J.Natn.Sci.Foundation Sri Lanka 2015 43 (4): 303 - 312
DOI: http://dx.doi.org/10.4038/jnsfsr.v43i4.7965

RESEARCH ARTICLE

Effect of Zn as soil addition and foliar application on yield and protein content of wheat in alkaline soil

Sabir Gul Khattak[1], Peter J. Dominy[2] and Wiqar Ahmad[3*]
[1] *Directorate of Soil and Plant Nutrition, Agriculture Research Institute, 24330 Tarnab, Peshawar, KPK, Pakistan.*
[2] *Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK.*
[3] *Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar, AMK Campus, Mardan, Pakistan.*

Revised: 28 February 2015; Accepted: 25 March 2015

Abstract: Zinc (Zn) deficiency in plant tissues is a reflection of both genetic and soil-related factors and is the most widespread problem in cereal crops worldwide, resulting in severe losses in the yield and nutritional quality. Developing cost-effective and quick solutions to Zn deficiency is, therefore, highly important. An experiment was conducted during 2009 − 2010, to assess the effects of various modes and concentrations of applied Zn on wheat yield and nutritional quality grown in alkaline soils.

Both soil addition and foliar spray of $ZnSO_4$ significantly increased grain yield, 1000 grain weight and grain protein content, while its effect on biological yield and grain protein composition was not significant. Foliar spray of 0.5 % and 1.0 % $ZnSO_4$ increased grain yield by 10 and 18.8 %, respectively while its soil application at the rate of 5, 10 and 15 kg ha^{-1} increased grain yield by 18, 32 and 41%, respectively over the control. The treatments receiving $ZnSO_4$ as 5 kg ha^{-1} soil + 1.0 % foliar, 15 kg ha^{-1} soil + 1.0 % foliar and 5 kg ha^{-1} soil + 0.5 % foliar application recorded 29.5, 29.0 and 27.5 % higher protein contents, respectively over the control. Comparing the value cost ratios (VCR) for the treatments showing higher grain yield and protein content, the VCR for 5 kg ha^{-1} $ZnSO_4$ as soil + 1.0 % $ZnSO_4$ as foliar (10.23) was three times higher than the VCR for 15 kg ha^{-1} $ZnSO_4$ as soil + 1.0 % $ZnSO_4$ as foliar (3.46), thus confirming the superiority of the former over the latter in terms of effectiveness. The results further revealed that despite presumably sufficient native Zn concentration in the soils under study (1.95 mg kg^{-1}), the crop responded positively to Zn treatment and therefore the Zn level of sufficiency (1 mg kg^{-1}) should be reconsidered in accordance with the nature and type of soils.

Keywords: Alkaline soil, foliar application of Zn, modes of Zn application, soil application of Zn, Zn effect on protein content, Zn effect on yield.

INTRODUCTION

Zinc (Zn) deficiency appears to be the most widespread and frequent micronutrient deficiency problem in crop and pasture plants worldwide, resulting in severe losses in yield and nutritional quality. This is particularly the case in cereal production areas and it is estimated that nearly half the soils on which cereals are grown have levels of available Zn low enough to cause Zn deficiency [50 % of soils in Turkey (Eyupoglu *et al*., 1994), 30 to 70 % of soils in India (Takkar, 1991), 70 % soils in Pakistan (Rashid *et al*., 1988), 2 m ha of paddy soils in Bangladesh and 8 m ha in China, Japan and the Philippines (Alloway, 2008; 2009)]. Since cereal grains have inherently low Zn concentrations, growing them on these potentially Zn-deficient soils further decreases the grain Zn concentration. It is therefore not surprising that the well-documented Zn deficiency problem in humans occurs predominantly in the countries/regions such as India, China, Pakistan and Turkey where soils are low in available Zn, and cereals are the major source of calorie intake (Alloway, 2008).

Although Zn is required in small amounts, its availability is critical for several key physiological functions in plants including growth regulation, photosynthesis and sugar formation, seed production, and defense mechanisms against various diseases. Its deficiency adversely affects these functions, thus resulting in lower yield and frequently in poor quality crop products (Tahir *et al*., 2009). Humans consuming crops grown on Zn deficient soils may suffer Zn deficiency

* Corresponding author (wiqar280@yahoo.co.uk)

(on average, one-third of the world's population, ranging from 4 to 73 % in different countries), and related health problems such as impairment in physical development, stunting in children, susceptibility to infectious diseases, increased morbidity and mortality, poor birth outcome in women and poor immune system and brain function (Hotz & Brown, 2004; Black *et al.*, 2008; Cakmak, 2008).

Alkaline soils with an apparently sufficient Zn level (>1 mg kg^{-1}) may show reduced Zn availability to crops due to (a) Zn affinity towards adsorption/fixation on the clay adsorption sites, and (b) the high pH of soil (pH 8.0), which might have helped in the formation of unavailable forms of Zn as hydroxides. Zn has greater affinity for adsorption on clay and also it tends to make unavailable zinc hydroxides due to increased pH (Khattak & Pulford, 1999). In such soils crops have responded positively to Zn application by showing increased protein content in grain as well as soil residual Zn concentration (Khattak *et al*., 2006). A portion of additional Zn probably helps in satisfying the adsorption sites and some of it might be retained as available Zn to plants in solution form. Besides fixation at adsorption sites and the formation of zinc hydroxide in alkaline conditions, low availability of Zn is also attributed to a number of soil and environmental factors including low soil organic matter, calcareous nature of soil, water logging and arid climate (Morvedt *et al*., 1991; Tandon, 1995; Cakmak *et al*., 1998).

