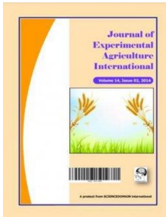

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Enzymatic and Nutritive Evaluation of Zinc and Organic Fertilization on Field Maize Yields in Islamabad - Pakistan

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Authors' contributions

This research was carried out mainly by the principal author MS as part of his doctoral studies, and wrote the first draft of the manuscript. Author GJ was his research supervisor, while other authors were members of his supervisory committee and all of them contributed by one way or the other in the planning of experiment, guidance in data analysis, write-up and discussion of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Low fertility and organic matter contents in soil limit the nutrient bio-availability for crops, potentially leading to poor nutrition in the animals and human beings that consume those crops. This field study on maize production employed integration of organic and chemical fertilizers to determine 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

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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}.Conjunctive use of FN and ON with the ratio of 75+25 along with Zn application at the rate of 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.

Keywords: Nutrient deficiencies; chemical nitrogen fertilizer; organic manure; conjunctive use; nitrate reductase; superoxide dismutase.

1. INTRODUCTION

Pakistani soils exhibit multiple nutrient deficiencies including that of nitrogen (N), phosphorus (P) and zinc (Zn) due to their alkaline-calcareous nature and low organic matter content, nutrient mining by previous intensive cropping, and inadequate and imbalanced fertilizer use. Particularly, low N contents in soil as well as crop are exhibited even under the application of recommended N fertilizer dose, which is mainly caused by N losses (40-60%) through NH_3 volatilization triggered by high temperature and alkaline soil pH in the country. Low soil organic matter in cultivated lands is a major cause of soil degradation; making these soils infertile and thus not capable of sustaining crop production [1]. Nutrient mining has also raised concerns about the sustainability of agricultural systems. Continuous use of imbalanced synthetic fertilizers over time can lower crops yield [2] and also contaminates the environment [3]. 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 etc., and thus raise the concentration of these nutrients in plant tissues [4]. Degraded lands can be ameliorated by conjunctive use of organic manures and inorganic fertilizers [5]. Appropriate blending of chemical fertilizers and organic manures varies with land-use pattern, agro-ecology, and socio-economic circumstances. Similarly, an organo-mineral combined fertilizer blend could be a promising alternative for sustaining the crop yields [6]. Improved management of crop residues / livestock manure and nutrients may also reduce the environmental impact of agriculture on climate change [7].

Nutrient deficiencies directly affect enzymatic activities in plants. Zinc deficiency in crops is a common disorder in most of the cropped lands in Pakistan [8]. It is mainly due to calcareous-alkaline soils, low organic matter, nutrient depletion, and injudicious fertilizer application. Zinc is a cofactor for one class of superoxide dismutase enzymes, which are important for detoxifying reactive oxygen species (ROS) and controlling free radicals [9,10] especially under drought stress. Similarly nitrate reductase is an important enzyme of N metabolism: its activity in plants is inducible by nitrate [11]. Therefore, addressing N deficiency by the use of nitrate 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 crop yields [1]. With these understandings, a field experiment on integrated nutrient management was conducted on rain-fed maize.

This paper reports the maize crop response (yield, nutrient uptake and enzyme 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. A 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

The field experiment was conducted using maize cv. Swan at the research farm of National Agricultural Research Centre (NARC), Islamabad (latitude 33º43´ N, longitude 73º04´ E, and altitude 490 m). The soil is classified in the 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 is located in a sub-humid to humid zone with medium to high rainfall, with annual rainfall ranging from 517 to 1550 mm. Mean maximum temperature during summer ranges from 33 to 40ºC [12]. Analysis revealed that original field soil was 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^{-l}), available P (3.0 mg kg^{-l}), with extractable K (80 mg kg^{-l}) and Zn (0.27 mg kg^{-l}).

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 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 at the rate of 4 kg ha^{-1}
Zn8 = Zinc fertilizer application at the rate of 8 kg ha^{-1}

Nitrogen was applied at the rate of 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 analysis showed 1.85% N, 0.43% P and 1.14% K contents. It was applied before planting at the rate of 3.3 t ha^{-1} for replacing 50% of recommended mineral N, and at the rate of 1.82 t ha^{-1} for 25% replacement of N fertilizer. 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 0-15 cm depth, air dried, ground and passed through 2 mm sieve. Soil samples were analyzed for pH [13], AB-DTPA P [14], electrical conductivity (ECe), [15], organic matter [16] NH_4OAC extractable K [17], NO_3-N and Zn [14]. At blooming stage, recently fully matured leaves from selected plants were collected. The leaves were washed, cleaned, air dried and then oven dried at 65°C to a constant weight. The samples were ground, sieved and stored in small plastic bags for determination of N, P and Zn. Nitrogen was determined using the Kjeldahl method as described by Jackson [18]. Phosphorus was determined by wet digestion as described by Benton and co-workers [19]. Zinc was determined using atomic absorption spectrometer as described by Wright & Stuczynski [20].

