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RESEARCH ARTICLE

Starch-based nanoformulation of rhizobium enhancing growth, nodulation and yield in black gram

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Abstract

Black gram (*Vigna mungo* (L.) Hepper) is a significant pulse crop due to its nutritional value and productivity within the Indian subcontinent. Pulses possess the inherent ability to fix atmospheric nitrogen into the soil through a symbiotic association with the *Rhizobium,* a genus of essential soil microorganisms that facilitate nitrogen fixation in legumes. However, conventional biofertilizers encounter challenges related to limited shelf life, reduced cellular viability and inefficacy of carriers. This research investigates the encapsulation of *Rhizobium* using starch nanoparticles and sodium alginate to mitigate these drawbacks. The nanoformulations was assessed, with a mean droplet size and polydispersity index of 292 nm and 0.056, respectively. FTIR analysis confirmed the successful incorporation of all functional components. SEM imaging illustrated a uniform distribution of the formulation over the seed coat. Release kinetics displayed an initial burst release, followed by controlled and sustained release phases. The nanoformulations effectively protects the cells from adverse conditions in soil with different pH levels. A pot culture experiment with Black gram was conducted to evaluate the efficacy of the nanoformulations. The findings indicated significant enhancements in growth parameters, nodulation and yield characteristics compared to the control and conventional treatments. The highest dosage of nanoformulation at the rate of 15ml/8Kg (T_6) consistently surpassed other treatments, demonstrating improved shoot and root lengths, chlorophyll content, soluble protein and enzyme activities. Treatment T_6 gains the highest nodule count (approx. 100/plant) and maximized yield parameters. This investigation highlights the potential of *Rhizobium* nanoformulations in promoting plant growth, nodulation and yield in black gram. It offers a promising strategy for sustainable agricultural practices and addresses the limitations of traditional biofertilizers.

Keywords

Rhizobium; nanoformulations; black gram; enzyme activity; SEM; controlled release

Introduction

Black gram (*Vigna mungo* (L.) Hepper) is a nutrient-rich legume commonly cultivated in South and Southeast Asia, with India being the largest producer. However, the crop faces several biotic and abiotic stresses, leading to significant yield losses. It plays a vital role in vegetarian diets in India due to its high

nutritional value. Mature black gram seeds contain approximately 60% carbohydrates, 24%-26% protein, 1.3% fats, along with several essential amino acids, nutrients and vitamins (1, 2). India produced about 20.55 lakh tons in 2023-2024, with Tamil Nadu being the fourth-largest producer of black gram (3).

In modern agriculture, chemical fertilizers have become prevalent, especially after the Green Revolution, where farmers increasingly relied on chemical fertilizers, pesticides, insecticides and herbicides to boost crop yield (4). Synthetic or inorganic fertilizers are commonly employed in agriculture due to their quick nutrient delivery, significantly enhancing plant growth and productivity (5). However, excessive use of chemical fertilizers poses environmental hazards, including groundwater contamination and eutrophication of water bodies (6, 7). High nitrogen fertilization can inhibit mycorrhizal colonization of plant roots and interfere with the symbiotic nitrogen fixation process . It also accelerates the decomposition of soil organic matter, resulting in the deterioration of soil structure (8).

Pulses have the unusual capacity to use soil bacteria (*Rhizobium*) to fix nitrogen from the atmosphere in a natural process known as biological nitrogen fixation. These helpful bacteria mobilize essential nutrients for plant metabolism (9, 10). Biofertilizers are an affordable substitute for chemical fertilizers that improve soil fertility and plant growth by stimulating microbial interactions and increasing the availability of nutrients. They also reduce environmental pollution, protect against soil-borne diseases and promote plant growth under stress conditions (11-13), with lower production costs compared to synthetic fertilizers (14).

Biofertilizers face several technological challenges, including degradation risks due to limited shelf life and potential mutations during fermentation or storage. Their development is complicated by the inability to form spores. Careful handling and specialized transport are required to mitigate the effects of external factors. Additionally, selecting a suitable carrier for biofertilizer formulation presents further difficulty (14, 15). The introduction of nano-biofertilizers resolves the problems associated with conventional biofertilizers by encapsulating viable cells in a biodegradable matrix, which extends their shelf life and effectiveness under various conditions with reduced mass (16).

Microbial inoculants can be applied through seed treatment or soil application. Seed treatment is more cost-effective and has proven to be efficient in the rhizosphere soil. By delivering the inoculum directly to the seed, a high concentration of viable microbes can quickly colonize the roots as the seedlings emerge (17).