Estimates suggest that 25 % of the world's population is at risk of Zn deficiency (Maret & Standstead, 2006). Several approaches have been made to overcome Zn deficiency in humans and these include Zn supplementation, food diversification as well as food fortification (Ahmad *et al.*, 2012). Increasing the Zn content in food crops may be a good strategy to overcome its deficiency in people in developing countries. Many researchers endeavoured to chalk out various strategies of its better supplementation, which included; Zn application to soil in the form of $ZnSO_4$, Zn chelates, soil and foliar spray with different Zn compounds and seed priming (Singh & Abrol, 1985; Yilmaz *et al.,* 1997; Cakmak *et al.,* 1998; Amar *et al*., 2000; Khattak *et al*., 2006; Khan *et al*., 2008; Maqsood *et al*., 2009). Realising the importance of Zn in plant and human nutrition and the problems associated with its availability to plants in alkaline soils, the present study was conducted to assess the effect of various modes and levels of Zn application on wheat yield and protein content in order to evolve a cost effective strategy to address Zn deficiency in cereals grown in alkaline soils.

METHODS AND MATERIALS

The present experiment was conducted in the greenhouse of the Institute of Biological and Life Sciences (IBLS), University of Glasgow, UK during 2009 – 2010. Wheat variety 'Siran 2008' obtained from the Institute of Biotechnology and Genetic Engineering (IBGE), University of Agriculture, Peshawar, Pakistan was used for testing the effect of 0, 5, 10 and 15 kg Zn ha^{-1} (0.0, 2.5, 5.0 and 7.5 mg Zn kg^{-1} soil in the form of $ZnSO_4$) as soil addition and 0, 0.5 and 1 % $ZnSO_4$ solution (0, 5 and 10 g $ZnSO_4$ per litre of de-ionised water) as foliar application on wheat yield and protein content in alkaline soil. A total of 12 treatment combinations (Table 1) arranged in RCB design with 4 replications were applied in pots filled with 2 kg sandy loam soil of known physico-chemical characteristics (Table 2).

Table 1: Details of Zn treatments as soli application and foliar spray applied to wheat crop during experiment

Serial No.	Treatments	$N:P_2O_5:K_2O$ (kg ha^{-1})	Zn as soil treatment (kg ha^{-1})	As foliar spray ($ZnSO_4$) (%)
1	Zn S0 F0 (Control)	120:65:110	0.0	0.0
2	0S-0.5F	120:65:110	0.0	0.5
3	0S-1.0F	120:65:110	0.0	1.0
4	5S-0F	120:65:110	5.0	0.0
5	5S-0.5F	120:65:110	5.0	0.5
6	5S-1.0F	120:65:110	5.0	1.0
7	10S-0F	120:65:110	10.0	0.0
8	10S-0.5F	120:65:110	10.0	0.5
9	10S-1.0F	120:65:110	10.0	1.0
10	15S-0F	120:65:110	15.0	0.0
11	15S-0.5F	120:65:110	15.0	0.5
12	15S-1.0F	120:65:110	15.0	1.0

S: soil treatment; F: foliar spray

Initially, seed grains were soaked in distilled water on a double layered towel paper pad tray and kept in the growth chamber under 18 – 22 ^oC, 9 hrs light/15 hrs dark, 60 – 70 % humidity and light 150 µMole m^{-2} S^{-1}. After germination, 6 healthy seedlings were transferred to each pot and kept in a glass house under day and night lengths of 16 and 8 hrs, respectively with mercury reflector lighting of 400 watts/base E40.

Fertiliser at recommended levels i.e. $N:P_2O_5:K_2O$ at the rate of 120:65:110 kg ha^{-1} (60:32.5:55 mg kg^{-1} soil) was added to each treatment in solution form in two splits. Zn as soil treatment or foliar spray was applied in required amount in the form of $ZnSO_4$. To assess the yield trend with Zn application at lower doses of NPK, the same layout was repeated with half the recommended NPK levels ($N:P_2O_5:K_2O$ at the rate of 60:32.5:55 kg ha^{-1}). Water was provided through plastic trays beneath the pots to avoid nutrient leaching. At boot stage, two healthy plants were cut at the base for analysis of ion retention by plants. At maturity, spikes were removed for grain yield and 1000 grain weight determination, while the remaining plants were harvested to determine the total biomass yield.

Soil analysis

The composite soil sample was subjected to various physical and chemical analyses. Existing standard methods were followed for the determination of soil pH (McClean, 1982) and electrical conductivity (EC) (Rhoades, 1996) using 1:5 soil and water suspension. The texture and organic matter content were determined by Bouyoucous method (Bouyoucous, 1962) and Black procedures (1965), respectively.

The nutrient ions were determined by Induced Coupled Plasma-Optical Emission Spectrometer (ICP-OEC; Perkin Elmer Optima 4300 DV). A 10 g soil sample was taken in triplicate in 250 mL Erlenmeyer flask to which 20 mL of ammonium bicarbonate di-ethylene triamine penta acetic acid (AB-DTPA) solution was added. Flasks were shaken on a reciprocating shaker at 180 rpm per min for 15 min. The suspension was filtered through a filter paper No. 42 and 5 mL of the AB-DTPA extract from each sample was transferred into 15 mL falcon tubes to which 0.5 mL concentrated HCl was added and mixed on a rotary shaker for 15 min to drive off CO_2. After this, 4.5 mL of distilled water was added to each tube in order to bring the pH of the sample to be in accordance with the pH of the background electrolyte. The solution in the tubes was then analysed by the ICP (OES).

Grain protein

To assess the wheat grain protein content, 0.5 g of wheat flour samples were taken in triplicate to which, 0.25 mL protein buffer was added, mixed by Vortex Mixer, and then centrifuged for 15 min at 13 K rpm. The supernatant was removed for spectroscopic analysis at E-280 nm by ultraviolet spectrophotometer in mg/mL i.e. approximately equal to 1 optical density (OD). The absorbance was multiplied by the dilution factor and the protein percentage was calculated as follow;

$$\% \text{ protein} = \text{absorbance reading} \times \text{dilution} \times \frac{100}{1000}$$

The effect of Zn on the composition of proteins in wheat grain was studied using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

RESULTS AND DISCUSSION

Soil samples were analysed before sowing for various physico-chemical characters. The results (Table 2) revealed that the soil texture was a sandy loam, free from excess salinity and alkaline in reaction (pH > 7.0). The soil was moderate in organic matter content (OM > 2 %), and sufficient in macro (N, P, K, S, Ca and Mg) and micronutrients (Fe, Zn, Mn, Cu and B contents) (Table 2).

