PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.9821

eISSN 2348-1900

RESEARCH ARTICLE

Evaluating the efficacy of nano-NPK liquid fertilizer on rice: Impact on physiology, grain quality and soil microbial communities

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Received: 04 June 2025; Accepted: 16 July 2025 ; Available online: Version 1.0: 29 August 2025

Cite this article: Asha SG, Manivannan V, Parasuraman P, Kannan P, Thirukumaran K, Sivakumar R, Sivakumar U. Evaluating the efficacy of nano-NPK liquid fertilizer on rice: Impact on physiology, grain quality and soil microbial communities. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.9821

Abstract

Nano-fertilizers offer a sustainable strategy to improve nutrient-use efficiency while reducing reliance on conventional fertilizers. This study investigates the efficacy of foliar-application of nano-NPK liquid fertilizer in rice. The effects of fertilizers were examined through changes in physiological responses, grain quality and soil microbial communities. A field experiment was conducted during the *Rabi* 2023-24 and *Kharif* 2024 seasons with nine treatments in which different concentrations (50, 75 and 100 %) of Recommended Dose of Fertilizers (RDF) were used along with one or two foliar sprays of 1 % nano-NPK at the tillering and panicle initiation stages. Key parameters assessed included chlorophyll content, NDVI, amylose, crude protein content and microbial populations. Results indicated that the application of 100 % RDF, combined with a foliar spray of 1 % nano-NPK at the tillering stage, significantly enhanced physiological traits and grain quality. This performance was statistically comparable to 100 % RDF alone and 75 % RDF with two nano-NPK sprays at tillering and panicle initiation stages. Notably, nano-NPK foliar spray without basal NPK led to substantial increases in soil microbial populations. These findings suggest that reduction (about 25 %) in conventional NPK inputs can be maintained through supplementation of nano-fertilizers. This was evident through rice productivity and improved soil health via enhanced nutrient-use efficiency. The findings suggest that nano-fertilizers can act as a sustainable alternative to conventional fertilizers and help in coping up seasonal variability in rice production.

Keywords : chlorophyll; foliar spray; grain quality; nano-NPK; soil health; sustainability

Introduction

Rice (*Oryza sativa* L.) is one of the most widely consumed cereals globally, serving as a staple food for a significant portion of the population, particularly in Asia. Alongside wheat, it plays a vital role in ensuring food security across diverse regions (1). Rice is a good source of carbohydrates and also provides sufficient amounts of dietary fiber, vitamins and essential minerals which are vital for nutritional security (2). In India, rice is crucial for food security. According to Ministry of Agriculture and Farmers Welfare, India's rice production is estimated to be around 119.93 million tonnes in 2024 with an average yield of 2.74 t ha^{-1}. However, the need of growing population can be met if the production reaches 130 million tonnes by 2030 for which the significant improvement in rice productivity is required (3).

Since the amount of cultivable land is limited, the agricultural productivity and yield per unit area of the land needs to be increased to meet the future demand. This goal can be achieved via the adoption of advanced agronomic practices and efficient input technologies (4). Fertilizers play an important role in modern agriculture. This is because they directly influence both crop yield and grain quality (5). In the fiscal year 2022-23, fertilizer consumption in India reached 20.21 million tonnes for nitrogen (N), 7.92 million tonnes for phosphorus (P_2O_5) and 1.72 million tonnes for potassium (K_2O) with a total of 29.84 million tonnes for NPK nutrients. The imbalanced application of these nutrients is a major concern. In many regions, farmers apply fertilizers in imbalanced ratios such as 10.9:4:4 (N:P:K), deviating from locally recommended norms like 4:2:1, which are based on general crop nutrient requirements. This imbalance often arises from misconceptions about nutrient functions and crop responses, leading to inefficient nutrient absorption and a decline in soil health (6). Nutrient-use efficiency in Indian agriculture remains critically low, only 30-35 % for nitrogen, 18-20 % for phosphorus and 35-40 % for potassium. These inefficiencies lead to significant nutrient losses through leaching and

volatilization, ultimately reducing crop productivity and contributing to environmental degradation. Therefore, innovative strategies are urgently needed to improve nutrient uptake while minimizing losses and ecological impact (7).

Nanotechnology offers an innovative and promising solution for improving nutrient management in agriculture by utilizing materials at the nanoscale, typically less than 100 nm in size (8). Nano-fertilizers are characterized by their high surface-area-to-volume ratio, which enhances nutrient solubility, controlled release and increases their availability to crops. Their extremely small particle size allows them to penetrate plants through stomatal openings and trichome bases, particularly when applied as a foliar spray (9). This targeted delivery system improves nutrient uptake efficiency, allowing plants to exhibit favorable responses even at lower application rates compared to conventional fertilizers (10). In addition to potentially enhancing nutrient absorption, nano-fertilizers may reduce nutrient losses through leaching and minimize fixation in the soil due to their controlled release properties; however, long-term field studies are still needed to confirm these effects under real-world conditions. Their unique physicochemical characteristics can also stimulate metabolic activity and improve physiological responses. From an economic perspective, nano-fertilizers may offer cost advantages due to lower application rates; however, factors such as production costs, scalability and potential ecological risks like nanoparticle toxicity must be carefully considered. Furthermore, foliar-applied nano fertilizer has shown to reduce disease incidence while enhancing both crop yield and grain quality (11). Therefore, nano-fertilizers provide a sustainable and efficient alternative to traditional fertilization practices in modern agriculture. Although individual nano-formulations of nitrogen, phosphorus and potassium have been widely studied, integrated nano-NPK formulations remain relatively underexplored in field-level applications.

The novelty of this study lies in optimizing the rate, method and timing of integrated nano-NPK liquid fertilizer to enhance rice productivity. The present study was conducted to investigate the effects of nano-NPK foliar application, in combination with reduced conventional NPK inputs, on physiological traits, grain quality and soil microbial dynamics across different seasonal conditions. By adopting a system-level approach, this study contributes to the understanding of plant-soil-nutrient interactions under nano-fertilization and offers insights for sustainable and efficient rice production.

