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MORPHOLOGICAL CHARACTERISATION, ANTI-OXIDANT, ANTIMICROBIAL ANALYSIS OF ISOLATED NANO-FERTILIZER AND ITS APPLICATION ON SOIL FOR THE GROWTH OF SPINACH *(Spinacia oleracea)*

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ABSTRACT----------
Plants are known as multicellular creatures. Photosynthesis is an important process used by plants to produce their own food for their sustainability. Plants play an important role in food chains as plants are eaten by many living organisms. Oxygen, which plays a major role in living, is produced by plants. By giving living things food and oxygen, plants are essential to the energy and environmental ecology. Research of the impact of nanoparticles in environmental sectors (particularly in agriculture) has become more important because of the growing use of nanoparticles in the last ten years, important. In this study, we showed how iron oxide (Fe2O3) nanoparticles are synthesized by self-combustion method and their morphological characteristics and particles size. Furthermore, this study also focuses on how ingested iron oxide (Fe2O3) nanoparticles were absorbed by spinach and investigated how this affected the plant's growth and productivity. The results of experiments on plant development (stem and root length) showed that the uptake of Fe2O3 led to a dose- and time-dependent increase. Bacterial count analysis was done for Total Plate Count (Nutrient Agar), Yeast and Mold Count (YGCA), and Coliform count (MCA) was done for the Nano-fertilizers isolated from banana peels and orange peels. Antioxidant analysis was also done for the Nano-fertilizers by analyzing the DPPH (a, a-diphenyl-picrylhydrazyl) activity of the Nano-fertilizers. Iron, Nitrogen, Potassium and Phosphorus content was analyzed for spinach grown on soil containing Nano-fertilizers and comparative study was done with the spinach grown on normal soil.
KEY WORDS: *Antioxidant, Bacterial count, Nano-fertilizers, Nanoparticles, Spinach, DPPH*----------

1. INTRODUCTION

Nanosized material plays a vital role in human welfare society, and it provides great opportunities for industrial products in research and development sector. Numerous biological uses, including anti-bacterial, biofilm inhibition, cancer detection and therapy, and dentistry applications, have made use of nanomaterials with sizes between 1 and 100 nm6. Additionally, the use of nanoparticles in the production of consumer goods including paints, textiles, solar roofs, and cosmetics indicates that these materials may be released into the environment. Nanotechnology is the management and control of shape and size at the nano meter scale used in the synthesis and application of devices. It has opened the path and made it possible to employ "smart fertilizer," or materials with nanostructures, as fertilizers. Furthermore, the efficient nutrient uptake, soil fertility restoration, ultra-high absorption, increased photosynthesis, increased production, decreased soil toxicity, decreased frequency of application, increased plant health, and decreased environmental pollution can all be made possible by the composition of nano-fertilizers *(A. Haleem, et.al.2023)*

NANO-FERTILIZERS

To permit regulated release and subsequent gradual diffusion into the soil, nutrients are encapsulated or coated with nanomaterial to create nano fertilizers. Utilizing nanoscale fertilizers could prevent nitrogen loss through leaching and runoff and cut down on Fast decomposition and volatility improve the soil's fertility and nutrient quality, boosting crop output over time. Furthermore, nano fertilizers may be a good substitute for chemical fertilizers due to their high surface area to volume ratio and strong penetration capabilities. Additionally, the use of nano fertilizers, also known as "nano biofertilizers," might significantly lessen the environmental hazard. (*A. Nongbet, et.al.2022)*

NEED FOR NANO-FERTILIZERS

The need for environmentally friendly, effective, and non-toxic nano fertilizer synthesis has increased demand for the bio-fabrication of NPs using biological processes technologies. Nanoscale coating fertilizers, nanoscale additive fertilizers, and nano porous materials are the three different ways that nano fertilizers can be made, depending on the nutrient requirements for plant growth and development. Nutrient delivery systems that are nano enabled, have a high surface-area-to-volume ratio, and can provide both calcium and phosphorus

to plants are hydroxyapatite-containing nano fertilizers. Potential nano encapsulated fertilizers for the gradual releasing of nitrogen includes urea-loaded hydroxyapatite nanohybrids. Mesoporous silica NPs have the potential to improve crop quality and yield due to their exceptional properties, including their large surface area, mesoporous architecture, biocompatibility, and non-toxicity. Because of the growing commercialization of agriculture and the rising demand for food grains to feed an expanding population, the Indian seed market is predicted to expand at a rapid rate throughout the projected period. Over the next five years, it is anticipated that the production of food grains will increase and that government spending on agriculture will rise. Additionally, a growing number of farmers are becoming aware of the benefits of using certified seeds and rising disposable income, which is further boosting demand for seeds, especially hybrid seeds—across the nation. The seed market in India is divided into segments depending on the kind of product, crop, availability, sales channel, and geography *(K.Tara Meghan., et.al., 2021)*

2. MATERIALS AND METHODS

2.1 Sample Preparation

2.1.1 Isolation of Nano-fertilizers from Banana and orange peels

Nano-fertilizers were isolated from the peel of banana and orange. The banana and orange peels were collected from the local juice market in Lucknow for the extraction of Nano-fertilizers. 0.40kg of banana peels and orange were taken and soaked for 2-3 weeks. Then both the peels were washed thoroughly and shredded into small pieces and then they are subjected to blending by using high-speed mechanical blender. For banana peels the slurry was mixed with potassium hydroxide in specified amount and continuous mixing was done for 1 minute. This will make the slurry alkaline based and then heated for 30 minutes. The slurry was subjected to cooling at room temperature, and the citric acid was added in the mixture, dropwise till it acquires the pH of 5. Then, the slurry was dried in hot air oven and blended to make fine powder. For the isolation of nano-fertilizers from orange peels the slurry of blended orange peels are allowed to filter by using filter cloth. For maintain the pH up to 5, an alkaline solution is added in the acquired filtrate, and it is subjected to drying in hot air oven at 110°C and ground to get fine powder.

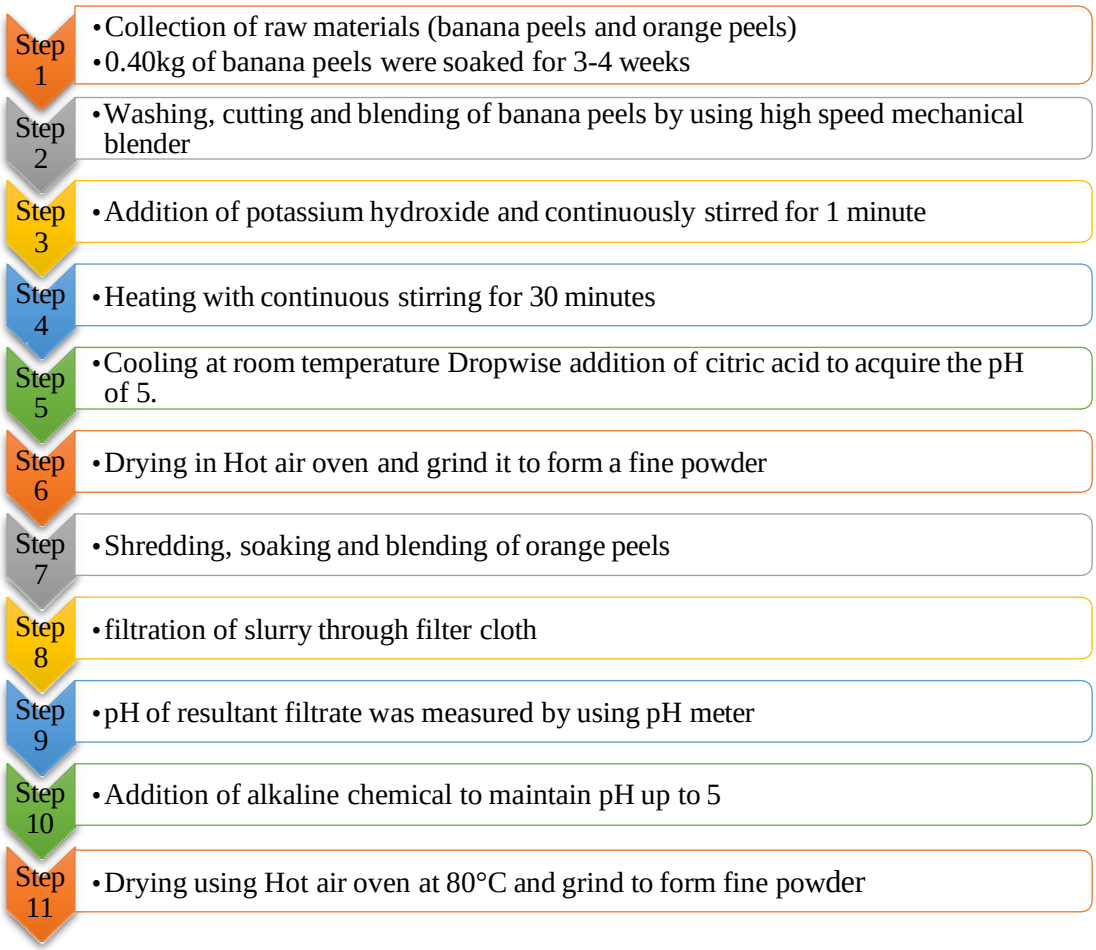

Fig 2.1 Flow chart of isolation of Nano-fertilizers

2.1.2 Synthesis of Iron Oxide Nanoparticles

Ferric (III) Nitrate, also known as Iron Nitrate with chemical formula Fe (NO3)3 is used for synthesis of Iron Oxide Nanoparticles for the

