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Synthesis and Characterization of Urea- doped Calcium, Magnesium and Manganese Phosphate Nanoparticles and Their interaction with Phospho-Bacteria for Sustainable Agriculture Application

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KEYWORDS

Urea-doped calcium, magnesium, manganese phosphates nanoparticles ($CMM-PO_4$ NPs), Phosphobacteria, Scanning Electron Microscopy (SEM) and Electron Dispersive X-Ray Analysis (EDX), Proline, Photosynthetic efficiency, Superoxide dismutase, Peroxidase.

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Abstract

Sustainable agricultural production worldwide requires the development of innovative fertilization strategies that enhance agricultural production. The effective utilization of microorganisms in nitrogen fixation can provide a bearable alternative to chemical fertilizers. the design of nano fertilizers that can control nutrient release and delivery is a promising solution offered by nanotechnology. This research investigates the synthesis, characterization, and application of urea-doped calcium, magnesium, and manganese phosphate nanoparticles ($CMM-PO_4$ NPs) as nanofertilizers, both independently and in conjunction with Phosphobacteria, for sustainable agriculture. The nanoparticles were synthesized via a co-precipitation method and characterized using FT-IR, EDX, and SEM, confirming the presence of relevant functional groups and elemental composition, as well as their nanoscale morphology. The study assesses the impact of these nanofertilizers on *Vigna mungo* (black gram) plants, focusing on water holding capacity of soil, chlorophyll content, proline accumulation, photosynthetic efficiency, and the activity of antioxidant enzymes (SOD, POD, and LPO). Comparative analysis was performed across treatments: Urea-doped $CMM-PO_4$ Nps with Phosphobacteria, Nano-urea alone, bulk urea, and a control. Agronomic assays measured proline (0.6600 $\mu g g^{-1}$), photosynthetic efficiency of soluble protein (0.9780 $mg g^{-1}$), superoxide dismutase (0.7022 Ug^{-1}FW), peroxidase (0.3546 Ug^{-1}FW) and lipid peroxidase (-0.8322Ug^{-1}FW). Particularly when combined with Phosphobacteria, enhance water retention in soil, increase chlorophyll production, promote proline synthesis (indicating stress tolerance), improve photosynthetic efficiency, and modulate antioxidant enzyme activity in *Vigna mungo*. Specifically, the combined treatment of Urea-doped $CMM-PO_4$ Nps with Phosphobacteria demonstrates superior performance compared to individual nano fertilizer application or traditional urea treatment. This suggests a synergistic effect that could lead to enhanced nutrient uptake, improved plant growth, and reduced environmental impact, highlighting the potential of these nanofertilizers as a sustainable alternative to conventional fertilizers.

1. INTRODUCTON

Nanotechnology holds excessive potential for addressing agricultural challenges such as soil deprivation, nutrient deficiencies, low harvests, and nutrient leaching. Nano fertilizers enable more efficient nutrient absorption by plants due to their enlarged surface area, bestowing viable solutions [1]. Fertilizers $A = \pi r^2$ considered to be significant components of soil nutrients that improve plant development and efficiency. Over the past 50 years, farmers have used many commercial or traditional fertilizers, which have balanced the circulation of the primary minerals required for the best growth of plants: potassium, nitrogen, and phosphorous [2,3,4].

The global population is growing, as is the consumption of fertilizers. Despite the fact that plants can only absorb approximately 42% of the applied phosphorus, farmers are currently utilizing approximately 85% of the world's total mined phosphorus as fertilizer [5]. The use of these fertilizers causes significant economic losses in terms of 40–70% leaching-related concerns [6]. Additionally, heavy metals can cause serious harm to the ecosystem as well as to the soil microbial flora, soil structure, and plant life [7]. Therefore, the development of a special compost that gradually and economically discharges supplements is required so that the plants can effectively ingest the supplements [8].

It is essential to develop safer, more dependable, easy-to-use, non-toxic, and environmentally friendly methods for their manufacture [9,10]. Nanotechnology is a promising technique with tremendous potential for resolving agricultural-based issues such as land degradation, poor crop yield, nutrient shortage, and leaching losses [11,12]. The promising application of nanotechnology to enhance plant nourishment and reduce unfavorable ecological consequences is attracting much interest [13,14].

Although fertilizers are essential for agriculture to feed the growing population, the excessive use of large amounts of chemical fertilizers leads to environmental pollution [15]. The soil is a system full of life, particularly symbiotic relationships with plants. Plant–microbe interactions are mutually beneficial, where rhizosphere microorganisms such as rhizobacteria

solubilize minerals and mycorrhizal fungi transport these nutrients to the plant [16].

