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Short Communication

Influence of Varying Levels of Zinc on Yield and Zinc Biofortification in Hybrid Rice (*Oryza sativa* L.) Grown in Moderate Zinc Soil

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Zinc (Zn) is regarded as the 4th most significant yield-limiting nutrient after nitrogen (N), phosphorus (P) and potassium (K). The average total Zn in Indian soil is 55 mg kg^{-1} and the available Zn is 0.54 mg kg^{-1}. Singh *et al.* (2016) reported that 33.1% soils of Eastern Uttar Pradesh were deficit in Zn and DTPA-extractable Zn content in soils of Varanasi district ranged from 0.03 to 5.36 mg kg^{-1}. Zinc bioavailability in soil not only affects plant uptake but also human and animal nutrition. Approximately one-third of the world's population suffers from Zn deficiency and is ranked 5th largest risk factor for disease in developing countries (Cakmak and Kutman 2018). In early life stages, Zn deficiency may influence embryogenesis, growth retardation, impaired brain development, increased susceptibility to infectious diseases. Nutrient fortification is a preferred way of addressing the problem of the undernourished rural population as majority of them are unable to obtain a variety of diets, supplements and commercially fortified foods. Bio-fortification is a method of enhancing the bio-availability of vital elements in edible crop portions through agronomic action or genetic selection (White and Broadly 2011). Zinc may be applied as seed coating, soil application or foliar spray for fortification of crops with Zn and for better translocation of Zn to grain. Das *et al.* (2018) noted that Zn application not only corrects crop Zn deficiency but also enhances crop yield and productivity. The information on Zn fertilizers application for high yielding hybrid rice in soils with moderate Zn is limited. Soils with available Zn above the critical limits have also been reported to respond to Zn application (Varshney *et al.* 2008). The present study was undertaken to investigate the effect of bio-fortification of Zn through various levels of Zn on yield and availability of Zn in hybrid rice using a moderate Zn containing soil of middle Gangetic plain of Eastern Uttar Pradesh, India.

A pot experiment was conducted using a bulk soil (0–15 cm) with moderate in Zn content (1.20 mg kg^{-1}) collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The experiment was conducted during 2018-19 in completely randomised design (CRD) involving ten treatments *viz.,* T_1: control (no fertilizer), T_2: recommended dose of fertilizer (RDF), T_3: RDF + Zn @ 1.25 mg kg^{-1}, T_4: RDF + Zn @ 2.5 mg kg^{-1}, T_5: RDF + Zn @ 3.75 mg kg^{-1}, T_6: RDF + Zn @ 5.0 mg kg^{-1}, T_7: RDF + Zn @ 6.25 mg kg^{-1}, T_8: RDF + Zn @ 7.5 mg kg^{-1}, T_9: RDF + Zn @ 8.75 mg kg^{-1}, T_{10}: RDF + Zn @ 10.0 mg kg^{-1} and each treatment was replicated thrice. The soil sample was air-dried, ground and passed through 2-mm sieve. The soil was homogeneously mixed and each polythene lined pot was filled with 10 kg of soil. Early maturing hybrid rice variety Arize® H 6444 Gold was taken as a test crop. The recommended doses of N, P and K for hybrid rice applied were 75, 30 and 30 mg kg^{-1}, respectively. Urea, di-ammonium phosphate (DAP) and muriate of potash (MOP) was used to supply the NPK to the crop. Half dose of N and full dose of P and K were applied in solution form before transplanting of rice and remaining half-dose of N was applied at 30 and 60 days following trans-plantation (DAT) in two equal splits. Zinc was applied through zinc sulphate ($ZnSO_4.7H_2O$) in solution form to each Zn treated pots in required quantity. Five rice plants were transplanted and grown up to maturity and 5 cm of water level was maintained continuously in each pot up to physiological maturity of rice.

Rice was harvested at 120 DAT with the help of sickle and cut was made 5 cm above the soil surface to avoid contamination from soil. Plant samples were washed in detergent solution (0.2% liquid) followed

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by 0.1 *N* HCl solution and de-ionized water. The plant samples were kept in hot air oven at 60 °C till the constant weight. The grain and straw yield per pot were recorded and harvest index (HI) was calculated as follows:

Harvest index (%) =

(Economic yield)/(Total biological yield) ×100

Soil reaction (pH) and electrical conductivity (EC) of post-harvest soil samples were analyzed in 1:2.5 soil: water suspension (Tandon 2001). Organic carbon (OC) was determined by method of Walkley and Black (1934), while micronutrients were extracted using the DTPA solution (Lindsay and Norvell 1978) and measured by atomic absorption spectrophotometer (Agilent FS 240). The hot water extractable boron (B) was estimated using the method outlined by Berger and Truog (1939). Di-acid mixture (HNO_3: $HClO_4$:: 9:4, v/v) was used to digest the plant samples (grain and straw) and Zn content was determined by atomic absorption spectrophotometer following the procedure as outlined by Tandon (2001). The data acquired were analyzed using statistical software SPSS 16.0 for ANOVA (Complete Randomized Design). Duncan Multiple Range Test (DMRT) at *p≤0.05* levels of significance was used to evaluate the significant differences among treatment mean values.

