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Effect of Methods of Nitrogen Application on Nitrogen Recovery from ^{15}N–labeled Urea Applied to Paddy Rice (*Oryza sativa* L.)

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Nitrogen efficiency of two methods of application of urea at topdressing of paddy rice (*Oryza sativa* L. cv. Koshihikari) before panicle initiation stage (BPIS) as well as recovery of basally applied nitrogen and topdressed nitrogen BPIS were studied in a pot experiment. Three N rates (200 (N_{200}), 400 (N_{400}) and 600 (N_{600}) mg kg^{-1} soil) and equal P_2O_5 and K_2O rates (150 mg kg^{-1} soil) were applied. Topdressing BPIS was made by broadcasting ^{15}N–labeled urca and by injection of ^{15}N–labeled urea solutions in depth of 8 cm, while the basal fertilization was made with unlabeled urea. For other three treatments with the same N rates, ^{15}N–labeled urea only for basal application was used. Concentrations of the ammonium in leaching water varied from 45 mg NH_4–N L^{-1} (N_{200}) to 192 mg NH_4–N L^{-1} (N_{600}) one week after transplanting date (TD) of rice and dropped to zero for about a month. The amount of urea determined a week after TD was 3, 9 and 13 mg urea–N per pot at the three N rates, respectively. The concentration of potassium in the leaching water depended on N rates (higher N rate led to higher leaching of K) and plant growth. No positive effect was found of the injection method of urea application into the soil on the grain yield and the nitrogen accumulation in the plants in comparison with the broadcasting method of urea application BPIS. The differences in ^{15}N recovery among studied N rates were less with injected urea BPIS where ^{15}N recovery was from 68% at the lowest N rate to 75% at the highest rate, while that was from 57 to 79%, respectively with broadcast urea BPIS. The recovery of basally applied nitrogen was less than topdressed nitrogen and ranged from 39 to 44%. There was no significant difference between methods of N application BPIS on the apparent nitrogen recovery, which varied between 58 and 68%.

INTRODUCTION

The nitrogen is an essential nutrient and has the strongest effect on the plant growth and the yield of the rice crop. The nitrogen fertilizers account for 67% from the whole amount of mineral fertilizers applied to rice (Vleck and Byrnes, 1986). Above all the urea accounts for more than 75% of the total consumption of nitrogen fertilizers in Asia (Schnier, 1995). Mineral nitrogen fertilizers undergo different transformations in the paddy field leading to the loss of considerable amounts of applied nitrogen. Ammonia volatilization, denitrification, leaching, immobilization, ammonium fixation and runoff are the main causes for the nitrogen losses. The strongest factor to nitrogen losses from applied urea is ammonia volatilization. Depending on the method of application and environmental conditions the losses vary from 11 to 47% of applied nitrogen (Fillery *et al.*,

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1986a; Fillery *et al.*, 1986b; Mikkelsen *et al.*, 1978). De Datta (1995) established that ammonia losses are higher with transplanted rice (49%) in comparison with broadcast sowed rice. Two ways for reducing these losses were studied–the proper time and the method of nitrogen application (Cao *et al.*, 1984a, 1984b; Mohanty *et al.*, 1999; Schnier, 1995; Soliman and Mohemed, 1996).

The results from all the field experiments studying the methods of application of nitrogen fertilizers showed the advantages of deep placement of urea super granules (USG) and band placement of urea solution compared to broadcasting of urea as basal and topdressing application (Cao *et al.*, 1984a and 1984b; Mohanty *et al.*, 1999; Schnier *et al.* 1988; Schnier *et al.*, 1990; Schnier *et al.*, 1993; Schnier, 1995). In experiments conducted in farmers' fields in three regions in Bangladesh and four regions in Indonesia, Schnier *et al.* (1993) established that the grain yields from band placement of liquid urea solution were significantly ($p<0.01$) higher than basal broadcast and incorporation of urea.

The main reason for the higher efficiency of deep placement techniques of nitrogen fertilizers is the diminishing of nitrogen losses by ammonia volatilization. Savant and De Datta (1982) established that these losses were less than 1% from applied nitrogen.

