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Assessment of Genotypic Variability for Nitrogen Use Efficiency (NUE) and Improving NUE through Urease Inhibitors in Irrigated Rice

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Abstract

Field experiments (*Kharif* 2021 and *Rabi* 2021-2022) were conducted on a deep black clayey vertisol at the ICAR-Indian Institute of Rice Research farm in Hyderabad to identify efficient rice genotypes for their use of soil N (no external application) and response to applied N (100 kg N/ha) and to improve NUE using urease inhibitors (UIs). Twenty-one popular high-yielding genotypes were tested under two nitrogen levels: N_0 (no nitrogen) and N_{100} (100 kg N/ha). Significant differences were observed among the genotypes in terms of grain yield and various nitrogen use efficiency indices, including agronomic efficiency (AE), physiological efficiency (PE), recovery efficiency (RE), internal efficiency (IE), partial factor productivity (PFP), N requirement (NR), and nitrogen harvest index (NHI). Based on the grain yield data and NUE indices, the top-performing genotypes were Varadhan, Rasi, PSV 181, MTU 1010 and PUP 221 during the wet season, while KRH 4, PSV 181, PSV 344, PSV 190, and PUP 221 excelled during the dry season. Notably, PSV 181 and PUP 221 consistently ranked among the top 5 genotypes in both seasons. Additionally, the application of two urease inhibitors (NBPT and allicin) resulted in a significant increase in grain yield while reducing nitrogen levels by 15-20%.

Key words: Genotypes, Nitrogen levels, Nitrogen use efficiency, Ranking, urease inhibitors.

Introduction

Among the cereal food crops, rice is the most important staple food crop in Asia. Also, it is the livelihood for one fifth of the world's population who depend on rice cultivation as an income source. Among the nutrients, nitrogen is an evergreen essential plant nutrient and its use efficiency is very low (30-40%) in flooded environment. Urea applied to soils undergoes rapid hydrolysis, producing ammonia (NH_3), which can be lost to the atmosphere.

Nitrogen use efficiency (NUE) not only depends on efficient fertilizer management but also on the cultivar that is used. While efficient fertilizer management practices can enhance NUE, their adoption by farmers is limited unless the cultivar exhibits responsiveness. Varieties vary in their capacity to absorb and utilize nutrients, and previous studies (Ladha *et al.,* 1998; Singh *et al.,*1998; Hiroshi, 2003; Surekha *et al.,* 2018) have reported genetic variations in NUE among rice genotypes.

Urease inhibitors are commonly employed to mitigate nitrogen losses in fields and enhance NUE by delaying urea hydrolysis. NBPT, N-(butyl) thiophosphoric triamide is the most efficient and commonly used chemical urease inhibitor worldwide (Cantarella *et al.,* 2018) and their availability is limited. Therefore, natural plant-origin inhibitors such as allicin ($C_6H_{10}OS_2$), an organosulfur compound obtained from garlic (*Allium sativum* L.) extracts were found to exhibit inhibitory properties against urease (Juszkiewicz *et al.*, 2004 and Modolo *et al.,* 2015).

Hence, the present study was undertaken to evaluate the NUE of some existing popular rice varieties and to improve it using urease inhibitors in irrigated rice.

Materials and Methods

Experimental site and soil characteristics

Field experiments were conducted over two seasons: the wet season (*Kharif* 2021) and the dry season (*rabi* 2021-2022) at the Indian Institute of Rice Research farm in Hyderabad on a deep black clayey vertisol (*Typicpellustert*). The study aimed to assess genotypic differences in NUE, identify efficient rice genotypes in terms of soil N utilization and responsiveness to applied N and explore the potential for improving NUE using urease inhibitors (UIs). The experimental soil exhibited slightly alkaline conditions (pH 8.2), was non-saline (EC 0.65 dS/m) and calcareous (with 5.21% free CaCO3). The soil had a cation exchange capacity (CEC) of 42.3 C mol (p+)/kg soil and a medium soil organic carbon content (0.62%). Available nitrogen (N) in the soil was low (220 kg/ha), while available phosphorus (50 kg P/ha), potassium (470 kg K/ha), and zinc (10.5 ppm) were relatively high.

