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Original Research Article

Zinc and organic fertilization in maize augments enzymatic activities and nutrient bio-availability rendering higher production

Abstract

Low fertility and organic matter contents in soil limit the nutrient bio-availability for crops leading to poor nutrition in the animals and human beings. This field study on maize production employed integration of organic and chemical fertilizers to find out their impact on plant enzymatic activities in relation to crop production, and nutrient dynamics in plants and soil. The treatments compared were: control (no application of N from any source); 100% N from fertilizer (FN); 75% FN + 25% N from organic manure (ON); and 50% FN + 50% ON. Further, all these treatments were superimposed by three levels of zinc (Zn) fertilizer, viz., 0, 4, 8 kg Zn ha^{-1}, applied each one separately. Conjunctive use of FN & ON with the ratio of 75+25 along with Zn application @ 4 kg ha^{-1} rendered the highest maize grain yield as compared to sole N application from chemical fertilizer. Similarly, enzymatic activities of nitrate reductase (NR) and superoxide dismutase (SOD) were significantly affected with combined use of N and Zn. Nitrogen, phosphorus and zinc contents in diagnostic leaves of maize were also higher in organically substituted treatments. So, integration of FN and ON was proved to render better nutrient supply to the plants throughout the growing season.

Keywords: Nutrient deficiencies; Chemical nitrogen fertilizer; Organic manure; Conjunctive use; Nitrate reductase; Superoxide dismutase.

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1 Introduction

Pakistani soils exhibit multiple nutrient deficiencies including that of zinc (Zn) due to their alkaline-calcareous nature and low organic matter content, nutrient mining with intensive cropping, and inadequate and imbalanced fertilizer use. Declined soil organic matter in cultivated lands is a major cause of soil degradation; converting these soils infertile and thus not sustaining the crop production (Diacono and Montemurro 2010). Nutrient mining has also gained concerns about the sustainability of agricultural system. Continuous use of synthetic fertilizers for long time not only turns the land infertile and decreases crops yield (Sortino *et al.* 2013) but also contaminates the environment (Hu and Qi 2013). Achieving a balance between nutrient requirements of the plants and nutrient supplies from the soil is essential for attaining and maintaining high crop yields and good soil fertility. Manures enhance the bio-availability of macro- and micro-nutrients, viz., N, P, K and Zn, and thus concentration of these nutrients in plant tissues (Masti *et al.* 2003). Degraded lands can be ameliorated by conjunctive use of organic manures and inorganic fertilizers (Saleque *et al.* 2004). Appropriate blend of chemical fertilizers and organic manures varies with land-use pattern, agro-ecology, and socio-economic circumstances. Similarly, organo-mineral combined fertilizer blend could be a promising alternative for sustaining the crop yields (Deeks *et al.* 2013). Improved management of crop residues / livestock manure and nutrients may also reduce the environmental impact of agriculture on climate change (Stavi and Lal 2013).

Nutrient deficiencies directly affect the enzymatic activities in plants. Zinc deficiency in crops is a common disorder in most of the cropped lands in Pakistan (Rafique *et al.* 2006). It is mainly due to calcareous-alkaline soils, low organic matter, nutrient depletion, and injudicious fertilizer application. Zinc is integral part of superoxide dismutase enzyme, which is important for detoxifying the reactive oxygen species (ROS) and controlling the free radicals (Cakmak 2000; Wang and Jin 2005) under drought stress. Similarly nitrate reductase is an important enzyme of N metabolism, its activity in plant is

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inducible by nitrate (Shankar *et al.* 2001). Therefore, N deficiency affects the activity of nitrate reductase in plants. Under rain-fed conditions maize production is lower than in irrigated areas, which is mainly due to water stress and poor soil fertility status. Continuous additions of organic amendments to cropland increase soil fertility, organic carbon, microbial biomass and enzymatic activities, and enhance the crop yields (Diacono and Montemurro 2010). With these understandings, a field experiment on integrated nutrient management was conducted on rain-fed maize crop.

This paper reports the maize crop response (yield, nutrients uptake and enzymes activity) to nitrogen and zinc fertilizers application along with organic manure. It was hypothesized that there will be differences in these crop attributes between organic-N fertilizer combinations and sole mineral N fertilizer treatments. Major objective of this study was to reduce the reliance on chemical fertilizers and enhance the use of organic amendments for sustainable soil health and crop production.

