Effect of nitrogen applied through nano clay polymer composites on nitrogen use efficiency of threeelite rice genotypes

Comment [U1]: Revise to reflect the main objective of study.

ABSTRACT

Nitrogen (N) is a crucial nutrient for crop growth, and its efficient utilization is imperative for global food production. However, excessive nitrogen fertilizer application leads to environmental losses. In this study, an attempt was made to reduce the nitrogen losses by loading of urea and urea ammonium nitrate (UAN) into Nano clay polymer composites (NCPCs) to act as slow release N fertilizers. To achieve the objectives of the present study, five N fertilizer treatments (T1-control, T2- N applied through urea, T3-N applied throughUAN, T4-N applied throughNCPC loaded with urea and T5-N applied throughNCPC loaded with UAN) were assessed using threeeliterice genotypes namely IR-64, Nagina 22 and MTU 1010. Results of the greenhouse study indicated that the treatment NCPC loaded with UAN was more effective in increasing the grain yield, nitrogen uptake, agronomic use efficiency and apparent nitrogen recovery by 36.9%, 51.0%, 64.2%, and 92.0% over urea, respectively. Amongst the genotypes, MTU 1010 responded efficiently in having highest percentage grain yield by 9.58 and 91.97 over IR-64 and Nagina-22 respectively.

Comment [U2]: Revise to represent the nitrogen effect.

Comment [U3]: What concept of loading were adapted as treatment(s)??

Comment [U4]: Localized variety. Avoid using superlatives throughout the manuscript.

Comment [U5]: 9.58 and 91.97? high variation. Not clear what the author means. Revise.

Key words:*Nitrogen use efficiency, Nano clay polymer composites, Genotypes, Urea ammonium nitrate*

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1. INTRODUCTION

Comment [U7]: Introduction need to emphasis on NUE and NCPC. Please revise.

Nitrogen (N) is one of the major essential plant nutrients required for crop growth and development. It's animportant constituent for the nucleotides, amino acids, proteins and chlorophyll and play a major role in cell structure and energy metabolism [1, 2].In order to meet the global food production, 50% of human population relies on nitrogen fertilizers [3]. The excess use of nitrogen fertilizer in agriculture has led to degradation of environment and atmosphere.Rice (*Oryza sativa* L.) is an important staple food crop and increasing its productivity necessitates use of excessive fertilizer N in large quantities asit is the important nutrient element required for their growth [4]. The effect of heavy nitrogen fertilization results in lower nitrogen use efficiency due to the quick N losses in various ways *viz.*, denitrification, ammonia volatilization, leachingand surface runoff in the soil-floodwater system [5]. In India, the major N fertilizer used isurea and it contributes 80% to the total consumption. Urea contributes fifty-five percent of the world total fertilizer consumption. Estimate results that by 2050 about 250 million tons of fertilizer nitrogen may be necessary

[6]. But N use efficiency of urea is very low due to rapid hydrolysis followed by nitrification-denitrification activities, more than 60% of N is lost to the environment [7].

In this direction several slow and controlled release fertilizers have been developed and tested for enhancing N use efficiency. Nano clay polymer composite (NCPC) based N fertilizers are one of the possible option to minimize the N losses and enhancing N use efficiency. As compared to urea, NCPCs loaded with N fertilizers have shown promising results *i.e.*, higher crop yields, enhancing N use efficiency and reduced nitrous oxide emissions [8, 9].As rice is a heavy feeder of N, selection of N efficient genotypes is also needed to improve productivity and N use efficiency. In this regard, ongoing efforts are necessary to achieve the highest yields possible with the provision of ideal nitrogen complementing the compromised yield with additional environmental and economic benefits [10].In present studyattempt has been made to study the effect of NCPC based slow release N fertilizers for enhancing N use efficiency under three elite rice genotypes. The study recognizes the benefits of using nano clay polymer composites in enhancing N use efficiency and highlights N efficient variety of rice.

2. MATERIALS AND METHODS

2.1 Location and collection of soil sample

~~To accomplish the objectives of present study, b~~Bulk soil sample(TypicHaplustepts) was collected from Main Block 4B ~~e~~Experimental ~~F~~farm, Division of Plant Physiology ICAR-IARI, New Delhi following standard protocol. The soil sample were ~~after collection was~~ air dried for how long??, ground, sieved to pass through 2mm sieve. The prepared soil were ~~and~~ used for green houseexperiment. ~~studies.~~

2.2 Synthesis of nanoclay polymer composite (NCPCs) fertilizers

NCPCs were synthesized by copolymerization reaction of acrylic acid and acrylamide with partial neutralization with ammonia. During the synthesis, bentonite (10% by weight) was added to provide mechanical strength to the polymer, N,Ńmethylenebis-acryamide as crosslinker and ammonium persulphate (APS) as free radical initiator[11].

