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Physiological, biochemical and soil microbial responses of green gram (*Vigna radiata* L.) to foliar nutrition of nano-fertilizers

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Abstract

A field experiment was conducted at Tamil Nadu Agricultural University (TNAU), Coimbatore, during the summer of 2024 to determine the physiological, biochemical and microbial responses of green gram to foliar nutrition of nano-fertilizers. The experiment was carried out in black heavy clay soil (vertisol). Treatments included recommended doses of fertilizer (RDF) at 100%, 75% and 50%, each combined with two rounds of foliar sprays (FS) using nano urea and nano DAP (di ammonium phosphate) (first spray and a second spray 15 days later), along with conventional urea and DAP sprays and a TNAU pulse wonder spray. Ten treatments were tested, each replicated three times in a randomized block design (RBD). Physiological (chlorophyll content), biochemical (soluble proteins, nitrate reductase activity) and microbiological (nodule number, microbial population) parameters were recorded at critical growth stages.

The combination of 100% RDF with two nano DAP sprays resulted in significantly higher total chlorophyll concentration (increases of 21.4%, 10.7%), soluble protein content (increases of 30.5%, 15.7%) and nitrate reductase activity (increases of 30.1%, 14.7%), with values at par with 75% RDF + nano DAP foliar sprays twice, as well as 100% RDF + TNAU pulse wonder in comparison to 100% RDF with conventional DAP sprays, respectively observed after 1st spray. Notably, the 50% RDF + nano DAP significantly increased nodule number and microbial population at critical stages.

Overall, the data demonstrated that 75% RDF + FS of nano DAP (twice) has improved physiological and biochemical changes in green gram plants, indicating a potential saving of phosphorus (P) fertilizers by up to 25%. Physiological responses were more pronounced with nano DAP than conventional DAP, likely due to its rapid absorption, quick assimilation and improved use efficiency.

Keywords

foliar spray; nano DAP; nano urea; root nodules; soil microbes; summer irrigated green gram

Introduction

Pulses play an important role in the Indian diet, especially for the predominantly vegetarian population. They are rich sources of carbohydrates (60-70%) and protein (20-25%) along with various vitamins, sugars and dietary fibers (1). Phytochemicals found in pulses, including flavonoids, phenolics, tannins, phytates, saponins, lectins, oxalates, phytosterols, peptides and enzyme inhibitors, all of these offer a wide range of health benefits (2).

In addition to their contribution to food grain production, cultivating pulses in rotation with other agricultural and horticultural crops enhances soil fertility. It also provides resilience against the negative effects of climate change in rainfed agricultural systems. This practice positively impacts soil properties, promotes efficient resource use, supports biological nitrogen fixation (BNF), improves nitrogen (N) management and contributes to the sustainability of food production (3, 4).

India is the largest producer (25.5 million tons) and consumer of pulses as of the 2021-2022 financial year (5-7). To meet the growing demand, India imports over 3.0 million tonnes of pulses annually, costing the country nearly USD 3.74 billion, and this figure is expected to rise in the coming years (8, 9). This issue emanates from the low production of pulses, mostly attributed to multi-nutrient shortages, climate change, rainfed farming, pests, diseases and a diminished source-sink ratio (10-12). Despite decades of efforts to improve pulse productivity, these strategies, due to limited adaptability, continue to be regarded as a “poor man’s crop” (13).

In intensive cropping systems, it was observed that commercial fertilizers and their management contribute approximately 35-40% of crop productivity potential. Recognizing this significance and aiming to sustain and enhance our country's annual food grain production, the Government of India provides substantial subsidies for major fertilizers, particularly nitrogenous fertilizers (urea). This subsidy policy has led to imbalanced N fertilization across India’s agro-climatic zones and the indiscriminate usage of N-based fertilizers led to groundwater nitrate pollution, especially in regions with intensive, irrigated agriculture.

Over recent decades, the use efficiencies for N, P and potassium fertilizers have remained stagnant at 30-35%, 18-20% and 35-40%, respectively. This poor efficiency of soil-applied fertilizers resulting in nutrient accumulation in the soil profile, which leaches into aquatic systems and contributes to eutrophication, resulting in water pollution across the major river basin of India. To mitigate the adverse effects of indiscriminate and imbalanced N fertilizer applications, developing nano-based fertilizer formulations with multiple functions is essential. These formulations can help address challenges related to low fertilizer use efficiency, imbalanced fertilization, multi-nutrient deficiencies and declining soil organic matter (14).

