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DEVELOPMENT AND CHARACTERIZATION OF BIOFERTILIZER FROM CORN COB (*Zea mays*) WASTE: A SUSTAINABLE APPROACH FOR SOIL ENRICHMENT AND ENVIRONMENTAL MANAGEMENT

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ABSTRACT

This study developed and characterized a corn cob based biofertilizer as a sustainable alternative to chemical fertilizers and a means of agricultural waste valorization. Corn cobs were dried at 40 °C, milled to 20 µm, sterilized at 100 °C for 10 min, and inoculated with *Bacillus* spp., followed by fermentation at 37 °C for 744 h under conditions optimized using Central Composite Design (CCD). The design variables included pH (4.5–9.5), inoculum concentration (0.1–30%) and moisture content (25–100%). Proximate and mineral analyses confirmed that corn cobs provided a nutrient-rich substrate containing 43.5% carbohydrates, 3.4% crude protein, 35.6% fibre, 0.55% nitrogen, 1.5% potassium and 0.65% phosphorus. Optimization produced a biofertilizer containing 1.55% nitrogen, 1.63% potassium and 1.78% phosphorus, with a high microbial load of *Bacillus* (2.1×10^6 CFU ml^{-1}). Soil trials conducted across sandy, loamy, and clay soils revealed significant improvements in soil fertility indices: There was an observed increase in pH, nutrient levels (nitrogen, phosphorus and potassium), Organic carbon, cation exchange capacity and also *Bacillus* counts in all the soil types before and after treatment, indicating robust microbial proliferation. Overall, these findings establish corn cobs as a viable carrier matrix for biofertilizer production. The resulting formulation is nutrient-enriched, microbially active, cost-effective and environmentally friendly, demonstrating significant potential for improving soil fertility and promoting sustainable agricultural practices.

Keywords: Biofertilizer, Corn Cob, Fermentation, Optimization and Soil

INTRODUCTION

Global agriculture faces the dual challenge of meeting rising food demands while mitigating the environmental costs of input-intensive production systems. Synthetic fertilizers, once central to yield enhancement, are increasingly associated with soil acidification, nutrient leaching, groundwater pollution, and greenhouse gas emissions. In many developing regions, including Nigeria that has agriculture as the main source of livelihood (Musa *et al.*, 2023), smallholder farmers struggle with both the ecological consequences of fertilizer overuse and the economic burden of their high cost and limited availability. These realities underscore the urgent need for sustainable nutrient management strategies that reconcile productivity with ecological integrity (Mohammad *et al*., 2004).

Biofertilizers have emerged as a promising alternative, leveraging the natural capacities of beneficial microorganisms such as nitrogen-fixing bacteria, phosphate-solubilizing microbes, and plant growth-promoting *rhizobacteria* (Olade, 2019). Through mechanisms including biological nitrogen fixation, mineral solubilization, phytohormone production, and pathogen suppression, biofertilizers enhance nutrient cycling, improve soil structure, and increase crop resilience to abiotic stresses (Haruna *et al*., 2017). Critically, they offer long term soil fertility benefits without the ecological trade-offs of synthetic fertilizers. Yet, the performance of biofertilizers is strongly influenced by their formulation, particularly the carrier matrix, which affects microbial viability, nutrient release, and field efficacy. Developing low-cost, locally adapted carriers is therefore essential to unlock their full potential in smallholder farming systems (Republic, 2014).

In parallel, the shift toward a circular economy has intensified interest in valorizing agricultural residues as substrates for biofertilizer production. Corn cobs, a lignocellulosic by-product of maize cultivation, exemplify such residues: they are generated in abundance yet often discarded or openly burned, contributing to environmental degradation (Vassilev *et al*., 2015). Their high carbohydrate, fibre, and organic carbon content make them particularly suitable for microbial colonization and fermentation. Transforming corn cobs into biofertilizers not only addresses waste management challenges but also creates an affordable input that aligns with climate-smart and resource-efficient agriculture (Basu *et al*., 2017).

Despite growing recognition of these opportunities, critical research gaps persist. Most biofertilizer formulations rely on imported or synthetic carriers, which are poorly suited to local agro-ecological conditions and economically inaccessible to small-scale farmers (Zych, 2008). Moreover, few studies have systematically optimized the production parameters of corn cob-based biofertilizers to maximize nutrient enrichment and microbial viability. The use of advanced statistical approaches, such as Response Surface Methodology (RSM), to refine key variables including pH, inoculum concentration, and moisture content remains limited, constraining scalability and field performance (Harsanti *et al*., 2019).

Among candidate microbial inoculants, *Bacillus* species stand out for their versatility, robustness, and proven commercial relevance. These gram-positive, spore-forming bacteria not only fix nitrogen and solubilize phosphorus and potassium but

also produce growth-promoting substances such as indole-3-acetic acid and antimicrobial compounds that suppress soil-borne pathogens (Ngampimol & Kunathigan, 2008). Their capacity to withstand adverse conditions and maintain long shelf life makes them ideal for biofertilizer applications in resource-limited environments (Ibrahimpašić *et al*., 2021).

Accordingly, the aim of this study is to develop and characterize a corn cob–based biofertilizer enriched with *Bacillus* species, integrating agro-waste valorization with soil fertility restoration to advance sustainable crop productivity and environmental management. To achieve this aim, the study pursues three objectives: (i) to characterize the nutrient composition of corn cobs as a substrate for biofertilizer production, (ii) to optimize the production process using Central Composite Design (CCD) to enhance microbial growth and NPK enrichment, and (iii) to evaluate the effects of the formulated biofertilizer on soil properties across different soil types.

MATERIALS AND METHODS

This chapter outlined the materials and methods used to aerobically produce liquid organic biofertilizer through the fermentation process.

Table1: List of Materials

S/N	Name of Materials	Manufacturer	Source
1	Organic Matter		Kasuwa Laraba Daura Katsina State
2	Nutrient Agars	Sigma-Aldrich	Department of Science Laboratory Technology Federal Polytechnic Daura, Katsina State
3	Cetrimide Agar	Himedia Laboratories	Department of Science Laboratory Technology Federal Polytechnic Daura, Katsina State
4	Citric Acid	Merck.	Department of Science Laboratory Technology Federal Polytechnic Daura, Katsina State
5	Calcium Carbonate	Sigma-Aldrich	Department of Science Laboratory Technology Federal Polytechnic Daura, Katsina State
6	Peptone Water	Himedia Laboratories	Department of Science Laboratory Technology Federal Polytechnic Daura, Katsina State
7	Nutrient Brought	Oxoid	Department of Science Laboratory Technology Federal Polytechnic Daura, Katsina State

Methodology

The process of developing biofertilizer from corn cob waste was carried out through several systematic steps, each contributing to the preparation, optimization, and preservation of a nutrient-rich, microbially active product. The flow diagram provides a visual representation of these stages:

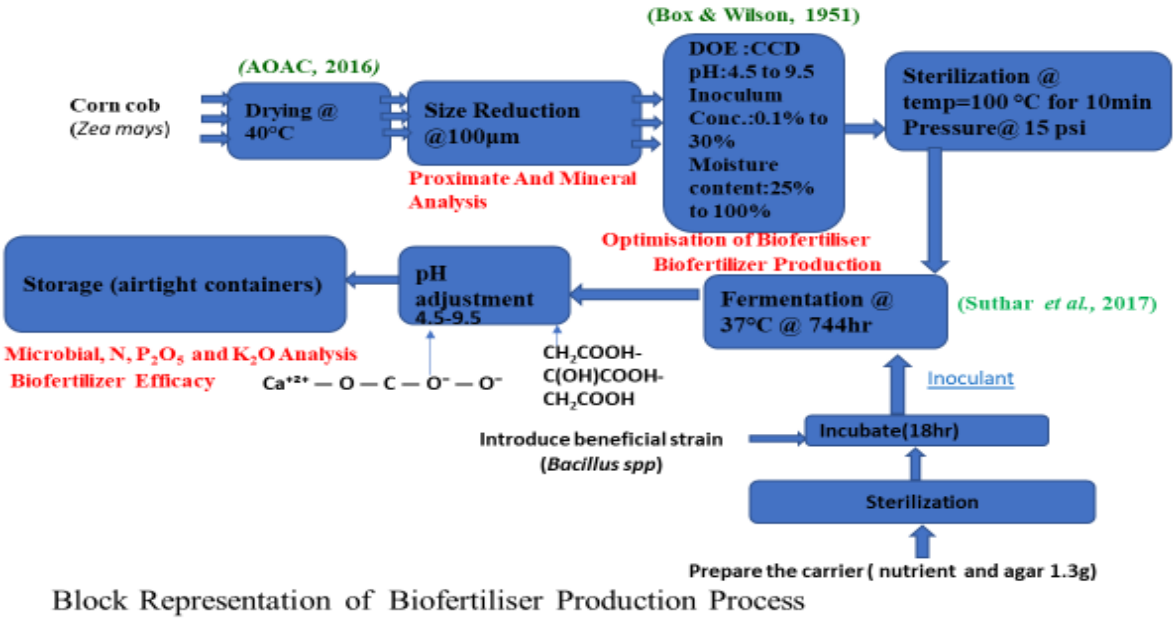

Figure 1: Flow Diagram for Biofertilizers Production from Corn Cob

Raw Material Preparation (Corn Cob)

i. ***Drying at 40 °C***:

Initially, corn cobs were dried at 40 °C (AOAC, 2016) to reduce moisture content, which facilitates easier grinding and prevents microbial contamination during early processing.

ii. ***Size Reduction to 20 µm***:

The dried cob was milled to a fine particle size (~20 µm) (Kumar *et al*., 2020) to enhance surface area, improve microbial colonization, and ensure uniform fermentation. This stage is also critical for consistency in chemical and microbiological analysis.