2.2 Measurement of Enzyme Activities

Samples of second youngest fully expanded leaves at booting stage (diagnostic leaves) were taken, immediately frozen in liquid N, ground to make fine powder, and refrigerated at –80ºC. Activity of superoxide dismutase (SOD) was assayed spectrophotometrically by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT). One unit of enzyme activity (expressed as U g^{-1} of fresh biomass weight) is the amount of SOD causing inhibition of photoreduction rate of NBT by 50% recorded at 560 nm absorbance [21]. For nitrate reductase activity (μmol g^{-1} of fresh biomass weight), fresh diagnostic 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 samples were 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, and the vials kept for 20 min for colour development. Absorbance values of these samples was measured spectrophotometrically at 540 nm as described by Jaworski [22].

2.3 Statistical Analysis

Data on growth, yield and nutrient uptake attributes of the crop were subjected to analysis of variance [23]. Data showing significant difference at $P \le 0.05$ 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.

3. RESULTS AND DISCUSSION

3.1 Maize Grain Yield

Pakistani soils exhibit multiple nutrient deficiencies including that of Zn [8] 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 shown in Table 1. Treatment with a combination of fertilizer and organic N (FN75+ON25) produced significantly higher grain yield as compared to the 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 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 and co-workers [24] 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 and co-workers [25] 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 the maize diagnostic leaf (third leaf below tassel) is given in Table 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 nutrient: in various studies on integrated plant nutrient management (IPNM), it has been observed that integration of N sources, viz., organic and inorganic origin increased NPK concentration in alfalfa and maize [26,27]. Under the combined application, the decomposition process is enhanced by microbial activity, and energy is readily available from carbon for release of nutrients. Sial and co-workers [27] 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 the maize diagnostic leaf over control, but Zn application rates of 4 and 8 kg ha^{-1} were statistically similar. As referenced below, different physiological processes are inhibited in plants due to Zn deficiency. As a result, plant growth and yield were severely affected. Zinc plays fundamental role in critical cellular functions like protein metabolism, gene expression, bio membranes integrity, and IAA metabolism [28]. It has also been reported that zinc application significantly increased protein and nitrogen content of maize [29,30].

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 and co-workers [31] 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

Effects of N and Zn treatments on P concentration in the diagnostic leaf of maize are given in Table 3. Nitrogen and Zn treatments significantly affected P contents. The highest P content was recorded in FN75+ON25 treatment, which was statistically higher than that with FN50+ON50 or FN100. Similar to N, Zn treatments have also increased the P content in the diagnostic leaf. Zinc application at 4 and 8 kg ha^{-1} gave significantly higher P content than in control but were statistically alike with each other. Akinrinde and co-workers [32] also reported that Zn application increased the P contents in maize tissues.

Table 1. Maize grain yield (t ha^{-1})

Treatment	Nitrogen source input (%)		Zn levels (kg ha^{-1})			Means (N)
			Zn1	Zn2	Zn3	
	Mineral	Organic	0	4	8	
N1	00	00	2.51 g	3.07 f	3.28 e	2.95 D
N2	100	00	4.27 d	4.88 b	4.89 b	4.68 C
N3	75	25	4.88 b	5.18 a	5.05 ab	5.04 A
N4	50	50	4.66 c	5.01 ab	5.00 b	4.89 B
Means (Zn)			4.08 B	4.54 A	4.55 A	

Means of N sources, Zn levels or their interactions bearing same letter(s) are statistically alike at p ≤ 0.05

Table 2. Nitrogen concentration (g/100 g) in maize leaf

Treatment	Nitrogen Source Input (%)		Zinc Levels (kg ha^{-1})			Means (N)
			Zn1	Zn2	Zn3	
	Mineral	Organic	0	4	8	
N1	00	00	0.95 f	0.97 f	0.98 f	0.97 C
N2	100	00	1.50 e	1.56 d	1.58 d	1.55 B
N3	75	25	1.71 e	1.75 abc	1.78 a	1.75 A
N4	50	50	1.72 bc	1.77 ab	1.80 a	1.76 A
Means (Zn)			1.47 B	1.51 A	1.53 A	

Means of N sources, Zn levels or their interactions bearing same letter(s) are statistically alike at p ≤ 0.05

Table 3. Phosphorus concentration (g/100 g) in maize leaf

Treatment	Nitrogen source input (%)		Zinc levels (kg ha^{-1})			Means (N)
			Zn1	Zn2	Zn3	
	Mineral	Organic	0	4	8	
N1	00	00	0.18 e	0.19 e	0.18 e	0.18 C
N2	100	00	0.21 d	0.26 ab	0.24 bc	0.24 B
N3	75	25	0.24 cd	0.26 bc	0.26 ab	0.25 A
N4	50	50	0.23 abc	0.24 ab	0.25 a	0.24 B
Means (Zn)			0.22 B	0.24 A	0.23 A	

Means of N sources, Zn levels or their interactions bearing same letter(s) are statistically alike at p ≤ 0.05

Interaction of N and Zn treatments was highly significant with respect to P concentration in diagnostic leaf samples. 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 [33].

