



Effect of precision nitrogen management on growth, yield and yield components of rice in Khumaltar

Sangita Kaduwal^{*}, Himal Prasad Timalsina¹, Reshama Neupane¹, Rajendra Kumar Battarai¹, Bhimsen Chaulagain¹, Prakash Ghimire²

¹National Agronomy Research Centre, Khumaltar, Lalitpur, Nepal

²Institute of Agriculture and Animal Sciences, Paklihawa, Tribhuvan University, Nepal

*Corresponding author email: sangkaduwal@gmail.com or smily_kaduwal@yahoo.com

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ABSTRACT

Enhancing the nitrogen use efficiency (NUE) is essential for sustainable rice production, especially in areas where general fertilizer recommendations don't take field-specific variability into account. Rice (var. NR11105) was the plant material of a two-year field experiment (2023 and 2024) at the National Agronomy Research Centre, Khumaltar, Nepal, to assess the impact of precision nitrogen management practices on rice growth and yield parameters. Three replications of the experiment were set up in a randomized complete block design. N omission (control), farmer practices, the recommended fertilizer dose (RDF: 100:30:30 kg N:P₂O₅: K₂O ha⁻¹), N management based on Leaf Color Chart (LCC), SPAD meter-based N management, NDVI-based N management, and Nutrient Expert®-Site Specific Nutrient Management (NE-SSNM) were among the treatments. Pooled analysis showed significant treatment effects of treatments were found for tiller number, grains per panicle, thousand-grain weight, sterility %, biomass yield, and grain yield. While SPAD (5.49 t ha⁻¹), RDF (5.47 t ha⁻¹), and LCC (5.19 t ha⁻¹) were statistically similar, NDVI- and NE-SSNM-based treatments yielded the maximum grain production (5.70 t ha⁻¹). The control (1.78 t ha⁻¹) and farmer practice (3.38 t ha⁻¹) yields were the lowest. Comparing precision-based N management to traditional methods, the former improved biomass accumulation and decreased the sterility percentage. The yield improvement with NE-SSNM and NDVI was 4.2% more than that under RDF. The results show that N management strategies based on sensors and decision assistance improve rice productivity and nitrogen use efficiency more than general advice. The sustainable intensification of rice systems in Nepal and other agro-ecological settings can be supported by the use of site-specific nutrient management techniques.

Keywords: LCC, SPAD, NDVI, NESSNM, nitrogen, rice.

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INTRODUCTION

Rice is the most important staple crop of Nepal and accounts for about 20% AGDP of Nepal. In recent years rice production has increased compared to earlier years, with production of ~57.24 lakh tons in recent years whereas rice cultivation area has declined by ~0.8% annually due to land conversion and labor shortages (migration). Nepal's rice self-sufficiency ratio (SSR) has declined from ~92.7% to ~82.0% over the past decade thus increasing reliance on imports. Current productivity growth (~1.47% per year) is not sufficient to achieve self-sufficiency by 2050 unless productivity increases. While production has grown, the self-sufficiency ratio is declining due to rising demand and slow productivity improvements. Nitrogen deficiency significantly reduces yield due to poor vegetative and reproductive growth. Poor nitrogen management leads to poor nitrogen use efficiency often below 50% thus drastically reducing yield. Rice is one of the input contributes approximately 20–25% to the total production costs of rice. There exists yield gap between potential yield and farmer field yield of around 3.0 t ha⁻¹ for monsoon rice (MOALD 2025).

Nitrogen Use Efficiency (NUE) is the measure of how effectively a rice crop uses applied nitrogen fertilizer to produce grain yield. NUE is important in rice field as it improves fertilizer efficiency, reduces production cost, prevents environmental pollution. Major factors for such yield gap include: poor soil nutrient management declining soil fertility due to low applications of mineral and organic fertilizers and imbalanced blanket fertilizer application (Devkota et al 2016). The fertilizer recommendations in the country are soil-test based (Pandey et al 2018), however, soil testing facilities are scarce in Nepal, and it is difficult to develop farm-specific fertilizer recommendation with the present infrastructure and facilities. Besides, the national recommendations have not

