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# Effect of source and methods of zinc application on corn productivity, nitrogen and zinc concentrations and uptake by high quality protein corn (*Zea mays*)

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## Abstract

Results of a field study conducted at the Indian Agricultural Research Institute, New Delhi, India showed that the combined application of soil + foliar (in two sprays at tasseling and initiation of flowering) produced significantly more grain and stover yields than either soil or foliar applications alone. Application of Zn-coated urea was better than soil application of Zn sulphate with regard to grain and stover yields. The combined application also recorded the highest Zn concentration in corn grain as well as in stover, with the treatments falling in the following order: combined > foliar > soil through Zn-coated urea > soil. This is an important finding for the agronomic biofortification of Zn in corn.

**Keywords**: Crude protein; foliar application of zinc, zinc biofortification, zinc-coated urea, zinc sulphate.

## Introduction

Corn (*Zea mays* L.) is a major cereal crop widely grown and consumed in developed as well as in developing countries. In India it is cultivated on an area of 8.55 million hectares with a production of 22.50 million Mg and an average productivity of 2.54 Mg $ha^{-1}$ (Fertilizer Statistics 2011-12). Widespread deficiencies of zinc (Zn) have been reported right through East Asia, to the tune of 50-70 % in India and Pakistan. Cereal grains are known to be inherently low in Zn, particularly in regions where soils are low in plant-available Zn (Shivay & Prasad 2012). Nearly 50 % of cereal-growing areas in the world have soils with low plant-available Zn (Graham & Welch 1996, Cakmak 2002), resulting in Zn concentrations in cereal grains of as little as 5–12 mg $kg^{-1}$ against a requirement of 40–60 mg $kg^{-1}$ (www.harvestplus.org, Pfeiffer & McClafferty 2007). Since the introduction of the Green Revolution in Asia, cultivation of high yielding genotypes, improved agricultural mechanization and production of macronutrient fertilizers with low impurities of trace elements has resulted in higher crop production per unit area and greater depletion of plant-available micronutrients (Cakmak 2008, Khoshgoftarmanesh *et al.* 2009). Most micronutrient deficiency problems are exacerbated by the cultivation of high-yielding crop cultivars that quickly deplete the limited soil nutrients (Cakmak *et al.* 1996, Martens & Lindsay 1990). This presents a further challenge for addressing Zn deficiency in cereal-based cropping systems.

Zn is required for structural and functional integrity of about 2,800 proteins, contributes to protein biosynthesis and is a key defence factor in detoxification of highly toxic oxygen-free radicals (Cakmak 2000, Broadley *et al.* 2007). Therefore Zn deficiency in cultivated soils, as documented at global level (Alloway 2004), poses a serious threat to crop production and human nutrition. Cereal-based diets are the major source of nutrients for the majority of the world's population, but over the past two to three decades, concentrations of essential minerals such as Zn have been found to be on a downward trend, far below the required 25–50 mg Zn $kg^{-1}$ (FAO 1996). Although meat is known to have a high Zn concentration, it is not readily available to many resource-constrained households who often constitute more than 60 % of the population in developing countries (Paul *et al.* 1998, Cakmak *et al.* 1999). Zn deficiency in humans was rated by WHO (2002) as fifth of the ten leading causes of illness and disease, especially in women and children in low-income countries. Health problems associated with

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Zn deficiency include pregnancy complications, low birth weight, impairments in brain development and function, and growth faltering in infants and children (Gibson 1994).

Approaches for improving the nutritional wellbeing of humans such as food diversification, supplementation with capsules or syrups, molecular biology and industrial food fortification still require much investment and social acceptance (Ruel & Bouis 1998, White & Broadley 2005). Conventionally, the use of inorganic Zn fertilizers and synthetic chelates provide avenues to alleviate Zn-deficiency-related problems both in human nutrition and crop production. It is better to increase the Zn content in cereals, the staple food in many developing countries, through Zn fertilization. Biofortification of cereals can be achieved either by developing crop cultivars with high concentrations of Zn in the grain, or by adequate Zn fertilization of crops grown on Zn-deficient soils. Agronomic fortification of corn by increasing Zn concentration and its bioavailability has great potential to alleviate its deficiency. The present study was conducted to evaluate the effect of source and methods of Zn application on corn productivity, nitrogen and Zn concentrations and uptake by high-quality protein corn.

## Materials & Methods

The field experiment was conducted at the Research Farm of the Indian Agricultural Research Institute, New Delhi, India (28$^o$ 38’ N, 77$^o$ 10’ E, altitude 228.6 m) during the rainy season (July to mid-November) of 2008 on a sandy-loam soil (‘ustrochrept’). The soil had 165 kg ha$^{-1}$ alkaline permanganate oxidizable N (Subbiah & Asija 1956), 18.2 kg ha$^{-1}$ available P (Olsen *et al.* 1954), 315 kg ha$^{-1}$ 1 *N* ammonium acetate exchangeable K (Hanway & Heidel 1952) and 0.378% organic C (Walkley & Black 1934). The pH of the soil was 8.3 (1:2.5 soil: water ratio) (Prasad *et al.* 2006) and the DTPA-extractable Zn (Lindsay & Norvell 1978) in the soil was 0.36 mg kg$^{-1}$ soil.

