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Isotope-Aided Micronutrient Studies in Rice Production with Special Reference to Zinc Deficiency (I)

—Efficiency of ^{65}Zn labelled fertilizers under flooded soil condition—

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Abstract

Using tracer technique of ^{65}Zn, a field experiment has been carried out to evaluate the efficiency of zinc fertilizer by rice plant grown under flooded conditions. The treatments include zinc slufate mixed throughout the soil with and without organic matter, combined urea-zinc fertilizer (N: 37.7%, Zn: 3.1%), and surface application at transplanting and two weeks after transplanting at the rate of 5 kg Zn/ha respectively. Other treatments were zinc sulfate mixed throughout the soil at the rate of 10 kg and 20 kg Zn/ha respectively. Root dipping in 2 % ZnO suspension, only organic matter added, and control were also included.

There was not much difference in rough grain yield between zinc levels and different application methods, but the highest yield was obtained from the treatment of the root dipping in 2% ZnO suspension.

Among the 5 kg Zn/ha treatments, the highest total zinc yield was observed from the zinc mixed throughout the soil. The organic matter treatment seemed to reduce the zinc fertilizer efficiency.

In case of the zinc fertilizer levels, 5 kg Zn/ha mixed throughout the soil showed the highest zinc fertilizer efficiency as compared with 10 kg and 20 kg Zn/ha treatments.

요　　약

담수(湛水) 상태에서 수도(水稻)에 의한 아연비료의 이용율을 평가하기 위해서 ^{65}Zn 표지아연비료를 사용하여 포장(圃場) 실험을 했다.

처리방법은 유기물 시용(施用)과 무시용 상태에서 $ZnSO_4$ (5 kg Zn/ha) 전층시비, 이앙(移映)시와 이앙 2 주후 $ZnSO_4$ (5 kg Zn/ha) 표층(表層) 시비, $ZnSO_4$ 다량(10 kg Zn/ha, 20 kg Zn/ha) 전층시비, 2 % ZnO 용액에의 묘(苗) 뿌리침적, 유기물과 대조구로 하였다.

수량은 2 % ZnO 용액에의 묘 뿌리침적후 이앙한 것이 가장 많았다. 기타처리에

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서는 아연 시비량과 시비방법에 따른 수량차는 크지 않았다. 아연흡수량은 $ZnSO_4$ 5 kg Zn/ha 전층시비에서 가장 많았다. 유기물 시용은 아연비료의 이용율을 저하시키는 것 같았다. 아연비료 이용율은 5 kg Zn/ha 전층시비가 다량 전층시비보다 높았다.

I. Introduction

Since higher yielding rice varieties have been introduced, rice plant requires much more macro and micronutrients as compared with local varieties for their good growth and yield.

Zinc was recognized as an essential micronutrient as early as 1926 by Sommer and Lipman[1], and numerous instances of zinc deficiency in upland crop were reported subsequently[2,3,4]. However zinc deficiency in the low land rice was recognized for the first time as a problem in India 1966[5]. The zinc deficiency problems of the low land rice were intensively studied at IRRI for last few years[6]. In Korea, there are some part of paddy fields derived from lime stone which might occur the zinc deficiency problem in the field. We have conducted experiments on the zinc status in rice growing field soils and the efficiency of the zinc fertilization for flooded rice under glass house condition using calcareous paddy soil.

The significance of this observation has not been adequately evaluated in terms of agronomic effectiveness of the previous study under field condition. So that the objectives of this experiment were to evaluate the efficiency of zinc fertilizer utilization by rice plant grown in the field under various methods of zinc application using ^{65}Zn and to determine the yield response to zinc application.

II. Materials and methods

1. Variety and nursery

One high yielding local rice variety, Jinheung was chosen for this experiment. For seedling preparation the seeds were chosen by the gravimetric method using salt solution (S. G. 1.06) and sterilized with 0.1 % $HgCl_2$ solution for 7 hours.

The nursery bed was prepared on April 18, by the routine method and the seeds were placed on the bed. During the nursery period the seedling received fertilizers at the rate of 300 kg N/ha, 200 kg P_2O_5/ha, and 250 kg K_2O/ha excluding zinc fertilizer.

2. Soil

The experimental site was the Experimental Farm of the Korea Atomic Energy Research Institute located at Geumgok-Ri, Yang ju-Gun, Geonggi-Do, Korea. For last three years only rice plants were cultivated at this experimental site.