been disseminated well to reach large numbers of farmers, leading to large nutrient related yield gaps (Timsina et al 2018). Management of fertilizer nitrogen in rice developed for large areas or zones having similar climate and land forms in Nepal. It cannot help to increase the nutrient-use efficiency beyond a limit. Further improvement can be achieved only by planning strategies for fertilizer nitrogen management in rice (*Oryza sativa L.*). Gadgets like green seeker, chlorophyll-meter (SPAD meter) and inexpensive leaf color chart (LCC) have proved quick and reliable tools to decide the time when fertilizer N needs to be applied to the crop. LCC, NDVI is useful in estimating the health of the canopy and its nitrogen level in large regions, SPAD offers accurate measurements of the chlorophyll content in the leaf, which helps in managing nitrogen application on a specific area. These tools have the potential to enhance better and efficient ways of handling rice through its integration that can provide farmers with practical information on improved nitrogen management and improved crop yields under climate change (Azhar et al 2025).

The current situation demands nutrient management recommendation guidelines for rice that are scientifically robust, easy to understand and simple to use by farmers and their advisors. Moreover, fertilizer recommendations need to be farm resource sensitive to provide farmers the option to decide their own investments on fertilizers based on their financial resources. The associated higher cost of under fertilization relative to over fertilization drives farmers to apply imbalanced rates. This uncertainty can be addressed by providing more accurate location and time specific recommendations that increase accuracy in nutrient recommendations (Clune et al 2013). The precise application of nutrients through the use of these tools can raise the profitability of the production system and may reduce environmental pollution. Very little work has been done to use these improved computer based tools for nutrient management in Nepal. Poor N use efficiency is also due to unproductive splitting of N doses in excess of crop demand (Ecarnot et al 2013). Enabling efficient management of N in rice under situations that face diversity in field, season and variety. Blanket fertilizer recommendation does not consider the spatial and temporal variation of crop demand. The synchronization of nitrogen fertilizer application with plant demand is necessary to reduce the losses, optimize the nutrient use efficiency and minimize the environmental pollution (Kumar et al 2013). This could be done with the help of precision N management practices.

In dry direct seeded rice, LCC based nitrogen management increase the nitrogen use efficiency, total N uptake and grain yield (Subedi et al 2018). NDVI, LCC, SPAD, NE-SSNM can respond to the plants in question, yet, to convert the spectral results into practical fertilizer application recommendations, it is necessary to perform calibrations and further research at different agro-ecological regions. Therefore, these tools shows potential routes to sustainable rice production based on additional study is required to standardize the approaches, test findings in different settings, and address uncertainty that restricts the applicability of this approach on the ground. Despite the promising applications of these tools in nitrogen management, varying results among rice varieties, climate zones and sensor types restrict the extrapolation of results. However, in Nepal, most research works have so far been focused mostly on the rate and timing of N application without considering the initial soil nitrogen and crop demand. So, this study was needed on crop-demand based N management.

Specific Objective

To identify most suitable optical sensor for nitrogen management in rice

MATERIALS AND METHODS

Experimental site and climatic condition

A field experiment was carried out in upland research block of National Agronomy Research Centre (NAgRC) in 2023 and 2024. Geographically the station lies at 27°40' north latitude and 85°20' east longitude at an elevation of 1360 masl with temperate climate. The meteorological data was taken from meteorological station of NAgRC. The total rainfall during the crop growing season was 1012.8 mm and 1499.6 mm in 2023 and 2024 respectively (Fig. 1, 2).

