



Synthesis and Characterization of Urea-doped Calcium, Magnesium and Iron Phosphate Nanoparticles and Their Interaction with *Azospirillum* for Sustainable Agriculture Application

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ABSTRACT

Nanotechnology offers new solutions for agriculture. Nanofertilizers are especially useful because they allow controlled release of nutrients and targeted delivery. The present study investigates the synthesis and comprehensive characterization of urea-doped calcium, magnesium, and iron phosphate nanoparticles (CMI-PO₄ NPs) and evaluates their physicochemical properties, water-holding capacity, and agronomic performance in *Vigna mungo* cultivation. The interaction of these nanofertilizers with the biofertilizer *Azospirillum* was also examined to assess their synergistic effects on plant growth and stress physiology. Advanced analytical techniques, including Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray analysis (EDX) were employed to confirm successful synthesis of urea-doped calcium, magnesium, and iron phosphate nanoparticles (CMI-PO₄ NPs). The characteristic peaks of urea-doped CMI-PO₄ NPs were recognized from FTIR. The SEM images showed irregular and agglomerated particles, while XRD confirmed their crystalline nature. The EDX analysis verified the elements present in the nanoparticles. A comparative assessment was carried out among different treatments: urea-doped CMI-PO₄ NPs combined with *Azospirillum*, urea-doped CMI-PO₄ NPs alone, conventional urea, and an untreated control. Agronomic and biochemical parameters, including proline content (0.3883 mg g⁻¹), soluble protein associated with photosynthetic efficiency (0.9343 mg g⁻¹), superoxide dismutase activity (0.7022 mg g⁻¹ FW), peroxidase activity (0.1370 mg g⁻¹ FW), and lipid peroxidation (-0.9048 mg g⁻¹ FW), were analysed to evaluate plant stress responses. The results demonstrated that urea-doped CMI-PO₄ NPs, particularly when integrated with *Azospirillum*, significantly enhanced nutrient availability, water-holding capacity (up to 80%), and plant physiological performance compared to conventional treatments.

Keywords: Urea-doped nanoparticles; *Azospirillum*; Proline; Photosynthetic efficiency; Superoxide dismutase; Peroxidase.

1. INTRODUCTION

The 21st century faces an unprecedented challenge in feeding an exploding global population amid declining cultivable land, soil degradation, and environmental pressures from conventional agricultural practices. Fertilizers, particularly those supplying nitrogen, phosphorus, and potassium, have long been central to boosting crop productivity. However, their excessive and inefficient use has resulted in nutrient leaching, environmental pollution, and suboptimal nutrient use efficiency, with plants often absorbing less than half of the applied phosphorus and nitrogen (Waheed *et al.* 2024; Raliya *et al.* 2018).

Nanotechnology, through the development of nano fertilizers (NFs), provides a transformative avenue for enhancing nutrient delivery and plant uptake. Nano fertilizers are defined as materials, either in the nanoscale

(1–100 nm) or as composites with nanoscale structures, designed for the targeted and/or controlled release of nutrients (Fellet *et al.* 2021). These materials offer advantages such as increased surface area, high reactivity, tunable release profiles, and the potential for loading with multiple nutrients or bioactive molecules (Monreal *et al.* 2016).

Among the major macronutrients, nitrogen (frequently applied as urea) is especially prone to losses through volatilization and leaching. Phosphate minerals, including calcium, magnesium, and iron phosphates, are naturally abundant and biocompatible, serving as suitable matrices for nutrient doping. Doping these phosphates with urea can form nanocomposites (nano-urea) that release nitrogen slowly and in a targeted way. However, there are very few studies on their effectiveness, which shows a clear research gap (Kumar *et al.* 2024; Epple, 2018). Integration of these NFs with plant growth-

promoting rhizobacteria, such as *Azospirillum*, may further enhance nutrient uptake, soil fertility, and crop resilience.

This research aims to synthesize urea-doped Calcium, Magnesium, and Iron phosphate nanoparticles (Urea-doped CMI-PO₄ NPs), characterize their structural and functional properties, and assess their agronomic efficacy in *Vigna mungo* (black gram) cultivation. The study also evaluates the synergistic effects of combining urea-doped CMI-PO₄ NPs with *Azospirillum*, focusing on plant physiological and biochemical parameters relevant to growth, stress tolerance, and photosynthetic function

1.1 Calcium Nanoparticles

Crystalline and nanocrystalline calcium phosphate compounds (CaP) are found in biological systems, where they precipitate under mild conditions of pressure and temperature. In contrast, in the environment, they occur as mineral deposits formed over thousands of years under more extreme pressure and temperature conditions (Krasilnikov *et al.* 2022). Calcium phosphates are the most important inorganic constituents of biological hard tissues in living organisms. In its various crystal forms, CaP has been widely used in a broad range of applications (Pahalvi *et al.* 2021).

1.2 Iron Nanoparticles

Fe content in soil is usually high, but a large proportion is fixed to soil particles. Fe is mainly in the form of insoluble Fe³⁺, especially in high-pH and aerobic soils; therefore, these soils are usually deficient in the available form, Fe²⁺. In plants, Fe participates in many physiological processes including chlorophyll biosynthesis, respiration, and redox reactions.