Materials and Methods

Experimental site

The field experiment was conducted at M4, Wetland Farms, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, during the *Rabi* 2023-24 and *Kharif* 2024 seasons. The experimental site was located at 11°01' N latitude, 76°92' E longitude and 426.7 m above mean sea level (Fig. 1). The soil of the experimental field was classified as clay loam with a pH range of 8.20 and 8.07; and EC was 0.35 and 0.31 dsm^{-1}. The soil nutrient status showed available nitrogen levels of 174 and 186 kg ha^{-1}, available phosphorus levels of 31.2 and 33.9 kg ha^{-1} and available potassium levels of 448 and 475 kg ha^{-1} during the two respective seasons.

Treatments

The treatments were as follows: T_1-100 % RDF (150:50:50 NPK kg ha^{-1}); T_2-100 % RDF + foliar spray of nano-NPK liquid at 1 % during panicle initiation stage; T_3-75 % RDF; T_4-75 % RDF + foliar spray of nano-NPK liquid at 1 % during tillering and panicle initiation stages; T_5-75 % RDF + foliar spray of nano-NPK liquid at 1 % during panicle initiation stage; T_6- 50 % RDF; T_7-50 % RDF + foliar spray of nano-NPK liquid at 1 % during tillering and panicle initiation stages; T_8-No NPK + foliar spray of nano-NPK liquid at 1 % during tillering and panicle initiation stages; T_9-absolute control.

The plot size was 7 x 5 m. Rice (var. ADT 53) seeds were sown at a seed rate of 20 kg ha^{-1} with a spacing of 20 cm x 10 cm. Nitrogen and potassium were applied in three equal splits (before transplanting, active tillering and panicle initiation stages) as per the treatment schedule. Phosphorus was applied entirely at before transplanting. Nano-NPK liquid fertilizer was applied as a foliar spray at 10 mL L^{-1} of water during tillering and panicle initiation stages as per treatment using a battery-operated sprayer with a 10 L tank capacity.

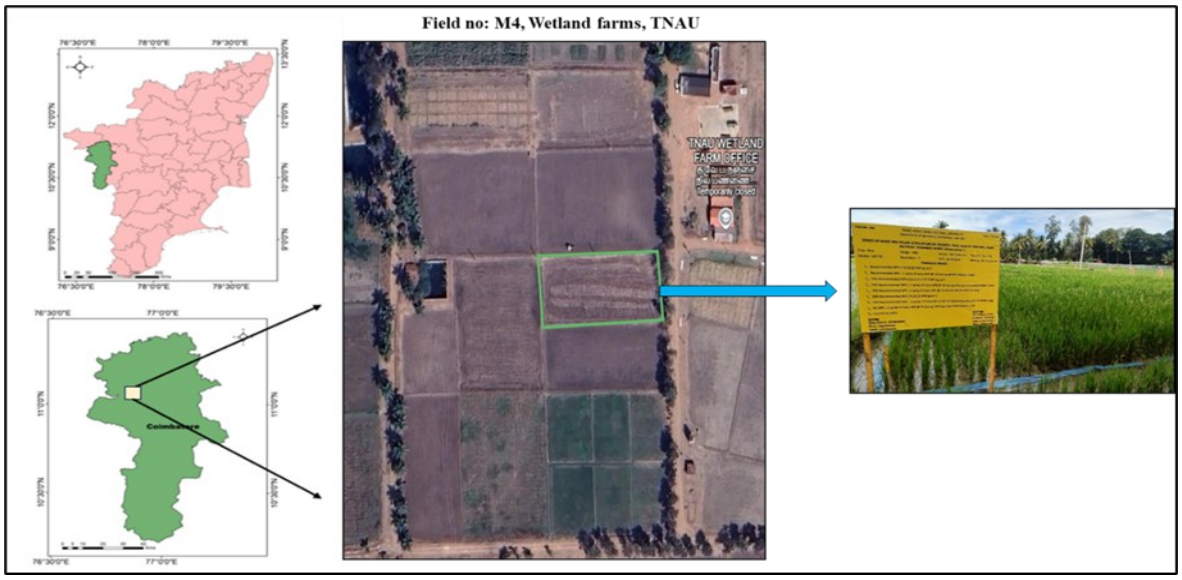

Fig. 1. Location of the experimental field of rice.

Photosynthetic pigment analysis

Chlorophyll a, chlorophyll b and total chlorophyll were extracted using 80 % acetone and measured at three stages: 20, 40, 60 DAT and at harvest stage. Optical Density (OD) values were recorded using a spectrophotometer (Microprocessor UV Single Beam) and expressed in mg g^{-1} of fresh weight. The values of chlorophyll a, b and total chlorophyll content were calculated by using the following formula and mentioned in mg g^{-1} fresh weight (12).

$$\text{Chlorophyll a} = (12.7 \times OD \text{ at } 663) - (2.69 \times OD \text{ at } 645) \times \frac{V}{1000 \times W} \quad \text{(Eqn. 1)}$$

$$\text{Chlorophyll b} = (22.9 \times OD \text{ at } 645) - (4.68 \times OD \text{ at } 663) \times \frac{V}{1000 \times W} \quad \text{(Eqn. 2)}$$

$$\text{Total chlorophyll} = (8.02 \times OD \text{ at } 663) - (20.2 \times OD \text{ at } 645) \times \frac{V}{1000 \times W} \quad \text{(Eqn. 3)}$$

Where,

OD - Optical density

V - Final volume of chlorophyll extract in 80 % acetone

W - Weight of the leaf sample taken in gram

NDVI

The GreenSeeker™ handheld sensor operates on the principle of measuring the reflectance of light from plant surfaces in both the Visible (VIS) and Near-Infrared (NIR) regions of the electromagnetic spectrum to evaluate vegetation health. It calculates the Normalized Difference Vegetation Index (NDVI), which is a widely used indicator of crop vigor and photosynthetic activity. To measure NDVI, the GreenSeeker™ sensor was positioned 60 cm above the crop canopy and readings were taken at 20, 40 and 60 DAT from various locations within each plot.

Yield

The crop harvested manually from the net plot of each treatment was threshed and cleaned separately. The grain weight was expressed in kg ha^{-1}.