Chemical Characterization and Analysis
Proximate and mineral analyses were carried out on the dried corn cob to determine its nutritional potential as a carrier substrate for microbial growth.

Optimization of Biofertilizer Conditions
Design of Experiment (DOE) - Central Composite Design (CCD)
The fermentation parameters were optimized using statistical methods (Box & Wilson, 1951), targeting a pH range of 4.5–9.5, an inoculum concentration of 0.1–30%, and a moisture content between 25–100%. This ensures optimal microbial growth and metabolic activity during fermentation.

Sterilization
The processed corn cob material was sterilized at 100 °C for 10 minutes under 15 psi pressure to eliminate unwanted microorganisms, ensuring aseptic conditions for the subsequent microbial inoculation.

Biofertilizer Production
Fermentation at 37 °C for 744 hours (31 days):
The sterilized substrate was inoculated with a 1:1 ratio of beneficial microbial strains, specifically *Bacillus spp.*, known for their plant growth-promoting properties. The long duration aerobic fermentation supports microbial colonization and enzymatic breakdown of organic matter.

pH Adjustment
The pH of the fermenting medium was maintained between 4.5 and 9.5 using buffering agents like acetate (CH_3COOH) and carbonate compounds [Ca^{2+}, CO_3^{2-}], as the pH is crucial for microbial metabolism and nutrient availability.

Inoculation and Incubation
After fermentation, the biofertilizer was mixed with a carrier medium (nutrient and agar at 1.3 g) and re-inoculated to ensure robust microbial presence. The mixture is incubated for 18 hours to allow microbial stabilization.

Final Sterilization and Carrier Preparation
A secondary sterilization step was carried out to ensure the products hygiene and shelf-stability. The final formulation includes the prepared carrier material and active microbial cultures, ready for storage or application.

Storage and Evaluation
The final biofertilizer was stored in airtight containers at 4 °C with a bulk density of 0.9 g/cm³. During this phase, key parameters such as microbial viability, nitrogen (N), potassium (K) and phosphorus (P) content are analyzed to assess product quality.

Characterization of Samples
Proximate Analysis
Proximate revealed the moisture content, ash content, crude fibre, crude protein, fat crude, carbohydrates, lignin and hemicellulose while the mineral analysis includes nitrogen, phosphorus, calcium, magnesium, iron, sodium, zinc, copper and potassium

Determination of Moisture Content
In this work, the standard procedure based on AOAC official method 930.15 was employed. This procedure makes use of over drying method. Cleaned Porcelain crucibles were weighed and dried in an oven for 30 minutes a 105°C to a constant weight (W_o) 2g of the sample were placed in pre-weighed crucibles after cooling and weighed (W_1), which were placed in an oven for 4 hours at 108°C after which it was removed, cooled in a desiccator and then weighed (W_2). The moisture content was calculated as a Percentage loss in weight using the formula below:

$$\text{Moisture content (\%)} = \frac{\text{loss in weight due to drying}}{\text{weight of the sample taken}} \times 100 \quad (1)$$

$$\text{Moisture content (\%)} = \frac{w1-w2}{w1-wo} \times 100 \quad (1)$$

Where:
W_0 = weight of the empty crucible (g)
W_1 = weight of crucible + sample before drying (g)
W_2 = weight of crucible + sample after drying (g)

Determination of Ash Content
In this work, the standard procedure based on ASTM D3174 was employed. It is a procedure employed based on the determination of coal samples. Porcelain crucibles were ignited in a muffle furnace at 550°C cooled in the desiccator and (W_o) 2g of the sample were accurately weighed into the crucible and weighed (w_1) the crucible containing the sample were heated in a muffle furnace at 550°C for four (4) hours to burn off all the organic matter? The crucible was removed cooled in the desiccator and weighed (w_2). The percentage of ash was calculated using the formula below.

$$\text{Ash Content (\%)} = \frac{\text{weight of ash}}{\text{weight of the sample}} \times 100 \quad (2)$$

$$\text{Ash Content (\%)} = \frac{w2-wo}{w1-wo} \times 100 \quad (3)$$

Where:
w_o=empty crucible(g),
w_1=weight of the sample +crucible(g),
w_2= final weight (g)

Determination of Crude Protein
Crude protein was determined using the Kjeldahl digestion method as described by AOAC (1999), where concentrated sulfuric acid (H_2SO_4) was used for sample digestion.

Determination of Crude Fibre
The Association of Official Analytical Chemists [AOAC] official method 962.09 for crude fibre determination was adopted (Cunniff, 1995; Kirk & Sawyer, 1991).To determine the crude fibre of a biofertilizer sample, weigh out 2g of the sample into a crucible, add 200mL of distilled water and stir to mix, then add 20mL of H_2SO_4 and stir gently; heat the mixture on a hot plate at 200°C for 30 minutes, filter the mixture through filter paper into a clean crucible, and was washed with distilled water. Next, add 20mL of NaOH and stir gently, heat the mixture again on a hot plate at 200°C for 30 minutes, filter the mixture through filter paper into a clean crucible, and wash the residue with distilled water once more. Finally, dry the crucible in a muffle furnace at 550°C for 2 hours, allow it to cool, and weigh the crucible to obtain the results.
Calculation

$$\text{Crude protein (\%)} = \frac{W2-W1}{W} \times 100 \quad (4)$$

Where:
W = Initial weight of the sample (2g),
W1 = Weight of crucible before drying
W2 = Weight of crucible after drying

Carbohydrates Determination
Carbohydrate content was determined using the phenol-sulfuric acid method, following the standard procedure described by Dubois *et al*. (1956). In this method, 1 g of biofertilizer sample was treated with 5% phenol and

concentrated sulfuric acid (H_2SO_4), the mixture was incubated at 25 °C for 10 minutes to allow color development. After cooling to room temperature, the absorbance was measured at 490 nm using a spectrophotometer. A glucose standard curve was used to quantify carbohydrate concentration.

$$\text{Carbonhydrate}(\%) = \frac{\text{Absorbance x Standard Curve Slope}}{\text{Sample Weight}} \quad (5)$$

Determination of Lignin Content

Lignin content was determined using a modified Klason lignin method, following procedures outlined in TAPPI T 222 and ISO 21436:2020 standards. Finely ground sample of corn cob was subjected to solvent extraction using either ethanol to remove extractives. After extraction, the residue was dried and lignin content was determined by gravimetric analysis, where the weight of the acid-insoluble lignin was measured and compared to the initial sample weight. The method was calibrated using known standards to ensure accuracy. This step is essential for the reliable quantification of lignin in biomass samples and contributes to understanding the structural properties of the biofertilizer components.

Determination of Hemicellulose in Biofertilizer

Hemicellulose content was determined using the Neutral Detergent Fiber (NDF) method as described by Van Soest (1963). The procedure involved treatment of the biofertilizer sample with neutral detergent solution, alpha-amylase, and sodium sulphite, followed by drying in a muffle furnace. The final mass was used to compute hemicellulose based on fiber content.

$$\text{Hemicellulose } \% = \pm 0.95 \text{ x Sum of NDF} - \text{ADL} \quad \text{Equation (7)}$$

Where:

NDF = Neutral Detergent Fibre content

ADL = Acid Detergent Lignin content

This method provides an estimate of hemicellulose content, which contributes to understanding the structural and nutritional quality of the biofertilizer.

Mineral Analysis (Elemental Analysis)

Mineral analysis was conducted to quantify the concentrations of calcium (Ca), nitrogen (N), sodium (Na), potassium (K), phosphorus (P), zinc (Zn), iron (Fe), magnesium (Mg) and copper (Cu) present in the sample, with results expressed as percentage composition.

Determination of Nitrogen Content

Nitrogen content was determined using the Kjeldahl method in accordance with the AOAC Official Method 988.05. Approximately 0.5 g of the sample (corn cob) was digested using concentrated sulfuric acid in the presence of potassium sulfate and copper sulfate as catalysts. The digest was distilled with sodium hydroxide, and the released ammonia was absorbed in hydrochloric acid (HCl) and back-titrated with a standard sodium hydroxide solution.

The nitrogen content was calculated using the following formula:

$$\%\ \text{N} = \frac{(1.4 \times \text{N} \times \text{V})}{\text{W}} \quad (6)$$

Where:

N = Normality of HCl (mol/L)

V = Volume of HCl used for neutralization (mL)

W = Weight of the sample (g)

Determination of Calcium Content

Calcium content was determined using the AOAC Official Method 985.01. One gram of the biofertilizer sample was digested with hydrochloric acid (HCl), followed by heating at 80 °C to facilitate calcium extraction. After dilution with distilled water and filtration, the solution was analyzed using an atomic absorption spectrophotometer (AAS) at a wavelength of 422.7 nm. A calibration curve was prepared using calcium standard solutions (0–100 ppm) to determine calcium concentration in the samples.

$$\text{Calcium } \% = \frac{(1.4 \times \text{NSample Absorbance x Standard Curve Slope}) \times \text{V})}{\text{Sample WeightW}} \quad (7)$$

Where:

V = Volume of the sample solution (mL)

W = Weight of the sample (g)

Determination of Potassium Content

Potassium content was determined using flame photometry in accordance with the AOAC Official Method 999.10. One gram of the organic sample was digested in distilled water and heated at 80 °C to extract potassium ions. After filtration, the resulting solution was analyzed using a flame photometer at a wavelength of 766.5 nm, which corresponds to potassium emission. A standard curve was prepared using potassium standard solutions ranging from 0 to 100 ppm.

$$\text{potassium } \{\%\} = \left(\frac{\text{sample emmission intensity} \times \text{standard curve slope}}{\text{Sample Weight.}}\right) \quad (8)$$

Where:

W = Weight of the sample (g)

This method allows for accurate quantification of potassium concentration in the organic sample and contributes to the nutrient characterization of the biofertilizer.