2 Materials and Methods

Field experiment was conducted on maize cv. Swan at the research farm of National Agricultural Research Centre (NARC), Islamabad (latitude 33º 43´ N, longitude 73º 04´ E, and altitude 490 m). Soil belongs to Nabipur soil series (coarse loamy, mixed, hyperthermic Udic Ustochrept). It is deep, well drained, and moderately calcareous developed on level to nearly level deposition of the flood plain. It lies under sub-humid to humid and medium to high rainfall zone with annual rainfall ranging from 517 to 1550 mm. Mean maximum temperature during summer ranges from 36 to 42°C. Soil is alkaline (pH, 7.8), calcareous ($CaCO_3$ equiv., 4.3 %), low in organic matter (0.50 %), and deficient in NO_3-N (3.5 mg kg^{-1}), available P (3.0 mg kg^{-1}), with extractable K (80 mg kg^{-1}) and Zn (0.27 mg kg^{-1}).

The experiment comprised of two factor treatments, viz., nitrogen sources combinations and zinc fertilization. The N application was made in main plots, while Zn was applied in sub-plots. Experimental

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design was split-plot under randomized complete block design (RCBD) with three replications. Plot size for main-plot treatments was 5 m × 9 m and each main-plot was divided into three equal sub-plots measuring 3 m × 5 m. Treatments detail is as follows:

Main plots - Nitrogen combinations

Control = No application of N from any source

FN100 = Whole N from chemical fertilizer (FN)

FN75+ON25 = 75% FN + 25% N from organic manure (ON)

FN50+ON50 = 50% FN + 50% ON

Sub plots - Zinc levels

Zn0 = No application of zinc fertilizer

Zn4 = Zinc fertilizer application @ 4 kg ha^{-1}

Zn8 = Zinc fertilizer application @ 8 kg ha^{-1}

Nitrogen was applied @ 120 kg ha^{-1} as recommended dose for the maize crop in rainfed areas using different combinations of organic (farm yard manure) and fertilizer N (urea) sources. Fertilizer N (FN) was applied in two splits, viz., at seed bed preparation and at booting stage. Farm yard manure containing 1.85% N was applied before planting @ 3.3 t ha^{-1} for replacing 50% recommended mineral N and 1.82 t ha^{-1} for 25% replacement. Phosphorus fertilizer was equally applied at the rate of 50 kg ha^{-1} in all the treatments at the time of sowing.

2.1 Soil and plant analysis

Before sowing the crop, composite soil samples were collected from 0-15cm depth, air dried, ground and passed through 2 mm sieve. Soil samples were analyzed for pH (McLean 1982), AB-DTPA P (Soltanpour and Workman 1979), Electrical conductivity (ECe), (Rhoades 1982), Organic matter (Nelson and Sommer 1982) NH_4OAC extractable K (Knudsen *et al.* 1982) NO_3-N and Zn (Soltanpour and Workman 1979). At blooming stage recently fully matured leaves from selected plants were collected.

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The leaves were washed, cleaned, air dried and then oven dried at 65°C to a constant weight. The samples were grinded, sieved and stored in small plastic bags for determination of N, P and Zn. Nitrogen was determined using Kjeldahl method as described by Jackson (1973), P was determined by wet digestion as described by Benton *et al.* (1991). Zinc was determined using Atomic Absorption Spectrometry as described by Wright and Stuczynski (1996).

2.2 Measurement of enzymes activity

Samples of 2nd youngest fully expanded leaves at booting stage (diagnostic leaves) were taken, immediately frozen in liquid N, ground to make fine powder, and refrigerated at –80°C. Extraction of antioxidant enzymes in these leaves was performed through the procedure as described by Sankhla *et al.* (1985). Activity of superoxide dismutase (SOD) enzyme was assayed spectrophotometrically at 560 nm by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (Dhindsa *et al.* 1981). For nitrate reductase activity, fresh leaf samples were weighed and shifted to plastic vials. In each vial, 2.5 mL phosphate buffer (pH 7.5), 0.2 M KNO_3 and 5% isopropanol solutions were added, and incubated for 2 h in dark at 30°C. After incubation, 1% sulphanilamide and 0.2% NED-HCl (N-1-naphthylethylene-diamine dihydrochloride) were added in to vials, and kept for 20 min for colour development. Absorbance in samples was measured spectrophotometrically at 540 nm as described by Jaworski (1971).

2.3 Statistical analysis

Data on growth, yield and nutrient uptake attributes of the crop were subjected to analysis of variance (Sokal and Rohlf 1997). Data showing significant difference at $P \leq 0.05$ were put to comparison of treatment means by least significant difference (LSD) test. All the data were processed using MSTAT-C software for statistical analysis.