1.3 Magnesium Nanoparticles

The concentrations of Mg in the cereals crops displays a clear decline over last 60 years, most possibly due to Mg dilution associated with increases in the grain yield as well as due to imbalanced fertilization without knowing crop demand for the magnesium. Foliar application of magnesium plays a very important role such as enzymes activation, protein synthesis, metabolism of carbohydrates and transferring of energy. It also performs as catalyst in oxidation and reduction reactions inside the tissues of plant and increases the crop resistance to drought (Adnan *et al.* 2020).

1.4 Nanoparticles as Nano Fertilizer

Soils are composed of micro-pores and macropores. During transport through these pores, single NPs are absorbed into mobile colloids, and their mobility

through micropores is improved, maintaining mega complexes of NPs in the macropores. However, the mobility of single NPs is inhibited when absorbed on non-mobile particles. Humic acids or organic matter in the soil and the ionic strength of water influence NP mobility (Xu *et al.* 2020). Once released into the environment, engineered NFs are aggregated to some extent (Nowack and Bucheli, 2007) Therefore, the organic content of the soil, the environmental conditions, and the chemical characteristics of NFs can improve or inhibit NP mobility (Kah *et al.* 2014).

It is well known that soil microbes play a key role in maintaining the balance in the biosphere because they are closely in contact with plants (Maldonado *et al.* 2021). It was demonstrated that metallic NPs such as silver NPs affect the dehydrogenase activity of bacteria and suppress growth when present in the range of 0.1–0.5 mg kg⁻¹ in soil. Throbäck *et al.* (2007) reported that denitrifying bacteria present in the soil are more susceptible, which disturb the nitrogen cycle through blocking the denitrification of nitrates to nitrogen. Similarly, other metallic NPs, like copper NPs, iron NPs, etc., and CNTs are also reported to exert some adverse effects on beneficial soil microflora.

The present study aims to synthesize urea-doped calcium, magnesium, and iron phosphate nanoparticles (Urea-doped CMI-PO₄) using a standard protocol and to characterize the synthesized nanoparticles through techniques such as FT-IR spectroscopy, XRD, EDX, and SEM analysis. It further seeks to evaluate the water holding capacity of the prepared nanoparticles and to investigate their application in the growth of *Vigna mungo*. In addition, the study intends to analyze key physiological and biochemical parameters in the plant, including chlorophyll content, proline levels, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), lipid peroxidation (LPO), and photosynthetic efficiency. Finally, the research aims to compare the growth performance of *Vigna mungo* treated with Urea-doped CMI-PO₄ nanoparticles against treatments with *Azospirillum*, urea alone, and a control, in order to assess the effectiveness of the synthesized nanomaterial as a potential nutrient source.

2. METHODOLOGY

2.1 Synthesis of Urea Doped Ca, Mg, Fe Phosphate Nano Particles

2.1.1 Materials

Analytical grade reagents, including calcium nitrate tetrahydrate, magnesium nitrate hexahydrate, ferric nitrate nonahydrate, sodium citrate, and urea, disodium hydrogen phosphate, sodium carbonate and potassium nitrate were procured from standard suppliers.

Deionized water was used for all preparations. All glassware and reaction vessels were cleaned with nitric acid and rinsed with deionized water before use.

The preparation of Urea-doped CMI-PO₄ NPs was carried out according to previous literature (Feil *et al.* 2021).

2.1.2 Synthesis Protocol

The preparation of Urea-doped CMI-PO₄ NPs was carried out according to previous literature (Feil *et al.* 2021). The preparation of urea-doped CMI-PO₄ nanoparticles (NPs) was carried out following the method reported by Feil *et al.* (2021). Initially, precursor solutions were prepared separately, including 0.4 M solutions of the respective metal nitrates, along with 0.24 M disodium hydrogen phosphate, 0.2 M sodium carbonate, and 0.9 M sodium citrate.

Two working solutions were then formulated. Solution A consisted of calcium nitrate (18.75 mL), magnesium nitrate (18.75 mL), iron nitrate (5 mL), and sodium citrate (37.5 mL). Solution B was prepared by combining disodium hydrogen phosphate (25 mL), sodium carbonate (25 mL), and potassium nitrate (25 mL).

Solution A and Solution B were subsequently mixed together under continuous stirring. The resulting reaction mixture was maintained at 35°C and allowed to age for 15 minutes to promote nucleation and particle growth.

Following this, the precipitate formed was separated by centrifugation at 4500 rpm for 10 minutes. The slurry obtained from two such preparations was combined and treated with a urea solution (1 g in 6 mL). The mixture was stirred vigorously to ensure homogeneity and effective incorporation of urea into the structure.

During the co-precipitation process, it was observed that Solution B initially had a pH of 11.2, which decreased to approximately 7.6 upon the addition of Solution A, indicating rapid precipitation of the urea-doped calcium magnesium phosphate.

The resulting precipitate was then collected and washed sequentially with deionized water and ethanol to remove any unreacted species and impurities. The washed product was subsequently dried, followed by freezing and lyophilization.

Finally, the dried material was gently ground using an agate mortar to obtain fine nanopowders of urea-doped CMI-PO₄ nanoparticles.