Traditional synthetic fertilizers often result in low nutrient use efficiencies (NUEs) because of their rapid release rates, which can surpass the plants nutrient absorption capacity. This causes nutrients to convert into other forms that are not easily accessible to crops (15). Unlike conventional fertilizers, nano-fertilizers slow the nutrient release rate while improving stability through mechanisms like aggregation or adsorption. Notably, these changes occur without altering the chemical composition, as they are influenced by factors such as pH-dependent processes, redox potential and the presence of ligands in the soil (16).

To address the multiple constraints in pulse cultivations, scientists have explored multiple strategies to promote pulse productivity using cutting-edge technologies. Nanotechnology, which involves atomic manipulation, allows for the precise design and development of products to deliver nutrients or inputs to the target site without any loss of nutrients (17-19). Several nano-based interventions have been attempted to promote pulses productivity. For instance, a recent study demonstrated that green gram seeds encapsulated with polyvinyl alcohol nano-fibers, fortified with macro and micronutrients, significantly improved productivity while drastically reducing fertilizer inputs (20). Interestingly, the nano-fiber coating with nutrients was comparable to the 100% RDF. In another study, nano-fibers were used to deliver the fungicide (tebuconazole) to control root rot, a severe disease in pulses (21). Further, a multi-nutrient capsule was developed using nano-zeolites fortified with essential nutrients for pulse cultivation (22). All of these literature clearly demonstrate that nanotechnology-enabled inputs improve crop productivity.

Commercial production of nano-enabled agricultural inputs has yet to materialize despite these data being published. The delay in their commercial production can be attributed to several factors. Regulatory hurdles, including safety assessments and environmental impact evaluations, remain significant barriers. Additionally, the high cost of scaling up nano fertilizer production and the lack of standardized protocols for large-scale agricultural use pose challenges. Farmers' reluctance to adopt new, unfamiliar technologies without long-term field trials further contributes to the slow transition from research to commercial application.

Applications of nanotechnology concepts and their product utilities are gaining momentum in various sectors, including energy production, conversion and storage; agricultural productivity enhancement; wastewater treatment and remediation; disease diagnosis and screening; drug delivery systems; food processing and storage; air pollution remediation; and vector and pest detection and control. To accelerate the adoption of nanotechnology and support nanotechnology-based inputs, the Government of India has initiated several schemes, like the intensification of research in high priority areas (IRHPAS), the national programme on smart materials (NPSM), nano science and technology initiative (NSTI) and the nano science and technology mission (NSTM) (23).

India is the first country to develop a DBT (Department of Biotechnology) regulatory guideline for evaluating nano-agricultural inputs, launched in 2020 (24). These guidelines served as the base document for notifying nano-fertilizers in the country. The first liquid IFFCO (Indian Farmers Fertiliser Cooperative Limited) nano urea was notified in 2021 (FCO, 2021) and subsequently, IFFCO liquid nano DAP was introduced for commercial application in 2023 (FCO, 2023). This innovative product, created using nanotechnology, reduces the particle size of urea, resulting in nano-scale urea particles. These smaller particles offer several benefits, including improved nutrient utilization efficiency, controlled-release properties and reduced N losses compared to conventional urea (25-28). In recent years, several studies have shown that nano-fertilizers improve the growth and yield of various crops, i.e., maize (29), pigeon pea (30) and green gram (31).

After introducing nano urea and nano DAP in the country, many insightful questions were asked, many of which remain unanswered. It is hypothesized that nano-fertilizers have distinctive characteristics, such as a high surface mass ratio, and that the dimensions of the micellar nanoparticles are less than 100 nm (32). Due to their extremely small size, nano-fertilizers get into the plant system rapidly and assimilate into the N and P pathways, which assist in minimizing the loss of nutrients and improving nutrient use efficiency (33, 34).