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fortification in Nano-fertilizers by using Self-combustion method. The ferric nitrate was taken in the amount of 20g, and it was dissolved in minimum amount of distilled water. The citric acid was added in the mixture having the ratio of (1:1.1). To maintain the pH of solution up to 9, 25% of Ammonia solution was taken and added dropwise by using a dropper. Now the complete evaporation of the solvent was performed by heating the resultant alkaline solution on a hot plate at 80°C until brown-red ash is formed. This brown-red ash was kept in muffle furnace for 7 hours at 600°C. The resultant powder is our Iron Oxide Nanoparticles. For further processing, isolated nano-fertilizers from banana peels and orange peels are mixed along with Iron Oxide Nanoparticles for the fortification process. The three isolated nano-sized materials were mixed in the ratio of 4:4:2 for banana peels, orange peels and Iron oxide Nanoparticles respectively.

$$10\ CH_2NH_2COOH + 6\ Fe\ (NO_3)_3 \rightarrow 3\ Fe_2O_3 + 14\ N_2 + 20\ CO_2 + 25\ H_2O$$

$$5\ CO(NH_2)_2 + 2\ Fe\ (NO_3)_3 \rightarrow Fe_2O_3 + 8\ N_2 + 5\ CO_2 + 10\ H_2O$$

$$5\ C_6O_7H_8 + 6\ Fe\ (NO_3)_3 \rightarrow 3\ Fe_2O_3 + 9\ N_2 + 30\ CO_2 + 20\ H_2O$$

Estimated final equation of reactions by observing weight measurements and evaporating gases of the product (Ondrej Seibert et.al., 2019)

Preparation- The ratio is considered as 40% of nano-fertilizers isolated from banana peels, 40% of nano-fertilizers isolated from orange peels, and 20% of iron oxide nanoparticles isolated from ferric nitrate.

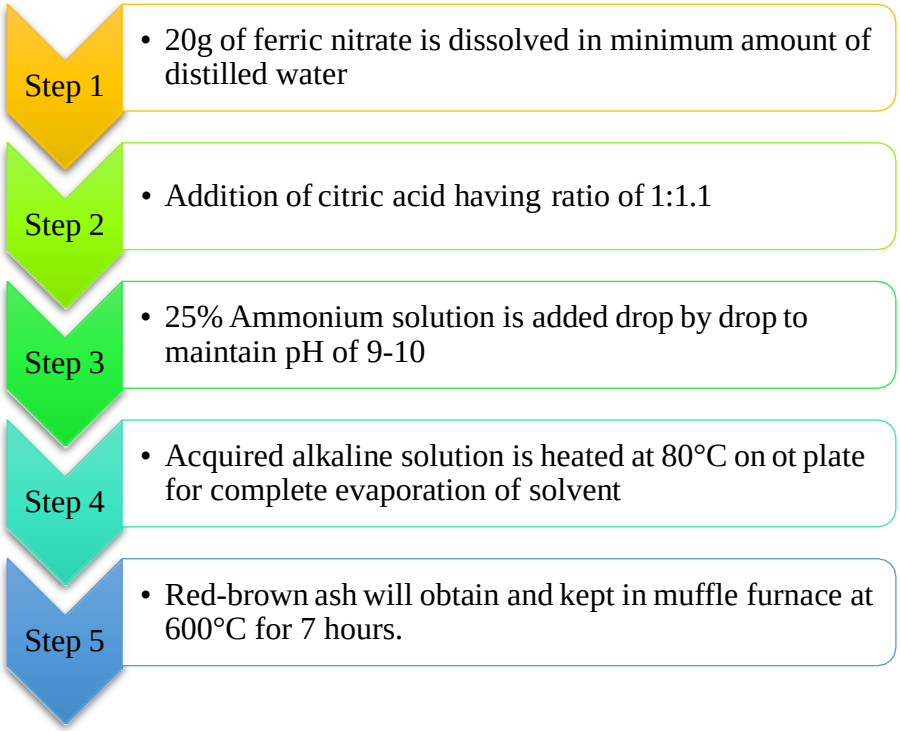

Fig 2.2 Flow chart of synthesis of Iron oxide nanoparticles from ferric nitrate using self-combustion method

2.2 Characterization of Iron Oxide Nanoparticles

2.2.1 Fourier-transform infrared spectroscopy (FTIR) analysis of Iron-oxide Nanoparticles

The FTIR analysis of the Iron Oxide Nanoparticles is done to study the vibrational and rotational properties of the NP molecules. It helps to identify the molecular binding and their structural changes between microbes and metal atoms, to find out the nature of their association and interaction. Functional groups of iron oxide nanoparticles are analyzed. The FTIR analysis was performed at the Instrumentation Research Centre of Babasaheb Bhimrao Ambedkar University, Lucknow. 4000-500 cm^{-1} was the spectral range and the sample with a particle size of <0.2mm was undertaken for the analysis.

2.3 Characterization of Extracted Nano-fertilizer

2.3.1 Scanning Electron Microscopy (SEM) Analysis of Extracted Nano-fertilizers

SEM analysis is done to know the particle- size of the by-product-based nano-fertilizer and to conclude whether their size is <100nm or not. SEM is a powerful and innovative technology that is used to study the chemical composition and morphological structure and

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texture of Nano- fertilizers. SEM analysis was performed at the USIC lab of Babasaheb Bhimrao Ambedkar University, Lucknow. Using a dehydrator, the sample was dried at 50°C for 6-7 hours. Keep the sample in Eppendorf micro-centrifuge tubes to protect the sample from relative humidity. Coating of 2-4 mm of dried sample was done by using a sputter coater of J.E.O.L. Analysis of the sample was done at 15 K.V. *(Fonseca et al., 2021).* Observation of images is done at high magnification, and the representative parts of the images were taken.

2.3.1 EDS Analysis of Nano-fertilizers

EDS stands for Energy-dispersive Spectroscopy which is a very powerful and non-destructive technique undertaken without sample removal. EDS material identification is performed by a combination of morphology via SEM imaging and elemental composition via EDS. It yields a qualitative spectrum displaying the components present in the sample. Additional analysis can be performed to quantify the discovered components to refine the material identification.

2.4 Free Radical Scavenging Activity (DPPH) of prepared Nano-fertilizers

The UV visible spectrophotometer with small modifications was used for the calculation of antioxidant potential of DPPH for nano-fertilizers. The color of DPPH was dark blue in methanol. The antioxidant compound changes its color from purple to yellow, in its reduced form, by allowing DPPH to gain electrons. At 517nm, DPPH shows strong absorption determined by 1,1-diphenyl-2-pyridyl hydroxylase (DPPH). DPPH was prepared in a 0.1 m/mol/L methanol solution. 1 ml of this solution can be combined with 3-5 ml of sample at varying concentrations (0, 50, 75, 100, 150 µg/ml). A control sample of 1 ml of methanol was prepared and incubated in the darkroom for 30 minutes at ambient temperature. A UV Visible spectrophotometer was used to measure absorbance at a wavelength of 517 nm. To calibrate the UV spectrophotometer, methanol is used as a blank. Reduction in the absorption value shows high activity in scavenging free radicals. It was measured as a percentage of DPPH Scavenging by using the following formula given below:

$$\% \text{ DPPH Scavenging activity} = \frac{\text{OD control - OD sample} \times 100}{\text{OD control}}$$

OD=Optical density

Note: The test tube was covered with brown paper as DPPH is very sensitive to light.

2.5 Antimicrobial analysis of prepared nano-fertilizers

An antimicrobial is an agent that kills microorganisms (microbicide) or stops their growth (bacteriostatic agent). Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria, and anti-fungal are used against fungi. They can also be classified according to their function. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.*(Merriam-Webster Online Dictionary).* The total number of yeasts, mold, bacteria and coliform count determined by using standard method *(Wehr and Frank 2004).*

2.5.1 Media Preparation-

2.5.1.1 Media preparation for Total Plate Count-

Nutrient Agar is type of media that helps to grow various types of microorganisms. Beef extract, Peptone and Agar are three main constituents of NA media. It is a very simple and common formulation which provides the nutrients for the replication of generous number of microbes.15g of Nutrient Agar is taken in the conical flask. Then add 0.3g of beef extract and 0.5g of peptone. Mix and dissolve the solution completely by heating it on a hot plate in case of lumps. The solution along with the conical flask is subjected to sterilization by using Auto-clave at 121°C for 15 minutes at 15 pounds pressure. Pour the liquid into a petri dish and solidify the media.