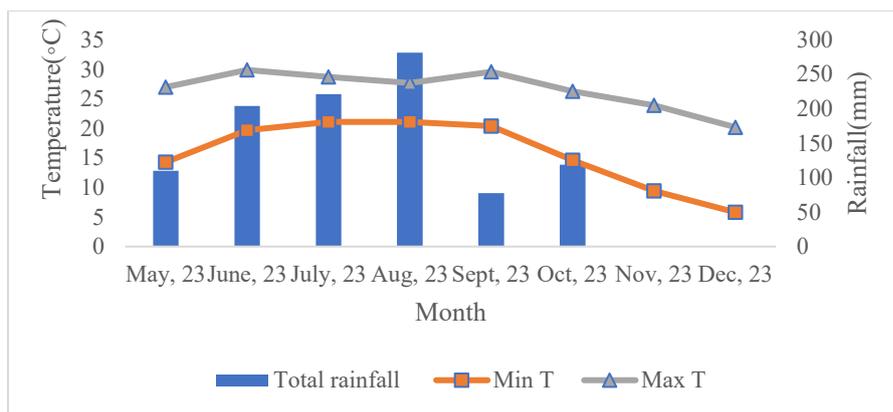


Fig. 1. Monthly total rainfall, mean maximum and minimum temperatures during the experiment (2023)

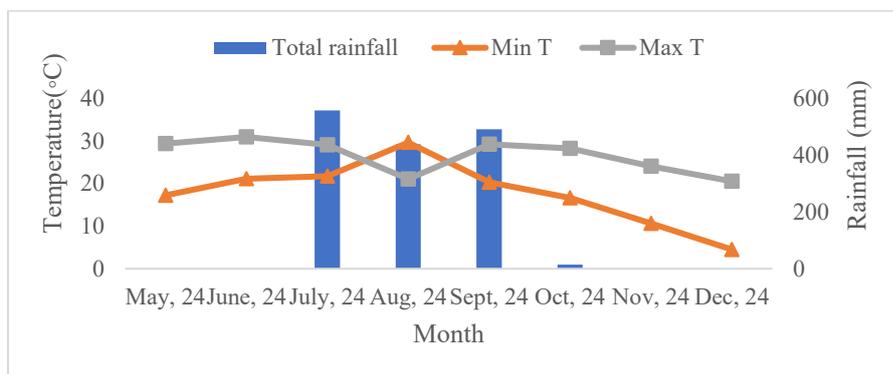


Fig. 2: Monthly total rainfall, mean maximum and minimum temperatures during the experiment (2024)

Chemical properties of soil (0-15 cm) at the experimental site

Composite soil samples were taken randomly from three different spots of each replication from 0-15cm soil depth using tube auger and the samples were air dried, ground and sieved through 2mm sieve and sent to laboratory for testing before planting. Total N was determined by Kjeldahl Digestion method, total phosphorous by Vanadomolybdate method, total potassium by flame photometric method, soil pH by Potentiometric (1:2.5) metric method, soil organic matter by Walkley and Black method and sand, silt, clay percentage by hydrometer method. Chemical properties of the experimental field revealed that soil pH was moderately acidic with medium organic matter content. Total nitrogen content and total available potassium was medium while soil was high in total available phosphorous with loam soil type. Farmyard manure (FYM) applied in experimental plot was slightly acidic with pH 6.35, high in nitrogen 1.39%, high in potassium content 1.70% and low in phosphorous 0.41% (Table 1). The soil sample analysis was done in Agricultural Technology Centre (ATC) Pvt Ltd, Lalitpur, Nepal.

Table 1. Physio-chemical properties of the soil

Physical properties	Content (kg ha ⁻¹)	Category
Sand%	36.16	Loam
Silt%	49.88	
Clay%	13.96	
Chemical properties	Content (kg ha ⁻¹)	Category
Soil organic matter%	2.53	Medium
Total Nitrogen %	0.23	Medium
Available Phosphorous (P ₂ O ₅ kg ha ⁻¹)	568.6	High
Available Potassium (K ₂ O kg ha ⁻¹)	212.02	Medium

Experimental design, treatment details and cultural practices

The experiment was laid out in randomized complete block design (RCBD) with three replications. The variety used was NR11105. The source of the variety is National Plant Breeding and Genetics Research Centre, NARC, Khumaltar. This variety is chosen as it is pipeline iron rich genotype and its fertilizer recommendation is necessary before release. Plot size was 3m × 3m with a spacing of 20 cm × 20cm. Fertilizer was applied