2.2 Water Holding Capacity of Soil (Percolation Method)

Four soil samples were collected and approximately 25 g of each sample was accurately weighed. The samples were then placed in separate containers and labeled appropriately for identification. To set up the percolation apparatus, four circular filter papers were folded into cone shapes and placed inside separate funnels. Each funnel was arranged over a graduated cylinder, and the setups were labeled to correspond with the respective soil samples. The pre-weighed soil samples were carefully transferred into the filter paper cones in each funnel. Subsequently, 50 mL of water was measured using a measuring cylinder and poured into four separate labeled beakers. The water from each beaker was then slowly poured into the corresponding funnel containing the soil sample, ensuring that the water level did not exceed the edge of the filter paper. As the water percolated through the soil, it passed through the filter paper and funnel, and the filtrate was collected in the graduated cylinders placed below. The setups were left undisturbed for several minutes until no further dripping was observed, indicating that the soil samples had become fully saturated. Finally, the volume of water collected in each graduated cylinder was recorded for further analysis.

2.3 Estimation of Chlorophyll Content

In this study, the plant *Vigna mungo* leaves are used to estimate the Chlorophyll A (Chlo A) and Chlorophyll B (Chlo B) content by using method (Arnon, 1949). About 1 g of finely cut fresh leaves was taken and ground with 20-40 mL of 80% acetone. Then, it was centrifuged at 5000-10000 rpm for 5 minutes. The supernatant liquid was collected and the procedure was repeated until the residue became colourless. The absorbance of the solution was determined by spectrophotometer at wavelengths 663 nm and 645 nm against the solvent (acetone) as a blank. The concentrations of Chlorophyll A and Chlorophyll B in a leave tissue were calculated by using the following equation:

$$\text{Chlorophyll A} = (12.7 \times A_{663}) - (2.69 \times A_{645}) \times \frac{V}{100 \times W} \quad \dots (1)$$

$$\text{Chlorophyll B} = (22.9 \times A_{645}) - (4.68 \times A_{663}) \times \frac{V}{100 \times W} \quad \dots (2)$$

A = Absorbance at specific wavelength, V = Final volume of chlorophyll extract in 80% acetone, W = Fresh weight of extracted tissue (g).

2.4 Estimation of Proline Content

Proline content was estimated by using the method of Bates (1973). About 0.5 g of leaf sample was macerated with 10 mL of 3% sulfosalicylic acid and

centrifuged at 3000 rpm for 10 minutes. 2 mL aliquot of the supernatant solution was taken in a test tube, to which 2 mL of acid ninhydrin, 2 mL of glacial acetic acid, and 2 mL of 6 M orthophosphoric acid were added. The reaction mixture was thoroughly mixed and incubated in a hot water bath for one hour to allow complete color development. After incubation, the test tubes were cooled under running tap water to bring the reaction mixture to room temperature. The cooled solution was then transferred to a separating funnel, and 4 mL of toluene was added. The mixture was shaken uniformly for approximately 30 seconds, resulting in the formation of two distinct layers. The lower aqueous layer, which was colorless, was discarded, while the upper pink-colored toluene layer containing the chromophore was carefully collected. The absorbance of the extracted solution was measured at 520 nm using a spectrophotometer. The proline content in the samples was determined by comparing the absorbance values with a standard calibration curve. The results were expressed in mg g^{-1} .

2.5 Superoxide Dismutase Activity

Superoxide dismutase (SOD) is a crucial antioxidant enzyme responsible for catalysing the dismutation of superoxide radicals, thereby safeguarding plant cells from oxidative damage. Fresh leaf samples (0.5 g) were homogenized in 5 mL of 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA. The homogenate was then centrifuged at 12,000 rpm for 20 minutes at 4°C, and the resulting supernatant was collected and used as the enzyme source.

The reaction mixture was prepared by combining 2.6 mL of 50 mM phosphate buffer (pH 7.8), 0.1 mL of 20 mM L-methionine, 0.1 mL of 1.1 mM nitro blue tetrazolium (NBT), 0.1 mL of 0.1 mM EDTA, and 0.1 mL of the enzyme extract. The reaction was initiated by the addition of 0.1 mL of 0.1 mM riboflavin.

The reaction mixtures were then exposed to fluorescent light for 15 minutes to facilitate the photochemical reaction. Following incubation, the absorbance of the samples was measured at 560 nm using a spectrophotometer. The enzyme activity was determined based on the absorbance values as described by Kono (1978).

$$\text{SOD activity (U/g FW)} = \frac{(\text{Absorbance at } 560\text{nm}) \times (\text{Total volume of reaction})}{(\text{Extinction coefficient}) \times (\text{Sample weight})} \dots (3)$$

2.6 Peroxidase Activity

Peroxidase (POD) functions as an antioxidant enzyme, catalysing the oxidation of various substrates such as guaiacol, utilizing hydrogen peroxide as an electron acceptor, thus detoxifying H_2O_2 and shielding plant cells from oxidative damage.

Fresh leaf samples (0.5 g) were homogenized in 5 mL of 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA. The homogenate was then centrifuged at 12,000 rpm for 20 minutes at 4°C, and the resulting supernatant was collected and used as the enzyme extract for the assay.