Starch, a renewable polymer, is synthesized by many plants to store energy and is the second most abundant biomass material in nature (18). Starch is a complex carbohydrate (polysaccharide) with numerous glucose units. Polymers like starch, sodium alginate and other biopolymers are widely studied for microbe encapsulation due to their biodegradable and eco-friendly nature (19). However, native starch's water solubility and weak mechanical properties limit its applications. Nano starch, with higher solubility and increased surface area, enhances encapsulation efficiency, while sodium alginate is a protective barrier for encapsulated microbes. Starch nanoparticles (SNPs) with diameters of 300-400 nm are produced through the nanoprecipitation method, using a sodium hydroxide and urea solvent system to reduce starch size (20).

Nanotechnology has found numerous applications in various industries and has driven improvements and innovations in agriculture through low-mass fertilizers. Nanoencapsulation provides a possible solution by extending shelf life and controlling microbial release, overcoming the constraints of traditional formulations and increasing application efficiency in the field (21). This study explores the formulation of *Rhizobium* with nano starch, characterizes the nanoformulations, and tests its efficacy through a pot culture experiment on black gram.

Materials and Methods

Materials

The formulation comprised sago starch, sodium alginate, Tween 80, Span 80, glycerol, and palm oil. Vamban 8 black gram seeds were obtained from the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore. *Rhizobium spp*. (Cog 15) pure culture slants were sourced from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore.

Methods

Preparation of nano starch-based formulation

A novel nanoformulations was prepared using nano starch, sodium alginate, surfactant (Tween 80, Span 80), stabilizer (Glycerol) and palm oil. The aqueous phase was prepared by mixing 2% nano starch, 1% sodium alginate, 2 ml concentrated *Rhizobium* cell mass (10^9 CFU/ml) and Tween 80 in distilled water under constant stirring. The organic phase was prepared by combining 9% palm oil, 8% glycerol and Span 80 under continuous magnetic stirring. The nanoformulations were produced by gradually adding the organic phase (19%) to the aqueous phase (81%) at 600 rpm. After mixing the two phases, the formulation was subjected to high-shear mixing in the magnetic stirrer.

Characterization of *Rhizobium*-based nanoformulations

The particle size of the formulation was analyzed using a particle size analyzer (Horiba Scientific Nano Partica SZ-100), which operates on the principle of dynamic light scattering. The surface of the nanoformulation-coated seed was examined using a scanning electron microscope (Quanta 250). The treated seeds were sputter-coated with gold to enhance conductivity. The seeds were mounted on double-sided conductive carbon tape, which was then attached to a stub and placed in the chamber. Images were captured upon interaction with the electron beam. Fourier Transform Infrared (FTIR) spectroscopy (JASCO FT-IR-6800) was performed to analyze the possible physicochemical interactions among the various components of the formulation. The prepared nanoformulations were irradiated and scanned in the 4000 cm $^{-1}$ to 400 cm^{-1} wave range to obtain the FTIR spectra, which were compared with the spectra of the functional

components.

Release kinetics and viability test

The release kinetics and viability of *Rhizobium* microbes from the nanoformulations under saline conditions were assessed by placing 2 ml of the nanoformulation inside a dialysis membrane, which was then submerged in phosphate buffer (pH 7.4) under stirring conditions for 30 days. Samples were taken regularly and plated onto Congo Red Yeast Extract Mannitol Agar media. Colonies were counted after incubation at room temperature (25°C) (22).

Viability of the *Rhizobium* nanoformulations at different pH levels

The viability of *Rhizobium* in the nanoformulations at different pH levels was determined by inoculating the nanoformulations into the soils with varying pH levels. The different pH soils (3, 5, 7, 9, 11) were prepared by adding HCl and NaOH to sterilized soils. A 10g sample of each soil pH was mixed with 1 ml of the nanoformulations in separate containers. These soils were left undisturbed for 30 days. After 30 days, 1g of soil sample from each treatment was serially diluted and plated onto Congo Red Yeast Extract Mannitol Agar Media. The viability of the cells was determined by the number of colonies formed in the plates.

Pot culture experiment in black gram

A black gram pot culture experiment was conducted at the Centre for Agricultural Nanotechnology, Tamil Nadu Agricultural University, Coimbatore, focusing on *Rhizobium* nanoformulations. The experiment utilized genetically pure Vamban 8 seeds. The experiment was conducted with seven treatments and three replications, as shown in Table 1. The pots with a diameter of 30 cm were chosen. The seeds were surface sterilized with 2% sodium hypo chloride solution and washed repeatedly with distilled water. Eight seeds were sown for each pot and then thinned to six plants after ten days. Growth parameters, including shoot and root length, were observed at 15, 30 and 45 days after sowing (DAS) using a centimeter scale. For these measurements, plants were meticulously pulled and rinsed with water and all nodules were enumerated on the 30th DAS. Biochemical attributes, such as total chlorophyll (23), soluble protein (24), nitrate reductase (25) and dehydrogenase activity (26) were measured at 30 and 45 DAS. Yield parameters were measured manually, including pods per plant, seeds per pod and total yield. The data collected from multiple tests were statistically evaluated using OPSTAT. The critical differences (CD) were determined using a 5% probability level.

