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Nutrient fortification approaches for enrichment of zinc enrichment in rice grain

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ABSTRACT

Zinc is considered as fifth major nutrient deficiency after protein, calorie, Iron, vitamin A and Iodine, according to the International Zinc Nutrition there is a large mismatch between the zinc requirement and supply that is happening as of now in the state or elsewhere in the country. Present study was taken up to fortify the rice grain with zinc through soil and foliar sprays at different intervals and with different concentrations at Regional Agricultural Research Station, Anakapalle, Andhra Pradesh, ANGRAU during kharif, 2012 and 2013. Results revealed that highest grain yield of rice 5.41 and 5.26 t/ha during 2012 and 2013, respectively was recorded with application of 100 % chemical fertilizers + 12.5 kg zinc sulphate/ha as soil application + two foliar sprays of zinc sulphate at 21 and 60 days after transplanting. Zinc enrichment in rice grain also followed that same trend as it was high in soil and foliar application of zinc fertilizer to the crop (15.60 % increase over control).

Key words: *Zinc fortification, rice, zinc deficiency, soil application*

INTRODUCTION

World's 90% rice is grown and consumed in Asia. On average, 30% of calories come from rice and this can go up to more than 70% in some low-income countries. Rice is a rich source of macro and micronutrients in its unmilled form. During rice milling the fat and micronutrient-rich bran layers are removed to produce the commonly consumed starch-rich white rice. Indians especially suffer from zinc and Iron malnutrition, resulting in poor health particularly in women and younger children due to the majority of them being vegetarian and depending mostly on cereals, which are inherently low in these minerals. Zinc is considered as fifth major nutrient deficiency after protein, calorie, Iron, vitamin A and Iodine, according to the International Zinc Nutrition there is a large mismatch between the zinc requirement and supply that is happening as of now in the state or elsewhere in the country. Zinc in blood serum of legume consuming populations is also not adequate due to its poor adsorption (15 % of the total zinc) owing to its high phytate content. Zinc plays an important role in regulating large number of physiological functions in all living systems by contributing in protein synthesis and gene expression. In addition it helps in maintenance of structural and functional integrity of biological membranes and detoxification of highly toxic oxygen free radicals (Biswapati Mandal, 2014). It deficiency, therefore results in diverse impairments in biological systems (Shukla, 2012). The staple food of India and in particular Andhra Pradesh being cereals, any attempt to provide adequate supply of zinc to the population need to be worked out through staple food grains of the geographical region. Observations made at All India Coordinated Research Project on micronutrient trials also indicated that the concentration in rice grain can increase in the range of 10 to 90 per cent in addition to normal increase in yield due to zinc application in a large number of varieties that were evaluated. Rice is likely to suffer from zinc deficiency at higher levels of availability compared to wheat (RK Rattan, 2015). However, the intervention required to fortify the crops with zinc without any compromise on yield and across the initial soil available zinc status need to be worked out for which specific trials are scarce. Hence present study was taken up to fortify the rice grain with zinc through soil and foliar sprays at different intervals and with different concentrations at Regional Agricultural Research Station, Anakapalle during *kharif*, 2012 and 2013.