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3. Results and Discussion

3.1 Maize grain yield

Pakistani soils exhibit multiple nutrient deficiencies including that of Zn (Rafique *et al.* 2006) due to their alkaline-calcareous nature and low organic matter content, nutrient mining with intensive cropping, and inadequate and imbalanced fertilizer use. Effect of experimental treatments on maize grain yield is given Fig 1. Treatment with combination of fertilizer and organic N (FN75+ON25) produced significantly higher grain yield as compared to FN50+ON50 combination and sole chemical N source (FN100). It shows a synergistic effect of organic and inorganic N sources. Secondly, the results of the study also showed positive interaction of N sources and Zn fertilization on maize productivity. The yield increase might be due to enhanced hormonal and enzymatic activities with the application of Zn, and due to balanced and sustained supply of nutrients from organic manure. In case of 100 % chemical N, the nutrients availability might not be sufficient for longer period. (Yadav *et al.* 2006) reported that compared to their sole application, integrated use of N, FYM and Zn is the best choice in term of crop yield, nutrient uptake, and economic return and benefit cost ratio. Shaheen *et al.* (2010) also observed enhanced crop yield and nutrient uptake through synergistic and improved fertilization practices.

3.2 Nitrogen concentration in diagnostic leaf of maize

Nitrogen concentration in maize diagnostic leaf (third leaf below tassel) is given in Fig 2. Compared to that in control, N application significantly increased N concentration. Regarding the comparison of N sources, organic treatments viz. FN75+ON25 and FN50+ON50 showed higher N contents than with sole N application (FN100). It reflects that N was available for long period to plants from organically substituted treatments. It might be due to synergistic effect of organic and inorganic sources on mineralization and sustained supply of essential nutrients. In various studies on IPNM, it has been observed that integration of N sources viz. organic and inorganic origin increased NPK concentration in

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alfalfa and maize (Lioveras *et al.* 2004; Sial *et al.* 2007). Under the combined application, decomposition process is enhanced by microbial activity, and energy is readily available from carbon for release of nutrients. Sial *et al.* (2007) also recorded higher N and P concentration in maize leaves and grain, when mineral N was applied in combination with organic sources.

Zinc fertilization also enhanced N concentration significantly in maize diagnostic leaf over control, but Zn application rates of 4 and 8 kg ha^{-1} were statistically similar. Different physiological processes are inhibited in plants due to Zn deficiency. As a result plant growth and yield are severely affected. Zinc plays fundamental role in critical cellular functions like protein metabolism, gene expression, bio membranes integrity, and IAA metabolism (Marschner 1995). It has also been reported that zinc application increased significantly protein and nitrogen content of maize plant (Tahir *et al.* 2009; Adiloglu and Saglam 2005).

Organically substituted N treatments along with Zn4 or Zn8 showed higher N contents in the diagnostic leaf as compared to that in sole N treatments with zinc, viz., FN100+Zn4 and FN100+Zn8. Overall, there was positive interactive effect on N content in maize. Verma *et al.* (2005) also recorded positive interactive effect of N and Zn on the growth, yield, quality and nutrient uptake of fodder sorghum.

3.3 Phosphorus concentration in diagnostic leaf of maize

Effect of N and Zn treatments on P concentration in diagnostic leaf of maize are given in Fig 3. Nitrogen and Zn treatments significantly differ in P contents. The highest P content was recorded in FN75+ON25 treatment, which was statistically higher than that under FN50+ON50 and FN100. Similar to N, Zn treatments have also increased the P content in diagnostic leaf. Zinc application at 4 and 8 kg ha^{-1} gave significantly higher P content than in control but statistically alike with each other. Akinrinde *et al.* (2006) also reported that Zn application increased the P contents in maize tissues.

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Interaction of N and Zn treatments was highly significant with respect to P concentration in diagnostic leaf . The highest P contents were recorded in organically substituted combinations of N and Zn, viz., FN75+ON25+Zn4, FN75+ON25+Zn8, FN50+ON50+Zn4 and FN50+ON50+Zn8. These treatments were statistically superior to all others including that of 100% fertilizer N application. These findings support the phenomena of higher nutrient availability under combined use of organic and inorganic fertilizers. Balanced application of plant nutrients and IPNM enhance crop yields (Dong *et al.* 2006).

3.4 Zinc Concentration in diagnostic leaf of Maize

Influence of N and Zn application on Zn content in diagnostic leaf is reflected in Fig 4. Zinc contents in diagnostic leaf were significantly increased with N and Zn application. The treatment containing 50% N gave significantly higher Zn content than with FN75+ON25 and FN100, which were statistically alike. Similarly, Zn application also affected Zn content of plant tissues compared to that in control. All the three levels of Zn application differed statistically with each other. Maximum contents were observed in Zn8 level, which were significantly higher than in Zn4 level. Interaction of N and Zn was also found significant that might be due to organic sources of N. The highest Zn concentration was observed in FN50+ON50+Zn8 followed by FN50+ON50+Zn4, which are statistically similar but differed with all other combinations of Zn and N fertilization. Other researchers also reported that zinc content and uptake by maize increased with Zn application as it attributed to better root growth (Obrador *et al.* 2002). Concentrations of N and Zn were also increased in maize plant with FYM application (Gondek and Mazur 2005). Akinrinde *et al.* (2006) also found that application of cow manure + $ZnSO_4$ produced the highest plant shoot biomass and gave the highest Zn uptake by maize.

