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RESEARCH ARTICLE

Nano-diammonium phosphate enhances grain yield by modulating gas exchange traits and nutrient use efficiency in rice

R Roshil Ben Sweety[1], P Boominathan[1*], A Senthil[1], D Jegadeeswari[2] & G Prabu Kumar[3]

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

[2]Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[3]Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

* Email: boominathan.p@tnau.ac.in

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Abstract

Nutrients are primarily applied to crops through the soil to enhance productivity while maintaining crop and soil health. However, high doses of soil -applied nutrients often reduce nutrient use efficiency, increase cultivation costs, and contribute to environmental pollution. With their high surface area-to-volume ratio, nano-formulated nutrients have emerged as effective alternatives, requiring lower doses and demonstrating superior nutrient use efficiency (NUE) compared to conventional fertilizers. This experiment was conducted to study the influence of nano-diammonium phosphate (nano-DAP) on physiological and yield-associated traits in rice and to assess the extent of replacement of soil application of nitrogen (N) and phosphorous fertilizers with foliar application of nano-form of DAP. Field trials included nine treatments, comprising 50% and 75% of the recommended dose of nitrogen and phosphorus (RDNP) and 100% of the recommended dose of fertilizers (RDF), and foliar application of conventional and nano-DAP. Foliar treatments were applied during the active tillering and panicle initiation stages. Among the treatments, 75% RDF and two foliar sprays of nano-DAP performed better in terms of morpho-physiological parameters, particularly leaf area, dry matter production, photosynthetic rate, stomatal conductance, and number of productive tillers compared to 100% RDF. Furthermore, the NUE of nano-DAP treatments was significantly higher, reflecting an improved ability of the rice plants to utilize the nanoform nutrients, resulting in enhanced yield effectively.

Keywords

nano-DAP; nanofertilizer; nutrient use efficiency; phosphorus use efficiency

Introduction

Nutrient requirements of crops are primarily met through the soil application of high doses of conventional fertilizers. However, this practice reduces soil fertility, damages the flora, and causes soil erosion, drastically reducing NUE. Among the essential nutrients, N and phosphorus (P) are significant elements required for metabolic processes that regulate the growth and development of plants. These two nutrients are crucial for protein synthesis, energy transfer, and root development. While N promotes healthy foliage and overall plant vigor, P is vital for root establishment, flowering, and grain formation, ultimately enhancing crop yield (1). A N deficiency can cause stunted growth, reduced chlorophyll content, and poor yields due to its role in protein synthesis and chlorophyll formation (2). Although N is abundant in the atmosphere, only a small portion is accessible to plants in nitrate or ammonium, necessitating careful management to ensure adequate nutrient supply (3). Insufficient P can result in plant membrane breakdown and hinder energy transfer within the plant (4). Although soils may hold P reserves greater than what plants need, only a small fraction of P is soluble and accessible for plant uptake (5).

Applying N to soil presents multiple challenges, including inefficient plant uptake and substantial losses through leaching, volatilization, and denitrification. These losses diminish N-fertilizers' effectiveness and exacerbate environmental problems such as water pollution and greenhouse gas emissions (6). P application in soil frequently faces challenges, including limited availability as P binds with soil particles, which impedes plant uptake. Moreover, P losses through runoff and erosion can lead to waterway eutrophication and environmental degradation (7). Hence, applying N and P gradually through foliar application allows plants to absorb and utilize them effectively for metabolic processes.

Improving N and P use efficiency is crucial for maximizing crop productivity and minimizing environmental impact by reducing fertilizer loss and improving plant uptake, leading to better crop performance (8). This issue is addressed through techniques such as split N application, N localization, precision farming, and foliar sprays designed to optimize NUE and reduce environmental concerns (8). Similarly, improving Phosphorus use efficiency (PUE) is essential for addressing environmental issues and decreasing reliance on non-renewable resources. Methods to enhance PUE, such as using P-solubilizing microorganisms or adopting precision farming techniques, can significantly improve nutrient uptake and crop performance (9).

Most rice cultivation, mainly under wetland conditions, is characterized by applying high N and P, which leads to significant nutrient losses. Nutrient loss in wetland rice cultivation occurs due to leaching, runoff, and volatilization, where N and P are washed away or lost as gases. Additionally, anaerobic conditions promote denitrification and P fixation, reducing plant nutrient availability. In nitrogenous fertilizers, ammonia (NH_3) fluxes represent a major N loss pathway, with higher emissions linked to increased N fertilization rates in wetland rice systems (10). In India, rice accounts for approximately 37% of total fertilizer N consumption, with significant losses (60-70%) occurring through various processes (11). Therefore, nano-form fertilizers as a foliar application have been given a major thrust to increase their efficiency. Using nano fertilizers can lower cultivation costs by decreasing the amount of fertilizer needed for cultivation (12) and provide a controlled release of nutrients, reducing losses associated with conventional fertilizers (13). Nano-fertilizers provide controlled nutrient release by encapsulating nutrients in nanomaterials, reducing losses through leaching, volatilization, and denitrification. For example, nano-urea releases N gradually, improving NUE and minimizing environmental impact. This controlled release enhances crop yields while reducing the need for frequent fertilizer applications (14).