The field soil used for this experiment belongs to Pung-cheon soil series which has moderately rapid drainage and permeability characteristics. It is alluvial deposit soils derived from the banded gneiss, a silty clay loamy soil, and abundant in mica.

Table 1. Chemical and physical properties of the soil

Depth cm	Texture	Total N %	NH_4 ppm	NO_3 ppm	pH H_2O (1 : 5)	pH $CaCl_2$ (1 : 2)	C %	Organic matter %	C. E. C. m. e. / 100g
0—20	SiCL	0.23	210.0	2090.0	5.6	4.7	2.0	3.4	9.4
20—40	L	0.20	280.0	1720.0	5.4	4.2	1.9	3.2	8.6
40—60	L	0.14	245.0	1155.0	5.1	4.2	1.0	1.7	5.7

Depth cm	Texture	Exchangeable cations m. e. /100g			Total exchangeable bases m. e. /100g	Free $CaCO_3$	Olsen P ppm	DTPA Zn ppm	0. 1N-HCl Zn ppm
		K	Ca	Mg					
0—20	SiCL	0. 41	5. 4	2. 0	8. 3	1. 8	21. 4	2. 31	5. 16
20—40	L	0. 12	3. 6	1. 9	7. 5	1. 2	19. 6	2. 10	4. 81
40—60	L	0. 13	2. 7	1. 1	4. 6	0. 7	16. 2	2. 05	4. 63

The chemical and physical properties of the soil are shown in Table 1.

The organic matter content and exchangeable potassium in the surface soil show somewhat higher values than their mean value respectively. On the other hand the DTPA extractable zinc concentration is rather high as comparing with the zinc deficient soil.

3. Experimental design

Randomized block design with four replications were adopted for this experiment.

3-1 Teatment

Basal nitrogen was applied as urea at 60 kg N/ha just before transplanting and mixed throughout the soil. Additional 40 kg N/ha was applied at the primordial initiation stage for all treatments except treatment No. 3. In case of treatment No. 3, no additional nitrogen was applied as a basal nitrogen. When 5 kg Zn/ha was applied as combined urea—zinc fertilizer, nitrogen also include at the rate of 60 kg N/ha. But nitrogen top dressing was applied at the rate of 40 kg N/ha as in all other treatments at the primordial initiation stage. The triple super phosphate was applied at the rate of 80 kg P_2O_5/ha as basal dressing. Also potassium chloride was applied at the rate of 80 kg K_2O/ha, 50 kg of that as basal and the rest 30 kg as top dressing at the primordial initiation stage. Overall treatments of this field experimen tis shown in Table 2.

Table 2. Overall treatments for field experiment

Treatment No.	Treatments
1*	5 kg Zn/ha as zinc sulfate mixed throughout the soil (MX-5)
2*	5 kg Zn/ha as zinc sulfate plus organic matter compost mixed throughout the soil (MX-5-OM)
3**	5 kg Zn/ha as combined urea-zinc fertilizer mixed throughout the soil (COMB-5)
4*	5 kg Zn/ha as zinc sulfate at transplanting on surface application (SURF-5)
5*	5 kg Zn/ha as zinc sulfate surface application 2 weeks after transplanting (SURF-5-2W)
6*	10 kg Zn/ha as zinc sulfate mixed throughout the soil (MX-10)
7***	Root dipping in 2% ZnO suspension (ROOT-DIP)
8****	Organic matter compost only (no zinc) (O. M.)
9	Control (no zinc) (CONTROL)
10*****	20 kg Zn/ha as zinc sulfate mixed throughout the soil (MX-20).

* Zn-65 labelled zinc sulfate were used for radioactive sub-plot

** Zn-65 labelled combined urea-zinc fertilizer (N: 37.7%, Zn: 3.1%) was used for radioactive sub-plot. Rest of sub-plot received unlabelled combined urea-zinc fertilizer

*** 0.336 kg Zn/ha: equivalent to the amount of zinc applied to one hill, 2.1 mg Zn/hill

**** 3 ton rice straw/ha was applied. The straw contains 1.8% N, 0.09% P, 1.7% K, 120 ppm Fe, 10ppm Cu, 20ppm Zn respectively.