2.5.1.2 Media preparation for Coliform Count-

For the coliform count the media is prepared by using MacConkey agar. MacConkey agar is a type of agar that only shows the growth of gram-negative bacteria. Based on their lactose metabolism it can differentiate the gram-negative bacteria species. It was prepared by suspending 49.53g of agar in 1000ml of distilled water in a conical flask.

2.5.1.3 Media preparation for Yeast and Mold count-

The media preparation for yeast and mold count has been done by using Chloramphenicol Yeast Agar (YGCA) media. The composition of YGCA agar is Yeast extract 0.5%, glucose 20.0%, chloramphenicol 0.1% and agar-agar 14.9% *(Merck Microbiology Manual 12th edition).* Dissolve 40g of media in 1000ml of distilled water and in case of lumps the solution can be subjected to heating on hot plate along with continuous stirring.

2.5.1.4 Preparation of Buffer peptone water-

BPW is basically used for serial dilution and is a type of broth medium. It is used as a base for determination of carbohydrate fermentation patterns of microbes and is used to grow microbes. It is prepared by the hydrolyzation of animal protein sources along with proteolytic enzymes and the resultant mixture is consist of peptides and amino acids. Peptone and sodium chloride water are the two main ingredients

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used. The composition constitutes of 10.0gm/l of peptone and 5.0gm/l of Sodium chloride water. Dissolve 20gm of BPW in 1000ml of distilled water and sterilize it at 121°F for 15 minutes at 15 pounds of pressure *(biologynote.com).*

Homogenization of the 5gm of nano fertilizer sample is done for 1 minute in 45 ml of BPW solution, with the same diluent. Furthermore, dilutions were made by serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} concentrations).

Sterilization of petri-plates is done in autoclave for 24 hrs. From this 1ml of solution is taken by using micropipette and 15-20ml media of MacConkey agar was taken and poured it in a petri plate by using Pour plate method inside a laminar flow. Incubate the prepared media with petri-plate at 37°C for 24 hrs. For the determination of yeast and mold count the diluent was taken in the amount of 1 ml in four petri-plates and 20-25ml of Yeast Glucose Chloramphenicol Agar media was poured by using Pour plate technique. Incubate the media at 25° C for 24 hrs. For Total Plate Count 15-20ml of nutrient agar media along with 1ml of prepared diluent has been taken and poured in the petri plate and incubate it.

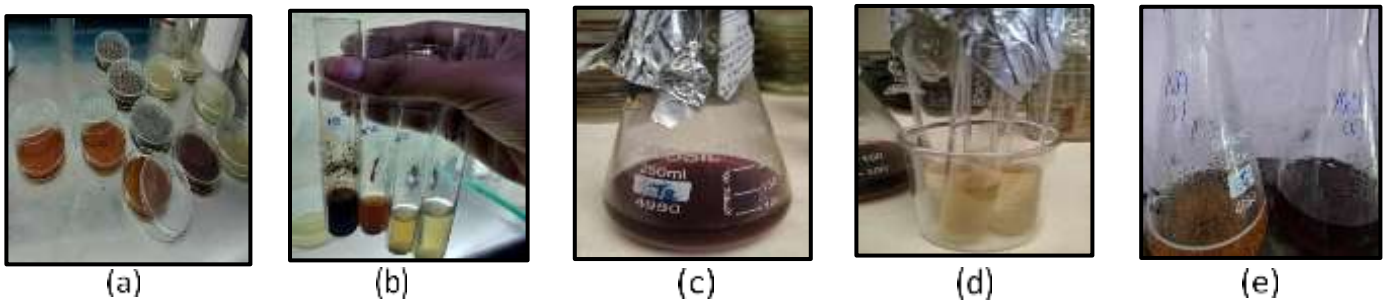

Fig 2.4.1 (a) shows media poured by poured plate technique inside laminar flow, Fig 2.4.1 (b) shows the sample with BPW at different concentration by serial dilution, Fig 2.4.1 (c) Media of Coliform count (MCA), Fig 2.4.1 (d) shows the BPW solution, Fig 2.4.1 (e) shows the media for TPC (NA) and Yeast and mold count (YGCA)

2.6 APPLICATION OF NANO-FERTILIZERS ON SOIL AND PLANT GROWTH

The application of nano-fertilizer was done in the soil by Broadcasting Top dressing method. Broadcasting method of nano fertilizer application refers to the uniformly spreading of fertilizer on soil. By broadcasting methods powdered, rock fertilizers are used. They help the permeation in plant roots to the whole volume of the soil. Large doses of fertilizers are used. Top dressing method usually refers to application of fertilizers on the top layer of the soil.

2.6.1 Preparation of soil

The soil was collected from local land of BBAU, Lucknow. Small sized pots were purchased from local markets and vermicompost and manures are also collected from fertilizer shop in Lucknow. Loosening of soil is done for better penetration effect. Then, manures and compost are added in the soil and mixed thoroughly. Manures are usually used as they prevent leaching and soil erosion.

2.6.2 Sowing of seeds and application of prepared nano fertilizer

Two pots were taken one was considered as control sample (P_1) without any growth promoter chemical or fertilizers. The second pot (P_2) was taken in which prepared Nano-fertilizer was added by using broadcasting top dressing method. To study the effect of nano-fertilizers the spinach *(Spinacia oleracea)* chosen for growth. Seeds were purchased from local seed shop from Ameena bad Market, Lucknow and were soaked overnight for sprouting of seeds. Then the seeds were shown in both the pots and regular watering is done and proper caring of plant. The plants were observed on the daily basis. Results were recorded.

2.6.3 Characterization of soil after the plant growth

Soil analysis of both the samples P_1 and P_2 was done to study the effect of nano-fertilizers on soil. Mineral content was analyzed by using standard methods given below-

- Nitrogen content
- Potassium content
- Phosphorus content
- Iron content
- Calcium content

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2.6.4 Characterization of grown crop

Analysis of both the spinach samples S_1 and S_2 was done to study the effect of nano-fertilizers on growth and germination period. Mineral content was analyzed by using standard methods given below-

- Potassium content
- Phosphorus content
- Iron content
- Calcium content

NOTE: Mineral content analysis of potassium, phosphorus, iron and calcium for both soil samples and spinach samples are done by same standard methods given below.

2.7 ASSESSMENT OF MINERAL CONTENT IN SOIL AND GROWN CROP

Assessment of mineral content in soil and grown crops is done to analyses the effect of applied Nano fertilizers to the soil. The study will show the comparative analysis between the sample S1 (soil sample do not contain any type of plant growth promoter chemical) and S2 (soil sample contain the isolated nano fertilizers from fruit peels fortified with Iron oxide nanoparticles). Similarly, the assessment of mineral content of grown crops will help us to find out about the effect of nano fertilizers in nutrients enhancement in plants by comparing it with plant grown on soil which do not contain any type of fertilizers.

2.7.1 Analysis of Potassium and Phosphorus content

The most common and tremendous technology for the analysis of phosphate and potassium content in the given sample is UV visible spectroscopy. In case of application of NPK-based derived nano fertilizers, total phosphorus and potassium content should be analyzed of the soil and grown crops to find nutrient enhancement due to addition of the Nano fertilizers. For the analysis Molybdate reagent is prepared by 25g Ammonium Molybdate is added in 400ml of distilled water. Dry ashing of molybdate in the amount of 5ml is done to obtain the ash solution. Then, resultant ash solution is taken in the amount of 5ml and addition of 2ml of amino naphthol sulphuric acid solution is done to make up the volume up to 50ml. In the place of a sample a blank solution is prepared with the addition of distilled water. A solution for potassium phosphate is used for the standard. The formula for the calculation is (Rangana, 2005)-

$$P\ (mg/100g) = \frac{mg\ of\ P\ in\ aliquot\ of\ ash\ sol.\ taken\ for\ estimation \times total\ vol.\ of\ ash\ sol. \times 100}{ml\ of\ ash\ sol.\ Taken\ for\ estimation \times wt.\ of\ a\ sample\ taken\ for\ ashing}$$

2.7.2 Analysis of Nitrogen content

The Nitrogen content of the sample was determined by the Kjeldahl method at the Food and Nutrition Department of Babasaheb Bhimrao Ambedkar University, Lucknow. There are three steps for the estimation of nitrogen content i.e., Digestion in which samples are digested with catalyst mixture (SeO_4-2.5g, K_2SO_4-100g. $CuSO_4.5H_2O$-20g) and concentrated sulphuric acid. The second step is Distillation in which 100ml of digested samples were taken and 10ml of aliquot was subjected to distillation with 20ml of 30% sodium hydroxide. The ammonium which gets liberated is collected in boric acid containing mixed indicators constitute of methyl-red and bromo-cresol green of ethyl alcohol. The last step includes Titration in which the collected ammonia is titrated against 0.1 N solution of Hydrochloric acid. The formula is (Rangana, 2005)-

$$\%\ Nitrogen = \frac{[(sample\ titer - blank\ titer)\ x\ Normality\ of\ HCL \times 14 \times 100\ x\ 100]}{(Wt.\ of\ sample\ x\ aliquot\ taken\ for\ distillation) \times 100}$$

