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Impact of bacteria with biofertilizing potentials and water hyacinth (Eichhornia crassipes) compost on plant growth parameters

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Abstract

Background

The purpose of this study is to analyze the impact of bacteria with biofertilizing potential from forest environment and water hyacinth (*Eichhornia crassipes*) compost on plant growth parameters. Soil was collected from selected forests in (Etche, Ogbogoro and Choba) from various soil horizons (0–15, 15–30 and 30–45 cm) depth. Soils parameters (bacteria count and physicochemical properties) were analysed, afterwards soils from different depth were pooled together for further analysis. Soil samples from the three forests were equally mixed to form a single soil in the ratio of 1:1:1 and used to make five treatments as follow: only mixed soil (control), 2 kg of soil + 0.1 kg microbial inoculant (MI), 2 kg of soil + 0.1 kg Water Hyacinth Compost (WHC), 2 kg of soil + 0.1 kg MI + 0.1 kg WHC, and 2 kg of soil + 0.1 kg chemical fertilizer Nitrogen Phosphorus and Potassium (NPK: 20-10-10). The efficacy of the bacterial inoculant and WHC product, were tested on 3 different plants (*Abelmoschus esculentus, Zea mays and Cucurbita pepo*) in different experimental set up.

Results

Results of the microbial count ranged from $26.00 \pm 4.50 \times 10^8$ to $100.66 \pm 2.07 \times 10^9$ cfu/g for the Total Heterotrophic Bacteria (THB), Phosphate Solubilizing Bacteria (PSB) and Nitrogen Fixing Bacteria (NFB). *Pseudomonas* sp and *Bacillus* sp were the abundant PSM and NFB isolated from the soil. There was an overall increase in the growth of the plant species as indicated in their shoots, leaves area, total chlorophyll content, germination rate, height, yield and rate of nutrients absorption after 60 days of planting with the best performance recorded in the treatment with combination of MI + WHC. Also, the content of indole-3-acetic acid (IAA) and gibberellin A_4 (GA_4) in shoots and leaves of crops was higher in soil treated with combination of MI + WHC than other treatments.

Conclusion

These studies have shown that biofertilizer should be adopted in crop production to increase agricultural growth performance and reduce dependency on chemical fertilizers.

Introduction

Forests are sizable tract of agricultural land that has been left untouched for several decades and is covered with trees and plants. A region of earth with a substantial tree coverage or a sizable tract of land covered with trees that serves as a habitat for untamed creatures is another way to describe it. Every forest is either man-made or indigenous, whereas constructed forests are engineered and maintained by humans, forests in nature grow naturally. Trees are the predominant life type in this intricate biological ecosystem. Forests have a lot of lateral division and are one of the planet's complicated environments. Since the advent of agricultural renaissance methods, farming today has become increasingly reliant on an ongoing source of artificial inputs, mostly nutrients. Nevertheless, the overuse and disproportion of such artificial ingredients has been observed to have negative impacts. The detrimental impacts that are being shown progressively include seeping out; this will leads to high death rates in aquatic organisms and drastically lowering quality of aquatic environment, eliminating beneficial insects and microorganisms, and increasing crop susceptibility to disease attacks. Considering previously described negative impacts of chemical-based fertilizers, biological fertilizers and other substitutes are now required (Britannica et al. 2019).

Biofertilizers are inexpensive, efficient, and environmentally benign agricultural products that improve plant growth, output, and soil health, they have been fervently promoted as the best alternative to chemical fertilizer use (Ammar et al. 2023). It is widely acknowledged as a crucial part of the integrated nutrient supply management system and has the potential to increase crop yields by using more ecologically friendly fertilizer sources. By releasing growth-promoting hormones and converting complicated nutrients into simple nutrients that plants can use, biofertilizers increase root proliferation (Ammar et al. 2023). Although they are free of any residues of toxic or dangerous substances, they aid in mitigating the negative impacts of chemical fertilizers. They are also reasonably priced, environmentally beneficial and save to use. Chemical fertilizers must be applied to crops on a regular basis to replace the nutrients lost during crop growth, while biofertilizers provide nutrients continuously all through the plant development in the field under favorable conditions. Continuous application of chemical fertilizers degrades agricultural activities, while the application of biofertilizers improves soil structure and crop product quality, increases agricultural produces by 22–32%, and replaces inorganic by 27%. According to Gyaneshwar et al. (2002), additional application of inorganic fertilizers in excess cause plant death whereas biofertilizers have no negative effects.

According to Kumar et al. (2017), the realization of the negative environmental impacts caused by the overuse and incorrect utilization of chemical fertilizers has led to a recent surge in interest in the development and implementation of microbial-based fertilizers. Through biological processes like stabilization of nitrogen, phosphate in the solubilization, excretion of substances that promote plant growth, and biodegradation in soil, various species of microorganisms may transfer economically essential compounds from non-useful to feasible forms, resulting in biofertilizers. They are live microbial inoculants that include fungi, bacteria, or algae individually. This resulted from the immense efforts to identify and select

strains of bacteria that have the ability to support plant growth, as well as from a greater knowledge of the relationships that occur in the roots between plant tissue and soil microbes. Microorganisms from the soil have long been used in crop cultivation. The recycling of nutrients, especially carbon into the atmosphere, nitrogen, phosphorus, and sulphur, is strongly associated with bacteria, the primary class of microorganisms that keep soils healthy (Thornbro 2022).