This research aims to synthesize urea-doped calcium, magnesium, and iron phosphate nanoparticles (urea-doped CMM-PO_4 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 CMM-PO_4 Nps with Phosphobacteria, focusing on plant physiological and biochemical parameters relevant to growth, stress tolerance, and photosynthetic function.

1.2. NANOPARTICLES OF ESSENTIAL ELEMENTS

*1. **Calcium Nanoparticles***

Calcium plays a fundamental role as an intracellular signaling molecule in living systems. Variations in cytosolic calcium concentration regulate many cellular functions, including muscle contraction, neurotransmitter release, and cardiac activity. Calcium signaling involves membrane channels, pumps, exchangers, intracellular calcium stores, and calcium-binding proteins [17]. These signaling components are widely distributed in most cell types, including somatic and germ cells. In oocytes, eggs, and early embryos, calcium signals are especially important as they regulate developmental transitions [18]. These signals occur in a precise, non-repetitive sequence and act as markers of changes in cellular state during early development.

*2. **Magnesium Nanoparticles***

Magnesium is an essential macronutrient required for normal plant growth and development. It plays a key role in the formation of seeds and roots and significantly influences crop productivity. Magnesium is involved in enzyme activation, protein synthesis, carbohydrate metabolism, and energy transfer in plants. Deficiency of magnesium leads to reduced germination, poor seedling establishment, and lower starch accumulation. Foliar application of magnesium improves physiological and biochemical processes in plants. Long-term studies have shown a decline in magnesium content in cereal crops due to yield dilution and imbalanced fertilization practices. Therefore, appropriate magnesium fertilization is necessary to

enhance agronomic efficiency and sustain crop yields [19].

*3. **Manganese Nanoparticles***

Manganese is an essential micronutrient that supports various physiological and metabolic processes in plants. Different inorganic and organic manganese sources are used to correct manganese deficiency in agricultural soils. Soil application studies indicate that manganese sulfate and manganese oxysulfate are more effective than manganese oxide in improving crop yield. Organic manganese chelates and complexes show comparable effectiveness when applied at suitable rates. Foliar application of both inorganic and organic manganese sources has also been shown to increase crop yield [20]. The effectiveness of manganese fertilization depends on the source, application method, and soil conditions.

1.3. NANOPARTICLES AS NANO FERTILIZER

Soils are composed of micro-pores and macro-pores. 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 [21].

Once released into the environment, engineered NFs are aggregated to some extent [22]. The interaction of NF and soil molecules can be favored by the traits of the particles and the surrounding environment. Therefore, the organic content of the soil, the environmental conditions, and the chemical characteristics of NFs can improve or inhibit NP mobility [23].

Throbäck et al. [24] reported that denitrifying bacteria present in the soil are more susceptible, which disturbs 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 have some adverse effects on beneficial soil microflora.

2. OBJECTIVES

- To Synthesis Urea-doped Calcium, Magnesium, Manganese Phosphate Nano particles (Urea-doped -

CMM-PO_4) by standard protocol.

- To Characterize the synthesized nano particles by FT-IR Spectroscopy and SEM.
- To analyse its Water Holding capacity.
- To apply the synthesized Nano particles in plant "*Vigna Munga*".
- To Analyse Chlorophyll, Proline, SOD, POD, COD, LPO and Photosynthetic Efficiency content in plant "*Vigna Munga*".
- To Compare the plant growth in Urea-doped Calcium, Magnesium, Iron Phosphate Nano particles (Urea-doped - CMI-PO_4) with Biofertilizer Phosphobacteria, Nanourea, Urea and control.

3. METHODOLOGY

3.1 Synthesis of Urea doped Ca, Mg, Mn Phosphate Nano particles

Materials

Analytical grade reagents, including Calcium Nitrate tetrahydrate [Ca $(NO_3)_2 \cdot 4H_2O$], Magnesium Nitrate hexahydrate [Mg $(NO_3)_2 \cdot 6H_2O$], Manganese Nitrate hexahydrate [$Mn(NO_3)_2 \cdot 6H_2O$], Sodium Citrate [$Na_3C_6H_5O_7$], and urea [$CO(NH_2)_2$], Disodium Hydrogen Phosphate [Na_2HPO_4], Sodium Carbonate [Na_2CO_3] and Potassium Nitrate [KNO_3] 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 CMM-PO_4 Nps was carried out according to the protocol reported by Gaiotti et al. [25].

