



RESEARCH ARTICLE

Role of nano urea plus and nano DAP for the improvement of nitrogen use efficiency in rice

A S Priyanka¹, M K Kalarani^{2*}, A Senthil¹, R Karthikeyan², M Umaphathi¹, M Prasanthrajan³ & D Selvi⁴

¹Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

²Directorate of Crop Management, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

³Department of Environmental Science, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

⁴Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Correspondence email - kalarani.mk@tnau.ac.in

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Abstract

Efficient nitrogen (N) management is vital for sustainable rice production, given the heavy reliance on urea and substantial environmental losses associated with its use. This study evaluated the integrated use of nano urea plus [n-UP; 20 % N (w/v)] and nano DAP [n-DAP; 8 % N (w/v) and 16 % P (w/v)] to improve growth, physiology, biochemical, yield and nitrogen use efficiency (NUE) of rice under a reduced recommended dose of nitrogen (RDN). A field experiment was conducted during rabi 2023 using the rice variety ADT 53 in a randomised block design (RBD) with 8 treatments and 3 replications. The results revealed that 75 % RDN along with foliar applications of n-UP at the active tillering (AT) stage and n-DAP at the panicle initiation (PI) stage significantly enhanced physiological traits such as photosynthetic rate ($35.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($0.568 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), F_v/F_m ratio (0.836) and biochemical traits such as soluble protein (SP) (24.1 mg g^{-1}) and nitrate reductase (NR) activity ($135.5 \mu\text{g NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$). This treatment also recorded the highest grain yield of 6.3 t ha^{-1} , grain N (0.75 %), straw N (1.44 %) and improved NUE indices, including N uptake efficiency (41.0), AE (25.9), physiological efficiency (32.1) and $\text{NUE}_{(\text{yield-based})}$ (17.7). Correlation and cluster analyses revealed strong positive associations between NUE indices and yield, demonstrating that 75 % RDN with nanofertilisers was equally effective as 100 % RDN. The study confirms that staggered foliar application of n-UP and n-DAP can achieve 25 % fertiliser savings while sustaining yield and enhancing NUE.

Keywords: nano DAP; nano urea; nitrogen management; nitrogen use efficiency; rice

Introduction

Rice is the staple food for more than half of the world's population, making it crucial for global food security (1). India is the world's largest exporter of rice, with 150 million metric tons of production from about 51 M ha^{-1} and an average productivity of 2.8 t ha^{-1} , accounting for about 28 % of global production during the 2024-2025 agricultural year (2, 3). However, this intensive production largely relies on excessive use of chemical fertilisers, particularly urea, resulting in suboptimal nitrogen use efficiency (NUE) and lower yields than those achieved by countries exceeding 5 t ha^{-1} . India consumed 14.6 Mt of nitrogen (N) fertilisers in 2024-2025, driven largely by subsidised urea use, where farmers pay only about ₹242 per 45 kg bag while the actual production/import cost is around ₹2200 per bag (4). This subsidised price structure contributes to economic inefficiency and environmental issues like soil acidification, greenhouse gas (GHG) emissions and eutrophication. In paddy fields, substantial N loss occurs through ammonia volatilisation, denitrification, leaching and runoff (5), making urea the major source of NH_3 (93.85 %) and N_2O (96.44 %) emissions in India (6). These losses emphasise the urgent need to enhance NUE for sustainable rice production.

Nitrogen management in rice includes site-specific

application, controlled-release fertilisers, deep urea placement, nitrification inhibitors and integrated nutrient and water management practices, along with advanced tools like leaf colour chart (LCC) and chlorophyll meter-based N management to synchronise N supply with crop demand and enhance NUE (7). However, these strategies still face limitations in achieving optimal efficiency and minimising environmental losses (8). In this context, nanofertilisers, which contain plant nutrients with particle sizes less than 100 nm, can enhance targeted nutrient delivery and uptake and reduce nutrient loss. Because of the unique properties of higher surface area to volume ratio, reactivity, tunable pore size, desired particle morphology and stability in the environment, nanofertilisers will be a promising and sustainable alternative option for efficient N delivery and to reduce the negative effects of conventional fertilisers (9). Currently, nano urea and nano DAP have emerged as promising alternatives to conventional fertilisers, with the potential to reduce nutrient losses and enhance nutrient use efficiency in crop production.

Numerous studies have investigated the application of nano urea and nano DAP in various crops across different regions of India, reporting that these nanofertilisers can elicit positive physiological responses and yield gains, though optimal dose reductions remain debated (10, 11). Overall, evidence suggests that

foliar application of nano urea and nano DAP can influence plant growth, physiological traits and N metabolism more effectively than conventional fertilisers in major cereals such as rice, wheat and maize (12). In rice, nano urea has been shown to stimulate early vegetative growth by increasing tiller production, leaf area, chlorophyll accumulation and photosynthetic carbon assimilation (13). In wheat, foliar application of nano urea under reduced basal N increased chlorophyll content, sugars, proline, total phenols, flavonoids and antioxidant enzyme activities, which indicates improved N metabolism. Thus, nano urea acts primarily as a NUE enhancer rather than a direct N supplier (14). Meanwhile, nano DAP enhanced root growth and P translocation to the developing panicles, which resulted in a higher seed set percentage in rice (15). Application of two foliar sprays of nano urea with 75 % recommended dose of nitrogen (RDN) reduced energy input by 8–11 %, GHG emissions by 164–416 kg CO₂-eq ha⁻¹ and increased energy use efficiency by 6–9 % across maize-wheat and pearl millet-mustard systems (16). Similarly, application of nano DAP under 75 % RDN in rainfed maize reduced GHG emissions by 24.5 % and energy consumption by 15.4 %. This integrated N management indicating its potential for sustainable production (17). Most studies indicate that a 25 % reduction in RDN, supplemented by two foliar applications of nano urea at critical growth stages, improved physiological traits and yield in cereals, whereas a 50 % reduction in RDN resulted in yield decline (18, 19). Similarly, reducing the RDP by 25 % and supplementing with foliar spray of nano DAP at 5 mL L⁻¹ applied at 20–25 and 40–45 days after sowing (DAS) significantly enhanced the yield and nutrient use efficiency in wheat (20). Recent studies suggest that achievable N savings through nanofertiliser spray might be lower, closer to 25 %, particularly in cereal crops such as maize, wheat, as well as pearl millet and mustard (16).

It is important to note that in rice cultivation, nano urea is conventionally applied at the active tillering (AT) stage of rice, a critical period for N demand (21), while nano DAP is applied later at the panicle initiation (PI) stage of rice to address P requirements essential for reproductive development (22). In this study, a sequential foliar application of nano urea plus (n-UP) at the AT stage and nano DAP (n-DAP) at the PI stage was adopted, rather than repeated application of either nanofertiliser alone. This staggered approach aims to synchronise the nutrient supply with crop demand, thereby optimising nutrient availability during key critical

growth stages of rice. Hence, the objective of this study was to evaluate the effectiveness of staggered foliar application of n-UP at AT stage and n-DAP at PI stage in improving nutrient use efficiency, physiological traits and yield of rice.

Materials and Methods

Materials

Nanofertilisers like n-UP and n-DAP were provided by Indian Farmers Fertiliser Cooperative Limited (IFFCO), New Delhi, India. Both n-UP and n-DAP are <100 nm in size (23). Seeds of the rice variety ADT 53 were collected from Tamil Nadu Rice Research Institute, Aduthurai.

Experiment and treatment details

A field experiment was conducted during the rabi 2023 at the Wetland Farms, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu (11°00' N, 76°92' E; 426.7 m above mean sea level), to study the impact of marginal reduction of conventional N application with subsequent foliar application of n-UP and n-DAP during critical growth stages of rice on morpho-physiological, biochemical, yield and NUE. The experiment was laid out in a randomised block design (RBD) with 8 treatments and 3 replications. The experimental site experienced a temperature range of 22.2–31.4 °C with a mean precipitation of 95.5 mm during the crop growing period. Before sowing, soil samples were collected and analysed for physico-chemical properties (Table 1).

The eight treatments of the experiment included T₁: 0 % RDN, T₂: 50 % RDN, T₃: 75 % RDN, T₄: 100 % RDN, T₅: 50 % RDN + 2 sprays of n-UP and T₆: 50 % RDN + spray of n-UP and n-DAP, T₇: 75 % RDN + 2 sprays of n-UP, T₈: 75 % RDN + spray of n-UP and n-DAP (Table 2). The foliar sprays were applied at the AT stage (35–40 DAS) and PI stage (45–50 DAS) of rice. The recommended dose of fertiliser (RDF) (N, P, K) for rice in this region is 150:50:50 kg ha⁻¹. The entire RDP and recommended dose of potassium (RDK) was applied to all treatments. Phosphorous was applied as single super phosphate (SSP) at basal, while N (as urea) and K (as muriate of potash, MOP) were applied in four equal splits such as basal, AT, PI and heading stages, according to treatment requirements.

