



1st INTERNATIONAL CONFERENCE ON
Climate Change & Sustainable Development
“Shaping a Sustainable Future: Global Solutions for Climate Change”
25th to 26th Feb 2025
SINES, NUST Islamabad

Synthesis of Nano-Biofertilizers for Enhanced Phosphorus Use Efficiency in Crops

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Abstract

Climate change and overpopulation significantly impact soil and plant systems, affecting plant productivity, soil quality, and environmental quality. Phosphorus deficiency in soil is a significant challenge in agricultural ecosystems, limiting crop growth and yield. This research study aims to improve phosphorus availability for plants by synthesizing nano-biofertilizer by combining phosphate-solubilizing microbes (PSMs) containing *Pseudomonas* spp with Titanium dioxide-coated green Graphene oxide nanoparticles (TiO₂-GO) coated on diammonium phosphate (DAP) fertilizer acting as a nutrient carrier. Each component was prepared individually using suitable respective methodologies, and then diammonium phosphate fertilizer was coated with these components individually using a drum rotatory coater. Nanoparticles were characterized via X-ray diffraction and scanning electron microscope analysis, which confirmed their optimum synthesis and potential to be used as an innovative fertilizer or conventional fertilizer enhancer by improving DAP efficiency and providing maximum phosphorus for plant growth and development.

Keywords: Nano-biofertilizer, sustainable agriculture, Phosphate fixation, Phosphate solubilizing microbes (PSMs), Coated fertilizers.

1. Introduction

By 2100, the world's population is predicted to have grown to between 8.5 and

12 billion people. With the escalation of the world population, the demand for food production amplifies, resulting in increased agricultural activity and associated

nutritional depletion (Singh et al., 2016). Numerous macronutrients and micronutrients, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), and iron (Fe), are essential for plants, which are unable to complete their life cycles or perform normal physiological functions without any of these nutrients (Kalaji et al., 2016).

The depletion of essential nutrients caused by agricultural demands driven by overpopulation leads to diminished soil fertility, decreased crop yields, and heightened vulnerability to pests and diseases. Furthermore, nutrient imbalances can have a negative impact on soil health, impacting its structure and microbial activity. This degradation amplifies the difficulties of maintaining sustainable agriculture methods. Presence of macronutrients (Nitrogen, phosphorus, and potassium (NPK)), in soil is for the optimal plant growth and development. Although these nutrients like potassium do occur naturally in average soils, but their concentration, bioavailability and stability vary within different geographical locations (Zorb et al., 2014). In some Asian regions, the useable concentration of potassium and phosphate ions for plants is lower as compared to other regions (Sinha & Tandon, 2020). Organic fertilizers can enhance the number of macronutrients in soils. However, Nitrogen is best obtained via natural sources such as decomposition of organic matter and nitrogen fixation by legumes as an essential part of nitrogen cycle. Nitrates are highly mobile as compared to phosphates which limits their availability in average soils. (CH 19. Management of Nitrogen and Phosphorus - SARE, 2021).

Morocco has the world's largest phosphate reserves of around 50 billion

metric tons, making almost 70% of the world's total phosphate supply. Egypt, along with Algeria, comes at 2nd and 3rd place, followed by Tunisia, China, South Africa, Brazil, and Saudi Arabia. (Phosphate Reserves by Country 2025, n.d.). With this uneven distribution and climate change challenges, only a small fraction of soluble Phosphorus left for plant uptake (Bindraban et al., 2020). Currently, average soil contains about 400-1000mg/kg of total phosphorus, out of which only 1-2.5% in plant available, causing high dependency on P-fertilizers (Pan & Cai, 2023). Generally, to overcome P deficiency, farmers apply P-fertilizers without taking soil composition and plants' needs. The low P-use-efficiency (PUE) of the plant in soil, which is approximately 15-20% indicates that most applied phosphorus fertilizers are not fully utilized by plants and cause phosphate fixation. leading to environmental issues like eutrophication (Hoque et al., 2023b).

Phosphate fixation in soil is one major problem that limits the availability of phosphorus (P) to plants, lowering the effectiveness of phosphate fertilizers. Phosphate fixation occurs when phosphate combines with soil minerals, producing insoluble molecules that plants cannot absorb. In acidic soils, phosphorus binds with iron (Fe) and aluminum (Al) oxides, but in alkaline soils, it combines with calcium (Ca) to produce calcium phosphates (Johan et al., 2021). This mechanism is highly pH-dependent and can render a significant amount of the applied phosphorus unavailable to crops. Phosphate fertilizers' inefficiency is exacerbated by environmental issues, since excess phosphorus can lead to eutrophication of water bodies, generating algal blooms and consequent aquatic ecosystem degradation (Penuelas et al., 2023).