To test this hypothesis, a field experiment was conducted using gradient levels of conventional RDFs (100%, 75%, 50%) in combination with FS of nano-fertilizers (nano urea and nano DAP) and conventional fertilizers (urea and DAP), along with TNAU pulse wonder sprays. Physiological, biochemical and microbiological measurements were taken at various critical growth stages to gain insights into how nano-fertilizers promote pulse productivity.

Materials and Methods

A field experiment was conducted in the wetlands (11° N latitude and 77° E longitude, at an altitude of 426.7 m above MSL) of Tamil Nadu Agricultural University, Coimbatore, to assess the combined application of conventional fertilizers and foliar nano-fertilizers (nano urea and nano DAP) on physiological parameters, biochemical parameters, root nodulation in green gram (*Vigna radiata* L.) and soil microbial population during the summer season (March-May, 2023). The experimental soil was classified as clay loam, with a pH of 8.02 and an electrical conductivity (EC) of 0.30 dSm^{-1}. The soil organic carbon content was measured at 0.58%, with available N at 179.0 Kg ha^{-1}, P at 19.0 Kg ha^{-1} and potassium at 653.0 Kg ha^{-1}. The experiment was conducted in a RBD with 10 treatments; each replicated three times.

Treatments

The treatments were as follows:

T_1- 100% RDF (25:50:25:20 Kg N: P_2O_5 (phosphorus pentoxide): K_2O (potassium oxide) & S (sulphur) ha^{-1}); T_2 - 100% RDF + FS of TNAU pulse wonder at 5Kg ha^{-1} at peak flowering stage; T_3 - 100% RDF + FS of DAP at 2%; T_4 - 100% RDF + FS of urea at 1%; T_5 - 100% RDF + FS of nano DAP at 2 mL L^{-1}; T_6 - 100% RDF + FS of nano urea at 2 mL L^{-1}; T_7 - 75% RDF + FS of nano DAP at the rate of 2 ml L^{-1}; T_8 - 75% RDF + FS of nano urea at 2 mL L^{-1}; T_9 - 50% RDF + FS of nano DAP at 2 mL L^{-1}; T_{10} - 50% RDF + FS of nano urea at 2 ml L^{-1}.

The plot size was 3.9 x 3.0 m. green gram (var. Co. 8) was sown at 20 Kg per ha^{-1}, with a spacing of 30 cm x 10 cm. According to the treatment schedule, the necessary amounts of urea, single superphosphate and muriate of potash were determined and uniformly administered as basal fertilizers. Foliar nutrition applications were carried out at flower initiation (FI) and 15 days later. After that, the required quantities of nano DAP and nano urea liquid fertilizer (at the rate of 500 mL per acre), as well as conventional DAP (2%) and urea (1%) fertilizers, were applied at the rate of 500 liters of water ha^{-1} and sprayed at FI and 15 days after that. To ensure better nutrient absorption, FS were administered in the morning hours (before 10 AM). The crop was irrigated six times during the cropping period.

Chlorophyll measurement

Chlorophyll a, chlorophyll b and total chlorophyll were extracted using 80% acetone and measured at three stages: before FS, after the 1st spray and after the 2nd spray (35). Optical density (OD) values were recorded at 645 and 663 nm using a spectrophotometer (Microprocessor UV Single Beam) and expressed in mg g^{-1} of fresh weight.

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

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

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

Where,

OD - Optical density

V - Final volume of chlorophyll extract in 80% acetone

W - Weight of the leaf sample taken (g)

Soluble protein and nitrate reductase activity

The soluble protein content was estimated, with results expressed in mg g^{-1} of fresh weight (36). A standard experimental procedure was used to calculate the nitrate reductase activity (37) and the data were expressed in µg NO_2 (nitrogen dioxide) g^{-1} hr^{-1}.

Nodule count and microbial population

Total nodule count, effective nodules count plant^{-1} and nodules dry weight plant^{-1} were observed after the 1st spray and after the 2nd spray. The serial dilution plate technique was used to assess the soil microbial population (38). Freshly prepared nutrient agar media, Martins Rose Bengal agar media, and Ken Knight agar media were used to determine the soil bacterial, fungal and actinomycetes populations, respectively. Observations were taken before FS, after the 1st spray, after the 2nd spray, and at the post-harvest stage. The results are expressed as the mean colony-forming units (CFU).