2.7.3 Analysis of Calcium content

Visual titration was used to determine the calcium concentration of the samples. In a beaker, combine 20 ml of the ash solution (obtained previously) with 30 ml of distilled water and 10 ml of saturated ammonium oxalate. Using diluted ammonia (1:1) and diluted acetic acid (1:4) solution, the pH of the mixture was brought down to 5.0 before 2 drops of the methyl red indicator were added. The mixture was cooked before spending the night at room temperature. The content was run through Whatman no. 42 filter paper the following day. The residue that was thus produced was thoroughly cleaned with hot distilled water until it was devoid of oxalate. A pointed glass rod accidentally shattered the filter paper, causing precipitate to collect inside. To remove the precipitate, wash the filter paper with 10 ml of hot, diluted H_2SO first. (1:4) after which distilled water get released. To achieve a stable pink coloration, the substance was heated to 80°C and titrated against 0.01N KMnO. The titration was finished after dropping the filter paper into the solution as well. The calculations for calcium were as follows:

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$$Ca(mg/100g) = \frac{titer \times 0.2 total\ volume\ of\ ash\ solution \times 100}{aliquot\ taken\ for\ titration \times wt.\ of\ sample\ taken\ for\ ashing}$$

2.7.4 Analysis of Iron content

The procedure as stated by adding 5 ml sample ash solution, 0.5 ml concentrated H_2SO_4, 1.0 ml saturated potassium per sulphate solution, and 2 ml of 3 N potassium thiocyanate was used to measure the iron concentration in the samples. The capacity was filled with distilled water to a total of 15 ml. In place of the sample, 5 ml of distilled water was obtained for the blank, while 1.0 ml of standard iron sulphate (0.702 g ferrous ammonium sulphate in 1 liter water) was taken for the standard. A spectrophotometer (Evolution 600) was used to measure the developed color at 480m. The sample's iron concentration was calculated as follows: -

$$Iron\ (mg/100g) = \frac{OD\ of\ sample \times 0.1 \times total\ volume\ of\ ash\ solution \times 100}{OD\ of\ standard \times 5 \times wt.\ of\ sample\ taken\ for\ testing}$$

3. RESULTS AND DISCUSSION

3.1 FTIR analysis of Iron-oxide Nanoparticles

The infrared spectrum of Iron-oxide Nanoparticles is shown in Figure 3.1. The peak at 3375.7 cm^{-1} shows the O-H and C-H stretching takes place and the intensity is very strong and broad. The peak at 2916.4 cm^{-1} shows the presence of carboxyl acid and asymmetric stretching of –C-H (CH_2). At 1742.3 cm^{-1}, –C=O (ester) stretching takes place. The peak at 1517.7 cm^{-1} shows the presence of Diketones in the sample. At 1172.2 cm^{-1} presence of ester carbonyl takes place. The peak of 1077.9 cm^{-1} shows the presence of polysaccharides, and the peak of 532.6 cm^{-1} shows the presence of Halogen compounds *i.e.*, C-I.

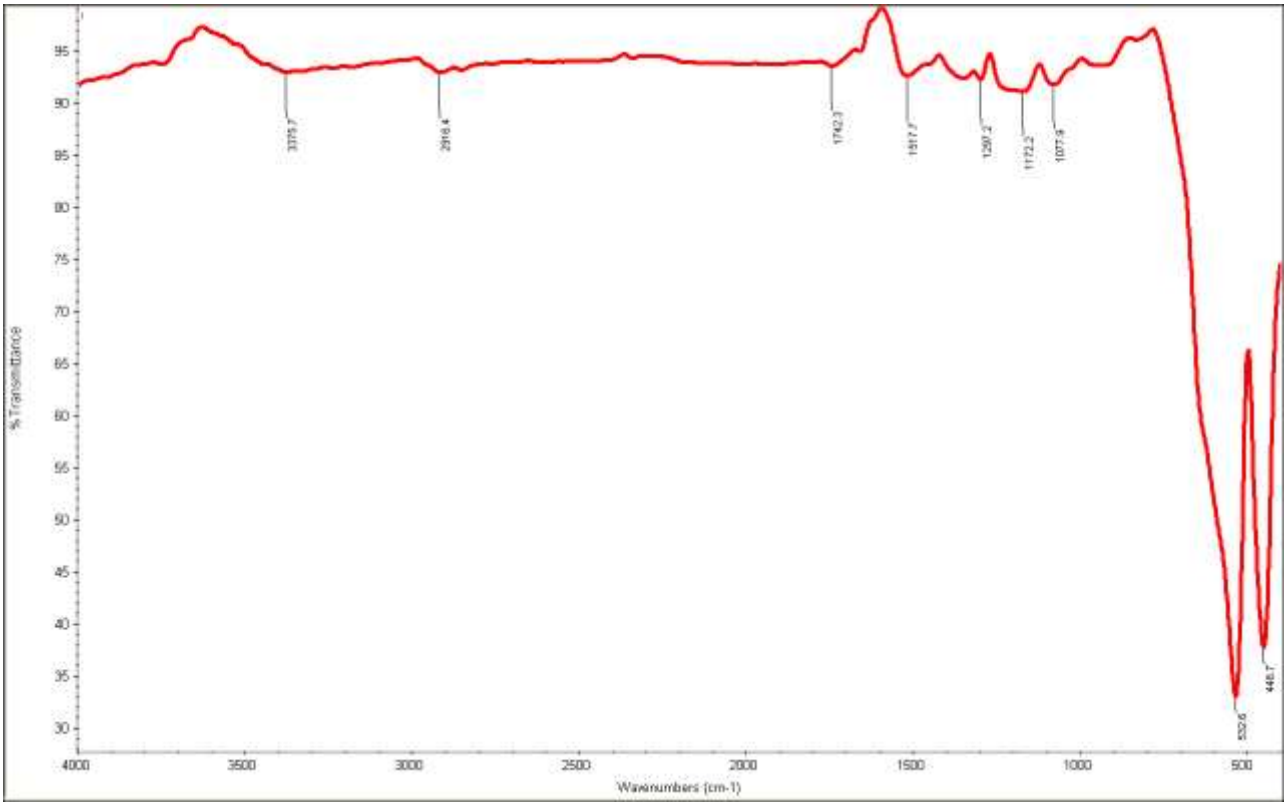

Fig 3.1: FTIR spectrum of extracted Iron-oxide Nano-particles

**peaks at* 3375.7 cm^{-1}, 2916.4 cm^{-1}, 1742.3 cm^{-1}, 1517.7 cm^{-1}, 1297.2 cm^{-1}, 1172.2 cm^{-1}, 1077.9 cm^{-1},532.6 cm^{-1}, 448.7 cm^{-1}

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3.2 SEM analysis of Nano-fertilizers

3.2.1 Morphological analysis of Nano-fertilizers extracted from banana peel

Fig 3.2.1(a) and Fig 3.2.1 (b) show the morphological properties of nano-fertilizers extracted from banana peels at the magnification of ×27,000 and ×10,000 at 15Kv respectively. Figure (B) shows the amorphous nature due to which it shows an uneven geometry of the particles. At ×27,000 magnification the sample shows a particle size of 0.05µm which is equal to 50nm. The surface of the sample is also smooth. However, there is no clear geometrical shapes depicted by the resultant images. At the ×10,000 magnification, the sample shows a particle size of 0.1µm which is equal to 100nm.

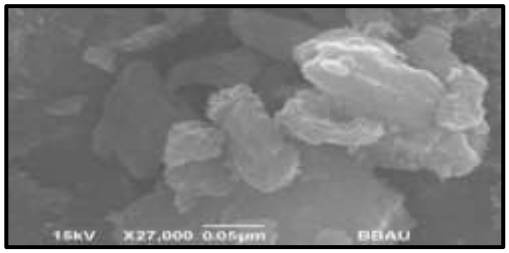

Fig 3.2.1 (a) shows the morphological properties of nano-fertilizers extracted from banana peels at ×27,000 magnification

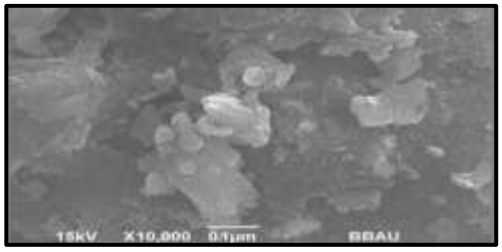

Fig 3.2.1 (b) shows the morphological properties of nano-fertilizers extracted from banana peels at ×10,000 magnification

3.2.2 Morphological analysis of Nano-fertilizers extracted from orange peel

Fig 3.2.2 (a) and Fig 3.2.2 (b) show the morphological properties of nano-fertilizers extracted from orange peels at the magnification of ×27,000 and ×10,000 at 15Kv respectively. At ×27,000 magnification of the sample depicts the particle size of 0.05µm which is equal to the 50nm. The resultant images show the highly smooth and even surface of the particles. At ×10,000magnification the sample shows a particle size of 0.1µm which is equal to 100nm. However, the images depict highly uneven surfaces without any shapes or geometry. The image shows rods and uneven oval shapes.