Seeds were sown in the nursery at 60 kg ha⁻¹. Seedlings aged 22 days were transplanted at a spacing of 20 × 10 cm spacing, with

Table 1. Physico-chemical properties of the pre-sowing soil of experimental site

pH	Electrical conductivity (dS m ⁻¹)	Organic carbon (%)	Available nitrogen (kg ha ⁻¹)	Available phosphorus (kg ha ⁻¹)	Available potassium (kg ha ⁻¹)
7.70	0.76	0.54	224	14.2	289

Table 2. Treatment details

Treatments	Application stage			
	Seedling stage (22 DAS)	Active tillering stage (35–40 DAS)	Panicle initiation stage (45–50 DAS)	Heading stage (65–75 DAS)
T ₁ : 0 % RDN	NA	NA	NA	NA
T ₂ : 50 % RDN	12.5 % (urea)	12.5 % (urea)	12.5 % (urea)	12.5 % (urea)
T ₃ : 75 % RDN	18.75 % (urea)	18.75 % (urea)	18.75 % (urea)	18.75 % (urea)
T ₄ : 100 % RDN	25 % (urea)	25 % (urea)	25 % (urea)	25 % (urea)
T ₅ : 50 % RDN + 2 sprays of n-UP	12.5 % (urea)	12.5 % (urea) + 1250 mL of n-UP	12.5 % (urea) + 1250 mL of n-UP	12.5 % (urea)
T ₆ : 50 % RDN + sprays of n-UP and n-DAP	12.5 % (urea)	12.5 % (urea) + 1250 mL of n-UP	12.5 % (urea) + 1250 mL of n-DAP	12.5 % (urea)
T ₇ : 75 % RDN + 2 sprays of n-UP	18.75 % (urea)	18.75 % (urea) + 1250 mL of n-UP	18.75 % (urea) + 1250 mL of n-UP	18.75 % (urea)
T ₈ : 75 % RDN + sprays of n-UP and n-DAP	18.75 % (urea)	18.75 % (urea) + 1250 mL of n-UP	18.75 % (urea) + 1250 mL of n-DAP	18.75 % (urea)

two seedlings per hill. The plot size was 35 m² (7 m × 5 m). To prevent lateral movement of irrigation water and fertilisers between plots, the edges were lined with 0.15 mm-thick polyethylene sheets, extending 0.15 m above and 0.25 m below the soil surface.

Sampling and data recording

The growth, physiological and biochemical traits were observed at two stages of rice including 45 DAS (5 days after 1st spray) and 55 DAS (5 days after 2nd spray). Five plants were randomly tagged in each replication of all treatment plots for growth traits.

Estimation of morphological traits

The growth traits such as plant height (cm), root length (cm), number of tillers per plant, leaf area per plant (cm²) and total dry matter accumulation per plant (g), were recorded from tagged plants. Plant height was measured from the base of the stem at the soil surface to the tip of the topmost leaf by using a ruler, while the plants were uprooted and roots were washed to measure the root length from the base of the stem to the tip of the primary root using a ruler. The number of tillers was determined manually by counting all the tillers emerging from the base of the stem. Leaf area was measured using the LICOR leaf area meter (LI-3100C, Lincoln, Nebraska, USA). Uprooted plants were oven dried at 80 °C and allowed to attain a constant weight and then weighed to calculate total dry matter accumulation per plant.

Estimation of physiological traits

Physiological traits were measured from the fully expanded, physiologically active leaves of the tagged plants following the procedure described in the previous studies (24). The chlorophyll index (soil plant analysis development (SPAD) reading) was measured using a chlorophyll meter [SPAD; Model 502, Minolta, Japan]. The normalised difference vegetation index (NDVI) values were measured using a GreenSeeker handheld crop sensor (Trimble, Thaus Co. Ltd., Bangkok, Thailand). The photosynthetic rate (μmol CO₂ m⁻² s⁻¹), transpiration rate (mmol H₂O m⁻² s⁻¹) and stomatal conductance (mol H₂O m⁻² s⁻¹) were measured using an infrared gas analyser (CI-340 portable photosynthesis system, CID Inc., Camas, WA, USA). Maximum quantum yield of photosystem II (F_v/F_m ratio) and potential activity of photosystem II (F_o/F_m ratio) were measured using a chlorophyll fluorometer (OS30p+, Opti Sciences, Hudson, NH, USA).

Estimation of total chlorophyll content

A fresh leaf sample of 250 mg was taken and macerated with 10 mL of 80 % acetone. Centrifuged the contents at 3000 rpm for 10 min. After centrifugation, collected the supernatant and made up to the volume of 25 mL with 80 % acetone. Measured the optical density at 480, 510, 645, 652 and 663 nm by a spectrophotometer. The total chlorophyll contents were expressed as mg g⁻¹ of fresh weight (25).

Estimation of nitrate reductase activity

Fresh leaf tissue of 500 mg was incubated with 10 mL of 0.2 M phosphate buffer (pH 7.5) containing KNO₃ and isopropanol. The contents were placed in a desiccator for 5 min, followed by an hour-long incubation period in dark to allow the reaction to proceed. After the incubation period, 2 mL of the aliquot was collected, to which 1 mL of 1 M zinc acetate was added, followed by 2 mL of 70 % ethanol to terminate the reaction. The mixture was filtered through Whatman No. 1 filter paper and to the filtrate, 1 mL of 1 % sulphanilamide in 1.5 N HCl and 1 mL of 0.02 % N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) were added. The optical

density (OD) was subsequently measured at 540 nm using a spectrophotometer. Enzyme activity was expressed as μmol NO₂ g⁻¹ h⁻¹ and potassium nitrite (KNO₂) utilised as the standard (26).

Estimation of soluble protein

Soluble protein (SP) content was determined in the previous study (27). Fresh leaf samples of 250 mg were homogenised in 10 mL of 0.2 M phosphate buffer and centrifuged at 3000 rpm for 10 min. The supernatant was used to estimate protein. To 1 mL of the extract, 5 mL of alkaline copper tartrate (ACT) reagent and 0.5 mL of Folin-ciocalteu reagent were added. The mixture was incubated for 30 min in the dark for colour development. The OD was measured at 660 nm using a spectrophotometer. Bovine serum albumin (BSA) was used as a standard and the SP was expressed as mg g⁻¹ fresh weight.

Estimation of yield and yield-associated traits

At physiological maturity, five random plants per replication were used to determine yield attributing traits. The number of panicles was determined by counting the number of tillers with panicle-bearing. Panicles with spikelets were used for measuring panicle length and weight. After, the grains were separated from the panicles and grouped and counted the filled and unfilled grains. A thousand filled grains were counted and weighed to determine the 1000-grain weight. Rice plants were harvested from a 1 m² area in each replication. The grains were threshed and dried to attain a constant moisture content of 14 %. Therefore, grain weight was recorded and converted to t ha⁻¹ and same procedure was followed for straw weight.

Estimation of nitrogen content

The N content in straw and grain was estimated by the semi-micro Kjeldahl method (28). Plant samples collected at physiological maturity were initially shade-dried and subsequently oven-dried at 70 °C. One gram of oven-dried leaf and grain samples were ground to a fine powder and digested using a diacid mixture of H₂SO₄:HClO₄ (5:2 v/v). The digested contents were cooled, filtered and the volume was made up to 100 mL with distilled water. A 10 mL aliquot of the digest was transferred to the distillation chamber of a Kel Plus nitrogen analyser (DISTYL EM VA, Pelican Equipments, Chennai, India), followed by the addition of 10 mL of 40 % NaOH solution to liberate ammonia. The released ammonia was trapped in 25 mL of 2 % boric acid containing a mixed indicator (bromocresol green and methyl red) placed in an ice-cold receiving flask. The absorbed ammonia was titrated against standardised 0.01 N H₂SO₄ until the mixed-indicator end-point colour changed. A reagent blank was run simultaneously and N concentration was calculated based on the titrant volume after blank correction and expressed as a percentage.

Estimation of nitrogen use efficiency indices

Nitrogen use efficiency indices such as agronomic efficiency (AE), physiological efficiency (PE), partial factor productivity (PFP), nitrogen uptake efficiency (NUpE), nitrogen utilisation efficiency (NUE) and nitrogen use efficiency_{yield} (NUE_{yield}) were calculated as per the previous study (29).

Agronomic efficiency (AE) =

$$\frac{\text{Yield of fertilised plot} - \text{Yield of unfertilised}}{\text{Fertiliser N}}$$

Physiological efficiency (PE) =

$$\frac{\text{Yield of fertilised plot} - \text{Yield of unfertilised}}{\text{Plant N in fertilised} - \text{Plant N in unfertilised plot}}$$

Partial factor productivity (PFP) =

$$\frac{\text{Yield of fertilised plot}}{\text{Fertiliser N}}$$

Nitrogen uptake efficiency (NUpE) =

$$\frac{\text{Plant N}}{\text{Fertiliser N} + \text{Soil N}} \times 100$$

Nitrogen utilisation efficiency (NuTE) =

$$\frac{\text{Yield}}{\text{Plant N}}$$

Nitrogen use efficiency_{yield} (NUE_{yield}) = NUpE × NuTE

Statistical analysis

All the data were analysed using R statistical software (version 4.5.1). One-way analysis of variance (One-way ANOVA) was performed separately for the data collected from two stages of rice, such as 45 DAS and 55 DAS. Mean comparison was performed using Tukey's Honest Significant Difference (Tukey's HSD) test. A correlation analysis between NUE and grain yield was conducted and a simple linear regression model was developed to illustrate the relationship between NUE and yield. To understand the overall treatment response patterns, a clustered heatmap of the morpho-physiological traits was generated in RStudio to visualise similarities and differences among treatments. All graphs were prepared in GraphPad Prism (version 9.3.0), except the regression graph and the clustered heatmap, which were generated in RStudio. Results are presented as the mean of 3 replications.