There were five treatments: control (no Zn), soil application of 5 kg Zn ha$^{-1}$ as $ZnSO_4.7H_2O$, foliar application of 1 kg Zn ha$^{-1}$ as $ZnSO_4.7H_2O$ (in two sprays at tasseling and initiation of flowering), 5 kg Zn ha$^{-1}$ as $ZnSO_4.7H_2O$ (soil application) + 1 kg Zn ha$^{-1}$ as $ZnSO_4.7H_2O$ as foliar application (in two sprays at tasseling and initiation of flowering) and soil application of Zn-coated urea (1.0% Zn through ZnO amounting to an application of 2.83 Zn ha$^{-1}$) (soil). These treatments were tested in a randomized block design with 3 replications.

The experimental field was disk-ploughed twice, cultivated three times with a cultivator and levelled. Corn (‘High Quality Protein Maize 1’) was planted at a spacing of 45 cm x 20 cm in the first week of August. The plot size was 5.0 m x 2.7 m for each treatment. At final ploughing 26 kg P ha$^{-1}$ as single superphosphate was broadcast. Nitrogen at 130 kg N ha$^{-1}$ as PU or Zn-coated urea was band-applied in two equal splits, half at planting and the other half at tasseling stage. Soil application of Zn sulphate was made by banding in rows just before corn planting. Foliar application of Zn sulphate was made twice; first just before tasseling development and the second a week after flowering. For foliar application, 500 litres of 0.5% solution of Zn sulphate was used; thus two sprays supplied 5 kg Zn sulphate ha$^{-1}$. Corn was grown as per recommended practices.

To record growth, yield attributes and yields of corn, five plants were randomly selected in each plot, measuring various attributes before harvesting (cob length, cob girth, grain weight per cob and 1,000-grain weight etc.). At harvest, the grain yield and stover yield were recorded for each plot and expressed in Mg ha$^{-1}$.

For chemical analysis, at harvest samples of grain and stover were drawn from each plot of the experiment. Zn in grain and straw samples was analysed on a di-acid ($HClO_4$ + $HNO_3$ in 3:10 ratio) digest on an Atomic Absorption Spectrophotometer (Prasad *et al.* 2006). Total N was determined by Kjeldahl method (Prasad *et al.* 2006). Therefore, the uptake of the nitrogen and Zn was calculated by multiplying N and Zn concentrations with respective plot yield of grain and stover of corn. Crude protein content in corn was determined by multiplying the N concentration by 6.25.

Zn use efficiencies (Zn harvest index - ZnHI, agronomic efficiency - AE, recovery efficiency - RE, and Zn mobilization efficiency index - ZnMEI) of the applied Zn were computed using the following expressions as suggested by Shivay *et al.* (2010):

$$AE = (Y_t - Y_o) / Zn_a$$
$$RE = [(U_t - U_o) / Zn_a] \times 100$$
$$ZnHI = (Zn_s / Zn_t) \times 100$$
$$ZnMEI = [\text{Zn concentration in grain} \div \text{Zn concentration in straw}]$$

where, $Y_t$ = yield in the treatment (kg ha$^{-1}$); $Zn_a$ = amount of Zn added (kg ha$^{-1}$); $Y_o$ = yield of the control treatment (kg ha$^{-1}$); $U_t$ = uptake of Zn in test treatment (kg ha$^{-1}$); $U_o$ = uptake of Zn in control treatment (kg ha$^{-1}$); $Zn_s$ = Zn uptake by grain at harvest, and $Zn_t$ = Zn uptake by whole crop (grain + stover) at harvest.

All the data recorded during the experiment were subjected to statistical analysis using the *F*-test as per the procedure given by Gomez & Gomez (1984). Least significant difference (LSD) values at $P = 0.05$ were used to determine the significance of differences between treatment means.