For loading of polymers with N, the known amount of aqueous solution of urea and urea ammonium nitrate was prepared in distilled water separately. The pre-weighted dry ground gels were immersed into the solution to reach swelling equilibrium. Thereafter the swollen gels were dried at 60 °C until constant weight is reached and used for further analysis and application.

2.3 Greenhouse experiment

Comment [U8]: In rice or which crop?? Comparable with current study?? How??

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Comment [U10]: Can the author estimate the bulk soil collected. 100 kg/200kg/500kg??

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Comment [U12]: which standard protocol. Please state.

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The green house experiment was conducted using rice as a test crop at NanajiDeshmukh Plant Phenomics Centre, IARI, New Delhi during kharif 2018. Stated below are the fiev (5) treatments:

Total five N treatments (T1: Control (with no nitrogen)

, T2: 100% RDF-N in the form of urea,

T3: 100% RDF-N N in the form of UAN,

T4: 100% RDF-N - N in the form of urea loaded in NCPCs,

T5: 100% RDF-N N in the form of UAN loaded in NCPCs)

All treatments were replicated three times in combination with three rice varieties (IR-64, Nagina 22 and MTU 1010) as were replicated three times in afFactorial Ccompletely Rrandomized Ddesign. (thus state how much is the total treatments inclusive of replications). Rice seedlings were transplanted ……………………… Transplanting of rice seedlings was done along with application of with full dose of(state the doses) phosphorus and potassiumand one third dose of N with respective sources. Further, Remaining two split N application were conducted at was applied in two splits at 40 and 60 days after transplanting (DAT).

2.4 Soil and plant analysies

Soils were sampledafter harvest. Soils were thoroughly mixed, extracted with KCl (2N)and then analyzed formineral nitrogen content (NH_4 and NO_3-N) [12].After recording the dried biomass of crop, plant sample were ground and nitrogen concentrations in grain and straw samples were determined using Kjeldhal digestion and distillation method [13]. Nitrogen uptake in straw and grain was estimated by multiplying the concentration of N with corresponding grain and straw biomass respectively. The summation of N uptake in grain and straw gives the total uptake in plant. Various yield attributes like nitrogen content in plants, apparent nitrogen recovery, agronomic use efficiency and physiological use efficiency were recorded after the harvest of the crop.

2.5 Statistical analysis

All treatment and variety combinations were subjected to the two-way analysis of variance (ANOVA) appropriate based on to the experimental design (two factor completely randomized design) as stated given by Gomez and Gomez [14].

3. RESULTS

3.1 Grain yield of paddy

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Comment [U16]: Which season/month does this correspond to?

Comment [U17]: Nomenclature for treatment from T1 to T5 is stated here, however not used in the text itself.

Comment [U18]: State the dose

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Comment [U20]: Name the method

Comment [U21]: Revise the whole section 2.4 clearly. State the methods use and the references.

The significant highest grain yield was recorded under NCPC loaded UAN (29.3 g/pot) followed by NCPC loaded with urea (25.7 g/pot) whereas; urea and UAN treatments were statistically at par with each other in terms of grain yield (T~~t~~able 1). In case of varieties, MTU 1010 recorded significantly highersyntax grain yield (26.3 g/pot) followed by IR 64 (24.0 g/pot) and Nagina 22 (13.7 g/pot).The interaction effectof variety and treatments showed ~~statistically~~ significant differences when MTU1010 treated with NCPC loaded with UAN resulted in highest grain yield (35.4 g/pot) over other interaction combinations.

Table 1: Effect of N sources on the grain yield (g/ pot) of three elite rice genotypes

Sources of Nitrogen (Treatments)	Rice Varieties (V)			
	IR-64	Nagina 22	MTU1010	Mean
Control	10.0	5.84	10.5	8.79
100% Urea	25.3	12.4	26.5	21.4
100% UAN	24.2	13.3	27.0	21.5
100% NCPC loaded Urea	27.8	17.1	32.0	25.7
100% NCPC loaded UAN	32.8	19.8	35.4	29.3
Mean	24.0	13.7	26.3	
CD (5%)	V= 1.53 T= 2.00 V*T=3.47			
SEm (±)	V= 0.51 T= 0.659 V*T=1.14			

3.2 Straw yield of paddy

The straw yield of different rice genotypes studied under varied N-treatments showed significant differences. The NCPC loaded with UAN resulted in the highest straw biomass (45.5 g/pot) followed by NCPC loaded with urea (38.6 g/pot). The treatment of urea and UAN performed statistically at par with each other for straw biomass (table 2). All genotypes of rice showed statistically significant differences for straw biomass where IR 64 has the highest straw biomass (37.1 g/pot) followed by MTU 1010 and Nagina 22. The interaction was non-significant.

