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A ^{15}N Tracer Study to Evaluate the Effects of Nitrogen and Copper Fertilization on Fertilizer Nitrogen Efficiency in Rice Production

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Abstract. In the study of the effects of nitrogen and copper fertilization on rice yield when four rates of N (0, 60, 120 and 180 kg N/ha) as ^{15}N labelled urea and three rates of Cu (0, 5 and 10 kg Cu/ha) were applied, grain yield increased significantly with increasing N rates upto 120 kg N/ha. The recovery of fertilizer N was around 40% irrespective of N and Cu rates. Copper application at 10 kg/ha increased grain yield by 0.53 t/ha insignificantly. Cu content in the straw was below the critical deficiency level of 6 mg/kg. Thus higher rate of Cu fertilizer (above 10 kg/ha) in soil increase rice yield and fertilizer N efficiency if Cu is applied as basal. Alternately, Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil.

Keywords: ^{15}N tracer study, copper, rice, fertilizer nitrogen efficiency

Introduction

Rice is the main food crop of an estimated 40 percent of the world's population (Buresh and De Datta, 1990). The rice crop removes from 16 to 17 kg N and 8 g of Cu for the production of one ton of rough rice including straw (Choudhury and Kennedy, 2005; Sahrawat, 2000; Ponnamperuma and Deturck, 1993; De Datta, 1981). Most of the rice soils of the world are deficient in N and biological nitrogen fixation by cyanobacteria and diazotrophic bacteria meets only fraction of the N requirement (Sattar *et al*., 2008; Hashem, 2001; Baldani *et al*., 2000; Tran Van *et al*., 2000). Fertilizer N applications are thus necessary to meet the crops demand. In wetland rice soils, the availability of water soluble Cu decreases due to decrease in redox potential (Ponnamperuma, 1985, 1972). Cu deficiency in soil increases sterility in rice grain resulting in a decrease in the yield (Ambak and Tadano, 1991). This problem can be solved by applying proper amount of Cu fertilizer.

The largest rice growing area of Malaysia is located in the Muda Irrigation Scheme, Kedah that covers an area of about 95,000 ha. Recent investigations showed that there is a tendency of yield decline in many sites of this area due to Cu deficiency (Samy *et al*., 1992a). Investigations showed that soils of some locations of this irrigation scheme are deficient in Cu (Choudhury and Khanif, 2000a). Farmers are applying a single fertilizer dose of 80 kg N/ha, 30 kg P_2O_5/ha and 20 kg K_2O/ha in rice fields throughout the Irrigation Scheme (Samy *et al*., 1992b). Indiscriminate application of fertilizers throughout the irrigation scheme caused low yield in many locations due to Cu deficiency. Cu fertilization may result in increased rice yield as well as fertilizer N efficiency. The ^{15}N tracer technique is used as the precise method to estimate fertilizer N use efficiency (Cong *et al*., 2008; Fan *et al*., 2007; Kongchum *et al*., 2007; Nishida *et al*., 2007).

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The present study was undertaken to evaluate the effects of N and Cu fertilization on rice yield and fertilizer N efficiency using the ^{15}N tracer technique.

Materials and Methods

A greenhouse experiment was conducted at Universiti Putra Malaysia to evaluate the effects of N and Cu fertilization on rice yield and fertilizer N efficiency. The study was conducted in two soils (Idris and Tebengau series). In this paper the findings on one soil (Idris series) are discussed. The taxonomy of the soil is typic plinthaquept, very fine clayey, mixed, isohyperthermic, pallid (Paramananthan, 1998). The soil was collected from rice growing areas of the Muda Irrigation Scheme, Kedah, about 500 km north of Kuala Lumpur, Malaysia. Soil samples were collected from 0-15 cm depth, air dried, ground and sieved through 2 mm sieve. Soil was analysed for organic matter, pH, cation exchange capacity (CEC), total N and available Cu. Organic matter was analysed by potassium dichromate and sulphuric acid digestion method (Walkley and Black, 1934). Soil pH was measured by glass electrode (Peech, 1965). Total N was determined by sulphuric-salicylic acid digestion method (Bremner and Mulvaney, 1982). Cation exchange capacity was determined by ammonium acetate extraction method (Schollenberger and Simon, 1945). Available Cu was analysed by 0.05 N HCl extraction method (Ponnamperuma *et al*., 1981). The soil had a pH of 3.9 with

CEC 22.78 cmol/kg. It contained 3.65% organic matter, 0.14% total N and 0.09 mg/kg available Cu.