The simultaneous application of traditional and nanoparticle mineral fertilizers are required to maintain a continuous nutritional balance in the different growth stages of rice. This approach reduces reliance on chemical fertilizers, fostering a more favorable environment for nutrient absorption. Additionally, foliar application allows for faster nutrient uptake by leaves than root applications, as nutrients are directly available in solution form rather than depending on soil uptake (15). This method also improves nutrient use efficiency, reduces environmental impact, and may offer enhanced health benefits for consumers (16). Moreover, it can be applied even under unfavorable soil and weather conditions. In addition, it allows plants to directly uptake nutrients, reducing fertilizer wastage. Consequently, the foliar application of nanofertilizers is becoming viable for enhancing nutrient use efficiency and boosting crop growth.

Newly developed nano-fertilizers are smaller, possess a larger surface area, and exhibit increased absorption capacity with controlled-release kinetics at specific sites (17). They are highly reactive and can penetrate the plant cuticle, ensuring controlled release and targeted nutrient delivery (18). Indian Farmers Fertiliser Cooperative Limited (IFFCO) is a leading Indian cooperative that manufactures and supplies nano-fertilizers to enhance agricultural productivity and significantly supports farmers by providing high-quality and cost-effective fertilizers (19). One recent product of IFFCO is nano-DAP fertilizer, which offers a cost-effective alternative to conventional DAP aimed to enhance crop yield and provide significant long-term benefits.

Further, the use of this nano-DAP requires crop-specific dosage optimization compared with the RDF and analyzing rice's response regarding physiological and biochemical traits. Therefore, this experiment was designed to study the response of rice to various amounts of DAP nanofertilizer that alter physiological and biochemical traits, resulting in improved yields.

Materials and Methods

Study area and experimental design

A field experiment was conducted from December 2023 to April 2024 at wetland farms affiliated with Tamil Nadu Agricultural University, Coimbatore, India. The location is at 1° N latitude and 77° E longitude, with an altitude of approximately 426.72 meters above mean sea level. The experimental design employed was a randomized block design (RBD), consisting of nine treatments, each replicated three times. The experimental field covered a total area of 675 m^2, with dimensions of 53 meters in length and 25 meters in width. The field was divided into 27 plots, each measuring 25 m^2 with a spacing of 20 cm × 20 cm.

Treatment details

We imposed nine treatments with soil application of recommended N and P fertilizers (100%, 75%, and 50%) in which N as four splits and P as basal combined with foliar sprays of 2% DAP and 0.7% nano-DAP (Table 1). A uniform application of 100% potassium fertilizer was made in four splits across all the treatments. The stages of application of foliar sprays are active tillering and panicle initiation stage.

Observations were recorded after the 10th day of the spray. The growth and physiological parameters were recorded 10 days after the first and second sprays.

The rice variety ADT 53 was used for this experiment, and regular cultivation practices were followed except for the fertilizer application. A fertile land with optimal irrigation infrastructure was selected for nursery cultivation, and the main field was properly leveled and well-puddled for transplanting. The soil texture is clay loam with pH of 7.7, electrical conductivity of 0.74 dS m^{-1}, N of 231 kg ha^{-1}, P of 13.9 kg ha^{-1}, and potassium of 295 kg ha^{-1}. Fertilizers were applied as per the treatment schedule, and no inputs were added to the control while the RDF was applied in T1. Control represents 0% N, 0% P and 100% K without DAP and nano-DAP sprays. The growth and physiological parameters were recorded on the 10th day after the first and second sprays.

Measurement of morpho-physiological and biochemical traits

The plant height was recorded from the stem's base to the longest leaf's tip. Measurement of leaf area was done using a leaf area meter (LICOR, Model LI 3000, Lincoln, NE, USA). Leaf samples were collected from each replication for every treatment and placed into the leaf area meter, and the value was expressed as cm^2 $plant^{-1}$. The reading was taken during different stages of the crop, namely tillering and panicle initiation.

The chlorophyll index (SPAD value) was measured using a chlorophyll meter from Minolta (Model 502 Minolta Japan). Measurements were taken from the five physiologically active leaves for all replications, and then the average was calculated. The gas exchange parameters were recorded using a portable photosynthesis system (PPS; LI-6400 XT, Licor Inc., Lincoln, NE, USA). Three values were recorded for each replication, and an average was calculated. The photosynthetic rate, transpiration rate, and stomatal conductance were expressed as µmol of CO_2 m^{-2} s^{-1}, mmol H_2O m^{-2} s^{-1}, and mol H_2O m^{-2} s^{-1}.

Total chlorophyll content was estimated by a standardized protocol (20). About 25 g of fresh sample with 10 mL of 80% acetone was separated using centrifugation for 3000rpm for 10 minutes. The supernatant was taken, and the volume was made 25 mL using 80% acetone. The optical density (OD) values were taken at 652 nm in a spectrophotometer (Shimadzu; UV-1800) and expressed in mg/g.

Soluble protein was estimated using the a standardized protocol (21). The procedure includes maceration of 0.25 g of leaf sample using 10 mL of phosphate buffer. Centrifugation is done at 3000 rpm for 10 minutes and made the volume to 25 mL. 1 mL of the supernatant was taken, 5 mL of alkaline copper tartrate (ACT), and 0.5 mL of Folin–Ciocalteau reagent was added, and the color developed was measured after 30 minutes at 660 nm in a spectrophotometer (Shimadzu; UV-1800) and expressed in mg/g. The standard was prepared using 50 mg of bovine serum albumin (BSA), dissolved in 100 mL of distilled water with a stock of 500 ppm. Different concentrations of BSA stock solutions were prepared by diluting the stock solution (100, 200, 300, 400, and 500 ppm).