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

Data collection and analysis

Data collected from field experiments and laboratory analyses were statistically analyzed (39). The critical difference was calculated at the 5% probability level for treatments showing a significant level of difference, while treatments not showing significance at this probability level were denoted as non-significant (NS).

Results and Discussion

Physiological parameters

Chlorophyll concentrations (a, b, and total) were measured in treatments that received different fertilizer gradients (100%, 75% and 50% RDF) combined with a FS of either nano-fertilizer (nano urea or nano DAP) or regular fertilizer (urea or DAP). Table 1 represents the data on physiological parameters measured at various growth stages.

Before the FS, treatments with 100% RDF registered significantly higher chlorophyll a, b and total chlorophyll concentrations. These values were on par with the 75% RDF treatment but were considerably higher than in the 50% RDF treatment. The data correspond with the observations of several authors who have shown positive and beneficial effects of fertilizers on enhanced chlorophyll concentrations (40, 41). There is a strong correlation between N and chlorophyll concentrations, which has led to the development of handheld gadgets like SPAD (soil plant analyzer development), which is commonly used for indirect onsite detection of N (42, 43). Nitrogen, a growth element and a constituent of chlorophyll, it is obvious that chlorophyll concentrations increased with higher levels of RDF. Combined application of N & P is also known to increase chlorophyll a, b and total chlorophyll (44).

After the first spray, green gram plants treated with 100% RDF + nano DAP spray registered the highest chlorophyll a (1.42 mg g^{-1}), chlorophyll b (1.08 mg g^{-1}) and total chlorophyll (1.59 mg g^{-1}) concentrations, which were on par with those treated with 75% RDF + nano DAP and 100% RDF + TNAU pulse wonder. As explained, chlorophyll concentration is directly related to the N content of the leaf (45). The total chlorophyll concentration in the best treatment (100% RDF + nano DAP) increased by 27.2%. On the other hand, TNAU pulse wonder and 75% RDF + nano DAP registered an increase of 22.4% and 20.0%, respectively, compared to the 100% RDF.

Similarly, after the second spray, 100% RDF + nano DAP spray registered the highest chlorophyll a (1.09 mg g^{-1}), chlorophyll b (0.85 mg g^{-1}) and total chlorophyll (1.28 mg g^{-1}) concentrations, which were also on par with 75% RDF + nano DAP and 100% RDF + TNAU pulse wonder. Total chlorophyll concentration in the 100% RDF + nano DAP increased by 20.2%, while TNAU pulse wonder and 75% RDF + nano DAP increased by 18.2% and 17.0%, respectively, compared to 100% RDF.

Higher application rates of N & P fertilizers increased the chlorophyll concentrations due to their direct nutritional impact (46). Interestingly, RDF application combined with nano DAP FS could increase the chlorophyll concentrations comparable to 100% RDF.

The significant increase in photosynthetic pigments (chlorophyll a, b and total chlorophyll) can be associated with the enhanced uptake of N, P and potassium nutrients. The application of N fertilizer boosts the content of photosynthetic pigments, improves light energy capture, enhances photochemical efficiency, and promotes both quantum efficiency as well as the self-protection capabilities of photosystem II (PSII). This mechanism is likely linked to the catalytic effect of N fertilizer on the activity of light-activated enzymes in plant leaves, which enhances the energy capture efficiency of the PSII reaction center (47).

Nanomaterial reactivity enhances nutrient uptake, leading to greater utilization efficiency and reduced nutrient losses compared to conventional fertilizers. The absorption, distribution and accumulation of nano-fertilizers are dependent upon parameters such as soil pH, organic matter content and texture, alongside inherent nanoparticle characteristics, including particle size and coating techniques, which affect nutrient acquisition, distribution and utilization in crops (48-50).