Four N rates (0, 60, 120 and 180 kg N/ha) in the form of ^{15}N-labelled urea (8.378% atom excess) and three Cu rates (0, 5 and 10 kg Cu/ha) as copper sulphate ($CuSO_4$. $5H_2O$) were used in the experiment. The experiment was laid out in randomised complete block design with four replications. The soil used for the study was collected from the plough layer of the field and was filled into plastic pots of 15 litre capacity to 10 cm below the brim of the container. The height and diameter of the pots were 29 cm and 28 cm, respectively. The soil was flooded and preincubated for three weeks to stabilize the physicochemical properties before seed sowing. Sprouted rice seeds of variety MR185 were sown. The number of seeds needed per pot was calculated on the basis of surface area of the pot and a sowing rate of 40 kg/ha. Ten sprouted seeds were equally spaced in the puddle soil of each pot.

Phosphorus (30 kg P_2O_5/ha) and K (20 kg K_2O/ha) fertilizers as triple superphosphate (TSP) and muriate of potash (MOP), respectively, were applied as basal dressings to all pots. Nitrogen was applied in three splits (half as basal and one fourth each at active tillering stage and at panicle initiation stage). Copper application was done all as basal. Nitrogen and copper were applied in solution form. Amount of fertilizers was calculated on the basis of soil surface area of each pot. Rice crop was harvested at maturity. Grain and straw weights (g/pot) were recorded. Grain and straw yields were calculated as t/ha considering the surface area of each pot. About 10 g of representative grain and straw samples were ground to pass through 1 mm sieve and were kept in plastic containers for chemical analyses. Total N of the plant tissue was analysed by H_2SO_4 digestion followed by steam distillation method (Bremner and Mulvaney, 1982), and subsequently the ^{15}N content of the plant samples was analysed by using emission spectrometry (Hauck, 1982). Total N uptake by grain and straw were calculated from yield and N content data. The fertilizer N uptake and recovery percentage of added N by rice plant were calculated following the procedure of Panda *et al*. (1995). Copper content of the plant samples was analysed following 1 N HCl extraction method (Yoshida *et al*., 1976). Total Cu uptake by grain and straw was calculated from yield and Cu content data.

All the data were analysed through analysis of variance (ANOVA) and the means were compared using the Duncan's multiple range test (DMRT) where the F-test was significant. All the analyses were done following Statistical Analysis System (SAS Institute Inc., 1987).

Results and Discussion

Grain and straw yields. Grain yield ranged from 2.11 to 4.93 t/ha while straw yield ranged from 3.95 to 6.90 t/ha (Table 1). Effect of N on both grain and straw yields was significant, whereas the effect of Cu was not significant on either of the parameters. Grain and straw yields increased significantly due to N fertilization up to 120 kg N/ha which was expected as the soil was deficient in N and is in agreement with the previous findings (Choudhury and Bhuiyan, 1994, 1991). Copper application did not increase grain and straw yields although the soil was deficient in Cu. The non-significant effect of Cu was attributed to higher Cu adsorption in this soil (Choudhury and Khanif, 2000b). Plant analysis indicated that the Cu content in the straw was below the critical deficiency level of 6 mg/kg (Yoshida *et al*., 1976). This indicates that application of 5 or 10 kg Cu/ha was not sufficient to increase grain yield in this soil; higher rates of Cu over these rates might be useful. Further research is needed to get optimum Cu rate for this soil.

Table 1. Effect of N and Cu on grain and straw yields of rice

N rate (kg/ha)		Cu rate (kg/ha) 0	5	10	Mean
		Grain yield (t/ha)			
0		2.11	2.97	3.23	2.77[c]
60		3.70	4.04	3.73	3.82[b]
120		4.48	4.34	4.93	4.58[a]
180		3.75	4.11	4.26	4.04[b]
	Mean	3.51	3.87	4.04	
		Straw yield (t/ha)			
0		4.34	3.95	4.36	4.22[c]
60		5.12	5.95	5.08	5.38[b]
120		6.19	6.08	6.90	6.39[a]
180		6.28	6.15	6.86	6.43[a]
	Mean	5.48	5.53	5.80	

Effect of Cu was not significant on either grain or straw yield; values followed by different letters within a parameter in a column are significantly different at 5% level by Duncan's Multiple Range Test (DMRT)