Table 2: Physico-chemical characters of the experimental soil before sowing

Property	Mean values
Textural class	Sandy loam
pH (1:5)	8.52
EC (1:5) (dS m^{-1})	0.398
Organic matter (%)	2.34
N (%)	0.041
P (mg kg^{-1})	34.44
K (mg kg^{-1})	235.35
Ca (mg kg^{-1})	197.2
Mg (mg kg^{-1})	33.38
S (mg kg^{-1})	76.6
Zn (mg kg^{-1})	1.95
Cu (mg kg^{-1})	1.79
Mn (mg kg^{-1})	138.8
Fe (mg kg^{-1})	216.4
B (mg kg^{-1})	0.618

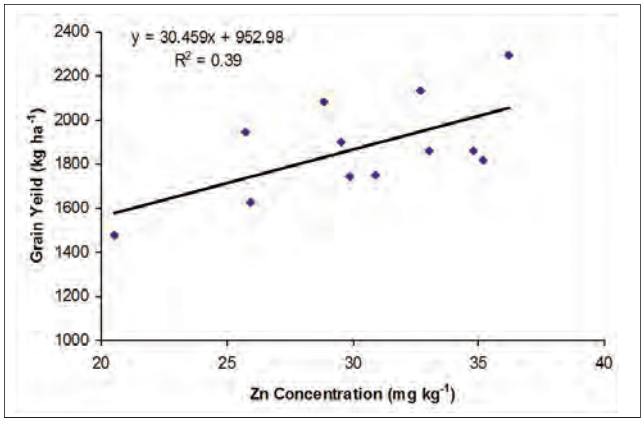

Figure 1: Correlation between Zn uptake by plant at boot stage and total grain yield of wheat

Application of Zn, both as soil and foliar treatments, significantly ($p < 0.05$) increased the wheat grain yield and 1000 grain weight while the effect on wheat biological yield was not significant. The treatment that received 5 kg ha^{-1} $ZnSO_4$ as soil treatment + 1.0 % $ZnSO_4$ as foliar spray recorded 55 % higher grain yield while that with 15 kg ha^{-1} $ZnSO_4$ as soil treatment + 1.0 % $ZnSO_4$ as foliar spray recorded 48 % higher grain yield over the control. However, the grain yield in both these treatments was not significantly different (Table 3). These results are in line with those reported by Alloway (2008) who states that Zn deficiency in wheat reduces grain yield. Kausar *et al*., (2001) have reported that despite a sharp increase in fertiliser use in Pakistan, the corresponding increase in yield was only 15 %, which was ascribed to imbalanced use of fertilisers and micronutrient deficiencies, specially of zinc and boron. Qayyum *et al*. (1987) have also reported a yield loss of 27.8 % in maize when Zn was omitted from the treatments involving NPK and micronutrients.

A positive correlation ($R^2 = 0.39$) was observed between the Zn uptake by the plant at boot stage and total grain yield (Figure 1). Takkar and Randhawa (1978) have reported a similar positive correlation between the Zn concentration and grain yield and suggested that a Zn concentration below 30 mg Zn kg^{-1} plant dry matter would result in yields not attaining their maximum potential for the site, and the value of 20 mg Zn kg^{-1} is the lower critical concentration. Previous studies (Viets,

Table 3: Effect of Zn application on grain yield (kg ha^{-1})

Serial No.	Treatments	Biomass yield (kg ha^{-1})	Grian yield (kg ha^{-1})	1000 weight grain (g)
1	Zn S0 F0 (Control)	3589.3 ± 041.7	1476 ± 054.4 ^{c}	47.44 ± 0.4 ^{d}
2	0S-0.5F	3815.5 ± 193.8	1626 ± 075.1 ^{bc}	46.1 ± 0.2 ^{ef}
3	0S-1.0F	3272.5 ± 260.2	1752 ± 060.9 ^{ab}	49.3 ± 0.4 ^{b}
4	5S-0F	3503.8 ± 279.7	1747 ± 125.7 ^{ab}	44.5 ± 0.7 ^{h}
5	5S-0.5F	3441.0 ± 082.9	1859 ± 139.2 ^{ab}	48.2 ± 0.4 ^{cd}
6	5S-1.0F	4239.8 ± 324.8	2294 ± 184.2 ^{a}	48.2 ± 0.2 ^{cd}
7	10S-0F	3697.0 ± 666.3	1947 ± 202.2 ^{a}	48.3 ± 0.3 ^{c}
8	10S-0.5F	3575.3 ± 301.6	1819 ± 209.0 ^{ab}	45.0 ± 1.0 ^{gh}
9	10S-1.0F	3894.3 ± 095.5	1861 ± 067.5 ^{ab}	45.5 ± 0.6 ^{fg}
10	15S-0F	4093.3 ± 333.6	2085 ± 180.9 ^{a}	46.5 ± 0.0 ^{e}
11	15S-0.5F	3595.5 ± 504.2	1901 ± 033.9 ^{a}	50.7 ± 0.3 ^{a}
12	15S-1.0F	3569.0 ± 374.8	2132 ± 137.9 ^{a}	47.8 ± 0.2 ^{cd}
	LSD ($p < 0.05$)	**ns**	**401.58**	**0.766**

S: soil treatment; F: foliar spray
Figures followed by similar letters are not significantly different at the $p < 0.05$ level

1966; Rashid & Fox, 1992) have suggested the critical value of 15 mg Zn kg^{-1} as a general value for the interpretation of grain analyses, and more recent work has shown a value of 10 mg Zn kg^{-1} (Brennan *et al*., 1993). The treatment of such deficiencies with Zn fertilisers or foliar sprays can increase the yield and also improve the plant's resistance to 'foot rot' fungus (*F. graminarum*) (Gooding & Davies, 1997).

