year	Adana-99	11.9	11.4	12.1	12.0	11.7	12.5	11.9^{c}
	Ceyhan-99	12.6	12.4	12.1	12.6	12.5	12.5	12.5^{bc}
	Pandas	12.5	10.9	12.3	12.8	12.5	12.9	12.3^{bc}
	Avr.	12.3	11.6	12.1	12.5	12.2	12.6	12.2^{bARC}
General Mean.	Adana-99	12.6	12.9	12.5	12.5	12.5	12.9	12.7
	Ceyhan-99	12.5	12.7	12.3	12.6	12.2	12.4	12.5
	Pandas	12.3	12.0	12.4	12.4	12.7	12.7	12.4
	Avr.	12.5	12.5	12.4	12.5	12.5	12.7	
CV (%)	7.3							
LSD	*year :0.37, cultivar (ns), **cultivar x year int.: 0.61, zinc (ns), zinc x year int. (ns), zinc x cultivar int. (ns), zinc x cultivar x year int. (ns), ARC: Average of rows and columns, *: P<0.05; **: P<0.01;ns: non significant							

Soil+foliar Zn treatments on grain P concentrations were found to be significant (1%) (Table 5). Compared to control treatment, soil+foliar Zn treatments decreased grain P concentrations. While grain P concentration was observed as 0.340% in control treatment, it decreased also with soil+foliar Zn treatments and the values varied between 0.292-0.308% (Table 5). Significant differences were observed in grain P concentrations of the years (1%). While grain P concentration was measured as 0.294% in the first year, the value was measured as 0.320% in the second year. Significant differences were not also observed between the cultivars (ns). Cultivar x year (1%), zinc x cultivar (5%) and zinc x cultivar x year interactions were also found to be significant (5%) with regard to grain P concentrations (Table 5). Differences in climate factors of the years and Zn doses resulted in significant interactions.

Phosphorus may restrict the Zn use in plant metabolism through various mechanisms. At high P concentration levels in plant tissues, P gets into complexes with Zn and consequently restricts Zn mobility (Cakmak and Marschner, 1987). Increasing P treatments may also increase the number of Zn-binding carboxyl groups in cell walls of plant tissues which result in Zn retention over cell walls and insufficient Zn use in metabolic processes (Younghdal et al., 1977). Increasing P treatments accelerate Zn deficiency symptoms under Zn deficient conditions. Significant decreases were observed in plant dry matter production with the emergence of Zn deficiency. Such a case indicates that P-Zn interaction commonly took place within the plant rather than outside of the plant (rhizosphere) (Cakmak and Marschner, 1986). High P doses may ultimately result in Zn deficiency in wheat through not hindering Zn uptake but restricting Zn use in physiological processes (Kalfa et al., 1988).

Cavdar et al. (1982) and Prasad (1984) indicated that Zn deficiency symptoms were commonly observed dominantly in cereals. Same researchers also pointed out that cereals had high levels of phytate, a P compound and phytic acid bound to Zn significantly restricted Zn bioavailability and ultimately result in Zn deficiency. In general, about 70-80% of seed P exists in the form of phytic acid. Thus, increasing phytic acid levels are observed in seeds with increasing P contents. Researchers indicated that if the phytic acid/Zn ratio of a diet in molls was over 25-30, then Zn bioavailability (nutritional value) of that diet would significantly decrease (Oberleas and Harland, 1981). Zn-poor food stuff are also poor in some organics and amino acids all improving bioavailability of Zn in human and animal cells. Insufficient levels of these substances were primarily resulted from the retard in protein synthesis.

Effects of treatments on thousand grain weight

Soil Zn treatments increased thousand grain weight significantly (1%) which was lowest in control (38.2 g) and highest (41.3 g) with 5 kg ha^{-1} Zn, but further increments in Zn dose did not have significant effects (Table 6). Also differences between cultivars were significant (1%) which were measured 40.2 g in Adana-99 cultivar, 40.0 g in Ceyhan-99 and 42.0 g in Pandas cultivars (Table 6).

Effects of soil+foliar Zn treatments were also resulted with significant (1%) increases on thousand grain weight (Table 6). While thousand grain weight was observed as 39.1 g in control treatment, 5 kg ha^{-1} soil+foliar Zn treatments increased this parameter to 42.2 g but further increments in Zn dose did not increase grain weights. Differences in cultivars were also significant (1%). The lowest thousand grain weight was 39.9 g in Adana-99 cultivar and it was followed by Ceyhan-99 cultivar with 40.5 g and Pandas cultivar with 42.4 g Cultivar x year interaction (1%) was also found to be significant with regard to thousand grain weight (Table 6). Differences in climate factors of the years resulted in significant interactions.

Togay et al. (2005) reported significant differences in thousand grain weight averages of the cultivars and indicated slight, but not significant, increases in thousand grain weights with Zn treatments. Some others reported insignificant increases in thousand grain weights with Zn treatments (Taban et al., 1998; Ceylan et al., 1998; Mungan and Duran, 2003). Increasing thousand grain weights were reported in different wheat genotypes with foliar Zn treatment (Mishra et al., 1989), unchanged values (El-Sayed et al., 1988) and decreasing values (Mandal and Singharoy, 1989) were also reported in previous studies.

Effects of treatments on grain protein contents

According to combined analysis results, the effects of soil Zn treatments on grain protein contents (%) were insignificant (Table 7). Also significant differences were not observed between years. On the other hand, the differences between cultivars were significant (1%). Grain protein content was measured as 12.1% in Adana-99 cultivar, 12.2% in Ceyhan-99 cultivar and 12.7% in Pandas cultivar. Feil and Fossati (1995) showed strong correlation between grain Zn and protein concentration (Table 7).

In soil+foliar Zn treatments, effect of treatments on grain protein contents were not significant. The differences between years were significant (1%) and respectively measured as 12.8 and 12.2% in first and second years. Significant differences were not observed between the cultivars, too (Table 7). Grain protein content was observed to be 12.7% in Adana-99 cultivar, 12.5% in Ceyhan-99 cultivar and 12.4% in Pandas cultivar (Table 7). Cultivar x year interaction (5%) was also found to be significant with regard to grain protein content. Differences in climate factors of the years resulted in significant interaction. Effects of zinc x year interaction on grain protein content were found to be in significant (Table 7). Grain Zn and Fe concentrations shows a significant positive correlation with grain protein content in a number of wheat collections studied by Cakmak et al. (2010a).

Conclusions

Results revealed that Zn treatments had significant effects on grain yield, grain Zn and P concentrations and thousand grain weights of bread wheat cultivars. Soil+foliar applications of Zn were more effective in improving grain Zn concentrations. It was concluded that application of 10-20 kg Zn per ha was quite effective on grain Zn concentrations.

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