Quantification of nutrient content and its use efficiency

Total N was measured by the Kjeldahl method using a 4:1 sulfuric acid to perchloric acid mixture (22). Total P was determined via a triple acid extraction with nitric, sulfuric, and perchloric acids in a 9:2:1 ratio using a spectrophotometer (23).

NUE was calculated using the formula (Eqn. 1) (24).

(Eqn. 1)

$$\text{Nitrogen use efficiency } (\text{kg kg}^{-1}) = \frac{\text{Dry yield}}{\text{Quantity of Nutrient applied} + \text{Nutrient in soil before transplanting}}$$

Measurement of yield components

The yield and yield components were observed during crop harvest. The number of productive tillers per plant, grain yield, straw yield, panicle length, panicle weight, number of total grains per panicle, number of filled grains per panicle, number of unfilled grains per panicle, 1000 seed weight all were recorded from randomly selected five plants average in each replication.

Statistical analysis

The experiment was conducted using a randomized block design, and the data collected was analyzed using SPSS software. A one-way analysis of variance (ANOVA) was performed on nine treatments and three replications, and significance was determined at the 5% level.

Results and Discussion

Impact of nano-DAP on morpho-physiological parameters

Nano-diammonium phosphate is a nanomaterial-based fertilizer that provides plants with a controlled release of nutrients, specifically N and P. It contains 8% N and 16% P. Its advantages over conventional fertilizers include improved nutrient use efficiency, reduced nutrient losses, and enhanced plant uptake due to its smaller particle size. Foliar application of nano-DAP significantly alters morphological traits, including plant height in rice variety ADT 53. The tallest plants (81.2 and 99.6 cm) were recorded in 75% RDNP with two nano-DAP sprays, while the shortest (66.8 and 82.8 cm) were observed in the control and application of 100% RDF, resulting in heights of 80.1 and 97.6 cm. nano-DAP spray significantly ($p \leq 0.05$) increased plant height by 20.2% over the control and 2% over 100% RDF (Table 2). Therefore, nano-DAP spray showed a marginal increase of 2% in plant height compared to RDF, suggesting that nanoform of N has a minimal impact on plant height, which may not hinder the yield enhancement crops such as rice. These results were similar to the findings of another study, which stated that DAP spray increased plant height by 2% (25). In cereal such as wheat, a reduction in plant height through peduncle length changes increases grain yield and grain number. Therefore, higher yields are not necessarily associated with increased plant height (26). Although few studies showed the increased plant height with nanofertilizer, our study result revealed that it was on par with an RDF.

Foliar application of nano-DAP significantly enhances leaf area and dry matter production

The treatment with 75% RDNP combined with two sprays of nano-DAP resulted in the highest leaf area, with 1553.2 cm^2 $plant^{-1}$ and 2053.9 cm^2 $plant^{-1}$ after the first and second sprays, respectively. This represents an 11 to 12% increase compared to 100% RDF. The response of plants from first to second spray for leaf area in case of 75% RDNP + 2 sprays of nano-DAP is 32.2% suggesting that two sprays of nano-DAP are essential for significant ($p \leq 0.05$) incremental increase in leaf area than single spray (Fig. 1). Reduced fertilizer doses (50% and 75% RDNP) without foliar sprays resulted in the lowest leaf area, indicating the importance of supplementing soil fertilization with foliar application. These results suggest that nano-DAP positively influences leaf area by supplying N and P in nano form, which enhances leaf production when applied at optimal concentrations. This reduces nutrient competition among plants, resulting in a larger leaf area. Besides, the efficiency of DAP application is predominantly due to N's role in increasing leaf area, as increasing the leaf area is important for light capture, which increases carbon assimilation in the leaves, leading to greater productivity. In addition, the nutrients stored in leaf tissues are also transported during seed formation, contributing to fuller seeds and greater weight gain (27).

In this study, the application of nanoform of N triggers chlorophyll production and, therefore, the rate of photosynthesis. N in nanoform is more effective than conventional forms because its smaller particle size allows for a controlled and gradual release, ensuring N remains available to plants over an extended period by minimizing N losses. The increased surface area of nano-N enhances its absorption by plant roots and leaves, improving NUE. This leads to better crop yields with fewer applications and a reduced environmental impact. This enhancement improves the translocation of assimilates and photosynthates to various plant parts, resulting in greater dry matter (DM) accumulation (28). Our study result revealed that 75% RDNP and two foliar sprays of nano-DAP showed significant ($p \leq 0.05$) higher dry matter production of 45.8 g/plant after the first spray and 98 g/plant after the second spray, which was 15.3% higher than the 100% RDF treatment (Table 1). It is understood from the data that foliar application of nutrients is highly effective when applied in nanoform, as evidenced by the high accumulation of dry matter due to various growth and physiological traits. Besides, the foliar application of major nutrients improves the accumulation and translocation of photosynthates, leading to greater biomass production (29). In addition, N and P, which are vital for photosynthesis and carbohydrate production, also significantly increase dry matter accumulation due to nano-DAP when applied through foliar spray (30).