Activity of superoxide dismutase

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Superoxide dismutase (SOD) is an antioxidant enzyme, which counters stress in plants. Zinc is the structural part of SOD, its activity was determined in leaves at booting stage to assess the effect of Zn application on SOD. Data regarding the SOD activity is mentioned in Fig. 5. The effect of N application on SOD activity was significant over control. However, N sources were statistically at par w.r.t. SOD activity. The highest SOD activity was recorded in N4 followed by N3 and sole application of N (FN100). The result is in line with the finding of (Alguacil *et al.* 2006), who reported that compost addition significantly enhanced the SOD and POD activities in the shoots of *J. oxycedrus*. Zinc application at 8 kg Zn ha^{-1} gave significantly higher activity than that with 4 kg Zn ha^{-1} and control. Interaction of Zn and N treatments was also significantly positive. The highest SOD activity was recorded in FN50+ON50+Zn8 followed by FN75+ON25+Zn8, and FN75+ON25+Zn4 treatments, being statistically similar.

Organic manure treatments with Zn significantly increased the SOD activity. It might be due to structural role of Zn in SOD enzyme. So, it has positive effect on the activity of SOD. Activity of SOD in wheat leaves significantly increased in NO_3 and NH_4 treated plants as compared to N deficient wheat plants (Polesskaya *et al.* 2004). Zn is a cofactor of 300 enzyme and proteins, and it is involved in cell division, nucleic acid metabolism and protein synthesis. Zinc application enhances the activities of antioxidant enzymes (Cakmak 2000). Wang and Jin (2007) also reported that Zn application raised the activity of SOD in maize leaves. Zinc enhances the SOD activity and reduces MDA contents, which is beneficial to counter the free radicals (Gao *et al.* 2001).

Nitrate reductase activity

Nitrate reductase is an important enzyme of N metabolism, its activity in plants is inducible by its substrate (NO_3). So, anything that affects nitrate availability will influence nitrate reductase activity (Pervaiz *et al.* 2002). Nitrogen application significantly enhanced nitrate reductase activity (NRA) over control even N sources significantly differ in NRA with each other (Fig 6). The highest NRA was recorded in FN50+ON50, where N was applied in equal ratio from organic and inorganic sources. Similar

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to N application, Zn also significantly affected the NRA. Zinc application at 8 kg ha^{-1} showed the highest NRA, which was significantly higher than with 4 kg Zn ha^{-1}. Zinc fertilization showed a positive effect on NRA. Maximum NRA was observed in manure supplemented treatments with Zn application.

Soil microbial biomass and enzymatic activities increase with integrated nutrient management (INM), which ultimately activates the micronutrients bio-availability (Tejada *et al.* 2008). Interactive effect of N sources and Zn was also significant in both crops. The highest NRA was recorded in FN50+ON50+Zn8 followed by FN50+ON50+Zn4 and FN75+ON25+Zn8 treatments. Alguacil *et al.* (2006) also found that under drought conditions, only inoculation with exotic arbuscular mycorrhizae fungi increased the shoot and root NRA by 188 and 38%, respectively as compared to the plants without inoculation or compost application. Nardi *et al.* (2004) also reported that NRA correlated positively with organic N, and humus extract of FYM treated soil contained 42% higher NRA as compared to control. Soil microbial biomass also increases enzymatic activities in soil.

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Figure Captions

Fig. 1. Effect of N sources and Zn levels on maize grain yield

Fig. 2. Effect of N sources and Zn levels on nitrogen concentration in maize leaf

Fig. 3. Effect of N sources and Zn levels on phosphorus concentration in maize leaf

Fig. 4. Effect of N sources and Zn levels on zinc concentration in maize leaf

Fig. 5. Effect of N sources and Zn levels on superoxide dismutase activity in maize leaf

Fig. 6. Effect of N sources and Zn levels on nitrate reductase activity in maize leaf