The provision of nutrients to crops, promotion of crop development and enhancement of soil structure and quality are some of the primary roles of bacteria in the soil. Bacteria like *Pseudomonas species*) are among the microorganisms utilized as biofertilizers (Seenivasagan et al. 2021). Biological nitrogen fixation (BNF), phytohormone synthesis, environmental stress alleviation, synergism with other microbial-plant interactions, inhibition of plant ethylene synthesis, increased availability of nutrients such as phosphorus, iron, and minor elements, and growth enhancement by volatile compounds are some of the microbial mechanisms of plant growth promotion (Ammar et al. 2023). Nevertheless, the manifestation of such bacterial activity in a lab setting does not ensure a symbiotic or reciprocal relationship with a host plant. This is particularly true for nitrogen fixation, which is widely expressed by a variety of bacterial and fungal species in culture medium polluted soils; beneficial microorganisms can also be employed for supplementation of soil nutrients (Atuchin et al. 2023).

Methods

Study mapped area Etche community is between latitudes 4° 04' 5" North and 5° 01' 7" North and longitudes 6° 05' 5" East and 7° 01' 7" East in the north eastern region, of Nigeria. Obio-Akpor is a local government area located in Rivers State, Nigeria, in the city of Port Harcourt; Ogbogoro community is a neighborhood in the Greater Port Harcourt City area of Rivers State, Nigeria; while Choba is located at coordinates 4° 89' 07" North, 6° 90’ 34” East with a total size of roughly 23.65 square kilometres (Fig. 1).

Insert Fig. 1 here please

Sample collection

Soil samples were collected randomly at three different depths (0–15, 15–30 and 30-45cm) from the various forests (Etche, Ogbogoro, and Choba) in Rivers state and Agricultural soil from the Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt as control with a soil Auger. The Soil Auger used was cleaned with ethanol after each collection to reduce contamination between samples. Each of the soil samples was mixed to form a composite sample and was sent in sterile containers and transported to the laboratory for further analysis within 24 hours. Sieving through a 5 mm mesh size sieve of the sample was done and they were stored at $4^{o}C$ for DNA analysis and others for physicochemical, soil composition and microbiological analysis. This was done according to the method of Abu et al. (2024).

Plant samples: okra (*Abelmoschus esculentus)*, maize *(Zea mays)* and pumpkin seeds *(Cucurbita pepo)* were purchased from River State Agricultural development Program (RSADP). Water hyacinth plant was gotten from new Calabar River at Choba. Samples collected from these areas include soil from different soil horizons. Sampling points were georeferenced (coordinates were taken using a GPS device). Samples were brought to the laboratory for analysis at the conclusion of the sampling process.

Physicochemical analysis of soil and biofertilizer samples

A portable, handheld, pH meter was used to potentiometrically measure the samples' pH according to Zhao et al. (2020); soil total nitrogen was analysed in compliance with ecological laws (LY/T 1237–1999 and LY/T 1228–2015); electrical conductivity was measured by dipping the electrode into the sample in the beaker and allowing the figures on the conductivity meter screen to stabilize before taking the conductivity reading (Zhao et al. 2020); total organic carbon was determined according to method by Allen 2019); nitrate and total nitrogen were determined following distillation method (Allen 2019); soil phosphate was measured using thermospectronic spectrophotometer; while soil potassium was analyzed following the digestion of $HF\text{-}HClO_4\text{-}HNO_3$.

Microbiological Analysis of Soil Samples

Preparation of soil sample To remove the organism from the soil particles, one gram (1g) of each soil sample was aseptically added to a separate test tube that contained nine milliliters of normal saline and shaken. To achieve varying dilutions up to 10^{-5}, 1 ml of each suspension was then serially added to 9 ml diluents. In duplicate, aliquots of the diluted soil suspension were transferred on mineral salt agar, nutrient agar, Pikovskaya (PVK) agar and Jensen’s Media agar,

Enumeration of heterotrophic bacteria Nutrient agar was plated with duplicate aliquots (0.1 ml) of serially diluted soil sample using spread plate techniques. The plates were then incubated at 37°C for 24 hours. After incubation, the colonies were counted to measure the number of colony forming units (CFU) per gram of soil sample. Discrete colonies were chosen and then sub cultured (Hema et al. 2017).