Table 1: Treatment details of Nanoformulated *Rhizobium* on black gram in pot culture experiment

T_1	Untreated seeds- Control
T_2	Starch-based *Rhizobium* Nano formulation at the rate of 3ml/8kg
T_3	Starch-based *Rhizobium* Nano formulation at the rate of 6ml/8kg
T_4	Starch-based *Rhizobium* Nano formulation at the rate of 9ml/8kg
T_5	Starch-based *Rhizobium* Nano formulation at the rate of 12ml/8kg
T_6	Starch-based *Rhizobium* Nano formulation at the rate of 15ml/8kg
T_7	*Rhizobium* biofertilizer at the rate of 50ml/8kg

Results and Discussion

Particle size and polydispersity index

The mean droplet size, polydispersity index and zeta potential of the nanoformulations were measured to assess the formulations' size and stability, as shown in Fig. 1. The mean droplet size was 292 nm, with a polydispersity index of 0.056, confirming that the emulsion contained nanosized, evenly distributed droplets along the matrix. The zeta potential value was -19.7 mV, showing that the formulation is moderately stable.

Fourier-transform infrared (FT-IR) spectroscopy

The FTIR spectrum of the nanoformulations demonstrates the successful incorporation of key functional groups from its components, as evidenced by the specific peak correlation shown in Fig. 2. The O-H stretching peak at 3370 cm^{-1} in the nanoformulations closely aligns with those in starch (3336 cm^{-1}) and sodium alginate (3305 cm^{-1}) (27). C-H stretching vibrations at 2922 cm^{-1} and 2859 cm^{-1} correspond to similar peaks in palm oil, span 80 and tween 80, indicating the presence of asymmetric and symmetric alkane groups, respectively. The C=O stretching at 1734 cm^{-1} closely matches carbonyl peaks in palm oil (1743 cm^{-1}), Span 80 (1736 cm^{-1}) and Tween 80 (1733 cm^{-1}), confirming the preservation of