Experiments and their treatment details

In the present study, there were three field experiments and one laboratory experiment. In the first experiment, detailed field studies were conducted for two seasons (*kharif* and *rabi*) at two nitrogen levels [without any external N application (N0) and with a recommended level (100 kg N/ha, N100) of N application] as the main treatments. Twenty-one (21) popular and high-yielding genotypes (varieties and hybrids) were tested as sub-treatments in a split-plot design. In the second experiment, two urease inhibitors (UIs, allicin and NBPT) along with neem-coated urea (NCU) were evaluated at graded levels of N (N0, N50, N75 and N100 kg/ha) in RBD. In experiment three, these two urease inhibitors were tested at 20% reduced N in comparison to 100% NCU in RBD. In all experiments, the recommended dose of fertilizers were given at the rate of 100-40-40-10 kg N, P_2O_5, K_2O and Zn/ha during both seasons through urea, single super phosphate, muriate of potash and zinc sulphate, respectively. Nitrogen was given in three equal splits at basal, maximum tillering and panicle initiation stages while P, K and Zn were given as basal doses only. Plant protection measures, irrigation and weeding operations were done as per the normal practice uniformly for all the experiments. In the laboratory experiment, urease activity in soil was estimated five times during crop growth period by Tabatabai and Bremner, (1972) method.

Observations and data recorded

Grain and straw yields were recorded at harvest and grain and straw samples were analysed for N content using standard procedure by micro Kjeldahl method. Nitrogen uptake by grain, straw and total (grain + straw) was calculated and different parameters of NUE indices *viz*; agronomic (AE), physiological (PE), recovery (RE) and internal efficiency (IE), N requirement (NR), N harvest index (NHI), partial factor productivity (PFP) etc. were computed using grain yield and nitrogen uptake data. Based on the grain yield data at N0 and N100, the genotypes were grouped into efficient (E), responsive (R) and efficient and responsive (ER) genotypes as per Fageria and Baliger, (1993). Based on their NUE indices, the genotypes were ranked based on their mean rank value for all indices as per the procedure followed by Singh *et al.,* (1998). All the data were subjected to standard statistical analysis, by applying analysis of variance for split plot and randomized block designs.

Results and Discussions

Experiment-1

Grain yield at two levels of N application

In the first season (*kharif* 2021), grain yield was significantly higher at N100 compared to N0 which was higher by 30% (**Table 1**) and all genotypes were superior at N100 over N0. With regard to genotypes, some performed very well at N0 showing their high efficiency in utilizing the soil available N in the absence of external N application. The genotypes PUP-221, PUP-223 and PSV-868 are coming in this category of efficient (E) group. Genotypes MTU 1010 and KRH 4 responded well to added N and these are considered as responsive (R) genotypes. Whereas, the genotypes Varadhan and PSV-344 performed well at both levels (N0 and N100) of N showing their efficient as well as responsive nature (ER).

In the second season (*rabi* 2021-22) also, grain yield was significantly higher at N100 compared to N0 by 48% and the per cent yield reduction in N0 over N100 was higher in *rabi* compared to *kharif* indicating high N requirement in dry season **(Table 1)**. With regard to genotypes, similar to the *kharif* season, all genotypes recorded higher yields at N100 than at N0. In this season, the genotypes PSV -344 and PSV-469 at N0; Varadhan at N 100; KRH 4 and PUP 221, PSV 190 at N0 as well as N100 recorded higher yields and are considered as efficient (E), responsive (R) and efficient plus responsive (ER) genotypes, respectively. Best performance of high yielding rice cultivars even at reduced N fertilizer rate was reported by Hiroshi (2003).