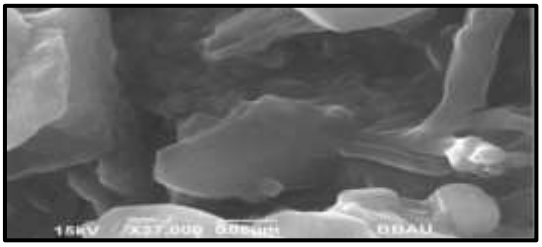

Fig 3.2.2 (a) shows the morphological properties of nano-fertilizers extracted from orange peels at ×27,000 magnification

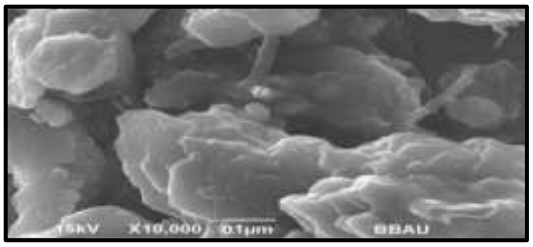

Fig 3.2.2 (b) shows the morphological properties of nano-fertilizers extracted from orange peels at ×10,000 magnification

Fig 3.2.3 (a) and Fig 3.2.3 (b) show the morphological properties of Iron oxide nanoparticles prepared from ferric III nitrate by self-combustion method at the magnification of ×27,000 and ×10,000 at 15Kv respectively. At ×27,000 magnification the sample depicts a particle size of 0.07µm which is equal to 70nm. The resultant images show the highly rough and uneven surface and structure of the nanoparticles. At ×10,000 magnification the sample shows a particle size of 0.1µm which is equal to 100nm. However, the images depict highly uneven, rough structures without any shapes or geometry. The image shows rods and uneven oval shapes and coiled particle shapes which show the amorphous nature of the nanoparticle.

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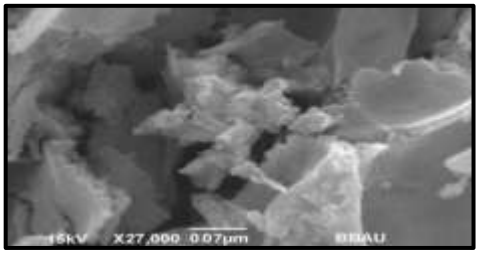

Fig 3.2.3 (a) shows the morphological properties of iron oxide nanoparticles

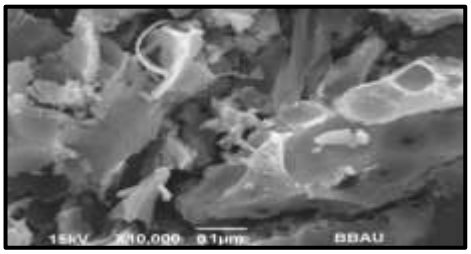

Fig 3.2.3 (b) shows the morphological properties of iron oxide nanoparticles

3.3 SEM-EDS analysis of Nano-fertilizers

Secondary and backscattered electrons employed in image formation for morphological analysis, as well as X-rays used for identification and quantification of compounds present at measurable amounts, are among the signals produced by an SEM/EDS system. In EDS, the detection limit is determined by the sample surface conditions; the smoother the surface, the lower the detection limit. EDS can detect major and minor elements with concentrations greater than 10 wt.% (major)and concentrations between 1 and 10 wt.% (minor). Because the detection limit for bulk materials is 0.1 wt.%, EDS cannot identify trace elements (concentrations less than 0.01 wt.%).

3.3.1 SEM-EDS analysis of Nano-fertilizers extracted from Banana peel

The EDS analysis of the sample in Fig 3.3.1(a) indicates the pure composition of potassium and calcium in the sample. The peak of C depicts the presence of organic components. There is a peak of O, Mg, Pt, and S showing the presence of Oxide, Magnesium, sulfur, and platinum. The total no. of iterations is 6.

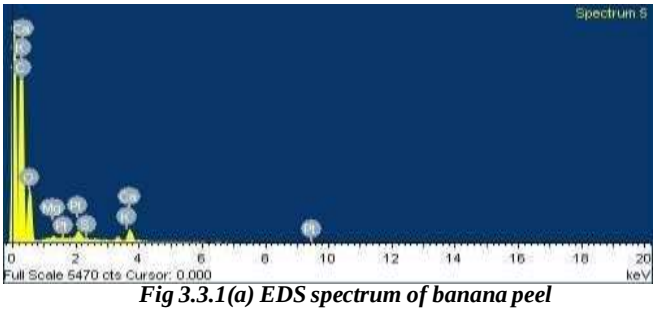

Fig 3.3.1(a) EDS spectrum of banana peel containdetectable Ca, K, C, O, Mg, Pt and S (calcium, potassium, carbon, magnesium, platinum and sulfur

Fig 3.3.1(b) Electron microscopic images of nano-fertilizer sample

Table 3.3.1: Element % of Nano-fertilizers extracted from banana peels as per EDS

Elements	Weight %	Atomic%
C K	51.17	60.10
O K	43.78	38.61
Mg K	0.32	0.19
S K	0.15	0.07
K K	0.59	0.21
Ca K	1.90	0.67
Pt M	2.09	0.15
Totals	100.00	

3.3.2 SEM-EDS analysis of Nano-fertilizers extracted from Orange peel

The EDS image of orange peels nano-fertilizers shows the pure and strong composition of Cl, Pt, and Al. The peak of K is also present which shows the presence of potassium. There is no peak of C which clearly indicates the absence of organic compound in the sample. The peaks possibly get omitted at 1.041 keV and the total number of iterations was 8.

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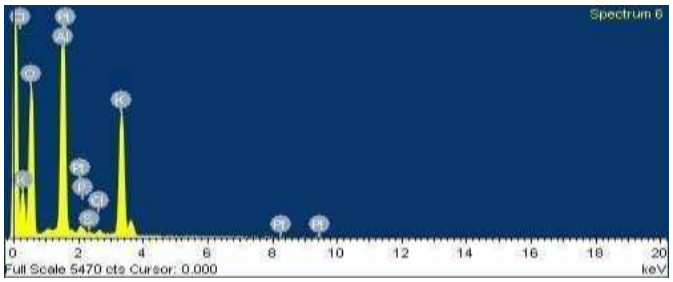

Fig 3.3.2(a) EDS spectrum of orange peels nanofertilizer Containing detectable Cl, Al, Pt, O, K, S (chlorine, Platinum, oxides, sulfur, potassium)

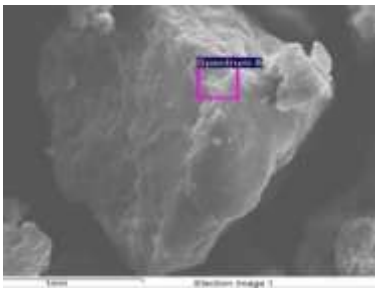

Fig 3.3.2(b) Electron microscopic images of orange peel sample

Table 3.3.2: Element % of Nano-fertilizers extracted from Orange peels as per EDS

Elements	Weight %	Atomic%
O K	63.32	77.81
Al K	19.04	13.87
P K	0.27	0.17
S K	0.13	0.08
Cl K	0.44	0.25
K K	15.23	7.66
Pt M	1.57	0.16
Totals	100.00	

3.3.3 SEM-EDS analysis of Iron-oxide Nanoparticle

The EDS analysis of Iron oxide nanoparticles derived from ferric III nitrate shows the pure composition and strong presence of Fe. Peak of C clearly indicates the presence of organic compounds. The peaks possibly omitted at 2.080KeV and 9.931KeV. Number of iterations is 7.