Nitrogen content and uptake. Nitrogen content ranged from 0.97 to 1.49% in the grain, and from 0.34 to 0.78% in the straw (Table 2). Nitrogen content in the grain increased significantly due to N fertilization up to 180 kg N/ha whereas Cu effect was not significant. Interactive effect of N and Cu was significant on N content in straw (Table 2). While application of Cu (at 5 kg Cu/ha) increased N content in straw significantly at 0 and 120 kg N/ha, its effect was not significant at 60 and 180 kg N/ha. Increase in N content in the straw due to Cu application was attributed to the decreases in straw yields at these levels of Cu application (Table 1) as Cu was less diluted in straw due to less straw yields. At 5 kg Cu/ha, N content in straw significantly increased over control due to N fertilization at 120 kg N/ha while the significant effect of N fertilization on this parameter was noticed at 180 kg N/ha at other Cu rates (0 and 10 kg Cu/ha). Total N uptake by the whole plant (grain and straw) ranged from 35.35 to 122.45 kg/ha (Table 2). Total N uptake increased significantly due to N fertilization up to 180 kg N/ha. Copper fertilization increased the total uptake significantly over the control at 5 kg Cu/ha; beyond this rate there was no further increase. The increase in total N uptake by the whole plant (grain and straw) due to N fertilization was attributed to the increase in grain and straw yield due to N fertilization as well as due to the increase in N content in grain and straw at higher N rates. Similar results were obtained in previous investigations (Cong *et al*., 2009; Choudhury *et al*., 1997; Panda and Mohanty, 1995). Significant increase in total N uptake due to Cu fertilization was attributed to the significant increase in N content in straw at higher Cu rates.

Table 2. Effect of N and Cu on N content in straw and grain and total N uptake by rice plant

N rate (kg/ha)	Cu rate (kg/ha) 0	5	10	Mean
	N content in straw (%)			
0	0.34^{bB}	0.47^{cA}	0.39^{bAB}	-
60	0.50^{bA}	0.55^{bcA}	0.49^{bA}	-
120	0.50^{bB}	0.68^{abA}	0.51^{bB}	-
180	0.68^{aA}	0.78^{aA}	0.75^{aA}	-
	N content in grain (%)			
0	0.97	1.06	1.06	1.03^{c}
60	1.11	1.08	1.07	1.09^{c}
120	1.16	1.32	1.16	1.21^{b}
180	1.48	1.49	1.41	1.46^{a}
Mean	1.18	1.24	1.18	-
	Total N uptake by whole rice plant			
0	35.35	49.51	50.79	45.22^{d}
60	65.57	73.83	64.05	67.82^{c}
120	81.97	97.48	91.67	90.37^{b}
180	105.62	122.45	122.19	116.75^{a}
Mean	72.13^{B}	85.82^{A}	82.18^{A}	-

[1]N and Cu interaction was significant on N content (%) in straw; Cu effect on N content (%) in grain was not significant; values followed by different small letters within a parameter in a column, and capital letters in a row are significantly different at 5% level by DMRT

^{15}N Atom excess. ^{15}N atom excess in the grain ranged from 2.588 to 5.244% and in straw, from 3.105 to 5.598% (Table 3). ^{15}N atom excess in grain and straw increased significantly at higher N rates, whereas Cu effect was not significant. Increase in ^{15}N atom excess in grain and straw with increasing N rates indicates that fertilizer contributed more in total N uptake at higher N rates. This finding is in agreement with the previous findings of Choudhury and Khanif (2001).

Table 3. Effect of N and Cu on ^{15}N atom excess in rice grain and straw

N rate (kg/ha)	Cu rate (kg/ha) 0	5	10	Mean
	^{15}N atom excess in grain (%)			
60	2.872	2.588	2.666	2.709^{c}
120	4.596	4.309	4.322	4.409^{b}
180	5.244	4.947	5.219	5.137^{a}
Mean	4.237	3.948	4.069	-
	^{15}N atom excess in straw (%)			
60	3.241	3.495	3.105	3.280^{c}
120	4.967	4.544	4.524	4.678^{b}
180	5.598	5.403	5.555	5.519^{a}
Mean	4.602	4.481	4.395	-

Effect of Cu on ^{15}N atom excess (%) in either grain or straw was not significant; values followed by different letters in a column within a parameter are significantly different at 5% level by DMRT

Fertilizer N uptake and recovery. Fertilizer N uptake by the whole plant (grain and straw) ranged from 21.73 to 78.92 kg/ha (Table 4) and increased significantly at higher N rates whereas the Cu effect was not significant. This was attributed to higher ^{15}N atom excess at higher N rates (Table 3) as well as due to higher total N uptake at higher N rates (Table 2); this is in agreement with the previous findings (Bandyopadhyay and Sarkar, 2005; Guindo *et al*., 1994b). Fertilizer N recovery (quan-

Table 4. Effect of N and Cu on fertilizer N uptake and recovery by rice plant

N rate (kg/ha)	Cu rate (kg/ha) 0	5	10	Mean
	Fertilizer N uptake by whole rice plant (kg/ha)			
60	23.60	25.89	21.73	23.74^{c}
120	46.28	51.35	48.22	48.62^{b}
180	68.44	75.96	78.92	74.44^{a}
Mean	46.11	51.07	49.62	-
	Fertilizer N recovery by rice plant (%)			
60	39.32	43.15	36.22	39.56
120	38.56	42.79	40.18	40.51
180	38.02	42.20	43.85	41.36
Mean	38.63	42.71	40.08	-