Results and Discussion

Morphological traits

One-way ANOVA revealed significant differences ($p \leq 0.05$) among the treatments for all recorded morphological traits, including plant height, root length, number of tillers per plant, leaf area and biomass at both 45 and 55 DAS (Table 3). A consistent increase in growth traits was observed with rising N levels from 0 % N to 100 % N at both growth stages. Among the treatments, 75 % RDN with 2 sprays of n-UP recorded the highest for most of the growth traits including plant height (71.8 cm and 86.5 cm), number of tillers per plant (22.7 and 26.3), leaf area (894.4 cm² plant⁻¹ and 1448.7 cm² plant⁻¹) and dry matter accumulation (62.4 g plant⁻¹ and 86.7 g plant⁻¹) at both 45 and 55 DAS respectively. Notably, at 55 DAS, the n-DAP containing treatments, such as 50 % RDN with sprays of n-UP and n-DAP and 75 % RDN with sprays of n-UP and n-DAP, recorded higher root lengths of 20.6 cm and 25.5 cm respectively, compared to the 50 % RDN and 75 % RDN alone treatments. Treatments including 50 % RDN with 2 sprays of n-UP and 50 % RDN with sprays of n-UP and n-DAP recorded higher values for most of the growth traits than 75 % RDN but lower than 100 % RDN. In contrast, 0 % RDN recorded the lowest values across all measured growth traits and growth stages.

Chlorophyll and greenness

Treatments differed significantly ($p \leq 0.05$) for total chlorophyll content, chlorophyll index (SPAD values) and NDVI at both 45 and 55 DAS (Fig. 1a–c). A gradual increase in all three traits was observed with increasing N levels at both growth stages. At 55 DAS, 75 % RDN with 2 foliar sprays of n-UP exhibited the highest total chlorophyll content (1.577 mg g⁻¹), SPAD value (48.4) and NDVI (0.85), followed by 75 % RDN with foliar sprays of n-UP and n-DAP, which recorded total chlorophyll content of 1.501 mg g⁻¹, SPAD value of 47.7 and NDVI of 0.84. Treatment including 50 % RDN with 2 foliar sprays of n-UP and 50 % RDN with foliar sprays of n-UP and n-DAP showed on par values of total chlorophyll content, SPAD values and NDVI with 75 % RDN in both the growth stages. In contrast, 0 % RDN consistently recorded the lowest values for chlorophyll content (0.893 mg g⁻¹), SPAD value (30.5) and NDVI (0.60) at 55 DAS.

Table 3. Effect of nano urea plus (n-UP) and nano DAP (n-DAP) on growth traits of rice at 45 and 55 days after sowing (DAS)

Treatments	Plant height (cm)		Root length (cm)		Number of tillers (plant ⁻¹)		Leaf area (cm ² plant ⁻¹)		Dry matter accumulation (g plant ⁻¹)	
	45 DAS	55 DAS	45 DAS	55 DAS	45 DAS	55 DAS	45 DAS	55 DAS	45 DAS	55 DAS
T ₁ : 0 % RDN	49.1 ^e	65.3 ^e	10.1 ^f	13.4 ^g	11.3 ^f	12.7 ^g	582.5 ^f	845.1 ^f	29.5 ^g	44.5 ^g
T ₂ : 50 % RDN	57.6 ^d	73.4 ^d	13.5 ^e	14.2 ^f	14.7 ^e	17.0 ^f	644.3 ^e	1009.2 ^e	36.8 ^f	56.2 ^f
T ₃ : 75 % RDN	62.4 ^c	78.6 ^c	16.2 ^d	18.4 ^e	17.3 ^d	19.7 ^e	729.4 ^d	1142.9 ^d	43.3 ^e	62.5 ^e
T ₄ : 100 % RDN	65.9 ^b	82.6 ^b	18.3 ^b	21.2 ^c	19.5 ^b	23.7 ^c	832.2 ^b	1389.2 ^b	53.9 ^b	79.4 ^c
T ₅ : 50 % RDN + 2 sprays of n-UP	62.6 ^c	82.3 ^b	17.1 ^c	18.5 ^e	18.7 ^c	21.3 ^d	775.7 ^c	1224.4 ^c	45.2 ^d	69.9 ^d
T ₆ : 50 % RDN + sprays of n-UP and n-DAP	63.0 ^c	81.0 ^b	16.5 ^d	20.6 ^d	18.5 ^c	21.0 ^d	771.9 ^c	1234.5 ^c	46.9 ^c	67.9 ^d
T ₇ : 75 % RDN + 2 sprays of n-UP	71.8 ^a	86.5 ^a	21.0 ^a	22.2 ^b	22.7 ^a	26.3 ^a	894.4 ^a	1448.7 ^a	62.4 ^a	86.7 ^a
T ₈ : 75 % RDN + sprays of n-UP and n-DAP	71.3 ^a	85.3 ^a	20.5 ^a	25.5 ^a	23.2 ^a	25.3 ^b	890.9 ^a	1456.6 ^a	61.1 ^a	83.4 ^b
CD ($p \leq 0.05$)	1.79*	2.23*	0.57*	0.59*	0.61*	0.70*	22.88*	36.86*	1.64*	2.24*

Values represent the mean of 3 replications.

*Means followed by different letters indicate significant differences at $p \leq 0.05$ (Tukey's HSD test).

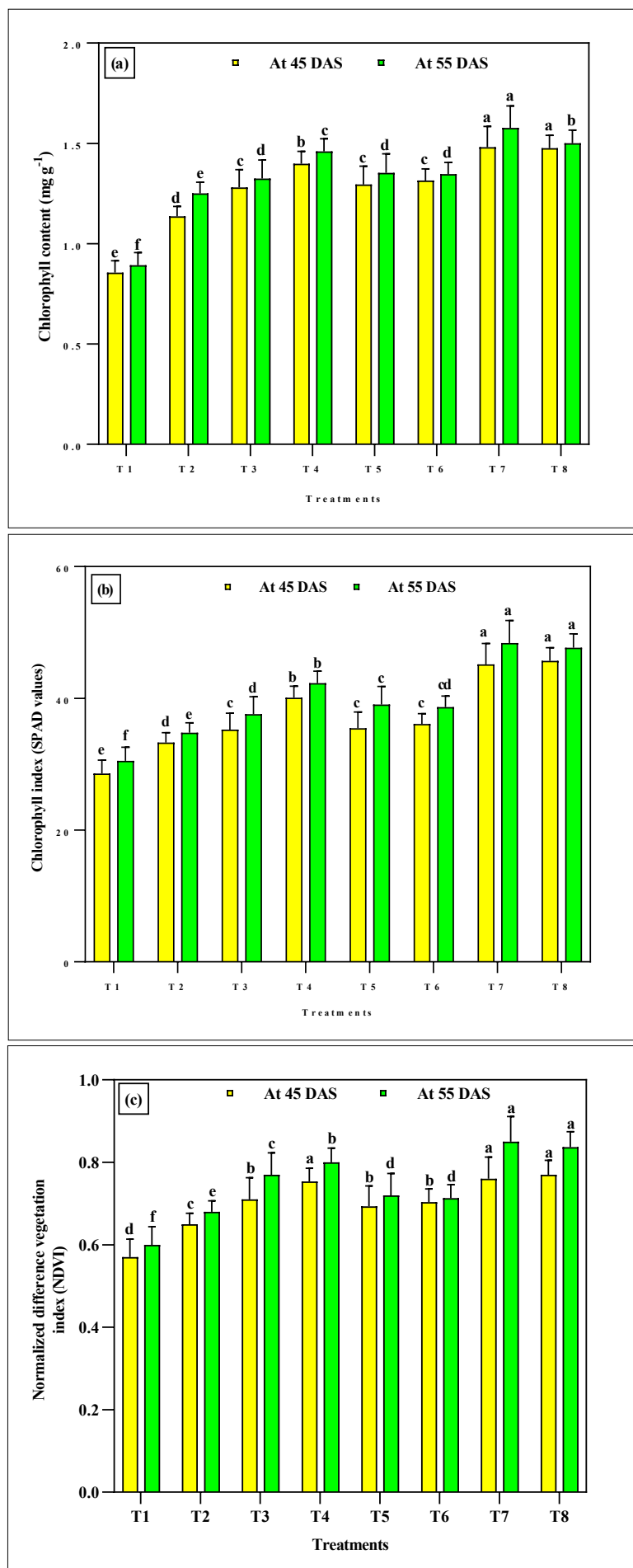


Fig. 1. Effect of nano urea plus (n-UP) and nano DAP (n-DAP) on (a) chlorophyll content (mg g⁻¹); (b) chlorophyll index (SPAD values) and (c) normalised difference vegetation index (NDVI) of rice at 45 and 55 days after sowing (DAS). Values represent mean $\pm$ SE of 3 replications and different letters indicate their relative significance at $p \leq 0.05$.