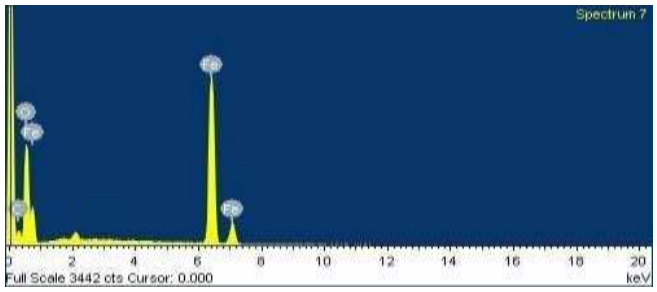

Fig 3.3.3(a) EDS spectrum of iron oxide nanoparticles Containing detectable Fe, O, and C (iron, oxides and carbon

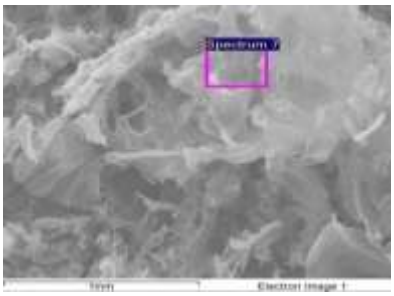

Fig 3.3.3(b) electron microscopic Image of Fe nanoparticles

Table 3.3.3: Element % of iron oxide nanoparticles as per EDS

Elements	Weight %	Atomic %
C K	10.46	22.65
O K	30.74	49.97
Fe K	58.80	27.38
Totals	100.00	

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3.4 N-P-K analysis of Nano-fertilizer

For analyzing Nitrogen, Phosphorus and Potassium content the nano fertilizers extracted from banana peels and orange peels are mixed and the fortification of iron oxide nanoparticles is done by adding desire number of nanoparticles in the mixture. The ratio for banana peel, orange peel and iron oxide nanoparticles was 4:4:2 respectively which means 40% of banana peels, 40% of orange peels and 20% of iron oxide nanoparticles constitute 100gm of final fertilizer.

3.4.1 Nitrogen content analysis of extracted Nano-fertilizers

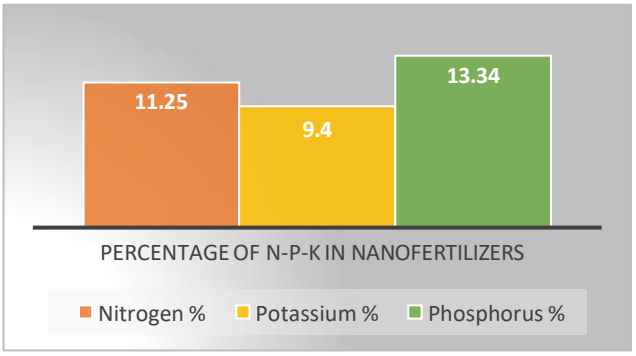
Nitrogen is a macronutrient that is required for plant activity and is a component of amino acids, which are the building blocks of plant proteins and enzymes. Proteins are the structural ingredients of all living things, and enzymes assist a wide range of biochemical events within a plant. Nitrogen is also a component of the chlorophyll molecule, which allows the plant to collect solar energy through photosynthesis, hence increasing plant growth and crop output. Nitrogen is essential within the plant to guarantee that energy is supplied when and where the plant requires it to maximize output. This essential component is even found in the roots, where proteins and enzymes assist control of water and nutrient intake. By using the Kjeldhal method the total nitrogen content was analyzed. The result shows that 11.25gm of Nitrogen is present in 100gm of nano fertilizer which indicates that 11.25 % of nitrogen is present in N-P-K-based nano-fertilizer.

3.4.2 Potassium content analysis of extracted Nano-fertilizers

In plant tissue, potassium is related to the flow of water, minerals, and carbohydrates. It is involved in enzyme activation within the plant, which impacts the creation of protein, starch, and adenosine triphosphate (ATP). The rate of photosynthesis may be controlled by the creation of ATP. Potassium also aids in the regulation of stomatal opening and shutting, which governs the exchange of water vapor, oxygen, and carbon dioxide. Plant development is stunted and yield is reduced when K is low or inadequately supplied. UV visible spectroscopy was used to determine the potassium content in the given sample. The results depict that 9.40gm of potassium is present in the given sample which indicates that near about 9% of potassium is present in the nano fertilizer.

3.4.3 Phosphorus content analysis of extracted Nano-fertilizers

In plants, phosphorus plays a critical role in absorbing, storing, and converting solar energy into biomolecules like adenosine triphosphate (ATP), which power biological activities (including photosynthesis) from germination through grain development to maturity. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), contain instructions on how plants should carry out everyday tasks including synthesizing proteins, lipids, nucleic acids, as well as sugar metabolism, both including phosphorus. Phosphorus encourages the development of early roots, winter hardiness, and seeds. The plants are often dark bluish green, with purple leaves and stems. The results depict that 13.34 gm of phosphorus is present in the given sample which indicates that near about 13 % of phosphorus is present in the N-P-K-based nano fertilizer.



3.5 Free Radical Scavenging Activity (DPPH)

The easiest method for figuring out a sample's antioxidant capacity is to use DPPH. The sample's color shifts from purple to yellow because of antioxidant chemicals scavenging DPPH free radicals. To determine the DPPH behavior in a sample, the optical densities of the sample and the control can be computed using a spectrophotometer. The DPPH value has very strong antioxidant properties if it is below 50 g/ml, strong antioxidant properties if it is between 50 and 100 g/ml, normal antioxidant properties if it is between 101 and 150 g/ml, and weak antioxidant properties if it is above 150 g/ml *(Fridriany et al. (2014)*. The results of an evaluation of the antioxidant activity of produced nano fertilizers at various concentrations (0,200,400,800, and 1000 g/ml) were presented in Figure 4.2.7 According to these results, nano fertilizers concentration increases up to 1000µg/ml. The activity of antioxidants at this concentration was the highest, that is 33.87%. At 400µg/ml lowest antioxidant activity was achieved which was 80.89%.

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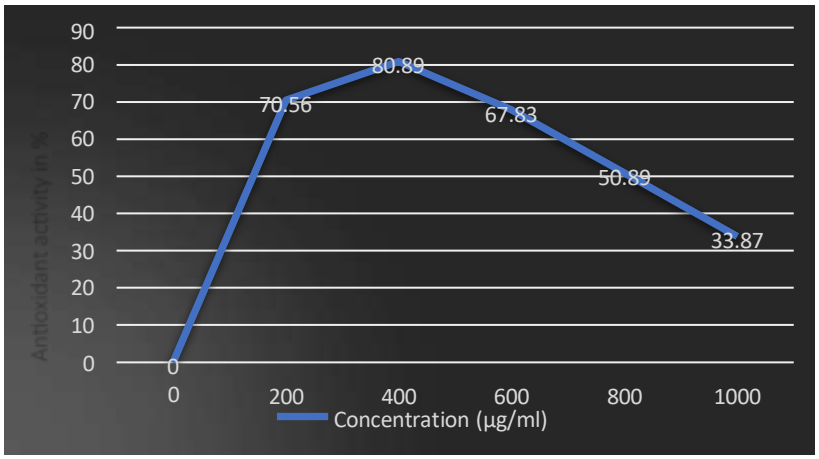


Fig 3.5 Free radical scavenging activity of nano fertilizers at different concentration

3.6 Antimicrobial activity analysis

3.6.1 Total Plate Count

On the first day there was no growth of any bacteria which is heterotrophic in nature in TPC petri plate. On the seventh day there was bacterial growth shown in TPC petri dishes of $3.76X10^2$ cfu/gm. After 14th days bacterial growth was shown up to 5.90X10 cfu /gm. After 21st days $6.89X10^3$ cfu/mg of growth was seen in the media. The colonies were counted by using Colony counter.

3.6.2 Yeast and Mold Count

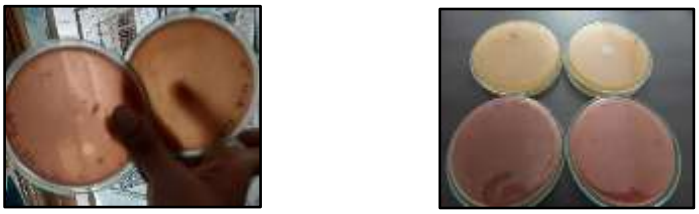
There was no growth of yeast and mold petri dishes after one day. There was no growth for yeast and mold after seventh days. After 14th day less than 10cfu/mg of growth is shown in the media. After 21st day less than 10cfu/mg of yeast and mold growth has been shown.

3.6.3 Coliform Count

There was no growth of coliform petri dishes. There was no growth for coliform after seven days.