The role of N in chlorophyll synthesis is implicated in many studies as it is a key component of the chlorophyll molecule. Nitrogen is essential for chlorophyll synthesis as it is a key component of the chlorophyll molecule. It aids in forming amino acids crucial for producing chlorophyll and related proteins. Adequate N levels enhance chlorophyll production, improving a plant's light absorption for photosynthesis and ultimately boosting growth and productivity. Adequate N increases chlorophyll content and enhances photosynthetic efficiency, while deficiency leads to chlorosis and reduced plant growth. Recent research highlights the importance of optimized N management to maintain optimal chlorophyll levels and improve crop performance (31). Optimising N dose is critical in crops such as rice where excessive application can lead to nutrient imbalances, increased susceptibility to pests and diseases, and environmental issues such as water pollution and greenhouse gas emissions. In addition, high N levels often result in excessive vegetative growth at the expense of yield and quality (31). In the study, the chlorophyll index showed significant differences among treatments. The result indicated that the first and second sprays of nano-DAP and DAP increased the chlorophyll index, with nano-DAP showing a higher increase than DAP. Among the treatments, 75% RDNP + 2 sprays of nano-DAP exhibited the highest values (41.7 and 46.2 SPAD units) after the first and second sprays, respectively, compared to 100% RDF.

From these results, the rate of chlorophyll content synthesis between the tillering and panicle initiation stage is significantly ($p \leq 0.05$) higher (10.5%) in 75% RDNP + two sprays of nano-DAP compared to 100% RDF (8.5%) (Table 3). This can be attributed to a slower release of N and P in nano-DAP in active tillering and panicle initiation stages. Besides, the increased surface area of nanoparticles enhances absorption efficiency and promotes enzyme activities that inhibit ethylene production, reducing chlorophyll degradation (32). Additionally, nanoparticles activate photosynthesis enzymes, improving chloroplasts' ability to absorb sunlight and convert it into stored energy, increasing chlorophyll content.

Nano-DAP elevates the soluble protein content and gas exchange parameters

Nitrogen and P significantly affect the enzymes involved in the application 75% RDNP and two applications of nano-DAP during critical growth stages, as indicated by the highest amount of total soluble protein, which was 17.74 mg/g -3.2% higher ($p \leq 0.05$) compared to100% RDF (Table 1). Generally, soluble protein serves as a surrogate, which indicates the performance of the Rubisco enzyme involved in carbon fixation due to enhanced photosynthetic activity. The soluble protein content is a crucial regulator of cell osmotic potential, with higher levels helping to maintain a low osmotic potential and strengthening the plant's resistance to stress-induced damage (33). In this study, soluble protein content increased with N levels, suggesting that an optimal amount of N can boost protein synthesis and soluble protein content in rice. These results corroborate the findings of a previous experiment (34).

In the case of gas exchange parameters, the application of 75% RDNP + 2 nano-DAP sprays exhibited the highest photosynthetic rates (24.8 and 28.4 µmol CO_2 m^{-2}-s^{-1}) (Fig. 2), transpiration rates (5.5 and 6.1 mmol H_2O_2 $m^{-2}s^{-1}$) (Fig. 3), and stomatal conductance (0.320 and 0.369 mol H_2O $m^{-2}s^{-1}$) (Fig. 4) after each spray. Conversely, the 2% DAP with reduced RDNP levels showed lower rates across all parameters, implying the beneficial effects of nano-DAP on key processes such as photosynthesis traits essential for growth and productivity. The percent increase in two nano-DAP sprays with 75% RDNP showed a significant ($p \leq 0.05$) increase in photosynthetic rate, transpiration rate, and stomatal conductance to the level of 12.2%, 7%, and 12.2%, respectively, compared to RDF signifying that nano-DAP has a major impact on photosynthesis and stomatal conductance compared to transpiration rate. From active tillering to panicle initiation, the percent increase in photosynthetic rate (14.5%) and stomatal conductance (15.3%) is higher with 75%

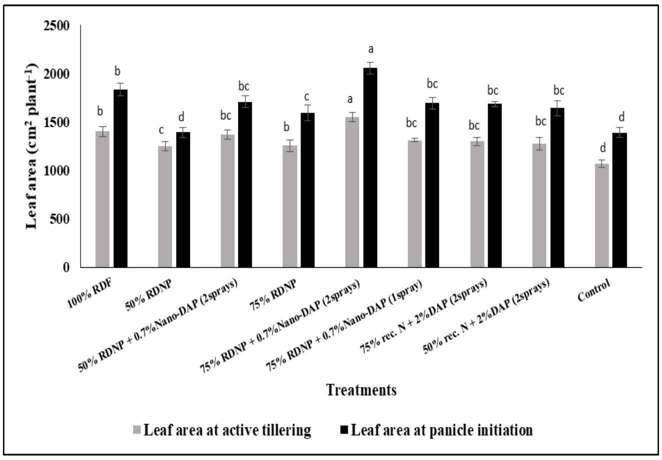

Fig. 1. Impact of nano-DAP on leaf area (cm^2 plant^{-1}).