The reaction mixture was prepared by adding 2.7 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 20 mM guaiacol, and 0.1 mL of 40 mM hydrogen peroxide (H_2O_2). To this, 0.1 mL of the enzyme extract was added to initiate the reaction. The increase in absorbance was recorded at 470 nm for a duration of 1 minute using a spectrophotometer. The peroxidase (POD) activity was determined based on the rate of change in absorbance, following the method described by Pütter (1974).

$$\text{POD activity (U/g FW)} = \frac{(\text{Change in absorbance per minute}) \times (\text{Total volume of reaction})}{(\text{Extinction coefficient}) \times (\text{Sample weight})} \dots (4)$$

2.7 Lipid Peroxidase Activity

Lipid peroxidation serves as an indicator of oxidative stress in plants, with malondialdehyde (MDA) often used as a marker. Leaf samples (0.5 g) were homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was then centrifuged at 12,000 rpm for 20 minutes, and the supernatant was carefully collected for further analysis.

An aliquot of 1 mL of the supernatant was mixed with 2 mL of 0.5% (w/v) thiobarbituric acid (TBA) prepared in 20% TCA. The reaction mixture was heated at 95°C for 30 minutes to facilitate the formation of the colored complex. After heating, the samples were rapidly cooled in an ice bath to terminate the reaction. The absorbance of the resulting pink-colored solution was measured at 532 nm using a spectrophotometer. The extent of lipid peroxidation was determined based on the absorbance values, following the method described by Heath and Packer (1968).

$$\text{MDA content (U/g FW)} = \frac{(\text{Absorbance at } 532\text{nm}) \times (\text{Total volume of reaction})}{(\text{Extinction coefficient}) \times (\text{Sample weight})} \dots (5)$$

2.8 Photosynthetic Efficiency

Leaf samples (0.25 g) were accurately weighed and macerated in 10 mL of phosphate buffer solution to obtain a uniform extract. The homogenate was then centrifuged at 3000 rpm for approximately 10 minutes, and the supernatant was carefully collected for further analysis.

An aliquot of 1 mL of the supernatant was transferred into a test tube, to which 5 mL of alkaline

copper reagent was added. The mixture was allowed to stand for 30 minutes to facilitate color development. Subsequently, 0.5 mL of Folin–Ciocalteu (phenol) reagent was added to the reaction mixture. The absorbance of the resulting solution was measured at 660 nm using a spectrophotometer. The soluble protein content of the leaf samples was determined based on the absorbance values and expressed as mg g⁻¹ of leaf sample, following the method described by Lowry *et al.* (1951).

$$\text{Amount of soluble protein} = \frac{X}{0.5} \times \frac{25}{500} \times 1000$$

Amount of soluble protein present in given sample is expressed in mg g⁻¹

3. RESULTS AND DISCUSSION

3.1 FTIR: Fourier Transform Infrared Spectroscopy

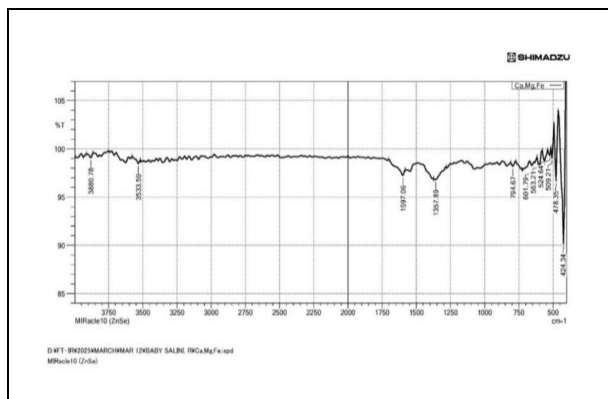


Fig. 1: FTIR spectrum of urea-doped -CMI-PO₄ nanoparticles

The FT-IR spectrum of urea-doped calcium, magnesium, and iron oxide nanoparticles reveals several characteristic absorption bands corresponding to both urea functional groups and metal–oxygen bonds, confirming successful doping and nanoparticle formation. A broad band observed at 3533.59 cm⁻¹ is attributed to N–H stretching vibrations, indicating the presence of amine groups from urea. The band at 1597.06 cm⁻¹ corresponds to N–H bending vibrations, while the peak at 1357.89 cm⁻¹ is assigned to the symmetric stretching of the NH₂ group, supporting the incorporation of urea into the nanoparticle matrix, consistent with previous reports (Sharma *et al.*, 2022). In the lower wavenumber region, distinct bands are observed that correspond to metal–oxygen bonds. The peaks at 794.67 cm⁻¹ and 601.79 cm⁻¹ are associated with Ca–O stretching vibrations, confirming the presence of calcium oxide components. Similarly, the bands at 478.35 cm⁻¹ and 424.34 cm⁻¹ are attributed to Mg–O vibrations, indicating magnesium oxide formation. The Fe–O bonds are identified by characteristic peaks at 593.21 cm⁻¹,

524.64 cm⁻¹, and 509.21 cm⁻¹, which are typical of iron oxide structures. Overall, the presence of these functional group vibrations and metal–oxygen bands confirm the successful synthesis of urea-doped calcium, magnesium, and iron oxide nanoparticles.

The elemental composition of urea doped calcium, magnesium and iron phosphate nanoparticles were characterized and quantified EDX analysis. The presence of Ca, C, Na and O as major elements while Fe, P, N, Mg and K as minor elements were confirmed by the EDX spectrum of urea-doped CMI-PO₄ nanoparticles (Waheed *et al.* 2024).