Higher grain yield and high response to N in dry season than in wet season in the tropics was also reported by De Datta and Malabuyoc (1976). Superior

Table 1: Grain yield (t/ha) of 21 genotypes at two nitrogen levels

***Kharif* 2021**				***Rabi* 2021-22**			
Variety/Hybrid	**N0**	**N100**	**Mean**	**Varieties**	**N0**	**N100**	**Mean**
Rasi	3.36	4.79	4.08	Rasi	2.90	4.29	3.60
Varadhan	4.10	5.50	4.68	Varadhan	3.81	5.85	4.83
Shanthi	3.69	4.72	4.21	Shanthi	2.86	4.22	3.54
MTU-1010	3.94	5.25	4.72	MTU-1010	3.63	5.61	4.62
Tellahamsa	2.80	3.84	3.32	Tellahamsa	2.90	4.32	3.61
KRH-4	3.95	5.14	4.55	KRH-4	4.07	6.27	5.17
CSR-23	3.33	4.44	3.89	CSR-23	2.84	4.63	3.74
PUP-221	4.14	5.00	4.62	PUP-221	4.05	6.12	5.09
PUP-223	4.07	4.96	4.52	PUP-223	3.82	5.15	4.29
PSV-56	3.81	5.04	4.53	PSV.56	3.37	4.45	3.91
PSV -167	2.83	4.37	3.60	PSV 167	3.81	5.90	4.86
PSV-181	3.81	5.06	4.44	PSV.181	3.44	5.56	4.50
PSV-190	3.46	4.47	3.97	PSV-190	4.20	6.27	5.24
PSV-344	4.20	5.15	4.68	PSV-344	4.10	5.92	5.01
PSV-469	3.46	4.54	4.00	PSV.469	4.00	5.98	4.99
PSV-414	2.97	4.03	3.50	PSV-414	3.52	5.31	4.42
PSV-703	3.43	4.28	3.86	PSV-703	3.53	5.28	4.41
PSV-868	4.04	4.95	4.50	PSV.868	3.67	5.1	4.39
PSV-1103-3	3.56	4.72	4.14	PSV.1103-3	3.99	5.46	4.73
PSV-1110	3.63	4.66	4.15	PSV.1110	3.87	5.42	4.65
PSV-1128	3.25	4.49	3.87	PSV.1128	3.71	5.53	4.62
Mean	3.64	4.73 (30%)		**Mean**	3.62	5.36 (48%)	
CD(0.05)	**Main -0.32; Sub - 0.50; MxS - NS**			**CD(0.05)**	**M-0.51; S- 0.55; MxS - NS**		

performance of genotypes at N100 over N0 could be attributed to the increased chlorophyll formation and photosynthesis thereby leading to increased plant growth, dry matter, yield and yield parameters (Kanade and Kalra, 1986; Tejeswara Rao *et al.,* 2014).

The variation in grain yield among different rice varieties due to their differential efficiency in converting dry matter into grain under different N levels in rice was also reported by Priyadarshini and Prasad (2003) and Srilaxmi *et al.,* (2005).

Nitrogen use efficiency (NUE) indices of genotypes

Some important NUE indices of the genotypes tested in two seasons are given in **Tables 2 and 3**. In general, the agronomic efficiency (AE), physiological efficiency (PE), internal efficiency (IE), recovery efficiency (RE) and partial factor productivity (PFP) are higher in the genotypes that recorded higher grain yield either with or without N addition and these values are in the range of optimum recommended values as suggested by Dobermann and Fairhurst (2000). N requirement was low at N0 due to limited N availability compared to N100 and NHI, that is, partitioning of N to grain was also high with N addition. If we see the seasonal variation, in general, all NUE indices were higher in dry season which could be attributed to better sunshine in dry season that might have helped for efficient utilization of the absorbed nitrogen and comparatively higher grain yield in dry season. NHI also serves as an indicator of the grain's protein content, thereby reflecting its nutritional quality (Sinclair, 1998). Genetic variation in NUE of irrigated rice was also reported by Gueye and Becker (2011).