Zinc fertilization showed a significant (*p≤0.05*) response with respect to the grain and straw yield and harvest index of rice (Table 1). The data showed that the grain and straw yields increased with soil application of Zn up to 6.25 mg Zn kg^{-1} (T_7) and beyond which, a significant reduction in yield was recorded. The grain and straw yield increased by 24.0 and 21.5 per cent, respectively in T_7 (6.25 mg Zn kg^{-1}) over RDF (T_2). The data revealed that harvest index (HI) varied from 52.2% in 8.75 mg Zn ha^{-1} (T_9) to 45.8% in control (T_1) (Table 1). The argument on the improved rice yield by Zn addition in the current research was strongly endorsed by the significant positive correlation (r = 0.73*, *p≤0.05*) between grain yield and grain Zn content. Higher yield due to Zn fertilization has been ascribed to its involvement in numerous metallic enzyme systems, regulatory functions, auxin manufacturing, carbohydrate synthesis and transport to the grain formation sites (Pedda Babu *et al.* 2007). Jatav and Singh (2018) also reported that application of Zn significantly increased the grain and straw yield of rice.

The concentration of Zn in rice grain varied from 17.5 to 28.2 mg kg^{-1} (Table 2). The maximum concentration was found in treatment receiving 6.25 mg Zn kg^{-1} (T_7), which showed 44.3 per cent increase over RDF (T_2). It was observed that Zn concentration in rice grain significantly increased up to soil application of 6.25 mg Zn kg^{-1} (T_7), above this level, a decrease in Zn concentration was noticed. This might be due to toxic effect of Zn on plant. The concentration of Zn in straw varied from 35.4 to 54.3 mg kg^{-1} (Table 3), the maximum being obtained with soil application of 6.25 mg Zn kg^{-1} (T_7), which recorded 18.2 per cent increase over RDF (T_2). Cakmak (2015) revealed that the enhanced concentration of Zn in brown rice resulted from enhanced accessibility, absorption, translocation, and deposition. Hussain *et al.* (2018) recorded a rise in Zn concentration in grain due to a gradual rise in Zn application (5, 10, 15 and 20 mg Zn kg^{-1}) having

Table 1. Effect of zinc application on grain yield, straw yield and harvest index of rice

Treatments	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})	Harvest index (%)
T_1 (Control)	19.9^{e}	23.5^{g}	45.8^{d}
T_2 (RDF)	29.2^{d}	31.0^{e}	48.4^{bcd}
T_3 (RDF + $Zn_{1.25}$)	29.5^{d}	31.3^{de}	48.5^{bcd}
T_4 (RDF + $Zn_{2.5}$)	33.1^{c}	33.3^{cde}	49.8^{abc}
T_5 (RDF + $Zn_{3.75}$)	33.9^{bc}	36.3^{ab}	48.3^{bcd}
T_6 (RDF + $Zn_{5.0}$)	35.5^{ab}	35.4^{abc}	50.1^{abc}
T_7 (RDF + $Zn_{6.25}$)	36.1^{a}	37.7^{a}	48.9^{bcd}
T_8 (RDF + $Zn_{7.5}$)	30.3^{d}	34.2^{bcd}	46.9^{cd}
T_9 (RDF + $Zn_{8.75}$)	29.5^{d}	27.1^{f}	52.2^{a}
T_{10} (RDF + $Zn_{10.0}$)	29.0^{d}	27.5^{f}	51.4^{ab}
SEm±	0.66	0.94	0.95
CD (p ≤ 0.05)	1.94	2.78	2.80

Different letters for each parameter show significant difference at *p≤0.05* by Duncan's Multiple Range Test.