Cost-effective, long-term, eco-friendly solutions are required to address nutrient deficiencies and fixation. Recently, Sustainable amendments such as the utilization of phosphate-solubilizing microorganisms (PSMs) and soil additions like biochar and wood ash have been investigated to enhance phosphorus availability and minimize fixation (Pang et al., 2024). Phosphate solubilizing microbes (PSBs) are significant to enhance phosphorus content in soil. They are categorized as organic and inorganic PSBs, which mineralize and convert insoluble phosphorus into organic phosphorus, which can ultimately, utilized by plants (Timofeeva et al., 2022). PSBs produce organic acids with low molecular weight, which can chelate metal ions (Fe^{3+} , Al^{3+} and Ca^{2+}) in acidic soil through hydroxyl and carboxyl groups. They compete for phosphate adsorption sites in soil, and their chelating capacity for Ca^{2+} is significant. This improves phosphate absorption and solubilization of inorganic phosphorus, raising overall phosphate concentration in soil (Pan & Cai, 2023b).

Nanoparticles (NPs) have been proposed to increase phosphorus (P) availability and soil P activation in agroecosystems (Zhang et al., 2023). Metallic nanoparticles, such as titanium dioxide (TiO_2) and iron oxide (Fe_3O_4), have been shown to improve soil phosphorus fractions by increasing variable phosphorus availability and reducing fixation (Zahra et al., 2020). Recently, (Soliman et al., 2025) investigated that TiO_2 nanoparticles increase P accumulation, improves chlorophyll content and growth in Apple plants. These results are aligned with the outcomes of other several studies indicating significant role of metallic nanoparticles for nutrient availability. Similarly in calcareous soil,

TiO_2 in conjunction with PSBs and Yeast peptides resulted in effective mobilization of phosphate at low dose and TiO_2 and PSBs in high dose. This happened due to decrease in soil Ph, improving nutrient uptake and enhancing growth of Bok choy (Peng et al., 2025).

Similarly, green nanoparticles perform well as nano fertilizers, improving plant health and development. Because of their environmentally favorable base, organic nanoparticles such as carbon nanotubes, graphene oxide, and carbon dots can repair soil while also providing nutrients to plants (Sharma et al., 2025). According to Andelkovic et al. (2018), cost effectively synthesized graphene oxide and Iron composite (GO-Fe) comparing to commercial monoammonium phosphate (MAP) fertilizers were more effective, holding up to 5% P to the surface, causing slow and impactful delivery for fertilizer. This indicates that GO-Fe-P composites have potential to act as efficient P transporters, which allows better utilization of nutrients without an adverse environmental impact.

Based on promising results of previous studies, this study is design to synthesize nano-biofertilizer containing green graphene oxide nanoparticles and TiO_2 nanoparticles and PSBs, which has potential to improve P concentration in soil and to remediate phosphorus fixation in soil ultimately, will reduce adverse environmental impact.

2. Materials and Methods

2.1. Materials

All the chemicals were of analytical grade purity and were utilized as received by Sigma Aldrich and Culture media without any additional purification. Standard

solutions were prepared using deionized water.