Table 2: Important nitrogen use efficiency (NUE) indices of genotypes (*Kharif* 2021)

Varieties	**AE**	**PE**	**RE**	**PFP**	**NR**		**IE**		**NHI**		**Rank**		
					N0	N100	N0	N100	N0	N100	N0	N100	Overall
Rasi	14	31	48	48	17.1	21.6	60	46	0.61	0.58	**2**	**1**	**2**
Varadhan	10	17	62	52	16.7	25.2	60	40	0.59	0.58	**1**	**4**	**1**
Shanthi	10	27	41	47	19.7	24.0	51	42	0.54	0.56	16	6	9
MTU-1010	16	22	70	55	18.0	25.6	56	39	0.50	0.58	9	**3**	**4**
Tellahamsa	10	26	41	38	18.6	24.0	55	42	0.53	0.55	18	13	17
KRH-4	11	20	54	50	18.8	25.4	54	39	0.57	0.55	6	**5**	7
CSR-23	11	20	49	44	18.0	24.4	55	41	0.49	0.60	15	8	13
PUP-221	8	17	48	51	17.7	24.3	57	41	0.53	0.55	**3**	11	**5**
PUP-223	8	17	44	50	18.4	24.1	54	42	0.53	0.55	7	14	8
PSV-56	9	16	57	50	19.5	27.2	51	37	0.47	0.55	20	15	16
PSV -167	15	30	50	44	20.1	24.4	50	41	0.52	0.53	21	7	19
PSV-181	12	22	56	51	17.3	24.1	58	42	0.51	0.55	**5**	**2**	**3**
PSV-190	10	21	47	45	19.0	25.1	53	40	0.54	0.55	17	19	20
PSV-344	8	16	52	51	18.9	25.8	53	39	0.52	0.52	13	17	14
PSV-469	11	19	57	45	17.2	25.6	58	39	0.53	0.51	**4**	18	12
PSV-414	11	22	46	40	18.2	24.9	55	40	0.54	0.52	11	20	18
PSV-703	9	17	48	43	18.9	26.3	53	38	0.53	0.53	19	21	21
PSV-868	9	21	43	49	19.8	24.8	51	40	0.55	0.50	14	16	15
PSV-1103-3	12	24	46	47	18.3	23.6	55	42	0.56	0.53	8	**9**	6
PSV-1110	10	22	44	47	18.7	23.9	54	42	0.54	0.51	12	12	11
PSV-1128	12	20	55	45	17.9	25.0	56	40	0.53	0.55	10	10	10
Mean	**10.8**	**21.3**	**50.4**	**47.2**	**18.4**	**24.7**	**55**	**41**	**0.53**	**0.55**			

AE- Agronomic efficiency (kg grain yield increase/kg N added); PE- Physiological efficiency (kg grain/ kg N uptake; RE- Recovery efficiency (% of N recovered); PFP- Partial factor productivity (kg grain/ kg N added); NR- N requirement (kg N/ton); IE - Internal efficiency (kg grain/ kg N taken up); NHI-Nitrogen harvest index

Ranking of genotypes based on nitrogen use efficiency (NUE) indices

Based on the NUE indices at both N levels, the genotypes were ranked **(Tables 2 and 3)**. Since no single genotype recorded maximum values for all indices and none of the genotypes possessed same rank for all NUE indices, the ranking was done based on the mean value of their ranks at N0 and N 100 and overall ranking was done as was also done as per Singh *et al.,* (1998) and Rao *et al.,* (2006). Thus, Varadhan, Rasi, PSV 181, MTU 1010 and PUP 221 in *kharif;* KRH 4, PSV 181, PSV 344, PSV 190 and PUP 221 in *rabi* stood in the top 5 out of 21 genotypes while PSV 181 and PUP 221 were in the top 5 in both seasons. The consistent performance of efficient genotypes over a range of soil and fertilizer N supply was also reported by Singh *et al.,* (1998). Grouping of genotypes based on grain yield and their ranking based on NUE indices indicated the emergence of same genotypes from both categories as the most N use efficient genotypes. Similar ranking system and genotype performance for NUE in rice was also given by Broadbent *et al.,* (1987).