Gas exchange traits

Significant differences ($p \leq 0.05$) were observed among the treatments for photosynthetic rate, transpiration rate and stomatal conductance at both 45 and 55 DAS (Fig. 2a–c). Photosynthetic rate ranged from 12.7 and 17.0 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in 0 % RDN to a maximum of 30.2 and 35.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in 75 % RDN with foliar sprays of n-UP and n-DAP at 45 DAS and 55 DAS respectively. Similarly, transpiration rate increased from 3.3 and 3.5 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in 0 % RDN to a maximum of 5.8 and 6.4 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in 75 % RDN with foliar sprays of n-UP and n-DAP at 45 DAS and 55 DAS, respectively. Stomatal conductance also showed a similar trend, ranging from 0.114 to 0.201 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in 0 % RDN to a maximum of 0.468 to 0.568 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in 75 % RDN with foliar sprays of n-UP and n-DAP at 45 DAS and 55 DAS respectively.

Photosynthetic efficiency

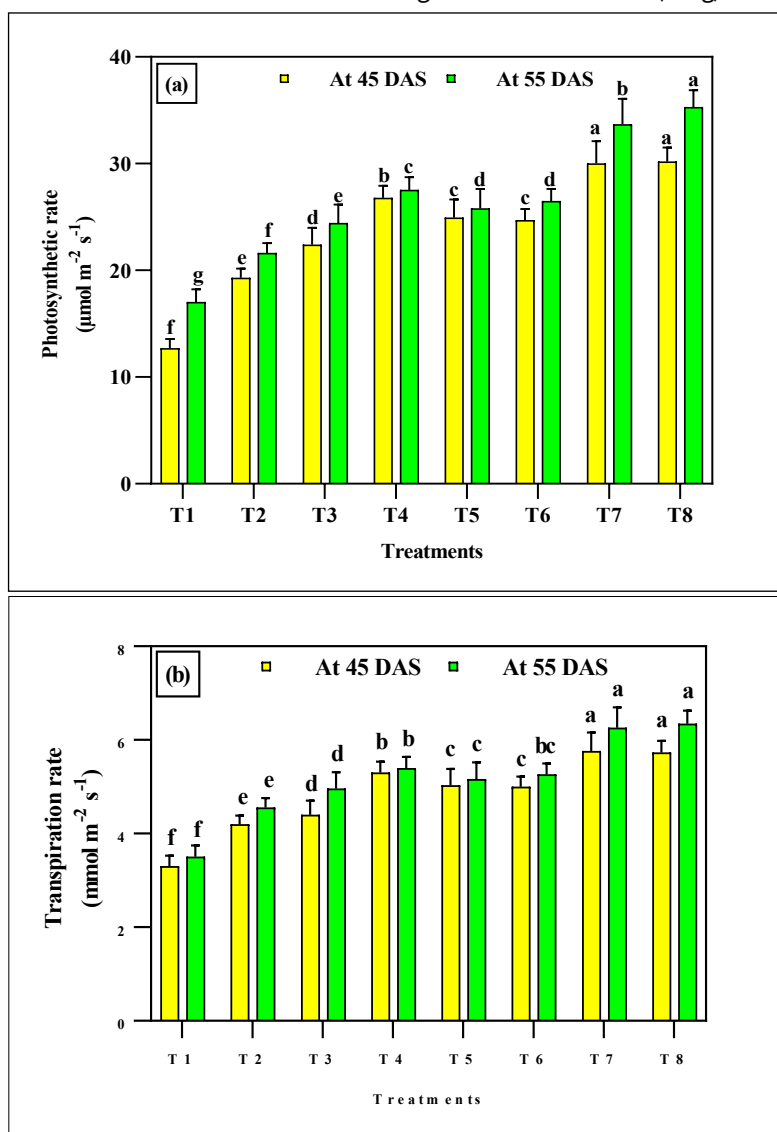
The maximum quantum yield of PS II (F_v/F_m) and the potential activity of PS II (F_v/F_o) differed significantly among treatments at both 45 and 55 DAS ($p \leq 0.05$) (Fig. 3a, b). A consistent increase in these traits was observed with higher N supply at both growth stages. At 55 DAS, the lowest F_v/F_m (0.667) and F_v/F_o (2.0) were recorded at 0 % RDN. In contrast, the highest values were observed in 75 % RDN with foliar sprays of n-UP and n-DAP, with F_v/F_m of 0.836 and F_v/F_o of 5.0, followed by 75 % RDN with 2 foliar sprays of n-UP (0.823 and 4.7), which exceeded the values recorded under 100 % RDN (0.814 and 4.3).

Soluble protein and nitrate reductase activity

Soluble protein and nitrate reductase (NR) were significantly influenced by the N supply through both conventional fertilisers and nanofertilisers at both 45 and 55 DAS ($p \leq 0.05$) (Fig. 4a, b). Both traits increased progressively with higher N supply at both growth stages. The lowest SP (13.3 and 14.9 mg g^{-1}) and NR (78.2 and 86.8 $\mu\text{g NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$) were observed in 0 % RDN at 45 and 55 DAS respectively. At both 45 DAS and 55 DAS, 75 % RDN with 2 foliar sprays of n-UP recorded the highest SP (21.3 and 24.1 mg g^{-1}) and NR (115.7 and 135.5 $\mu\text{g NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$), followed by 75 % RDN with foliar sprays of n-UP and n-DAP. Both the treatments exceeded 100 % RDN in SP (19.3 and 21.0 mg g^{-1}) and NR (104.2 and 121.2 $\mu\text{g NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$) at 45 and 55 DAS.

Yield associated traits

Significant differences were observed among the treatments with respect to yield associated traits ($p \leq 0.05$) (Table 4). All observed yield associated traits were increased gradually with N fertilisation and nanofertiliser supplementation. The longest panicles were recorded under 75 % RDN with foliar sprays of n-UP and n-DAP (25.8 cm) and 75 % RDN with 2 foliar sprays of n-UP (25.0 cm), which were significantly higher than 100 % RDN (24.3 cm), whereas the shortest panicles were observed in 0 % RDN (20.5 cm). Panicle weight followed a similar trend, with 75 % RDN with foliar sprays of n-UP and n-DAP recorded the highest panicle weight (3.5 g), significantly higher than 100 % RDN (3.3 g). The lowest panicle weight was



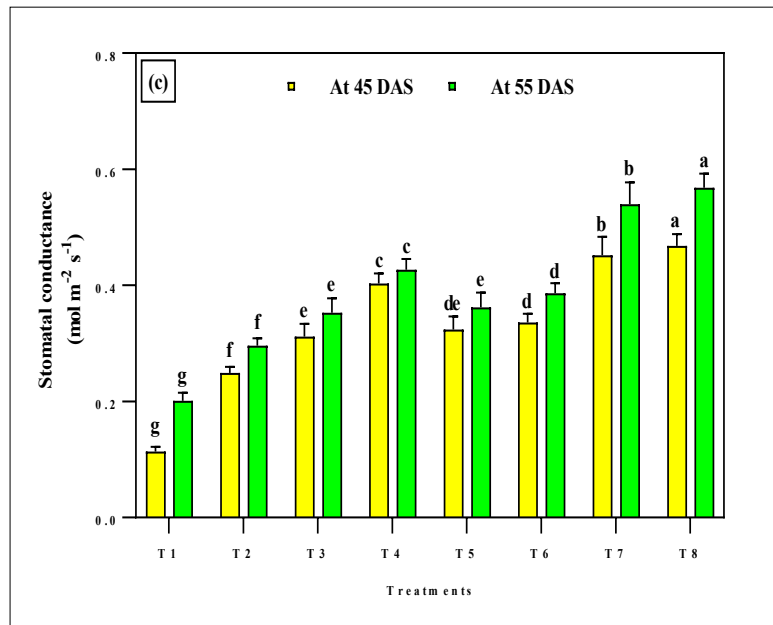


Fig. 2. Effect of nano urea plus (n-UP) and nano DAP (n-DAP) on gas exchange traits. [(a) Photosynthetic traits ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); (b) transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); (c) stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)] of rice at 45 and 55 days after sowing (DAS). Values represent mean $\pm$ SE of 3 replications and different letters indicate their relative significance at $p \leq 0.05$.