The main problem is the practical application of methods of deep placement in the fields. There is no technical method for deep placement of USG by now and these granules have to be applied manually, which has a high labor cost. An injector for deep band placement of urea (by injection of diluted urea solution) was developed (Schnier *et al.*, 1993; Schnier, 1995).

In the most of the publications cited above, the first application (diluted urea) was as a band placement after transplanting of rice, and the second application was as broadcasting before panicle initiation stage (BPIS). The aim of the present research was to study the recovery of topdressed nitrogen applied by broadcasting and direct injection of urea into the soil BPIS as well as the recovery of basal applied nitrogen.

MATERIALS AND METHODS

Cultivation of rice plant

The study was carried out with a pot experiment in four replications. Wagner's pots (a/5,000) of 3.5 liters were settled with a drainage system–sand and gravel bottom layer with a drain hole connected by rubber tubing. Futsukaichi soil (Inceptisol, gray lowland soil, soil texture: sandy loam, total N: 1 g kg^{-1}, CEC: 8 cmol (+) kg^{-1}) was used in the experiment. The treatments of the experiment are showed in Table 1. The fertilizers for basal application were mixed in the 3 kg soil before filling the pots. Phosphorus (450 mg P_2O_5 per pot) and potassium (450 mg K_2O per pot) were applied as KH_2PO_4 and K_2HPO_4. Urea was used as nitrogen fertilizer. Urea containing 7.72 atom% ^{15}N abundance for basal fertilizing of treatments 8–10 was used. The pots were flooded with tap water to minimize nitrogen losses immediately after the application of fertilizer, which was conducted 3 days before transplanting of rice seedlings.

One hill of three 25-day-old seedlings of the medium-early maturing rice variety Koshihikari was transplanted into flooded soil on June 1, 2001. Tap water was used for irrigation. Water level in the pots was kept at 4–5 cm depth above the soil surface. The topdressing with nitrogen was made on July 16, before panicle initiation stage (BPIS) at about 1 cm of the water level in the pots. This shallow water level was kept for two days. Urea solution was applied by broadcasting two 5 mL parts on opposite sides of the plant's hill on the soil surface for treatments 2–4. The diluted urea rates for treatments 5–7 were applied using a syringe in four 2.5 mL parts into four sides of the plant hill at 8 cm depth. Treatments 2–4 and 5–7 received labeled urea containing 10.5 atom% ^{15}N abundance.

Leachate sampling and analyses

Leachate (400 mL per pot) was collected every week (10 times) from June 6 to August 20. The contents of ammonium, urea, nitrate, potassium, calcium and magnesium in the leachate were determined. Ammonium was determined by the indophenol method, nitrate by the ultraviolet spectrophotometer method (Cawse (1967)), and potassium, calcium and magnesium by atomic absorption spectrophotometer (HITACHI Z–5300). Urea content in the leachate was determined as ammonium after transforming the urea by treatment with urease (Sigma, Tokyo).

Plant sampling and analyses

The chlorophyll content of the five leaves from each pot was measured by a chlorophyll meter (Minolta SPAD–502), weekly–from June 25 to September 3. The uppermost fully expanded leaf was measured up to panicle initiation of the plants and the flag leaf after that. The average value from four replications was used in Fig. 3.

The harvest of the plants was carried out at the maturity stage (September 6, 2001). The plants were dried at 70 °C for two days. They were separated into grain and straw and the oven dry weight of each part was recorded. Plant samples were analyzed for total N by Kjeldahl digestion and steam distillation. Labeled ammonium nitrogen in each Kjeldahl digest was collected into 0.1 M HCl by steam distillation. The ^{15}N abundance of ammonium nitrogen in the distillates was measured by emission spectrometer (Yamakawa and Haider, 1993).