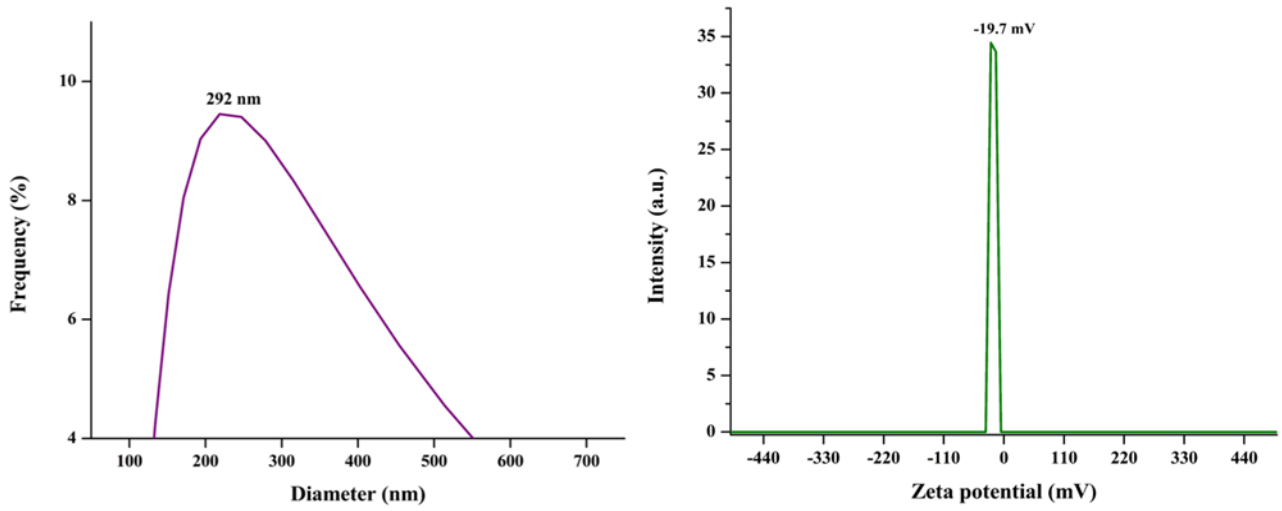

Fig. 1: Particle size determination and Zeta potential of the nanoformulations

ester groups in nanoformulations. These results are on par with (28). The related peaks at 1643 cm_{-1} (O-H stretching) correspond to similar vibrations in starch and sodium alginate (27). The CH_2 scissoring peak at 1458 cm^{-1} in nanoformulations aligns with peaks in palm oil, Span 80 and Tween 80. C-O stretching vibrations correlate with peaks between 1150 cm^{-1} and 1075 cm^{-1} in Tween 80, Span 80 and palm oil and these findings correlate with FTIR results of (29). Finally, the C-H out-of-plane bending at 943 cm^{-1} matches a peak in Tween 80. This comprehensive correlation of peaks demonstrate that the nanoformulations effectively integrate the chemical structures of all its components while suggesting potential interactions between them in the final formulation.

Release kinetics of the *Rhizobium* from the nanoformulations

The release dynamics of *Rhizobium* from the nanoformulations in phosphate buffer displayed a multi-phase release over the 30-day timeframe (Fig. 3). In the initial stage, a rapid release was observed on the second day, followed by a regulated release

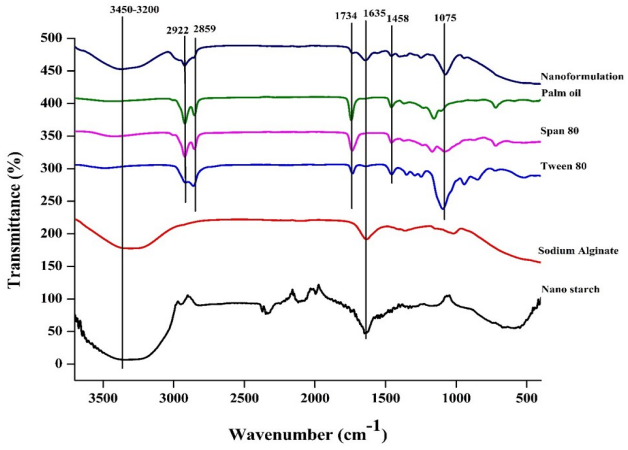

Fig. 2: FT-IR spectra of the nanoformulations and all its components

phase that continued for several days. After the 10th day, an increased release was observed, which evolved into a sustained release interval. A pronounced release occurred as the study neared the 28th day. The release kinetics of *Rhizobium* from the nanoformulations illustrate a sophisticated release profile that may offer significant benefits for agricultural applications. The initial surge in release on day 2 likely provides an immediate population of *Rhizobium* to facilitate colonization in plant roots. This is succeeded by a regulated release phase, which may aid in sustaining a consistent population of *Rhizobium* within the soil over an extended duration. Our results align with findings from a study (30), which reported that a microbe formulation with sodium alginate gives a more uniform sustained release profile. Additionally, the combination of sodium alginate and starch protects the bacterial cells and offers a sustained release, supporting our result that the starch and sodium alginate combination in the emulsion prevents the release of *Rhizobium* (31). The stabilized nanoformulations provide a more consistent release rate and improved survivability in phosphate buffer.

Viability of the *Rhizobium* nanoformulations at different pH levels

The impact of nanoformulation *Rhizobium* was assessed in pre-sterilized soils with different pH levels (3, 5, 7, 9 and 11)

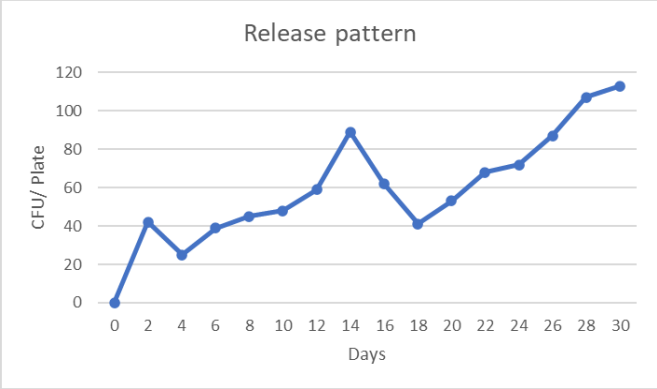

Fig. 3: Release pattern of the *Rhizobium* in phosphate buffer from the nanoformulations

over a 30-day incubation period. After 30 days, the soil samples were serially diluted and plated onto growth media at dilutions of 10^{-7}, 10^{-8} and 10^{-9}. Across all dilutions, cell colonies were observed on the plates, indicating the survival and proliferation of *Rhizobium* in varying pH environments. Soil with a neutral pH (7) demonstrated the highest number of cell colonies across all dilutions, indicating that neutral conditions were optimal for *Rhizobium* proliferation . In contrast, soils with extreme pH levels (3, 5, 9 and 11) showed reduced colony counts than pH 7. In acidic or highly alkaline conditions, the cell membrane integrity of *Rhizobium* can be compromised, leading to reduced survivability and nodulation. The nanoformulations, which coat the bacterial cells, protect them from the external environment and enhance the survivability of the bacteria in different pH conditions (32). These results were supported by the experiment on encapsulation of *Raoultella planticola* Rs-2 with alginate, starch and bentonite at various pH levels, which demonstrated controlled release and improved survival of the microbe in adverse soil conditions (31).