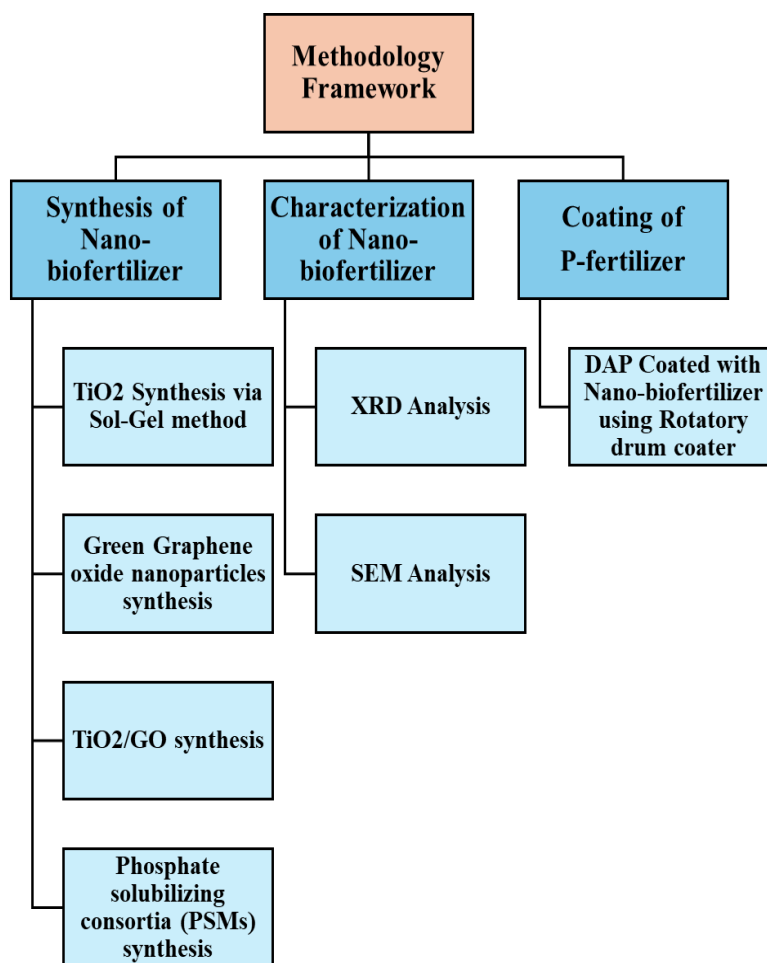


Fig: 1. Research Methodology Framework

2.2. Synthesis of titanium dioxide nanoparticles

Anatase nanoparticles have potential to effectively mobilize phosphate due to their high OH^- yield, facilitating photocatalytic activity and favorable surface chemistry (Kakuma et al., 2015). The Sol-gel method was followed for the synthesis of for the synthesis of Anatase TiO_2 nanoparticles and Titanium (IV)-n-butoxide ($\text{Ti}(\text{OBU})_4$) was used as a precursor. Initially, 5 ml $\text{Ti}(\text{OBU})_4$ and 50 ml ethanol were mixed and stirred for

one hour. Then HCl solution was added dropwise to the initial mixture until the white solution turns clear. The obtained sol was rested for 24hours to turn sol into gel. The gel was then dried at 80°C for 1 hour to fully evaporate water and organic materials. Finally, the dried gel was cured at 450°C for 3 hours using muffle furnace to achieve the TiO_2 nanoparticles (Karkare, 2014a).

2.3. Green synthesis of Graphene oxide nanoparticles

The green graphene oxide nanoparticles were synthesized using banana peels. The collected banana peels were cleaned, cut, and solar-dried for 2-3 days at maximum temperature. The samples were then dried in a hot air oven at 100°C for 2 hours. The fiber was crushed and processed thoroughly to generate powder (Kaviyarasan et al., 2023). The crushing and separating operation was done many times to produce fine powder. A crucible containing 0.5 g of pulverized banana peel powder and 0.1 g of Ferrocene was heated in a muffle furnace at 300°C for 10 minutes under air circumstances. The resulting black solid was then collected at ambient temperature and finally was characterized by X-ray diffraction analysis. (Somanathan et al., 2015a).

2.4. Synthesis of TiO₂/GO Nanocomposites

Green Graphene oxide (GO) was produced from banana peels using eco-friendly methodology, as detailed in (Somanathan et al., 2015b). For the preparation of TiO₂/GO nanocomposites in-situ sol-gel chemical technique was followed. Prepared GO nanoparticles with mixed with ethanol and Ultrasonicated for an hour to create homogeneous mixture. In parallel, 5ml of Ti (OBU)₄ was mixed with 50ml of ethanol and was stirred for several hour to create fine mixture (Karkare, 2014b).

Under steady stirring, the solution containing GO was gradually introduced to Ti (OBU)₄ over a few hours. The solution was heat-treated in an oven at 150 °C to produce the black TiO₂/GO complex. The complex was annealed at 300 °C for 4 hours to get TiO₂/GO nanocomposites (Khanna et al., 2021).

2.5. Synthesis of Microbial Consortia

Microbial consortia were synthesized using pre-isolated Phosphate solubilizing bacterial strains. Frozen microbial strains were revived and streaked on nutrient agar. The prepared plated were then placed in incubator for optimum growth. After the growth of 48hrs, bacterial culture was shifted into nutrient broth and solution was shifted into nutrient broth for 24hrs. Finally, optimal density of the solution was measured using UV Spectrophotometer at the wavelength of 600nm (Bhatia et al., 2018).