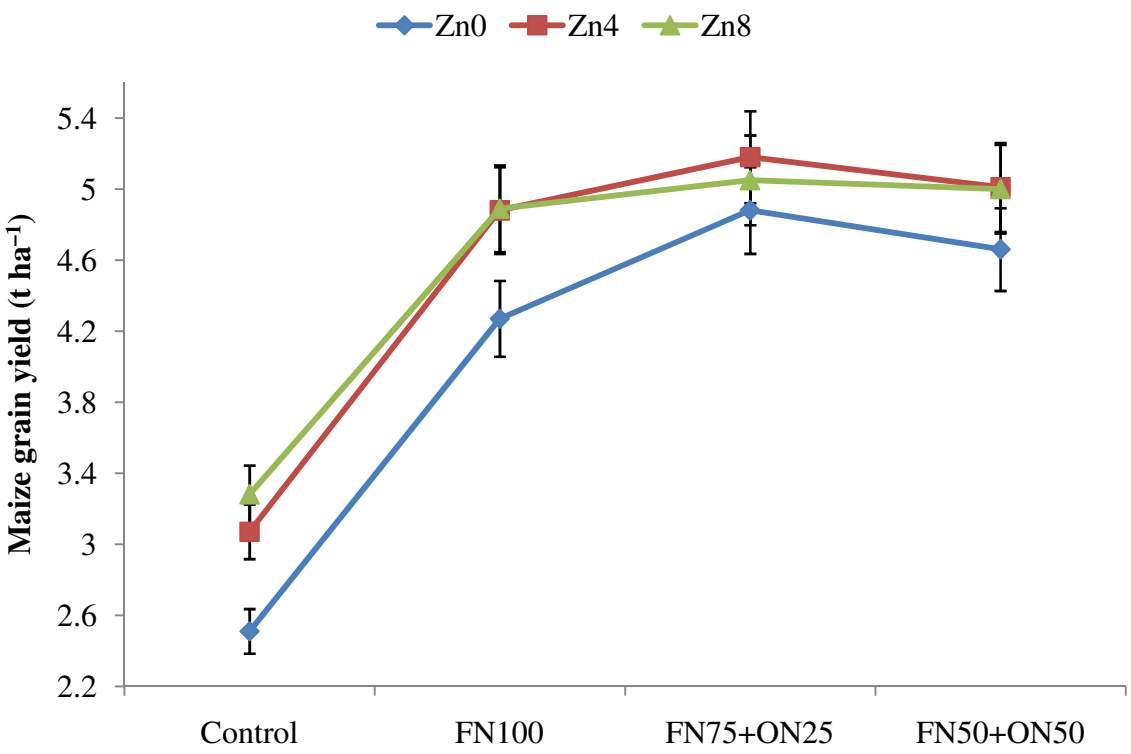

Fig. 1. Effect of N sources and Zn levels on maize grain yield

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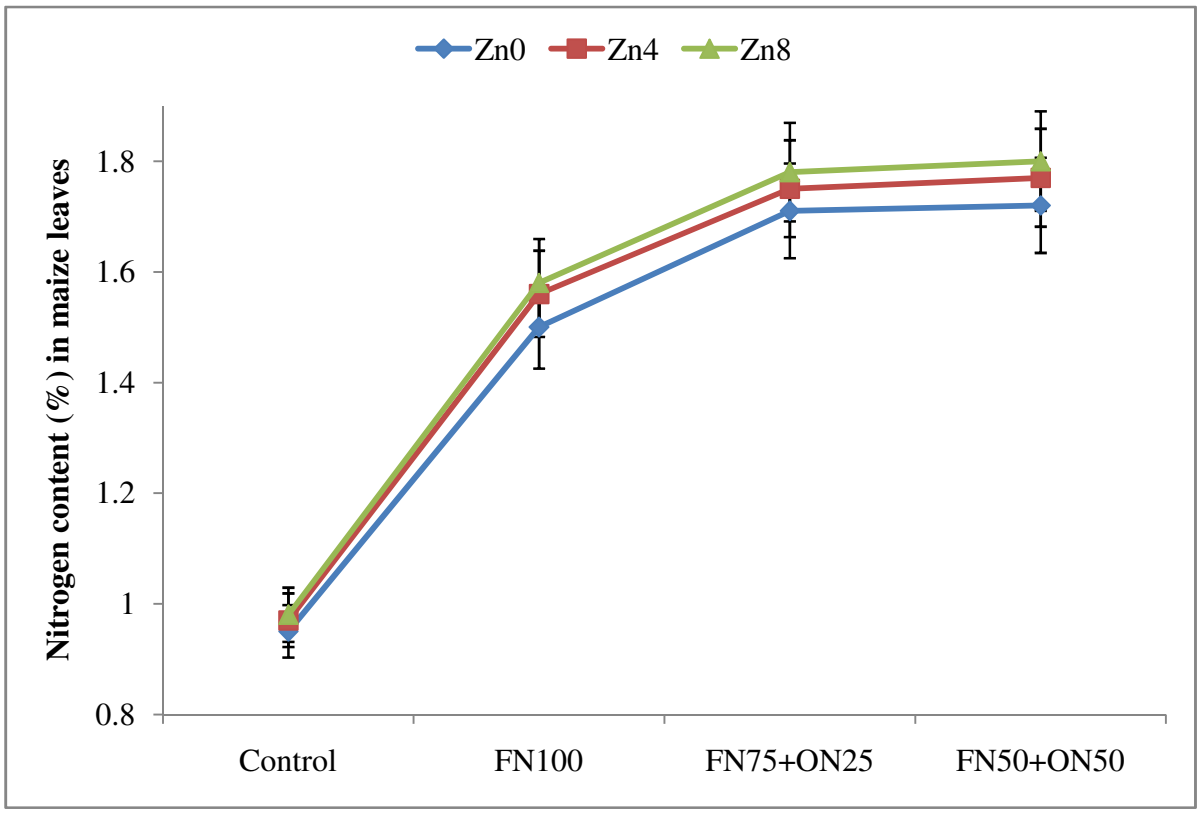