@100:30:30 kg ha⁻¹. Seeding was done in 29th May in 2023 and 2024 and transplanted on 27th June in 2023 and 2024. Two seedling per hill was maintained. Treatment comprised of control/N omitted (0N:30P₂O₅:30K₂O), NPK dose based on farmer practice (138:30:30 N: P₂O₅:K₂O kg ha⁻¹), recommended dose (RDF) (100:30:30 N: P₂O₅:K₂O kg ha⁻¹), N dose based on LCC, N dose based on SPAD, N dose based on NDVI and Nutrient expert-Site specific nutrient management i.e NE[®]-SSNM (113.5:30:30 N: P₂O₅: K₂O kg ha⁻¹). In RDF 1/3rd of N (33kg N) was applied as basal dose and remaining N was splitted twice at tillering and panicle initiation stage. In farmers second split (last) dose of urea was applied after 20 DAS. 1/3rd dose of RDF i.e 33 kg N was applied as basal in LCC, SPAD, NDVI and remaining N was applied when the reading fell below threshold limit for LCC (<4.0), SPAD (<37.5), NDVI (<0.5). P₂O₅, K₂O was kept constant for LCC, SPAD, NDVI, NE-SSNM, RDF. Urea, SSP and MOP was used as a source of fertilizer.

Data collection

Reading was taken for SPAD, LCC, NDVI after 25 days at 10 days interval. Middle seven rows were considered as net plot and 10 plants were randomly selected for taking growth, yield and yield attributing traits. All the agronomical practices were carried out when required. Using a portable Green Seeker sensor, NDVI values were taken throughout crucial stages of growth for nitrogen management treatments in order to direct nitrogen delivery. Site-specific fertilizer recommendations were generated using the Nutrient Expert[®] decision support tool, which was based on field-specific data such as crop history, soil properties, and yield targets.

Statistical Analysis

The collected data were processed by MS Excel 016 and analyzed by using Genstat15 ED. All the recorded data were subjected to analysis of variance (ANOVA) and Duncan's multiple Range Test (DMRT) at 5 % level of significance was used for mean comparison (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

The result (Table 2, 3) illustrated a significant difference in tiller number, grain number, thousand grain weight, sterility%, phenological traits, biomass and yield. Tiller number ranged from 10 to 15 per plant. Highest tiller number, thousand grain weight with lowest sterility% was recorded on N applied through NE-SSNM. Likewise significant increase in grain number was also in NE-SSNM followed by N application on the basis of NDVI and biomass (20.74 t ha⁻¹) was recorded in NE-SSNM over RDF (recommended dose of fertilizer) and it was at par with N applied on the basis of NDVI and lowest yield (1.80 t ha⁻¹) and biomass (6.13 t ha⁻¹) in control followed by N applied based on farmer practice. Similar effect of RDF, LCC and SPAD were recorded in all these mentioned. Higher and continuous supply of 'N' to rice resulted in increased production of growth parameters such as plant height, number of tillers m⁻² Gill and Walia (2023). The highest no of grains per panicle with green seeker-based N fertilization. Lowest no of grains per panicle was observed in control which may be due to lower nitrogen fertilization than recommended dose of fertilization. Similar results were reported by (Pateel et al 2017) traits. These sensor-determined topdressing of 'N' for rice increased the number of split applications as against two splits in conventional farmer practice. The increased availability of nitrogen at distinct physiological phases would have supported for better assimilation of photosynthates towards grain and also due to the favourable effect of accelerating the yield attributes like thousand gain weight, no of grains per panicle (Ravi et al 2017).

Table 2. Effect of nitrogen on growth and yield attributes of rice at Khumaltar condition in 2023

Treatment	Plant height (cm)	Tiller/hill	Panicle length (cm)	No of grains/panicle	TGW (g)
0:30:30NPK kg ha ⁻¹ (Control)	137.0	10 ^b	24.83	96 ^e	21.07 ^b
138:30:30NPK kg ha ⁻¹ (Farmer practice)	140.3	13 ^a	24.80	111 ^d	20.57 ^b
100:30:30NPK kg ha ⁻¹ (RDF)	141.5	14 ^a	25.53	156 ^c	23.80 ^a
N application on the basis of LCC	141.0	14 ^a	24.84	157 ^c	24.77 ^a
N application on the basis of SPAD	141.9	14 ^a	23.77	158 ^c	24.88 ^a
N application on the basis of NDVI	141.1	14 ^a	24.12	165 ^b	25.21 ^a
NE-SSNM	141.3	15 ^a	24.34	176 ^a	25.50 ^a
Mean	140.59	13.58	24.60	145.71	23.68
F test	NS	*	NS	*	NS
LSD	-	-	-	5.35	-
CV%	1.5	8.8	5.1	2.1	1.69