Determination of Phosphorus Content

Phosphorus content was determined using the vanadomolybdate method, following the AOAC Official Method 965.17. One gram of the organic sample was digested using nitric acid (HNO_3) and perchloric acid ($HClO_4$). After digestion, ammonium molybdate and vanadium pentoxide solutions were added to react with the released phosphate, forming a yellow-colored complex. The absorbance of the resulting solution was measured at a wavelength of 470 nm using a spectrophotometer.

A phosphorus standard curve (0–100 ppm) was prepared for calibration. Phosphorus content was calculated using the following formula:

$$\text{Phosphorus} = \left(\frac{\text{Sample Absorbance} \times \text{Standard Curve Slope}}{\text{Sample Weight}}\right) \quad (9)$$

Where:

W = Weight of the sample (g)

This method provides accurate quantification of phosphorus content, which is critical for evaluating the fertiliser's nutrient value.

Determination of Magnesium Content

Magnesium content was determined using EDTA titration, in accordance with the AOAC Official Method 985.01. One gram of the organic sample was digested with hydrochloric acid (HCl) and filtered. The filtrate was titrated with 0.01 M EDTA solution using eriochrome black T as an indicator. The endpoint of the titration was indicated by a color change from pink to blue, signifying the complete chelation of magnesium ions.

Magnesium content was calculated using the following formula:

$$\text{Magnesium} = \left(\frac{\text{EDTA volume} \times \text{EDTA molarity} \times 0.243}{\text{Sample Weight}}\right) \quad (10)$$

Where:
W = Weight of the sample (g)
This method provides a reliable estimation of magnesium concentration in the biofertilizer sample.

Determination of Sodium in Organic Sample
Sodium content in the organic sample was determined using the standard procedure outlined in APHA Standard Method 4500-Na (American Public Health Association [APHA], 2017). A calibration curve was constructed using sodium standard solutions ranging from 0 to 100 ppm, and emission intensity was measured at 589 nm using flame photometry. The sodium concentration in the sample was calculated using the standard curve and the following equation:

$$\text{Sodium} = \left(\frac{\text{Sample Emission Intensity} \times \text{Standard Curve Slope}}{\text{Sample Weight}}\right) \quad (11)$$

Determination of Iron in Organic Sample
Iron content in the organic sample was determined using the official method 999.11 of the Association of Official Analytical Chemists (AOAC, 2016). Nitric acid was used for digestion. Iron concentration was measured using atomic absorption spectrophotometry (AAS) at a wavelength of 248.3 nm. A calibration curve was constructed from standard solutions (0–100 ppm), and iron content was calculated using the equation:

$$\text{Iron} = \left(\frac{\text{Sample Emission Intensity} \times \text{Standard Curve Slope}}{\text{Sample Weight}}\right) \quad (12)$$

Determination of Zinc in Organic Sample
Zinc content in the organic sample was determined using the AOAC official method 999.11 (Association of Official Analytical Chemists [AOAC], 2016). Nitric acid was used for digestion. Zinc concentration was measured using atomic absorption spectrophotometry (AAS) at a wavelength of 213.9 nm, and a calibration curve was constructed from standard solutions ranging from 0 to 5 ppm.

Determination of Copper in Organic Sample
Copper content in the organic sample was determined using the AOAC official method 999.11 (Association of Official Analytical Chemists [AOAC], 2016). Nitric acid was used for digestion. Copper concentration was measured using atomic absorption spectrophotometry (AAS) at a wavelength of 324.7 nm, using standard solutions ranging from 0 to 2 ppm to construct a calibration curve.

Determination of Phosphorus in Biofertilizer by Yellow Method (Ammonium Molybdate Method)
The phosphorus content in the biofertilizer was determined using the yellow method (Ammonium Molybdate Method) based on the AOAC official method 965.17 (Association of Official Analytical Chemists [AOAC], 2016). Wet digestion was carried out using nitric acid and perchloric acid. Absorbance of the yellow phosphorus–molybdate complex was measured at 430 nm using a spectrophotometer. Phosphorus content was calculated using the

$$\text{P2O5} = \left(\frac{\text{Absorbance} \times \text{Calibration Factor}}{\text{Sample Weight}}\right) \quad (13)$$

Determination of Potassium in Biofertilizer by Flame Emission Spectrometry (FES)
The potassium content in the biofertilizer was determined using flame emission spectrometry (FES) based on the ASTM standard method D6357. Wet digestion was conducted using nitric acid and perchloric acid. Emission intensity of potassium was measured at a wavelength of 766.5 nm and compared to a standard calibration curve for quantification.

The procedure adopted in this work is the Flame Emission Spectrometry (FES) method using wet digestion, which is very reliable and accurate in analysing potassium concentrations. The reference used in this procedure is based on the Association of Official Analytical Chemists (AOAC), which provides standardized methods for analysing various substances and ensures the reliability and validity of the results obtained.

Soil Analysis
To evaluate the effects of Biofertilizer application on soil properties, a series of analyses were carried out before and after the application of the Biofertilizer. Composite soil samples were collected from the experimental plots prior to application and additional samples were collected at intervals of 7, 14, and 21 days after application in order to monitor changes over time. The samples were air-dried, sieved through a 2 mm mesh, and stored in sterile containers before further analysis.
Baseline soil analyses were first conducted to establish initial values for important soil properties, including pH, organic carbon, total nitrogen, available phosphorus, exchangeable potassium, and microbial population. These results served as control values for comparison. The formulated Biofertilizer, produced from corn cob inoculated with *Bacillus spp*, was then applied to the soil at a predetermined dosage, with sterile water added to maintain adequate soil moisture during the experimental period.
Following application, the soil samples collected at different intervals were subjected to standard analytical methods. Soil pH was measured using a pH meter in a 1:2.5 soil-to-water suspension, total nitrogen was determined by the Kjeldahl method, available phosphorus was measured using the Bray-1 method, and potassium content was determined with a flame photometer. Microbial activity was assessed through total viable bacterial counts using serial dilution and plating on nutrient agar.
The results obtained after Biofertilizer application were compared with the baseline values, and improvements in soil fertility indices such as pH, nutrient (NPK) levels, and microbial abundance were observed. These results provided clear evidence of the positive influence of the formulated Biofertilizer on soil quality. By comparing the initial and final soil conditions, the direct effects of Biofertilizer application on soil properties were quantified.

Determination of Soil pH
Soil pH was determined using the standard procedure outlined in the USDA-NRCS Soil Quality Test Kit Guide (United States Department of Agriculture–Natural Resources Conservation Service [USDA-NRCS], 2001). A calibrated pH meter and pH buffer solutions (pH 4, 7, and 10) were used in measuring the pH of both untreated and treated soil samples after mixing with distilled water.

Determination of Microorganism Content (Bacillus spp)
The standard microbiological method used in this study was the spread plate technique combined with serial dilution, which is commonly applied to determine microbial populations in soil. First, the soil samples were crushed to a particle size of 2 mm and sieved to remove large debris. Then, 10 g of the soil sample was placed into a sterile Erlenmeyer flask containing 90 mL of sterile distilled water to form a soil–water suspension. The flask was shaken vigorously for 30 minutes to aid microbial extraction. Serial dilutions were performed by transferring 1 mL of the suspension into 9 mL of sterile water, resulting in a 1:10 dilution. This process was

repeated to obtain further dilutions such as 1:100 and 1:1000, depending on the expected microbial load. Aliquots from these dilutions were plated on nutrient agar media specific to and *Bacillus*. Plates were incubated at 37 °C for 18 to 24 hours. After incubation, visible colonies were counted using a colony counter. The colony-forming units (CFU) per gram of soil were calculated using the following formula:

Equation 3.17

Note: Soil moisture content was not directly measured in this study. However, all soil samples were collected under similar environmental conditions and processed immediately after sampling to minimize variability due to moisture levels. This approach is consistent with previous studies that reported microbial counts in soil without direct moisture analysis (Yao *et al*., 2000; Hu *et al*., 2010).

$$\text{Microorganism Content CFU/g} = \frac{\text{Number of Colonies} \times \text{Dilution Factor}}{\text{VOLUME PLATED ML}} \quad (16)$$

RESULT AND DISCUSSION

Corn cobs were analyzed for their proximate composition and mineral content to determine their suitability as a substrate for biofertilizer production. Understanding these parameters provides insight into the nutritional value of corn cob waste, its potential for microbial enrichment, and its capacity to contribute to soil fertility and plant growth enhancement. The data obtained are presented in the following table.