3. Results and Discussion

The synthesized nano-biofertilizer (TiO₂, GO, TiO₂/GO, PSBs) have potential to improve overall phosphate availability in nutrient deficient soils. Based on the promising results of each component in the previous studies it can be estimated that application of this fertilizer will alleviate phosphate fixation, leading to soil remediation, cost reduction and optimal plant growth and development.

3.1. Characterization of Nanoparticles

X-ray diffraction analysis was performed on prepared samples to ensure successful synthesis of TiO₂, GO and TiO₂/GO nanocomposites. Positive results have been obtained ensuring further production of nanomaterials. Furthermore, Particles shape and structure were examined using field emission scanning electron microscopy (SEM) analysis which showed presence of TiO₂ nanoparticles (under 100nm) and graphene oxide (500nm) within required approximate size range. Finally, in TiO₂/GO nanocomposites, TiO₂ nanoparticles were seen to be attached on graphene oxide sheets. The resultant products can be utilized

individually and synergistically in optimum doses.

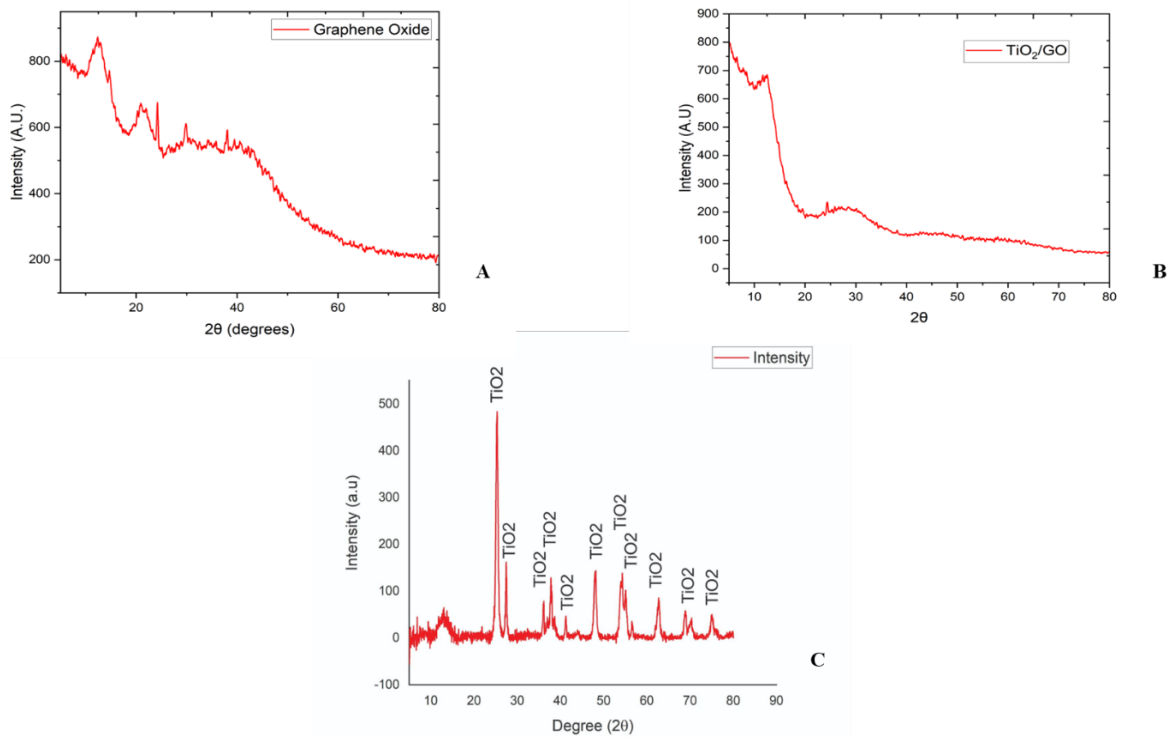


Fig. 2. XRD Analysis results. A) Graphene oxide, B) TiO₂/Graphene oxide, C) TiO₂ nanoparticles.