Preparation of Precursor Solutions: 0.4 M solutions of the respective metal nitrates, 0.24 M Disodium Hydrogen Phosphate, 0.2M Sodium Carbonate and 0.9M Sodium Citrate were prepared separately.

Solution A - Calcium Nitrate (V= 18.75ml), Magnesium Nitrate (V=18.75ml), Manganese Nitrate (V=5 ml) and Sodium Citrate (V=37.5ml).

Solution B - Disodium hydrogen phosphate (V=25ml), sodium carbonate (V=25ml) and potassium nitrate (V=25ml).

Aging: Solution A and Solution B were mixed together. The reaction mixture was

stirred, then aged at 35°C for 15 minutes to facilitate nucleation and growth.

Addition of Urea: The resultant precipitate was centrifuged at 4500 rpm for 10 minutes. The slurry obtained from two preparations was mixed with a solution of Urea at a predetermined molar ratio (optimized at 5:1 for solution: urea based on preliminary trials) and stirred vigorously to obtain a homogenous mixture.

Co-precipitation: Before precipitation, solution B has a pH of 11.2. After the addition of solution A, the fast precipitation of urea doped calcium magnesium manganese phosphate made the pH value drop down to 7.6.

Separation and Washing: The precipitate was washed sequentially with deionized water and ethanol to remove unreacted species, and dried.

Grinding: After freezing and lyophilizing (Telstar LyoQuest 55 Eco), the dried precipitate was gently ground using an agate mortar to obtain fine nanopowders of CMM-PO_4 NPs.

3.3 WATER HOLDING CAPACITY OF SOIL (PERCOLATION METHOD)

Materials Required

- Four soil samples (oven-dried for 24 hours and powdered)
- Four identical funnels
- Four graduated cylinders (100 mL or 250 mL capacity)
- Four beakers (100 mL or 250 mL capacity)
- Four filter paper circles
- Weighing scale (accurate to at least 1 g)
- Distilled water

Procedure

Take four oven-dried soil samples and weigh 25 g of each using a weighing scale. Label each sample as Soil A, B, C, and D and keep them aside.Prepare the percolation setup by folding a filter paper circle twice and opening one-fold to form a cone. Insert the filter paper cone into a funnel.Place the funnel securely into the mouth of a graduated cylinder. This forms one percolation setup.Prepare four such identical and appropriately labeled percolation

setups for the four soil samples.Carefully transfer each pre-weighed soil sample into the corresponding filter paper cone placed in the funnel.Measure 50 mL of water using four labeled beakers.Slowly add water in small increments into each soil-filled funnel. Ensure that the water level does not exceed the height of the filter paper cone.Allow the water to percolate through the soil. The excess water will trickle down through the funnel and collect in the graduated cylinder.After all the water has been added, leave the setups undisturbed for several minutes until no more water drips from the funnel and the soil becomes fully saturated.Measure and record the volume of water collected in each graduated cylinder.

Calculation

$$\text{Water Holding Capacity (\%)} = \frac{(Water\ added) - (Water\ collected)}{(Water\ added\ )} \times 100$$

3.3. ESTIMATION OF CHLOROPHYLL CONTENT

In this study, we use the plant *Vigna mungo* leaves to estimate the Chlorophyll A (Chlo A) and Chlorophyll B (Chlo B) content by using Arnon (1949) method [26]. One gram of finely cut fresh leaves was taken and ground with 20-40ml of 80% acetone. Then it was centrifuged at 5000-10000rpm for 5 minutes. The supernatant liquid was collected and repeat the procedure until the residue became colourless. The absorbance of the solution was determined by spectrophotometer at wavelengths 663nm and 645nm 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:

$$\text{Chlorophyll A} = (12.7 \times A663nm) - (2.69 \times A645nm) \times \frac{V}{100 \times W}$$

$$\text{Chlorophyll B} = (22.9 \times A645nm) - (4.68 \times A663nm) \times \frac{V}{100 \times W}$$

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

3.4. ESTIMATION OF PROLINE CONTENT

Proline content is estimated by using the method of Bates et al. (1973) [27]. 0.5g of leaf sample and macerate with 10ml of 3% sulfosalicylic acid and centrifuged at 3000rpm for 10 minutes. Take 2ml of supernatant solution in a test tube and add 2ml of acid ninhydrin, 2ml of glacial acetic acid and 2ml of 6M ortho phosphoric acid and keep the test tube in hot water bath for 1 hour and cool the test tube under tap water. Transfer the solution to the separating funnel and add 4ml of toluene and shake the separating funnel uniformly for 30 seconds and note the formation of two different layers. Discard the colourless bottom layer and collect the upper pink colour solution and measure the OD value at 520nm and the quantity of proline was calculated using a standard curve. The result was represented in μgg^{-1} and $mgg^{-1.}$

3.5. Superoxide Dismutase (SOD) Activity

Principle

Superoxide dismutase (SOD) is a crucial antioxidant enzyme responsible for catalysing the dismutation of superoxide radicals, thereby safeguarding plant cells from oxidative damage.