Amylose content

The amylose content was estimated using a previously described simplified method (13). A 0.1 g sample of milled rice flour was mixed with 1 mL of 95 % ethanol and 9 mL of 1 mol NaOH in a 100 mL volumetric flask and left overnight. The volume was then made up to 100 mL. For analysis, 5 mL of this solution was treated with 1 mL of 1 M acetic acid and 2 mL of iodine-potassium iodide solution. The solution was diluted to 100 mL. The absorbance was read at 620 nm using a spectrophotometer. A standard curve was prepared using known concentrations of potato amylose.

Crude protein content

Grain samples were oven-dried, ground and digested using concentrated sulfuric acid mixed with a catalyst mixture of potassium sulfate and copper sulfate. After digestion, the samples were distilled with 0.1 N sodium hydroxide and the released ammonia was captured in 0.1 N boric acid containing a mixed indicator. The distillate was then titrated against standardized 0.1 N sulfuric acid to determine the nitrogen content. The total nitrogen content was estimated using the Kjeldahl method, following the procedure of Humphries (14). The nitrogen value obtained was multiplied by 6.25 to calculate the crude protein content in the grain.

Soil microbial population

The dynamics of soil microbial populations were studied in an experimental plot at 40, 60 and 90 DAT using the serial dilution plate count method. 1 g of soil was measured and thoroughly mixed with 10 mL of sterile water to create a 10^{-1} dilution. Subsequent dilutions were prepared up to 10^{-6} by pipetting 1 mL of each suspension into 9 mL of sterilized water blanks. Nutrient agar, rose bengal and Kenknight's agar were used as culture media for bacteria, fungi and actinomycetes, respectively. These media were melted, cooled and then added to sterile petri plates using the pour plate method. Appropriate dilutions were made. The petri plates were incubated at 30 °C for 2 days for bacteria, 4 days for fungi and 7 days for actinomycetes. The viable count of soil microorganisms was calculated and expressed as Colony Forming Units (CFU) per gram of soil.

$$\text{No. of colony units (CFU)} = \frac{\text{No. of colonies}}{\text{Quantity of soil sample taken on dry weight basis}} \times \text{Dilution factor} \quad \text{(Eqn. 4)}$$

Data collection and statistical analysis

Data collected from field experiments and laboratory analyses were statistically analyzed. The significant differences among the 9 treatments was analyzed using R software version 4.5.0 (R Studio 2024.12.1 + 563). The significant differences were analyzed at 5 % level. The treatments not showing significance at this probability level were denoted as Non-Significant (NS). For multivariate visualization, a heatmap was generated using the “ggplot2” and “metan” packages in R. This enabled the graphical representation of treatment-wise variation across physiological, biochemical and microbial parameters.

Results

Physiological parameters

The 100 % RDF along with foliar spray of nano-NPK at 1 % during the panicle initiation stage (T_2) recorded higher the chlorophyll a, chlorophyll b and total chlorophyll content in rice at all the stages of plant growth i.e. before spray, active tillering, panicle initiation and harvest stage (Table 1-3). This treatment was statistically comparable to RDF alone (T_1) during tillering and panicle initiation stages (T_4). In contrast, the control plot (T_9), recorded the lowest values for chlorophyll content during the tillering and panicle initiation stages of rice in both *Rabi* 2023 and *Kharif* 2024 seasons. Compared to 100 % RDF (T_1), total chlorophyll content in T_2 increased by 3.2 %. The chlorophyll content in rice progressively increased from before spray (20 DAT) to the panicle initiation stage (60 DAT), followed by a gradual decline observed at the harvest stage.

Table 1. Impact of nano-NPK foliar application on chlorophyll a (mg g^{-1}) content in rice

Treatments	*Rabi* 2023-24				*Kharif* 2024			
	Before spray (20 DAT)	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At harvest stage	Before spray (20 DAT)	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At harvest stage
T_1 - Recommended NPK (150:50:50 NPK kg ha^{-1})	0.75	1.09	1.51	0.34	0.73	1.10	1.27	0.21
T_2 - RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	0.77	1.19	1.59	0.37	0.75	1.15	1.34	0.23
T_3 - 75 % RDF NPK	0.67	0.66	1.08	0.23	0.68	0.79	0.98	0.16
T_4 - 75 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.63	1.15	1.54	0.35	0.66	1.11	1.31	0.22
T_5 - 75 % RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	0.65	0.85	1.37	0.29	0.64	1.02	1.14	0.18
T_6 - 50 % RDF NPK	0.48	0.52	0.91	0.17	0.45	0.69	0.87	0.12
T_7 - 50 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.42	0.72	1.19	0.25	0.41	0.83	1.03	0.15
T_8 - No NPK in basal + 2 sprays of nano - NPK @ 1 % during tillering and PI stages	0.31	0.39	0.78	0.14	0.32	0.44	0.71	0.09
T_9 - Control (no NPK)	0.29	0.33	0.69	0.12	0.27	0.38	0.59	0.07
SE(d)	0.02	0.04	0.05	0.02	0.02	0.03	0.05	0.01
CD (P=0.05)	0.05	0.07	0.09	0.03	0.04	0.06	0.09	0.02

Table 2. Impact of nano-NPK foliar application on chlorophyll b (mg g^{-1}) content in rice

Treatments	*Rabi* 2023-24				*Kharif* 2024			
	Before spray (20 DAT)	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At harvest stage	Before spray (20 DAT)	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At harvest stage
T_1-Recommended NPK (150:50:50 NPK kg ha^{-1})	0.66	0.92	1.01	0.21	0.61	0.87	0.95	0.19
T_2-RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	0.65	0.97	1.09	0.25	0.59	0.91	1.02	0.21
T_3-75 % RDF NPK	0.56	0.71	0.72	0.09	0.48	0.69	0.71	0.11
T_4-75 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.54	0.95	1.05	0.23	0.51	0.89	0.98	0.20
T_5-75 % RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	0.51	0.84	0.89	0.16	0.49	0.79	0.84	0.15
T_6-50 % RDF NPK	0.41	0.62	0.61	0.08	0.38	0.59	0.61	0.07
T_7-50 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.39	0.75	0.76	0.11	0.37	0.72	0.74	0.12
T_8-No NPK in basal + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.28	0.54	0.59	0.06	0.25	0.51	0.49	0.05
T_9-Control (no NPK)	0.27	0.48	0.54	0.05	0.24	0.45	0.41	0.04
SE(d)	0.03	0.04	0.05	0.02	0.02	0.03	0.03	0.02
CD (P=0.05)	0.06	0.07	0.11	0.04	0.04	0.06	0.09	0.03