The grain and biomass yields were plotted (Figure 2) to observe their trends with the increasing levels of Zn application. The line for grain yield in the graph showed a significant response (R^2 = 0.51) to increasing levels of Zn, while the biomass was independent (R^2 = 0.06) of increasing levels of Zn. The biomass yield was not affected by 50 % Zn treatments, while the remaining 50 % increased positively. This is in conformity with the results obtained by Rengel and Graham (1996), who reported that the relative production of root and shoot dry matter at deficient compared to sufficient Zn supply for two wheat genotypes tested had no difference. Khattak *et al.,* (2006) have concluded from their experiment on maize tested with various application levels of Zn that there was no statistically significant difference among the treatments regarding fresh biomass yield; anyhow they observed 8 – 11 % increase in biomass yield in various treatments.

Different modes and levels of Zn demonstrated variable effects on 1000 grain weight, and both increase and decrease in 1000 grain weight was observed with respect to the control due to Zn application (Table 3). The highest 1000 grain weight (50.6 g) was recorded in the treatment receiving 15 kg $ZnSO_4$ as soil and 0.5 % $ZnSO_4$ solution as foliar spray. 1000 grain weight was statistically different among the levels of Zn applied as soil addition with the best results shown by 15 kg $ZnSO_4$ ha^{-1} (48.3 g), while on average over the levels of soil application, different concentrations of foliar application did not show any significant change in 1000 grain weight. Previous investigations (Khattak *et al*., 2006) have reported an increase in the grain yield of maize and wheat with Zn addition at the rate of 5 kg ha^{-1} or 2 times as foliar spray of 0.5 % $ZnSO_4$, and this treatment showed an economical value greater than all the other concentrations added to maize. Yilmaz (1997) has shown increased wheat grain yield by 260 % and 124 %, with Zn application as soil addition and foliar application, respectively.

The present study reveals that Zn with higher levels of NPK performed well (R^2 = 0.47) and almost an increasing trend in the grain yield was observed. With lower doses of NPK (half NPK levels to that of the recommended dose), application of Zn at lower levels both as soil and foliar spray recorded poor performance as compared to half NPK only treatment (Figure 3). This behaviour of Zn could be attributed to its ineffectivity at lower levels at the highly alkaline calcareous conditions in the soil under study (Table 1), its reaction with clay minerals (Abat *et al.*, 2012), or might be due to some negative interaction with P (Brown, 2008). With higher doses of Zn, although the yield improved, due to lack of major nutrients the total yield could not attain its maximum potential as in the case of full dose of NPK. Yet, the R^2 value (0.55) showed that Zn increment, irrespective of its mode of application, improved the overall yield of the crop better in the case of half NPK dose as compared to full NPK dose (Figure 3). Increased phosphorus supply enhances the symptoms of Zn deficiency (Brown, 2008), yet, nitrogen appears to affect the Zn status of

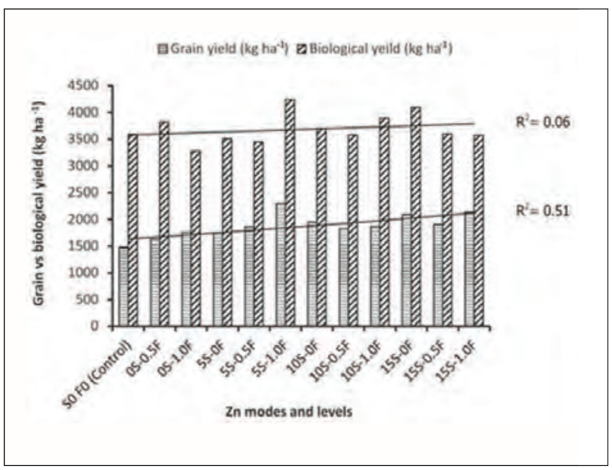

Figure 2: Effect of various modes and levels of Zn on grain *vs* biomass yield in the presence of full dose of NPK

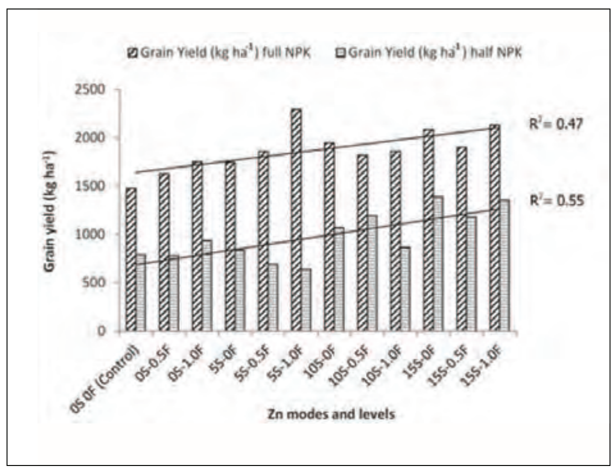

Figure 3: Effect of various modes and levels of Zn application on grain yield in the presence of full and half doses of NPK

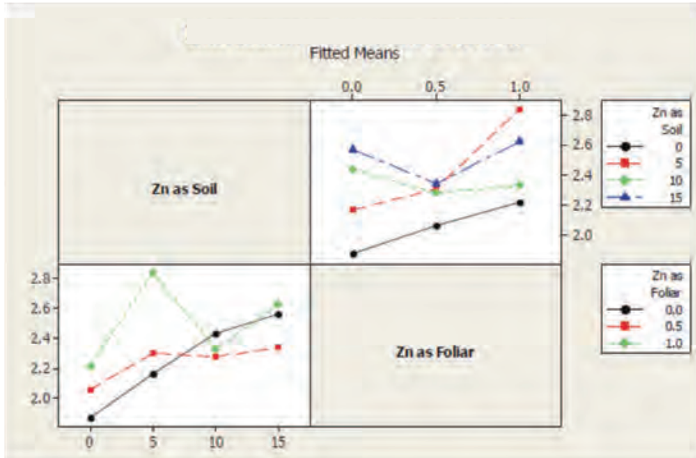

Figure 4: Interaction of various modes and levels of Zn treatment on grain yield in the presence of full dose of NPK

crops by promoting plant growth and by changing the pH of the root environment (Alloway, 2008). In many soils, nitrogen is the chief factor limiting the growth and yield and therefore in the case of full dose of NPK, improvements in yield have been found through positive interactions between nitrogen and Zn fertilisers.

