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MICRONUTRIENT STATUS AND EFFECTIVELY AMELIORATING ZINC DEFICIENCY IN RICE*

M. Tahir, M. Aslam Kausar and A. Saeed Bhatti

Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan

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Analysis of Basmati-370 and IR-6 rice leaves, collected at mid-tillering and panicle initiation growth stages from 36 different sites at farmers' field, showed that Zn was mainly deficient (63-69%) followed by Cu (30-45%) while Fe and Mn were sufficient in soils. Basmati-370 rice grown in pots to panicle initiation stage in Nankana soil produced 58-76% higher yield with ZnCl₂ and ZnCO₃ than the other Zn sources. But Zn concentration in plants was overall increased significantly (P=.05) and total uptake variably affected with added Zn. Increasing Zn rate, irrespective of its sources, irregularly affected yield and total Zn content but mostly significantly (P=.05) depressed Zn concentration in plants grown in Warburton soil. Rice significantly (P=0.05) gave higher yield and Zn uptake in plants with Zn, depending on its sources, applied either way to soils.

Key words: Micro-nutrient, Zinc deficiency, Rice.

Introduction

When N and P fertilizers failed to increase rice yield, the role of micronutrients in its production was looked into [1]. Rice being grown under submergence is strongly affected with micronutrient deficiency particularly with that of Zn [2-5]. Since micronutrient fertilizers are expensive materials and their excess or deficiency can severely reduce crop yields, an explicit information on micronutrients status of rice crop is urgently required. Various investigations [3,4] have been carried out in the past on this aspect of micronutrient research. They have, however, limited scope yet disclosed that rice is more widely and severely suffered with Zn deficiency which has drastically reduced yields [2,5].

Zinc applied at 100 ppm to nursery or 10 ppm after transplanting [1] or 10 kg/ha as top or basal dressing [6] proved effective in alleviating its deficiency in rice. But rice takes up more Zn from that applied after transplanting than from other applications [1]. Dipping rice seedling roots in 1% ZnO suspension is noticed as the most practical method for curing Zn deficiency in rice [1,6]. In a field study, ZnSO₄, ZnCl₂, ZnO and fritted trace elements appeared as effective materials for removing Zn deficiency in rice [7] and 10-100 kg/ha Zn application was suggested its optimum rate [7,8]. Rice growth was similarly affected with Zn applied as ZnO, ZnSO, and Zn-EDTA but Zn uptake in plants differed [9]. Zinc applied as ZnSO₄ to flood water surface, rice seed or the soil comparably affected yield and Zn uptake in rice [9]. Moreover, Zn applied to surface or mixed in soil affected yield and Zn uptake more favourably than that placed below the seed [10]. Rice yielded

more with Zn applied as ZnO than that applied as ZnCl_2 , ZnSO_4 or Zn-EDTA [11]. Soil incorporation of Zn prior to flooding was more effective than its flood water application for rice [12].

This study was carried out to know the micronutrient status of field rice and to compare the different methods, rates and sources of Zn fertilizer for effectively ameliorating Zn deficiency in flooded rice.

Materials and Method

Field experiment. Top-most fully expand leaves, detached at the culm, from Basmati-370 and IR-6 rice plants at mid-tillering and panicle initiation stages were collected from 33 (clay) and 3 (loamy clay-clay) sites respectively on the farmer's field. The leaves were collected from 100 plant hills at the various sites in the districts of Lahore, Sheikhupura and Gujranwala. Alongwith plant sampling at mid-tillering, soil sample (0-20 cm depth) were also collected from each site. After air-drying, soil samples were analysed for clay (30-67%), organic matter (0.86-3.64%) and Olsen's NaHCO₃-P (1-20 ppm) contents and pH (7.9-9.7) with their respective methods as explained by Jackson [13]. Zinc concentration in soil extracted by DTPA (Diethylene triamine penta acetic acid) [14] determined by atomic absorption spectrophotometery (AAS) was 0.42-3.44 ppm at mid-tillering and 0.28-4.48 ppm at panicle initiation growth stage.