Reagents

- 50 mM phosphate buffer (pH 7.8)
- 1 mM EDTA
- 20 mM L-methionine
- 1.1 mM NBT
- 0.1 mM EDTA
- 0.1 mM riboflavin

Procedure

Homogenize 0.5 g of leaf samples in 5 mL of 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA, followed by centrifugation at 12,000 rpm for 20 minutes at 4°C to obtain the supernatant as the enzyme source. Prepare the reaction mixture 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 NBT, 0.1 mL of 0.1 mM EDTA, and 0.1 mL of the enzyme extract. Initiate the reaction with the addition of 0.1 mL of 0.1 mM riboflavin and expose the tubes to a fluorescent lamp for 15 minutes. Finally, measure the absorbance at 560 nm to determine the activity (Kono *et al*., 1978)[28].

Calculation

SOD activity (U/g FW) =

$$\frac{(Absorbance\ at\ 560nm)\times(Total\ volume\ of\ reaction)}{(Extinction\ coefficient)\times(Sample\ weight)}$$

3.6. Peroxidase (POD) Activity

Principle

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.

Reagents

- 50 mM phosphate buffer (pH 7.0)
- 1 mM EDTA
- 20 mM guaiacol
- 40 mM H_2O_2

Procedure

Homogenize 0.5 g of leaf samples in 5 mL of 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA, followed by centrifugation at 12,000 rpm for 20 minutes at 4°C to obtain the supernatant as the enzyme source. Prepare the reaction mixture by combining 2.7 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 20 mM guaiacol, 0.1 mL of 40 mM H_2O_2, and 0.1 mL of the enzyme extract. Record the increase in absorbance at 470 nm for 1 minute to determine peroxidase (POD) activity (Putter, 1974)[29]

Calculation

POD activity (U/g FW) =

$$\frac{(Change\ in\ absorbance\ per\ minute)\times(Total\ volume\ of\ reaction)}{(Extinction\ coefficient)\times(Sample\ weight)}$$

3.7 Lipid Peroxidase (LPO) Activity

Principle

Lipid peroxidation serves as an indicator of oxidative stress in plants, with malondialdehyde (MDA) often used as a marker.

Reagents

- 0.1% (w/v) trichloroacetic acid (TCA)
- 0.5% (w/v) thiobarbituric acid (TBA) in 20% TCA

Procedure

Grind 0.5 grams of leaf specimens in 5 milliliters of a 0.1% (w/v) solution of trichloroacetic acid (TCA), then subject the resulting mixture to centrifugation at 12,000 revolutions per minute for 20 minutes. Collect the supernatant and combine 1 milliliter of it with 2 milliliters of a 0.5% (w/v) solution of thiobarbituric acid (TBA) in 20% TCA. Heat the mixture at 95°C for 30 minutes, followed by cooling on ice. Finally, determine the absorbance of the resulting pink-colored complex at 532 nano meters (Heath and Packer, 1968)[30].

Calculation

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

3.8. PHOTOSYNTHETIC EFFICIENCY

Weigh 0.25 g leaf sample, macerate with 10ml of phosphate buffer solution and centrifuge the leaf extract at 3000 rpm for about 10 minutes and collect the supernatant solution. Pipette out 1ml of the supernatant solution into a test tube and add 5ml of Alkali copper reagent keep the solution as such for 30 minutes for color development. Then, add 0.5 ml of Folin-Ciocalteau (phenol) reagent and read the absorbance of the sample at 660 nm in spectrophotometer. The soluble protein content is expressed as mg g^{-1} of leaf sample.

Calculation

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

Amount of soluble protein present in given sample is expressed in mg g^{-1}

4. RESULTS AND DISCUSSION

4.1. FTIR: Fourier Transform Infrared Rays

The characteristic peaks of urea doped Calcium, Magnesium, Manganese oxide nanoparticles can be recognized in the bands as to be: δ(NH) Stretching in 3194.12 cm^{-1}[31], $v(NH_2)$ in 1535.34 cm^{-1} , (Ca-O) in 586.36cm^{-1}, (Mg-O) in 447.49cm^{-1} ,(Mn-O) in 563.21cm^{-1}.