***** Only one replication received Zn-65 labelled fertilizer because shortage of the labelled compound.

3-2 Plot layout

Main plot size was 15 m (3m×5m) and the planting distance was 25cm×25cm. Radioactive zinc microplots were placed at the center of the plots at the size of 100cm×100cm=1m^2. The active microplot was separated from the plot by using plastic sheets of appropriate dimensions, maintaining a height of about 25 cm above the ground, and down to a depth of 30 cm below the soil surface to prevent the movement of water between radioactive microplot and the non-radioactive area of the main plot.

3-3. Procedure

Each plot was demarcated according to the plot size. The land for planting was prepared by ploughing and levelling 8 days before transplanting. In case of the plot receiving organic matter, the compost was spread uniformly over the soil surface before ploughing to incorporate well within the plough layer.

Basal dressings of N, P and K fertilizers were applied and incorporated into the soil by puddling into a depth of about 6 cm at transplanting. The zinc fertilizers application(both unlabelled and Zn-65 labelled) for the treatments 1, 2, 3, 6 and 10 were made simultaneously with the basal application of N, P and K.

In plots receiving treament 4, the surface application of zinc sulfate was done after the basal fertilizer dressings of N, P and K had been incorporated into the soil. The ^{65}Zn labelled zinc sulfate which was supplied by the IAEA was diluted and applied into the active microplot in the activity of 1 mCi/g zinc according to the treatments. Immediately after fertilizer application, the entire plot was irrigated up to a water level of 5 cm above ground on May 31. The rice seedlings were transplanted 24 hours after the irrigation and the field was irrigated as and when required to maintain the water level in plots continuously at a height of 5 cm.

Two weeks after transplanting, zinc sulfate was applied to the surface water of treatment 5. For top dressing, 40 kg N/ha with urea, and 30 kg K_2O/ha with potassium chloride to all plots on July 15. On July 3 herbicide, Machete (2-Chloro 2, 6′-diethyl-N-butoxymethyl acetanilide) was applied at the rate of 30 kg/ha to prevent weed. To control diseases 3 % of Reldan granule (O, O′-dimethyl-0-3, 5, 6, trichloro-2-pyridyl phosphrothioate) and Bla-S (Blasticidin-S-benzyl-amino-benzene sulfonate) were applied at the rate of 30 kg/ha and 20 kg/ha respectively. At hte milking stage, bird net was installed over the plots.

4. Harvest

On September 22, the upper parts of rice plant were cut 3 cm above the soil surface and separated into straw and grain. In case of radioactive microplot rice plant was harvested separately from unlabelled area for analysis of ^{65}Zn. Yield measurements were made for all the plants harvested from the plots including radioactive microplot except the border line. Moisture content on representative samples of straw, rough and hulled grain was also recorded by drying the samples in a 65°C oven to present the result as oven dried weight basis.

5. Analysis of the plant sample

The rice straw, rough grain and hulled grain harvested from each treatment including radioactive microplot were ground with the Wiley cutting mill for analysis of total zinc, nitrogen, phosphorus, and ^{65}Zn contents.

1) Total zinc in plant: Sample of 0.25 gram was treated with 5 ml of conc. HNO_3 and 3 ml of 70 % perchloric acid in a covered digestion tube heated gently until no visible solid material remained. The digested solution was adjusted to a desired volume and analyzed by

atomic absorption spectrophotometer.

2) Total nitrogen: Kjeldahl method

3) Phosphorus: Vanadomolybdate yellow color method[7]

4) ^{65}Zn activity measurement: To measure the gamma activity of ^{65}Zn, plant samples were pulverized again using vibrating mill to obtain fine powder. The activity of the finely ground powder was measured by 400 channels pulse height analyzer.

6. Analysis of soil sample

The routine soil physical and chemical analyses were made and followed by the soil analytical methods[8].

Ⅲ. Results and discussion

The effect of various zinc application methods on the plant height, number of tillers and panicles at harvesting stage is shown in Table 3. It is very difficult to find the differences of the plant height among the treatments. The same trends happened in the numbers of tillers and panicles per hill. Also 20 kg Zn/ha, higher level of the zinc application did not show good response in terms of the plant height, number of tillers and panicles per hill. However, number of tillers and panicles per hill seemed to be reduced by the treatment of organic matter as compared with zinc mixed throughout the

Table 3. Effects of various zinc application methods on plant height, number of tillers and panicles per hill at harvest