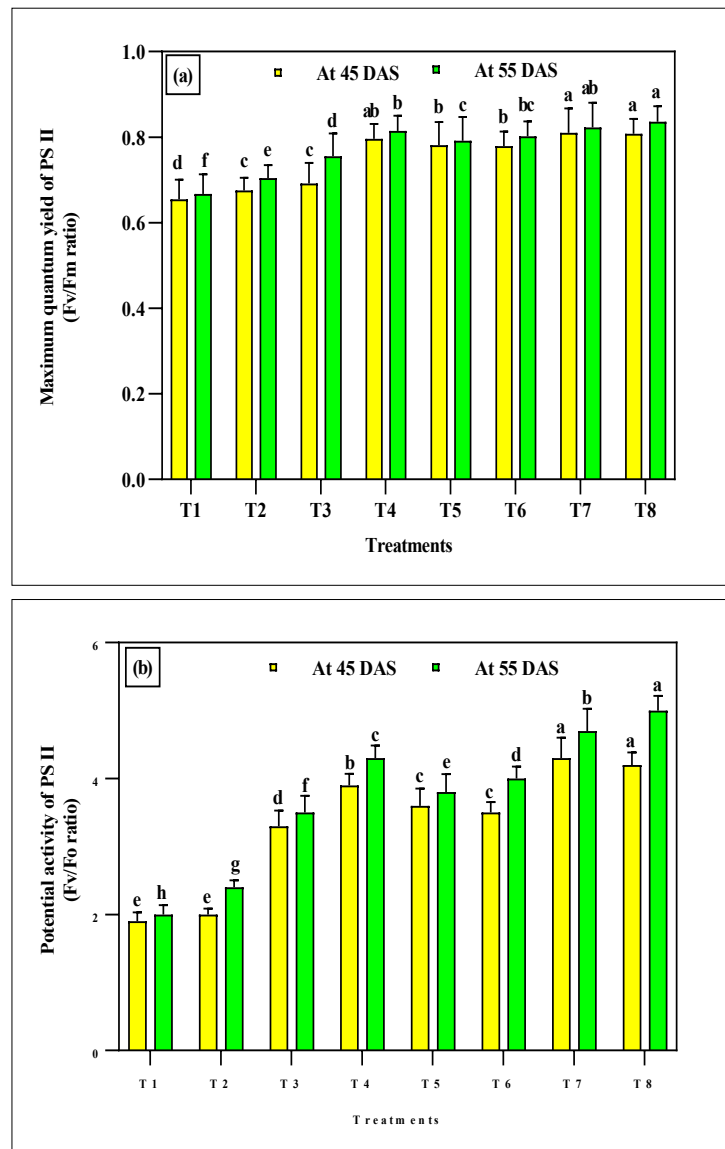


Fig. 3. Effect of nano urea plus (n-UP) and nano DAP (n-DAP) on photosynthetic efficiency. [(a) Maximum quantum yield of PS II (F_v/F_m ratio); (b) potential activity of PS II (F_v/F_o ratio)] of rice at 45 and 55 days after sowing (DAS). Values represent mean $\pm$ SE of 3 replications and different letters indicate their relative significance at $p \leq 0.05$.

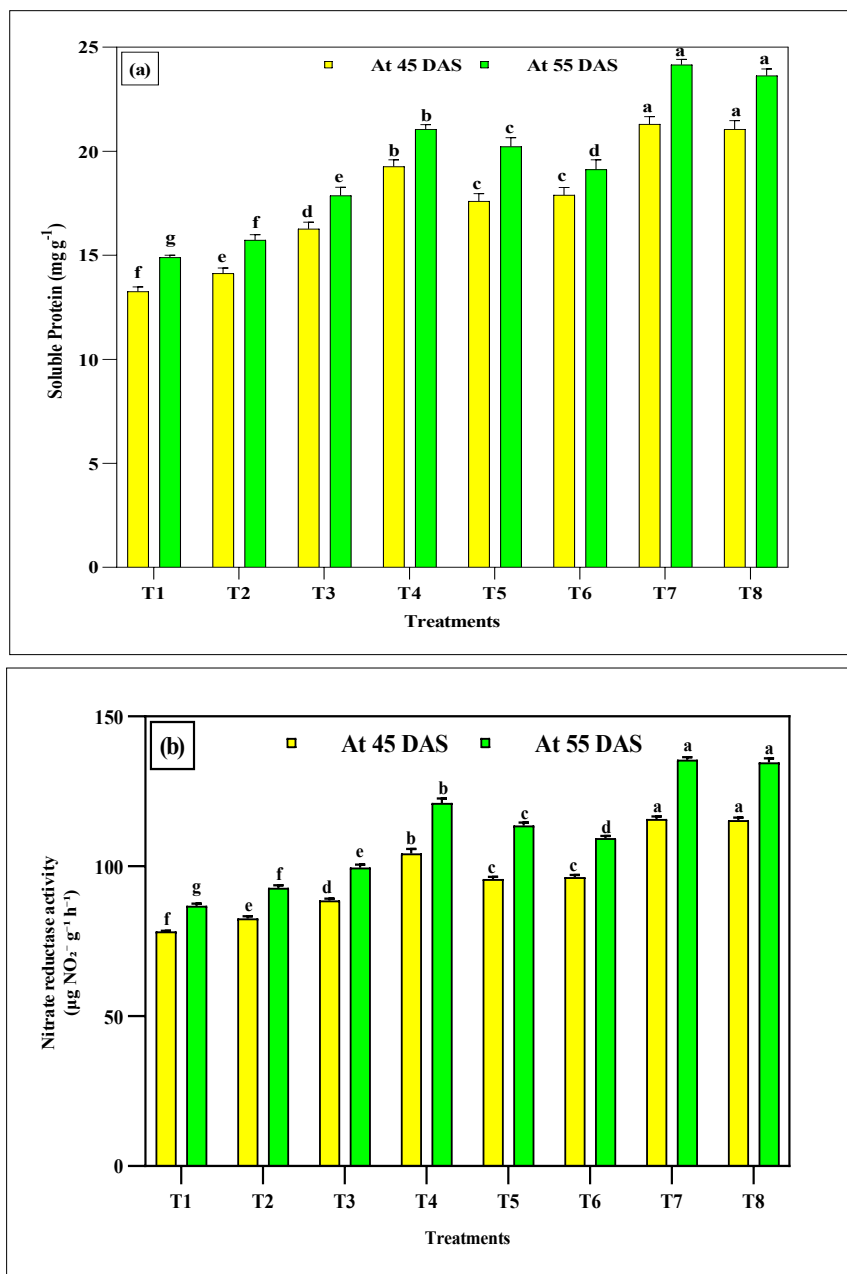


Fig. 4. Effect of nano urea plus (n-UP) and nano DAP (n-DAP) on (a) soluble protein (mg g⁻¹) and (b) nitrate reductase activity (μg NO₂⁻ g⁻¹ h⁻¹) of rice at 45 and 55 days after sowing (DAS). Values represent mean ± SE of 3 replications and different letters indicate their relative significance at $p \leq 0.05$.

Table 4. Effect of nano urea plus (n-UP) and nano DAP (n-DAP) on yield associated traits of rice

Treatments	Panicle length	Panicle weight	No. of panicle per plant	No. of filled grains per panicle	1000-seed weight
T ₁ : 0 % RDN	20.5 ^g	1.9 ^h	10.0 ^g	75.7 ^g	13.6 ^e
T ₂ : 50 % RDN	21.7 ^f	2.0 ^g	12.7 ^f	98.0 ^f	14.2 ^d
T ₃ : 75 % RDN	22.4 ^e	2.1 ^f	14.0 ^e	127.0 ^e	14.5 ^{cd}
T ₄ : 100 % RDN	24.3 ^c	3.3 ^c	22.3 ^b	204.7 ^b	15.3 ^b
T ₅ : 50 % RDN + 2 sprays of n-UP	23.6 ^d	2.8 ^e	17.0 ^d	191.0 ^d	14.6 ^c
T ₆ : 50 % RDN + sprays of n-UP and n-DAP	24.0 ^{cd}	3.0 ^d	18.7 ^c	197.0 ^c	14.8 ^c
T ₇ : 75 % RDN + 2 sprays of n-UP	25.0 ^b	3.4 ^b	24.0 ^a	209.7 ^a	15.5 ^b
T ₈ : 75 % RDN + sprays of n-UP and n-DAP	25.8 ^a	3.5 ^a	24.3 ^a	211.3 ^a	15.9 ^a
CD ($p \leq 0.05$)	0.63*	0.07*	1.30*	4.37*	0.41*

Values represent the mean of 3 replications.

*Means followed by different letters indicate significant differences at $p \leq 0.05$ (Tukey's HSD test).

recorded in 0 % RDN (1.9 g). Treatments including 75 % RDN with foliar sprays of n-UP and n-DAP and 75 % RDN with 2 foliar sprays of n-UP recorded the maximum number of panicles per plant (24.3 and 24.0 panicles plant⁻¹), significantly higher than 100 % RDN (22.3 panicles plant⁻¹). In contrast, 0 % RDN produced the least number of panicles (10.0 plant⁻¹).