3.2 Energy Dispersive X-ray Analysis (Edx)

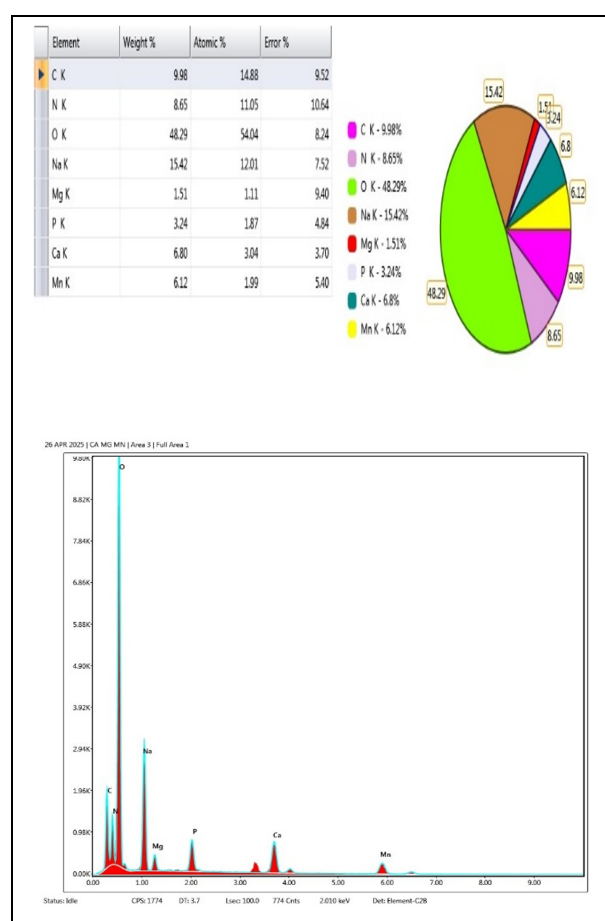


Fig. 2: EDX spectrum of urea doped CMI-PO₄ nanoparticles

3.3 X Ray Diffraction Analysis

The XRD pattern exhibits several diffraction peaks at specific 2θ values, indicating the crystalline nature of the sample. The presence of sharp and intense peaks confirms good crystallinity, while the slightly broad background suggests the presence of nano-sized particles or partial amorphous character (Mishra *et al.* 2023). The major diffraction peaks correspond to phases containing Ca, Mg and Fe as marked in the pattern. This

confirms the successful incorporation of these elements in the synthesized material. No extra impurity peaks are observed, indicating high phase purity, supporting its suitability for further functional application.

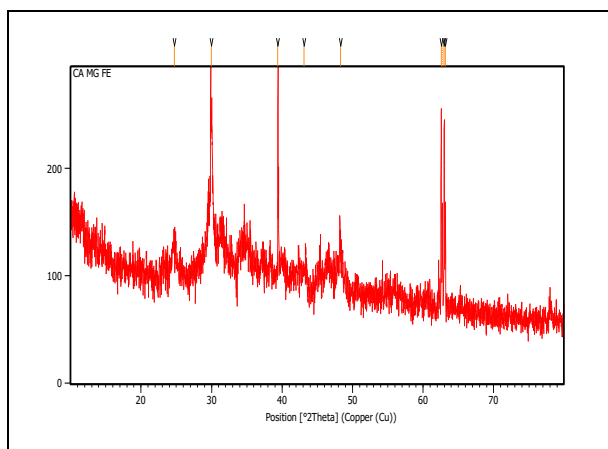


Fig. 3: Xray diffraction pattern of urea doped CMI-PO₄ nanoparticles

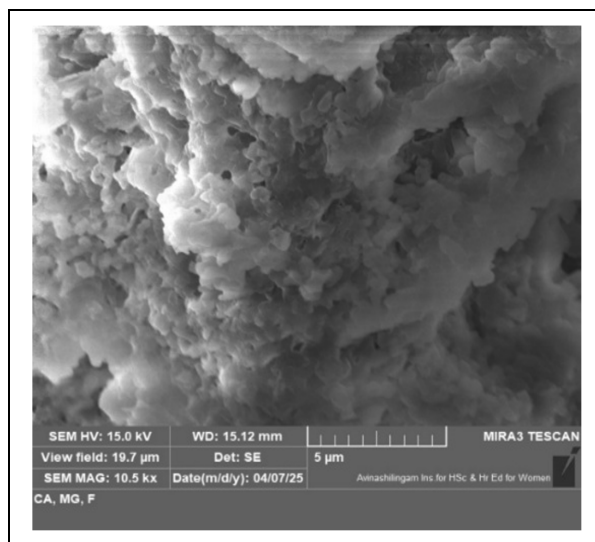


Fig. 4: SEM image of urea-doped -CMI-PO₄ nanoparticles

3.4 Fe-SEM Analysis

The SEM image of Ca, Mg, Fe oxide nanoparticles is given in figure 3.4. Irregular shaped agglomerated particles were present on the SEM image. The particles are well formed and the boundaries are clearly visible and the size range was approximately 50–300 nm. Accumulation of particles are also seen in some places. A previous study has reported the agglomerated nanoparticles (Sharma *et al.* 2022)