Screening for phosphate solubilizing bacteria Each sample had about 2g of dirt removed from its source and placed in sterile tubes with 2 ml of sterile deionized (DI) water. After carefully vortexing each test tube, 100 µl of each 10-fold dilution was made down to 10^{-9}. The dilutions were then plated onto nutrient agar, and later cultured in both Pikovskaya (PVK) and Jensen's media agar plates (Pikovskaya, 1948). Ten 10 glucose, 0.2 of yeast extract, 0.2 of $(NH_4)_2SO_4$, 0.1 $MgSO_4.7H_20$, $Ca(PO_4)$, 0.2 KCl, 0.002 $MnSO_4.2H_20$, 0.002 $FeSO_4.7H_20$, and 15 agar were all present in the PVK medium (in g L^{-1}). To get rid of the soluble phosphate impurities, the insoluble $Ca(PO_4)$ was centrifuged after being cleaned with diluted water. The wet $Ca(PO_4)$ was dried using a vacuum-flask device, and the supernatant was disposed of. The total number of rhizospheric bacteria in each plant sample was calculated from the colonies that developed on the Jensens media agar plates. In the PVK agar plates, the colonies that generated clearing zones were separated. For a more thorough examination of clearing zone creation, individual colonies from the isolation were subsequently cultivated onto fresh PVK plates. For a maximum of 3 days, all plates were incubated at 30°C (Hema et al. 2017).

Screening for nitrogen fixing abilities Five milliliter of Jensens media were used to inoculate the 48-hour-old cultures. The incubation period was 48 hours. 50 ml of semi-solid medium was inoculated with 1ml of this Jensens media. It was then incubated for fifteen days. Nitrogen estimate was performed using 10 ml of this culture and the conventional Jensens approach (Hema et al. 2017).

Experimental design According to Vincent-Akpu et al. (2024), the various soil treatments were loaded in pot according to pot experimentation procedure we have the following experimental set up. The soil samples from the various forests depth were mixed to form a single soil in the ratio of 1:1:1 and used to make five treatments as follow:

Only mixed soil (control)

2 kg of Soil + 0.1kg Microbial inoculant

2 kg of Soil + 0.1kg Water Hyacinth Compost

2 kg of Soil + 0.1kg Microbial inoculant + 0.1kg Water Hyacinth Compost

2 kg of Soil + 0.1kg Nitrogen Phosphorus and Potassium (NPK)

Analysis of Crop Growth Parameters

Seed variable test Using the "flotation method" in seed processing, which is essentially a straightforward technique to test seed viability by using their density difference in water, viable, heavier seeds sink to the bottom while less viable, lighter seeds float to the top. This allows for the separation and selection of high-quality seeds for planting.

Pot culture Experiment *Zea mays* maize, *Abelmoschus esculentus* okro and *Cucurbita pepo* pumpkin seeds were grown in a pot culture experiment to test the effectiveness of the treatments (MS = Mixed soil, MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK = Nitrogen, Phosphorus, Potassium Fertilizer). To determine whether the various prepared treatments improved plant growth, they were utilized in combination and ratio. The experiment was set up using several treatments. Plants were used in each treatment, and the entire procedure was repeated numerous times. Sterile distilled water was used to revive 0.1kilogram of the substrate, which was subsequently combined with 0.1kilogram of the different substrate in an aseptic setting. The seeds were planted straight into their containers. Forest mixed soil weighing 0.2 kg was placed in each pot. For 60 days with various treatments the plants' phenotypic characteristics are measured every five days. At the Green house across from the Basic Science building, the plants were housed in a poly house with a humidity level between 70 and 80%. The green house's temperature fluctuates based on local conditions and is not controlled (max: 32ºC, min: 15ºC) (Akintokun et al. 2019).

Determination of Shoot length We experimented with a method called mid-line tracking, which counts the number of points on the stem to determine shoot length. However, we found that the stem was often invisible during the plant's early growth stage when the shoot was quite short. Furthermore, the plant's shoot system may curve under natural circumstances, therefore a precise estimate of the actual shoot length using meter rule was equally done.

Determination of plant height This was done with meter rule measuring from the base of the crop to the top in cm according to Krzynanowski et al. (2020).

Determination of Leaves area After, rule was used to measure the width, (at the widest point) of the lead and length from the lead apex. The readings of three leaves were taken (both width and length). The leaf area was as follows:

$(L \times W) + 1.66$

Where leaf = L = length of leaf, W = width of leaf. 1.66 = constant (Suma and Srimathi 2014).

Determination of Leaf chlorophyll contents Contents of leaf chlorophyll (mg.100g^{-1} fresh weight): A representative fresh leaf sample from the middle of the shoots was collected in order to perform a calorimetric study of the chlorophyll content. The manufacturer's instructions were followed when calibrating the equipment. Five plants or replications of each species were then selected at random to determine the first ten mature leaves. Ten SPAD-502 measurements on average were used to determine each leaf (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan). It measured the transmittance of leaves in the infrared (940 nm) and red (650 nm) portions of the electromagnetic spectrum. The transmittance measurements were converted into a relative SPAD-502 meter value (from 0 to 99), which was proportionate to the sample's chlorophyll concentration (Soil Plant Analysis Development (SPAD) Markwell et al. 1995).