Table 3.6.1: TPC, Yeast and Mold and Coliform count at different stages

Days	TPC	Yeast and Mold	Coliform
0	-	-	-
7	$3.76X10^2$ cfu/gm	-	-
14	5.90X10 cfu/gm	Less than 10cfu/mg	-
21	$6.89X10^3$ cfu/mg	Less than 10cfu/mg	-



(a) **(b)**

Fig 3.6.3 (a) shows the coliform growth Fig 4.2.8 (b) shows the growth of TPC and Yeast and Mold

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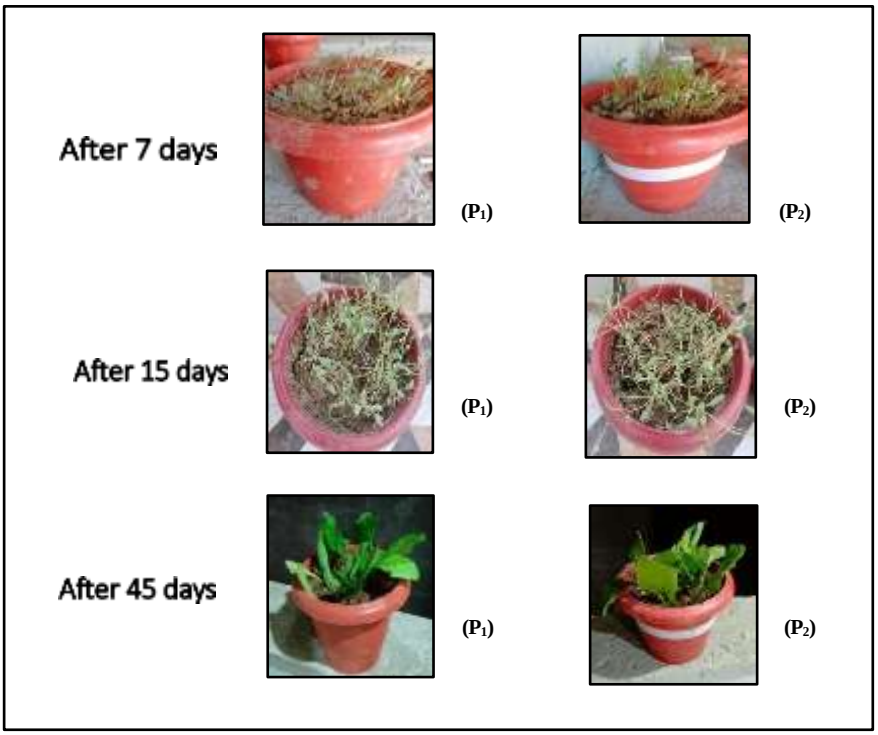
3.7 Optimization of effect of applied nano-fertilizer on plant growth



Spinach is used for studying the effect of nano fertilizers in plant growth because it takes minimum days for growth than the other crops as we have time boundation for this study. Also, the spinach was the comfortable crop according to the season in which the study was done. The optimization of the effect of nano fertilizers on the plant growth is done by visual method. From the above results it is clearly shown that after 7 days of sowing there is little growth in plants that can be seen. The P_1 which contains only soil has less growth than the P_2 which contains nano-fertilizers prepared. It clearly shows that the germination percentage has increased after the use of nano-fertilizers. After 15 days the sample P_1 shows the growth of leaves but the leaves are small as compared to the leaves grown in P_2. After 45 days full growth is shown in both the pots.

3.8 Characterization of soil after the plant growth

3.8.1 Analysis of Nitrogen content

The main source of nitrogen in soil is atmospheric nitrogen. It is present in the atmosphere as N2, and it must be changed before it can be used in the soil. Rhizobia, a bacterium that infects the roots of bean plants and gets a lot of food and energy from them, can fix a lot more nitrogen annually. After the analysis of soil sample P_1 which contains only soil, the resultant nitrogen content was 1040mg/100gm. For the sample P_2 which contains soil with nano fertilizers shows a little bit enhancement in nitrogen content which was around 1098mg/100gm.

3.8.2 Analysis of Phosphorus content

After nitrogen, phosphorus ranks as the second-most significant crop nutrient. In all crop metabolic processes, including photosynthesis, respiration, energy storage, transfer, cell division, cell enlargement, and nitrogen fixation, it is a crucial macronutrient. *(researchgate.com)*. After the analysis of soil sample P_1 which contains only soil, the resultant phosphorus content was 103.5mg/100gm. For the sample P_2 which contains soil with nano fertilizers shows a little bit enhancement in phosphorus content which was around 114.7mg/100gm

3.8.3 Analysis of Potassium content

Your plants will thrive and yield more flowers and fruit if you add potassium to your soil. Additionally, it enhances plants' ability to withstand pests and disease. Plant ailments like rust or powdery mildew are less likely to weaken a strong, healthy plant. After, the analysis of soil sample P_1 which contains only soil, the resultant potassium content was 200mg/100gm. For the sample P_2, which contain soil with nano fertilizers shows a little bit enhancement in potassium content which was around 280.6mg/100gm.

3.8.4 Analysis of Iron content

One of the most prevalent minerals in soils and an essential one, iron appears in the environment as ferrous and ferric ions. The speciation of iron in environmental studies is crucial because each oxidation state of iron plays a unique role in the environment. *(Gabriele Verônica et.al 2021)*. After, the analysis of soil sample P_1 which contains only soil, the resultant iron content was 13000mg/100gm. For the sample P_2 which contain soil with nano fertilizers shows a little bit enhancement in iron content which was around 34568mg/100gm.

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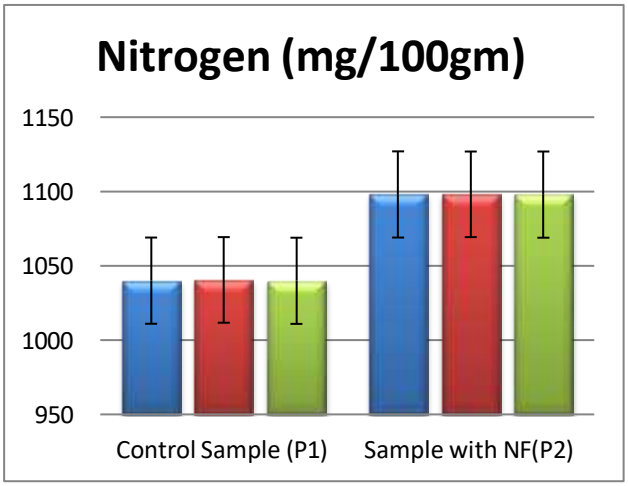
3.8.5 Analysis of calcium content

Calcium serves several functions in a plant. It helps plants build strong cell walls. Plant cell walls provide a rigid structure and protection a plant needs. Calcium plays a role in the permeability of the cell membrane. We must first comprehend how the vascular system of the plant, which consists of the xylem and phloem, works. After, the analysis of soil sample P_1 which contains only soil, the resultant iron content was 1275mg/100gm. For the sample P_2 which contains soil with nano fertilizers shows a little bit enhancement in iron content which was around 1980mg/100gm.

Table 3.8: result of analysis mineral content of both the soil sample

Parameters	Soil sample of control sample P_1	Soil containing nano fertilizers P_2
Nitrogen (mg/100g)	1040±0.32	1098±0.1
Phosphorus (mg/100g)	103.5±0.30	114.7±0.15
Potassium (mg/g)	200.9±0.1	280.6±0.25
Iron (mg/100g)	13000±0.2	34568.4±0.36
Calcium (mg/100g)	1275±0.32	1980±0.15

(a)



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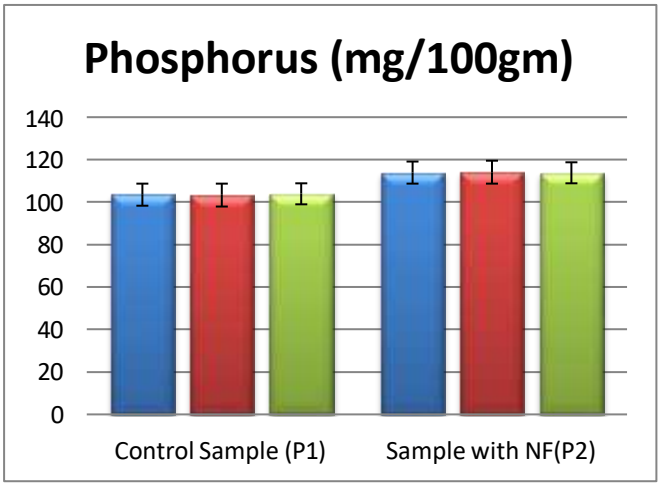

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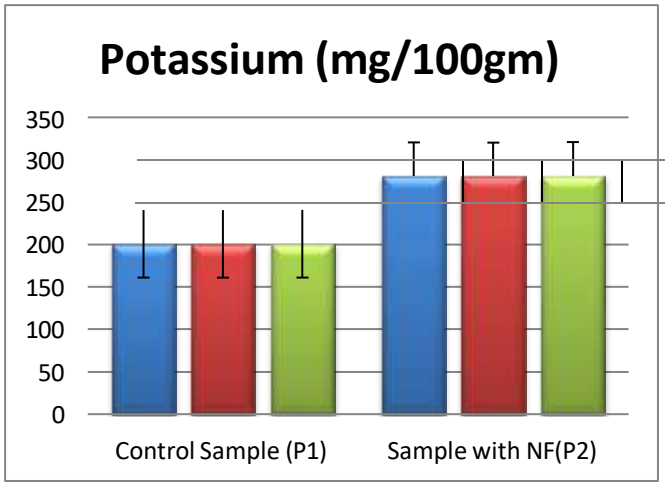