## Results

A significant improvement in yield attributes of corn was recorded with Zn fertilization (Table 1). The longest cob length was recorded with the combined soil+foliar application of Zn, significantly greater than the control. Mean cob girths were not significantly different among treatments, but slightly higher values were recorded with application of Zn compared to control. Significant improvements in the grain weight cob$^{-1}$ over the control were recorded for all Zn treatments except the foliar treatment, with the highest mean value recorded for the combined soil + foliar application, not statistically different from the soil and the Zn-coated urea treatments. Zn fertilization treatments did not influence the 1,000-grain weight significantly, but again the highest mean value was recorded for the combined treatment.

| **Treatment (all values are quantities of Zn ha$^{-1}$)** | **Cob length (cm)** | **Cob girth (cm)** | **Grain weight (g cob$^{-1}$)** | **1,000-grain wt (g)** | **Grain yield (Mg ha$^{-1}$)** | **Stover yield (Mg ha$^{-1}$)** |
|---|---|---|---|---|---|---|
| control (no added Zn) | 13.0 | 3.59 | 70.6 | 190.0 | 4.00 | 6.10 |
| 5 kg to soil | 14.0 | 3.70 | 74.9 | 199.3 | 4.70 | 6.68 |
| 1 kg foliar | 13.5 | 3.63 | 72.8 | 193.3 | 4.42 | 6.50 |
| 5 kg to soil + 1 kg foliar | 15.2 | 3.73 | 76.5 | 201.5 | 5.10 | 7.03 |
| 2.83 kg through Zn-coated urea (to soil) | 14.4 | 3.64 | 75.2 | 200.5 | 4.80 | 6.90 |
| sem | 0.6 | 0.06 | 1.3 | 3.9 | 0.12 | 0.19 |
| LSD (for p=0.05) | 2.1 | NS | 4.1 | NS | 0.38 | 0.62 |

**Table 1:** Effect of source and method of Zn application on yield attributes and yield of corn

As might be expected from the data on yield attributes, grain and stover yield of corn was the highest with the combined soil + foliar application of Zn (Table 1), significantly higher than all the other treatments (5-15%) and the control (27%). The mean stover yields were significantly different among treatments, all due to the control being below those of all the Zn treatments;

again the combined treatment recorded the highest mean value. In both grain yields and stover, Zn applied to the soil as Zn-coated urea had higher mean values than when applied as Zn sulphate, but the differences were not significant.

Nitrogen concentration and uptake in corn grain and stover and total uptake in corn grain was the highest with the combined soil + foliar treatment (Table 2). The mean values for the single soil applications of Zn sulphate or zinc-coated urea were very similar, and higher than those for the foliar application of Zn.

| Treatment (all values are quantities of Zn $ha^{-1}$) | N concentration | | N uptake | | | Crude protein content (%) |
|---|---|---|---|---|---|---|
| | Corn grain (%) | Corn stover (%) | Corn grain (kg $ha^{-1}$) | Corn stover (kg $ha^{-1}$) | Total (kg $ha^{-1}$) | |
| control (no added Zn) | 1.44 | 0.58 | 57.6 | 35.4 | 93.0 | 9.0 |
| 5 kg to soil | 1.60 | 0.64 | 75.2 | 42.8 | 118.0 | 10.0 |
| 1 kg foliar | 1.54 | 0.60 | 68.1 | 39.0 | 107.1 | 9.6 |
| 5 kg to soil + 1 kg foliar | 1.64 | 0.66 | 83.6 | 46.4 | 130.0 | 10.3 |
| 2.83 kg through Zn-coated urea (to soil) | 1.57 | 0.65 | 75.6 | 44.9 | 120.3 | 9.8 |
| sem | 0.03 | 0.01 | 1.7 | 1.3 | 2.0 | 0.3 |
| LSD (for p=0.05) | 0.09 | 0.03 | 5.4 | 4.1 | 6.4 | 1.0 |

**Table 2:** Effect of source and method of Zn application on N concentration in corn grain and stover and their uptake and also crude protein content in corn grain

Zn fertilization increased the crude protein content (Table 2), but this was only significant for treatments involving soil applications as Zn sulphate. The combined soil + foliar application recorded the highest mean value. Although higher, there were no significant differences between soils vs. foliar application, nor between application as Zn coated urea vs. Zn sulphate.

The mean values for Zn concentrations and uptake were all highest for the combined soil + foliar treatment, and always significantly superior to all other treatments (Table 3). Higher mean values were always seen for foliar than soil application, often significantly so. Soil application as Zn-coated urea was nearly always statistically higher than application as Zn sulphate. All Zn treatments for agronomic biofortification of Zn in corn grain as well as in stover were in the following order: 5 kg soil + 1 kg foliar > 1 kg foliar > 2.83 kg as Zn-coated urea to soil > 5 kg soil.