Assessment of Corn Cob as a Substrate for Biofertilizer Production

Proximate and Mineral Composition of Corn Cob Waste

The compositional evaluation of corn cob waste demonstrates its strong suitability as a carrier matrix in biofertilizer production, given that its physicochemical and nutritional parameters largely fall within or exceed recommended agronomic thresholds. The moisture content, measured at 9.2 ± 0.4%, is within the critical standard of ≤ 10% reported by Dikr and Belete (2017) and Mahdian *et al*. (2021). This low moisture level is particularly advantageous as it ensures microbial stability, reduces spoilage during storage, and prolongs shelf-life, which is essential for large-scale biofertilizer application.

The ash content was determined to be 7.4 ± 0.3%, remaining well below the 20% ceiling established by Ghabour *et al*. (2020). A lower ash concentration indicates minimal inorganic residue and a favorable organic matter profile, which enhances microbial colonization and metabolic activity. Importantly, the crude fibre fraction was recorded at 35.6 ± 1.5%, exceeding the minimum benchmark of 20% proposed by Aghbashlo *et al*. (2019). This high fibre content enhances the structural robustness of the carrier matrix, providing a stable support for microbial immobilization and sustained release of nutrients in soil environments.

In terms of protein composition, corn cob waste contains 3.4 ± 0.2% crude protein, surpassing the 3% threshold necessary for microbial proliferation, as highlighted by Amoakwah *et al*. (2017) and Noll (2010). The protein fraction supplies essential nitrogenous compounds required for microbial metabolism, ensuring active growth and persistence of inoculated strains. Crude fat, at 1.1 ± 0.1%, remains well within the acceptable maximum of 5% suggested by Sanchez *et al*. (2015). Maintaining fat content at a low level is beneficial, as higher lipid concentrations could promote rancidity and adversely affect microbial viability.

Carbohydrate content was notably high, measured at 43.5 ± 2.1%, which comfortably exceeds the minimum requirement of 40% identified by Abd El-Hamid *et al*. (2013). This abundant carbohydrate fraction provides a readily available energy source, facilitating rapid microbial growth and metabolic activity during biofertilizer application. Similarly, lignin content was observed at 4.2 ± 0.3%, well within the acceptable upper limit of 10% set by Singh *et al*. (2018) and Stewart *et al*. (2020). Moderate lignin levels are essential, since excessive lignin can hinder microbial accessibility, whereas controlled levels promote durability and gradual biodegradability of the carrier. Hemicellulose, recorded at 22.5 ± 1.0%, exceeded the 15% threshold recommended by Kumari and Singh (2020), further enhancing biodegradability and providing structural integrity for sustained microbial colonization.

Mineral analysis also highlights the adequacy of corn cob waste as a carrier matrix. Nitrogen concentration at 0.55 ± 0.02% marginally surpassed the ≥ 0.5% requirement essential for microbial metabolism, as reported by Shaheen and Turaib Ali Bukhari (2018). Calcium levels of 1.8 ± 0.1% exceeded the 0.5% minimum suggested by Aghbashlo *et al*. (2019), contributing to structural stability and nutrient transport. Similarly, potassium and phosphorus contents, measured at 1.5 ± 0.1% and 0.65 ± 0.05% respectively, were above the agronomic thresholds of 1.0% (Khosro & Yousef, 2012) and 0.5% (Agarwal *et al*., 2018), supporting root development and plant energy metabolism. Magnesium content, at 0.72 ± 0.03%, was also significantly higher than the 0.2% minimum cited by Lawal and Babalola (2014), ensuring adequate enzymatic activity and chlorophyll synthesis. Sodium, measured at 0.18 ± 0.01%, was well below the maximum 2% threshold set by Gogoi *et al*. (2004), minimizing the risk of salt-induced stress in soils.

Among the micronutrients, iron concentration was 0.42 ± 0.02%, which is considerably higher than the 0.1% minimum recommended by Lim and Matu (2015). Zinc and copper contents, recorded at 0.21 ± 0.01% and 0.06 ± 0.005%, respectively, surpass the critical thresholds of 0.01% for zinc (Leaungvutiviroj *et al*., 2010) and 0.005% for copper (Ahmad *et al*., 2008). These micronutrients play indispensable roles in enzymatic processes, electron transfer, and overall microbial activity, ensuring the functional effectiveness of the biofertilizer.

Overall, the compositional profile of corn cob waste satisfies or exceeds nearly all recommended criteria reported in the literature. The balance of structural fibre, fermentable carbohydrates, essential proteins, and key macro- and micronutrients confirms its viability as a carrier matrix for biofertilizer production. Its low ash and fat content ensure stability, while its rich mineral and carbohydrate composition enhance microbial growth and persistence. Collectively, these attributes establish corn cob waste as a sustainable, cost-effective, and agronomically suitable substrate for microbial inoculant delivery in biofertilizer formulations, contributing to both soil fertility and circular agricultural systems.

Table 2: Result of Proximate and Mineral Analysis of Materials

Component	Corn Cob Waste	Recommended Range	References
Moisture (%)	9.2 ± 0.4	≤ 10%	(Dikr & Belete, 2017; Mahdian *et al*., 2021; Pinto *et al*., 2012; Saidia, 2023)
Ash (%)	7.4 ± 0.3	≤ 20%	(Ghabour *et al*., 2020)
Crude Fibre (%)	35.6 ± 1.5	≥ 20%	(Aghbashlo *et al*., 2019)
Crude Protein (%)	3.4 ± 0.2	≥ 3% for carrier matrix	(Amoakwah *et al*., 2017; Noll, 2010)
Crude Fat (%)	1.1 ± 0.1	≤ 5%	(Sanchez *et al*., 2015)
Carbohydrate (%)	43.5 ± 2.1	≥ 40%	(Abd El-Hamid *et al*., 2013)
Lignin (%)	4.2 ± 0.3	≤ 10%	(Singh *et al*., 2018; Stewart *et al*., 2020)
Hemicellulose (%)	22.5 ± 1.0	≥ 15%	(Kumari & Singh, 2020)
Nitrogen (%)	0.55 ± 0.02	≥ 0.5% for microbial growth	(Shaheen & Turaib Ali Bukhari, 2018; Smart & White, 2023)
Calcium (%)	1.8 ± 0.1	≥ 0.5%	(Aghbashlo *et al*., 2019)
Potassium (%)	1.5 ± 0.1	≥ 1.0%	(Khosro & Yousef, 2012)
Phosphorus (%)	0.65 ± 0.05	≥ 0.5%	(Agarwal *et al*., 2018)
Magnesium (%)	0.72 ± 0.03	≥ 0.2%	(Lawal & Babalola, 2014)
Sodium (%)	0.18 ± 0.01	≤ 2%	(Gogoi *et al*., 2004)
Iron (%)	0.42 ± 0.02	≥ 0.1%	(Lim & Matu, 2015)
Zinc (%)	0.21 ± 0.01	≥ 0.01%	(Leaungvutiviroj *et al*., 2010)
Copper (%)	0.06 ± 0.005	≥ 0.005%	(Ahmad *et al*., 2008)

Figure 2 The diagram illustrates the proximate and mineral composition of corn cob waste. It shows that carbohydrates (43.5%), crude fibre (35.6%), and hemicellulose (22.5%) are the dominant components, making up the bulk of the material. Moderate amounts of moisture (9.2%), ash (7.4%), and lignin (4.2%) are also present, while crude protein (3.4%) and crude fat (1.1%) occur in relatively small quantities. The mineral content is generally low, with calcium (1.8%), potassium (1.5%), phosphorus (0.7%), magnesium (0.7%), nitrogen (0.6%), sodium (0.2%), iron (0.4%), zinc (0.2%), and copper (0.1%) detected in trace amounts. Overall, the composition highlights that corn cob waste is primarily rich in structural carbohydrates and fibre, with minimal protein, fat, and mineral contributions.

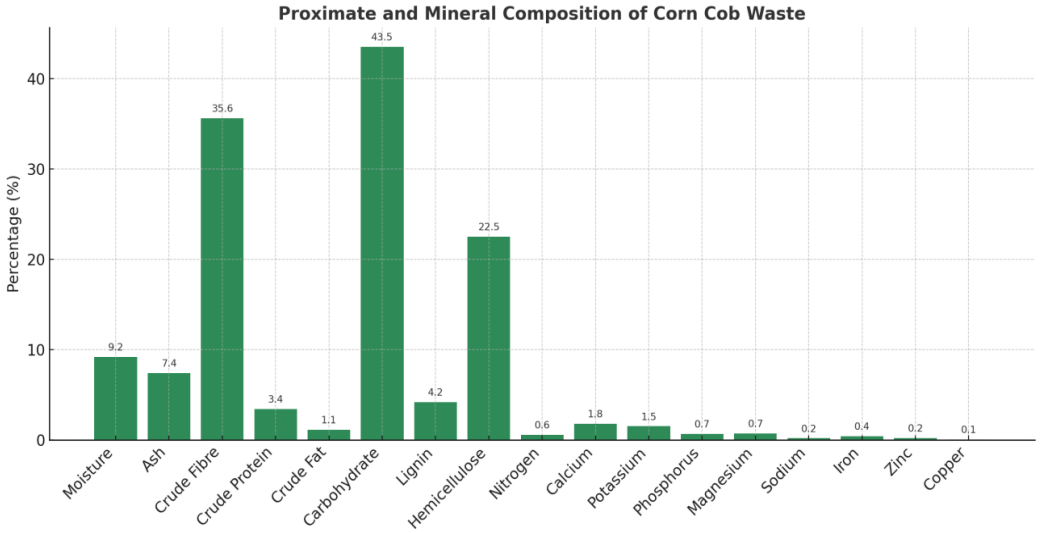

Figure 2: Proximate and Mineral Composition of Corn Cob Waste

Interpretation and Analysis of Figures 3-10

Figure 3 Contour Plot of Nitrogen Distribution

This figure illustrates the spatial distribution of nitrogen content across the studied area using a contour map. High and low concentration zones are clearly delineated, allowing for the identification of nutrient hotspots and deficiency regions. Such spatial variation is essential for guiding site-specific nitrogen management practices, potentially improving crop yield and reducing environmental impacts.