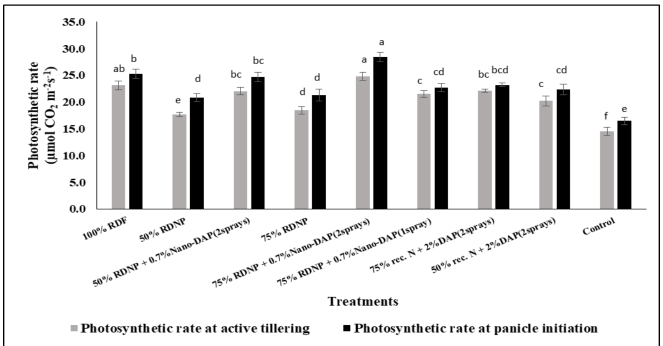

Fig. 2. Impact of nano-DAP on photosynthetic rate (μmol CO_2 m^{-2}s^{-1}).

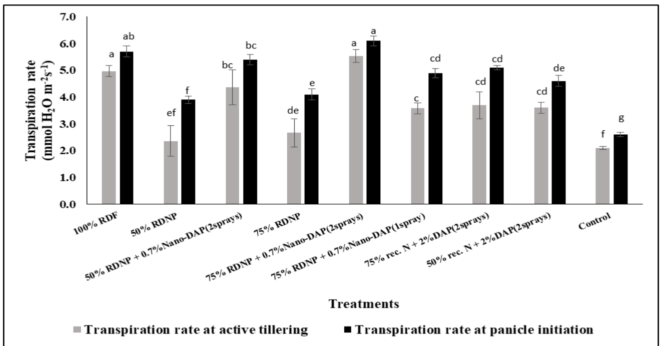

Fig. 3. Impact of nano-DAP on transpiration rate (mmol H_2O m^{-2}s^{-1}).

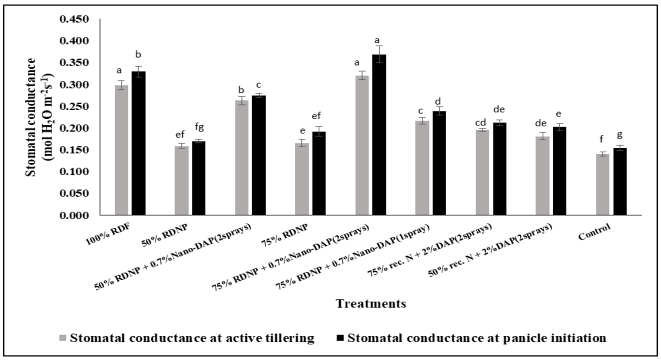

Fig. 4. Impact of nano-DAP on stomatal conductance (mol H_2O m^{-2}s^{-1}).

RDNP and 2 sprays of nano-DAP compared to 100% RDF. P is vital for key biomolecules involved in photosynthesis, including amino acids, nucleic acids, adenosine triphosphate (ATP), adenosine diphosphate (ADP), phospholipids, and phytic acid (35).

Plants treated with nano-KH_2PO_4 in Yoshida solution exhibit improved PUE compared to conventional fertilizers, as nano-fertilizers possess smaller particle sizes and larger surface areas that enhance metabolic processes (36). Nano N and P functions include the synthesis of chlorophyll and thylakoids, as well as the development of chloroplasts and also essential for energy transfer within the plant and support numerous enzymatic processes, including photosynthesis for promoting plant growth (37). Further, many studies suggest that applying nanoforms of N and P enhances nutrient absorption, promoting the optimal growth of plant parts and increasing metabolic processes, such as photosynthesis. This leads to increased accumulation of photosynthates and their efficient translocation to the plant's economic parts, thereby improving crop growth, development, and yield (38).

Nano-DAP increases grain yield primarily due to the number of productive tillers

Nano-DAP sprays enhanced yield primarily by enhancing the number of productive tillers, resulting in reduced soil application of fertilizers. Among the treatments, 75% RDNP + 2 nano-DAP sprays recorded the highest number of productive tillers, compared to 100% RDF and 50% RDNP + 2 DAP sprays (Table 4). This amounts to a 13.3% percent increase ($p \leq 0.05$) in 75% RDNP + 2 nano-DAP treated crops compared to all other parameters, which can be attributed to N and P role in reproductive development as deficiencies can restrict tiller initiation and development, disrupting normal growth processes and significantly impacting grain yield in paddy fields (30).

Treatment with 75% RDNP + 2 nano-DAP sprays recorded the highest 1000 seed weight of 14.98 g, although slight increase of 1.1% compared to 100% RDF. It is clear from the data that nano-DAP treatment has little influence; therefore, the 75% RDNP + 2 nano-DAP treatment is on par with the recommended dose of fertilizer (Table 4). These results corroborate with a previous study (39). Among the treatments, the highest grain yield was recorded in 75% RDNP + 2 nano-DAP sprays (6378 kg/ha), a 5.8% increase ($p \leq 0.05$) over 100% RDF (6028 kg/ha), and also the highest straw yield at 8414 kg/ha, marking a 10.3% ($p \leq 0.05$) improvement compared to 100% RDF (8164 kg/ha). In comparison, 50% RDNP + 2 nano-DAP sprays produced a grain yield of 5573 kg/ha, 8.2% decrease, and a straw yield of 7629 kg/ha, a 1.6% reduction relative to 100% RDF (Table 4). The higher grain yield in 75% RDNP + 2 nano-DAP sprays compared to 100% RDF is due to the enhanced absorption, interception, and utilization of P from nanoform. This slow P release provides a steady supply throughout the growth period, increasing photosynthesis and resulting in greater biomass accumulation (40).