Similarly, the number of filled grains per panicle was highest under 75 % RDN with foliar sprays of n-UP and n-DAP (211.3) and 75 % RDN with 2 foliar sprays of n-UP (209.7), significantly higher than 100 % RDN (204.7), while the lowest value observed in 0 % RDN (75.7). Treatments with 75 % RDN with foliar sprays of n-UP and n-DAP recorded the highest 1000-seed weight (15.9 g) than 100 % RDN (15.3 g). The lowest 1000-seed weight (13.6 g) was observed in 0 % RDN.

Yield and nitrogen content

Straw yield, grain yield and N content of rice responded significantly to different N levels and nanofertiliser supplementation ($p \leq 0.05$) (Fig. 5). Straw yield was maximised in 100 % RDN (8.3 t ha⁻¹), which was significantly higher than all other treatments, while the lowest straw yield was obtained in 0 % RDN (4.5 t ha⁻¹). The highest grain yield was observed in 75 % RDN with foliar sprays of n-UP and n-DAP (6.3 t ha⁻¹), followed by 75 % RDN with 2 foliar sprays of n-UP (6.1 t ha⁻¹), both of which were significantly higher than 100 % RDN (5.8 t ha⁻¹). In contrast, 0 % RDN produced the lowest grain yield (3.4 t ha⁻¹).

Nitrogen content in straw and grain also varied significantly among treatments. Straw N content was highest under 75 % RDN with 2 foliar sprays of n-UP (1.45 %) and 75 % RDN with foliar sprays of n-UP and n-DAP (1.44 %), both significantly higher than 100 % RDN (1.40 %). Grain N concentration followed a similar trend, with 75 % RDN with foliar sprays of n-UP and n-DAP recorded the maximum value (0.75 %), significantly higher than all treatments, including 100 % RDN (0.72 %). The 0 % RDN consistently showed

the lowest N content in both straw (0.82 %) and grain (0.61 %).

Nitrogen use efficiency indices

The spider heat map visualisation revealed that all observed NUE indices including AE, PE, PFP, NUpE, NUtE and NUE_{yield} exhibited significant variation among treatments (Fig. 6). Treatments including 75 % RDN + n-UP + n-DAP and 75 % RDN + 2 n-UP formed largest polygons with higher AE (25.9 and 23.9), PE (32.1 and 31.1), NUpE (41.0 and 39.8) and NUE_{yield} (17.7 and 17.0) compared to 100 % RDN. Intermediate polygons with higher PFP (65.8 and 64.0), NUtE (45.6 and 47.8) and moderate AE (20.8 and 19.2), PE (30.9 and 29.9), NUpE (33.2 and 32.5) and NUE_{yield} (15.1 and 15.5) were observed in 50 % RDN + n-UP + n-DAP and 50 % RDN + 2 n-UP. In contrast, 0 % RDN showed the lowest polygon with lowest values of NUE indices compared to all treatments.

Correlation of grain yield with nitrogen use efficiency indices

Significant positive correlations were found between grain yield and NUE indices such as AE, PE, PFP, NUpE and NUE_{yield} across treatments (Fig. 7). NUpE exhibited the highest positive correlation with grain yield ($R = 0.98$), followed by AE ($R = 0.88$), PE ($R = 0.83$) and NUE_{yield} ($R = 0.81$). PFP also showed a notable positive correlation ($R = 0.53$). In contrast, NUtE showed a moderate negative correlation with grain yield ($R = -0.55$).

Discussion

The present study demonstrated that staggered foliar application of n-UP at the AT stage and n-DAP at the PI stage significantly improved rice growth, physiological and biochemical traits and yield, even with a 25 % reduction in the RDN compared to conventional 100 % RDN. Additionally, foliar application of nanofertilisers along with 50 % and 75 % RDN enhanced plant growth compared to the sole application of conventional

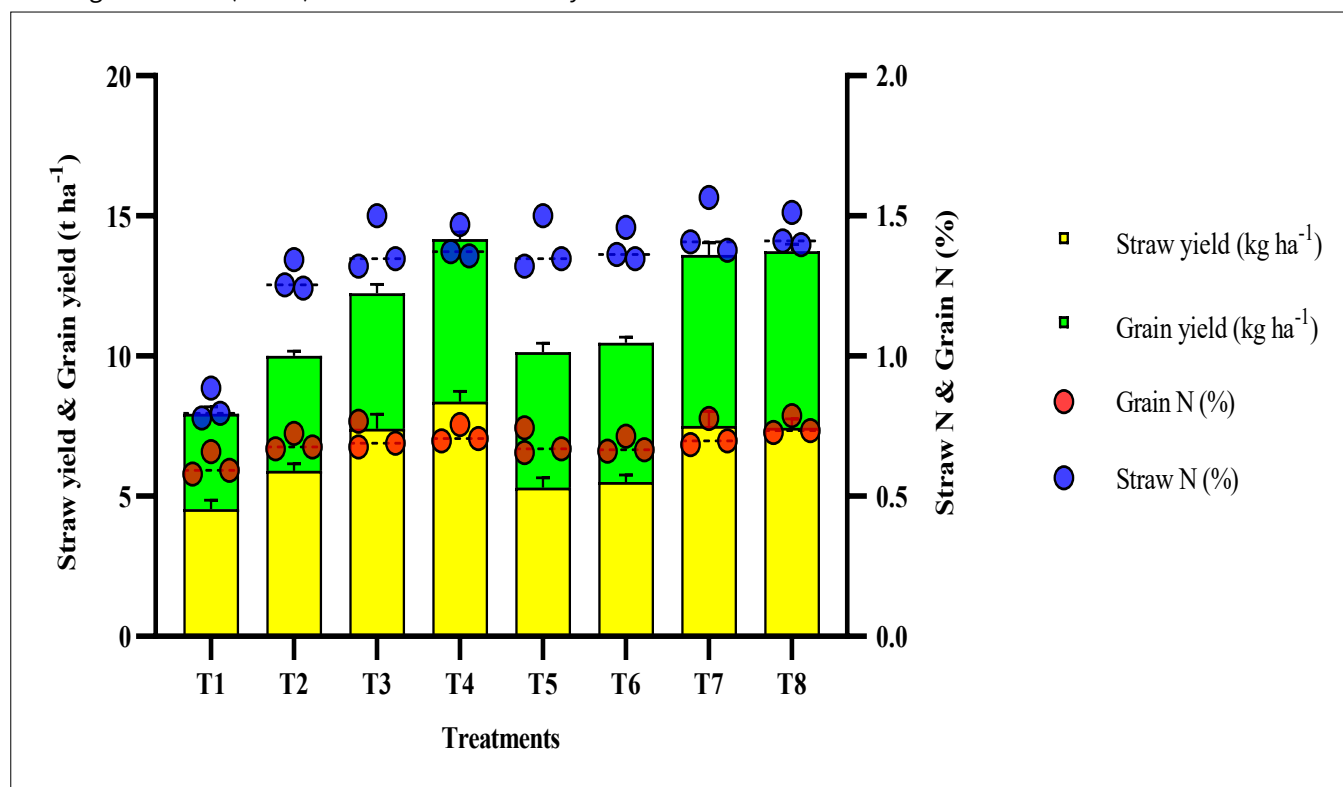


Fig. 5. Effect of nano urea plus (n-UP) and nano DAP (n-DAP) on rice straw yield, grain yield and nitrogen content of straw and grain. Values represent mean $\pm$ SE of 3 replications and significance at $p \leq 0.05$.

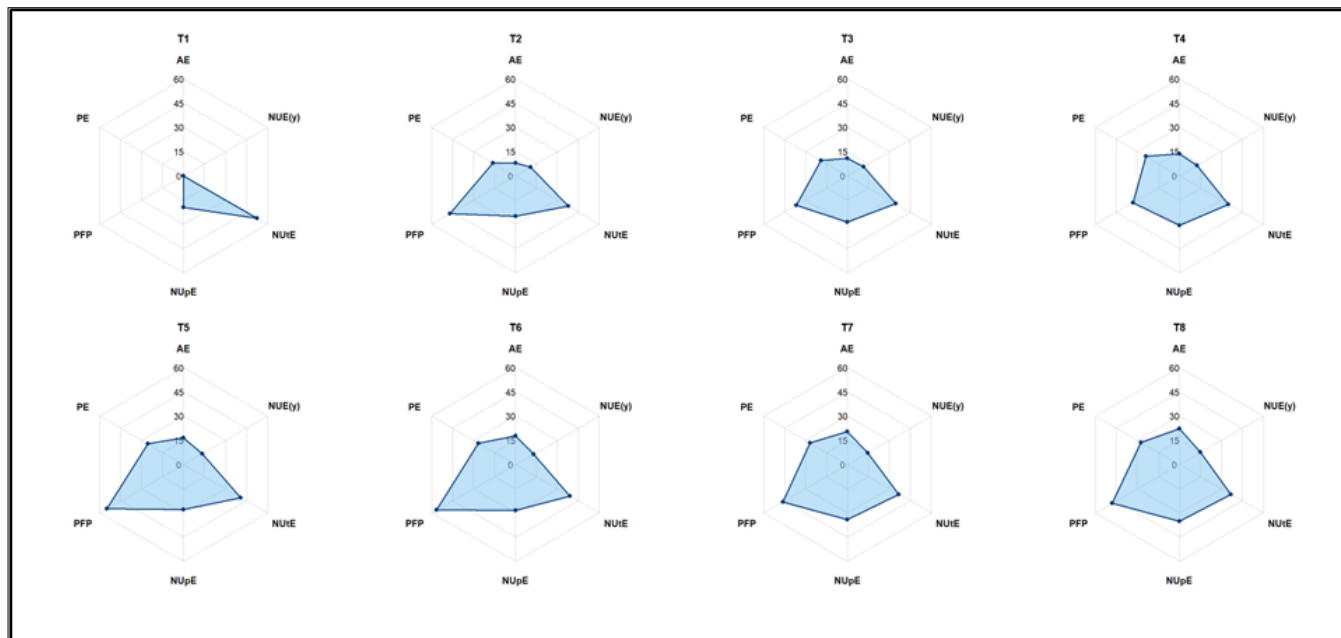


Fig. 6. Effect of nano urea plus (n-UP) and nano DAP (n-DAP) on nitrogen use efficiency (NUE) indices of rice. Values represent mean $\pm$ SE of 3 replications and significance at $p \leq 0.05$.