3.4 Zinc Concentration in Diagnostic Leaf of Maize

Influence of N and Zn application on Zn content in diagnostic leaf is reflected in Table 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 three levels of Zn application differed statistically. Maximum contents were observed in Zn8 level, which were significantly higher than in Zn4 level. Interactions of source of N and Zn were also found significant as related treatments with relative levels of 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 and attributed it to better root growth [34]. Concentrations of N and Zn were also increased in maize plants with FYM application

[35]. Akinrinde and co-workers [32] also found that application of cow manure + $ZnSO_4$ produced the highest plant shoot biomass and gave the highest Zn uptake by maize.

3.5 Activity of Superoxide Dismutase

Superoxide dismutase (SOD) is an antioxidant enzyme, which counters stress in plants. Zinc is the structural part of SOD, so activity was determined in leaves at booting stage to assess the effect of Zn application on SOD. Data regarding SOD activity is shown in Table 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 Z8 followed by Z4. The result is in line with the finding of Alguacil and co-workers [36], who reported that compost addition significantly enhanced the SOD and peroxidase (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 the structural role of Zn in SOD enzyme providing 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 [37]. Zn is a cofactor of 300 different enzymes and proteins, and it is involved in cell division, nucleic acid metabolism and protein synthesis. Zinc application enhances the activities of antioxidant enzymes (9). Wang & Jin [10] 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 [38].

3.6 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 [39]. Nitrogen application significantly enhanced nitrate reductase activity (NRA) over control even when N sources significantly differ in NRA with each other (Table 6). The highest NRA was recorded in FN50+ON50, where N was applied in equal ratio from organic and inorganic sources. Similar to N application, Zn also significantly affected the NRA. Zinc application at 8 kg ha^{-1}

Table 4. Zinc concentration (mg kg^{-1}) in maize leaf

Treatment	Nitrogen source input (%)		Zinc Levels (kg ha^{-1})			Means (N)
			Zn1	**Zn2**	**Zn3**	
	Mineral	**Organic**	**0**	**4**	**8**	
N1	**00**	**00**	15 NS	20	24	19 C
N2	**100**	**00**	16	22	27	21 B
N3	**75**	**25**	19	26	27	24 A
N4	**50**	**50**	20	25	30	25 A
Means (Zn)			17 C	23 B	27 A	

Means of N sources, Zn levels or their interactions bearing same letter(s) are statistically alike at $p \leq 0.05$
NS Means of N sources, Zn levels or their interactions have statistically non significant difference at $p \leq 0.05$

Table 5. Activity of SOD (U g^{-1} of fresh biomass weight) in maize diagnostic leaf

Treatment	Nitrogen Source Input (%)		Zinc Levels (kg ha^{-1})			Means (N)
			Zn1	**Zn2**	**Zn3**	
	Mineral	**Organic**	**0**		**8**	
N1	**00**	**00**	20.4 d	28.1 c	37.2 a	28.6 B
N2	**100**	**00**	25.8 c	33.5 b	37.9 a	32.4 A
N3	**75**	**25**	27.6 c	39.5 a	38.0 a	35.0 A
N4	**50**	**50**	28.8 c	36.8 a	38.6 a	34.7 A
Means (Zn)			25.6 C	34.5 B	37.9 A	

Means of N sources, Zn levels or their interactions bearing same letter(s) are statistically alike at $p \leq 0.05$

Table 6. Activity of nitrate reductase (μmol g^{-1} of fresh biomass weight) in maize diagnostic leaf

Treatment	Nitrogen Source Input (%)		Zinc Levels (kg ha^{-1})			
			Zn1	Zn2	Zn3	Means (N)
	Mineral	Organic	0		8	
N1	00	00	1.02 h	1.04 h	1.03 h	1.03 D
N2	100	00	1.42 g	1.46 f	1.48 e	1.45 C
N3	75	25	1.51 d	1.55 c	1.56 bc	1.54 B
N4	50	50	1.57 b	1.58 d	1.60 a	1.58 A
Means (Zn)			1.38 C	1.41 B	1.42 A	

Means of N sources, Zn levels or their interactions bearing same letter(s) are statistically alike at $p \leq 0.05$

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 [40]. 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 and co-workers [36] 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 and co-workers [41] 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.

4. CONCLUSIONS

This study compared different combinations of organic and mineral nitrogen fertilizers with and without zinc application for maize growth, yield, nutrient uptake and enzymatic activities. Farmyard manure (25% N-basis) + 4 kg Zn ha^{-1} performed better than N fertilizer alone (100%) for maize production. Integration of organic and inorganic sources of nitrogen improved N and P status in soil possibly because the farmyard manure also contained some quantities of nutrients other than nitrogen. Activity of antioxidative enzyme (superoxide dismutase) was enhanced with the application of zinc and FYM, indicating improved stress tolerance in plants. Further, enhanced activity of nitrate reductase under integrated N fertilization indicated balanced supply of nitrogen to the crop.

The study led to the conclusion that the synergistic use of nitrogen sources (FYM and chemical fertilizer at 25:75 N ratio) is advantageous over sole application of mineral fertilizer. Farm yard manure and zinc fertilization further enhance the crop growth, yield and antioxidative enzyme activity for stress tolerance. Twenty percent increased maize yield with the above mentioned IPNM strategy makes the system economically incentive based.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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