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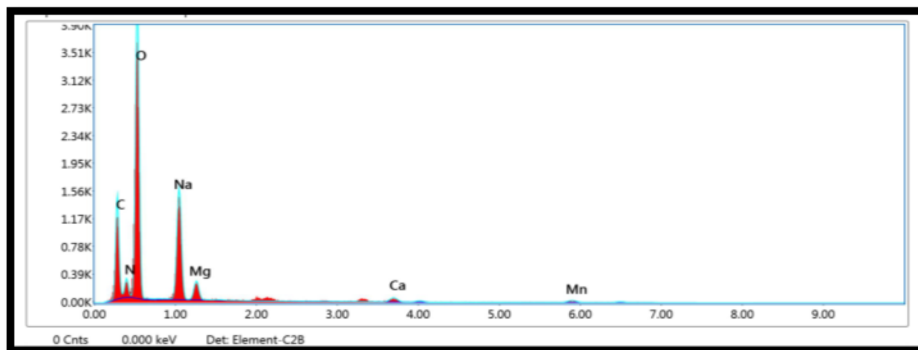
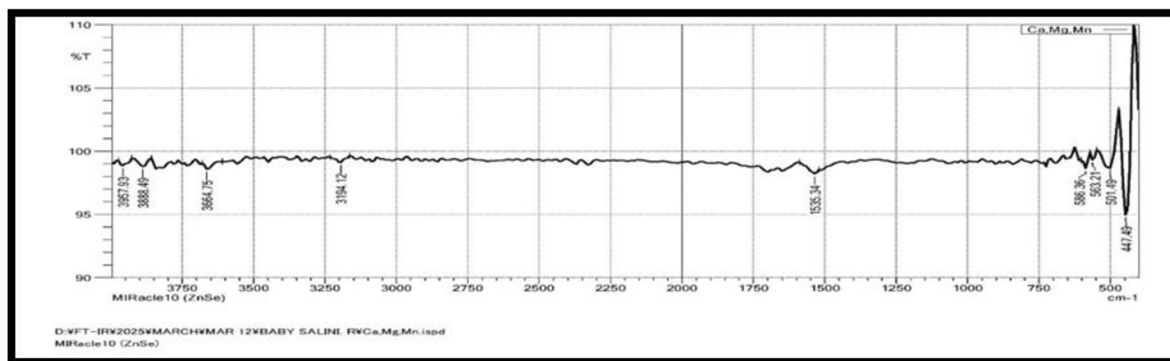


Figure 4.1.1. FTIR Spectrum of Urea-doped -CMM-PO_4 nanoparticles

4.2. ENERGY DISPERSIVE ANALYSIS



The EDAX Spectrum in Fig: 4.2.1. represents 8 major signals Ca (3.8 key), Mg (1.2 key) Mn (6.0 key), C (0.2kev), N (0.3 Kev), O (0.5 Kev), Na (1.0 Kev) indicated the elements Ca, Mg, Mn, O, Ca, K, Na, respectively. The formation of urea doped Ca, Mg, Mn phosphate nano particles are confirmed by the EDX Spectrum.

Figure 4.2.1. EDAX spectrum of Urea-doped -CMM-PO_4 nanoparticles

Element	LINE TYPE	WEIGHT %	ATOMIC %
Ca	**K Series**	**1.41**	**0.57**
Mg	**K Series**	**2.85**	**1.90**
Mn	**K Series**	**1.62**	**0.48**
C	**K Series**	**20.55**	**27.73**

N	**K Series**	**5.77**	**6.68**
O	**K Series**	**48.21**	**48.83**
Na	**K Series**	**19.59**	**13.81**

Table 4.2.1. Weight Percentage of elements found in EDAX Spectrum

4.3. FE-SEM ANALYSIS

The morphology of the fabricated urea doped calcium, magnesium and manganese phosphate nanoparticles was examined by SEM analysis. The figure 4.3.1. shown irregular shaped with broad size distribution of nanoparticles. Nano fertilizers were of various sizes, as confirmed by previous literature [32].