Table 1. Effect of varied doses of basal fertilizer and foliar application of nano-fertilizers on physiological parameters of summer irrigated green gram

Treatment	Chlorophyll-a (mg g^{-1})			Chlorophyll-b (mg g^{-1})			Total chlorophyll (mg g^{-1})		
	Before spray	After 1st spray	After 2nd spray	Before spray	After 1st spray	After 2nd spray	Before spray	After 1st spray	After 2nd spray
T_1	0.76	1.03	0.83	0.64	0.83	0.66	0.85	1.25	1.02
T_2	0.96	1.36	1.06	0.81	1.05	0.82	1.07	1.53	1.25
T_3	0.87	1.24	0.96	0.75	0.97	0.75	0.98	1.42	1.16
T_4	0.81	1.16	0.87	0.68	0.87	0.70	0.91	1.33	1.09
T_5	0.98	1.42	1.09	0.83	1.08	0.85	1.10	1.59	1.28
T_6	0.90	1.28	0.99	0.76	0.99	0.78	1.00	1.46	1.19
T_7	0.95	1.34	1.04	0.79	1.03	0.80	1.04	1.50	1.23
T_8	0.83	1.19	0.91	0.70	0.90	0.71	0.93	1.36	1.11
T_9	0.74	1.00	0.79	0.62	0.80	0.64	0.83	1.23	1.02
T_{10}	0.71	0.99	0.78	0.61	0.78	0.63	0.82	1.20	1.00
SE (Standard Error)	0.03	0.05	0.04	0.03	0.04	0.03	0.04	0.05	0.04
CD (Critical Difference) (p=0.05)	0.07	0.11	0.08	0.06	0.08	0.06	0.08	0.11	0.08

The physiological characteristics of plants also influence the absorption of nanoparticles. Typically, these particles enter through trichomes, stomata, stigmas and hydathodes, transported within the plant via the phloem and xylem. Nanoparticles can also enter through meristematic tissue at root tips or lateral roots, utilizing lesions in the Casparian strip. To reach the epidermal layer of the roots, nanoparticles pass via cell walls and plasma membranes before entering vascular bundles, particularly the xylem. Size plays a critical role in nanoparticle entry through the cell wall pores or stomata and is directly correlated with nanoparticle absorption. Although the pore size of 3 to 8 nm in the cell wall is generally considered too small for nanoparticle entry, studies show these pores can expand to accommodate them. Reducing conventional fertilizer's particle size to the nanoscale and modifying surface properties could reduce the required dosage compared to traditional fertilizers (51-53).

Foliar application of nano DAP facilitates easy and rapid absorption and assimilation, leading to quick entry into the plant metabolic pathway while minimizing the nutrient loss, a unique characteristic of nano-fertilizers (54). Several studies have reported similar increases in chlorophyll concentrations with nano DAP FS (55, 56). In comparison, plant response to urea was lower due to its single-nutrient content. Pulses, which have a higher P requirement, respond more effectively to DAP fertilization than to urea (57).

Biochemical attributes

Before the FS, treatments with 100% RDF had significantly greater soluble protein and nitrate reductase activity levels. These values were comparable with 75% RDF but much higher than 50% RDF. Table 2 represents the data on biochemical parameters observed at different crop growth stages.

After the first spray, green gram plants treated with 100% RDF + nano DAP spray showed the highest levels of soluble protein (14.2 mg g^{-1}) and nitrate reductase activity (152.4 µg NO_2 g^{-1} hr^{-1}), comparable to those treated with 75% RDF + nano DAP and 100% RDF + TNAU pulse wonder. Soluble protein and nitrate reductase activity in the 100% RDF + nano DAP treatment increased by 30.5% and 30.1%, respectively. In comparison, TNAU pulse wonder showed increases of 28.5% in soluble protein and 27.5% in nitrate reductase activity, while the 75% RDF + nano DAP showed increases of 26.7% in soluble protein and 25.4% in nitrate reductase activity, relative to 100% RDF. These changes are closely coincided with the absorption of nano DAP and the associated metabolic changes in the plants. Nitrate reductase is a substrate-inducible enzyme that facilitates nitrate reduction into nitrite (58). Since nano-fertilizers are absorbed more rapidly through the leaves, the substrate may have induced higher nitrate reductase activity, eventually resulting in higher activities (59). The soluble protein concentration reflects the total biochemical changes within the plant, enabled by the combined uptake of both N and P together (60, 61).