MATERIALS AND METHODS

Field experiments were conducted during *kharif* 2012 and 2013 as rice is the test crop (variety RGL 2537) at Regional Agricultural Research Station, Anakapalle. The soil was clay loam in texture with neutral in soil reaction (pH 7.22) with normal electrical conductivity (0.210 dSm^{-1}). The organic carbon content was 0.51 % and the available nitrogen content was low (241 kg ha^{-1}), available phosphorus (27.45 kg ha^{-1}) and exchangeable potassium (309 kg ha^{-1}) was medium in status. There were 12 treatments with randomized block design and three replications. The treatments include T_1 : Control (recommended dose of chemical fertilizers without zinc), T_2 : T_1 + 25 kg zinc sulphate ha^{-1} (soil application), T_3: T_2 + zinc sulphate foliar spay at 21 days after transplanting (@ 2 g lit^{-1}), T_4 : T_2 + zinc sulphate foliar spay at 60 days after transplanting (@ 2 g / lit, T_5 : T_2 + Foliar sprays of zinc sulphate at 21 and 60 days after transplanting, T_6 : T_1 + 12.5 kg zinc sulphate/ha through soil application, T_7 : T_6 + zinc sulphate foliar spray at 21 days after transplanting, T_8. 12.5 kg zinc sulphate/ ha as soil application + zinc sulphate foliar spray at 60 days after transplanting, T_9 : 12.5 kg zinc sulphate/ha through soil application + Foliar sprays of zinc sulphate (21 & 60 days after transplanting), T_{10} : Foliar Spray (@ 2 g / lit) at 21 days after transplanting, T_{11} : Foliar Spray (@ 2 g / lit) at 60 days after transplanting and T_{12} : Foliar Sprays (@ 2 g / lit) at 21 & 60 days after transplanting. Recommended doses of nitrogen, phosphorus and potassium were applied uniformly to all the treatments in the form of urea, single super phosphate and muriate of potash. Urea was applied in 3 equal splits (1/3rd basal, 1/3rd at tillering and 1/3rd at panicle initiation stages of the crop and single super phosphate was applied basally and muriate of potash at basal and flowering stage. All the package of practices recommended for growing rice were followed to ensure good crop growth and better yields. Representative soil sample from surface was collected from the field before laying out the experiment. Treatment wise soil and plant samples were collected at 50 percent flowering and at harvest. The samples were dried under shade, pounded, to pass through a 2 mm sieve and then were preserved in polythene bags for analysis of different characteristics. Soil reaction (pH) and electrical conductivity was determined in 1:2.5 soils: water suspension using pH meter and EC meter after shaking the sample for 30 minutes (Jackson, 1967). Organic carbon (%) was determined by wet digestion method by Walkley and Black (1934) as described by Jackson, 1967. Available nitrogen in the soil was determined by alkaline potassium permanganate method as described by Subbaiah and Asija (1956). Available phosphorus was extracted from soil by Olsen's reagent. The blue colour was developed following ascorbic acid method of Watanabe and Olsen (1965) and the intensity of blue colour was determined using spectrophotometer. Exchangeable potassium was extracted from soil by using neutral normal ammonium acetate (Murh et al., 1965) and was determined by using flame photometer as described by Jackson (1967). Available zinc were extracted from soil by using DTPA reagent as per the procedure of Lindsay and Norvell (1978) Total zinc from grain was determined as per Jackson 1967 and was determined using atomic absorption spectrophotometer. The grain and straw yields were recorded from net plot area and were computed to t ha^{-1}. The experimental data were analysed by the method of analysis of variance as suggested by Rao (1983). All the characters were analyzed in a randomized block design to list the variance of different treatments at 5 per cent level of significance.

RESULTS AND DISCUSSION

Physico-chemical properties of soil

There was no significant difference observed regarding soil pH and electrical conductivity within the treatments. Normal pH with non saline conductivity was observed in all the treatments. The organic carbon (OC) content ranged between 0.51 to 0.63 % among different treatments, lowest OC content was observed in T_4 *i.e.,* recommended dose of chemical fertilizers with zinc sulphate foliar spray at 60 days after transplanting and highest content of 0.63 % was recorded with recommended dose of chemical fertilizers with soil application of zinc sulphate @ 25 kg/ha, followed by 0.61 % was recorded with T_9 *i.e.,* recommended dose

Table 1. Initial properties of soil.

Parameter	
pH	7.50
EC (dS/m)	0.193
Organic Carbon (%)	0.55
Nitrogen	281
P_2O_5	72.36
K_2O	389
Available Zn (ppm)	0.77

of chemical fertilizers with soil application of zinc sulphate @ 12.5 kg ha^{-1} + two foliar spays at 21 and 60 days after transplanting. Even though significant difference between treatments were observed, there is no particular trend among zinc levels were observed during both the years.

Available nutrient status

Perusal of data presented in table 2 indicated that the initial values of available nitrogen, phosphorus and potassium was high is status. Available zinc status was sufficient is range. After the end of two crop cycles, pH and EC value were recorded normal in range in all the treatments. There was a build-up of organic carbon (%), there is no particular trend in case of available N, P and K status was observed, however significant build up was noticed in case of zinc status in the soils of experimental plots which received zinc fertilizers and it crosses the critical limits and categorized as sufficient.