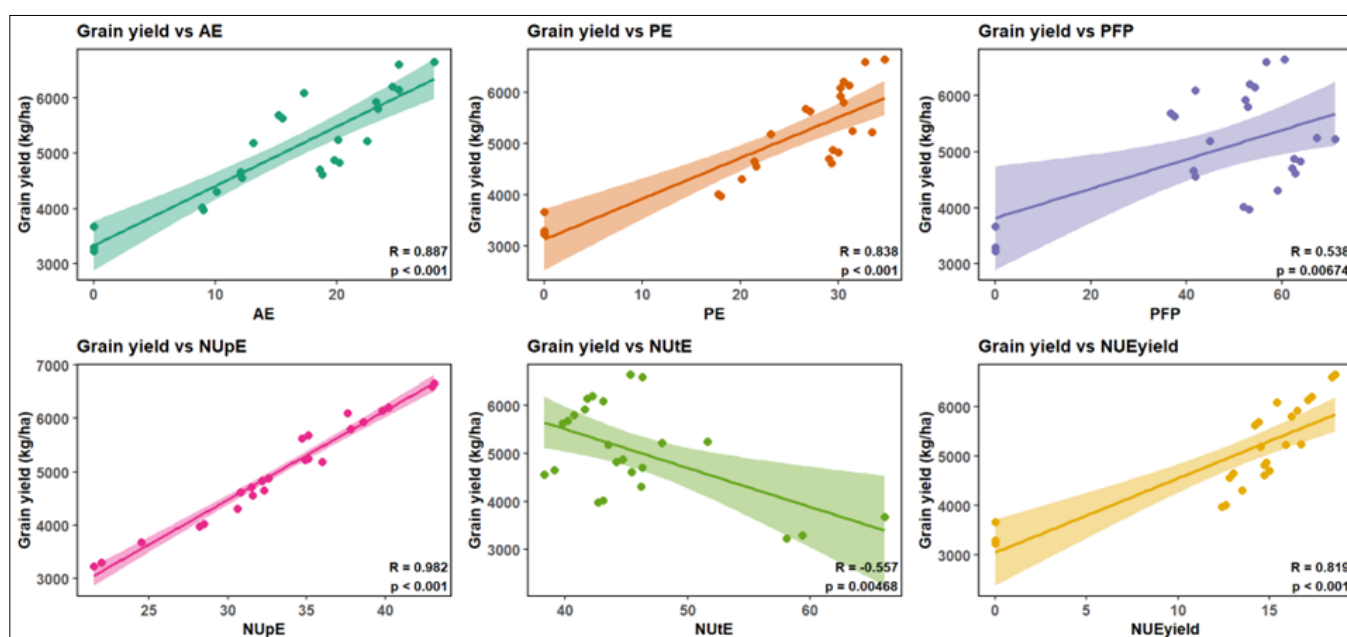


Fig. 7. Correlation between NUE and grain yield (kg ha^{-1}) of different nitrogen treatments.

fertilisers at 75 % RDN and 100 % RDN respectively.

Plant growth traits such as plant height, leaf area, tiller number and dry matter accumulation are the key indicators of crop vigour and nutrient status. These traits collectively determine canopy size, light interception and photosynthetic capacity, serving as early predictors of yield potential (30). The growth traits were higher in the treatments receiving 75 % RDN with two sprays of n-UP and 75 % RDN with sprays of n-UP and n-DAP. This indicates that nanofertiliser application effectively enhances vegetative growth. The readily available N improves protein synthesis and photosynthesis, thereby accelerating cell division and cell expansion (31). Particularly, tiller density is closely associated with grain yield. A higher number of tillers was observed under nanofertiliser-supplemented treatments. This implies better activation of basal nodes for tiller formation, which ultimately contributes to increased yield potential (32). Nano urea applications increased plant height and dry matter accumulation in rice by

providing sustained N availability and improved nutrient uptake, similar findings were reported in the previous study (19). Root growth also responded positively to nanofertilisers. Interestingly, root length was higher in the treatment containing 75 % RDN with sprays of n-UP and n-DAP. Enhanced root growth can be primarily due to adequate P supply at the PI stage through n-DAP, which stimulated root elongation, branching and overall root system architecture (33). Similarly, 75 % RDF along with foliar spray of nano urea and n-DAP showed higher root length compared to 100 % RDF in chickpea (34). In addition to P, enhanced N availability has also been reported to promote larger roots and higher biomass in rice (35).

Photosynthetic traits such as chlorophyll content, chlorophyll index and NDVI are reliable indicators of leaf N status and photosynthetic capacity, while gas-exchange traits and PSII efficiency indices reflect the functional efficiency of the photosynthetic apparatus (36). In this study, chlorophyll content,

chlorophyll index and NDVI were higher in the 75 % RDN treatment with two sprays of n-UP. Application of nano urea delivers nano-N for rapid foliar uptake and chloroplast translocation, thereby enhancing chlorophyll biosynthesis. The enhanced pigment concentration improved light interception and electron transport, indicating sustained chloroplast development and delayed senescence (37). Similarly, gas exchange and photochemical traits were maximised under the 75 % RDN treatment with sprays of n-UP and n-DAP. Nano-N from n-UP enhanced Rubisco carboxylation and stomatal conductance by providing a sustained N supply to photosynthetic enzymes (38), while n-DAP boosted ATP synthesis and PSII quantum yield (F_v/F_m) through P-mediated energy metabolism (39). Earlier, the improvements in gas exchange and photosynthetic-related traits in rice have been reported with nano-N and nano-P applications individually, indicating that their combined use could synergistically enhance CO_2 assimilation and overall photosynthetic efficiency (13, 40).

Soluble protein and NR are key indicators of the plant's N assimilation capacity. NR catalyzes the first rate-limiting step in nitrate reduction to nitrite, linking N uptake to amino acid synthesis, while SP reflects the efficiency of N incorporation into structural and enzymatic compounds. Enhanced NR and SP are therefore associated with improved NUE and photosynthesis (41). In this study, 75 % RDN + two foliar sprays of n-UP achieved the highest values of SP and NR, followed by 75 % RDN with sprays of n-UP and n-DAP. Continuous supply of readily available N from repeated nano urea application sustained enzyme activity and protein synthesis. The persistent N availability prevented feedback repression of NR and supported a steady flux through the nitrate assimilation pathway (42). Further, n-DAP supplementation enhanced the plant's energy metabolism, as P is crucial for ATP and NADPH production that supports N reduction and subsequent amino acid and protein synthesis (43). Thus, the synergistic N and P supply may upregulate NR gene expression and stabilise NR protein activity. The coupling of nutrient delivery with enzyme activation is recognised as a key mechanism through which nanofertilisers enhance metabolic fluxes (44).

Yield, yield-associated components and N content collectively represent the efficiency of assimilate partitioning, N remobilisation and source-sink regulation (45). In the present study, the combined nanofertiliser treatment (75 % RDN with sprays of n-UP and n-DAP) recorded the highest grain yield (6.3 t ha^{-1}), outperforming the conventional 100 % RDN treatment by approximately 9 %, while the 75 % RDN with 2 sprays of n-UP treatment achieved a 5 % yield advantage. In contrast, both the combinations of 50 % RDN with nanofertilisers resulted in 14–16 % yield reductions, underscoring that an adequate conventional N supply is prerequisite for realising the synergistic benefits of nano formulations. The superior performance of the 75 % RDN with foliar sprays of n-UP and n-DAP treatment can be attributed to spatiotemporally synchronised nutrient availability that aligned with key developmental stages. N supplied as n-UP during the AT stage promoted the survival of productive tiller and spikelet initiation (18), whereas P applied through n-DAP at the PI stage supported energy metabolism and panicle differentiation, thereby facilitating improved pollen viability and grain filling at later stages (15). This combination of 75 % RDN with foliar sprays of n-UP and n-DAP synchronised nutrient supply with developmental demand. Nano-N and nano-P

supplementation at 75 % of the recommended dose effectively sustained grain yield and NUE compared to 100 % RDN, consistent with previous findings (20, 46). In contrast, 50 % nano combinations significantly reduced yield, underscoring the necessity of adequate conventional N for achieving synergistic nano nutrient benefits (11).