Table 3: Important nitrogen use efficiency (NUE) indices of genotypes (*Rabi* 2020-21)

Varieties	AE	PE	RE	PFP	NR		IE		NHI		Rank		
					N0	N100	N0	N100	N0	N100	N0	N100	Overall
Rasi	11	24	46	43	14.0	21.8	71	46	0.49	0.64	**7**	19	13
Varadhan	16	24	66	58	14.6	22.5	68	44	0.59	0.66	**3**	14	7
Shanthi	11	27	40	42	15.5	21.5	64	46	0.53	0.65	12	18	15
MTU-1010	14	27	52	56	15.7	21.7	64	46	0.48	0.67	20	11	17
Tellahamsa	11	29	38	43	17.1	21.8	58	46	0.55	0.66	17	15	18
KRH-4	19	31	61	63	13.5	19.4	74	51	0.57	0.69	**1**	**1**	**1**
CSR-23	15	29	51	46	15.3	21.8	66	46	0.52	0.65	11	12	11
PUP-221	18	28	64	61	15.5	21.8	65	46	0.58	0.68	**6**	**5**	**5**
PUP-223	14	26	54	51	15.5	22.2	64	45	0.47	0.65	19	17	19
PSV-56	8	35	22	44	16.2	18.6	62	54	0.55	0.67	15	**5**	12
PSV -167	16	36	45	59	17.3	20.4	58	49	0.56	0.65	18	**6**	14
PSV-181	14	33	44	56	14.5	19.3	69	52	0.58	0.68	**2**	**3**	**2**
PSV-190	18	29	60	63	14.9	20.6	67	48	0.51	0.66	10	**2**	**4**
PSV-344	18	27	68	59	13.9	21.6	72	46	0.53	0.63	**4**	8	**3**
PSV-469	17	28	61	60	14.8	21.2	67	47	0.55	0.62	**5**	9	**6**
PSV-414	10	18	55	53	15.4	23.3	65	43	0.46	0.57	16	21	20
PSV-703	15	27	53	53	16.0	22.0	63	46	0.56	0.60	14	16	16
PSV-868	11	26	43	51	17.1	22.0	58	45	0.53	0.64	21	20	21
PSV-1103-3	12	26	45	55	14.6	20.0	69	50	0.50	0.70	8	10	8
PSV-1110	13	24	53	54	14.5	21.2	69	47	0.47	0.65	9	13	9
PSV-1128	15	33	46	55	15.4	19.6	65	51	0.49	0.61	13	7	10
Mean	**14**	**28**	**51**	54	**15.3**	**21.2**	**66**	**47**	**0.53**	**0.65**			

AE- Agronomic efficiency (kg grain yield increase/kg N added); PE- Physiological efficiency (kg grain/ kg N uptake; RE- Recovery efficiency (% of N recovered); PFP- Partial factor productivity (kg grain/ kg N added); NR- N requirement (kg N/ton); IE - Internal efficiency (kg grain/ kg N taken up); NHI-Nitrogen harvest index

Experiment-2

Grain yield at graded levels of N with urease inhibitors (UIs)

During *kharif* 2021, grain yield was maximum at N100 (5.68 t/ha) but was on par to N75 (5.41 t/ha). These two treatments were significantly superior to other N levels (N0 and N50 with 3.97 and