Table 2. Effect of zinc application on micronutrient concentration (mg kg^{-1}) in rice grain

Treatments	Zn	Cu	Fe	Mn	B
T_1 (Control)	17.5^{f}	4.7^{c}	36.3^{f}	14.1^{e}	15.5^{ef}
T_2 (RDF)	19.5^{ef}	4.8^{c}	56.2^{ab}	19.2^{bc}	17.6^{cd}
T_3 (RDF + $Zn_{1.25}$)	21.3^{de}	6.2^{a}	60.5^{a}	24.9^{a}	20.4^{b}
T_4 (RDF + $Zn_{2.5}$)	21.9^{d}	6.2^{a}	59.4^{ab}	20.4^{b}	22.0^{a}
T_5 (RDF + $Zn_{3.75}$)	23.5^{cd}	6.0^{ab}	54.3^{b}	18.5^{bcd}	16.3^{de}
T_6 (RDF + $Zn_{5.0}$)	26.0^{ab}	5.9^{ab}	47.2^{c}	16.5^{cde}	18.9^{c}
T_7 (RDF + $Zn_{6.25}$)	28.2^{a}	5.1^{c}	45.0^{cde}	17.6^{bcde}	14.3^{f}
T_8 (RDF + $Zn_{7.5}$)	27.6^{a}	4.8^{c}	46.2^{cd}	15.8^{cde}	14.1^{f}
T_9 (RDF + $Zn_{8.75}$)	25.2^{bc}	5.3^{bc}	41.5^{def}	16.1^{cde}	14.4^{f}
T_{10} (RDF + $Zn_{10.0}$)	23.2^{cd}	5.0^{c}	39.8^{ef}	15.0^{de}	14.9^{ef}
SEm ±	0.76	0.23	1.71	2.60	0.48
CD (p≤0.05)	*2.20*	*0.68*	*5.00*	*0.90*	*1.40*

Different letters for each parameter show significant difference at *p≤0.05* by Duncan's Multiple Range Test.

Table 3. Effect of zinc application on micronutrient concentration (mg kg^{-1}) in rice straw

Treatments	Zn	Cu	Fe	Mn	B
T_1 (Control)	35.4^{e}	3.80^{f}	246^{g}	197^{e}	15^{bc}
T_2 (RDF)	44.4^{d}	5.90^{c}	336^{b}	266^{b}	17^{a}
T_3 (RDF + $Zn_{1.25}$)	48.7^{c}	8.38^{a}	352^{a}	284^{a}	17^{a}
T_4 (RDF + $Zn_{2.5}$)	49.7^{c}	7.10^{b}	311^{c}	266^{b}	16^{ab}
T_5 (RDF + $Zn_{3.75}$)	51.7^{b}	6.21^{c}	293^{d}	260^{bc}	17^{a}
T_6 (RDF + $Zn_{5.0}$)	52.9^{ab}	5.89^{c}	288de	225^{d}	15^{bc}
T_7 (RDF + $Zn_{6.25}$)	54.3^{a}	5.15^{d}	288de	248^{c}	15^{bc}
T_8 (RDF + $Zn_{7.5}$)	47.8^{c}	4.71^{d}	280^{e}	219^{d}	13^{c}
T_9 (RDF + $Zn_{8.75}$)	47.8^{c}	4.15^{ef}	283de	204^{e}	14^{c}
T_{10} (RDF + $Zn_{10.0}$)	45.3^{d}	4.30^{ef}	267^{f}	202^{e}	15^{bc}
SEm ±	0.64	0.19	3.40	4.90	0.52
CD (p≤0.05)	*1.90*	*0.56*	*10.0*	*14.0*	*1.50*

Different letters for each parameter show significant difference at *p≤0.05* by Duncan's Multiple Range Test.

corresponding increase of 43.5, 71.5, 79.5, and 80.4 per cent over RDF.

Application of 1.25 mg Zn kg^{-1} (T_3) (Table 2) showed a respective increase of 7.65, 29.2 and 29.7 per cent in iron (Fe), manganese (Mn) and copper (Cu) concentration in rice grain over RDF (T_2). Increasing Zn levels further had a negative impact on Fe and Mn concentration (r = -0.12, $p \le 0.05$) in rice grain, due to competition with Zn on root absorption sites. Malakaouti (2007) also reported that Zn fertilizer application significantly decreased grain concentrations of Fe and Mn. The B concentration significantly increased by 26 per cent over RDF (T_2) due to application of 2.5 mg Zn kg^{-1}, thereafter, a significant reduction was observed. The B concentration significantly decreased as Zn concentration in grain increased (r = -0.68). It appears that Zn has created a protective mechanism in the root cell environment against excessive uptake of B thus it tends to reduce its uptake in plants. Singh *et al.* (1990) observed that the concentration of B in wheat crops reduced as Zn applied to soil increased.