3.5 Water Holding Capacity of Soil (Percolation Method)



Fig. 5: Estimation of water holding capacity

Water holding capacity (WHC) value was calculated for soil mixed with nano fertilizer, soil without fertilizer, soil with biofertilizer and soil mixed with urea. The WHC of soils varied with treatment. Soil amended with nano fertilizer showed a WHC of 76%, while soil mixed with urea had 48%. Soil treated with biofertilizer exhibited the highest WHC at 80%, whereas untreated soil recorded 56%. Thus, nano fertilizer-amended soil demonstrated a higher water holding capacity compared to urea-treated and untreated soils. Improved water holding capacity is important for conserving soil moisture and supporting plant growth.

Table 1. Analysis of water holding capacity

Sample	Weight of Sample	Volume of Water Poured Through (V1)	Volume of Water Collected in Cylinder (V2)	Volume of Water Retained by Soil (V1-V2)	Percentage of Water Holding Capacity
Soil-A (Water)	25	50	36	14	56%
Soil-B (Urea)	25	50	38	12	48%
Soil-C (Urea-doped CMI-PO ₄ NPs)	25	50	31	19	76%
Soil-D (Urea-doped CMI-PO ₄ NPs + Biofertilizer)	25	50	30	20	80%

3.6 Chlorophyll Estimation

The chlorophyll content of the leaves was determined for four plants (*Vigna mungo*) during two periods (after 30 days). The chlorophyll content was measured thrice in each replicate. The chlorophyll contents without fertilizer, with urea and urea-doped CMI-PO₄ and biofertilizer are given below. Significant differences were found in the Urea-doped CMI-PO₄ NPs with *Azospirillum* treatment compared to other treatments.

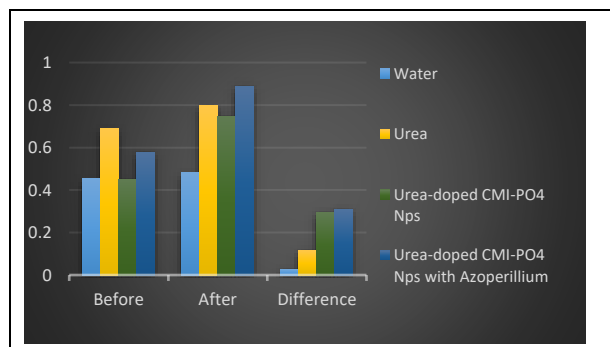


Fig. 6: Relative chlorophyll content



Fig. 7: Estimation of chlorophyll



Fig.8: (a)Water (b)Urea (c)Urea-doped CMI-PO₄ NPs (d)Urea-doped CMI-PO₄ NPs with *Azoperillum*

3.7 Proline

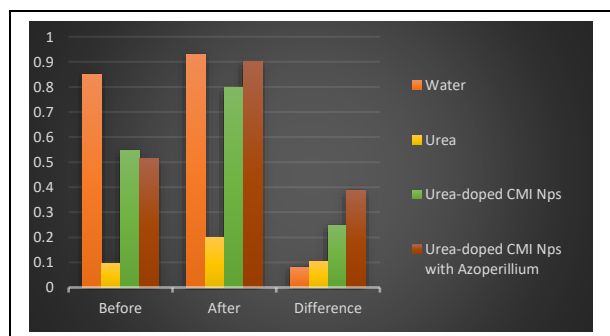


Fig. 9: Relative proline content

The study evaluated the impact of different fertilizers on proline content in *Vigna mungo* plants. Proline content was measured three times in each replicate. Urea-doped CMI-PO₄ NPs with *Azospirillum* significantly increased proline content, with a rise of 0.3883 mg g⁻¹FW, indicating enhanced stress tolerance. Urea-doped CMI-PO₄ NPs alone also increased proline content, but to a lesser extent (0.2479 mg g⁻¹ FW). In contrast, urea and control showed relatively smaller increases of 0.1026 mg g⁻¹FW and 0.0805 mg g⁻¹FW, respectively. These findings suggest that Urea-doped CMI-PO₄ NPs alone with *Azospirillum* is an effective fertilizer for *Vigna mungo*, potentially promoting stress resilience and plant health.