Further analysis of the data using minitab statistical package to find the significance of Zn as soil, foliar, and soil and foliar treatments revealed that Zn as soil treatment had a significant ($p = 0.031$) effect on the grain yield and nearly significant ($p = 0.084$) effect of foliar spray on grain yield, but no interaction effect of the two modes. These findings are supported by the findings of Yilmaz *et al.* (1997) who concluded that soil and foliar Zn increased the grain yield of wheat by 260 and 124 %, respectively in Zn deficient calcareous soils. The interaction plot (Figure 4) shows that increasing levels of soil application of Zn has an obvious increasing effect on grain yield.

The value cost ratio (VCR) for 5 kg ha^{-1} $ZnSO_4$ as soil treatment + 1.0 % $ZnSO_4$ as foliar spray (10.23) was three times higher over the VCR for 15 kg ha^{-1} $ZnSO_4$ as soil treatment + 1.0 % $ZnSO_4$ as foliar spray (3.46), confirming the superiority of the former over the latter

Table 4: Economic analysis of Zn application on grain yield of wheat

Treatment No.	Grain yield (kg ha^{-1})	Yield increase (kg ha^{-1})	Gross return (Pak Rs)	$ZnSO_4$ used (kg ha^{-1})	Cost of $ZnSO_4$ (Pak Rs)	Net return (Pak Rs)	% increase over control	VCR
1	1476	000	00000	00	0000	00000	00.0	00.0
2	1626	150	03607	05	0300	03307	10.2	12.0
3	1752	277	06640	10	0600	06040	18.8	11.1
4	1747	271	06510	22	1320	05190	18.4	04.9
5	1859	383	09200	27	1620	07580	26.0	05.7
6	2294	818	19634	32	1920	17714	55.4	10.2
7	1947	471	11314	44	2640	08674	32.0	04.3
8	1819	344	08247	49	2940	05307	23.3	02.8
9	1861	386	09260	54	3240	06020	26.2	02.9
10	2085	610	14638	66	3960	10678	41.3	03.7
11	1901	425	10210	71	4260	05950	28.8	02.4
12	2132	657	15763	76	4560	11203	44.5	03.5

Note: Wheat grain rate = Pakistan rupees (Pak Rs) 24 kg^{-1} and $ZnSO_4$ = Pakistan rupees (Pak Rs) 60 kg^{-1}

Table 5: Effect of Zn application on glutenin fraction of grain protein in wheat

Serial No.	Treatments	Protein (mg mL^{-1})	Protein (%)	% increase over control
1	Zn S0 F0 (Control)	20.00	2.00	0.0
2	0S-0.5F	21.90	2.19	9.5
3	0S-1.0F	24.50	2.45	22.5
4	5S-0F	25.05	2.51	25.5
5	5S-0.5F	25.45	2.55	27.5
6	5S-1.0F	25.90	2.59	29.5
7	10S-0F	24.50	2.45	22.5
8	10S-0.5F	23.45	2.35	17.5
9	10S-1.0F	23.60	2.36	18.0
10	15S-0F	23.80	2.38	19.0
11	15S-0.5F	23.70	2.37	18.5
12	15S-1.0F	25.75	2.58	29.0

S: soil treatment; F: foliar spray

in terms of effectiveness (Table 4). Although VCR for 0.5 % $ZnSO_4$ (VCR:12) and 1.0 % $ZnSO_4$ (VCR:11) as foliar application was the highest among the list of treatments (Table 4), yet, taking into account the yield per unit area of the treatments, the performance of 0.5 % $ZnSO_4$ (1626 kg ha^{-1}) and 1.0 % $ZnSO_4$ (1752 kg ha^{-1}) as foliar application only was not satisfactory as compared to 5 kg ha^{-1} $ZnSO_4$ as soil + 1.0 % $ZnSO_4$ as foliar (2294 kg ha^{-1}) and 15 kg ha^{-1} $ZnSO_4$ as soil + 1.0 % $ZnSO_4$ as foliar (2132 kg ha^{-1}). Thus in a country like Pakistan, which faces food deficit due to increased population, the growers must adopt the technology that could increase their production per unit area with acceptable margin of profit. Thus, this study suggested the best level of Zn as 5 kg ha^{-1} as soil treatment + 1.0 % $ZnSO_4$ as foliar application as a better option to improve the grain yield on alkaline soil (Table 4).

Application of Zn, both as soil treatment and foliar spray, to wheat crop in alkaline soil significantly ($p < 0.05$) increased the protein content in wheat grains over the control (Table 5, Figure 5). The treatments receiving 5 kg ha^{-1} $ZnSO_4$ soil application + 1.0 % $ZnSO_4$ solution as foliar application, 15 kg ha^{-1} $ZnSO_4$ as soil application + 1.0 % $ZnSO_4$ solution as foliar spray and 5 kg ha^{-1} $ZnSO_4$ as soil application + 0.5 % $ZnSO_4$ solution as foliar spray application recorded the highest increase in wheat grain protein content, which were 29.5, 29.0 and 27.5 % higher than the control treatment, respectively. It was thus concluded that protein in wheat grain could be increased either by low level of Zn as soil application + comparatively higher dose of foliar application or higher dose of Zn through soil application along with higher concentration of foliar spray. The bars (Figure 5) clearly indicate an initial gradual increase in protein with low Zn concentration levels, but with medium Zn concentration levels it tends to decrease irrespective of its application method. With higher application of Zn at 15 kg as soil treatment and 1.0 % $ZnSO_4$ as foliar spray, it shows an increase in the protein content. The regression coefficient indicated an overall increase of 21 % in grain protein content with increasing Zn level applied either as foliar application or as soil application (Figure 5).