The leaves were carefully washed with deionized water to remove dust and other contaminations and dried in an oven at 65°. The leaves were then ground to a 40-mesh powder in a Wiley micromill fitted with stainless steel blades. One gram portion of ground leaves was digested in HNO_3 - $HClO_4$ (1:1) mixture and Zn, Cu, Fe and Mn concentrations in the digests

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were determined by AAS. The grain yields were recorded by harvesting 2 square meter area from the various sites.

Pot experiment. Bulk soil samples (0-20 cm depth) were collected from Nankana and Warburton rice areas, air-dried and ground to a 20 mesh powder. Four kilograms of the ground soil were placed in polyethlene lined plastic pots. A uniform basal dose of N at 100 kg as urea and P at 60 kg. P_2O_g/ha as potassium dihydrogen phosphate was applied to the soils. Zinc was applied, except the control, at 5 and 10 kg/ha as ZnSO₄, ZnCl₂ and ZnCO₃ by soil mixing, soil surface, water surface, 14 days after transplanting and as seedling roots dipping for one min. in 1 and 2% ZnO suspension. All the fertilizers were added in solution form before transplanting. About 16 day old 6 rice seedlings (var: Basmati-370), thinned to 4 after a week, were transplanted. The experiment was replicated three times in a randomized block design. The pots were flooded throughout the growth period.

The plant were harvested at the panicle initiation growth stage. After recording oven-dried weights, plants were undergone through all the processes as mentioned above and their Zn concentrations were determined.

Various analyses showed pH as 8.78 and 9.00, clay as 68.5 and 38.0%, organic matter as 2.62 and 1.95%, Olsen's P as 25.2 and 6.6 ppm and DTPA-Zn as 1.02 and 0.08 ppm determined by their respective procedures [13,14] in Nankana and Warburton soil respectively.

Results and Discussion

FIELD EXPERIMENT

Concentration. Leave concentration of Zn ranged from 10.0 to 34.3 and 11.4 to 25.7 ppm, Cu from 4.5 to 20.8 and 3.0 to 13.4 ppm, Fe from 109 to 573 and 68 to 195 ppm and Mn from 136 to 945 and 49 to 886 ppm at mid-tillering and panicle initiation growth stages respectively in Basmati-370 rice (Table 1). The corresponding values in IR-6 were 15.5 to 21.4 and 20.0 to 24.3 ppm for Zn, 7.4 to 16.4 and 7.4 to 14.9 ppm for Cu, 191 to 273 and 109 to 136 ppm for Fe and 302 to 399 and 146 to 321 ppm for Mn (Table 1).

Considering plant critical level of Zn as 20, Cu as 6.5, Fe as 50 and Mn as 20 ppm [5,14], there were 10 and 12 sites out of a total 33 sites containing adequate amounts of Zn and 23 and 18 sites that of Cu at mid-tillering and panicle initiation growth stages respectively in Basmati-370 rice plants. In IR-6 rice, there was 1 out of a total 3 sites containing an adequate amount of Zn in plants only at the mid-tillering stage (Table 1). However, Fe and Mn both for Basmati-370 and IR-6 rice were sufficient while Cu for that only IR-6 was sufficient in all the soils. In this way, 23 and 21 sites appeared deficient in Zn and 10 and 15 sites that in Cu at mid-tillering and panicle initiation growth stages respectively of Basmati-370. Only 2 sites were deficient in Zn at mid-tillering stage of IR-6 rice. This showed about 69 and 63% sites deficient in Zn and 30 and 45% that in Cu at mid-tillering and panicle initiation growth stages respectively for Basmai-370. It was further noticed that only 66% sites deficient in Zn at mid-tillering stage for IR-6 rice (Table 1). These results indicate that rice naturally recuperates from micronutrients deficiency during later growth stages, as generally thought, is not absolutely true rather found dependent on sampling location, rice cultivar and the micronutrient being studied. The pH, and clay, organic matter and P content are the important soil factors which may induce or reduce the availability of micronutrients in soils or to plants [1,4,5]. Since none of the soil pH (-0.23 to 0.22), clay (-0.04 to 0.19), organic matter (-0.13 to 0.26) and available P (-0.22 to 0.18) showed significant correlation with micronutrients, they should not thus be considered during micronutrient fertilization of the rice soils studied here.