Table 3. Impact of nano-NPK foliar application on total chlorophyll content (mg g^{-1}) in rice

Treatments	*Rabi* 2023-24				*Kharif* 2024			
	Before spray (20 DAT)	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At harvest stage	Before spray (20 DAT)	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At harvest stage
T_1-Recommended NPK (150:50:50 NPK kg ha^{-1})	1.12	2.19	2.46	0.55	1.09	2.09	2.31	0.49
T_2-RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	1.15	2.29	2.59	0.59	1.11	2.19	2.45	0.52
T_3-75 % RDF NPK	1.05	1.76	2.01	0.36	0.95	1.75	1.85	0.35
T_4-75 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	1.06	2.21	2.51	0.57	0.96	2.15	2.36	0.50
T_5-75 % RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	1.01	2.02	2.25	0.47	0.94	1.94	2.12	0.42
T_6-50 % RDF NPK	0.84	1.54	1.75	0.29	0.81	1.61	1.66	0.29
T_7-50 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.81	1.85	2.05	0.39	0.78	1.78	1.94	0.36
T_8-No NPK in basal + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.69	1.35	1.54	0.23	0.62	1.45	1.50	0.23
T_9-Control (no NPK)	0.65	1.24	1.45	0.19	0.59	1.39	1.46	0.19
SE(d)	0.05	0.07	0.09	0.02	0.05	0.06	0.07	0.02
CD (P=0.05)	0.11	0.15	0.18	0.05	0.10	0.13	0.16	0.04

Application of foliar spray of nano-NPK increased the NDVI of rice, with significant differences observed across all treatments after the first spray (Table 4). The highest NDVI values were recorded in treatment T_2 (RDF NPK + 1 spray of nano-NPK liquid at 1 % during panicle initiation stage), reaching 0.82 in *Rabi* 2023-24 and 0.80 in *Kharif* 2024. This was followed by treatment T_4 (75 % RDF NPK plus two sprays of nano-NPK at tillering and panicle initiation stages) had NDVI values of 0.80 in *Rabi* 2023-24 and 0.76 in *Kharif* 2024. T_1 (100 % NPK) with values of 0.79 in *Rabi* 2023-24 and 0.73 in *Kharif* 2024 respectively, which were statistically comparable to T_4.

Yield

Application of 100 % RDF + 1 foliar spray of nano NPK @ 1 % during tillering and panicle initiation stage recorded the highest grain yield, which on par with 75 % RDF + 2 foliar spray of nano NPK @ 1 % during tillering and panicle initiation stage and 100 % RDF. Application of 50 % RDF + 2 foliar spray of nano NPK @ 1 % during tillering and panicle initiation stage results decline in yield compared to application of 100 % RDF during both *Rabi* 2023-24 and *Kharif* 2024 seasons (Fig. 2).

Quality parameters

Application of the recommended dose of NPK (100 %) along with foliar spray of nano-NPK at 1 % during the panicle initiation stage (T_2) recorded higher amylose content (24.3 and 24.1 %) of rice during *Rabi* 2023-24 and *Kharif* 2024 seasons, respectively. This was comparable with RDF alone (T_1) and 75 % RDF combined with two foliar sprays of nano-NPK at 1 % during tillering and panicle initiation stages (T_4). The lowest amylose content (13.9 and 13.8 %) was observed in the control (T_9) plot during both seasons, respectively. This was grouped under intermediate amylose content (Fig. 3).

Crude protein content also peaked in T_2 (9.19 % in *Rabi* and 9.12 % in *Kharif* season, respectively), which was statistically comparable to T_1 and T_4 (Fig. 4). The lowest protein levels were again recorded in T_9 (5.72 % and 5.75 % for *Rabi* and *Kharif*, respectively), reflecting the impact of nutrient deficiency on protein synthesis.

Soil microbial population

Soil microbial populations (bacteria, fungi and actinomycetes) were evaluated at active tillering, panicle initiation and harvest stages under different nano-NPK foliar treatments during both seasons (Table 5-7). In *Rabi* 2023-24, at active tillering, T_8 (no basal NPK + two nano-NPK sprays) recorded the highest counts of microbial population, which correspond to increases of 3.3 %, 15.7 % and 7.3 % over the 100 % RDF (T_1). After the second spray at panicle initiation, T_8 again led to marking increases of 11.3 %, 13.2 % and 8.8 % relative to T_1. By harvest, T_8 maintained superiority, outperforming T_1 by 12.6 %, 18.1 % and