Scanning Electron Microscope imaging

The SEM image demonstrates the even distribution of the formulation over the seed coat, with visible encapsulated microbes on the coated surface. When diluted, the microbes become more prominently visible on the seed coat (Fig. 4).

Growth Attributes of Black Gram

Shoot and Root length:

Rhizobium inoculation notably increased shoot length compared to the uninoculated control (Table 2). Across all treatments, shoot length increased significantly between 15 DAS and 45 DAS. T_6 consistently exhibited the longest shoot length at each measurement, followed closely by T_5 and T_4. The shortest shoot length was observed in T_1 throughout the experiment. Root length also increased steadily in all treatments between 15 DAS and 45 DAS. T_6 displayed the longest root length at each time point, indicating strong root system development, while T_1 showed the shortest root length at 15 DAS to 45 DAS (12.7, 15.80, 18.56), respectively. T_7 (*Rhizobium* biofertilizer) is on par with the T_5.

The application of the nanoformulations increased microbial activity, which increased the growth parameters (33, 34). During the vegetative stage, the nanoformulations promoted greater photosynthetic activity and accelerated

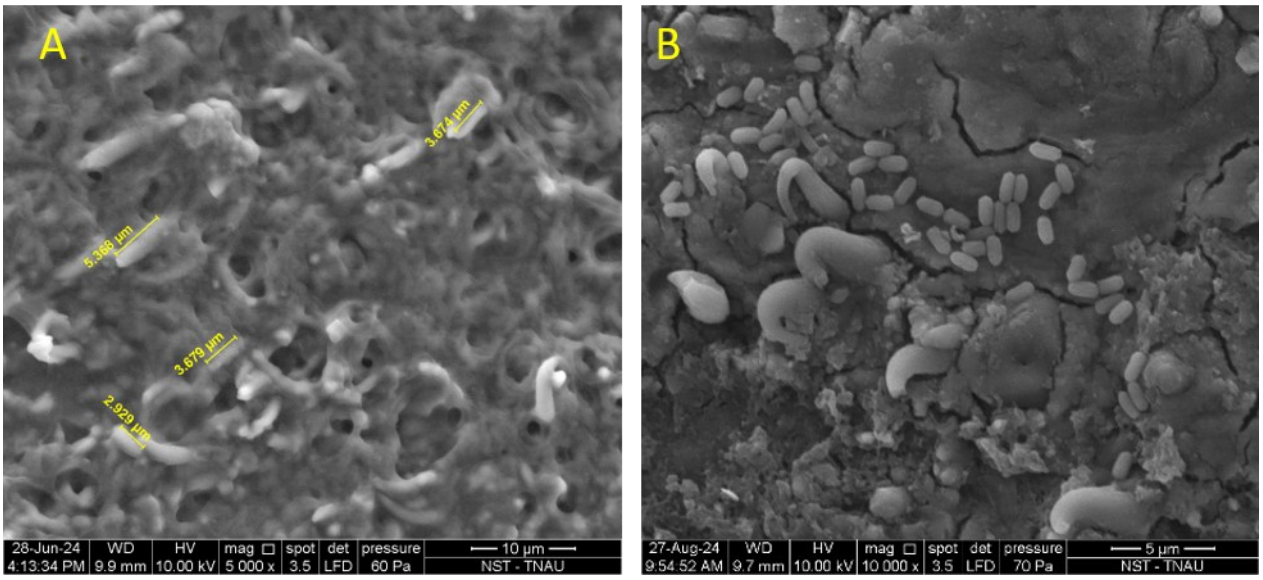

Fig. 4: SEM image of the nanoformulations coated black gram seed depicting the *Rhizobium* over seed coat. A) Direct application B) Diluted application

flowering onset, aligning with findings on plant growth promotion by *B. subtilis* nano-capsules (27).

Effect of nodulation

Nodulation signifies enhanced nitrogen fixation through the symbiotic relationship with the host, black gram.