LSD=Least significant difference, CV=Coefficient of variation, NS=Not significant at p<0.05, *=Significant at p<0.05, Means with same letter in column are not significantly different at p = 0.05 by DMRT

Table 3. Effect of nitrogen on phenology and yield of rice at Khumaltar condition in 2023

Treatment	Sterility%	Days to 80% heading	Days to 80% maturity	Biomass yield (tha ⁻¹)	Yield (tha ⁻¹)
0:30:30 NPK kg ha ⁻¹ (Control)	9.15 ^a	95	130	6.13 ^c	1.80 ^c
138:30:30 NPK kg ha ⁻¹ (Farmer practice)	8.06 ^a	96	130	8.08 ^c	2.27 ^c
100:30:30 NPK kg ha ⁻¹ (RDF)	1.45 ^b	94	129	13.60 ^b	4.70 ^b
N application on the basis of LCC	1.31 ^b	93	129	14.35 ^b	4.79 ^b
N application on the basis of SPAD	1.20 ^b	94	129	15.02 ^b	5.16 ^b
N application on the basis of NDVI	0.92 ^b	93	128	20.52 ^a	7.45 ^a
NE-SSNM	0.64 ^b	93	128	20.74 ^a	7.96 ^a
Grand mean	3.25	93.81	129.30	14.06	4.88
F test	*	NS	NS	*	*
LSD	1.41	-	-	3.37	1.07
CV%	24.4	1.6	1.2	13.50	12.40

LSD=Least significant difference, CV=Coefficient of variation, NS=Not significant at p<0.05, *=Significant at p<0.05, Means with same letter in column are not significantly different at p = 0.05 by DMRT

The results (Table 4, 5) illustrated a significant difference in tiller number, grain number, thousand grain weight, sterility%, biomass and yield. Tiller number ranged from 10 to 12 per plant. Significantly highest tiller number, thousand grain weight with lowest sterility% was recorded on N applied through NE-SSNM. Application of N in more number of splits upto reproductive phase as per optical sensor and NE-SSNM guidance was responsible for retaining more number of active leaves till the maturity in the above treatments. Similar reports were reported by (Peng et al 2012). Likewise significant increase in grain number was also in NE-SSNM followed by N application on the basis of NDVI.

Table 4. Effect of nitrogen on growth and yield attributes of rice at Khumaltar condition in 2024

Treatment	Plant height (cm)	Tiller/Hill	Panicle length (cm)	No of grains/Panicle	TGW (g)
0:30:30NPK kg ha ⁻¹ (Control)	143.61	10 ^b	23.95	94 ^b	21.65 ^c
138:30:30NPK kg ha ⁻¹ (Farmer practice)	145.77	11 ^a	24.18	182 ^a	22.46 ^c
100:30:30NPK kg ha ⁻¹ (RDF)	148.36	11 ^a	24.72	214 ^a	24.24 ^b
N application on the basis of LCC	146.48	11 ^a	24.37	212 ^a	24.11 ^b
N application on the basis of SPAD	146.99	11 ^a	24.61	218 ^a	24.13 ^b
N application on the basis of NDVI	148.60	11 ^a	24.79	223 ^a	25.33 ^{ab}
NE-SSNM	150.97	12 ^a	25.25	233 ^a	25.38 ^a
Mean	147.26	13.58	24.60	196.67	23.76
F test	NS	*	NS	*	*
LSD	-	-	-	87.6	1.96
CV%	2.4	8.8	1.4	2.50	2.5