Table 2: Effect of N sources on straw yield (g/ pot) of three elite rice genotypes

Sources of Nitrogen (Treatments)	Rice Varieties (V)			
	IR-64	Nagina 22	MTU1010	Mean
Control	18.0	10.8	11.9	13.6
100% Urea	33.7	26.5	29.6	29.9
100% UAN	39.9	26.2	31.1	32.4
100% NCPC loaded Urea	43.9	33.3	38.7	38.6
100% NCPC loaded UAN	50.2	41.0	45.3	45.5
Mean	37.1	27.6	31.3	
CD (5%)	V= 2.98 T= 3.85 V*T=NS			

Comment [U22]: Why combine the statement significant and highest in single sentence. Revise.

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Comment [U24]: what is the difference between interaction and effect?
It would be better… Interaction between rice variety and treatments were significantly different, as T5 (variety MTU1010) resulted highest grain yield at 35.4 g/pot compared to others (Table 1).

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SEm (±)	V= 0.98 T= 1.27 V*T=2.19

3.3 Uptake of nitrogen

Comment [U26]: Nitrogen uptake of paddy from all treatments

The NCPC loaded with UAN gave significant highest uptake (0.74 g/pot) of nitrogen over rest of the fertilizer treatments. The urea and UAN treatments were statistically at par with each other in respect of nitrogen uptake by rice and both these treatments were statistically inferior to the NCPC loaded with urea (0.62 g/pot).Both varieties, IR64 and MTU1010 recorded significantly higher nitrogen uptake over Nagina 22 (table 3).No significant differences in interactions were observed between variety and fertilizer treatments.The total nitrogen uptake of MTU 1010 was statistically similar with IR 64 in having the highest nitrogen uptake as these two genotypes produced more or less similar straw and grain yield as compared to Nagina 22.

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Table 3: Effect of N sources on the total uptake of N (g/pot) of three elite rice genotypes at harvest

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Sources of Nitrogen (Treatments)	Rice Varieties (V)			
	IR-64	Nagina 22	MTU1010	Mean
Control	0.23	0.13	0.19	0.18
100% Urea	0.55	0.36	0.55	0.49
100% UAN	0.57	0.37	0.57	0.50
100% NCPC loaded Urea	0.67	0.49	0.70	0.62
100% NCPC loaded UAN	0.81	0.60	0.82	0.74
Mean	0.56	0.39	0.57	
CD (5%)	V= 0.03 T= 0.04 V*T=NS			
SEm (±)	V= 0.01 T= 0.01 V*T=0.02			

3.4 Apparent nitrogen recovery

Apparent N recovery (ANR) increased with the increase in the uptake of nitrogen from soil compared to control. Highest ANR (53.2%) was found with NCPC loaded UAN treatment and lowest (27.7%) was obtained in urea treatment, statistically similar with UAN treatment (29.3%). Both urea and UAN treatments were statistically inferior to NCPC loaded treatments in terms of ANR. Among the varieties, both IR 64 (42.4%) and MTU 1010 (42.2%) gave similar ANR and these were significantly higher than~~over~~Nagina 22 (~~t~~Table 4).The highly responsive MTU1010 genotype being highest yielder absorbed ~~took up~~ more

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nitrogen when treated with NCPC loaded with UAN (T4) because ~~of~~ T4 was able to ~~its ability to~~ supply (release)~~more~~ nitrogen over extended period of time. Therefore acting as a slow release ertilizer.~~at required rates for long time.~~Interaction effect was non-significant between variety and treatment combinations.