RESULTS AND DISCUSSION

Leachate analyses

The concentration of the ammonium in the leachates depended on the N rate (Fig. 1). It varied from 45 mg (N_{200}) to 192 mg NH_4–N L^{-1} (N_{600}) on the first sample collecting date. For the first two weeks after transplanting, the ammonium concentration in the leachates remained almost the same. In the next few weeks the concentration decreased rapidly, mainly due to the fast growth of young plants. The ammonium concentration dropped to zero one month after rice transplantation with treatment N_{200} and one week later with treatments N_{400} and N_{600}. Obcemea *et al.* (1984) established that all NH_4–N in the soil was taken up by rice plants for 30 days after the date of transplanting in the wet season. The whole amount of leached NH_4–N per pot was 10%, 15%, 16% from basally applied nitrogen for treatments N_{200}, N_{400} and N_{600}, respectively.

First week after transplanting, the leached amount of urea–N per pot was 3, 9 and 13 mg for the three N rates, respectively. Three times lower content of urea–N per pot was detected in the second portion of leaching water one week later. The hydrolysis of urea

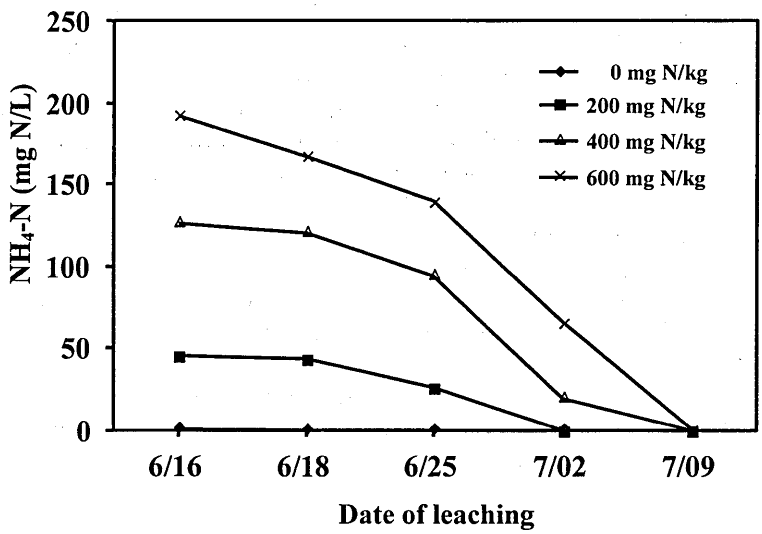

Fig. 1. Changes in NH_4–N concentrations in leachates from pot soil with different rates of nitrogen applied.

Table 1. Amounts of nitrogen applied and methods of nitrogen application in the experiment.

No.	Treatment	Basal N mg/kg	N for topdressing mg/kg	Method of topdressing
0	$N_0P_0K_0$	0	0	–
1	$N_0P_{150}K_{150}$	0	0	–
2	$N_{200}P_{150}K_{150}$	120	80[a]	BCT[b]
3	$N_{400}P_{150}K_{150}$	240	160[a]	BCT[b]
4	$N_{600}P_{150}K_{150}$	360	240[a]	BCT[b]
5	$N_{200}P_{150}K_{150}$	120	80[a]	IN[b]
6	$N_{400}P_{150}K_{150}$	240	160[a]	IN[b]
7	$N_{600}P_{150}K_{150}$	360	240[a]	IN[b]
8	$N_{200}P_{150}K_{150}$	120[a]	80	BCT[b]
9	$N_{400}P_{150}K_{150}$	240[a]	160	BCT[b]
10	$N_{600}P_{150}K_{150}$	360[a]	240[a]	BCT[b]

Phosphorus and potassium were applied at the rate of 150 mg kg^{-1} of P_2O_5 and K_2O. a) Labeled urea (^{15}N), b) BCT; broadcasting topdressed urea and In; injected urea before panicle initiation stage.

proceeds quite rapidly in paddy fields and the process depends mainly on the temperature (Mitsui, 1968; Savant and De Datta, 1982).