No.	Treatment	Plant height, cm	Tillers per hill	Panicles per hill
1	MX-5	84.7	16.2	15.7
2	MX-5-OM	85.2	14.9	14.2
3	COMB-5	84.9	15.3	15.0
4	SURF-5	83.5	14.3	13.9
5	SURF-5-2W	84.5	14.4	14.0
6	MX-10	83.3	13.5	13.2
7	ROOT-DIP	84.1	14.3	14.4
8	O. M.	85.0	14.7	14.4
9	CONTROL	82.6	15.5	15.1
10	MX-20	81.4	14.8	14.4

*Refer to table 2

Table 4. Effects of various zinc application methods on contents of nitrogen, phosphorus and zinc in rice plant

65°C oven dried basis

No.	Treatment	Straw			Rough grain			Hulled grain			Whole Plant		
		% N	% P	ppm Zn	% N	% P	ppm Zn	% N	% P	ppm Zn	% N	% P	ppm Zn
1	MX-5*	0.53	0.033	33.0	1.18	0.145	27.2	1.31	0.184	25.9	0.83	0.085	27.7
2	MX-5-OM	0.51	0.037	23.2	1.15	0.142	23.4	1.29	0.185	24.2	0.80	0.085	23.4
3	COMB-5	0.48	0.041	25.9	1.15	0.136	23.3	1.28	0.179	22.3	0.78	0.083	24.8
4	SURF-5	0.49	0.022	33.7	1.13	0.144	23.6	1.24	0.177	24.8	0.78	0.083	29.3
5	SURF-5-2W	0.50	0.030	36.6	1.10	0.140	24.1	1.27	0.185	23.6	0.78	0.081	30.8
6	MX-10	0.47	0.030	48.4	1.12	0.140	33.6	1.24	0.185	31.5	0.76	0.080	41.7

7	ROOT-DIP	0.45	0.033	40.2	1.11	0.167	25.4	1.21	0.195	26.9	0.73	0.100	32.8
8	O.M.	0.48	0.030	21.2	1.01	0.169	21.6	1.22	0.205	22.1	0.74	0.097	21.4
9	CONTROL	0.45	0.035	22.4	1.05	0.144	20.6	1.18	0.193	22.6	0.73	0.087	21.6
10	MX-20	0.52	0.034	55.0	1.10	0.164	35.2	1.27	0.174	43.7	0.78	0.094	46.0

*Refer to table 2

soil treatment.

Table 4 shows the contents of nitrogen, phosphorus and zinc in rice plant affected by various application methods. The content of nitrogen in straw, rough grain, hulled rice and whole plant seemed not to be affected by the treatments of zinc. However, the content of phosphorus in rice plant was reduced by increasing of zinc fertilizer. The content of zinc in rice plant was increased along with increase in zinc application. Generally, the zinc content seemed to be decreased by the application of organic matter. In case of combined urea-zinc fertilizer, organic matter treated, and the control treatments showed relatively lower content of zinc but surface treatments of zinc and root dipped using ZnO suspension showed as good treatment as zinc mixed throughout the soil without organic matter application.

The effect of various zinc application methods on absorbed amount of nitrogen, phosphorus and zinc of rice plant is shown in Table 5. There were larger differences of absorbed zinc between 5 kg Zn/ha mixed throughout the soil with and without organic matter. Also it was observed in the field that plant growing status of organic matter treatments showed poor growth at early stages. Table 6 and 7 show the yield of rice, total yield of zinc, specific activity of ^{65}Zn in the plant, zinc derived from fertilizer and zinc fertilizer efficiency as affected by various zinc application methods. There was not much difference in rough grain yield between zinc levels and different application methods. The highest rough grain was obtained from the

Table 5. Effects of various zinc application methods on absorbed amount of nitrogen, phosphorus and zinc in rice plant

65°C oven dried basis

No.	Treatment	Straw, mg/hill			Rough grain mg/hill			Hulled grain mg/hill			Total absorbed amount mg/hill		
		N	P	Zn	N	P	Zn	N	P	Zn	N	P	Zn
1	MX-5*	131	8.2	0.811	250	31	0.573	228	32	0.449	381	39.2	1.384
2	MX-5-OM	124	9.0	0.563	235	29	0.477	213	31	0.409	359	38.0	1.040
3	COMB-5	122	10.4	0.655	237	28	0.478	215	30	0.376	359	38.4	1.133
4	SURF-5	117	7.9	0.799	217	28	0.454	193	28	0.386	334	35.9	1.253
5	SURF-5-2W	117	7.0	0.858	219	28	0.483	207	30	0.384	336	35.0	1.341
6	MX-10	120	7.7	1.219	230	29	0.682	206	31	0.521	350	36.7	1.901
7	ROOT-DIP	99	7.2	0.888	238	36	0.547	213	34	0.471	337	43.2	1.435
8	O.M.	107	6.7	0.471	209	35	0.448	209	35	0.378	316	41.7	0.919
9	CONTROL	99	7.7	0.491	210	29	0.412	196	32	0.373	309	36.7	0.906
10	MX-20	117	7.7	1.234	207	31	0.660	197	27	0.688	324	38.7	1.894