In calcareous soils, Zn precipitates in unavailable forms for plants, and its uptake and transition to the shoot is inhibited by high concentrations of bicarbonate (Dogar & Van Haj, 1980). However, Zn deficiency in the plants grown in calcareous soils can be recovered fairly readily

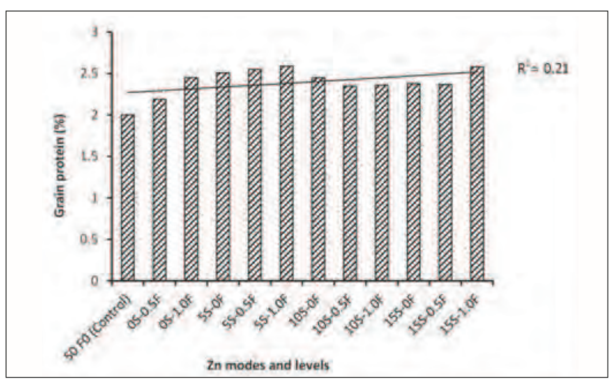

Figure 5: Effect of various modes of treatment and levels of Zn on percent protein content in wheat grain

by application of inorganic Zn salts such as $ZnSO_4$ to the soil (Nayyar & Takkar, 1980), and such application from external sources linearly increased the grain yield and protein content of two wheat genotypes (Morshedi & Farahbakhsh, 2010). Szakal (1989) and Fecenko and Lozek (1998) have reported similar findings. Khattak *et al.*, (2006) have reported a maximum increase in protein content in the grains of maize obtained from the treatment supplied with 0.5 % $ZnSO_4$ as foliar spray at the stage when the crop was only 20 – 25 days old. Several researchers have reported significant seed protein and dry matter yield response to Zn fertilisation (Biswas *et al.*, 1977; Tandon, 1992; Zaidan *et al.*, 2010.; Keram, 2014). Zn is required for the synthesis of auxin [a growth regulating compound indole acetic acid (IAA)] (Brown *et al.,* 1993). Tryptophan is the most likely precursor for the biosynthesis of IAA and Zn is required for the synthesis of tryptophan (Marschner, 1995). This includes an observed increase in the tryptophan content in rice grains after Zn fertilisation of plants growing on calcareous soil (Singh, 1991).

No major differences were observed in protein profiles of the samples isolated from different Zn treatments (Figure 6) despite the increase or decrease in protein content in various treatments. Alloway (2008) reported that in general the amount of protein in the grains of Zn deficient plants is greatly reduced, but the protein composition remains almost unchanged. In Zn deficient bean leaves, the concentration of free amino acids was 6.5 times greater than in the control but these decreased, and the protein content increased after the administration of Zn for 48 or 72 hours. The mechanism by which Zn deficiency affects protein synthesis is considered to be due to a reduction in RNA and the deformation and reduction of ribosome. The importance of Zn in protein synthesis suggests that a relatively high Zn concentration is required by meristematic tissues where cell division as well as the synthesis of nucleic acid and protein is actively taking place (Brown *et al*., 1993).

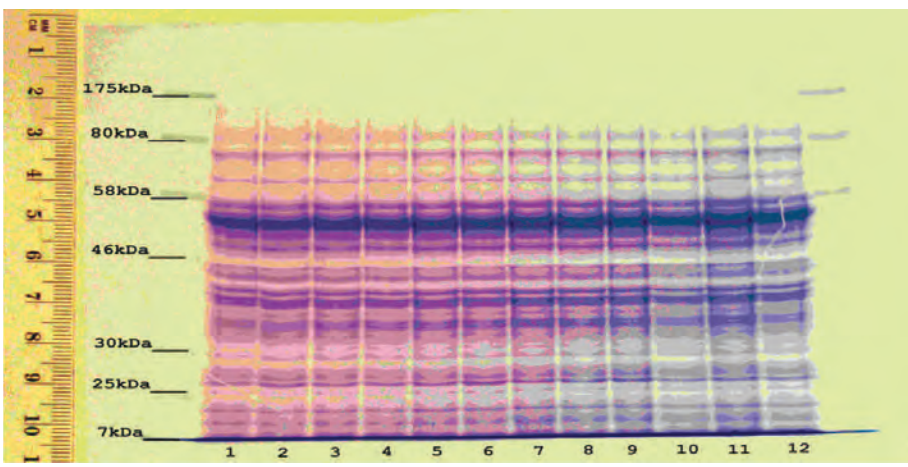

Figure 6: Effect of various modes of treatment and levels of Zn on composition of protein in wheat grain

Legends: Coomassie-blue strained SDS-PAGE gel of wheat grain proteins isolated from the 12 Zn treatments. Molecular weight standards are shown on the left.

Lane 1, S0F0: L2, S0F1: L3, S0F2: L4, S1F0: L5, S1F1: L6, S1F2: L7, S2F0: L8, S2F1: L9, S2F2: L10, S3F0: L11, S3 F1: L12, S3F2

S = soil Zn application at 0, 1 (5 kg ha^{-1}), 2 (10 kg ha^{-1}), 3 (15 kg ha^{-1})

F = foliar $ZnSO_4$ application at 0,1 (0.5 %), and 2 (1 %).