4.53 t/ha, respectively) (**Figure 1**). With regard to urease inhibitors (UIs), two UIs, allicin and NBPT recorded significantly higher yield than NCU by 17 and 25%, respectively. During *rabi* 2021-22, the trend was same showing no significant difference between N75 and N100 with 6.37 and 6.48 t/ha, respectively. Here also, UIs recorded higher yield by 12% over NCU (**Figure 2**). Overall, a 25% saving in N was observed in both seasons. Improved NUE in addition to 25% Nitrogen saving with INM was also reported by Lakshmi *et al.,* (2012). Similar findings were reported by Yang *et al.,* (2020) that the application of urea combined with Azolla and a urease inhibitor (NAUI) reduced NH_3 volatilization by 54.6% compared to plots treated with urea and Azolla alone (NA). Additionally, the NAUI-treated plots showed an increase in grain yield by 9.0-9.7%, primarily attributed to enhanced nitrogen uptake (35.8%). Carlos *et al.,* (2022) also highlighted the relevance of using the urease inhibitor NBPT to mitigate ammonia volatilization, improve agronomic efficiency, and enhance grain yield, especially when there are delays in irrigation.

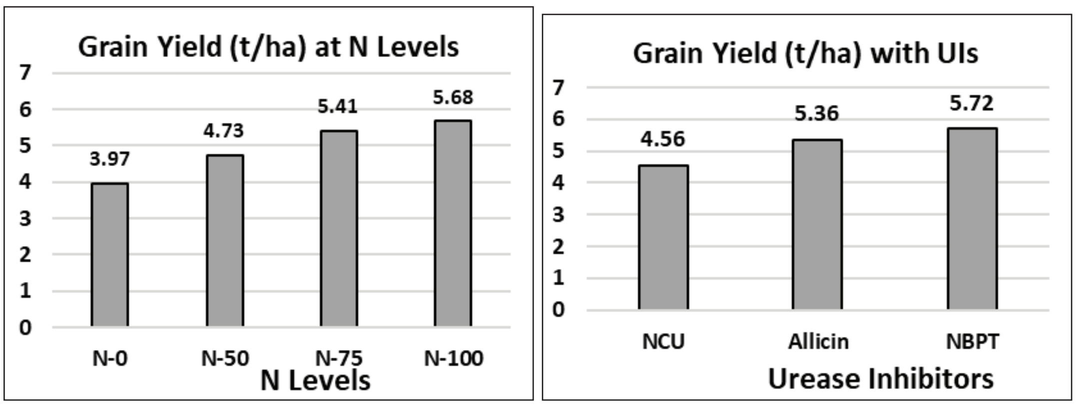

Figure 1: Grain Yield at graded levels of N and with Urease inhibitors (*kharif* 2021)

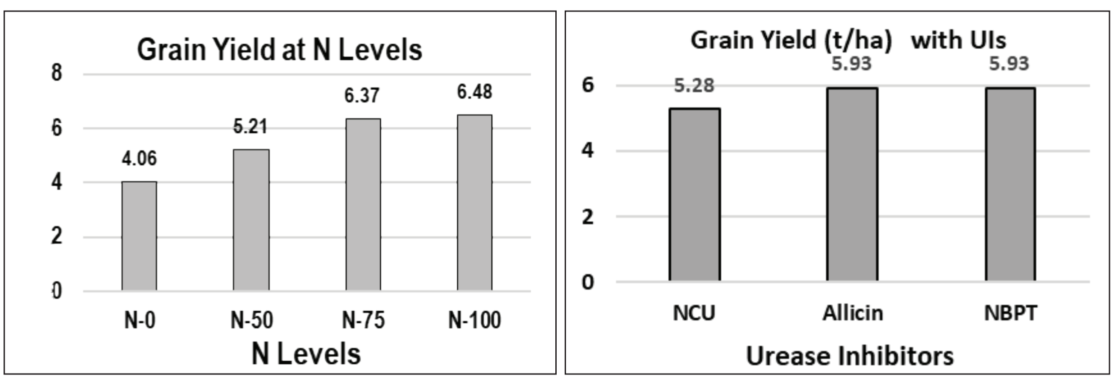

Figure 2: Grain Yield at graded levels of N and with Urease inhibitors (*rabi* 2021-22)