Effect of N was not significant on fertilizer N recovery (%); effect of Cu was not significant on both fertilizer N uptake and recovery (%); values followed by different letters in a column are significantly different at 5% level by DMRT tified by ^{15}N atom excess) by rice plant ranged from 36.22 to 43.85% (Table 4). Effects of N and Cu were not significant on fertilizer N recovery. In general, the recovery of fertilizer N by rice plant was around 40%. It is in agreement with the previous findings (Craswell and Vlek, 1979; Guindo *et al*., 1994a). Copper application did not increase fertilizer N recovery by rice plant significantly. This was due to the non-significant response of rice crop to added Cu. The available Cu content in the soil was below the critical deficiency level of 0.1 mg/kg (Ponnamperuma *et al*., 1981), and it was expected that Cu application might increase rice yield and thereby increase fertilizer N uptake. But due to higher adsorption of Cu in this soil the effect of Cu was not significant. Higher doses of Cu (above 10 kg Cu/ha) might be useful to increase grain yield and N uptake. Further research using various levels of Cu is needed to draw the inference.

Copper content and uptake. Copper content in grain ranged from 3.63 to 7.22 mg/kg while in straw, ranged from 4.58 to 5.99 mg/kg (Table 5). Copper content in grain and straw increased significantly due to both N and Cu fertilization. Nitrogen fertilization enhanced plant growth. This might contribute in increase in Cu content of both grain and straw due to higher Cu absorption capacity of the rice plants. Although Cu content in straw increased significantly due to Cu fertilization, it was below the critical deficiency level of 6 mg/kg (Yoshida *et al*., 1976). This indicates that the applied Cu rates were not enough in this soil to meet the demand of the rice plants. As a consequence, grain yield did not increase due to Cu fertilization. A follow-up laboratory experiment indicated that Cu adsorption capacity of this soil was high (Choudhury and Khanif, 2000b). Maximum Cu adsorption capacity (calculated from the Langmuir equation) in this soil was 588 mg/kg (Choudhury and Khanif, 2000b). So, higher rate of Cu (more than 10 kg Cu/ha) is needed to get response in this soil if Cu is applied as basal. Alternately, Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil. Copper uptake by the whole rice plant (grain + straw) ranged from 27.39 to 71.88 g/ha (Table 5). Copper uptake by rice plant increased significantly due to both N and Cu fertilization which was attributed to the increases in Cu content in grain and straw due to N and Cu fertilization. The increase in Cu content in grain and straw due to Cu fertilization is in agreement with some other findings (Ambak and Tadano, 1991; Choudhury and Khanif, 2002).

Table 5. Effect of N and Cu on Cu content in grain and straw, and total Cu uptake by rice plant

N rate (kg/ha)		Cu rate (kg/ha)			Mean
		0	5	10	
		Cu content in grain (mg/kg)			
0		3.63	3.85	4.73	4.07^{c}
60		4.51	5.23	5.57	5.10^{b}
120		5.45	6.76	6.54	6.25^{a}
180		5.87	6.85	7.22	6.65^{a}
	Mean	4.87^{C}	5.67^{B}	6.02^{A}	
		Cu content in straw (mg/kg)			
0		4.58	5.44	5.52	5.18b
60		5.74	5.74	5.99	5.82a
120		5.19	5.57	5.72	5.49a
180		5.64	5.79	5.97	5.80a
	Mean	5.29^{C}	5.64^{B}	5.80^{A}	
		Total Cu uptake by whole rice plant			
0		27.39	33.09	39.44	33.31^{c}
60		46.29	55.18	51.35	50.94^{b}
120		56.76	62.99	71.55	63.77^{a}
180		56.86	64.48	71.88	64.41^{a}
	Mean	46.83^{B}	53.94^{A}	58.56^{A}	

Values followed by different letters within a parameter in a column and different capital letters in a row are significantly different at 5% level by DMRT

Conclusion

The present study indicates that copper application did not increase grain yield and fertilizer N efficiency significantly although the soil was deficient in Cu. This was attributed to higher Cu adsorption in this soil. It indicates that application of 5 or 10 kg Cu/ha was not enough to increase grain yield and fertilizer N efficiency in this soil. Higher rates of Cu over these doses may increase grain yield and fertilizer N efficiency if Cu is applied as basal. Alternately Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil. Further research is needed to find out optimum Cu rate and method of application for this soil.

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