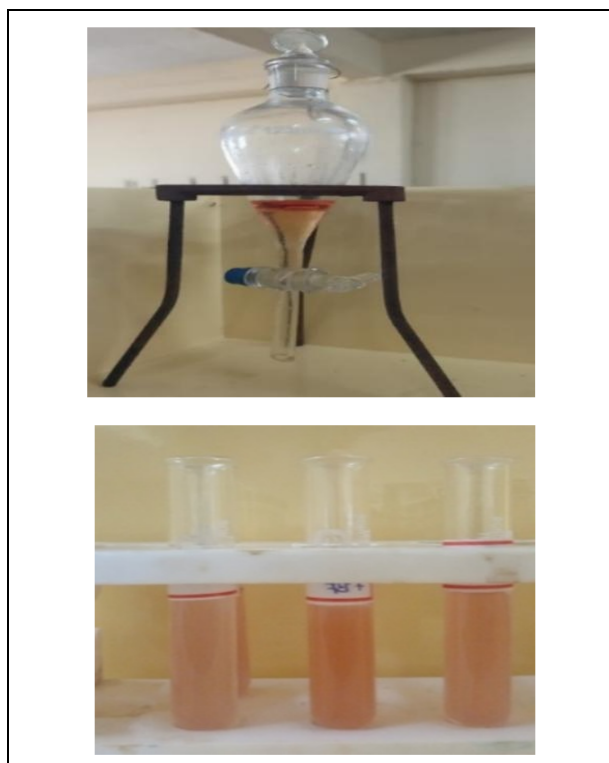


Fig. 10: Proline estimation

3.8 Photosynthetic Efficiency – soluble Protein (OD@660nm)

The study evaluated the impact of different fertilizers on photosynthetic efficiency in *Vigna mungo* plants. The soluble protein content was measured three times in each replicate. The results showed that Urea-doped CMI-PO₄ NPs with *Azospirillum* significantly enhanced photosynthetic efficiency, with an increase of 0.9343 mg g⁻¹FW, outperforming other treatments. Urea-doped CMI-PO₄ NPs alone also improved efficiency, with a rise of 0.7901 mg g⁻¹FW. In contrast, urea and control showed relatively lower increases of 0.4721 mg g⁻¹FW and 0.3873 mg g⁻¹FW, respectively. These findings suggest that Urea-doped CMI-PO₄ NPs with *Azospirillum* is the most effective fertilizer for *Vigna mungo*, boosting its ability to convert light energy into chemical energy and potentially leading to improve plant growth and productivity.

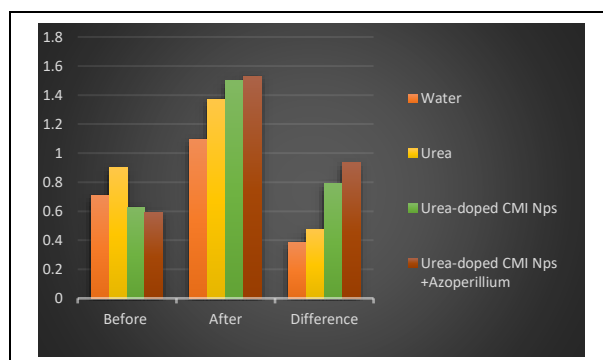


Fig. 11: Relative photosynthetic efficiency

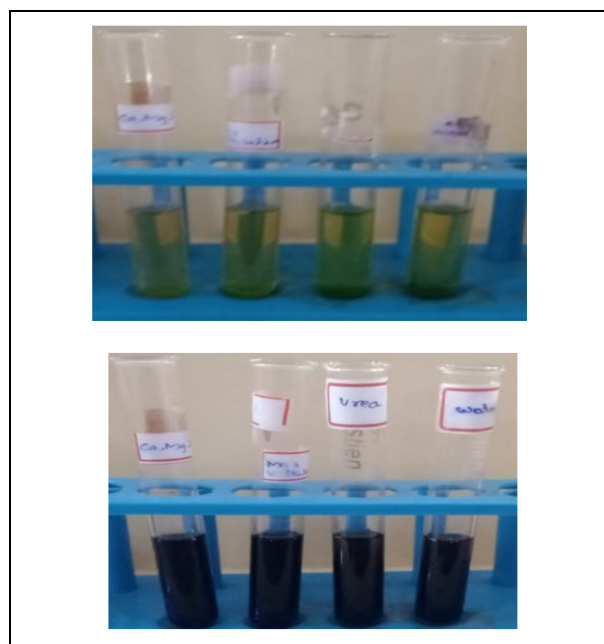


Fig. 12: Photosynthetic efficiency estimation

3.9 SOD

In this study, SOD activity in plants was estimated before and after application of Urea-doped CMI-PO₄ NPs with *Azospirillum* and Urea-doped CMI Nps followed by urea and control. After 15 days, in case of Urea-doped CMI-PO₄ NPs with *Azospirillum* SOD activity increases by 0.7022 mg g⁻¹FW followed by the application of Urea-doped CMI-PO₄ NPs the SOD activity increases by 0.1681 mg g⁻¹FW. In contrast, in the case of urea the SOD activity increases by 0.0966 mg g⁻¹FW followed by control with the increase of 0.0809. mg g⁻¹FW