Table 2: Effect of Nanoformulated *Rhizobium* on shoot and root length of black gram (Vamban 8) under pot culture condition

Treatments	Shoot length (cm)			Root length (cm)		
	15 DAS	30DAS	45DAS	15 DAS	30DAS	45DAS
T_1	12.561	23.89	32.56	12.70	15.80	18.56
T_2	17.60	28.57	44.50	16.56	21.83	25.36
T_3	18.73	29.30	45.53	17.10	23.36	25.06
T_4	18.70	31.23	44.86	17.60	23.06	26.53
T_5	19.60	31.45	46.30	17.96	23.60	28.10
T_6	21.22	33.53	49.23	19.46	25.20	29.46
T_7	18.90	29.37	45.36	17.33	23.10	25.93
SED	1.802	1.588	1.854	1.297	1.054	1.271
CD (0.05)	3.864	3.405	3.977	2.782	2.261	2.727

*cm-centimetre, DAS- Days after sowing, SED- standard error deviation, CD - Co-efficient of deviation, *-average of three replicates

Rhizobium is the soil bacterium responsible for forming nodules on the root hairs (35). The application of the *Rhizobium* nanoformulation as a seed treatment enhances growth parameters by colonizing the roots during the early stage of the vegetation. Treatment T6 exhibited the highest number of nodules, with nearly 100 nodules counted (Fig. 5). Followed by T_5 and T_7. The formulation allows for better interaction with plant roots, resulting more nodules than traditional biofertilizers and untreated controls.

The increased surface area and viable cell counts in the nanoformulations *Rhizobium* boost its performance in symbiotic nitrogen fixation. The yield is directly correlated with the number of nodules; further optimization could be achieved by co-inoculating with complementary biofertilizers (36-38).

Total chlorophyll content

The analysis of total chlorophyll content at 30 and 45 DAS

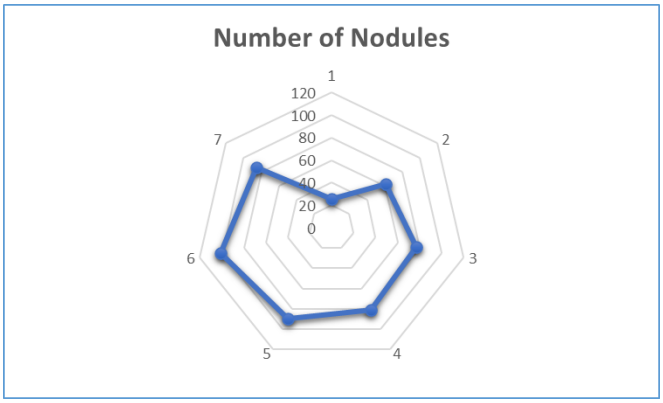

Fig. 5: Effect of nanoformulated *Rhizobium* on root nodule numbers in black gram (Vamban 8) under pot culture condition

reveals significant differences among the treatments (Table 3). Treatment T_6 consistently recorded the highest total chlorophyll content, with values of 0.952 mg g^{-1} at 30 DAS and 1.309 mg g^{-1} at 45 DAS, indicating its strong influence on enhancing the photosynthetic efficiency. The increased chlorophyll content in T_6 suggests that this treatment provides optimal nutrient conditions, particularly nitrogen, essential for chlorophyll synthesis. Higher chlorophyll content implies improved plant growth and biomass production. Enhanced photosynthetic rates boost nutrient absorption, leading to increased biomass during the vegetative stage and yield at the harvest stage, even under elevated CO_2 concentration (39) .

Treatments T_7 and T_5 also showed notable chlorophyll levels, indicating their positive influence on maintaining photosynthetic activity. The lowest chlorophyll content was observed in T_1, with values of 0.457 mg g^{-1} at 30 DAS and 0.814 mg g^{-1} at 45 DAS, suggesting that, without *Rhizobium*, reduced nitrogen fixation limited chlorophyll synthesis. The increase in chlorophyll content from 30 to 45 DAS across most treatments, particularly in T_6, highlights the sustained improvement in photosynthetic capacity over time, crucial for continuous growth and development as plants mature. Using *Rhizobium* biofertilizer could increase leaf chlorophyll content in the plants, as previously reported (40-42).