The concentration of NH_4–N in the leachate one week after top dressing was very low –about 0.5 mg N L^{-1}, irrespective of the method of urea application and N rates. Therefore the plants shortly before panicle initiation stage (BPIS) have an ability to absorb large amounts of nitrogen for a short period and to minimize the leaching losses of ammonium after topdressing in this way. Well–developed root mat of the rice plants absorbed very rapidly almost all of the amount of applied nitrogen. The results are in accordance with the results from previous study (Ikeda and Watanabe, 2002). Obcemea *et al.* (1984) found that downward movement of deep placed nitrogen fertilizer was minimal. Fertilizer placed at 10 cm depth in this study led to the peak concentrations of NH_4–N at the 8–10 cm depth, regardless of whether urea or ammonium sulfate was deep-placed.

The reduced soil conditions in paddy rice fields decreased the possibility of oxidizing of ammonium because of a very low concentration of NO_3– N in the leachate. The concentration was about 1 mg NO_3– N L^{-1} (data not shown).

The specific properties of the phosphorus leading to its low mobility in soil practically excluded losses in leachate. The concentration of P_2O_5 in leaching waters on the first date of their collecting ranged from 0.02 to 0.08 mg L^{-1}.

The results for the potassium concentration in the leachates (Fig. 2) showed that it depended mainly on nitrogen fertilizing rate at the beginning because NH_4–N obtained in result of urea transformation in soil is competing with K–ions for cation–exchangeable places in the soil and mainly from the plant's growth in the later stages when plants better supplied with nitrogen had higher growth and they absorbed larger amounts of nutrient elements including potassium. The concentration of potassium in leachate with treatments N_{400} and N_{600} dropped almost to zero 35–40 days after TD. The potassium from treatment N_{200} completely disappeared 2 weeks later. The weak growth of the plants with treatment 1 ($N_0P_{150}K_{150}$) caused the concentration of potassium in leachate to be almost constant during two months after TD (16.2–13.6 mg K L^{-1}). After that it started gradually decreasing. The amount of leached potassium was in the range from 16.4 to 21.1 mg K

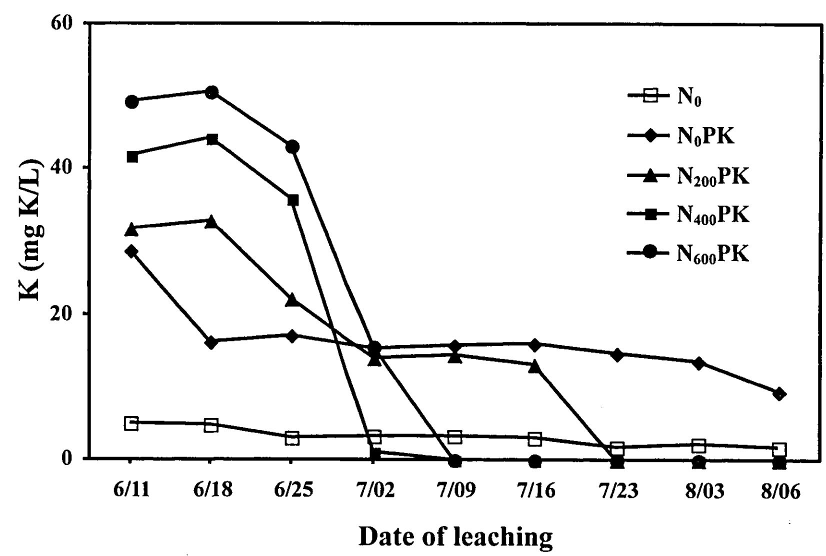

Fig. 2. Changes in K concentrations in leachates from pot soil with different rates of nitrogen applied.

per kg soil, or 14 to 16 % from applied potassium as basal fertilization. The split application of potassium like the nitrogen will be more effective practice in order to diminish these losses.

There was no clear dependence of Ca and Mg concentrations in leachate on N rate and method of its application (data not shown). Ca concentration varied between 80 and 110 mg L^{-1} and Mg from 19 to 22 mg L^{-1}. The total amounts of Ca and Mg leached were less than the amounts supplied by irrigation water.