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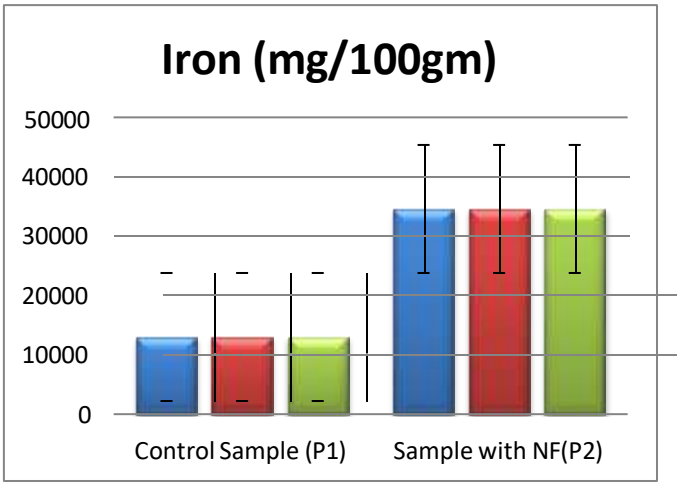

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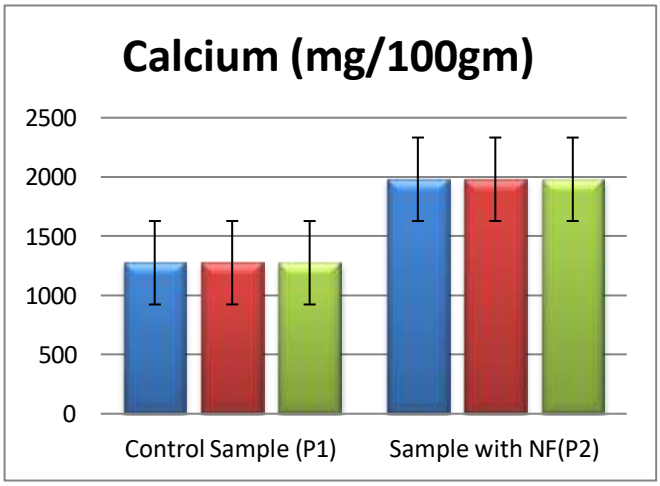

Fig 3.8 *Mean;* ***(a)*** *Nitrogen (mg/100g),* ***(b)*** *Phosphorus (mg/100g),* ***(c)*** *Potassium (mg/100g),* ***(d)*** *Iron (mg/100g),* ***(e)*** *Calcium (mg/100g)*

3.9 Characterization of grown crops (spinach)

3.9.1 Analysis of Phosphorus content

After the analysis of spinach grown in soil sample P1 which contains only soil, the resultant phosphorus content was 47mg/100gm. For the sample P2 which contains soil with nano fertilizers shows a little bit enhanced in phosphorus content of spinach which was around 65mg/100gm.

3.9.2 Analysis of Potassium content

After, the analysis of spinach grown in soil sample P1 which contains only soil, the resultant potassium content was 534mg/100gm. For the sample P2 which contains soil with nano fertilizers shows a little bit enhanced in potassium content of spinach which was around 596mg/100gm.

3.9.3 Analysis of Iron content

After the analysis of spinach grown in soil sample P1 which contains only soil, the resultant iron content was 2.84mg/100gm. For the sample P2 which contains soil with nano fertilizers shows a little bit enhanced in iron content of spinach which was around 4.56mg/100gm.

3.9.4 Analysis of Calcium content

After, the analysis of spinach grown in soil sample P1 which contains only soil, the resultant calcium content was 102 mg/100gm. For the sample P2 which contains soil with nano fertilizers shows a little bit enhanced in calcium content of spinach which was around 159.9mg/100gm.

Table 3.9: result of analysis of mineral content of both the spinach sample

Parameters	Spinach grown in control sample P1	Spinach grown in soil containing nano fertilizers P2
Phosphorus	47.8±0.30	65±0.32
Potassium	534.3±0.1	596±0.26
Iron	2.84 ±0.05	4.56±0.02
Calcium	102.1±0.32	159.9±0.3

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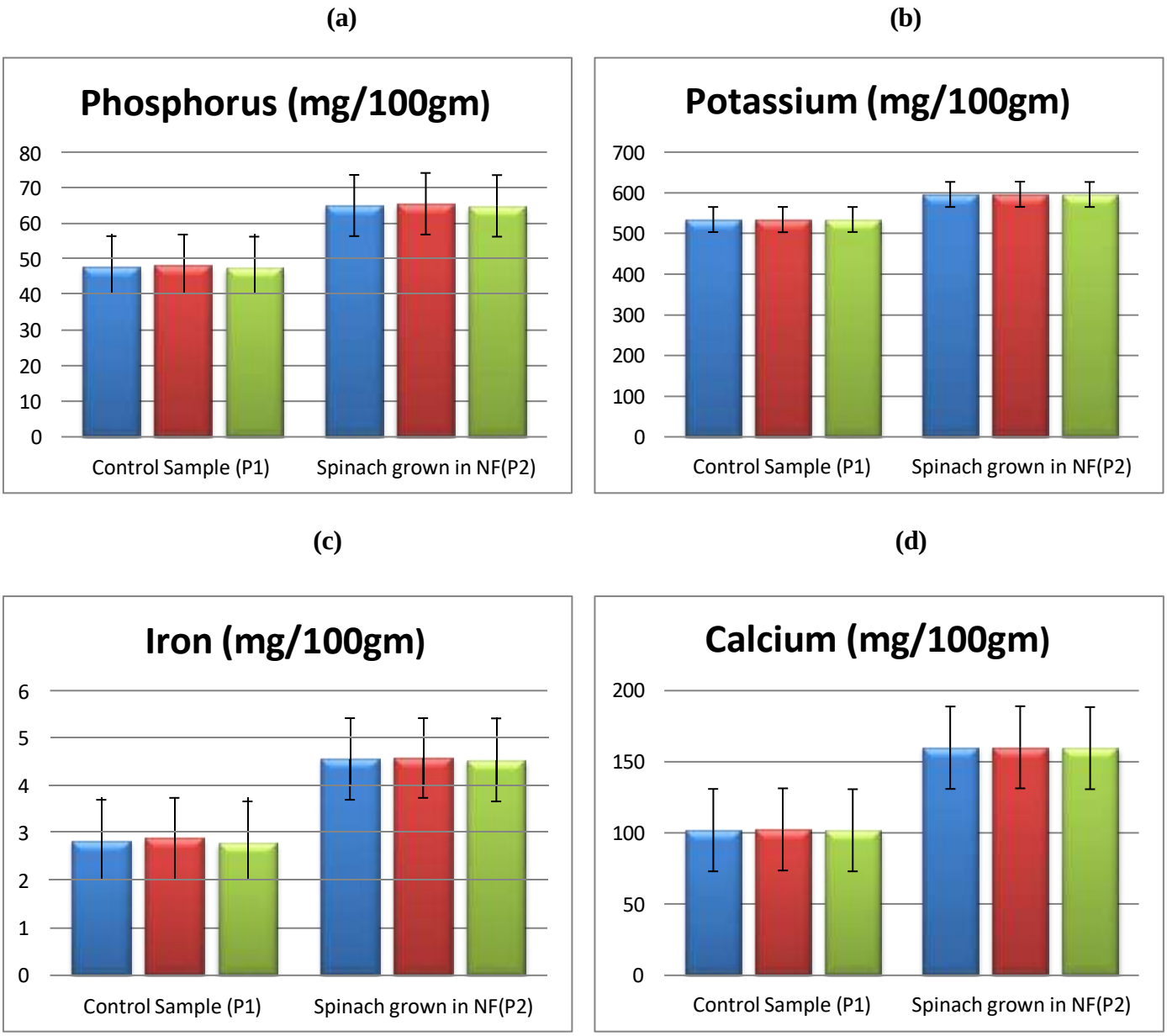

Fig 3.9 Mean: (a) Phosphoras (mg/100g), (b) Potassium (mg/100g), (c) Iron (mg/100g), (d) Calcium (mg/100g)

4. CONCLUSION

Nano fertilizers prepared from banana peels and orange reels are fortified with iron oxide nanoparticles are a fine source of Nitrogen, Potassium, Phosphorus and Iron. Along with these minerals there was also increase in small amount of calcium content. From the fig P_1 and P_2 of plant growth it is clearly visible that the soil containing prepared nano-fertilizers are showing more growth than the control soil. Germination percentage of seeds has also been enhanced.

There was also increase in nitrogen, potassium, phosphorus, calcium and iron percentage in spinach. It is suggested that there should be a need to conduct research on the nano fertilizers on a higher level so that we should be able to know more about the effect of nano fertilizers on plant growth. Further studies are necessary to find out the effect of extracted nano fertilizers on plant growth to find out whether it can provide enough nutrition to plants or not and to study the effect of iron oxide nanoparticles enriched fertilizer on plant growth.

DECLARATION

The authors affirm that they have no known financial or personal affiliations with competitors that could have an impact on the research presented in this paper.

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