Available nitrogen ranged between 228 to 315 kg/ha among different treatments. Highest status was observed with 100 % chemical fertilizers with 25 kg zinc sulphate as soil application + zinc sulphate foliar sprays (T_4 and T_5), where as lowest status was observed with 100 % chemical fertilizers with one foliar spray of zinc sulphate at 21 days after transplanting. Regarding available phosphorus there was no significant difference among different treatments however, slightly highest available phosphorus status (80.45 kg/ha) was recorded with 100 % chemical fertilizers with 25 kg zinc sulphate as soil application + zinc sulphate foliar spray and lowest status of 67.50 kg/ha was recorded with 100 % chemical fertilizers + zinc sulphate soil application (12.5 kg/ha). Available potassium status in post harvest soils of rice was ranged between 358 to 583 kg/ha. Highest potassium status was recorded 100 % recommended dose of chemical fertilizers + foliar spray of zinc sulphate @ 0.2 % at 60 days after platning, where as lowest status was recorded with 100 % recommended dose of chemical fertilizers + 25 kg zinc sulphate per ha as soil application + foliar spray of zinc sulphate (0.2 %) at 21 days after planting

These findings are in corroborative with Ali et al., 2012; Soomani, 2008. Zinc nutrition failed to influence on soil organic carbon content, however, available nutrients *viz.,* nitrogen and zinc were significantly improved. Improvement in fertility status leads to improvement in productivity status of the experimental plot (Sahaa et al., 2007; Muthukumararaja & Sriramachandrasekharan, 2012; Singh et al., 2011).

Table 2. Effect of zinc ferti-fortification on soil physico chemical properties in post harvest soils of rice.

Treatments	pH		EC (dS/m)		OC (%)	
	2012	2013	2012	2013	2012	2013
Initial	7.50		0.193		0.55	
T_1: Control (Only NPK but no zinc)	7.60	7.22	0.154	0.371	0.54	0.59
T_2: 25 kg Zinc Sulphate / ha (SA)	7.70	7.31	0.141	0.204	0.63	0.61
T_3: T_2 + 1 ZS Spray at 21 DAT (@ 2 g / lit)	7.52	7.23	0.122	0.314	0.53	0.60
T_4: T_2 + 1 ZS Spray at 60 DAT	7.53	7.42	0.135	0.206	0.51	0.59
T_5: T_2 + 2 Sprays of Zn (21 & 60DAT	7.49	7.43	0.145	0.259	0.57	0.61
T_6: 12.5 kg ZS /ha (SA)	7.74	6.94	0.213	0.646	0.49	0.62
T_7: 12.5 kg ZS /ha (SA) + ZS Spray at 21DAT(@ 2g/ lit	7.80	7.19	0.167	0.295	0.57	0.60
T_8: 12.5 kg ZS /ha (SA) + ZS Spray at 60 DAT	7.48	6.95	0.095	0.525	0.51	0.61
T_9: 12.5 kg ZS /ha (SA) + Sprays of Zn (21 & 60DAT)	7.50	7.20	0.129	0.393	0.61	0.61
T_{-10} Foliar Spray (@ 2 g / lit) at 21 DAT	7.20	6.78	0.161	0.591	0.54	0.60
T_{11}: Foliar Spray (@ 2 g / lit) at 60 DAT	7.21	7.26	0.182	0.159	0.56	0.62
T_{12}: Foliar Sprays (@ 2 g / lit) (at 21 & 60 DAT)	7.32	7.18	0.189	0.287	0.56	0.61
Mean	7.51	7.18	0.153	0.350	0.55	0.61
S Em+					0.016	0.019
CD (5 %)	NS	NS	NS	NS	0.039	0.042
CV (%)					8.9	8.7

Table 3. Effect of zinc ferti-fortification on soil nutrient status in post harvest soils of rice (*Kharif*, 2013).