Similarly, after the second spray, the 100% RDF + nano DAP FS recorded the highest soluble protein (9.47 mg g^{-1}) and nitrate reductase activity (98.1 µg NO_2 g^{-1} hr^{-1}). It was on par with 75% RDF + nano DAP and 100% RDF + TNAU pulse wonder. The best-performing treatment, 100% RDF + nano DAP, soluble protein increased by 29.9% and nitrate reductase activity by 31.1%. TNAU pulse wonder increased 25.6% in soluble protein and 29.7% in nitrate reductase. In comparison, the 75% RDF + nano DAP treatment showed increases of 24.5% in soluble protein and 29.2% in nitrate reductase activity, compared to the 100% RDF treatment. The foliar application of N as a nano-fertilizer improved N uptake by plant leaves, resulting in elevated leaf chlorophyll levels.

Glutamine synthetase (GS) is a key enzyme that converts amino acids into amides and facilitating the transformation of glutamate into glutamine. GS plays a crucial regulatory role in N assimilation and utilization in plants. Foliar applications of nano DAP at critical growth stages significantly enhanced GS activity, leading to improved N assimilation (62) in all green gram growth and development stages. A similar trend was observed after the second spray, indicating sustained effects of the nano DAP FS. This response was not observed when urea, conventional DAP, or nano urea were applied individually.

Soil microbial attributes

Root nodules: After the first spray, the treatment with 50% RDF + nano DAP spray registered the highest total nodules (10.4), effective nodules (6.13) and nodule dry weight (30.7mg $plant^{-1}$), on par with 50% RDF + nano urea treatment (Table 3). In the best treatment, 50% RDF + nano DAP, total nodules, effective nodules, and nodule dry weight increased by 26.2%, 17.9% and 25.0%, respectively, while the 50% RDF + nano urea registered an increment of 22.7%, 16.4 % and 20.1%, respectively, in comparison to treatment with 100% RDF only.

Table 2. Biochemical parameters influenced by the integrated application of various doses of basal fertilizer and nano-fertilizers foliar spray

Treatment	Soluble protein (mg g^{-1})			Nitrate reductase activity (µg NO_2 g^{-1} hr^{-1})		
	Before spray	After 1st spray	After 2nd spray	Before spray	After 1st spray	After 2nd spray
T_1	8.01	9.88	6.64	81.7	106.5	67.6
T_2	10.48	13.8	8.93	116.5	146.9	96.2
T_3	9.33	12.0	7.88	99.5	130.1	81.3
T_4	8.22	10.7	7.19	91.4	116.5	73.3
T_5	10.57	14.2	9.47	119.7	152.4	98.1
T_6	9.62	12.6	8.12	103.3	135.4	85.5
T_7	10.40	13.5	8.79	110.9	142.8	95.5
T_8	8.73	11.2	7.38	94.4	120.7	76.4
T_9	7.94	9.64	6.43	79.0	101.3	65.3
T_{10}	7.87	9.49	6.16	78.3	98.9	63.7
SE	0.38	0.63	0.41	5.24	6.78	4.33
CD (p=0.05)	0.80	1.32	0.87	11.02	14.25	9.09

Similarly, after the second spray, the 50% RDF + nano DAP spray recorded the highest total nodules (20.5), effective nodules (14.6), and nodules dry weight (104 mg plant^{-1}), again comparable to the 50% RDF + nano urea. In the 50% RDF + nano DAP treatment, total nodules, effective nodules, and nodules dry weight increased by 23.3%, 25.6% and 23.7%, respectively, while the 50% RDF + nano urea treatment registered an increase of 21.4%, 22.8% and 21.7%, respectively, in comparison to 100% RDF.

The application of a lower N dose to the soil considerably improved the plant's genetic nodulation potential and its effectiveness in growth and development, corroborating findings related to soybean (63). Whereas the addition of nanoscale fertilizer significantly increases root nodules by promoting lateral root formation and influencing the initial step of bacterial infection, as reflected in the increased weight of root nodules (64). At the molecular level, nano DAP and nano urea facilitate lateral root formation and bacterial infection by improving the bioavailability of essential nutrients like N and P. The smaller particle size of these nano fertilizers allows for more efficient uptake by root cells, stimulating auxin signaling, a key hormone in root development. Additionally, they promote better nutrient absorption, which supports symbiotic interactions with beneficial soil bacteria, facilitating more effective root colonization and infection

Soil microbial population

Soil microbial populations (bacteria, fungi and actinomycetes) were measured in treatments receiving various fertilizer gradients (100%, 75% and 50% RDF) in combination with either nano-fertilizer FS (nano urea or nano DAP) or conventional fertilizers (urea or DAP). The results are presented in Table 4. Before FS, the 50% RDF (T_9) treatments registered a significantly higher soil microbial population, on par with T_{10}.