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Table 4: Effect of N sources on the apparent nitrogen recovery (%) of three elite rice genotypes

Sources of Nitrogen (Treatments)	Rice Varieties (V)			
	IR-64	Nagina 22	MTU1010	Mean
100% Urea	32.5	19.4	31.3	27.7
100% UAN	33.9	20.6	33.3	29.3
100% NCPC loaded Urea	44.7	31.9	46.2	41.0
100% NCPC loaded UAN	58.6	43.1	57.8	53.2
Mean	42.4	28.8	42.2	
CD (5%)	V= 4.85 T= 5.60 V*T=NS			
SEm (±)	V= 1.56 T= 1.80 V*T=3.12			

3.5 Agronomic use efficiency OK

Agronomic use efficiency (AUE) is the increase in yield per unit nitrogen applied along with NCPC over urea and UAN treatments (T~~t~~able 5). The data (Table 5) showed significant differences among the treatments applied to three rice genotypes. Highest AUE (20.2 g/g N) was found with NCPC loaded UAN treatment a lowest in (12.3 g/g N) in urea which was statistically similar to UAN treatment. In case of different genotypes, MTU 1010 showed the significant highest AUE (18.8 g/g N)indicating its highly responsive nature for the applied nitrogen sources, which was statistically similar to IR 64 (17.5 g/g N) and both these varieties were statistically superior over Nagina 22 (9.8 g/g N). There were no significant differences observed among the various interactions of variety and treatment combinations.

Table 5: Effect of N sources on the agronomic use efficiency (g/g N) of three elite rice genotypes

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Sources of nitrogen (Treatments)	Rice Varieties (V)			
	IR-64	Nagina 22	MTU1010	Mean
100% Urea	15.2	6.60	15.1	12.3
100% UAN	14.2	7.50	15.6	12.4
100% NCPC loaded Urea	17.8	11.3	20.6	16.6
100% NCPC loaded UAN	22.7	14.0	24.0	20.2
Mean	17.5	9.80	18.8	
CD (5%)	V= 2.53 T= 2.92 V*T=NS			

SEm (±)	V= 0.81 T= 0.94 V*T=1.63

3.6 Physiological use efficiency

The physiological use efficiency (PUE) increases with increase in the difference of yield between treated and control pots and decrease in the difference of uptake of nitrogen between treated and control pots (Ttable 6). PUE in this study ranged from 37.5 to 42.9 g/g N under various treatment applications. The maximum PUE (42.9 g/g N) was observed with urea and minimum (37.5 g/g N) in NCPC loaded UAN treatment. The highest being for urea treated pots signifying some losses occurred in the soil and thus resulting in low uptake and thus giving highest physiological use efficient treatment. In case of different genotypes, highest PUE (45.2 g/g N) was obtained with MTU 1010 and which was statistically at par with IR 64.

Table 6: Effect of N sources on the physiological use efficiency of three elite rice genotypes

Sources of Nitrogen (Treatments)	Rice Varieties (V)			
	IR-64	Nagina 22	MTU1010	Mean
100% Urea	46.9	34.1	47.8	42.9
100% UAN	41.9	36.3	46.9	41.7
100% NCPC loaded Urea	39.8	35.4	44.6	39.9
100% NCPC loaded UAN	38.8	32.4	41.4	37.5
Mean	41.8	34.6	45.2	
CD (5%)	V= 3.63 T= NS V*T=NS			
SEm (±)	V= 1.17 T= 1.35 V*T=2.33			

3.7 Soil mineral nitrogen

The mineralnitrogen content in soil was determined after the harvest of rice crop.The treatment of NCPC loaded with UAN showed significantly higher amount of mineral nitrogen content (55.2 mg/kg) followed by NCPC loaded with urea, urea, UAN, and control treatments (table 7). The NCPC might have retained and regulated both nitrogen forms of ammonium as well as nitrate (show the form) and released slowly to rice crop. The genotypeNagina 22 retained significant highest amount of mineral nitrogen content (50.1 mg/kg) in the soil followed by IR64 and MTU1010. To quote about the interaction effect, there were no significant differences.

Table7: Effect of N sources on the soil mineral nitrogen (mg/kg) of three elite rice genotypes at harvest

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Comment [U31]: Days? Timeline.

Comment [U32]: Genotype or variety. The author intermix the term usage. Choose one and standardize in text.

Comment [U33]: Revise.