Yield. Grain yield ranged from 1130 to 4185 kg/ha in Basmati-370 and 1850 to 5460 kg/ha in IR-6 rice (Table 1). At the lowest Zn concentration as 11.4 ppm in plants at midtillering stage at site-19 the grain yield was 3525 kg/ha and at the highest Zn concentration as 34.3 ppm at site No. 17 the grain yield was 3376 kg/ha in Basmati-370 rice. Same was true for rest of the micronutrients. But the maximum grain yield as 4185 kg/ha was obtained at site-15 containing 20 ppm Zn in plants at mid-tillering stage. Similar trends were also observed in IR-6 rice. These results indicate that yield is not dependent only on Zn availability but on various other factors also [1,7,15,16].

POT EXPERIMENT

Effect of Zn sources. Dry matter yield of Basmati-370 rice was significantly (P=0.05) reduced with various Zn sources applied to rice soil, water or seedling roots in the Nankana soil (Table 2). The crop yields are sometime unaffected or reduced [2,17,18] with Zn fertilization just like Cu-deficient wheat plants, which produced secondary tillers from the base of old shoots, gave increased dry matter yield without Cu over with Cu fertilization [17][. No doubt, the dry matter produced by Basmati-370 rice with various Zn sources did not significantly (P=0.05) vary, nevertheless, with Zn Cl, and ZnCO₃, depending on their application rates, produced 58-76% higher dry matter than the other sources (Table 2). Yield response of rice to Zn fertilizers may, in addition to the rate and method of application of Zn [1,6,8,9,10,12], rice cultivars [2,5,19] and various soil characteristics [1,7,15,16,20,21], depend on Zn sources also [2,5,11,17,18].

The control plants of Basmati-370 rice on Nankana soil contained Zn (25.7 ppm) higher than the critical level (20 ppm) of Zn but applications of Zn still significantly (P=0.05) increased Zn concentration in plants from a minimum of about

41% with ZnO to a maximum of about 63% with ZnSO₄, 61% with ZnCl₂ and 52% with SnCO₃ over that in control plants (Table 2). Other workers [9,22] reported higher Zn uptake by rice from ZnO, possibly due to different cultivar [2,5,17,19] as well as the method of application [1,2,6,9,10,12] used, than from ZnSO₄, and Zn-EDTA. But some investigators observed that ZnSO₄ applied to soil or flood water was superior to Zn-

EDTA [12] as well as ZnO [23] though Chatterjee and Mandal [24] reported opposite results. Zinc concentration data, however, indicated Zn absorption effeciency of rice from the various Zn sources (Table 2) as $ZnSO_4 = ZnCl_2 > ZnCO_3 >$ ZnO. However, other workers [9,22] recorded comparatively higher Zn uptake, due to different soil [1,4,5], crop cultivar [2,5,17,19], rate and method of application [1,2,6,9,10,12]

TABLE 1. GRAIN YIELD AND CONCENTRATION OF Zn, Cu, Fe AND Mn IN BASMATI-370 AND IR-6 RICE PLANTS ON FARMERS FIELD.