LSD=Least significant difference, CV=Coefficient of variation, NS=Not significant at p<0.05, *=Significant at p<0.05, Means with same letter in column are not significantly different at p = 0.05 by DMRT

Table 5. Effect of nitrogen on phenology and yield of rice at Khumaltar condition in 2024

Treatment	Sterility%	Days to 80% heading	Days to 80% maturity	Biomass yield (t ha ⁻¹)	Yield (t ha ⁻¹)
0:30:30 NPK kg ha ⁻¹ (Control)	13.52 ^a	91	131	9.14 ^b	1.76 ^c
138:30:30 NPK kg ha ⁻¹ (Farmer practice)	12.48 ^a	92	131	13.21 ^a	4.49 ^b
100:30:30 NPK kg ha ⁻¹ (RDF)	1.69 ^b	93	132	14.04 ^a	5.51 ^a
N application on the basis of LCC	1.65 ^b	93	132	13.31 ^a	5.59 ^a
N application on the basis of SPAD	1.62 ^b	93	132	13.35 ^a	5.81 ^a
N application on the basis of NDVI	0.71 ^b	93	132	14.35 ^a	5.94 ^a
NE-SSNM	0.51 ^b	93	132	15.32 ^a	5.98 ^a
Grand mean	4.60	92.71	129.30	13.24	5.07 ^a
F test	*	Ns	Ns	*	*
LSD	1.96	-	-	2.35	0.6
CV%	23.9	1.90	0.61	10.0	6.2

LSD=Least significant difference, CV=Coefficient of variation, NS=Not significant at p<0.05, *=Significant at p<0.05, Means with same letter in column are not significantly different at p = 0.05 by DMRT

Significantly highest yield (5.98 t ha⁻¹) and biomass (15.32 t ha⁻¹) was recorded in NE-SSNM but was statically similar to recommended dose (5.91 t ha⁻¹) and lowest yield (1.76 tha⁻¹) and biomass (9.14 t ha⁻¹) in control followed by N applied based on farmer practice. Similar effect of RDF, LCC, SPAD, NDVI, NE-SSNM were recorded in terms of yield. Higher straw yield with NDVI value and NE-SSNM might be owing to favorable N influence on

vegetative growth that promoted more dry matter production resulting in significant increase in straw yield. Positive correlations were recorded among leaf N content and available soil N status at critical crop growth stages. The derived soil N status at critical growth stages (active tillering, panicle initiation and flowering stages, respectively) and the leaf N content at those stages clearly indicated that soil available N and leaf N status. Thus, optimizing these the values at active tillering, panicle initiation and flowering that could help in maintaining the desired soil available N status and leaf N content for maximizing grain yield of transplanted rice (Ghosh et al 2013).

Significant difference among treatment and over the year was observed in number of tiller per hill, no of grains per panicle (Table 6). Thousand grain weight was significantly influenced by different treatment and treatment × year effect. Highest sterility% was observed in control and farmer practice. Significant difference in yield among treatments and over year was observed. Statistically similar yield was recorded in RDF (5.47 t ha⁻¹), LCC (5.19 t ha⁻¹), SPAD (5.49t ha⁻¹), NDVI (5.70 t ha⁻¹), NE-SSNM (5.70 t ha⁻¹) and lowest in control (1.78 t ha⁻¹) followed by farmer practice (3.38 t ha⁻¹) (Table 6). These results are in line with previous studies by Sen et al. (2011), who showed that real-time nitrogen control increased rice output. Previous research findings showed that precision nitrogen management outperforms uniform blanket recommendations by better matching nitrogen supply with crop demand (Pampolino et al 2012, Bhattarai et al 2024). In N was applied at different DAT as indicated through green seeker which might have coincided exactly with crop ‘N’ demand and that might be the reason for the increase in yield over treatments with NDVI value 0.7and NDVI value 0.9. This sensor-determined topdressing of ‘N’ for rice increased the number of split applications to three as against two splits in the conventional blanket method of fertilization. The increased availability of nitrogen at distinct physiological phases would have supported for better assimilation of photosynthates towards grain and also due to the favourable effect of accelerating the yield attributes. These types are designed to take into account the diversity of space and time in the supply of nutrients and to ensure nutritional management. Similar finding has been reported by Sen et al (2011).