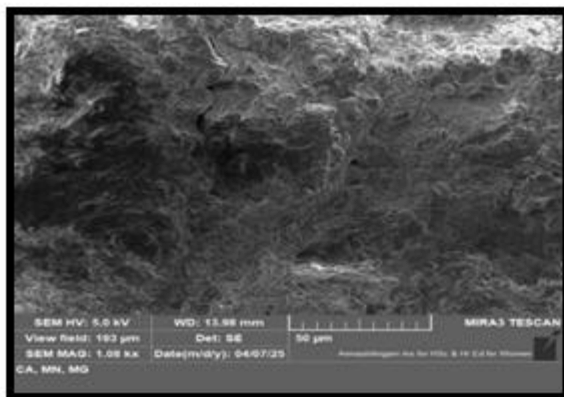


Figure: 4.3.1. SEM image of Urea-doped -CMM-PO_4 nanoparticles

4.4. WATER HOLDING CAPACITY OF SOIL (PERCOLATION METHOD)

Water holding capacity (WHC) value was calculated for soil mixed with nano fertilizer, soil without fertilizer, soil with biofertilizer and soil mixed with urea. It was observed that the WHC capacity of Nano fertilizer mixed soil was 72% for soil mixed urea 56%, soil mixed with biofertilizer was 88% and soil without fertilizer 48%. The water holding capacity for Nano fertilizer mixed soil was higher water holding capacity rate. The higher water holding capacity is essential for saving water to improve the plant's health.

FORMULA:

$$\%WHC = \frac{(V1 - V2)100}{\text{Weight of sample}}$$

SAMPLE	WEIGHT OF SAMPLE	VOLUME OF WATER POURED THROUGH (v1)	VOLUME OF WATER COLECTED IN CYLINENDER (v2)	VOLUME OF WATER RETAINED BY SOIL (V1-V2)	PERCENTAGE OF WATER HOLDING CAPACITY
Soil A(Water)	25	50	38	12	48%
Soil b (Urea)	25	50	36	14	56%
Soil C (Urea-doped CMM-PO_4 Nps)	25	50	32	18	72%
Soil D (Urea-doped CMI-PO_4 Nps + Bio Fertilizer)	25	50	28	22	88%

Table 4.4.1. Analysis of Water Holding Capacity

Figure: 4.4.1. Estimation of water holding capacity

4.5. CHLOROPHYLL ESTIMATION

The chlorophyll content was recorded at four chosen *Vigna Munga* plants was determined within the interval of 30 days. The difference of chlorophyll content without fertilizer with urea, urea doped calcium magnesium and manganese phosphate nanoparticles with phospho bacteria and urea doped calcium, magnesium and manganese phosphate nanoparticles $A = \pi r^2$ tabulated below. We found significant differences in the Urea-doped CMM-PO_4 Nps with phosphobacteria

treatment compared with other three. This significantly increases the chlorophyll content of plant.

Fig 4.5.1. Water Urea Urea-doped CMM-PO_4 Nps Urea-doped CMM-PO_4 Nps with Phosphobacteria

Fig 4.5.2. Estimation of chlorophyll

4.6. PROLINE

In this study, proline content in plant *Vigna Munga* was estimated before and after 15 days of application of Urea-doped CMM-PO_4 Nps with Phosphobacteria, Urea-doped CMM-PO_4 Nps, Urea and control manner. In the case of Urea-doped CMM-PO_4 Nps with Phosphobacteria the value of proline increased by 0.6600 followed by Urea-doped CMM-PO_4 Nps increases by 0.5882. In contrast, in case of

Urea and control the value increased by 0.2479 and 0.1026. From the table, the significant difference between control (water) and treated plants were observed clearly.

PROLINE	BEFORE	AFTER	DIFFERENCE
Water	0.0954	0.1980	0.1026
Urea	0.5482	0.7961	0.2479
Urea-doped CMM-PO_4 Nps	0.1071	0.6954	0.5882
Urea-doped CMM-PO_4 Nps with Phospho Bacteria	0.1056	0.7656	0.6600