Treatments	Available nutrients(kg ha^{-1})			Micronutrient(ppm)
	N	P_2O_5	K_2O	Zn
Initial	281	72.36	389	0.77
T_1: Control (Only NPK but no zinc)	276	76.60	403	0.76
T_2: 25 kg Zinc Sulphate / ha (soil application)	258	76.35	426	0.88
T_3: T_2 + 1 ZS Spray at 21 DAT (@ 2 g / lit)	271	80.45	358	0.79
T_4: T_2 + 1 ZS Spray at 60 DAT	315	76.68	426	0.81
T_5: T_2 + 2 Sprays of Zn (21 & 60DAT	315	72.76	448	0.83
T_6: 12.5 kg ZS /ha (SA)	258	67.50	459	0.69
T_7: 12.5 kg ZS /ha (SA) + 1 ZS Spray at 21DAT(@ 2g/ lit	298	75.58	437	0.76
T_8: 12.5 kg ZS /ha (SA) + 1 ZS Spray at 60 DAT	297	79.68	482	0.86
T_9: 12.5 kg ZS /ha (SA) + 2 Sprays of Zn (21 & 60DAT)	263	76.60	426	0.81
T-$_{10}$: Only 1 Foliar Spray (@ 2 g / lit) at 21 DAT	228	75.58	414	0.73
T_{11}: Only 1 Foliar Spray (@ 2 g / lit) at 60 DAT	286	67.89	583	0.76
T_{12}: Two Foliar Sprays (@ 2 g / lit) (at 21 & 60 DAT)	258	74.55	448	0.73
Mean	274	75.05	443	0.78
S Em+	7.25		14.50	0.027
CD (5 %)	16.8	NS	31.50	0.055
CV (%)	8.70		9.20	7.70

Effect of zinc on grain and straw yield of rice

Highest grain yield of rice (5.41 and 5.26 t/ha during 2012 and 2013, respectively) was recorded with application of 100 % chemical fertilizers + 12.5 kg zinc sulphate/ha as soil application + two foliar sprays of zinc sulphate at 21 and 60 days after transplanting, whereas, lowest grain yield of rice (4.71 and 4.63 t/ha during 2012 and 2013, respectively) was recorded with control plots where no zinc application was done during entire experimentation period (Table 4). Similar results were reported by Mondal et al., 2004 and Singh et al., 2011. However, this was on par with all the treatments which received zinc fertilizers through soil application and foliar sprays over foliar sprays alone. These results suggest that for better output and for balanced nutrition combined application which is also advocated by Mondal et al., 2004; Sahaa et al., 2007; Singh et al., 1997. Bodruzzaman et al. (2000); Mahendra and Singh (1981); Tisdale et al. (1993) and Mehla et al. (2006).

Straw yield also followed the same trend as it was highest in the zinc applied treatments compared to control treatment. Straw yield varied from 5.80 to 6.68 t/ha during first year of experimentation and from 6.08

Table 4. Effect of zinc ferti-fortification on Grain yield, straw yield and total yield (t/ha) of rice.

	Grain			Straw			Total Yield		
	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
T_1	4.71	4.63	4.67	5.80	6.08	5.94	10.51	16.45	13.48
T_2	4.93	5.19	5.06	6.00	6.86	6.43	10.93	17.36	14.15
T_3	5.16	5.43	5.30	6.54	7.17	6.86	11.70	18.56	15.13
T_4	5.20	5.27	5.24	6.45	6.96	6.71	11.65	18.36	15.00
T_5	4.85	5.52	5.19	6.23	7.29	6.76	11.08	17.84	14.46
T_6	5.02	4.96	4.99	6.25	6.55	6.40	11.27	17.67	14.47
T_7	5.16	5.21	5.19	6.30	6.88	6.59	11.46	18.05	14.76
T_8	5.29	4.90	5.10	6.68	6.47	6.58	11.97	18.55	15.26
T_9	5.41	5.26	5.34	6.63	6.95	6.79	12.04	18.83	15.44
T_{10}	4.72	5.22	4.97	5.84	6.90	6.37	10.56	16.93	13.75
T_{11}	4.79	5.05	4.92	5.88	6.67	6.28	10.67	16.95	13.81
T_{12}	4.89	5.25	5.07	6.17	6.80	6.49	11.06	17.55	14.30
Mean	5.02	5.16	5.09	6.23	6.80	6.52	11.25	17.77	14.51
S.Em+	0.1365	0.178		0.226	0.210				
CD (5 %)	0.386	0.415		0.498	0.457	-	-	-	-
CV (%)	7.9	8.4		8.1	8.3	-	-	-	-

Table 5. Effect of zinc ferti-fortification on mean zinc content of rice grain.