In rice straw, increased application of Zn had both positive and negative impact on the concentration of micronutrients (Table 3). The concentration of Fe, Mn, Cu and B was highest up to soil application of 1.25 mg Zn kg^{-1} (T_3) and above this dose, their concentrations significantly decreased. Soil application of 1.25 mg Zn kg^{-1} (T_3) enhanced the Fe, Mn and Cu concentration by 4.54, 6.33 and 42 per cent over RDF (T_2), respectively whereas, no significant changes in B concentration was recorded. Sharma and Bapat (2000) reported decreased Mn concentration of wheat with the application of Zn and argued that interaction of Zn with Cu, Fe and Mn in plants was antagonistic due to competition for same carrier protein in the plasma membrane or interference in chelation process or competitive inhibition during unloading in xylem.

In post-harvest soil, the DTPA extractable Zn content gradually increased with a gradual rise in Zn application doses (Table 4). As DTPA extractable Zn in soil increases, it affects other cationic micronutrient antagonistically with Fe (r = -0.89*), Mn (r = -0.84*) and Cu (r = -0.73*). The DTPA extractable Fe, Mn and Cu and hot water extractable B content in post-harvest soil varied from 56.8 to 35.4, 8.41 to 4.17, 4.82 to 3.50 and 0.27 to 0.20 mg kg^{-1}, respectively. The result showed that lower doses of Zn initially increased micronutrients content in soil, whereas,

Table 4. Effect of zinc application on post-harvest soil properties

Treatments	pH	EC (dS m^{-1})	OC (g kg^{-1})	DTPA extractable micronutrients (mg kg^{-1})				Hot water extractable B (mg kg^{-1})
				Zn	Cu	Mn	Fe	
T_1 (Control)	8.09	0.07	8.88	1.18^{f}	4.00	6.25^{cd}	53.6^{ab}	0.20^{d}
T_2 (RDF)	8.20	0.10	9.73	1.27^{f}	4.82	8.46^{a}	56.9^{a}	0.24^{bc}
T_3 (RDF + $Zn_{1.25}$)	8.30	0.09	8.84	1.58^{ef}	4.61	7.71^{ab}	51.9^{bc}	0.25^{ab}
T_4 (RDF + $Zn_{2.5}$)	8.23	0.09	9.14	1.96^{def}	4.34	8.39^{a}	46.7^{bcd}	0.24^{bc}
T_5 (RDF + $Zn_{3.75}$)	8.28	0.08	8.95	2.43^{cde}	3.68	6.91^{bc}	44.5^{cde}	0.27^{a}
T_6 (RDF + $Zn_{5.0}$)	8.35	0.08	9.57	2.64^{cd}	3.45	6.09^{cde}	43.2^{de}	0.27^{a}
T_7 (RDF + $Zn_{6.25}$)	8.38	0.08	9.25	3.29^{c}	3.73	5.19^{ef}	43.6^{de}	0.25^{ab}
T_8 (RDF + $Zn_{7.5}$)	8.36	0.08	9.56	4.69^{b}	3.71	5.86^{de}	43.4^{de}	0.21^{cd}
T_9 (RDF + $Zn_{8.75}$)	8.39	0.08	9.67	5.31^{bc}	3.39	4.26^{fg}	37.0^{ef}	0.22^{cd}
T_{10} (RDF + $Zn_{10.0}$)	8.38	0.09	9.75	5.87^{a}	3.50	4.17^{g}	35.4^{f}	0.21^{cd}
SEm ±	0.07	0.01	0.44	0.29	0.36	0.32	2.35	0.01
CD (p≤0.05)	*NS*	*NS*	*NS*	*0.85*	*NS*	*0.94*	*6.93*	*0.02*

Different letters for each parameter show significant difference at *p≤0.05* by Duncan's Multiple Range Test.

higher doses decreased their contents in post-harvest soil except Zn. Mollah *et al*. (2015) noted that concentrations of B, Zn, Cu, Fe and Mn in post-harvest soil varied considerably as a result of Zn fertilizer being applied as $ZnSO_4$ in rice cultivation.

It may be concluded that application of 6.25 mg Zn kg^{-1} resulted in highest yield and also bio-fortification of Zn in grain and straw. Thus, application of Zn is required even in soil having Zn content above critical limit for achieving twin target of higher yields and Zn content in rice grain and straw which would be beneficial for humans and cattle. However, this is a pot culture study of fundamental nature which needs further verification under field conditions for its practical applicability.

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