Determination of Germination rate (%) To measure the germination rate with fluorescent lighting, four replicates of one hundred seeds were planted in sand medium and maintained at room temperature and relative humidity. The normal seedlings were counted after the seven-day test period, and the mean values were translated into a percentage of the total number of seeds planted for germination (Suma and Srimathi 2014).

Determination of yield For calculation of crop yield, 16 samples each of maize cobs, okra pods and pumpkin leaves were picked randomly. The respective crops after topped by hand were taken to the laboratory in rice bag well-perforated for air movement. Soil particles and other debris were carefully removed prior to weighing on a weighing balance to avoid error in calculation. The total weight obtained in kg was noted and utilized for the calculation of crop yield which was expressed in tonnes per hectare (t ha^{-1}).

Molecular Identification

The National Institute for Medical Research (NIMR) Molecular Biology Laboratory in Yaba, Lagos, Nigeria, performed DNA extractions, PCR amplification of the partial bacterial 16S rRNA genes, and gel electrophoresis of the isolates. The European Genome and Diagnostics Centre, GATC Biotech AG, Jakob Stadier-Platz 7, 78467 Constance, Germany, received the PCR products for 16S rRNA sequencing. Following the manufacturer's instructions, a QIAGEN QIAamp DNA extraction kit was used to extract DNA directly from the samples. The PCR amplification of the partial 16S rRNA genes was carried out using the primer set 27F-5'-AGA GTTTG ATYMTGG CTC AG-3', and 515R 5'-TACCGCGGCKG CTGGCA C-3'. The procedure outlined by Ezebuiro et al. (2015) was followed for conducting the reaction. The reaction mixture was made up of 20 microliters of 1× PCR buffer (Solis Biodyne), 1.5 mM magnesium chloride (Solis Biodyne), 0.2 mM of each dNTP (Solis Biodyne), 2 U Taq DNA Polymerase (Solis Biodyne), 20 pMol of each primer, and sterile water. The following cycling conditions were used for doing PCR in an Eppendorf Nexus heat cycler: a 5-minute initial denaturation step at 95°C, 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45s, and extension at 72°C for 1 min. A final extension was then performed for 10 minutes at 72°C. Following the PCR, a 1.5% agarose gel was used to separate the PCR products. Solis Biodyne's 150 base-pair (bp) DNA ladder was employed as a measure of DNA molecular weight. The gel was examined under UV light following ethidium bromide staining, and electrophoresis was carried out at 80V for one hour and thirty minutes.

Determination of Plant Hormones

Purchase of hormone standards Analytical standards of four plant hormones were purchased from commercial stores as stated. Santa Cruz Biotechnology, Inc. (CA, USA) supplied gibberellin A_4 (GA_4), Tokyo Kasei Kogyo Co. (Tokyo, Japan) provided abscisic acid (ABA), Wako Pure Chemical Industries Ltd. (Osaka, Japan) provided indole-3-acetic acid (IAA), and Santa Cruz Biotechnology, Inc. supplied *cis*-Zeatin (cZ). Tochu Co. (Aichi, Japan) provided the quartz sand.

Preparation of crop samples With a few minor adjustments, a previously described procedure has been used for the sample preparation and plant hormone extraction (Simura et al. 2018). In short, a TissueLyser II (Thermo Fisher Scientific) was used to individually homogenize the leaves, roots, and stems in liquid nitrogen. A total of one milliliter of cold 50% acetonitrile was used to extract fifty milligrams of the substance. After that, the extract was purified using an Oasis HLB cartridge (Waters) on a non-selective reversed-phase solid-phase extraction (RP-SPE). 100% methanol and ultrapure water were used to activate the column, and 50% acetonitrile was used to equilibrate it. After loading the sample onto the cartridge, the flow-through was recorded. One milliliter of 30% acetonitrile was then used to elute the target hormone residues. The flow-through and eluted fractions were combined, evaporated for three hours in a vacuum concentrator, and the dried residues were dissolved in 100 µl of 30% acetonitrile. Standard addition was used to quantify plant hormones (Cheng et al. 2017; Garcia-Rodrıguez et al. 2014). Standard solutions were fortified with samples at concentrations ranging from 1.0 ng/ml to 250.0 ng/ml. To create matrix calibration curves in the range of 1.0 ng/ml to 250.0 ng/ml to equalize matrix effects among samples, actual samples were diluted (dilution factor 1.05). The same matrix was used to create calibration curves for every phytohormone for every analyte. The peak areas of A and B were then used to calculate matrix effects (ME = A—B/A*100), where A was recognized as an analyte peak area in a standard solution and B as an analyte peak area in a matrix (Zhou, Yang and Wang 2017). The sample was analyzed in advance, and the peak area of an analyte derived from the sample was subtracted from B. By comparing the peak areas of each phytohormone found in the sample spiked before SPE and the sample spiked after SPE, the recovery rate was determined. The signal-to-noise ratios of 3:1 and 10:1 were used to define the limits of detection (LOD) and quantification (LOQ), respectively.