To optimize grain yield in paddy fields, providing sufficient P is crucial for promoting healthy and abundant grain production (41). The foliar application of nano fertilizer

Table 1. Details of treatment

Treatments	Details of treatments
T_1	Recommended dose of fertilizer (RDF) + all package of practices
T_2	50% RDNP
T_3	50% RDNP + 2 sprays of nano-DAP at 0.7% during active tillering and panicle initiation
T_4	75% RDNP
T_5	75% RDNP + 2 sprays of nano-DAP at 0.7% during active tillering and panicle initiation
T_6	75% RDNP + 1 spray of nano-DAP at 0.7% during tillering (for cost effectiveness)
T_7	75% recommended dose of N through fertilizer + 2 sprays of DAP at 2% during active tillering and panicle initiation
T_8	50% recommended dose of N through fertilizer + 2 sprays of DAP at 2% during active tillering and panicle initiation
T_9	Control (No nitrogen and phosphorus)
	The recommended dose of K were applied in the all treatments

enhances nutrient absorption and translocation more rapidly, increasing photosynthesis and dry matter accumulation, eventually leading to a higher straw yield. These findings align with the results of previous studies where it was reported that spraying nano di-ammonium phosphate on leaves improves plant metabolism and photosynthesis, which increases the number of flower clusters and grain development, leading to a substantial increase in crop yield (42, 43). Applying nano di-ammonium phosphate 30 days after planting and one week before flowering activates metalloprotease and enzyme functions essential for enhancing grain and straw yield. This approach ensures efficient nutrient delivery throughout the crop's growth cycle (44).

The highest total grains per panicle was recorded at 75% RDNP + 2 nano-DAP sprays (278), compared to 100% RDF (Table 4). The increase in the number of grains per panicle may be due to timely N delivery, which stimulates grain production and increases the overall count. Additionally, foliar spraying of nano urea improves photosynthate assimilation and translocation from source to sink, enhancing plant growth and development (45). Similarly, the number of filled grains per panicle was also higher in 75% RDNP + 2 nano -DAP sprays (264) compared to 100% RDF (Table 4). The higher number of filled grains per panicle in 75% RDNP + 2 sprays of nano-DAP is due to initial N from soil-applied urea, which supports biomass accumulation and a strong source-sink relationship. Foliar application of nano fertilizer during active tillering and panicle initiation further enhances N availability, boosting photosynthate production and translocation. This increases the number of filled grains per panicle (46). The increased number of filled grains is due to improved enzymatic activity, which enhances the formation and transport of photosynthates. This process likely contributes to more grains per panicle (47). 75% RDNP + 2 nano-DAP sprays had the fewest unfilled grains per panicle (14) (Table 4). A sufficient amount of N and P supply to the crop through nano fertilizer reduces the unfilled grains, which may be due to increased P content that helps in grain

Table 2. Effect of foliar application of nano-DAP on the growth and biochemical traits of rice grown during Navarai, 2024

Treatments	Plant height (cm plant^{-1})		Dry matter production (g plant^{1})		Total chlorophyll (mg g^{-1})		Soluble protein (mg g^{-1})	
	Active tillering	Panicle initiation	Active tillering	Panicle initiation	Active tillering	Panicle initiation	Active tillering	Panicle initiation
Recommended dose of fertilizer (RDF) + all package of practices	80.1^{ab}	97.6^{ab}	41^{b}	85^{b}	1.31^{a}	1.36^{ab}	16.09^{a}	17.12^{a}
50% RDNP	69.4^{cd}	86.2^{cd}	27^{f}	57^{d}	0.99^{c}	0.97^{de}	12.39^{de}	11.52^{d}
50% RDNP + 2 sprays of nano-DAP at the rate of 0.7% during active tillering and panicle initiation stage	75.5^{abc}	94^{abc}	40^{b}	83^{b}	1.28^{b}	1.32^{ab}	15.97^{a}	16.71^{ab}
75% RDNP	70.4^{cd}	88.8^{bcd}	31^{e}	67^{c}	1.06^{c}	1.02^{c}	12.83^{cde}	12.26^{d}
75% RDNP + 2 sprays of nano-DAP at the rate of 0.7% during active tillering and panicle initiation stage	81.23^{a}	99.6^{a}	46^{a}	98^{a}	1.36^{a}	1.43^{a}	16.83^{a}	17.74^{a}
75% RDNP + 1 spray of nano-DAP at the rate of 0.7% during tillering stage	74.97^{abcd}	93.3^{abc}	38^{bc}	79^{b}	1.26^{bc}	1.30^{ab}	15.44^{ab}	16.37^{ab}
75% recommended dose of N through fertilizer + 2 sprays of DAP at the rate of 2% during active tillering and panicle initiation stage	72.13^{bcd}	91.8^{abcd}	36^{cd}	80^{b}	1.19^{c}	1.22^{b}	14.23^{bc}	15.11^{bc}
50% recommended dose of N through fertilizer + 2 sprays of DAP at the rate of 2% during active tillering and panicle initiation stage	70^{cd}	90.3^{abcd}	34de	68^{c}	1.18^{c}	1.19^{bc}	13.46^{cd}	14.15^{c}
Control (No nitrogen and phosphorus)	66.8^{d}	82.8^{d}	17^{g}	33^{e}	0.91^{bc}	0.85^{e}	11.72^{e}	10.93^{d}
CD ($p \leq 0.05$)	8.47	9.64	3.34	8.40	0.09	0.14	1.65	1.68

Means present within the column with same letters are not significantly different at $p \leq 0.05$ and different letters represent the significant differences.