			Concentration, ppm								
S.No.	Sampling sites G	Grain yield	Zr	Zn		Cu		Fe		Mn	
in the second		kg/ha	MT	PI boz	MT	PI	MT	PI	MT	PI	
Basmo	ati-370	al belone (canep	Mixed in a	*. ei b	biY (Es	idai) "Oʻ	with ZnC	allocted	on bas .C	th ZnSC	
1.	Goawa, Lahore	1254	18.6	11.4	14.9	10.4	245	95	234	49	
2.	Burj, Lahore	n.a.	10.0	17.1008	13.4	8.9	245	68	633	224	
3.	Ladheyke, Lahore	n.a.	17.1	21.4	20.8	8.9	354	95	284	886	
4.	Chambrupur, Lahore	2127	17.1	n.a.	11.9	n.a.	450	n.a.	828	n.a.	
5.	Ali Razabad, Lahore	3150	17.1	24.3	16.4	10.4	573	136	380	467	
6.	Niaz Baig, Lahore	2912	22.9	18.6	13.4	7.4	464	82	945	536	
7.	Shahdara, Sheikhupura	n.a.	21.4	17.1	13.4	11.9	354	109	721	789	
8.	Khori, Sheikhupura	n.a.	10.0		7.4	7.4	218	109	555	195	
9.	Kamoke, Gujranwala	1130	15.5	18.6	8.9	7.4	218	109	243	243	
0.	Eminabad, Gujranwala	3780	15.5	24.3	6.0	4.5	123	195	243	146	
1.	Kot Shahan, Gujranwala	2184	20.0	22.9	6.0	6.0	164	95	516	526	
2.	Baddo, Sheikhupura	n.a.	18.6	22.9	11.9	8.9	191	95	594	351	
3.	Nabipur, Sheikhupura	3147	17.1	17.1	7.4	6.0	109	82	263	370	
4.	Harianwala, Sheikhupura	2055	18.6	20.0	7.4	4.5	136	82	399	224	
5. 0	Churkana, Sheikhupura	4185	20.0	15.7	11.9	8.9	136	95	672	390	
6.	Rorianwala, Sheikhupura	2517	17.1	12.9	6.0	6.0	204	123	360	458	
7.	Dhirdhey, Sheikhupura	3376	34.3	25.7	10.4	6.0	218	173	584	458	
8.	Sakham, Sheikhupura	1890	14.3	18.6	7.4	6.0	204	136	273	253	
9.	Chak-28, Sheikhupura	3525	11.4	12.9	6.0	6.0	245	136	360	360	
20.	Klure, Gujranwala	1990	14.3	14.3	4.5	3.0	177	123	136	146	
21.	Klaske, Gujranwala	2945	17.1	17.1	4.5	3.0	164	82	351	282	
22.	Gondalanwala, Gujranwala	3650	17.1	17.1	4.5	3.0	164	95	166	204	
23.	Aroope, Gujranwala	2912	18.6	20.0	6.0	6.0	231	109	253	195	
24.	Kot Pano, Gujranwala	2075	28.6	22.9	6.0	6.0	204	82	351	321	
25.	Kot Nisarshah, Gujranwala	3830	24.3	21.4	6.0	8.9	218	95	292	487	
26.	Herdev, Sheikhupura	n.a.	15.6	22.2	13.4	13.4	136	82	195	97	
27.	Nain Sukh, Sheikhupura	1820	27.0	22.2	13.4	11.9	259	82	243	253	
28.	Tadiali, Sheikhupura	n.a.	22.2	16.5	11.9	7.4	291	82	390	331	
29.	Toale, Sheikhupura	1845	20.3	16.5	12.3	7.7	273	95	477	516	
30.	Rehanwala, Sheikhupura	n.a.	18.4	16.5	10.8	7.7	218	95	390	390	
31.	Khiarey, Sheikhupura	n.a.	16.5	17.5	12.3	9.3	282	123	204	399	
2.	Mahammadwala, Sheikhupun	ra 1820	16.5	n.a.	10.8	n.a.	313	n.a.	224	n.a.	
3.	Warburton, Sheikhupura	n.a.	17.5	18.4	9.3	7.7	464	173	282	243	
R-6											
34.	Chak-38, Sheikhupura	3185	21.4	22.9	16.4	14.9	273	82	302	146	
35.	Chak-37, Sheikhupura	5460	18.6	24.3	10.4	14.9	177	109	321	321	
36.	Monnoabad, Sheikhupura	1850	15.5	20.0	7.4	7.4	191	136	399	282	

n.a. = not available, MT = Mid-tillering and PI = Panicle initiation growth stage.

used, from ZnO in rice. Total Zn uptake in plants from Zn SO₄ and ZnCl₂ was not significantly (P=0.05) affected but that from ZnCO₃ and ZnO was significantly (P=0.05) affected but that from ZnCO₃ and ZnO was significantly (P=0.05) depressed from that in plants grown in control or with ZnSO₄ and ZnCl₂ (Table 2). Zinc uptake in plants may vary with its source, rate and method of application [1,6-12], type of soil [1,4,5] and crop cultivars [2,5,17,19].