Chlorophyll content

The chlorophyll content in the leaves of nitrogen fertilized plants was almost equal in the first month (June 25, 2001) after transplanting (Fig. 3). The different rates of exhausting of the available nitrogen in the treatments were the reason for established differences between chlorophyll content of the leaves during the last three weeks before topdressing of nitrogen. The applied nitrogen at TD led to increases in the chlorophyll content of the leaves after the first week. This content gradually decreased in the following weeks up to mature stage.

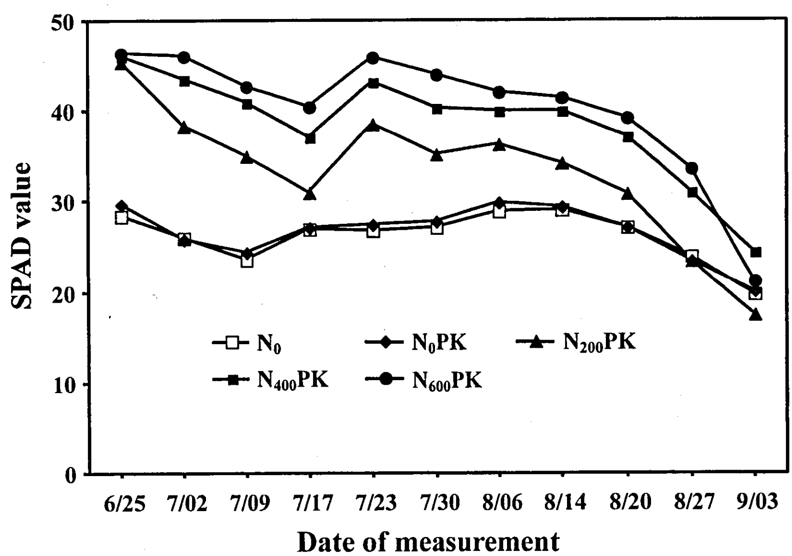

Fig. 3. Changes in chlorophyll content (SPAD value) of leaves of rice plants with different rates of nitrogen applied.

Yields and yield structure

There was positive dependence of the weight of rice straw and grain, as well as the number of ears and grains per pot on N rate (Table 2). The correlation coefficient between N rate and total biomass was +0.91. The effect of N rate on the number of grain

Table 2. Effect of amounts of nitrogen applied and timing and methods of nitrogen application on yields and yield components of paddy rice.

No.	Method of topdressing	Dry weight			Number			Mass of
		Straw	Grains	Total biomass	ears	grains	grains	1000 grains
		g/pot	g/pot	g/pot	per pot	per pot	per ear	g
0	–	4.8a	2.4a	7.3a	3a	133a	44a	18.2a
1	–	5.1a	2.8a	7.8a	3a	147a	49a	18.3a
2	BCT*	36.0b	25.6b	62.3b	20b	1299b	68b	20.3b
3	BCT	64.5d	37.3c	101.8d	30d	1891c	76b	19.7b
4	BCT	75.4e	55.3f	125.8f	38f	2552c	69b	19.6b
5	IN*	38.1b	25.7b	63.3b	18b	1237b	70b	20.4b
6	IN	61.5d	46.9e	103.9d	29d	2106cd	76b	20.2b
7	IN	64.4d	46.6e	108.5d	34e	2152cd	69b	20.5b
8	BCT*	36.5b	24.7b	61.8b	19b	1292b	72b	19.6b
9	BCT	51.8c	40.9d	90.8c	24c	1978cd	87c	19.7b
10	BCT	62.2d	46.3e	112.6d	32de	2336de	75b	19.9b
LSD 5%		6.1	3.2	10.3	3	353	8	0.1

The figures followed the same letter are not significantly different according to DMRT analysis.
* BCT; broadcasting topdressed urea and In; injected urea before panicle initiation stage.

per ear and mass of 1000 grains was low. There was no significant difference in these parameters between treatments with different N rates. The effect of method of application of topdressed nitrogen was expressed only with decreasing of effectiveness of the highest nitrogen rate at injected urea on the production of biomass (grain and straw).