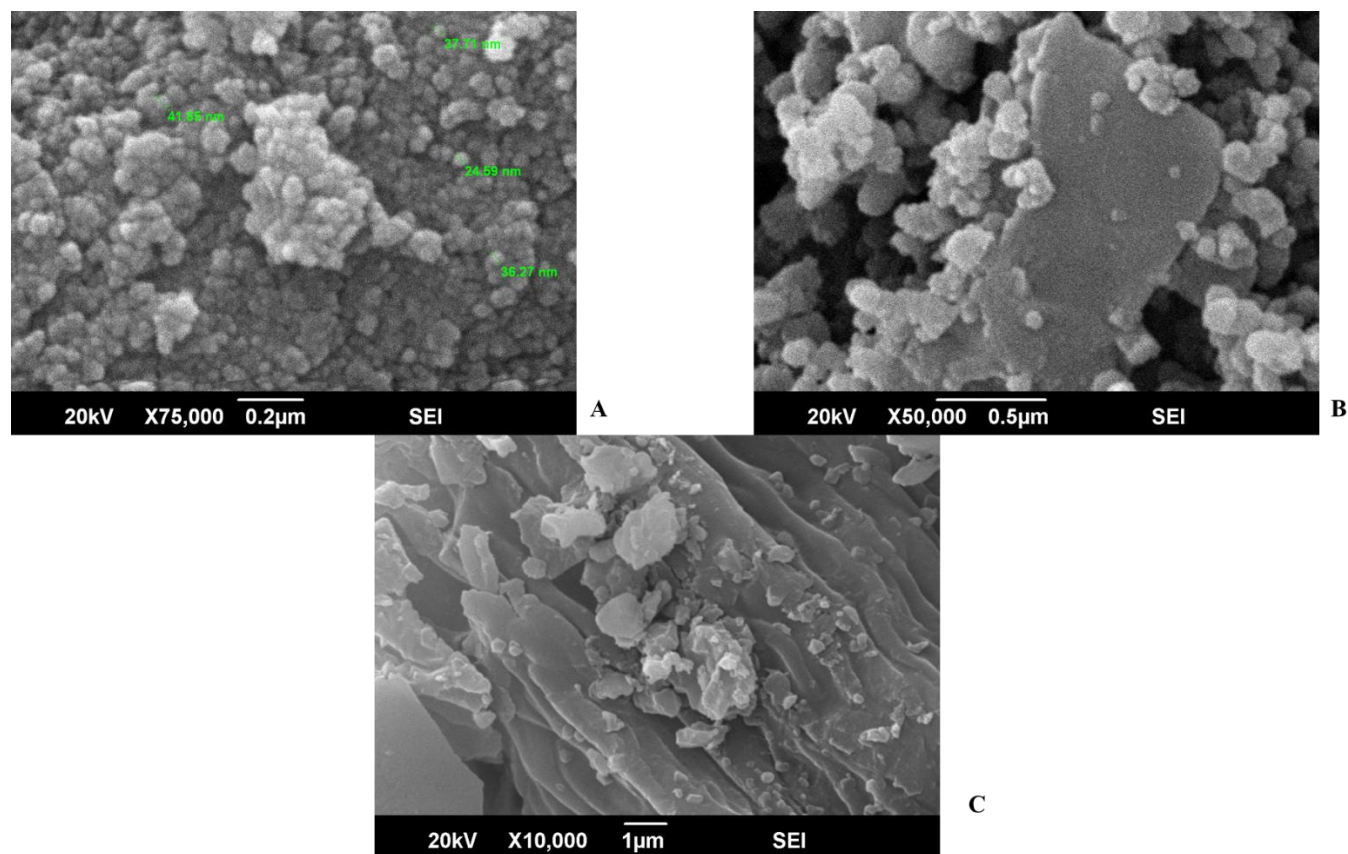


Fig: 3. SEM Analysis results. A) TiO₂ nanoparticles., B) TiO₂/Graphene oxide, C) Graphene oxide.

3.2. Testing of Microbial Consortia

Multiple pre-isolated microbial strains were revived and tested for their optimum growth pattern and phosphate solubilizing abilities. Pikovskaya's Agar was used for P-Solubility test. After various testing few bacterial strains, *Pseudomonas* spp showed hallow zones (ranging 2mm-3mm) ensuring the positive test results. Optimal environment was provided with the pH ranging (6.8-8.8) and temperature These strains when grown at a wide range of ecological factors showed maximum growth at pH between 6.8 and 8.8, temperature between 28 °C and 37 °C (Gupta et al., 2022). Furthermore, Optimal densities were measured using spectrophotometer

ranging values between (0.5-0.7), (Sharma & Lamsal, 2025). After that bacterial consortium was prepared using *Pseudomonas* spp strains in nutrient broth and stored for further use