Measurement of plant hormones Ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (LC-MS/MS) was used to analyze plant hormones. Purified extracts were separated, in short, using a linear methanol gradient of 1–100% for 10 minutes at a flow

rate of 0.5 ml/min and a column oven set at 40˚C on a 2.6 µm Accucore C18 LC column (150 mm × 2.1 mm) (Thermo Fisher Scientific). Using an electrospray ionization Orbitrap Q-Exactive (Thermo Fisher Scientific) connected to an UltiMate 3000 RSLC (Thermo Fisher Scientific), MS data were obtained in targeted selected ion monitoring (t-SIM) mode.

Polarity, positive and negative ionization modes, spray voltages of 3.5 kV and 2.0 kV, sheath gas flow rate of 50, auxiliary gas of 10, sweep gas of 0, heated capillary temperature of 380˚C, S-lens RF level of 50, and auxiliary gas heater temperature of 350˚C were the conditions for mass spectrometry. Then, 70,000 was chosen as the resolution and 5E4 was the AGC objective. For all plant hormones, the isolation window was 10 m/z, and the maximal ion injection period was 200 ms.

Data Analysis

The software utilized was the Statistical Package for the Social Sciences (SPSS) Inc. 21.0.For physicochemical and microbiological parameters tracked over the study period, mean values (M) ± SD computed were then subjected to two-way analysis of variance (ANOVA) for multiple comparison to find out whether there are any notable difference between the different soil horizons and the physicochemical properties of the soil, as well as between the different production substrates of soil amendments and plant growth. A value (P) was deemed statistically significant if it was less than 0.05. That is ($P < 0.05$).

Results

Microbial count

Tables 1 and 2 showed the total heterotrophic bacterial (THB) count and total hydrocarbon utilizing bacterial (THUB) of soil bacteria in the forest soils. THB in the soil ranged from 31.0×10^8 – 100.66×10^9 cfu/g while THUB in the soil ranged from 32.00×10^8 – 35.20×10^9 cfu/g. The number of total phosphate solubilizing bacteria (PSB) and nitrogen fixing bacteria (NFB) suggest abundance with the highest population of NFB and PSB as showed in Tables 3 and 4. THBC, THUB, PSB and NFB significantly differed ($p < 0.05$) in most of the soils obtained from the three forests when compared to control.

Table 1
Total Heterotrophic Bacterial Count (cfu/g) (THB)

Soil depth	Control soil	Etche forest	Ogbogoro forest	Choba forest
0-15cm	$75.00 \pm 1.30 \times 10^{9a}$	$69.33 \pm 3.50 \times 10^{8b}$	$100.66 \pm 2.07 \times 10^{9c}$	$68.00 \pm 8.88 \times 10^{8b}$
15-30cm	$51.00 \pm 4.00 \times 10^{8a}$	$60.00 \pm 7.50 \times 10^{8b}$	$81.00 \pm 1.37 \times 10^{9c}$	$63.00 \pm 8.14 \times 10^{8b}$
30-45cm	$31.00 \pm 2.08 \times 10^{8a}$	$43.30 \pm 3.50 \times 10^{8b}$	$39.33 \pm 7.09 \times 10^{8c}$	$39.66 \pm 3.51 \times 10^{8c}$

Values are mean ± standard deviation (M ± S.D) of triplicate determinations (n = 3).

Values bearing different superscript alphabets “a, b, c” across the rows differ significantly ($p < 0.05$).

Table 2
Total Hydrocarbon Utilizing Bacterial Count (cfu/g) (THUB)

Soil depth	Control soil	Etche forest	Ogbogoro forest	Choba forest
0-15cm	$35.20 \pm 6.08 \times 10^{9a}$	$32.33 \pm 1.52 \times 10^{8b}$	$55.33 \pm 5.50 \times 10^{8c}$	$43.66 \pm 3.21 \times 10^{8d}$
15-30cm	$44.00 \pm 9.53 \times 10^{8a}$	$35.66 \pm 1.15 \times 10^{8b}$	$44.33 \pm 5.13 \times 10^{8a}$	$42.33 \pm 2.50 \times 10^{8c}$
30-45cm	$32.00 \pm 1.00 \times 10^{8a}$	$33.30 \pm 2.64 \times 10^{8a}$	$34.00 \pm 3.60 \times 10^{8b}$	$34.66 \pm 4.50 \times 10^{8b}$

Values are mean ± standard deviation (M ± S.D) of triplicate determinations (n = 3)

Values bearing different superscript alphabets “a, b, c, d” across the rows differ significantly ($p < 0.05$).