Fig. 2. Effect of N sources and Zn levels on nitrogen concentration in maize leaf

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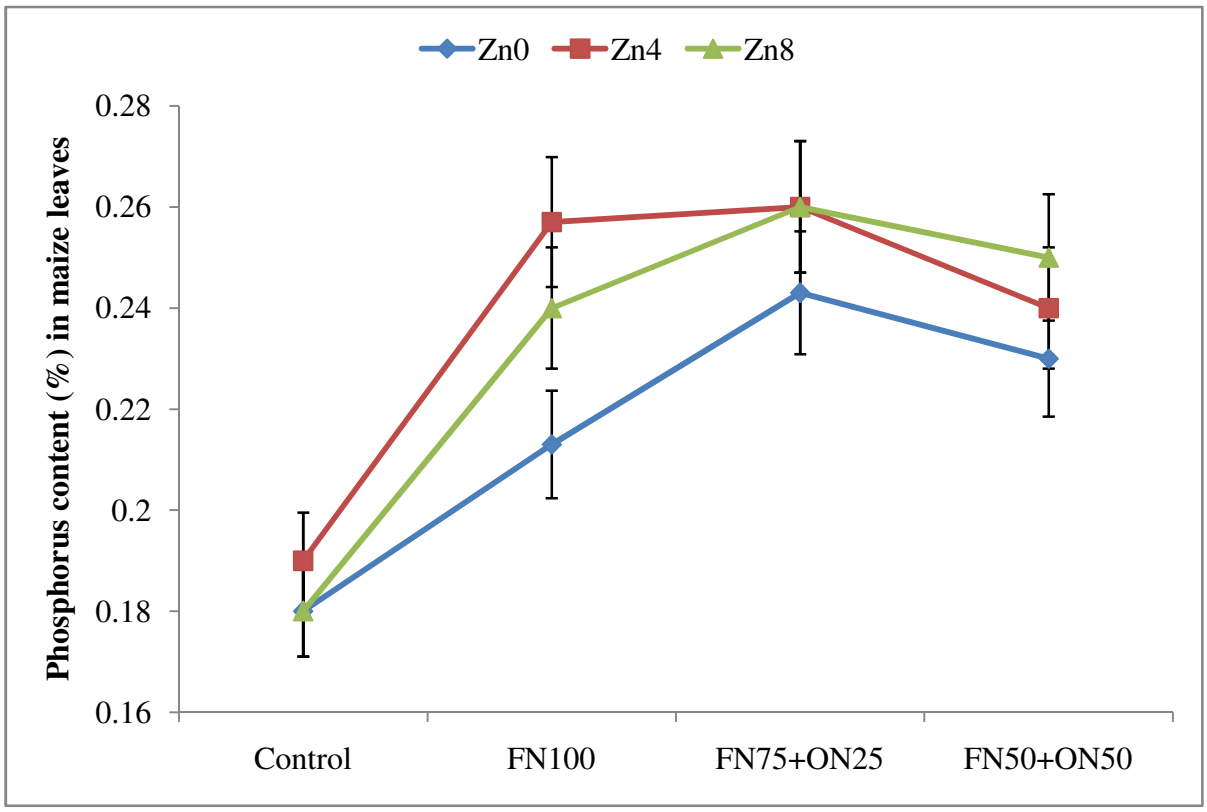

Fig. 3. Effect of N sources and Zn levels on phosphorus concentration in maize leaf