Cao *et al.* (1984a) did not find significant differences between grain yields after band placed urea and broadcast urea for basally application in field conditions. The data for significant difference of grain yields between band and broadcast application of urea in field conditions reported by Schnier *et al.* (1993) and Schnier (1995), were obtained after split band application of urea 14 days after transplanting and 5–7 days BPIS.

Nitrogen accumulation

The increasing the N rate led to increasing of accumulated nitrogen in the plants (Table 3). The difference between N rates was proved. The differences between respective N rates applied by the two methods of urea application BPIS in most cases was not proved. The injected nitrogen in the soil before panicle initiation of the rice plants has shown about the same effectiveness as topdressed nitrogen. Probably keeping up the water level at a depth of 1 cm for two days after surface application of urea allows a fast absorption of nitrogen by well developed root mat near the soil surface, which is in practice difficult to be made in field conditions. This process might be facilitated from the capability of the rice roots to absorb urea directly into plant cells on the base of osmotic absorption through cell membrane (Mitsui, 1968). The process of absorption was very fast and the nitrogen losses by ammonia volatilization were minimized.

The ^{15}N accumulation in the rice grain and straw followed total N accumulation in the plants (Table 3). There was no significant difference in ^{15}N accumulation between treatments of two methods of urea application with the same N rate.

Table 3. Effect of amounts of nitrogen applied and timing and methods of nitrogen application on nitrogen accumulation and ^{15}N accumulation in the grains and straw of paddy rice.

No.	Method of topdressing	Nitrogen accumulation			^{15}N accumulation		
		Grains	Straw	Total	Grains	Straw	Total
			mg/pot			mg/pot	
0	–	19a	24a	44a	–	–	–
1	–	21a	22a	43a	–	–	–
2	BCT*	227b	203b	430b	72a	65a	137a
3	BCT	365cd	467c	833c	150b	176b	326b
4	BCT	541e	689d	1230e	275c	312c	587d
5	IN*	213b	238b	450b	79a	85a	164a
6	IN	386cd	443c	829c	161b	190b	351b
7	IN	451d	636d	1087d	224c	316c	540d
8	BCT*	194b	203b	398b	71a	81a	152a
9	BCT	331c	421c	752c	134b	181b	315b
10	BCT	424d	601d	1025d	151b	274c	425c
LSD 5%		79	93	134	33	55	69

The figures followed the same letter are not significantly different according to DMRT analysis.
* BCT; broadcasting topdressed urea and In; injected urea before panicle initiation stage.

Nitrogen recovery

The differences between N rates for recovered nitrogen topdressed on the soil surface were higher than for the nitrogen injected directly into the soil (Table 4). In the first case total nitrogen recovered in the plant varied from 57% (the lowest N rate) to 79 % (at the highest N rate), while in the latter, the differences were between 68 and 75%, respectively (Table 4). There was no significant difference in recovery of urea nitrogen among N rates applied directly into the soil by injection (treatments 5 to 7). Therefore the injection method of application of urea BPIS increased the recovery of N with the lower fertilizer rates (68% recovery–N_{200}) in comparison with the traditional way of urea application (57% recovery–N_{200}). Schnier *et al.* (1988) reported for similar values of crop ^{15}N recovery (57–65%) of band placed (injected) liquid urea in field conditions.

Recovery of nitrogen from the applied urea as basal fertilization was clearly lower than that from urea applied BPIS (Table 4). There were no difference in the percentage of recovered ^{15}N among N rates. As leached nitrogen from the control was negligible (Fig. 1), it can be assumed that leached nitrogen with the other treatments was derived from applied urea. In this case the total ^{15}N recovery for basal applied nitrogen was 53, 61 and 57 % for N_{200}, N_{400} and N_{600} rates, respectively. Plant recovery of topdressed nitrogen is always higher than basal applied nitrogen because it is applied when the root system is well developed (Savant and De Datta, 1982).