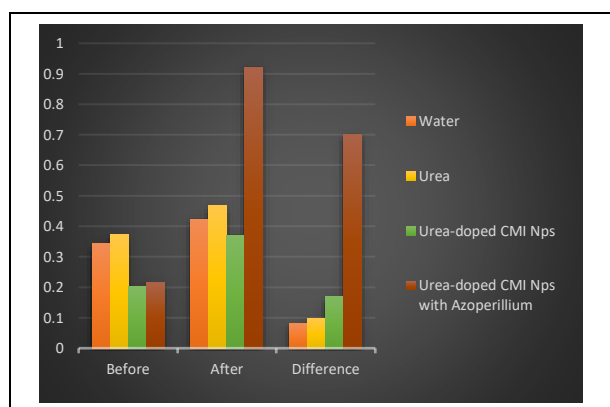


Fig. 13: Relative SOD content

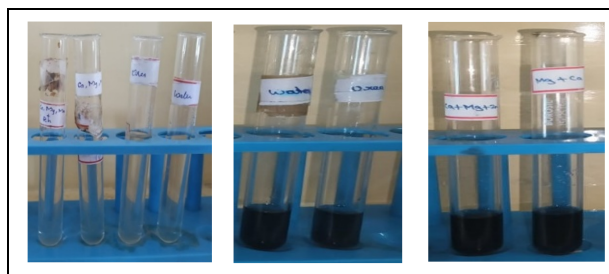


Fig. 14: SOD estimation

3.10 POD

The study assessed POD activity in plants treated with different fertilizers. The results showed that Urea-doped CMI-PO₄ NPs with *Azospirillum* led to the highest increase in POD activity, with a rise of 0.1370 mg g⁻¹FW, followed closely by Urea-doped CMI-PO₄ NPs alone, which increased POD activity by 0.1184 mg g⁻¹FW. In contrast, urea and control showed relatively minor increases in POD activity, with rises of 0.0686 mg g⁻¹FW and 0.0170, mg g⁻¹FW respectively. These findings suggest that Urea-doped CMI-PO₄ NPs, especially with *Azospirillum*, enhances POD activity, potentially boosting plant defense mechanisms.

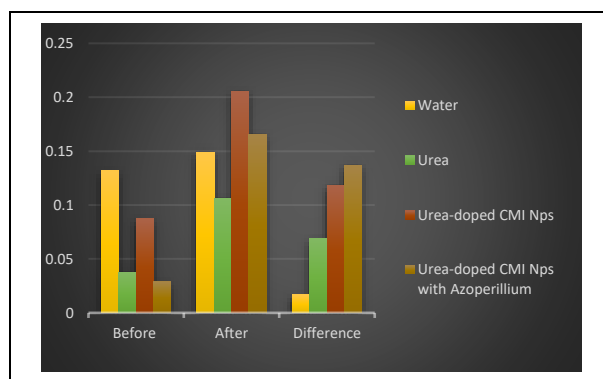


Fig. 15: Relative POD content

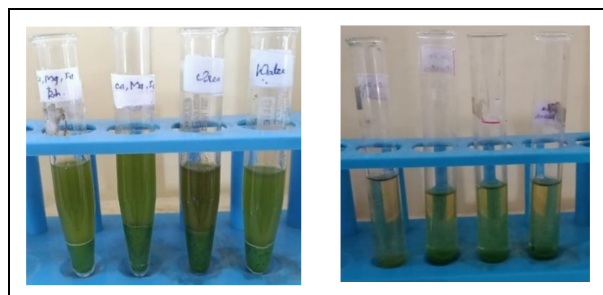


Fig. 16: POD estimation

3.11 Lipid Peroxidation

The study evaluated Lipid Peroxidation (LPO) in plants treated with different fertilizers. The LPO content was measured three times in each replicate. Urea-doped CMI-PO₄ NPs with *Azospirillum* showed the most significant reduction in LPO, decreasing by 0.9048 mg g⁻¹FW, indicating effective stress mitigation. Urea-doped CMI-PO₄ NPs alone showed a decrease of 0.5824 mg g⁻¹FW. In comparison, urea and control showed decreases of 0.4999 mg g⁻¹FW and 0.3156 mg g⁻¹FW, respectively. These findings suggest that nano urea with *Azospirillum* is a promising fertilizer, potentially enhancing plant health by reducing oxidative stress.

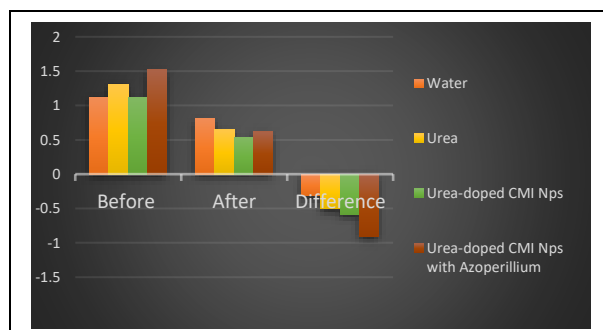


Fig. 17: Relative LPO content

4. CONCLUSION

This study demonstrates the successful synthesis and multifunctional characterization of Urea-doped CMI-PO₄ NPs establishing their potential as next-generation nanofertilizers. The integration of these NPs with *Azospirillum* biofertilizer in *Vigna mungo* cultivation resulted in marked improvements in plant growth, photosynthetic efficiency, stress physiology, and nutrient use efficiency, surpassing the performance of bulk urea and control treatments. Agronomic and biochemical parameters, including proline content (0.3883 mg g⁻¹), soluble protein associated with photosynthetic efficiency (0.9343 mg g⁻¹), superoxide dismutase activity (0.7022 mg g⁻¹ FW), peroxidase activity (0.1370 mg g⁻¹ FW), and lipid peroxidation (−0.9048 mg g⁻¹ FW), were analysed to evaluate plant stress responses.

Future studies should evaluate these nanoparticles under field conditions, particularly under environmental stress, to better understand their effects on yield and nutritional quality. Emphasis should also be placed on field validation, long-term ecological impact, and scalable production. Integrating nanofertilizers with biofertilizers offers a promising approach for sustainable agriculture and food security.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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