Soluble protein content

The soluble protein content varied significantly across

Table 3: Effect of nanoformulated *Rhizobium* on soluble protein and total chlorophyll in black gram (Vamban 8) under pot culture condition

Treatments	Soluble protein (mg g^{-1})		Total chlorophyll (mg g^{-1})	
	30 DAS	45 DAS	30 DAS	45 DAS
T_1	8.6	9.2	0.457	0.814
T_2	10.6	11.2	0.733	1.090
T_3	12.4	12.5	0.866	1.223
T_4	13.2	13.9	0.628	0.985
T_5	14.1	14.6	0.762	1.119
T_6	15.3	15.7	0.952	1.309
T_7	12.7	13.5	0.646	1.003
SEd	0.2488	0.4474	0.0074	0.0233
CD (0.05)	0.5336	0.9595	0.0160	0.4999

*mg-Milligram, g-Gram, DAS-Days after sowing, SED-standard error deviation, CD - Co-efficient od deviation, *-average of three replicates

treatments, serving as an indicator of nitrogen assimilation and overall plant metabolic activity (Table 3). Treatment T_6 was the most effective, with the highest soluble protein content recorded at both 30 DAS (15.3 mg g^{-1}) and 45 DAS (15.7 mg g^{-1}). The elevated protein levels in T_6 suggest that this treatment likely enhanced nitrogen uptake, promoting protein synthesis, a critical component of plant growth. Treatments T_5 and T_7 also showed relatively high protein levels, indicating their effectiveness in supporting nitrogen metabolism, which is essential for vegetative growth (43).

Treatment T_1 exhibited the lowest soluble protein content at both time points, with 8.6 mg g^{-1} at 30 DAS and 9.2 mg g^{-1} at 45 DAS, suggesting limited nitrogen availability and reduced protein synthesis. The increase in protein content from 30 to 45 DAS in most treatments indicates that the plants continued to benefit from the treatments over time, resulting in enhanced metabolic activity.

Enzyme activities

Fig. 6 and 7 illustrate the effects of different treatments on nitrate reductase and dehydrogenase activities in black gram at 30 and 45 DAS, respectively. Dehydrogenase activity increased over time in all treatments. At 30 DAS, treatment T_6 exhibited the highest dehydrogenase activity (11.31 µg TPF released/g soil/h), followed by T_5 (11.06 µg TPF released/g soil/h). By 45 DAS, T_6 again showed the highest activity (14.47 µg TPF released/g soil/h) followed by T_5 (13.22 µg TPF released/g soil/h). The control (T_1) recorded the lowest dehydrogenase activity at 30 and 45 DAS.

Similar trends were observed for nitrate reductase. Treatment T_6 had increased nitrate reductase activity at both 30 DAS (54.29 µg NO_2/g soil/h) and 45 DAS (96.59 µg NO_2/g soil/h), followed by T_5 (48.27 µg NO_2/g soil/h at 30 DAS and 90.25 µg NO_2/g soil/h at 45 DAS). The control treatment (T_1) exhibited the lowest nitrate reductase activity.

The observed increase in dehydrogenase and nitrate reductase activities with various treatments suggests that biofertilizers or nutrient treatments significantly enhance microbial enzyme activities in the soil. The highest dehydrogenase activity in T_6 indicates enhanced microbial respiration and soil biological activity, essential for nutrient cycling. The improved dehydrogenase activity was on par with the results of (44). The improved nitrate reductase activity in T_6 also suggests efficient nitrogen assimilation, which leads to better plant growth and productivity.

The lower enzyme activities in the control treatment (T_1) highlight the limited microbial activity and nutrient availability without adding biofertilizers or nutrients. These findings align with previous studies showing that *Rhizobium* stimulates soil microbial activities, enhances enzyme production and improves overall soil fertility, contributing to higher crop yields (45). The gradual increase in enzyme activity from 30 DAS to 45 DAS across all treatments indicates that the effects of the treatments are sustained over time, benefiting the crop's growth stages.

Yield attributes

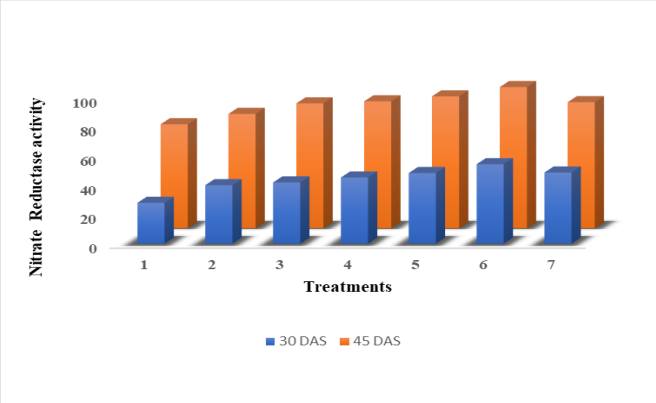

Fig. 6: Effect of nanoformulated *Rhizobium* on nitrate reductase activity in black gram (Vamban 8) under pot culture condition