3.3. Coating of P-fertilizers using Nano-biofertilizer

Coating procedure was initiated with the preparation of uniform solutions of TiO₂ nanoparticles, green Graphene oxide nanoparticles and TiO₂/GO nanocomposites in deionized water individually. Optimum concentrations were added of each material based on the methodologies followed before. Next, all three solutions were kept in sonication bath for one hour to ensure homogenous mixture (Beig et al., 2023). Required amount of diammonium phosphate fertilizer was then placed in rotatory drum coater and using spray nozzle solutions of nanoparticles were coated individually. Heat

was provided throughout the coating process. After the completion of coating process, coated granules were placed in hot air oven for further curing at 80°C (Seddighi et al., 2024). Finally, the cured fertilizer was further sprayed with microbial consortia and prepared product was stored for further use. Based on the previous studies, characterization results and coating results of present study, this nano-biofertilizer coated P-fertilizer can be efficiently tested in the laboratory and finally apply in the field for optimal phosphate mobilization and nutritional balance in soil for plant growth.

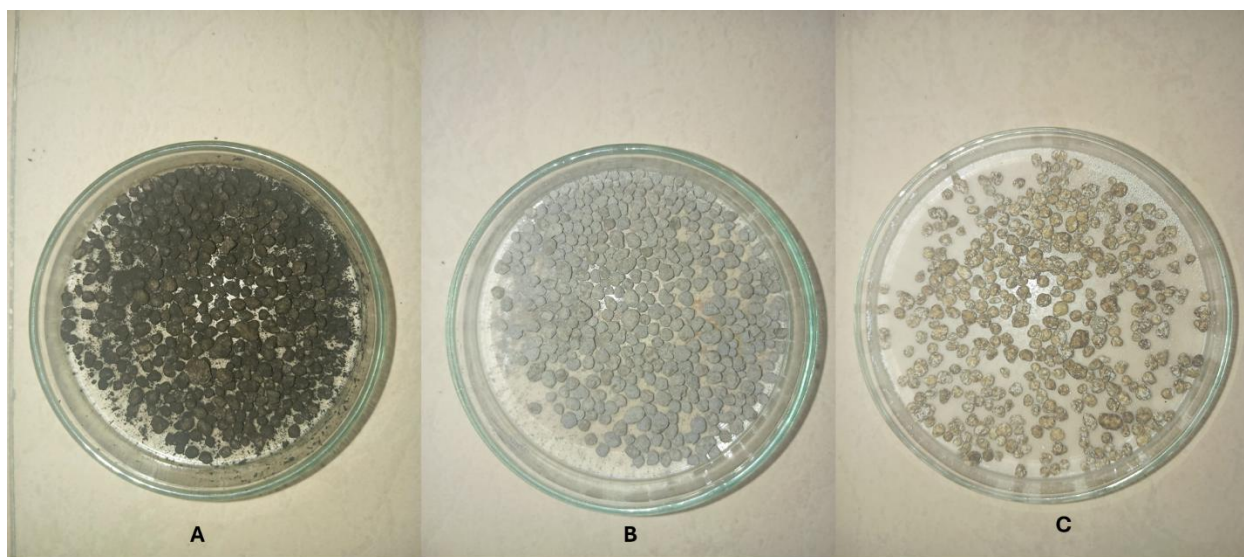


Fig:4. Samples of DAP Coated with Nano-biofertilizer. A) Graphene oxide NPs, B) TiO₂/GO NPs, C) TiO₂ NPs

4. Conclusion

Nano-biofertilizers are presented in this paper as a multidimensional approach to sustainable agriculture. By combining inorganic and organic materials, these supplements can improve the nutritional profile in agricultural soils. They enhance the availability of nutrients, promote plant growth and support environmental objectives including solid waste management and

wastewater treatment. Improved microbial interactions in the rhizosphere, decreased leaching losses, and targeted nutrient delivery are made possible by the accuracy and bioactive synergy of the nano-fertilizers. Global initiatives to reduce chemical inputs, improve soil health, and ensure food security in the face of climate change are in line with the precision agriculture strategy. Their function in reducing abiotic and biotic stresses, improving carbon sequestration and

combining with smart farming technology could all be investigated in future studies. Agronomic efficiency and environmental protection are connected by these nano-biofertilizers, which are crucial instruments for regenerative agriculture.

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