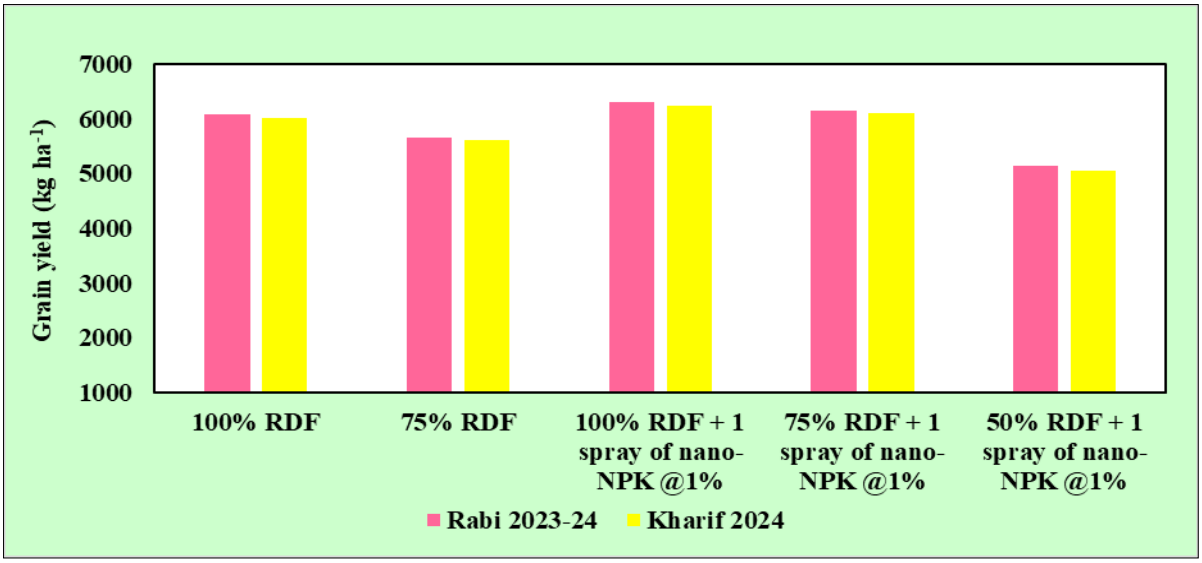

Fig. 2. Impact of nano-NPK foliar application on grain yield (kg ha^{-1}) of rice.

Table 4. Impact of nano-NPK foliar application on NDVI in rice

Treatments	*Rabi* 2023-24			*Kharif* 2024		
	Before spray (20 DAT)	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	Before spray (20 DAT)	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)
T_1 - Recommended NPK (150:50:50 NP kg ha^{-1})	0.42	0.70	0.79	0.41	0.69	0.73
T_2 - RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	0.44	0.76	0.82	0.43	0.74	0.80
T_3 - 75 % RDF NPK	0.40	0.49	0.51	0.39	0.47	0.51
T_4 - 75 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.42	0.72	0.80	0.37	0.72	0.76
T_5 - 75 % RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	0.41	0.61	0.68	0.36	0.59	0.64
T_6 - 50 % RDF NPK	0.37	0.41	0.43	0.35	0.39	0.41
T_7 - 50 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.36	0.52	0.57	0.34	0.51	0.54
T_8 - No NPK in basal + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	0.32	0.39	0.41	0.31	0.35	0.39
T_9 - Control (no NPK)	0.31	0.35	0.39	0.28	0.30	0.35
SE(d)	**0.02**	**0.04**	**0.04**	**0.02**	**0.03**	**0.04**
CD (P=0.05)	**0.04**	**0.07**	**0.09**	**0.03**	**0.06**	**0.08**

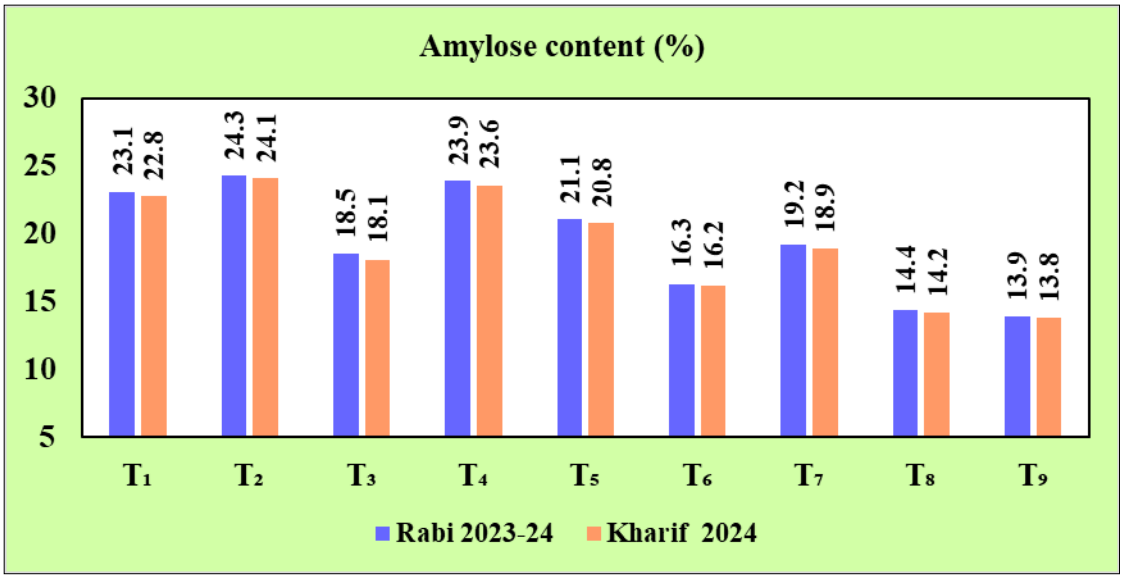

Fig. 3. Impact of nano-NPK foliar application on amylose content (%) of rice.

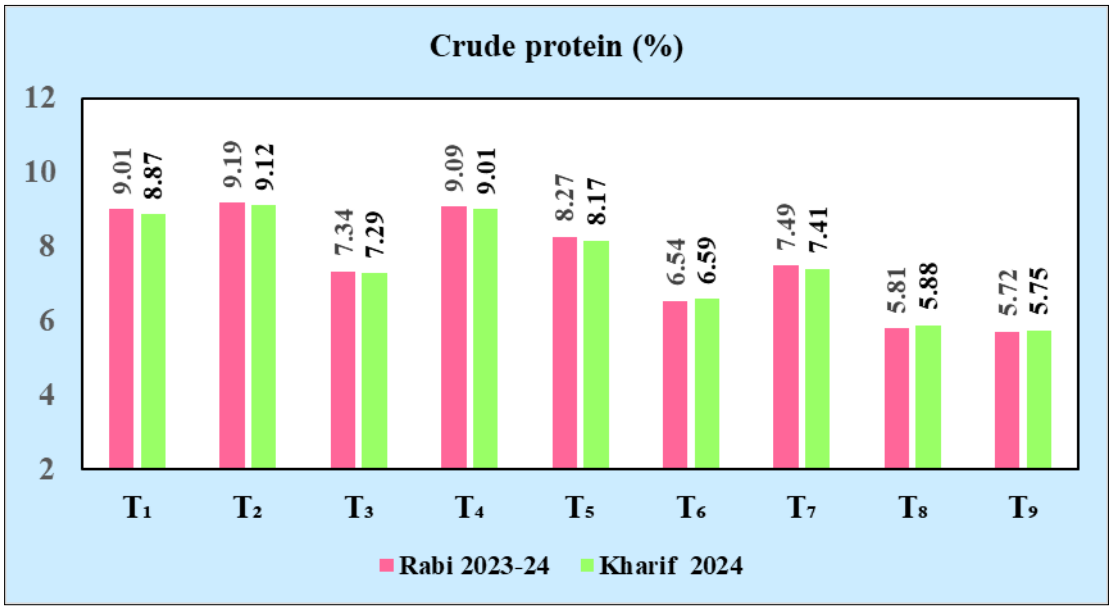

Fig. 4. Impact of nano-NPK foliar application on crude protein (%) of rice.