Table 3. Effect of foliar application of nano-DAP on chlorophyll index (SPAD units) at different growth stages

Treatments	Chlorophyll index (SPAD units)				
	Before spray	10 days after 1st spray	15 days after 1st spray	10 days after 2nd spray	15 days after 2nd spray
Recommended dose of fertilizer (RDF) + all package of practices	34.6[a]	38.6[ab]	40[ab]	43.2[ab]	43.4[ab]
50% RDNP	35[a]	33.2[c]	33.0[c]	31.9[d]	31.3[c]
50% RDNP + 2 sprays of nano-DAP at the rate of 0.7% during active tillering and panicle initiation stage	36.8[a]	38[ab]	39.7[ab]	41.2[ab]	42.0[ab]
75% RDNP	35.8[a]	35.4[bc]	35.9[bc]	34.6[cd]	34.8[c]
75% RDNP + 2 sprays of nano-DAP at the rate of 0.7% during active tillering and panicle initiation stage	35.5[a]	41.1[a]	41.8[a]	45.3[a]	46.2[a]
75% RDNP + 1 spray of nano-DAP at the rate of 0.7% during tillering stage	36.6[a]	38.9[ab]	39.1[abc]	41.2[ab]	41.8[ab]
75% recommended dose of N through fertilizer + 2 sprays of DAP at the rate of 2% during active tillering and panicle initiation stage	36.5[a]	38.1[ab]	38.7[ab]	41.1[ab]	41.7[b]
50% recommended dose of N through fertilizer + 2 sprays of DAP at the rate of 2% during active tillering and panicle initiation stage	36.8[a]	38[ab]	37.1[bc]	39[bc]	39.5[b]
Control (No nitrogen and phosphorus)	37.9[a]	35.6[c]	35.91[c]	30.8[c]	30.47[c]
CD ($p \leq 0.05$)	NS	4.31	4.35	4.43	4.48

Means present within the column with same letters are not significantly different at $p \leq 0.05$ and different letters represent the significant differences.

formation.

Among the treatments, 75% RDNP + 2 nano-DAP sprays had the highest weight of 3.3g, compared to 100% RDF and treatment with 50% recommended N with two nano-DAP sprays at 2% during critical growth stages (Table 4). The increased panicle weight is primarily due to enhanced photosynthetic activity, dry matter production, and efficient translocation of photosynthates facilitated by the foliar application of nano fertilizers. These fertilizers improve nutrient delivery by penetrating stomata and providing a timely nutrient supply (48).

Nitrogen and phosphorus use efficiency significantly improved by nano-DAP

The study showed nano-DAP treatments recorded higher N and P content in leaves than 100% RDF. 75% RDNP + 2 sprays of nano-DAP at the rate of 0.7% during active tillering and panicle initiation recorded higher N (1.19%) and phosphorous content (0.34%) content compared to 50% RDNP + 2 sprays of nano-DAP at the rate of 0.7% during active tillering and panicle initiation and 75% RDNP + 1 spray of nano-DAP at the rate of 0.7% during tillering (Fig. 5). This could be because nano fertilizers have a large surface area and small particle size that enhances their ability to penetrate plants from the applied surface, improving both nutrient uptake and use efficiency. This increased contact increases penetration and nutrient uptake, resulting in higher nutrient content and improved absorption (40). A 75% RDNP + 2 sprays of nano-DAP at the rate of 0.7% recorded increased N and PUE of 53.3 and 54.4 kg/kg, respectively, compared to 50% RDNP + 2 sprays of nano-DAP at the rate of 0.7% (52.5 and 53.4 kg/kg) and 100% RDF application (Fig. 6).

The higher NUE and PUE in rice may be due to increased biomass production associated with higher uptake of N and P. Rice generally has higher NUE for biomass (NUEb) and grain (NUEg) compared to other C3 crops like soybean and wheat, due to its lower N content in straw and grain. While rice's efficiency in N remobilization is lower than maize due to its C3 nature, improving grain yield and N concentration through physiological strategies can enhance NUE in rice (49). Phosphorus use efficiency in crop plants, measured as dry matter production per unit of P uptake, is typically higher than that of N and potassium (K). nano-DAP increases PUE by enhancing P availability and uptake with its larger surface area and controlled release features, which leads to improved crop yields and minimizes P losses compared to traditional fertilizers.