After the first spray, significantly higher bacterial (40.8 CFU), fungal (31.9 CFU), and actinomycetes (20.7 CFU) populations were observed in treatments receiving the 50% RDF + nano DAP spray, at par with 50% RDF + nano urea FS. In the best treatment, 50% RDF plus nano DAP, bacterial, fungal and actinomycetes populations increased by 27.8%, 21.8% and 24.1%, respectively. In comparison, the 50% RDF plus nano urea treatment showed increases of 23.9%, 20.2% and 22.1%, respectively, compared to 100% RDF.

Similarly, after the second spray, the 50% RDF + nano DAP spray registered the highest bacteria (46.8 CFU), fungi (37.6 CFU) and actinomycetes (28.6 CFU) populations and were on par with the 50% RDF + nano urea. In the best treatment, 50% RDF plus nano DAP, bacteria, fungi and actinomycetes population increased by 23.4%, 23.4% and 26.8%, respectively, while the 50% RDF + nano urea registered an increase of 20.8%, 21.8% and 24.8%, respectively, in comparison to 100% RDF.

In post-harvest soil analysis, treatments that received 50% RDF + nano DAP spray recorded the highest bacteria (50.8 CFU), fungi (40.0 CFU) and actinomycetes (32.6 CFU) population, which were comparable with 50% RDF + nano urea. Bacterial, fungal, and actinomycetes populations in the 50% RDF + nano DAP treatment increased by 22.2%, 22.7% and 26.6%, respectively, while the 50% RDF + nano urea registered an increase of 20.4%, 19.5% and 25.0%, respectively, in comparison to 100% RDF.

Table 3. Influence of various levels of basal fertilizer and foliar application of nano-fertilizers on root nodules of summer irrigated green gram

Treatment	No. of nodules		No. of effective nodules		Nodules dry weight (mg plant^{-1})	
	After 1st spray	After 2nd spray	After 1st spray	After 2nd spray	After 1st spray	After 2nd spray
T_1	7.68	15.8	5.03	10.9	23.0	79.4
T_2	7.85	16.3	5.17	11.2	23.8	81.0
T_3	8.37	16.7	5.29	11.4	24.9	84.7
T_4	8.45	17.0	5.32	11.8	25.8	85.3
T_5	8.73	17.3	5.38	12.1	26.2	87.8
T_6	8.82	17.6	5.46	12.3	26.4	89.3
T_7	9.58	18.7	5.80	13.3	28.2	95.6
T_8	9.26	18.2	5.62	12.8	27.1	93.2
T_9	10.4	20.5	6.13	14.6	30.7	104.0
T_{10}	9.93	20.0	6.02	14.1	28.8	101.4
SE	0.34	0.65	0.15	0.51	1.01	3.15
CD (p=0.05)	0.71	1.37	0.31	1.07	2.11	6.62

Table 4. Influence of different doses of basal fertilizer and foliar application of nano-fertilizers on soil microbial population (cfu g^{-1} of soil) of summer irrigated green gram