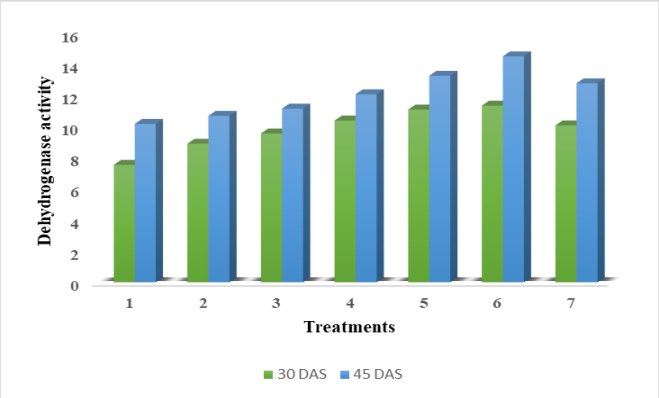

Fig. 7: Effect of nanoformulated *Rhizobium* on dehydrogenase activity in black gram (Vamban 8) under pot culture condition

The application of *Rhizobium* nanoformulations significantly impacted plant yield, as reflected in the total grain weight per plant (Table 4). A significant variation was observed in the number of seeds per pod across treatments. T_6 exhibited the highest number of seeds per pod (8.33), followed by T_5 (7.66), while the control treatment (T_1) recorded the lowest value of 5.66 seeds per pod. The number of pods per plant followed a similar trend, with T6 recording the highest value (33.66) and T1 the lowest (14.66), while the treatments T_2 to T_5 exhibited intermediate values.

Test weight, an indicator of grain quality, gradually increased with increasing treatment levels. T6 recorded the highest test weight (5.257 g), while T_1 had the lowest (3.893 g). The control treatment (T_1) yielded an average of 6.821 g per

plant. In contrast, applying of *Rhizobium* nanoformulation (T_6) led to a remarkable yield increase, with plants producing 12.891 g of grain, representing an 89.01% enhancement over the control. This positive trend in yield across treatments (T_2-T_5), with yields ranging from 8.464 g to 11.174 g per plant, further supports the positive correlation between the concentration of the nanoformulations and grain production.

The improved nodulation and nitrogen fixation facilitated by the nanoformulations undoubtedly contributed significantly to increased plant growth and subsequent grain output. *Rhizobium* inoculation has demonstrated an increase in yield, as evidenced by the findings of Marimuthu et al. (33).The interaction between plant and microbe increases nutrient availability and enhances plant yield (2). The Nanoformulation's ability to optimize these symbiotic processes leads to increased nitrogen availability and improved crop productivity due to the high cell mass and increased survivability of microbes in the rhizosphere soil.

Table 4: Effect of nanoformulated *Rhizobium* on yield parameters of black gram (Vamban 8) under pot culture condition

Treatments	Seeds Per Pod*	Pod Per Plants*	Test Weight (g)	Yield per plant (g)
T_1	5.667	14.66	3.893	3.067
T_2	6.333	21.33	4.273	4.007
T_3	7.000	25.66	4.552	5.460
T_4	7.333	29.33	4.637	7.220
T_5	7.667	30.33	4.608	8.567
T_6	8.333	33.66	5.257	10.197
T_7	7.333	28.33	4.739	8.267
SEd	0.617	1.391	0.226	0.226
CD (0.05)	1.323	2.984	0.486	0.485

g-Gram, DAS-Days after sowing, SED-standard error deviation, CD-Co-efficient od deviation, *-average of three replicates

Conclusion

In conclusion, the present investigation demonstrated the potential of *Rhizobium* nanoformulations as a promising approach to enhance plant growth, nodulation and yield parameters. The progressive increase in nanoformulation concentration led to a corresponding improvement in plant performance, as evidenced by the significant results in growth parameters. The highest dosage of the nanoformulation T_6 (15ml/8kg of black gram seeds) exhibited the most pronounced effects, promoting efficient nodulation and increased yield compared to the lower dosage of biofertilizer (50ml/8kg of black gram seeds). These findings underscore the crucial role of nanotechnology in optimizing *Rhizobium* nanoformulation concentration to enhance agricultural productivity.

The present study provides a promising foundation for developing a sustainable and eco-friendly nano-biofertilizer using starch, which is abundant in nature and is an effective encapsulant. Sustainable agriculture can be achieved using nano-biofertilizer and organic manure, such as FYM and vermicompost, which ensures soil nutrient status. Future research should focus on optimizing the formulation, evaluating its efficacy under diverse agro-climatic conditions and developing a consortium of biofertilizers using nanotechnology to enhance the microbial diversity in rhizosphere soil.

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Authors' contributions

Authors LT and GV framed the work plan and all other authors contributed equally with writing the manuscript and doing the research work.

Compliance with ethical standards

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

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