Sources of nitrogen (Treatments)	Rice Varieties (V)			
	IR-64	Nagina 22	MTU1010	Mean
Control	34.0	39.7	32.7	35.5
100% Urea	45.6	49.7	41.2	45.5
100% UAN	43.8	46.3	41.8	44.0
100% NCPC loaded Urea	51.2	55.9	47.0	51.3
100% NCPC loaded UAN	56.3	59.0	50.3	55.2
Mean	46.2	50.1	42.6	
CD (5%)	V= 1.30 T= 1.68 V*T=NS			
SEm (±)	V= 0.43 T= 0.55 V*T=0.96			

4. DISCUSSION

4.1 Plant growth and Nitrogen use efficiency

Maximum plant biomass (grain + straw) was recorded under NCPC treated soils both with urea and UAN which ~~ultimately~~resulted ~~in to~~ higher N uptake and use efficiencies. It might be due to synchronized release of N from the NCPC based N fertilizers, so that the plants getmore opportunity to absorb nitrogen and subsequently increase in production [15].Shoji [16]also synthesized different controlled release N fertilizers and confirmed the enhancement of crop yield and N use efficiencies. Among varieties, IR 64 gave the highest straw yield followed by MTU 1010 when treated with NCPC loaded with UAN. The delayed panicle initiation of the variety IR 64 having more time for its vegetative growth resulting in the second highest number of tillers next to MTU 1010 might be the reason for having highest straw yield at harvest, as the panicle and effective tillers are a major indicators of production per plant [4].

Kaneta *et al*. [17] found that the N uptake was notably increased by the use of polyolefin-coated urea which can release N in a pattern that matches the N demand of the rice during the whole growing season. Similarly, Zhao *et al*., [18] studied the effect of fertilizer loaded nano clay polymer composites on the chlorophyll content in maize crop and observed the enhanced photosynthetic rate after anthesis in maize. Since nutrient uptake is the product of nutrient concentration and yield, hence, uptake generally followed the yield trend. Which ultimately leads to enhanced recovery and agronomic use efficiency of nitrogen. The increase in N uptake is due to better availability and absorption of N in balanced quantity because of good proliferation of root system [19].

4.2 Mineral nitrogen availability

Comment [U34]: Earlier stated as T4, but the author doesnot use these nomenclature, despite that, referring the by treatment name in full. Please standardize.

Comment [U35]: Not suitable for scientific write-up.

Comment [U36]: When the uptake is high, does it nor indicate the NUE is also high. If so, why is the necessity to repeat both?

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Comment [U37]: Syntax error.

Comment [U38]: NUE.

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At same level of N fertilizer application, ammonium N content was significantly higher under NCPC treatments compared to conventional fertilizer. This indicated that NCPC could maintain a gradual release of loaded nutrients in soil for longer period. On the other hand, rapid hydrolysis of urea under conventional fertilizer and subsequent nitrification – denitrification resulted in losses of N from the soil system (20, 21). Total mineral N content was also recorded under NCPC treated soils as under rice crop major portion of mineral N remain in ammonical form due to reduced conditions. Jatav *et al.*, [22] also reported an increase in total mineral nitrogen in soils on application of fertilizer loaded NCPCs over conventional fertilizer due to slow release pattern of N from the NCPC based fertilizers.

5. CONCLUSION

Study data indicates that T5 (~~The~~ NCPC loaded UAN)treatment gavesignificantly higherresults for various yield and yield attributes followed by NCPC loaded Urea.Among genotypes,MTU 1010 recorded ~~gave~~ highest yields and ~~the same variety~~ respondeds well to the nitrogen sources, irrespective of treatments. ~~any fertilizer treatment.~~Meanwhile, ~~Whereas,~~ Nagina 22 wasisathe tolerant variety tolerant to? and based on what?. ~~irrespective of any nitrogen sources it was not influenced much by the different fertilizer sources.~~Study found, increase in ~~Higher~~ grain yield (by how much??), NUE, ~~N use efficiency and higher~~N uptake fromnano clay polymer composite (NCPC)streated fertilizers were supportive of ~~were obtained in this study because of the~~ N availability (release/supply) from NCPCs. ~~closely matched the N~~ Plant N requirement ~~needs of the crops which might be due t~~o sSynchronized supply of N into the soil for plant uptake by NCPC fertilizers are one plausible scenario.Thus, Ffurther research has to be conducted on the usage of NCPC fertilizers at graded doses under field conditions.

Comment [U42]: Can the author justify this statement based on the soil data collected from different paddy growth stage? If any.

Thanks.

Comment [U43]: ammoniacal

Comment [U44]: soil data analysis before and after harvest or even better, soil data analysis based on growth stage would strongly support this section. However, no soil data were observed here.
Please consider.

Thanks.

Comment [U45]: This is T5.

Comment [U46]: In many sentences the author used significantly higher.
If the confidence level were set at 99%, and the outcome was significant than it would be suitable, however at CS %, likely the confidence level is 95%. Thus using significantly higher can be non-suitable.
Please consider.

Thanks.

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Comment [U48]: Table indicates varity, and here again, it is genotypes.
Select one and standardize.

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Comment [U49]: NUE sums up N uptake?

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