Table 6. Effect of nitrogen on growth and yield attributes of rice at Khumaltar condition, 2023 and 2024

Treatment	PH (cm)	NTPH (No.)	PL (cm)	NGPP	TGW (g)	Sterility (%)	D50H (%)	D50 M (%)	BY (t ha ⁻¹)	GY (t ha ⁻¹)
Control	140.3 ^b	10 ^c	24.39	9 ^c	21.36 ^c	11.33 ^b	93	131	7.63 ^c	1.78 ^c
Farmer Practice	143.1 ^{ab}	12 ^b	24.49	14 ^b	21.51 ^c	10.27 ^b	94	131	10.65 ^b	3.38 ^b
RDF	144.9 ^a	13 ^{ab}	25.12	18 ^{5ab}	24.02 ^b	1.58 ^a	93	131	13.82 ^a	5.47 ^a
LCC	143.8 ^a	13 ^{ab}	24.6	18 ^{5ab}	24.44 ^{ab}	1.41 ^a	93	131	13.83 ^a	5.19 ^a
SPAD	144.4 ^a	13 ^{ab}	24.29	18 ^{8ab}	24.51 ^{ab}	0.72 ^a	94	131	14.18 ^a	5.49 ^a
NDVI	144.9 ^a	13 ^{ab}	24.45	19 ^{4ab}	24.77 ^{ab}	0.72 ^a	93	130	17.44 ^a	5.70 ^a
NE-SSNM	146.1 ^a	14 ^a	24.79	20 ^{4a}	25.44 ^a	0.67 ^a	93	130	18.03 ^a	5.70 ^a
Grand mean	143.92	12.36	24.58	17.19	23.72	3.92	93	131	13.65	5.80 ^a
F test (T)	*	*	ns	*	*	*	Ns	Ns	*	*
F test (Y)	*	*	ns	*	Ns	*	*	*	ns	*
F test (T × Y)	Ns	Ns	ns	Ns	*	*	Ns	Ns	*	Ns
LSD (T)	3.23	1.2		44.14	1.01	1.15	2.1	1.8	3	0.55
LSD (Y)	1.76	0.64		23.6	-	0.61	1.1	1	26	0.3
LSD (T × Y)	4.7	-		62.43	1.43	1.63	2.9	2.6	2.86	
CV%	1.9	8.2	4.2	21.7	3.6	24.8	1.9	1.2	12.5	9.9

PH=Plant height, NTPH=Number of tillers per hill, PL=Panicle length, NGPP=Number of grains per panicle, TGW= Thousand grain weight. D50H= Days to 50% heading, D50M= Days to 50% maturity, BY=Biomass yield, GY= Grain yield LSD=Least significant difference, CV=Coefficient of variation, T=Treatment, Y=Year, Means with same letter in column are not significantly different at p = 0.05 by DMRT

CONCLUSION

Compared with the nationally recommended fertilizer dose, NE-SSNM and NDVI-based nitrogen management increased grain yield by 4.2%. This indicates that optimal rice productivity may not be achieved through fixed-time nitrogen applications based solely on generalized recommendations. The findings suggest that existing nitrogen fertilizer guidelines require revision to better align with crop demand and field-specific variability. Site-specific nutrient management approaches significantly enhanced nitrogen-use efficiency and yield. Overall, the study clearly demonstrates the superiority of SSNM (Nutrient Expert®) over blanket recommendations and conventional farmer practices.

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AUTHORS' CONTRIBUTION

Sangita Kaduwal: Writing manuscript, editing, analysis and conceptualization. Dr Himad Prasad Timalina, Reshama Neupane, Rajendra Kumar Bhattarai, Bhimsen Chaulagain, Prakash Ghimire: Data curation, visualization, methodology development, supervision, conceptualization. Subindra Balami: Layout preparation Swastika Giri, Mina Mahatara, Asha Rana Magar: Conduction of experiment, data collection.

CONFLICT OF INTEREST

The authors declare no competing interests relevant to the content of this article.

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