Treatments	Zinc content(ppm)	% increase over control
T_1: Control (Only NPK but no zinc)	13.88	-
T_2: 25 kg Zinc Sulphate / ha (SA)	14.94	7.64
T_3: T_2 + 1 ZS Spray at 21 DAT (@ 2 g / lit)	15.46	11.88
T_4: T_2 + 1 ZS Spray at 60 DAT	15.21	9.58
T_5: T_2 + 2 Sprays of Zn (21 & 60DAT)	17.05	15.63
T_6: 12.5 kg ZS /ha (SA)	14.53	4.68
T_7: 12.5 kg ZS /ha (SA) + 1 ZS Spray at 21DAT(@ 2g/ lit	14.76	6.34
T_8: 12.5 kg ZS /ha (SA) + 1 ZS Spray at 60 DAT	14.81	6.70
T_9: 12.5 kg ZS /ha (SA) + 2 Sprays of Zn (21 & 60DAT)	14.70	5.91
T_{10}: Only 1 Foliar Spray (@ 2 g / lit) at 21 DAT	15.17	9.29
T_{11}: Only 1 Foliar Spray (@ 2 g / lit) at 60 DAT	14.33	3.24
T_{12}: Two Foliar Sprays (@ 2 g / lit) (at 21 & 60 DAT)	14.93	7.56
Mean	14.98	-
CD (5 %)	1.08	-
CV (%)	8.4	-

to 7.29 t/ha during second year of experimentation. Lowest straw yields were recorded with control (recommended chemical fertilizers alone) whereas highest straw yield was recorded with recommended chemical fertilizers + soil and foliar application of zinc sulphate. However most of the zinc treated plots are on par with each other. These results are in support with Singh et al., 2011 and Sahaa et al., 2007.

Zinc content in rice grain

Results showed that soil as well as foliar application of $ZnSO_4$ significantly enhanced grain Zn concentration in rice. The significant increases in grain Zn concentration were found in the case of combined application of soil (25 kg zinc sulphate per ha) and foliar Zn fertilizers (0.2 % foliar spray) that caused 6 to 15 % increase in grain Zn (Table 5) under different combinations and levels of zinc sulphate. Irrespective of soil Zn status, foliar Zn applications resulted in significant increases in grain Zn, especially in the case of late-season foliar Zn application. In view of the above results, providing Zn to plants (for example, by applying Zn-fertilizers to soil and/or to foliar) appears to be important to ensure success of breeding efforts for increasing Zn concentration in grain. Fertilizer strategy could be a rapid solution to the problem and can be considered an important complementary approach to the on-going breeding programs. Fertilizer studies focusing specifically on increasing Zn concentration of grain (or other edible parts) are, however, very rare, although a large number of studies are available on the role of soil and foliar applied Zn fertilizers in correction of Zn deficiency and increasing plant growth and yield (Martens and Westermann, 1991; Mortvedt and Gilkes, 1993; Rengel et al., 1999; Kumar et al., 2017). Zinc sulfate ($ZnSO_4$) is the most widely applied inorganic source of zinc due to its high solubility and low cost. Zinc can also be applied to soils in form of ZnO, Zn-EDTA and Zn-oxysulfate. The agronomic effectiveness (*e.g.,* magnitude of the crop response per unit applied micronutrient) of zinc fertilizer is higher with Zn-EDTA than the inorganic Zn fertilizers (Mortvedt, 1991; Martens and Westermann, 1991). However, due to its high cost, use of Zn-EDTA in cereal farming is limited.

CONCLUSION

It can be summarized that rice zinc content was significantly increased due to soil application of zinc sulphate @ 25 kg/ha and foliar spray of zinc sulphate @ 0.2 % at 21 and 60 days after transplanting. Rice is a staple food consumed by more than half of the world's population, thus rice fortification provides an avenue to help combat micronutrient deficiencies (Muthayya et al., 2012; Food Fortification Initiative, 2014). When rice fortification is implemented on a large scale, more of the population can receive the health benefits, because rice loses a large percentage of several key nutrients during the milling process, individuals that rely heavily on rice for their energy needs often do not receive adequate nutrition. This suggests that in areas where it is a staple food, rice has the potential to be a good vehicle for fortification as even small increases in nutrient levels could have a positive health impact

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