Treatment	Bacteria (x 10^6 g^{-1} of soil)				Fungi (x 10^4 g^{-1} of soil)				Actinomycetes (x 10^3 g^{-1} of soil)			
	Before spray	After 1st spray	After 2nd spray	Post-harvest	Before spray	After 1st spray	After 2nd spray	Post-harvest	Before spray	After 1st spray	After 2nd spray	Post harvest
T_1	12.8	29.4	35.8	39.5	12.0	25.0	28.8	30.9	8.43	15.7	20.9	23.9
T_2	13.0	30.7	36.0	40.2	12.0	25.6	29.5	31.5	8.65	16.5	21.9	24.4
T_3	13.7	33.0	38.0	41.4	12.7	27.1	31.2	33.0	8.81	17.0	22.9	25.9
T_4	13.8	33.7	38.5	42.8	12.8	27.5	32.0	33.2	8.94	17.4	23.2	26.2
T_5	14.0	34.1	39.8	43.8	12.9	27.9	32.1	33.8	9.03	17.6	23.8	26.8
T_6	14.4	34.3	40.4	44.2	13.1	28.2	32.5	34.2	9.17	17.9	24.4	27.1
T_7	15.3	37.2	43.4	47.4	13.8	30.0	35.0	37.1	9.64	19.2	26.0	29.7
T_8	14.8	36.6	42.9	46.1	13.6	29.1	34.2	36.3	9.46	18.8	25.9	28.9
T_9	16.3	40.8	46.8	50.8	14.7	31.9	37.6	40.0	10.28	20.7	28.6	32.6
T_{10}	15.7	38.7	45.2	49.7	14.2	31.3	36.9	38.3	9.95	20.2	27.8	31.9
SE	0.43	1.37	1.48	1.56	0.35	0.85	1.15	1.33	0.24	0.63	0.97	1.20
CD (p=0.05)	0.91	2.87	3.11	3.27	0.73	1.80	2.42	2.78	0.51	1.33	2.04	2.53

Various factors, including soil type and properties (pH, organic matter content, texture and ionic strength) influence the impact of nano fertilizers on microbial communities. Additionally, nanoparticles type, size and concentration play a crucial role in sustaining microbial populations by interacting with soil microorganisms, which can have either beneficial or toxic effects on the soil microbial community (65, 66). N fertilization may have contributed to increased root biomass, which enhanced the overall population of soil microorganisms, including fungi and bacteria (67). Applying nanoparticles, such as nano clay, nano chitosan and nano zeolite, can enhance soil health, leading to improved microbial activity (68).

Due to their smaller size and larger surface area, nanoparticles can penetrate microbial cells, impacting the growth and development of microorganisms. However, beneficial soil microorganisms that break down organic matter and sustain long-term soil fertility may be vulnerable to the broad-spectrum antimicrobial product. Over time, repeated exposure to these nano-formulations could negatively impact soil fertility and crop yields (69). Nanomaterials may pose toxicity to beneficial soil microbes depending on the nature of the nanomaterial, particle size, dose, concentration, soil type and soil moisture levels. When the concentration of nanomaterials exceeds a certain threshold level, the growth of several beneficial soil microbes may be inhibited, significantly affecting both microbial biomass and community structure (70). Recent studies suggest that biogenic nanomaterials are less harmful to soil microbial communities than their chemically synthesized counterparts, leading to the advocacy for their use to mitigate nanotoxicity in soils (71).

Conclusion

The data demonstrated that soil application of 75% RDF in combination with two rounds of nano DAP FS at FI and 15 days thereafter, assisted in retaining higher chlorophyll concentrations, nitrate reductase and soluble proteins that facilitated the plants to grow better than their counterpart with either lower or higher doses of fertilizers. On the other hand, effective nodules and soil microbial population were higher under 50% RDF in combination with nano DAP. Hence, it can be concluded that up to 25% of the RDF can be saved by applying nano fertilizers at critical growth stages, without significantly impacting plant growth, yield, or profitability.

Additionally, the reduced use of synthetic chemical fertilizers not only maintains higher yields but also contributes to reducing environmental and soil pollution. Despite these promising results, additional research is needed to validate these findings across different pulse crops grown in various soil types and agro-ecosystems and to evaluate the long-term impacts on soil health and microbial diversity. Further, field experiments should optimize the dosage and timing of nano-fertilizer applications to maximize crop productivity, profitability and sustainability.

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Authors' contributions

BR conducted field experiments and prepared the manuscript. MS formulated the research project and served as the chairman of the advisory committee. GP assisted in formulating the research project and facilitated field experiments. MG assisted in microbiological studies and analysis. VBRP contributed to physiological studies and analysis. KSS provided technical guidance and inputs for manuscript preparation and modification. All the authors have approved the final manuscript.

Compliance with ethical standards

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

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