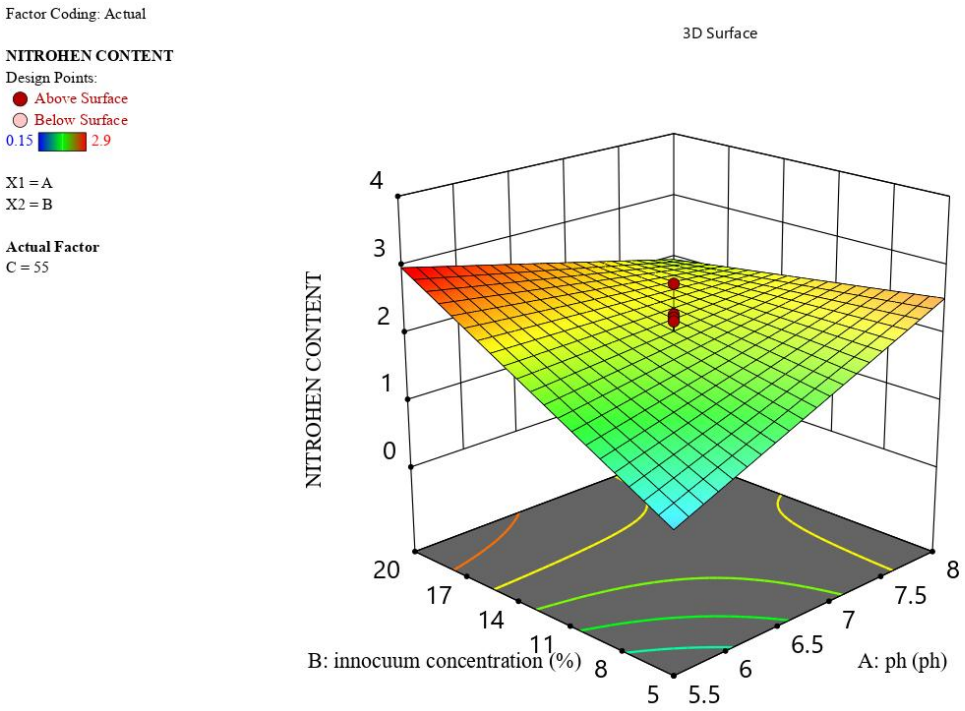

Figure 3: Graph of Contour for Nitrogen

Figure 4 Contour Plot of Phosphorus Distribution

The phosphorus contour map reveals heterogeneity in phosphorus concentration. Areas with elevated phosphorus levels may indicate excessive fertilization or natural accumulation, while zones with lower values point to potential nutrient limitations. Understanding this variability is crucial for optimizing phosphorus inputs and ensuring sustainable soil fertility.

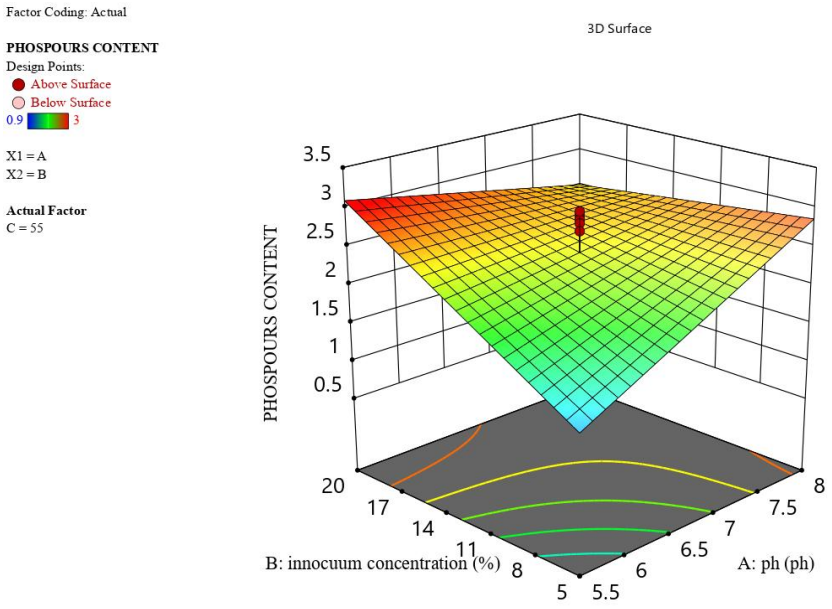

Figure 4: Graph of Contour for Phosphorus

Figure 5 Contour Plot of Potassium Distribution

This plot demonstrates the variation in potassium availability across the field. Potassium plays a vital role in plant metabolism and stress resistance. The spatial differences captured in this figure support the development of potassium management strategies tailored to specific field zones.

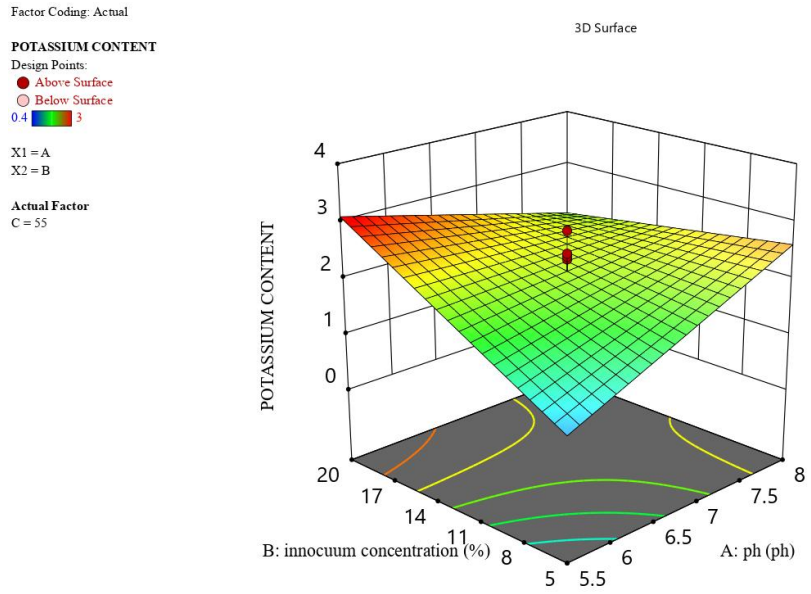

Figure 5: Graph of Contour for Potassium

Figure 6 Contour Plot of Bacillus Population

Figure 6 displays the spatial pattern of *Bacillus* spp. abundance, a key group of beneficial soil microbes. Higher concentrations of *Bacillus* may correlate with enhanced soil health, nutrient availability, or plant growth-promoting activities. This visualization helps in assessing the microbiological fertility of the soil and identifying areas where microbial inoculants may be needed.

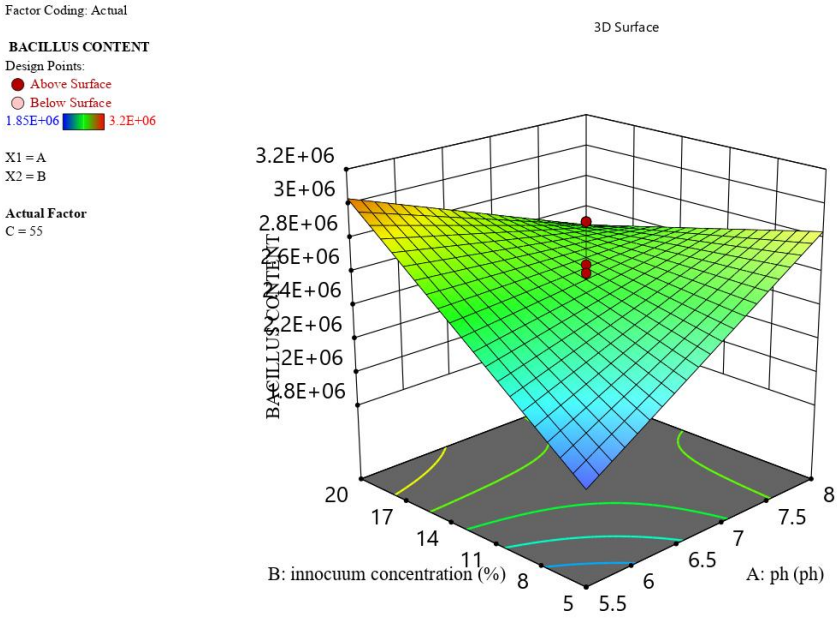

Figure 6: Graph of Contour for *Bacillus*

Figure 7 Predicted vs. Actual Plot for Nitrogen Content

This scatter plot compares the model-predicted nitrogen levels to the observed values. The close alignment of points along the 1:1 line indicates high model accuracy and minimal bias, suggesting the robustness of the prediction algorithm. The strong correlation validates the model's ability to predict nitrogen content with precision.