Table 5. Impact of nano-NPK foliar application on soil bacterial population (cfu g^{-1} of soil) of rice

Treatments	*Rabi* 2023-24			*Kharif* 2024		
	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At harvest stage	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At harvest stage
T_1-Recommended NPK (150:50:50 NPK kg ha^{-1})	44.7	42.6	41.2	42.7	43.8	40.3
T_2-RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	45.3	43.7	43.1	44.3	45.3	44.1
T_3-75 % RDF NPK	36.4	35.1	33.6	35.7	37.7	34.2
T_4-75 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	43.1	40.7	37.2	40.1	41.8	39.7
T_5-75 % RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	41.7	38.2	36.5	39.7	40.6	37.3
T_6-50 % RDF NPK	33.9	34.7	32.6	34.4	35.1	33.8
T_7-50 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	39.2	37.3	35.1	36.2	38.2	36.5
T_8-No NPK in basal + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	46.2	47.4	46.4	46.5	47.2	46.2
T_9-Control (no NPK)	47.4	48.9	47.2	47.2	48.7	47.3
SE(d)	**2.35**	**2.30**	**2.21**	**2.28**	**2.35**	**2.25**
CD (P=0.05)	**4.98**	**4.88**	**4.67**	**4.89**	**4.99**	**4.76**

Table 6. Impact of nano-NPK foliar application on soil fungi population (cfu g^{-1} of soil) of rice

Treatments	*Rabi* 2023-24			*Kharif* 2024		
	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At Harvest stage	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At Harvest stage
T_1-Recommended NPK (150:50:50 NPK kg ha^{-1})	12.7	12.9	11.6	13.9	13.2	12.6
T_2-RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	13.6	13.7	12.1	14.1	14.3	13.1
T_3-75 % RDF NPK	10.9	10.7	9.60	10.7	10.3	9.10
T_4-75 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	12.3	12.2	11.2	13.2	12.8	11.9
T_5-75 % RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	11.7	11.4	10.7	12.5	11.4	10.3
T_6-50 % RDF NPK	9.80	9.90	8.80	9.60	9.60	8.60
T_7-50 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	11.2	11.0	10.1	11.8	10.9	9.70
T_8-No NPK in basal + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	14.7	14.6	13.7	14.8	15.1	14.2
T_9-Control (no NPK)	15.1	15.8	14.8	15.3	15.7	14.7
SE(d)	**0.70**	**0.70**	**0.6**	**0.73**	**0.72**	**0.67**
CD (P=0.05)	**1.48**	**1.5**	**1.37**	**1.54**	**1.52**	**1.41**

Table 7. Impact of nano-NPK foliar application on soil actinomycetes population (cfu g^{-1} of soil) of rice

Treatments	*Rabi* 2023-24			*Kharif* 2024		
	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At Harvest stage	Active tillering stage (40 DAT)	Panicle initiation stage (60 DAT)	At Harvest stage
T_1-Recommended NPK (150:50:50 NPK kg ha^{-1})	20.6	21.6	19.7	20.6	21.9	19.2
T_2-RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	21.7	22.7	20.9	21.7	22.1	20.6
T_3-75 % RDF NPK	17.3	18.4	16.2	16.8	18.1	17.2
T_4-75 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	19.8	20.3	18.3	19.7	20.1	18.7
T_5-75 % RDF NPK + 1 spray of nano-NPK @ 1 % during PI stages	18.4	19.7	17.4	18.2	19.4	18.1
T_6-50 % RDF NPK	16.9	17.5	15.4	16.1	17.7	16.3
T_7-50 % RDF NPK + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	17.8	19.2	16.7	17.4	18.7	17.9
T_8-No NPK in basal + 2 sprays of nano-NPK @ 1 % during tillering and PI stages	22.1	23.6	21.6	22.8	23.7	21.9
T_9-Control (no NPK)	23.5	24.8	22.7	23.2	24.8	22.4
SE(d)	**1.11**	**1.17**	**1.05**	**1.11**	**1.16**	**1.07**
CD (P=0.05)	**2.35**	**2.47**	**2.24**	**2.34**	**2.46**	**2.26**

9.6 %. A similar pattern was observed in *Kharif* 2024 at active tillering, T_8 increases of 8.9 %, 15.5 % and 10.7 % compared to 100 % RDF. At panicle initiation, T_8 again led with 8.0 %, 8.6 % and 8.2 % above T_1. Post-harvest, T_8 recorded, reflected increases of 14.6 %, 12.6 % and 14.1 % over T_1. Overall, foliar nano-NPK application without basal NPK (T_8) consistently enhanced soil microbial proliferation more than the standard 100 % RDF (T_1) across both seasons.

Clustered heatmap

The heatmaps for *Rabi* 2023-24 and *Kharif* 2024 illustrate the Z-score standardized clustering of physiological traits, grain quality parameters and soil microbial populations under different treatments (Fig. 5 & 6). In *Rabi* 2023-24, microbial populations (bacteria, fungi and actinomycetes) formed a distinct cluster, separate from chlorophyll content, NDVI, protein and amylose. This indicates that higher microbial activity at 40 and 60 DAS did not directly align with improved plant physiological traits, suggesting a delayed or indirect response during the *Rabi* season. In contrast, the *Kharif* 2024 heatmap revealed better integration between microbial and physiological traits. Treatments T_1 (100 % RDF), T_2(100 % RDF + foliar spray of nano-NPK at panicle initiation stage) and T_4(100 % RDF + foliar spray of nano-NPK at active tillering and panicle initiation stages) showed consistently higher Z-scores across NDVI, chlorophyll, protein and amylose, indicating stronger plant responses and effective nutrient assimilation under nano-NPK foliar application. These treatments clustered closely, while T_6, T_8 and T_9 showed negative trends, reflecting poorer performance. The close grouping of NDVI with chlorophyll at multiple stages further supports the link between photosynthetic efficiency and crop vigor.