The higher unaccounted fraction for losses of ^{15}N occurred with the low N rates with both methods of urea application BPIS (Table 4). This could be due to the weaker development of plants, which led to slower absorption of applied nitrogen. Unaccounted nitrogen for losses included the ^{15}N nitrogen remaining in the plant roots (not determined), losses by ammonia volatilization, and denitrification. Probably better–developed

Table 4. Effect of amounts of nitrogen applied and timing and methods of nitrogen application on the recovery of ^{15}N–urea nitrogen in soil and plant at harvest.

No.	Method of topdressing	Recovery of ^{15}N–urea nitrogen				
		Straw	Grains	Total(Plant)	Soil	Unaccounted
				Percent of applied N		
2	BCT*	27a	30c	57b	7	36
3	BCT	37b	31c	68bc	4	28
4	BCT	43b	36c	79c	9	12
5	IN*	35b	33c	68bc	3	29
6	IN	40b	34c	73c	3	23
7	IN	44b	31c	75c	5	20
8	BCT*	23a	20b	42a	10	48
9	BCT	25a	19ab	44a	9	48
10	BCT	25a	14a	39a	6	54

The figures followed the same letter are not significantly different according to DMRT analysis.
* BCT; broadcasting topdressed urea and In; injected urea before panicle initiation stage.

Table 5. Effect of amounts of nitrogen applied and timing and methods of nitrogen application on nitrogen balance and apparent nitrogen recovery.

No.	Method of topdressing	Applied N	Leached N	Taken–up N	Sum of leached and taken–up N	Unaccounted N	Apparent N recovery
				mg/pot		%	%
1	–	0	0.3	43	43	–	–
2	–	600	40	430	470	22	65
3	BCT*	1200	123	833	956	20	66
4	BCT	1800	193	1230	1423	21	66
5	BCT	600	40	450	490	18	68
6	IN*	1200	123	829	952	21	66
7	IN	1800	193	1087	1280	29	58
8	IN	600	40	398	438	27	59
9	BCT*	1200	123	752	875	27	59
10	BCT	1800	193	1025	1218	32	55

* BCT; broadcasting topdressed urea and In; injected urea before panicle initiation stage.

plants and their root system faster absorbed nitrogen applied BPIS, diminishing the N losses. This is not valid for the basal applied nitrogen, because all the plants were equally developed immediately after TD. There was no difference in plant height and stage of development between basal N rates. They suffered from transplanting shock, which could be a reason for almost equal losses determined with the three N rates.

The nitrogen balance of the total applied nitrogen (Table 5) showed that the difference between applied and determined nitrogen was almost the same among the treatments. This difference was a little higher only with the treatments with the highest

N rate. Therefore the ratio of the applied nitrogen to nitrogen remaining in both the soil and plant roots was similar irrespective of the applied N rates. Apparent recovery of nitrogen from applied nitrogen was calculated by the subtraction method. There was no significant difference between methods of nitrogen application BPIS in the apparent N recovery, which varied between 58 and 68% (Table 5).

CONCLUSIOS

The leaching losses of nutrient elements from paddy rice soil were restricted mainly to NH_4–N and potassium. The highest ammonium losses were during the first month after the rice transplantation and depended on the N rate. The ammonium losses were negligible after topdressing of urea BPIS even at the highest N rate, independently of the method of the urea application, due to well–developed root mat in the surface soil layers. The highest potassium leaching was determined also during the first month after TD. It depended mainly on the N rate and the growth vigour of the rice plants. Splitting of potassium rate on two parts–one for basal application and second for topdressing BPIS could decrease the potassium losses.

The nitrogen accumulation in rice and the grain yield were affected by N rates. An effect of the method of urea application BPIS on the both parameters was not observed in the present experiment. The injection of urea into the soil increased, mainly, the ^{15}N recovery of lower N rates in comparison with topdressing of urea. The ^{15}N recovery of injected urea for the three studied N rates ranged from 68 to 75%. The recovery of broadcast nitrogen BPIS was the lowest (57%) at N_{200} rate and the highest (79%) at N_{600} rate. The nitrogen recovery of the basal applied nitrogen was lower than the topdressed nitrogen. It was almost the same (about 42%) for the three different N rates.

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