Table 3
Phosphate Solubilizing Bacterial Count (cfu/g) (PSB)

Soil depth	Control soil	Etche forest	Ogbogoro forest	Choba forest
0-15cm	$50.00 \pm 4.58 \times 10^{9a}$	$34.33 \pm 4.10 \times 10^{8b}$	$69.00 \pm 4.35 \times 10^{8c}$	$37.33 \pm 6.65 \times 10^{8b}$
15-30cm	$38.00 \pm 9.16 \times 10^{8a}$	$34.33 \pm 4.93 \times 10^{8b}$	$38.00 \pm 11.00 \times 10^{8a}$	$34.66 \pm 7.23 \times 10^{8b}$
30-45cm	$32.33 \pm 2.08 \times 10^{8a}$	$33.30 \pm 3.21 \times 10^{8a}$	$36.00 \pm 2.51 \times 10^{8b}$	$32.00 \pm 3.40 \times 10^{8a}$

Values are mean ± standard deviation (M ± S.D) of triplicate determinations (n = 3)

Values bearing different superscript alphabets “a, b, c” across the rows differ significantly ($p < 0.05$).

Table 4
Nitrogen fixing Bacterial Count (cfu/g) (NFB)

Soil depth	Control soil	Etche forest	Ogbogoro forest	Choba forest
0-15cm	$32.00 \pm 3.50 \times 10^{9a}$	$27.10 \pm 2.03 \times 10^{8b}$	$30.00 \pm 5.20 \times 10^{8a}$	$40.00 \pm 1.00 \times 10^{8c}$
15-30cm	$30.00 \pm 2.50 \times 10^{8a}$	$30.10 \pm 4.93 \times 10^{8a}$	$27.00 \pm 3.50 \times 10^{8b}$	$35.00 \pm 5.20 \times 10^{8c}$
30-45cm	$28.00 \pm 2.20 \times 10^{8a}$	$23.30 \pm 3.21 \times 10^{8b}$	$26.00 \pm 4.50 \times 10^{8a}$	$31.00 \pm 3.30 \times 10^{8c}$

Values are mean ± standard deviation (M ± S.D) of triplicate determinations (n = 3)

Values bearing different superscript alphabets “a, b” across the rows differ significantly ($p < 0.05$)

Physicochemical Properties

The physicochemical analysis of the various soil samples and soil treatments (after pooling them together and after adding soil amendments) are shown in Tables 5 and 6 respectively. In Table 5: pH of the various forest soils ranged from 5.20–7.23. For the rest of the physicochemical parameters, electrical conductivity which ranged from 104.65-121.52 µS/cm was the highest while mercury 0.01 mg/kg in all the soil samples was the least.

In Table 6 which showed the physicochemical characteristics of the soils in the proportion mixture of 1:1:1 with various substrates (MS = Mixed soil, MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK = Nitrogen, Phosphorus, Potassium Fertilizer) before and after adding the treatment: pH of all soils treatment were alkaline except soil treated with NPK fertilizer (5.80 ± 0.11); there was a significant difference ($p < 0.05$) in all the physicochemical parameters of soil treatments using various amendments when compared with mixed soil.

Table 5
Physicochemical characteristics of the various forest soils

Parameter	Control soil	Etche forest	Ogbogoro forest	Choba forest
pH	7.23 ± 0.05^{a}	5.85 ± 0.02^{b}	6.58 ± 0.07^{a}	5.20 ± 0.11^{b}
E/C (µs/cm)	117.46 ± 0.05^{a}	104.65 ± 0.02^{b}	108.38 ± 0.06^{b}	121.52 ± 0.15^{c}
Nitrate (mg/kg)	2.70 ± 0.17^{a}	3.79 ± 0.58^{b}	3.13 ± 0.03^{a}	3.64 ± 0.26^{b}
Potassium (mg/kg)	0.38 ± 0.05^{a}	0.74 ± 0.02^{a}	0.84 ± 0.05^{a}	1.68 ± 0.08^{b}
Total organic carbon (%)	2.78 ± 0.11^{a}	3.87 ± 0.02^{b}	2.63 ± 0.16^{a}	3.80 ± 0.01^{b}
Total organic matter (%)	3.62 ± 0.08^{a}	4.76 ± 0.06^{b}	5.45 ± 0.23^{c}	5.38 ± 0.08^{c}
Phosphate Po_4^{-3}(mg/kg)	0.38 ± 0.05^{a}	1.24 ± 0.02^{b}	1.84 ± 0.05^{b}	1.68 ± 0.08^{b}
Magnesium (mg/kg)	1.23 ± 0.50^{a}	1.90 ± 1.00^{b}	1.40 ± 0.50^{a}	$1.55 \pm .0.50^{a}$
Calcium(mg/kg)	0.60 ± 0.01^{a}	0.20 ± 0.01^{a}	0.20 ± 0.00^{a}	0.20 ± 0.00^{a}
Sodium(mg/kg)	0.40 ± 0.01^{a}	0.30 ± 0.01^{a}	0.10 ± 0.00^{a}	0.20 ± 0.00^{a}
Zinc (mg/kg)	2.54 ± 0.31^{a}	2.63 ± 0.06^{b}	1.48 ± 0.05^{b}	1.93 ± 0.04^{a}
Manganese (mg/kg)	0.30 ± 0.01^{a}	0.60 ± 0.01^{b}	0.50 ± 0.00^{b}	0.20 ± 0.00^{a}
Iron (mg/kg)	1.45 ± 0.12^{a}	2.89 ± 0.06^{b}	2.67 ± 0.78^{b}	2.23 ± 0.05^{b}
Mercury (mg/kg)	0.01 ± 0.00^{a}	0.10 ± 0.00^{b}	0.01 ± 0.01^{a}	0.01 ± 0.00^{a}
Lead (mg/g)	0.50 ± 0.21^{a}	0.55 ± 0.19^{b}	0.45 ± 0.30^{b}	0.70 ± 0.01^{b}
Copper (mg/kg)	0.20 ± 0.05^{a}	0.10 ± 0.06^{b}	0.45 ± 0.50^{b}	0.15 ± 0.49^{a}