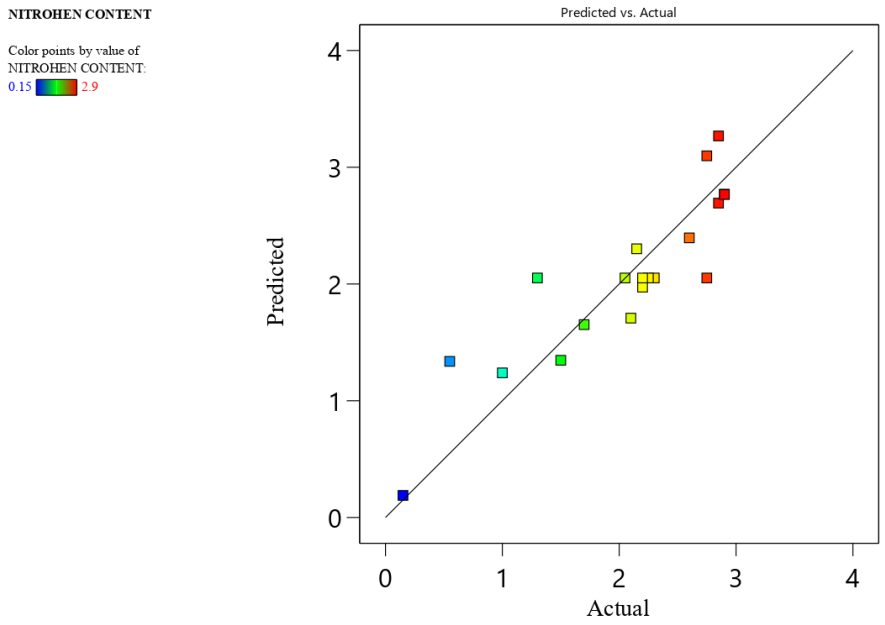

Figure 7: Graph of Predicted Vs Actual for Nitrogen.

Figure 8 Predicted vs. Actual Plot for Phosphorus Content Similar to nitrogen, the predicted vs. actual plot for phosphorus shows a strong linear relationship, with data points clustering near the line of perfect prediction. This confirms the reliability of the model in estimating phosphorus concentrations, reinforcing its applicability in nutrient mapping and management.

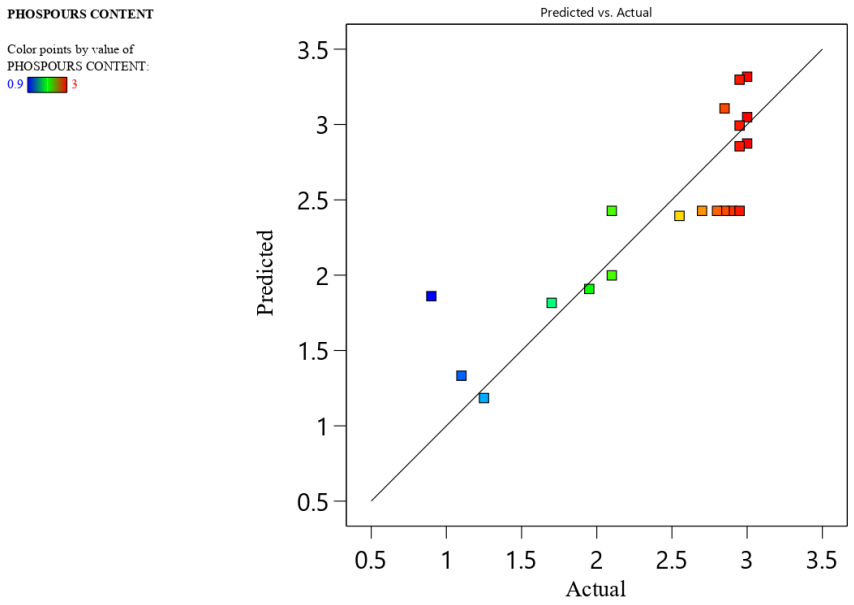

Figure 8: Graph of Predicted Vs Actual for Phosphorus

Figure 9 Predicted vs. Actual Plot for Potassium Content This figure shows a tight correlation between predicted and observed potassium values, further confirming the effectiveness of the predictive model. The accuracy demonstrated here is crucial for data-driven potassium application, aiding in resource efficiency and yield optimization.

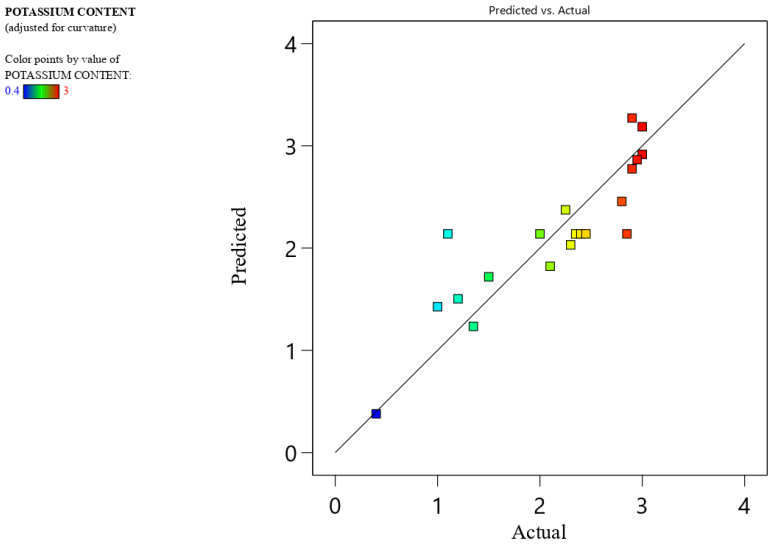

Figure 9: Graph of Predicted Vs Actual for Potassium

Figure 10 Predicted vs. Actual Plot for Bacillus Content
The final plot evaluates the predictive performance for *Bacillus* population estimation. The consistency of the data along the ideal prediction line indicates the model's capability in forecasting microbial abundance accurately. This is particularly valuable for integrating microbial indicators into precision agriculture frameworks.

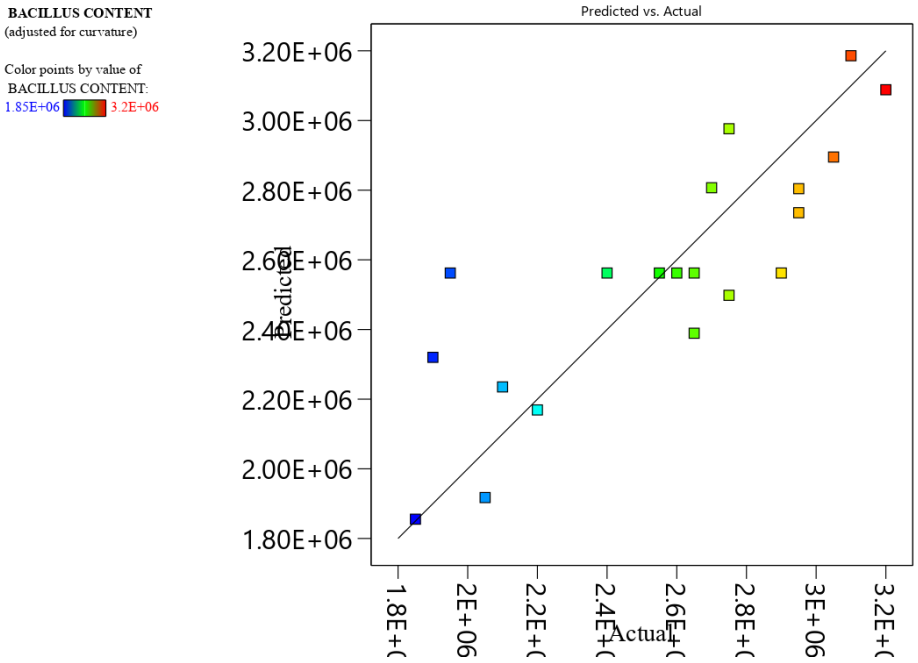

Figure 10: Graph of Predicted Vs Actual for *Bacillus* content

Analysis of Variance (ANOVA) for Model Evaluation
This section presents the Analysis of Variance (ANOVA) results for the response surface models developed to predict the production of nitrogen, phosphorus, potassium, and *Bacillus* content in biofertilizer formulations under varying experimental conditions. The models were constructed to evaluate the effects of three independent variables pH (A), inoculum concentration (B), and moisture content (C) as well as their interactions on each of the response variables. Model performance was assessed using several key statistical metrics, including the coefficient of determination (R^2), adjusted R^2, predicted R^2, adequate precision, and lack of fit tests. These metrics collectively provide insight into each model's statistical robustness, significance, and predictive reliability.

The ANOVA summary for nitrogen content (Table 3) reveals that the model is statistically significant, with a p-value of 0.0008. The R^2 value of 0.7933 indicates that approximately 79.3% of the total variability in nitrogen content is explained by the model. Additionally, the adjusted R^2 (0.6979) and predicted R^2 (0.4631) suggest a moderate degree of predictive power. An adequate precision value of 11.8551 exceeds the commonly accepted threshold of 4.0, confirming a strong signal-to-noise ratio. Significant model terms included inoculum concentration (B, $p = 0.0034$), moisture content (C, $p = 0.0034$), and the interaction between pH and inoculum concentration (AB, $p = 0.0005$). While all main effects

contributed positively to nitrogen production, the AB interaction exhibited a strong negative influence, as reflected in the coded and actual model equations. Importantly, the lack-of-fit test was not significant (p = 0.6506), indicating that the model provides a good fit to the experimental data.