CONCLUSION

This study concludes that alkaline soils are Zn deficient in terms of availability to plants despite the apparent Zn levels in the sufficient range (> 1.0 mg kg^{-1}). Therefore, this range should be revised in accordance with the nature and type of soils. Foliar application of Zn up to 1.0 % $ZnSO_4$ solution and 5 kg ha^{-1} as soil addition increased the yield, while regardless of the method, Zn application increased the protein content in grains. Thus, for higher yield and better quality Zn should be added by farmers cultivating alkaline soils in Pakistan to the extent of 5 kg ha^{-1} $ZnSO_4$ as soil addition and 1.0 % $ZnSO_4$ solution as foliar application.

REFERENCES

1. Abat M., Michael J.M., Jason K.K. & Samuel P.S. (2012). Adsorption and desorption of copper and zinc in tropical peat soils of Sarawak, Malaysia. *Geoderma* **176**: 58 – 63.
   DOI: http://dx.doi.org/10.1016/j.geoderma.2012.01.024
2. Ahmad W., Watts M.J., Imtiaz M., Ahmad I. & Zia M.H. (2012). Zinc deficiency in soils, crops and humans. *Agrochimica* **104**(2): 65 – 97.
3. Alloway B.J. (2008). Zn in Soil and Crop Nutrition. 2nd edition, pp. 33. International Zinc Association (IZA) and International Fertilizer Association (IFA) Brussels, Belgium and Paris, France.
4. Alloway B.J. (2009). Soil factors associated with zinc deficiency in crops and humans. *Environmental Geochemestry and Health* **31**: 537 – 548.
   DOI: http://dx.doi.org/10.1007/s10653-009-9255-4
5. Amar S., Vyas A.K., Singh A.K. & Singh A. (2000). Effect of nitrogen and zinc application on growth, yield and net returns of maize (*Zea mays* L.). *Annals of Agricultural Research* **21**: 296 – 297.
6. Biswas C.R., Rajinderjit S.G. & Sekhon S. (1977). Zinc availability to maize and wheat in relation to P and K status of the soil in a long-term fertility experiment. *Journal of the Indian Society of Soil Science* **25**: 414 – 421.
7. Black C.A. (1965). *Methods of Soil Analysis (part II).* American Society of Agronomy, Inc., Madison, Wisconsin, USA.
8. Black R.E., Lindsay H.A., Bhutta Z.A., Caulfield L.E., De Onnis M., Ezzati M., Mathers C. & Rivera J. (2008). Maternal and child under-nutrition: global and regional exposures and health consequences. *Lancet* **371**: 243 – 260.
9. Bouyoucos G.J. (1962). Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal* **53**: 464 – 465.
10. Brennan R.F., Armour J.D. & Reuter D.J. (1993). Diagnosis of zinc deficiency. *Zinc in Soils and Plants,* chapter 12 (ed. A.D. Robson), p. 206. Kluwer Academic Publishers, Dordrecht, The Netherlands.
    DOI: http://dx.doi.org/10.1007/978-94-011-0878-2_12
11. Brown P.H. (2008). Micronutrient use in agriculture in the United States of America: current practices, trends and constraints. *Micronutrient Deficiencies in Global Crop Production*, chapter 11 (ed. B.J. Alloway), pp. 267 – 286. Springer, New York, USA.
    DOI: http://dx.doi.org/10.1007/978-1-4020-6860-7_11
12. Brown P.H., Cakmak I. & Zhang Q. (1993). Form and function of Zn in plants. *Zn in Soil and Plants* (ed. A.D. Robson), pp. 90 – 106. Kulwer Academic Publisher, Dordrecht, The Netherlands.
13. Cakmak I. (2008). Enrichment of cereal grains with zinc: Agronomic or genetic bio-fortification. *Plant Science* **302**: 1 – 17.
    DOI: http://dx.doi.org/10.1007/s11104-007-9466-3
14. Cakmak I., Torun B., Eronoglu B., Ozturk L., Marschner H., Kalayci M., Ekiz H. & Yilmaz A. (1998). Morphological and physiological differences in the response of cereals to zinc deficiency. *Euphytica* **100**: 349 – 357.
    DOI: http://dx.doi.org/10.1023/A:1018318005103
15. Dogar M.A. & Van Haj T. (1980). Effect of P, N and HCO_3^- levels in the solution on rate of Zn absorption by rice roots and Zn content in plants. *Zeitschrift für Pflanzenphysiologie* **98**: 203 – 212.
16. Eyupoglu F., Kurucu N. & Sanisa U. (1994). Status of plant available micronutrients in Turkish soils. *Annual Report,* pp. 25 – 32. Turki Soil and Fertilizer Research Institute, Ankara, Turkey.
17. Fecenko J. & Lozek O. (1998). Maize grain yield formation in dependence on applied zinc doses and its contents in soil. *Rslinna Vyroba* **44**: 15 – 18.
18. Gooding M.J. & Davies W.P. (1997). *Wheat Production and Utilization: Systems, Quality and Environment.* CAB International, Oxford, UK.
19. Hotz C. & Brown K.H. (2004). Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutrition Bulletin* **25***:* 94 – 204.
20. Kauser M.A., Hussain F., Ali S. & Iqbal M.M. (2001). Zinc and Cu nutrition of two wheat varieties on a calcareous soil. *Pakistan Journal of Soil Science* **20**: 21 – 26.
21. Keram K.S. (2014). Response of zinc fertilization to wheat on yield, quality, nutrients uptake and soil fertility grown in a zinc deficient soil. *European Journal of Academic Essays* **1**(1): 22 − 26.
22. Khan M.A., Fuller M.P. & Baloch F.S. (2008). Effect of soil applied zinc sulphate on wheat (*Triticum aestivum* L.) grown on a calcareous soil in Pakistan. *Cereal Research Communication* **36**: 571 – 582.
    DOI: http://dx.doi.org/10.1556/CRC.36.2008.4.6
23. Khattak S.G. & Pulford I.D. (1999). Adsorption and desorption behaviour of zinc in some selected soils of United Kingdom in water background. *Pakistan Journal of Soil Science* **17**: 73 – 78.
24. Khattak S.G., Rohullah A., Malik A., Perveen Q. & Ibrar M. (2006). Assessing maize yield and quality as affected by Zn as soil or foliar applications. *Sarhad Journal of Agriculture* **22**: 465 – 472.
25. Maqsood M.A., Rahmatullah, Ranjha A.M. & Hussain M. (2009). Differential growth response and zinc utilization efficiency of wheat genotype in cultivars buffered nutrient solution. *Soil and Environment* **28**: 174 – 178.
26. Maret W. & Sandstead H.H. (2006). Zinc requirements and the risks and benefits of zinc supplementation. *Journal of Trace Elements and Medical Biology* **20**(1): 3–18.
    DOI: http://dx.doi.org/10.1016/j.jtemb.2006.01.006
27. Marschner H. (1995). *Mineral Nutrition of Higher Plants,* 2nd edition, p. 890. Academic Press, London, UK.