Experiment-3

Grain yield at reduced levels of N with urease inhibitors (UIs)

For the confirmation of benefit from UIs, in this separate experiment conducted simultaneously with 20% reduced N with UIs and 100% N with NCU, UIs recorded higher yield by 9 and 13% in *kharif* and 20 and 28% in *rabi* with allicin and NBPT, respectively over NCU (**Figure 3**). Thus, a 20% saving can be achieved when UIs are used in both seasons. Drulis *et al.,* (2022) found that Urease inhibitors along with biologics have showed effective increase in maize yield and also showed decreased usage of nitrogen fertilizers. Cui *et al.,* (2024) reported combined use of Controlled release urea (CRU) and Urease Inhibitor (UI) treatment achieve higher yields than with CRU at same N level and at 20% reduction of N use, one-time application of CRU + UI recorded same high yield as the conventional split application of urea.

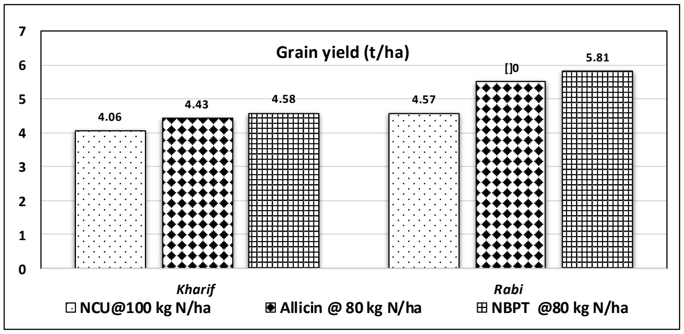

Figure 3: Grain Yield with urease inhibitors at reduced levels of N

Urease activity in soil with urease inhibitors

Urease activity was estimated five times during crop growth period *viz*; after basal, before and after first split application and before and after second split application to know the pattern of N release from urea with and without urease inhibitors and presented in **Figure 4**. Urease activity was high with NCU and low when UIs were used for coating on NCU. Urease inhibition was high with NBPT compared to allicin but these two exhibited higher inhibition than NCU. Similar results from a laboratory study by Ranitha Mathialagan *et al.,* (2017) demonstrated the potential of allicin as a viable urease inhibitor and higher inhibition by NBPT compared to allicin. This indicated the slow and gradual release of N over a period of time as per the crop needs when UIs are used and this might have reflected in higher yield. Further, the loss of N through many ways might have been reduced by keeping the N in amide form for longer period and the released NH4-N was also retained in the soil for a longer period due to clayey texture of the soil and was made available to the crop. Greater adsorption of NH4-N on the clay complex in fine textured soils with higher clay content was also reported by Suraya *et al.,* (2007).

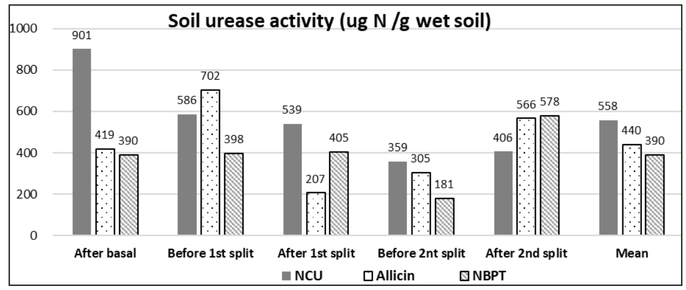

Figure 4: Urease activity (ug N /g wet soil) during *kharif* 2022

Conclusion

The conclusions that can be drawn from the present study are: significant genotypic variation with regard to grain yield and various nitrogen use efficiency (NUE) indices under reduced levels as well as at recommended N conditions; urea coating with urease inhibitors can save about 20-25% N and N release was slow and gradual throughout the crop growth period when urease inhibitors are used thus reducing the soil, water and environmental pollution.

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