Values are mean ± standard deviation (M ± S.D) of triplicate determinations (n = 3).

Values bearing different superscript letters “a, b, c” are significantly different ($p < 0.05$) when compared to control.

Table 6
Physicochemical properties of soil before and after adding the various treatments

Before treatment	After adding substrates				
Parameters MS		**MI**	**WHC**	**MI + WHC**	**NPK**
pH	6.50 ± 0.02^{a}	8.10 ± 0.02^{b}	9.60 ± 0.03^{b}	9.90 ± 0.03^{b}	5.00 ± 0.03^{a}
E/C (µs/cm)	112.00 ± 0.10^{a}	115.00 ± 0.10^{a}	113.0 ± 0.06^{a}	114.00 ± 0.03^{a}	123.00 ± 0.03^{a}
Nitrate (mg/kg)	7.00 ± 0.01^{a}	25.0 ± 0.03^{b}	28.0 ± 0.03^{b}	37.0 ± 0.03^{b}	22.0 ± 0.03^{b}
Potassium (mg/kg)	6.10 ± 0.03^{a}	20.0 ± 0.02^{b}	18.10 ± 0.00^{b}	24.20 ± 0.05^{b}	19.90 ± 0.05^{b}
Total organic carbon (%)	3.50 ± 0.01^{a}	4.00 ± 0.01^{a}	6.90 ± 0.00^{b}	9.00 ± 0.04^{c}	6.30 ± 0.02^{a}
Total organic matter (%)	3.68 ± 0.13^{a}	6.84 ± 0.23^{b}	7.15 ± 0.14^{b}	9.94 ± 0.26^{c}	5.15 ± 0.02^{d}
Phosphate Po_4^{-3}(mg/kg)	6.20 ± 0.01^{a}	13.20 ± 0.02^{b}	16.00 ± 0.01^{b}	20.20 ± 0.03^{b}	14.50 ± 0.02^{b}
Magnesium (mg/kg)	2.10 ± 0.03^{a}	2.90 ± 0.02^{a}	1.00 ± 0.01^{a}	2.30 ± 0.03^{a}	3.00 ± 0.02^{a}
Calcium(mg/kg)	1.20 ± 0.03^{a}	8.70 ± 0.01^{b}	10.10 ± 0.01^{b}	27.30 ± 0.03^{b}	8.60 ± 0.02^{b}
Sodium(mg/kg)	3.50 ± 0.02^{a}	3.50 ± 0.02^{a}	1.10 ± 0.01^{b}	1.10 ± 0.03^{b}	1.60 ± 0.02^{b}
Zinc (mg/kg)	2.10 ± 0.02^{a}	3.50 ± 0.02^{a}	3.10 ± 0.02^{a}	6.50 ± 0.02^{b}	2.50 ± 0.02^{a}
Manganese (mg/kg)	1.50 ± 0.02^{a}	3.00 ± 0.02^{b}	2.90 ± 0.02^{b}	5.50 ± 0.02^{b}	3.50 ± 0.02^{b}
Iron (mg/kg)	0.50 ± 0.02^{a}	1.10 ± 0.00^{b}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.02^{a}
Mercury (mg/kg)	0.10 ± 0.02^{a}	0.10 ± 0.00^{a}	0.20 ± 0.02^{a}	0.30 ± 0.01^{a}	0.10 ± 0.03^{a}
Lead (mg/g)	0.10 ± 0.02^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.10 ± 0.05^{a}	0.10 ± 0.01^{a}
Copper (mg/kg)	0.00 ± 0.00^{a}	0.10 ± 0.02^{b}	0.00 ± 0.00^{a}	0.10 ± 0.20^{b}	0.10 ± 0.02^{b}

Keys: MS = Mixed soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK = Nitrogen, Phosphorus, Potassium Fertilizer. Values bearing different superscript letters “a, b, c, d” are significantly different ($p < 0.05$) when compared to mixed soils.