The increased N content in both grain and straw under nanofertiliser supplemented treatments indicates improved N uptake and translocation efficiency, reflecting better nutrient assimilation and remobilisation. Foliar nano urea ensures direct N delivery to photosynthetically active tissues, reducing loss pathways such as volatilisation and leaching, while n-DAP enhances root nutrient foraging and ATP production needed for N transport and reduction (46). These processes collectively contribute to higher grain N content, a vital indicator of both grain quality and NUE. Nitrogen use efficiency indices provide critical insights into how effectively plants acquire, assimilate and utilise N to support growth and yield. In the present study, nanofertiliser supplemented treatments, particularly 75 % RDN with sprays of n-UP and n-DAP and 75 % RDN with two sprays of n-UP, recorded the highest agronomic efficiency (AE), physiological efficiency (PE) and nitrogen uptake efficiency (NUpE), compared to conventional 100 % RDN. The improved NUE indices under these treatments indicate that staggered foliar nano nutrient application enhanced both N acquisition and its conversion into grain biomass, likely by reducing unproductive N losses and sustaining metabolic activity (13, 16). Treatments such as 50 % RDN with nanofertiliser supplemented treatments recorded moderately efficient values, which suggest that foliar application of nanofertilisers partially compensates for lower soil-applied N by improving uptake and utilisation efficiency. Correlation analysis revealed strong positive relationships between grain yield and NUE indices, with NUpE ($R = 0.98$) emerging as the most influential determinant of yield, followed by AE, PE and NUE_{yield} . This demonstrates that efficient N uptake from reduced fertiliser input was the key driver of yield improvement under nano urea and n-DAP integration, consistent with previous findings (6, 9). The negative association between NUpE and yield suggests that treatments achieving higher yield prioritise N uptake and allocation rather than maximising utilisation efficiency per unit N absorbed.

Hierarchical clustering analysis was carried out using the UPGMA linkage method and visualised through a heatmap to group the treatments based on their morpho-physiological and biochemical responses (Fig. 8). Treatments with similar morpho-physiological and biochemical responses to different levels of N supply were grouped together within the same clusters. Eight treatments were grouped into three distinct clusters. Eight treatments were grouped into three distinct clusters. Cluster I (0 % RDN, 50 % RDN) exhibited strongly negative z-scores across all the observed morpho-physiological and biochemical traits, reflecting a low N condition. Low N is known to limit chlorophyll biosynthesis, reduce PSII efficiency, restrict leaf expansion and suppress nitrate assimilation-ultimately lowering carbon fixation and overall biomass accumulation. The reduced NR and SP in this cluster confirm impaired N assimilation capacity, a key determinant of NUE. Thus, Cluster I reflects poor morpho-physiological functioning and low NUE. Cluster II (75 % RDN, 50 % RDN + 2 sprays of n-UP, 50 % RDN + sprays of n-UP and n-DAP) displayed moderate, near-zero z-scores for most traits, indicating partial N sufficiency, leading to moderate NUE. In contrast, treatments comprising 100 % RDN, 75

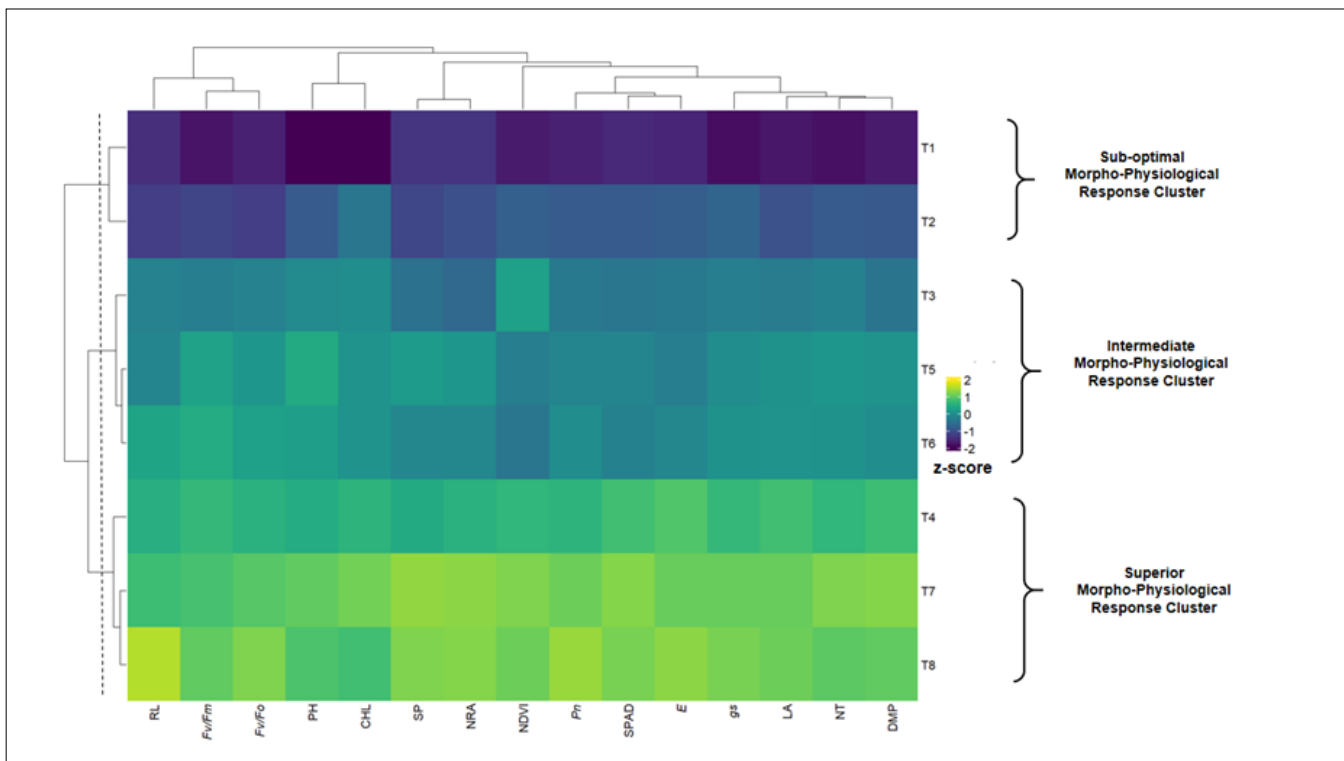


Fig. 8. Clustered heat map of different nitrogen treatments based on morpho-physiological and biochemical traits observed at 55 DAS.

% RDN + 2 n-UP and 75 % RDN + n-UP + n-DAP were grouped under Cluster III, which exhibited the highest positive standardised values (z-scores) for key traits such as NR, photosynthetic rate, leaf area and SP. This association reinforces the strong interrelationship between N assimilation, photosynthetic efficiency and NUE-driven yield response. The higher expression of these traits confirms that nano urea and n-DAP supplementation, particularly under 75 % RDN, substantially enhances physiological N utilisation, thereby improving NUE and elevating yield potential.

Overall, the staggered foliar application of n-UP at the AT stage and n-DAP at the PI stage with 75 % RDN synchronised nutrient supply with rice demand, enhancing photosynthetic activity, N assimilation and yield components while saving 25 % of conventional N fertiliser. This integrated nano-nutrient strategy represents a promising sustainable approach to mitigate N losses and enhance NUE in irrigated rice systems.

Conclusion

The present study demonstrates that staggered foliar application of n-UP at the AT stage and n-DAP at the PI stage with 75 % RDN significantly improves yield, photosynthetic rate, nitrate reductase activity, SP content and NUE indices of rice. This approach enables a 25 % reduction in RDN without compromising yield. Synchronising nutrient supply with crop demand not only promotes plant growth but also enhances soil equilibrium and fosters synergetic effects for crop production. Nevertheless, there is a pressing need for comprehensive, quantitative research on the key aspects of nanofertiliser use, such as the long-term efficacy, energy and resource consumption and ecological impacts, to ensure their stability and sustainability. Future research should emphasise ¹⁵N isotopic tracing to quantify nano-nutrient uptake and translocation, while multi-omic approaches can elucidate N assimilation and signalling pathways. Additionally, long-term multi-location trials are needed to validate yield and NUE stability and life-cycle

assessments and closed-chamber GHG flux measurements should be conducted to assess energy and carbon footprints. Detailed profiling of grain and straw will further establish dose-response relationships, supporting evidence-based recommendations for sustainable nanofertiliser adoption.

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Authors' contributions

ASP contributed to software, data curation and writing-original draft preparation. MKK and AS contributed to conceptualization. AS and MU contributed to methodology. MKK, AS, RK, MP and DS contributed to validation. RK, MP and DS conducted the investigation. ASP and MU contributed to data curation. MKK, AS, MU, RK, MP and DS contributed to writing-review and editing and MKK supervised the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors declare that they have no conflict of interest.

Ethical issues: None

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