Discussion

Physiological parameters

The application of nano-NPK, particularly in combination with 100 % RDF and 75 % RDF, registered higher chlorophyll a, b and total chlorophyll content in rice across both seasons. This can be attributed to the nanoscale size and high surface reactivity of nano-NPK, which facilitates efficient foliar absorption through stomatal openings and cuticle pores. Once absorbed, these nano-nutrients are rapidly translocated and assimilated, improving nitrogen metabolism and stimulating chlorophyll synthesis (15). Similar findings were reported, that observed improved leaf greenness and photosynthetic activity with nano -fertilizer applications (16). The increased chlorophyll content from 20 DAT to 60 DAT followed by a natural decline at harvest, aligns with the remobilization of nitrogen from leaves to

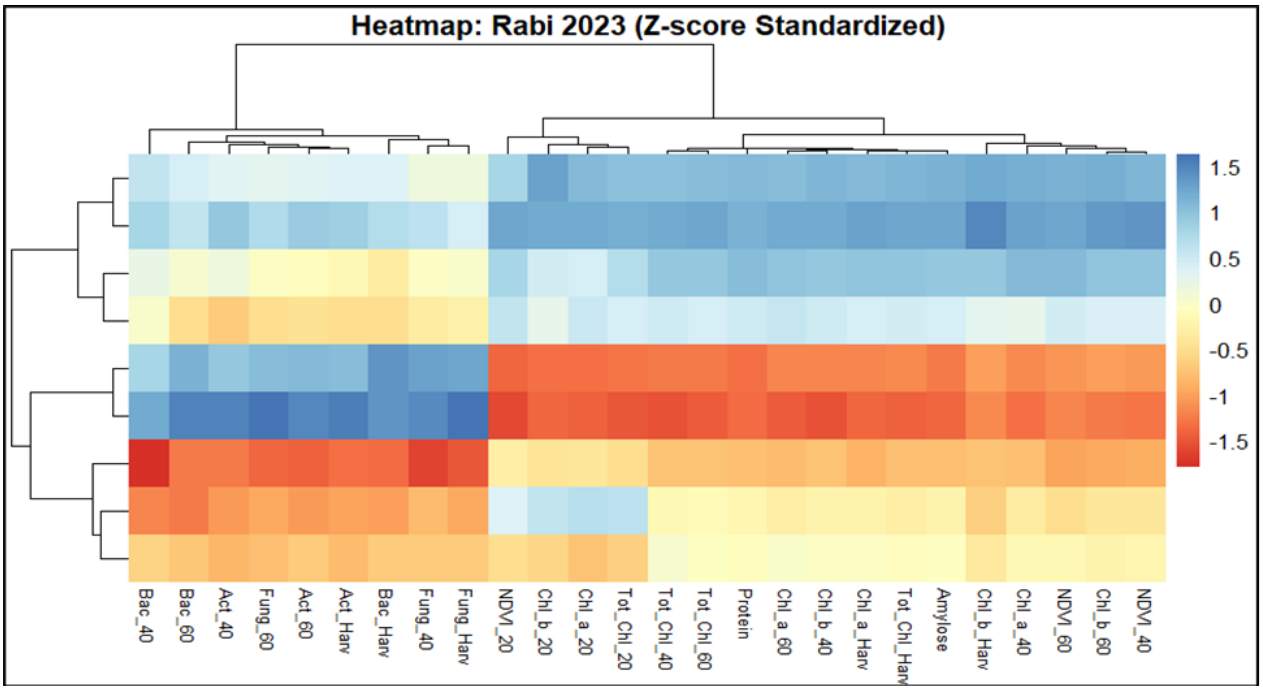

Fig. 5. Heatmap of treatment effects on rice traits (*Rabi* 2023-24).