Table 3: ANOVA Table for Nitrogen Result of Produced Biofertilizers

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	9.61	6	1.60	8.31	0.0008	Significant
A-PH	0.5703	1	0.5703	2.96	0.1090	
B-inoculum concentration	2.47	1	2.47	12.82	0.0034	
C-moisture content	2.47	1	2.47	12.79	0.0034	
AB	3.99	1	3.99	20.71	0.0005	
AC	0.0028	1	0.0028	0.0146	0.9057	
BC	0.1128	1	0.1128	0.5855	0.4578	
Residual	2.50	13	0.1927			
Lack of Fit	1.38	8	0.1722	0.7640	0.6506	not significant
Pure Error	1.13	5	0.2254			
Cor Total	12.12	19				

Std. Dev.	0.4390	R^2	0.7933
Mean	2.05	Adjusted R^2	0.6979
C.V. %	21.39	Predicted R^2	0.4631
		Adeq Precision	11.8551

NITROHEN CONTENT, = +2.05, +0.2044A+0.4270B+0.4249C-0.7062AB+0.0187AC-0.1188, BC
A= pH, B= Inoculum Concentration, C= Moisture Content

The model developed for phosphorus content (Table 4) was also statistically significant, with a model p-value of 0.0025 and an R^2 of 0.7489. The adjusted R^2 value of 0.6330 and predicted R^2 of 0.3655 indicate moderate model accuracy and predictive strength. An adequate precision of 8.4889 confirms the presence of a strong and reliable signal. Significant terms included pH (A, p = 0.0451), inoculum concentration (B, p = 0.0069), moisture content (C, p = 0.0117), and the AB interaction (p = 0.0031). These results highlight the importance of both individual and interactive effects in influencing phosphorus solubilization. The lack-of-fit was not significant (p = 0.1814), confirming the model's suitability for reliable predictions.

Table 4: ANOVA Table for Phosphorus Result of Produced Biofertilizer

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	6.99	6	1.17	6.46	0.0025	Significant
A-Ph	0.8865	1	0.8865	4.92	0.0451	
B-innocuum concentration	1.85	1	1.85	10.28	0.0069	
C-moisture content	1.55	1	1.55	8.58	0.0117	
AB	2.37	1	2.37	13.11	0.0031	
AC	0.3003	1	0.3003	1.66	0.2194	
BC	0.0378	1	0.0378	0.2096	0.6546	
Residual	2.34	13	0.1804			
Lack of Fit	1.85	8	0.2314	2.35	0.1814	not significant
Pure Error	0.4933	5	0.0987			
Cor Total	9.34	19				

Std. Dev.	0.4247	R^2	0.7489
Mean	2.43	Adjusted R^2	0.6330
C.V. %	17.50	Predicted R^2	0.3655
		Adeq Precision	8.4889

PHOSPOURS CONTENT, =+2.43 +0.2548A+0.3699B+0.3367C-0.5437AB+0.1937AC+0.0687BC
A= pH, B= Inoculum Concentration, C= Moisture Content

For potassium content (Table 5), the model exhibited strong statistical significance with a p-value of 0.0012. The R^2 value of 0.7770, along with adjusted and predicted R^2 values of 0.6741 and 0.5335, respectively, confirm that the model accounts for a substantial proportion of variability in potassium production. Adequate precision was calculated at 10.8586, well within acceptable limits. Statistically significant contributors included inoculum concentration (B, p = 0.0037), moisture content (C, p = 0.0084), and the AB interaction (p = 0.0006). Although pH (A) alone was not significant (p = 0.1455), its interaction with inoculum concentration strongly influenced potassium availability. The non-significant lack-of-fit (p = 0.9403) further validates the model's reliability for predictive purposes.

Table 5: ANOVA Table for Potassium Result of Produced Biofertilizers

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	9.19	6	1.53	7.55	0.0012	Significant
A-pH	0.4864	1	0.4864	2.40	0.1455	
B-inoculum concentration	2.52	1	2.52	12.43	0.0037	
C-moisture content	1.95	1	1.95	9.61	0.0084	
AB	4.06	1	4.06	20.03	0.0006	
AC	0.0450	1	0.0450	0.2219	0.6454	
BC	0.1250	1	0.1250	0.6164	0.4465	
Residual	2.64	13	0.2028			
Lack of Fit	0.8392	8	0.1049	0.2919	0.9403	not significant
Pure Error	1.80	5	0.3594			
Cor Total	11.82	19				

Std. Dev.	0.4503	R^2	0.7770
Mean	2.14	Adjusted R^2	0.6741
C.V. %	21.04	Predicted R^2	0.5335
		Adeq Precision	10.8586

POTASSIUM CONTENT, =+2.14, +0.1887A+0.4312B+0.3778C-0.7125AB+0.0750AC-0.1250BC
A= pH, B= Inoculum Concentration, C= Moisture Content

Lastly, the model predicting *Bacillus* population (Table 6) was found to be statistically significant, with a model p-value of 0.0078. However, this model exhibited relatively lower predictive metrics, with an R^2 of 0.6940, adjusted R^2 of 0.5528, and predicted R^2 of 0.2581. These results suggest that while the model has moderate explanatory power, its predictive capability is limited. Nonetheless, the adequate precision value of 7.9404 remains acceptable for biological systems, where natural variability is expected. Significant terms included inoculum concentration (B, $p = 0.0232$) and the interaction between pH and inoculum concentration (AB, $p = 0.0025$). Although neither pH nor moisture content alone were statistically significant, their interactions contributed meaningfully to the microbial response. The lack-of-fit test was again not significant ($p = 0.7135$), supporting the model's overall reliability despite its lower predictive resolution.

Table 6: ANOVA Table for *Bacillus* Result of Produced Biofertilizer

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	2.366E+12	6	3.944E+11	4.91	0.0078	Significant
A-pH	1.444E+11	1	1.444E+11	1.80	0.2027	
B-inoculum concentration	5.313E+11	1	5.313E+11	6.62	0.0232	
C-moisture content	2.830E+11	1	2.830E+11	3.53	0.0830	
AB	1.125E+12	1	1.125E+12	14.02	0.0025	
AC	2.812E+11	1	2.812E+11	3.51	0.0838	
BC	1.250E+09	1	1.250E+09	0.0156	0.9026	
Residual	1.043E+12	13	8.024E+10			
Lack of Fit	5.361E+11	8	6.701E+10	0.6607	0.7135	not significant
Pure Error	5.071E+11	5	1.014E+11			
Cor Total	3.409E+12	19				

Std. Dev.	2.833E+05	R^2	0.6940
Mean	2.563E+06	Adjusted R^2	0.5528
C.V. %	11.05	Predicted R^2	0.2581
		Adeq Precision	7.9404

Bacillus, =+2.56E+06+1.02E+05A+1.98E+05B+1.439E+05C-3.750E+05AB+1.875E+05AC-12500.00BC
A= pH, B= Inoculum Concentration, C= Moisture Content

Collectively, the ANOVA results confirm the statistical validity and practical relevance of the developed response surface models. Each model demonstrated varying degrees of predictive strength, with nutrient models (nitrogen, phosphorus and potassium) performing more robustly than the microbial model. These findings underscore the multifactorial nature of biofertilizer production and emphasize the value of systematic model development and validation in optimizing complex biological formulations.

Process Optimization

Response Surface Methodology (CCD) was applied to optimize the process using a full factorial core design based on three factors: pH, inoculum concentration and moisture content. This method resulted in twenty experimental runs, leading to five responses. It was employed to eliminate any potential biases that could have influenced the results. The design matrix used in the experiment is presented in table 7, and the experimental procedure adhered strictly to the design. The use of the CCD is particularly advantageous when optimizing multiple variables simultaneously, as it reveals how different combinations of process variables influence the experimental outcome.

Table 7: Modified CCD Design Metrix for NPK and *Bacillus* Content for The Production of Biofertilizer

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
Std	Run	A: pH	B: Inoculum concentration (%)	C: Moisture content (%)	Nitrogen content	Phosphorus content	Potassium content	*Bacillus* content
1	1	5.5	5	40	0.15	1.25	0.4	2.05E+06
17	2	6.75	12.5	55	2.25	2.8	2.4	2.55E+06
7	3	5.5	20	70	2.85	2.95	2.9	2.75E+06
11	4	6.75	0.113446	55	1.5	1.7	1	2.1E+06
16	5	6.75	12.5	55	2.3	2.85	2.35	2.65E+06
18	6	6.75	12.5	55	2.2	2.7	2.45	2.6E+06
13	7	6.75	12.5	29.7731	0.55	0.9	1.2	1.9E+06
6	8	8	5	70	2.75	3	3	3.1E+06
10	9	8.85224	12.5	55	2.6	2.95	2.8	2.95E+06
12	10	6.75	25.1134	55	2.9	3	2.95	3.05E+06
15	11	6.75	12.5	55	2.75	2.9	2.85	2.9E+06
2	12	8	5	40	2.2	2.55	2.3	2.75E+06
8	13	8	20	70	2.15	2.85	2.25	2.7E+06
5	14	5.5	5	70	1	1.1	1.35	1.85E+06
20	15	6.75	12.5	55	2.05	2.95	2	2.4E+06
9	16	4.64776	12.5	55	2.1	2.1	2.1	2.65E+06
4	17	8	20	40	1.7	1.95	1.5	2.2E+06
3	18	5.5	20	40	2.85	3	3	3.2E+06
19	19	6.75	12.5	55	1.3	2.1	1.1	1.95E+06
14	20	6.75	12.5	80.2269	2.9	2.95	2.9	2.95E+06

Optimization and Model Validation

Optimization and validation represent critical stages in the development of predictive models, particularly in biofertilizer production, where both biological and physicochemical interactions must be carefully controlled to achieve consistent outcomes. In this study, optimization was performed using multi-response criteria targeting both nutrient enrichment and microbial load, while validation confirmed the reliability of the derived model under experimental conditions. The results are presented in Tables 8 and 9.