| Treatment (all values are quantities of Zn $ha^{-1}$) | Zn concentration | | Zn uptake | | |
|---|---|---|---|---|---|
| | Corn grain (mg $kg^{-1}$ grain) | Corn stover (mg $kg^{-1}$ DM) | Corn grain (g $ha^{-1}$) | Corn stover (g $ha^{-1}$) | Total (g $ha^{-1}$) |
| control (no added Zn) | 40.2 | 45.0 | 160.8 | 274.5 | 435.3 |
| 5 kg to soil | 44.2 | 49.2 | 207.7 | 328.5 | 536.4 |
| 1 kg foliar | 46.0 | 59.2 | 203.2 | 384.8 | 588.0 |
| 5 kg to soil + 1 kg foliar | 49.2 | 64.5 | 250.9 | 453.4 | 704.3 |
| 2.83 kg through Zn-coated urea (to soil) | 45.8 | 58.2 | 219.8 | 401.6 | 621.4 |
| sem | 0.6 | 0.8 | 3.4 | 3.4 | 3.8 |
| LSD (for p=0.05) | 2.0 | 2.7 | 11.1 | 11.1 | 12.5 |

**Table 3:** Effect of source and method of Zn application on Zn concentration in grain and stover of corn and its Zn uptake by corn

Zn use efficiencies were influenced significantly by Zn treatment (Table 4). The highest harvest index was recorded for the single soil application Zn sulphate, significantly higher than all other Zn treatments, but not the control. Similar results were also observed with the mobilization efficiency index. All treatments were significantly different from one another with respect to agronomic efficiency, and as expected, this was much the highest with the foliar treatment and lowest with the soil treatment (as Zn sulphate). The recovery index varied from substantially due to different Zn treatments. All Zn treatments for agronomic efficiency and recovery efficiency were in the following order: 1 kg Zn $ha^{-1}$ (foliar) > 2.83 kg Zn $ha^{-1}$ through Zn-coated urea (soil) > 5 kg Zn $ha^{-1}$ (soil) + 1 kg Zn $ha^{-1}$ (foliar) > 5 kg Zn $ha^{-1}$ (soil).

| **Treatment (all values are quantities of Zn $ha^{-1}$)** | **harvest index (%)** | **mobilization efficiency index** | **agronomic efficiency** | **recovery efficiency (%)** |
|---|---|---|---|---|
| control (no added Zn) | 36.9 | 0.89 | - | - |
| 5 kg to soil | 38.7 | 0.90 | 140 | 2.02 |
| 1 kg foliar | 34.6 | 0.78 | 420 | 15.27 |
| 5 kg to soil + 1 kg foliar | 35.6 | 0.76 | 183 | 4.48 |
| 2.83 kg through Zn-coated urea (to soil) | 35.4 | 0.79 | 283 | 6.58 |
| sem | 0.6 | 0.01 | 3 | 0.02 |
| LSD (for p=0.05) | 2.1 | 0.03 | 9 | 0.08 |

**Table 4:** Effect of source and method of Zn application on Zn use efficiencies in corn

## Discussion

In the present study most yield attributes, grain and stover yield, N and Zn concentrations and uptake by corn were the highest with the combined soil + foliar application of Zn sulphate, and partly this could be due to the higher amount of Zn sulphate (6 kg Zn $ha^{-1}$) involved. In general, soil application of Zn was superior to foliar application in respect of yield attributes, grain and stover yield, and N concentration and uptake by corn, which again may partly be due to the application of a larger amount (5 kg Zn $ha^{-1}$) through soil as opposed to foliar application (1 kg Zn $ha^{-1}$), and partly due the fact that soil application was made at planting, while foliar application was made much later at tasseling and flowering. Nevertheless, foliar application of Zn resulted in high Zn concentrations in corn grain and stover, and also higher Zn uptake in corn stover. Zee & O'Brian (1970) reported that in wheat and barley a large portion of Zn comes through its remobilization from leaves. Xue *et al.* (2012) also observed that remobilization of Zn from leaves to grain contributed to the Zn content of grain. Thus foliar-applied Zn that then easily moves to corn grain should be adopted as a practice for agronomic biofortification of corn.

From the viewpoint of biofortification of corn grain, Zn-coated urea was as good as foliar application. Further, Zn-coated urea applied to the soil resulted in significantly higher Zn uptake in corn grain and stover than foliar allocations. The Zn-coated urea, supplying nearly half the Zn as compared to soil application of Zn sulphate, was significantly superior to the latter from the viewpoint of Zn biofortification of corn grain and stover. This could be due to concomitant availability of Zn and N to the crop roots. This is supported by the fact that Zn fertilization of corn increased the N concentration and uptake by corn grain and stover. A positive N x Zn interaction has been reported in rice (Lakshmanan *et al.* 2005, Pooniya & Shivay 2012) and wheat (Kutman *et al.* 2012, Wu *et al.* 2010). In these studies increased Zn uptake due to N fertilization has been reported. The present study shows that the reverse (increased N concentration due to Zn fertilization) also occurs.

The results of the present study shows that for agronomic Zn biofortification of corn grain and stover, foliar application of 1 kg Zn sulphate $ha^{-1}$ (in two sprays at tasseling and initiation of flowering) or application of Zn-coated urea is better than soil application of Zn sulphate.

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