Table 4.6.1. Analysis of Proline in plant *Vigna mungo*

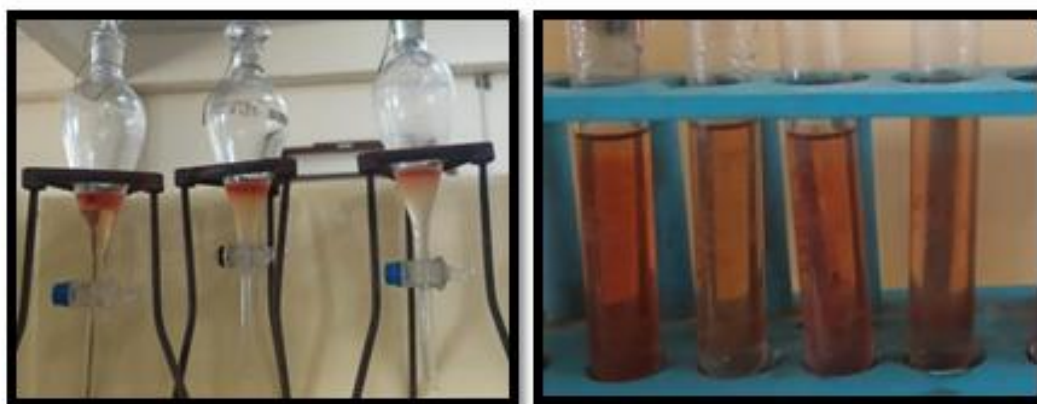


Figure 4.6.1. Proline Estimation

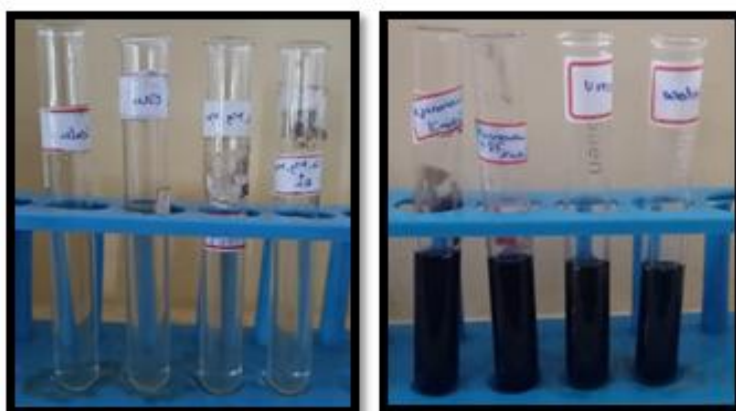
4.7. Photosynthetic efficiency – soluble protein (OD@660nm)

In this study, the photosynthetic efficiency for plant *Vigna mungo* was estimated before and after 15 days of application of Urea-doped CMM-PO_4 with Phospho Bacteria, Urea-doped CMM-PO_4, Urea and control manner. In the case of Urea-doped CMM-PO_4 with Phospho Bacteria the value of photosynthetic efficiency increased by 0.9780 followed by Urea-doped CMM-PO_4 0.8060. In contrast, in case of Urea and control the value of photosynthetic

efficiency increased by 0.4721and 0. 3873. Thus, Urea-doped CMM-PO_4 with Phospho Bacteria serves as the best fertilizer for plants and increases their photosynthetic efficiency.

Photosynthetic efficiency	Before	After	Difference
Water	0.7098	1.0971	0.3873
Urea	0.8992	1.3713	0.4721
Urea-doped CMM-PO_4Nps	0.6155	1.4215	0.8060
Urea-doped CMM-PO_4 Nps +Phosphobacteria	0.6912	1.6692	0.9780

Table 4.7.1. Analysis of in Photosynthetic efficiency plant *Vigna mungo*



4.7.1. Photosynthetic efficiency Estimation

4.8. SOD

The results showed that Urea-doped CMM-PO_4 Nps with Phospho Bacteria led to the highest increase in SOD activity, with a rise of 0.9587 after 15 days. Urea-doped CMM-PO_4 Nps also significantly increased SOD activity, with an increase of 0.3823. In contrast, urea and control showed relatively minor increases in SOD activity, with rises of 0.09 and 0.0809, respectively. These findings suggest that Urea-doped CMM-PO_4 with Phospho Bacteria has a substantial

impact on SOD activity, potentially enhancing plant stress tolerance.

SOD	Before	After	Difference
Water	0.3427	0.4236	0.0809
Urea	0.3715	0.4681	0.0966
Urea-doped CMM-PO Nps	0.2018	0.3699	0.1681
Urea-doped CMM-PO_4 with Phospho Bacteria	0.2143	0.9215	0.7022