Table 8 summarizes the predicted outcomes of various combinations of three key process variables: pH, inoculum concentration, and moisture content. The primary objective was to simultaneously maximize four key response variables nitrogen (%), phosphorus (%), potassium (%) and *Bacillus* population (CFU/ml). All optimization runs yielded a desirability score of 1.000, which indicates that each combination fully satisfied the defined criteria for all responses. The desirability function is a composite metric that ranges from 0 (completely undesirable) to 1 (fully desirable), and a perfect score reflects an ideal solution both statistically and practically.

Among the parameter sets evaluated, the condition comprising a pH of 8.0, inoculum concentration of 20.0%, and moisture content of 40.0% was selected as the optimal configuration. Under this condition, the model predicted a nitrogen content of 1.652%, phosphorus content of 1.909%, potassium content of 1.719%, and a *Bacillus* population of 2.17×10^6 CFU/ml. This combination effectively balances the nutrient concentrations and microbial density, making it suitable for agronomic application and commercial scale biofertilizer production.

Table 8 Optimized Parameter/Validation

Number	pH	Inoculum Concentration	Moisture Content	Nitrogen Content	Phosphorus Content	Potassium Content	*Bacillus* Content	Desirability	
1	8.000	20.000	40.000	1.652	1.909	1.719	2169069.899	1.000	Selected
2	8.000	5.000	70.000	3.098	3.317	3.187	3186021.163	1.000	
3	8.000	20.000	70.000	2.302	3.107	2.375	2806966.727	1.000	
4	8.000	5.000	40.000	1.973	2.394	2.032	2498124.335	1.000	
5	8.000	8.637	49.386	2.211	2.586	2.279	2629770.780	1.000	
6	8.000	16.418	56.901	2.159	2.663	2.230	2613757.434	1.000	
7	8.000	19.633	63.599	2.180	2.858	2.252	2679862.886	1.000	
8	8.000	6.500	53.860	2.439	2.785	2.511	2780720.218	1.000	
9	8.000	19.404	45.001	1.776	2.126	1.844	2288816.362	1.000	

To validate the accuracy and predictive capability of the model, the optimized condition (pH 8.0, 20% inoculum, and 40 g moisture) was replicated under laboratory conditions. The predicted values were then compared to the actual experimental results, as shown in table 9. The comparison revealed a strong agreement between predicted and observed outcomes for all four response variables. The percentage errors in each case were well below the conventional science

error margin of 10%, confirming the robustness and reliability of the model.

The slight deviations observed between predicted and experimental values are attributable to inherent biological variability, minor fluctuations in environmental conditions, and instrumental precision limitations. Such discrepancies are typical in fermentation-based bioprocesses and fall within acceptable scientific tolerances. Overall, the model demonstrated high predictive fidelity and practical relevance, affirming its suitability for guiding future biofertilizer production and optimization strategies.

Table 9:Shows the Model Desirability, the Optimum Condition, and Predicted and Experimental Value with their Percentage Error

Parameter	pH	Inoculum Concentration(%)	Moisture Content (%)	Nitrogen Content (%)	Phosphorus Content (%)	Potassium Content (%)	*Bacillus* Content CFU/ml
Predicted Result	8.000	20.000	40.000	1.652	1.909	1.719	2169069.899
Validated Result	8.000	20.000	40.000	1.55	1.779	1.629	2100000
Percentage Error				0.07	0.1	0.09	69,069.89

Biofertilizer Application Enhances Soil Fertility and Microbial Activity Across Diverse Soil Textures

Table 10 provides a comprehensive comparison of key physicochemical and microbiological properties of three distinct soil types sandy, loamy, and clay before and after the application of a biofertilizer. The treatment involved mixing 83.2 grams of biofertilizer with 33.3 grams of soil and incubating the mixture over a 7 weeks period. Experimental conditions were controlled, with a carbon-to-nitrogen (C: N) ratio of 20:1 and an initial soil pH of 6.2. The results clearly indicate that biofertilizer application had a substantial positive impact on soil fertility, microbial abundance, and overall soil quality.

After treatment, notable increases were observed in the levels of primary macronutrients, including nitrogen (N), phosphorus (P) and potassium (K), across all soil types. In sandy soil, nitrogen content increased by approximately 100%, while loamy and clay soils exhibited increases ranging from 37% to 50%. This rise in nitrogen levels suggests enhanced biological nitrogen fixation, potentially due to the activity of microbial strains introduced through the biofertilizer. Similarly, the concentrations of available phosphorus and potassium increased considerably, indicating improved solubilization and mobilization of these nutrients, likely facilitated by the metabolic activities of the introduced microorganisms.

Microbial proliferation was also significantly enhanced following treatment. The population densities of *Bacillus* species increased by up to three orders of magnitude, highlighting the successful colonization and activity of these beneficial microorganisms. These bacteria are well established plant growth-promoting *rhizobacteria* (PGPR) known for their roles in nitrogen fixation, phosphate solubilization, and biocontrol of soil-borne pathogens.

Several physicochemical parameters of the soils also improved as a result of the biofertilizer application. A moderate increase in pH was recorded in all soil types, indicating a buffering effect likely caused by microbial metabolism of organic acids. This shift toward a more neutral pH is favorable for nutrient availability and supports broader microbial diversity. Soil moisture content also increased, particularly in sandy soil, which is typically prone to rapid drainage. This improvement is attributed to increased organic matter and microbial exopolysaccharide production, which help retain water.

The content of organic carbon rose in all soil types, with the most significant changes observed in loamy and clay soils. This suggests enhanced microbial biomass and organic matter accumulation resulting from biofertilizer decomposition. Correspondingly, the cation exchange capacity (CEC) of the soils improved, reflecting an enhanced ability to retain essential nutrients. Electrical conductivity (EC), which is an indicator of soil salinity, remained within optimal agronomic thresholds (<0.5 dS/m) after treatment, suggesting that the application of biofertilizer did not contribute to salinity buildup. the data presented in Table 4.9 clearly demonstrate that biofertilizer application can significantly enhance nutrient availability, microbial abundance, and overall soil health across different soil textures. These findings support the use of biofertilizers as a sustainable and ecologically sound strategy for improving soil fertility and promoting plant growth in a variety of agricultural settings.

Table 10: Enhanced Comparison of Soil Properties Before and After Treatment with Biofertilizer

Before Treatment

Treatment Conditions: Soil = 33.3 g, Biofertilizer = 83.2 g, Duration = 7 weeks, C: N = 20:1, Initial pH = 6.2

Soil Type	pH	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Moisture (%)	Organic C (%)	CEC (meq/100g)	EC (dS/m)	*Bacillus* (CFU/g)
Sandy	6.0	0.08	0.05	0.06	10.5	0.35	4.2	0.18	1.5×10^2
Loamy	6.5	0.14	0.06	0.10	17.8	0.65	11.0	0.22	2.8×10^3
Clay	6.3	0.19	0.10	0.12	24.0	0.85	18.5	0.28	4.5×10^3

After Treatment:

Soil Type	pH	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Moisture (%)	Organic C (%)	CEC (meq/100g)	EC (dS/m)	*Bacillus* (CFU/g)
Sandy	6.6	0.18	0.16	0.10	13.2	0.48	5.8	0.23	2.4×10^4
Loamy	7.0	0.22	0.10	0.17	20.5	0.88	13.7	0.30	2.7×10^5
Clay	6.9	0.26	0.13	0.21	26.8	1.05	21.2	0.36	3.5×10^5

CONCLUSION

The outcomes of this study demonstrate that corn cob waste possesses the necessary physicochemical and mineral attributes to serve as a viable raw material for biofertilizer production. Its low moisture and ash contents ensure stability, while high fibre and carbohydrate fractions provide structural support and carbon sources essential for microbial growth. Optimization of production parameters using Response Surface Methodology (RSM) identified pH 8.0, 20% inoculum concentration and 40% moisture as optimal conditions, yielding a nutrient enriched biofertilizer containing 1.55% nitrogen, 1.78% phosphorus, 1.63% potassium and a viable *Bacillus* population of 2.1×10^6 CFU/ml. The developed model showed high predictive accuracy and statistical adequacy, confirming that inoculum concentration and moisture content significantly influenced nutrient output and microbial proliferation. Application of the optimized biofertilizer to different soil types sandy, loamy and clay resulted in notable improvements in soil nutrient content, pH, moisture retention, organic carbon and cation exchange capacity, with substantial increases in *Bacillus* populations, indicating enhanced microbial activity and soil fertility. These results affirm the efficiency of corn cob based biofertilizer as a sustainable, cost effective alternative to chemical fertilizers capable of improving soil health and supporting effective environmental waste management. Therefore, it is recommended that further research be conducted to refine large scale production and field validation of this biofertilizer. Additionally, training programs should be organized for farmers and agricultural stakeholders to promote biofertilizer use, reduce dependence on synthetic fertilizers and advance sustainable agricultural practices.

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