*Refer to table 2

Table 6. Yield of straw, rough and hulled grains, and total yield of zinc as affected by various zinc application methods

65°C oven dries basis

No.	Treatment	Yield, kg/ha				Total yield of Zn kg/ha
		Straw	Rough grain	Hulled grain	Whole dry matter	
1	MX-5	3951	3394	2775	7345	0.2215
2	MX-5-OM	3897	3256	2695	7153	0.1665
3	COMB-5	4062	3293	2683	7355	0.1814
4	SURF-5	3801	3070	2492	6871	0.2001
5	SURF-5-2W	3726	3176	2602	6902	0.2146
6	MX-10	3406	3272	2661	6678	0.3042
7	ROOT-DIP	3504	3422	2814	6926	0.2287
8	O. M.	3539	3318	2737	6857	0.1471
9	CONTROL	3535	3193	2655	6733	0.1445
10	MX-20	3590	3048	2502	6638	0.3032

*Refer to table 2

Table 7. Total yield of zinc, specific activity of ^{65}Zn in the plant, zinc derived from fertilizer, and zinc fertilizer efficiency as affected by various zinc application methods

No.	Treatment	Total yield of Zn kg/ha	Specific activity of ^{65}Zn in the plant mCi/g Zn	Zndff[1), %]		Zinc fertilize efficiency %r
				Straw	Rough grain	
1	MX-5*	0.2215	0.0746	13.89	14.71	0.630
2	MX-5-OM	0.1655	0.0793	13.60	16.91	0.504
3	COMB-5	0.1814	0.0254	4.74	5.05	0.174
4	SURF-5	0.2001	0.0583	10.18	12.31	0.439
5	RURF-5-2W	0.2146	0.0629	11.73	12.50	0.551
6	MX-10	0.3042	0.0853	16.02	16.56	0.494
7	ROOT-DIP	0.2287	—	—	—	—
8	O. M.	0.1417	—	—	—	—
9	CONTROL	0.1445	—	—	—	—
10	MX-20	0.3032	0.1075	17.20	28.6	0.360

* Refer to table 2

1) Zndff: Zinc derived from fertilizer

treatment of root dipping in 2 % ZnO suspension. Among the 5 kg Zn/ha Zn treatments, the highest total zinc yield was observed from the zinc mixed throughout the soil, but the lowest yield from the zinc with organic matter was shown compared with other treatments. Generally, reviewing of results, organic matter treatments showed less number of tillers and panicles, poor absorption of zinc, and poor yield of zinc. The reason might be considered that organic matter application enhanced the fixation of zinc by microorganisms. Some microorganism seemed to utilize organic matter as source of energy and produced organo-zinc complex resulting unavailable zinc compounds. There was no good zinc yield from the combined urea-zinc fertilizer treatment. The zinc derived from the fertilizer in whole plant was the highest at

5 kg Zn/ha mixed throughout the soil with organic matter and the lowest at the combined urea-zinc fertilizer treatment. This fact suggests that more native zinc of the used soil is inactivated by organic matter application so that fertilizer zinc may be utilized effectively by rice plant. In case of the combined urea-zinc fertilizer, fertilizer zinc seemed to be released slowly as compared with the other treatments.

Referring to the percentage zinc efficiency of zinc fertilizer, the highest value was obtained from the treatment of 5 kg Zn/ha mixed throughout the soil. This fact is well in agree with the finding of Mordvedt and Giordano[9]. However, the lowest zinc fertilizer efficiency was obtained from the combined urea-zinc fertilizer. Among the zinc levels, 5 kg Zn/ha mixed throughout the soil showed the highest zinc fertilizer efficiency as compared with 10 kg and 20 kg Zn/ha mixed throughout the soil. It seems to be quite natural thing that higher rates of zinc application bring about higher per cent zinc derived from fertilizer and less fertilizer efficiency. Root dipping in 2 % ZnO suspension showed good total zinc yield as compared with the other treatments.

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