Table 4.8.1. Analysis of SOD in plant *Vigna mungo*

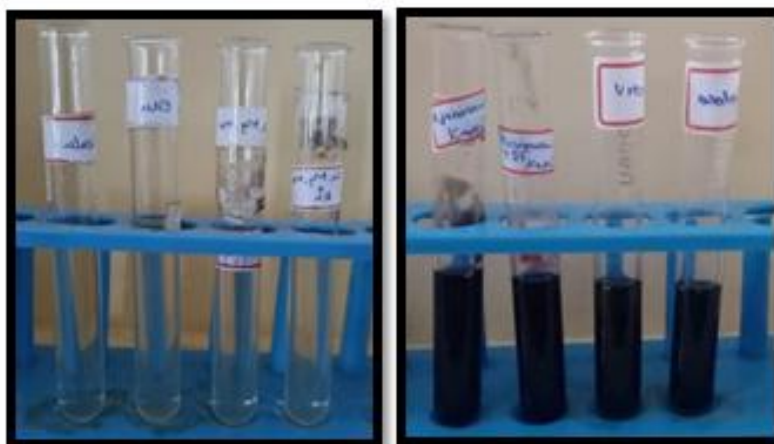


Figure: 4.8.1 SOD Estimation

4.9. POD

The study evaluated Peroxidase (POD) activity in plants treated with different fertilizers. The results showed that Urea-doped CMM-PO_4 Nps with Phospho Bacteria led to the highest increase in POD activity, with a rise of 0.3546. Urea-doped CMM-PO_4 Nps alone also increased POD activity, with an increase of 0.1347. In contrast, urea and control showed relatively minor increases in POD activity, with rises of 0.0686 and 0.0170, respectively. These findings suggest that Urea-doped CMM-PO_4 Nps with Phospho Bacteria has a significant

impact on POD activity, potentially enhancing plant defence mechanisms.

POD	Before	After	Difference
Water	0.1319	0.1489	0.0170
Urea	0.0378	0.1064	0.0686
Urea-doped CMM-PO_4 Nps	0.0982	0.2329	0.1347
Urea-doped CMM-PO_4 Nps with Phospho Bacteria	0.1089	0.4635	0.3546

Table 4.9.1: Analysis of POD in plant *Vigna mungo*

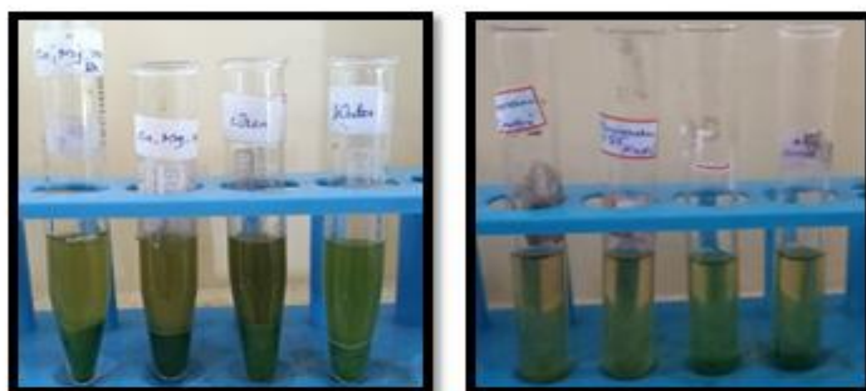


Fig: 4.9.1 POD Estimation

4.10. LIPID PEROXIDATION

LPO can refer to lipid peroxidation, which is a process that damages cell membranes in plants and animals. LPO is a series of reactions that involve free radicals attacking polyunsaturated fatty acids. LPO can damage cell membranes, which can lead to cell death. LPO can also activate signaling pathways that promote antioxidant defense mechanisms. LPO is a complex process that can both damage cells and regulate redox homeostasis. Urea-doped CMM-PO_4 Nps with Phospho Bacteria proved to be a potent fertilizer, reducing Lipid Peroxidation (LPO) by 0.8332, and thereby minimizing oxidative stress in plants. This outperforme d Urea-doped CMM-PO_4 Nps (0.6801 decrease),

urea (0.4999 decrease), and control (0.3156 decrease), showcasing its effectiveness in promoting plant health.

LPO	BEFORE	AFTER	DIFFERENCE
Water	1.1218	0.8062	-0.3156
Urea	1.3061	0.6437	-0.4999
Urea-doped CMM-PO_4 Nps	1.1654	0.04853	-0.6801
Urea-doped CMM-PO_4 Nps with Phospho Bacteria	1.2139	0.3817	-0.8322

Table 4.10.1 Analysis of LPO in plant *Vigna mungo*

5. CONCLUSION

The study showcases the potential of nano fertilizers in sustainable agriculture, leveraging their high surface area and targeted nutrient delivery to boost crop production. Successful synthesis of Urea-doped CMM-PO_4 Nps was confirmed through FTIR, EDX, and SEM analyses. When combined with Phospho Bacteria biofertilizer and applied to *Vigna mungo*, the hybrid significantly enhanced plant enzymes activities SOD, POD and also increases chlorophyll, proline and photosynthetic efficiency. It also reduced stress-related enzyme like LPO, promoting plant growth and health. The hybrid outperformed Urea-doped CMM-PO_4 Nps with Phosphobacteria, urea, and control showed improved water-holding capacity. Future research will focus on evaluating the safety, toxicity, and bioavailability of these nano fertilizers, exploring their potential to support sustainable agriculture.

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