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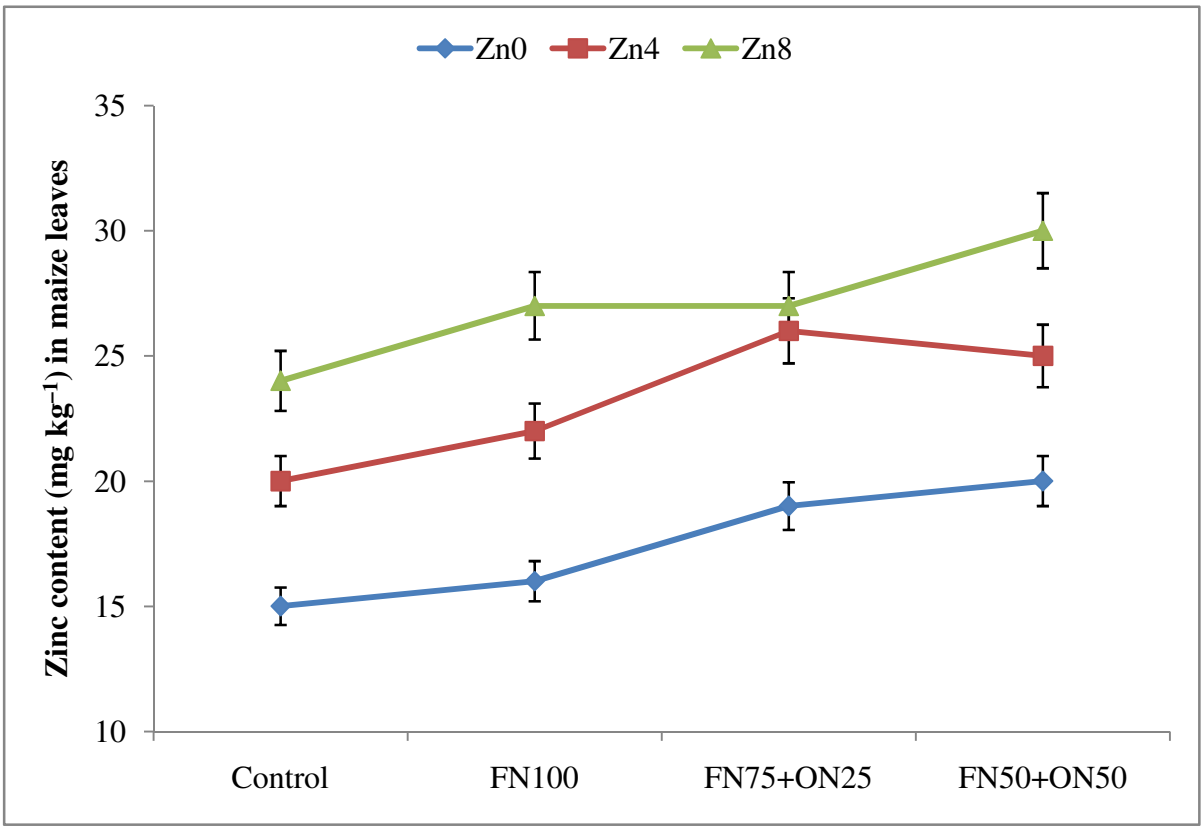

Fig. 4. Effect of N sources and Zn levels on zinc concentration in maize leaf

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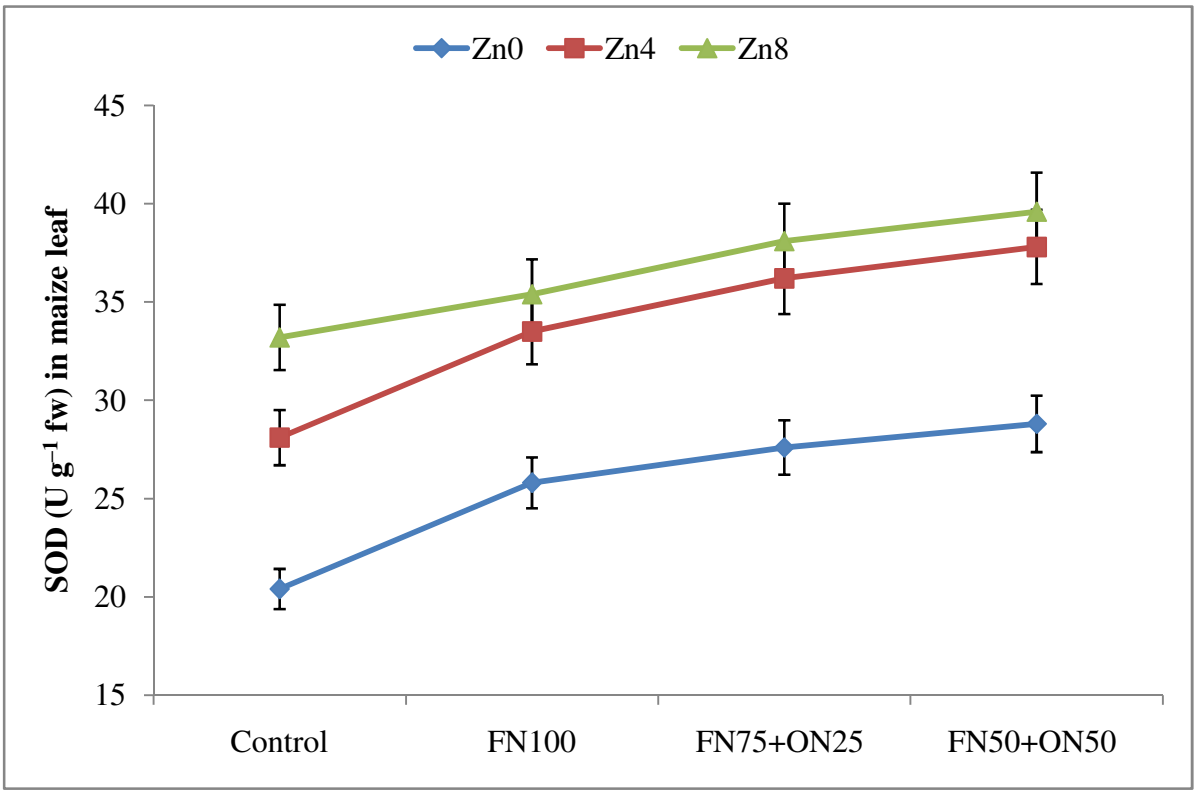