28. McClean E.O. (1982). Soil pH and lime requirement. *Methods of Soil Analysis*, part 2, 2nd edition (eds. A.L. Page, R.H. Miller & D.R. Keeney), pp. 209 – 223. American Society of Agronomy, Madison, Wisconsin, USA.
29. Morshedi A. & Farahbakhsh H. (2010). Effects of potassium and zinc on grain protein contents and yield of two wheat genotypes under soil and water salinity and alkalinity stresses. *Plant Ecophysiology* **2**: 67 – 72.
30. Morvedt J.J., Cox F.R., Shuman L.M. & Welch R.M. (1991). *Micronutrients in Agriculture,* 2nd edition, Soil Science Society of America, Madison, Wisconsin, USA.
31. Nayyar V.K. & Takkar P.N. (1980). Evaluation of various zinc sources for rice grown on alkali soil. *Zeitschrift für Pflanzenernährung und Bodenkunde* **143**: 489 – 493. DOI: http://dx.doi.org/10.1002/jpln.19801430502
32. Qayyum F., Ashraf M. & Yasin M. (1987). Response of corn to micronutrients on Barani land. *Proceedings of the National Seminar on Micronutrients in Soils and Crops in Pakistan,* NWFP Agricultural University, Peshawar, Pakistan, December 13 – 15.
33. Rashid A. & Fox R.L. (1992). Evaluating internal zinc requirements of grain crops by seed analysis. *Agronomy Journal* **84**: 469 – 474.
34. Rashid A., Rahmatullah & Salim M. (1988). Soil conditions and crop factors inducing zinc deficiency in plants. *Proceedings of the National Seminar on Micronutrients in Soils and Crops in Pakistan,* NWFP Agricultural University, Peshawar, Pakistan, December 13 – 15.
35. Rengel Z. & Graham R.D. (1996). Uptake of zinc for chelated-buffered nutrient solutions by wheat genotypes differing in zinc efficiency. *Journal of Experimental Botany* **47**: 217 − 226. DOI: http://dx.doi.org/10.1093/jxb/47.2.217
36. Rhoades J.D. (1996). Salinity: electrical conductivity and total dissolved salts. *Methods of Soil Analysis,* part 3 (ed. D.L. Sparks), pp. 417 – 436. American Society of Agronomy, Madison, USA.
37. Singh M.V. & Abrol I.P. (1985). Direct and residual effect of fertilizer zinc application on the yield and chemical composition of rice-wheat crops in an alkali soil. *Fertilizer Research* **8**: 179 – 191. DOI: http://dx.doi.org/10.1007/BF01048901
38. Singh M. (1991). Effects of zinc, phosphorous and nitrogen on tryptophan concentration in rice grains grown on limed and unlimed soils. *Plant and Soil* **62**: 305 – 308.
39. Szakal P. (1989). Effect of zinc and copper amine complexes on the yield and quality of maize. *XIX – HUNGAROCHEM. Konferencia Murtagyazas Novenyvedelem.* pp. 43 – 47.
40. Tahir M., Fiaz N., Nadeem M.A., Khalid F. & Ali M. (2009). Effect of different chelated Zn sources on the growth and yield of maize (*Zea mays* L.). *Soil and Environment* **28**: 179 – 183.
41. Takkar P. (1991). Zinc deficiency in Indian soils and crops. *Zinc in Crop Nutrition*. pp. 55 − 64. International Lead Zinc Research Organization Inc. and Indian Lead Zinc Information Centre, New Dehli, India.
42. Takkar P.N. & Randhawa N.S. (1978). Micronutrients in Indian agriculture. *Fertiliser News (Delhi)* **23**(8): 3 – 26.
43. Tandon H.L.S. (1995). *Micronutrients in Soils Crops and Fertilizers.* Fertilizer Development and Consultation Organization, New Delhi, India.
44. Tandon H. (1992). *Management of Nutrient Interactions in Agriculture*. Fertilizer Development and Consultation Organization, New Delhi, India.
45. Viets F.G. (1966). Zinc deficiency in the soil plant system. *Zinc Metabolism* (eds. A.S. Prasad & C.C. Thomas), pp. 90 – 127. Springfield, Illinois, USA.
46. Yilmaz A., Ekiz H., Torun B., Gultekin I., Karanalik S., Bagsi S.A. & Cakmak I. (1997). Effect of different zinc application methods on grain yield and zinc concentration in wheat cultivars grown on zinc-deficient calcareous soil. *Journal of Plant Nutrition* **20**: 461 – 471. DOI: http://dx.doi.org/10.1080/01904169709365267
47. Zeidan M.S., Mohamed M.F. & Hamouda H.A. (2010). Effect of foliar fertilization of Fe, Mn and Zn on wheat yield and quality in low sandy soils fertility. *World Journal of Agricultural Sciences* **6**(6): 696 − 699.