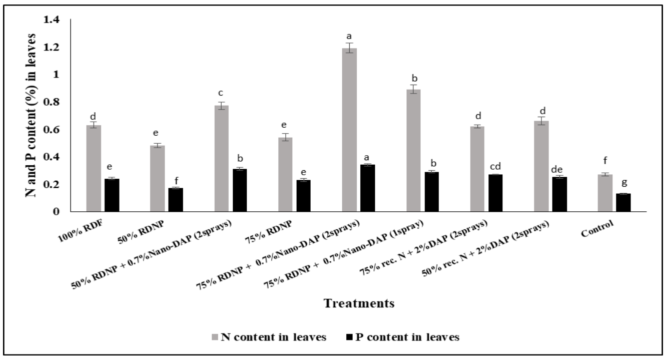

Fig. 5. Impact of nano-DAP on N and P content (%) in leaves of rice at harvest

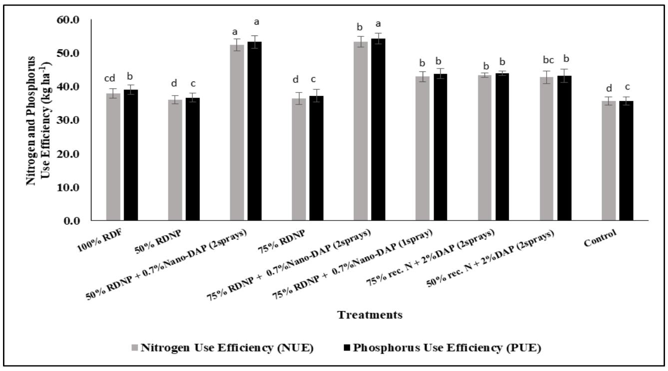

Fig. 6. Impact of nano-DAP on nitrogen and phosphorus use efficiency (kg kg^{-1}) in rice at harvest.

Table 4. Effect of foliar application of nano-DAP on yield attributes and yield of rice grown during Navarai, 2024

Treatments	Yield attributes						Yield	
	Number of productive tillers per hill	1000 seed weight (g)	Filled grains panicle^{-1}	Unfilled grains panicle^{-1}	Total grains panicle^{-1}	Panicle weight (g)	Grain Yield (kg ha^{-1})	Straw Yield (kg ha^{-1})
Recommended dose of fertilizer (RDF) + all package of practices	15^{b}	14.8^{a}	244^{a}	18^{d}	262^{a}	3.15^{ab}	6028^{ab}	8164^{a}
50% RDNP	12^{c}	12.7^{cd}	166^{c}	26^{b}	192^{cd}	2.34^{c}	4336^{e}	6294^{cd}
50% RDNP + 2 sprays of nano-DAP at the rate of 0.7% during active tillering and panicle initiation stage	15^{b}	14.5^{ab}	242^{a}	20^{c}	260^{a}	3.13^{ab}	5573^{bc}	7629^{ab}
75% RDNP	13^{bc}	13.0^{bcd}	176^{c}	24^{b}	200^{c}	2.36^{c}	4782de	6821^{bc}
75% RDNP + 2 sprays of nano-DAP at the rate of 0.7% during active tillering and panicle initiation stage	17^{a}	15.0^{a}	264^{a}	14^{d}	278^{a}	3.3^{a}	6378^{a}	8414^{a}
75% RDNP + 1 spray of nano-DAP at the rate of 0.7% during tillering	14^{b}	14.2^{abc}	208^{b}	23^{d}	231^{b}	2.85^{b}	5528^{bc}	7589^{ab}
75% recommended dose of N through fertilizer + 2 sprays of DAP at the rate of 2% during active tillering and panicle initiation stage	14^{b}	13.9^{abc}	182^{c}	26^{b}	208^{c}	2.53^{c}	5236^{cd}	7206^{b}
50% recommended dose of N through fertilizer + 2 sprays of DAP at the rate of 2% during active tillering and panicle initiation stage	13^{bc}	13.4^{abcd}	184^{c}	21^{c}	205^{c}	2.45^{c}	5048^{cd}	7180^{b}
Control (No nitrogen and phosphorus)	9^{d}	12.4^{d}	141^{d}	30^{a}	171^{d}	1.96^{d}	3348^{f}	5614^{d}
CD ($p \leq 0.05$)	1.73	1.59	23.46	2.93	25.57	0.31	596.45	834.23

Conclusion

nano-DAP application in rice cultivation has shown promising results in optimizing physiological and biochemical traits, resulting in enhanced crop performance, as indicated by higher yield compared to conventional soil application of fertilizers. This study demonstrates that nanotechnology-based foliar application, combined with a reduced recommended fertilizer dose, improved NUE and provided added benefits such as enhanced crop yield and sustainability compared to 100% soil application. .Among the nine treatment combinations tested, 75% of the recommended dose of fertilizer with two sprays of nano-DAP during the active tillering and panicle initiation stages proved the most effective in terms of morpho-physiological traits among them, leaf area (12.1%), dry matter production (15.3%) photosynthetic rate (12.3%), and stomatal conductance (12.2%) number of productive tillers (13.3%) were significantly impacted positively. The findings conclude that 25 % of soil applications of conventional fertilizers can be saved through two foliar applications of nano-DAP, in addition to achieving a higher yield in rice. Therefore, nano-DAP fertilizers can be effectively used at the field level to improve rice productivity by altering plant physiological parameters and making it a valuable asset for sustainable agriculture. However, further research is needed to understand the process at the molecular level and make this technology more viable and sustainable. Investigating the signal transduction pathways activated by nano-DAP can elucidate its effects on plant growth and photosynthesis. Additionally, studying the gene expression associated with nutrient transport will enhance our understanding of its impact on crop resilience and productivity.

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Authors' contributions

RRBS and PB participated in data collection and analysis. AS, DJ and GPK reviewed and composed the manuscript. All the authors read and approved the final version of the manuscript.

Compliance with ethical standards

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While preparing this work, the authors used ChatGPT for language assisting service. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publication’s content.

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