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339 **Fig. 5.** Effect of N sources and Zn levels on superoxide dismutase activity in maize leaf

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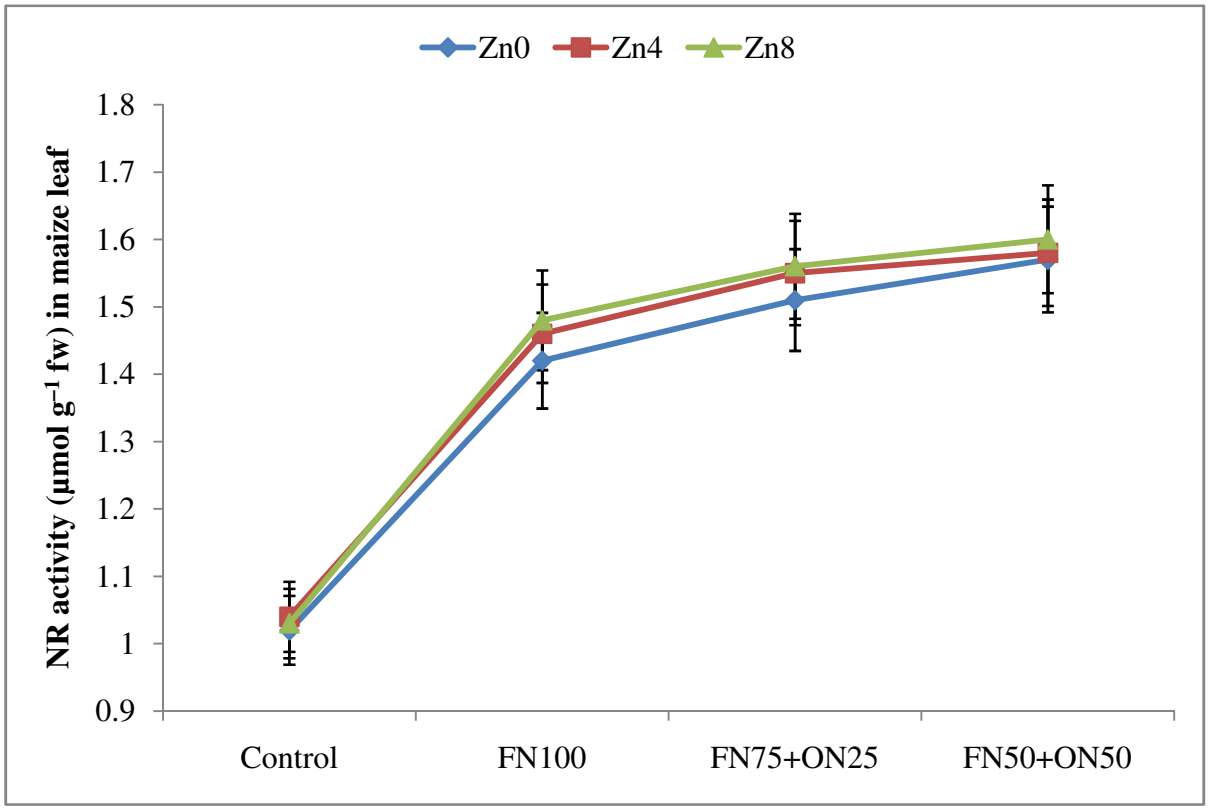

Fig. 6. Effect of N sources and Zn levels on nitrate reductase activity in maize leaf