Effect of zinc rates. Dry matter yield of Basmati-370 rice applied with different fertilizers of Zn at either rate was altogether significantly (P=0.05) depressed compared to that applied non-Zn (Table 3). However, yield was increased with higher rates of ZnCl₂ (29%) and ZnO (14%) and depressed with ZnSO₄ and not affected with ZnCO₃ (Table 3). Yield is dependent not only on the nutrient supply in the soil but also on various other factors which affect their availability and uptake by plants [1,7,15,16,21] as well as within plant processes contribute to yield [17].

Concentration of Zn in plants was altogether significantly (P=0.05) increased with Zn application in different fertilizer forms as compared to the non-Zn applied plants grown on Warburton soil (Table 3). Higher applications of Zn in various forms generally slightly depressed Zn concentration in plants from a minimum of 5.4% with ZnO to a maximum of 17.5% with ZnCl₂ (Table 3). As the control plants on Warburton soil contained 20.9 ppm Zn, slightly over the Zn critical level (20 ppm) in rice, it may be apprehended that 5 kg Zn/ha or 1% ZnO suspension application was sustained without adverse effect on growth, by the Basmati-370 rice plants. Higher Zn addition particularly in its ZnSO₄ form, due possibly to some toxic effects [17], depressed Zn concentration and the dry matter yield (Table 3), therefore repeated low addition of Zn fertilizers over a single high dose may be preferred [25]. Total Zn uptake in plants with Zn fertilization also overall increased, but non-significantly (P=0.05), from that applied non-Zn (Table 3). However, the effect of increase in rates of application of various Zn sources on total Zn uptake was not uniformally evidenced since each source may vary in its response limits [7-9].

Effect of Zn application methods. As mentioned before, dry matter yield was reduced with Zn fertilization (Table 4). However, among from the various Zn sources, rice produced higher yield with $ZnSO_4$ and $ZnCl_2$ mixed in Nankana soil than their application to soil or water surface (Table 4). Similarly on Warburton soil rice produced more dry matter with soil surface application of $ZnSO_4$, $ZnCl_2$ and $ZnCO_3$ than that their soil mixing or water surface application as well as than root dipping in ZnO suspension application. This indicates that soil surface application of these Zn fertilizers on Warburton soil, being low in clay, available P and Zn contents, may be TABLE 2. EFFECT OF ZN SOURCES ON DRY MATTER YIELD (DMY) AND ZN CONTENT IN BASMATI-370 RICE GROWN IN NANKANA SOIL IN POT.

*Zn	Zn Applied, kg/ha								
Sources	5	10	5	10	borl15 n.e.t.	10			
boviosdo g	DMY	g/pot	Zn Concn.	ppm	Zn Uptake	µg/pol			
Control	5.8	0	bool 25.	108.01	149				
ZnSO,	3.72	3.83	41.9	41.8	156	160			
ZnCL	4.47	3.30	44.8	41.4	200	138			
ZnCÓ,	3.78	4.02	36.2	39.1	137	157			
ZnO	3.46	3.67	28.6	35.2	99	129			
(Suspension)	(1%)	(2%)	(1%)	(2%)	(1%)	(2%)			
**L.S.D.(P=.0	05) 0.8	2	3.74		33.0	00			

* Mixed in soil before transplanting and seedling roots dipped in ZnO suspension., **LSD=Least Significant Difference and (P=0.05)=Significant at 5% level of probability.

TABLE 3. EFFECT OF RATES OF Zn APPLICATION ON DRY MATTER YIELD (DMY) AND Zn CONTENT IN BASMATI-370 RICE GROWN IN WARBURTON SOIL IN POTS.