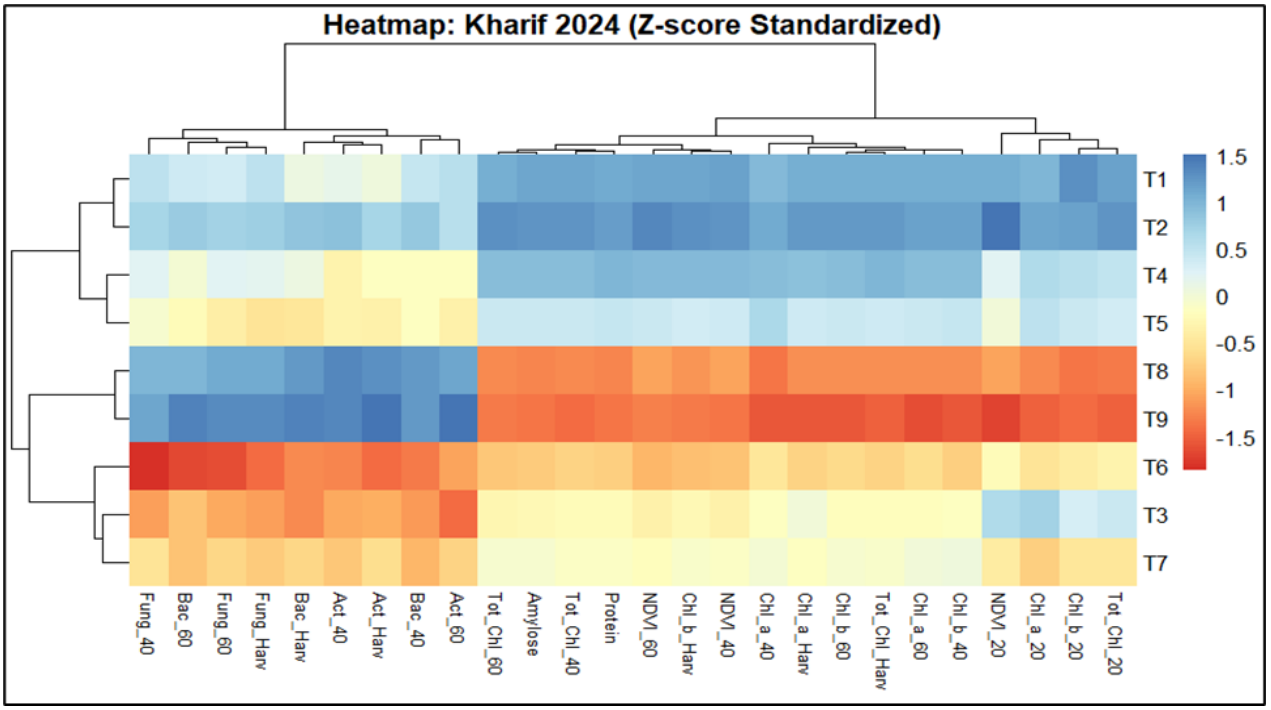

Fig. 6. Heatmap of treatment effects on rice traits (*Kharif* 2024).

developing grains (17). This transition reflects the shift from vegetative to reproductive growth stages, where leaves function as source organs supplying nutrients to the panicle (18,19).

Higher NDVI values indicate greater vegetation greenness due to nano NPK foliar application. This might be due to enhanced magnesium availability, better nitrogen assimilation and increased activity of RUBISCO enzyme all of which are influenced by nano-fertilizers (20). Nano-formulations not only improve photosynthetic activity but also reduce nitrogen losses by minimizing volatilization and leaching (21,22). Additionally, NDVI-guided nano fertilizer applications can reduce conventional NPK requirements by 25 %, promoting sustainable productivity with reduced environmental impact (23,24).

Yield

The combination of nano-fertilizers and conventional fertilizers enhances nutrient absorption in plants, promoting optimal growth and key processes like photosynthesis. This increases the accumulation and movement of photosynthates to economically important parts, resulting in higher yields (17). The effectiveness of both the leaves (source) and the economic parts (sink) improves. Applying nano-NPK foliar fertilizer during critical growth stages ensures a synchronized supply of nitrogen, phosphorus and potassium, further boosting crop yields (22).

Quality parameters

Amylose content in rice is strongly influenced by both the rate of NPK fertilization and the supplemental application of nano-NPK foliar sprays. Higher levels of conventional NPK increased amylose concentrations, which were further improved by nano

-NPK, indicating enhanced nutrient uptake and utilization efficiency. This supports earlier findings that nano-fertilizers boost the effectiveness of conventional inputs by improving nutrient delivery and assimilation (25,26). In contrast, reduced NPK levels led to a decline in amylose content, consistent with reports that nitrogen deficiency hampers starch biosynthesis in rice grains (27). However, the integration of nano-NPK sprays with reduced NPK applications showed a partial recovery in amylose levels, indicating the potential of nanofertilizers to mitigate the adverse effects of lower nutrient inputs and maintain grain quality (2).

Crude protein content in rice is significantly affected by both the amount and form of nutrient application. Full recommended NPK fertilization typically results in the highest protein levels, as nitrogen plays a central role in grain protein synthesis (28). Reducing the conventional NPK rate leads to a decline in protein content. This reflects the direct relationship between nitrogen availability and grain protein accumulation (29). However, supplementing reduced NPK rates with nano-NPK sprays, especially when applied at critical growth stages such as tillering and panicle initiation, can compensate for lower basal fertilizer inputs and help maintain or even enhance grain protein levels (2,30). Nano-fertilizers are known to improve nutrient uptake efficiency and provide a more sustained release of nutrients, which supports protein synthesis even under reduced conventional fertilizer regimes. This approach not only sustains grain quality but also contributes to more sustainable and resource-efficient rice production systems.

Soil microbial population

The impact of nano-fertilizers on soil microbial communities is influenced by soil properties such as pH, organic matter content, texture and ionic strength, along with the type, size and concentration of nanoparticles. Nanoparticles like nano clay, chitosan and zeolite can improve soil health by enhancing microbial activity (31). Due to their small size and large surface area, these particles can interact with microbial cells, leading to either beneficial or harmful effects. While they may promote microbial growth at optimal levels, excessive or repeated application can harm beneficial microbes that decompose organic matter and maintain soil fertility. This disruption may reduce microbial biomass, alter community structure and negatively impact long-term soil productivity (32). Actinomycetes are essential for breaking down complex organic materials and producing antibiotics that inhibit soil-borne pathogens. However, they may be sensitive to nanoparticles, which can disrupt their populations and hinder nutrient cycling and soil stability. Sensitivity varies with nanoparticle composition and some types may inhibit actinomycetes activity more than other microorganisms. The toxicity of nanoparticles depends on their composition, particle size, dose, soil type and moisture conditions. High concentrations can suppress the growth of important soil microorganisms. However, biogenic nanomaterials are considered less harmful than chemically synthesized ones and are recommended to minimize nanotoxicity in soil ecosystems (33).

Conclusion

Application of 75 % recommended dosage of NPK fertilizer along with two foliar sprays of nano-NPK at tillering and panicle initiation stages significantly improves the physiological traits and grain quality in rice. Nano-NPK alone maintained the soil microbial populations suggesting its positive impact on soil health. The research findings suggest that nano-fertilizers can reduce use of conventional fertilizer (up to 25 %) without compromising productivity and promoting environmental sustainability. Further studies need to be conducted to validate the results across diverse agro-ecological zones and with an aim for broader applicability of these results.

Acknowledgements

The authors would like to sincerely thank the IFFCO (No: F37APS) for the funding. The authors acknowledge the Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India for providing resource facilities for conducting the research.

Authors' contributions

ASG conducted field experiments and prepared the original draft of the manuscript. MV and PP provided supervision and contributed to the writing through review and editing. KP provided technical guidance and inputs for manuscript preparation and modification. TK assisted in formulating the research project and facilitated field experiments. SR contributed to physiological studies and analysis. SU assisted in microbiological studies and analysis. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare .

Ethical issues: None

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Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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