Zn Tr	eatments	DMY	Zn Content		
Sources	*Rate kg Zn/ha	g/pot	Concn. ppm	Uptake µg/pot	
Control	0	4.41	20.9	92	
ZnSO	5	3.14	40.9	128	
0.81	10	2.60	39.0	102	
ZnCl,	5	2.63	40.0	105	
6.81	10	3.40	33.0	112	
ZnCO,	5	2.87	36.7	105	
17.1	10	2.33	32.8	76	
ZnO	1% suspension	3.73	27.7	96	
	2% suspension	4.27	26.2	112	
**LSD (1	P=0.05)	1.53	7.81	NS	

* mixed in soil before tansplating and seedling roots dipped in ZnO suspensions,, **LSD=Least Significant Difference and NS=non-Significant.

TABLE 4. EFFECT OF METHODS OF Zn Application on Dry Matter Yield (DMY) and Zn Concentration

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*Zn treatments		Nank	ana soil	Warburton soil		
Sources	Application methods	DMY g/pot	Zn Concn. ppm	DMY g/pot	Zn concn. ppm	
Control	- 10.01	5.80	25.7	4.41	20.9	
ZnSO,	Soil mixing	4.25	41.9	3.14	40.9	
9,61	Soil surface	3.72	41.4	4.77	32.6	
	Water surface	3.77	36.2	2.98	40.0	
ZnCl	Soil mixing	4.47	44.8	2.63	40.0	
17.5	Soil surface	3.16	39.1	5.76	36.2	
ZnCO,	Soil mixing	3.31	36.2	2.87	36.7	
	Soil surface	3.57	35.7	3.60	29.0	
ZnO	Root dip	3.13	28.6	3.73	25.7	
**LSD (P=0.05)		1.04	3.71	1.28	6.43	

*ZnSO₄, ZnCl₂ and ZnCO₃ applied at 5 kg Zn/ha and roots dipped in 1% ZnO suspension and **LSD=Least Significant Difference.

preferred. Some workers have also shown that Zn applied to surface or mixed in soil has responded better than its flood water application or placement below the seed on different soils [10,12]. Over and above, the methods of application of Zn fertilizers somewhat similarly affected, as reported by other workers [7,9], dry matter yield particularly on the Nankana soil (Table 4).

Zinc concentration in the non-Zn applied plants on Nankana soil was higher (25.7 ppm) than, but that on Warburton soil was similar (20.9 ppm) to, the critical Zn level (20 ppm) in rice (Table 4). Zinc concentration in plants, irrespective of source and application method of Zn, still significantly (P=0.05) increased with Zn fertilization due to soil and plant characteristics [1,5,7,15,16] which favoured Zn uptake under its higher supply. The Zn concentration in plants significantly (P=0.05) increased from a minimum of about 11% with root dipping in ZnO suspension application at Nankana soil or water surface application of ZnSO₄ at Warburton soil to a maximum of about 91-95% with mixing of ZnSO₄ or ZnCl₂ in Warburton soil or 63-74% with mixing or surface application of ZnCl, or ZnSO, at the Nankana soil (Table 4). The results indicate that Zn uptake is dependent, besides Zn fertilizers [2,7,9,11,17] and soil type [1,4,5,17,20], also on the methods of application as reported by other workers [1,6,10,12].

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Ravours during preparation of soymille. Sosked beans were ringed in hot water and blanched for 15 mins in boiling water containing 0.25% sodium bicarbonate (based on weight of

original beans). Further processing is described in Fig. 1. Preparation of soynals voghurt. As reported by H. Kanda [3], yoghurt made from low protein content soymilk is soft in matter and one made from 3.5-4.5% protein had a desirable texture. Total solid content, therefore, in milk was adjusted to 12% instead of 10% so as to obtain a protein content of 4.2%. Soundle, thus, memored, was holied with 2% snear

and food grade coloue. Cooled to 45° and few drons of

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Youghurt is the only item lifety to be acceptable by Pakistanis among various stybean based products, because of its close affarity to the one prepared from cow's milk. Besides, protein and lipid commus of seymilk and its yoghan can also be adjusted to values equivalent to cow's milk. Moreover, fermentation masks the understrable beauty odour to a great extent. Since yoghart is most commonly used milk product in Pakistan by people of all walks of life, this study was underinden to develop a high proteim low cost soymilk yoghurt. The process is simple and can be adopted at household level without destroying its nuclifive value. Organoloptic evalu-