Eefect of Biofertilizers on Crop Growth Parameters

Table 7
Shoot length in (cm) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	5.20 ± 0.05^{a}	5.40 ± 0.07^{a}	7.90 ± 0.04^{b}	15.90 ± 0.09^{c}	6.40 ± 0.02^{d}
Abelmoschus esculentus	2.90 ± 0.06^{a}	4.60 ± 0.03^{b}	6.20 ± 0.07^{c}	11.20 ± 0.12^{d}	4.90 ± 0.06^{b}
Cucurbita pepo	3.90 ± 0.05^{a}	4.90 ± 0.04^{b}	6.70 ± 0.00^{c}	13.40 ± 0.03^{d}	6.84 ± 0.02^{c}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c, d” across the rows differ significantly ($p < 0.05$).

Table 8
Leave area in (cm^2) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	1122.00 ± 0.00^{a}	1233.00 ± 0.24^{b}	1222.00 ± 0.00^{b}	1343 ± 0.03^{c}	1314 ± 0.16^{d}
Abelmoschus esculentus	1120.00 ± 0.20^{a}	1240.00 ± 0.22^{b}	1212.00 ± 1.20^{c}	1338 ± 0.36^{d}	1311 ± 2.47^{d}
Cucurbita pepo	1152.00 ± 0.42^{a}	1234.00 ± 0.43^{b}	1312.00 ± 3.16^{c}	1385 ± 2.05^{d}	1345 ± 3.68^{e}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c, d, e” across the rows differ significantly ($p < 0.05$).

Table 9
Total chlorophyll content in ($mg.100g^{-1}$) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	30.36 ± 2.23^{a}	31.43 ± 1.55^{a}	33.40 ± 1.94^{a}	36.67 ± 1.41^{b}	33.23 ± 2.04^{a}
Abelmoschus esculentus	31.34 ± 2.40^{a}	30.34 ± 1.48^{a}	31.27 ± 1.06^{a}	37.45 ± 1.86^{b}	32.24 ± 2.14^{a}
Cucurbita pepo	30.20 ± 2.12^{a}	32.30 ± 1.83^{b}	32.10 ± 0.76^{a}	35.15 ± 1.35^{b}	34.12 ± 2.06^{b}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b” across the rows differ significantly ($p < 0.05$).

Table 10
Germination rate in (%) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	50.00 ± 0.00^{a}	60.00 ± 5.00^{b}	80.00 ± 4.50^{c}	100.00 ± 2.50^{d}	60.00 ± 3.50^{b}
Abelmoschus esculentus	60.00 ± 0.00^{a}	50.00 ± 4.00^{b}	70.00 ± 2.50^{c}	90.00 ± 3.00^{d}	60.00 ± 4.00^{a}
Cucurbita pepo	40.00 ± 0.00^{a}	50.00 ± 0.00^{b}	50.00 ± 4.00^{b}	95.00 ± 2.00^{c}	50.00 ± 2.50^{b}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c, d” across the rows differ significantly ($p < 0.05$).

Table 11
Height in (cm) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	48.00 ± 0.00^{a}	50.50 ± 0.45^{a}	58.40 ± 0.40^{b}	65.00 ± 0.00^{c}	56.00 ± 0.60^{b}
Abelmoschus esculentus	47.80 ± 1.50^{a}	65.50 ± 1.00^{b}	68.60 ± 1.80^{b}	75.00 ± 0.00^{c}	51.00 ± 0.46^{a}
Cucurbita pepo	40.80 ± 0.40^{a}	49.40 ± 0.50^{b}	50.10 ± 1.10^{b}	54.60 ± 1.40^{c}	51.40 ± 0.80^{b}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c” across the rows differ significantly ($p < 0.05$).

Table 12
Yield in ($t\ ha^{-1}$) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	550.00 ± 45.40^{a}	650.00 ± 75.60^{b}	825.00 ± 50.70^{c}	1100.00 ± 40.40^{d}	670.00 ± 60.50^{b}
Abelmoschus esculentus	660.40 ± 90.00^{a}	720.00 ± 80.40^{b}	880.00 ± 70.80^{c}	1080.00 ± 80.60^{d}	730.00 ± 70.90^{b}
Cucurbita pepo	520.60 ± 85.50^{a}	610.00 ± 60.90^{b}	625.00 ± 85.00^{b}	980.00 ± 70.90^{c}	615.20 ± 65.60^{b}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c, d” across the rows differ significantly ($p < 0.05$).

Effects of Biofertilizer on Nutrient Levels

Table 13
Nitrogen level in (mg/kg) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	3.50 ± 0.60^{a}	14.80 ± 2.30^{b}	16.30 ± 2.91^{b}	18.20 ± 2.50^{c}	13.20 ± 1.72^{b}
Abelmoschus esculentus	3.00 ± 0.70^{a}	9.00 ± 1.60^{b}	8.80 ± 0.80^{b}	13.20 ± 2.07^{c}	9.10 ± 1.60^{b}
Cucurbita pepo	3.10 ± 0.45^{a}	9.00 ± 1.80^{b}	8.70 ± 0.66^{b}	13.80 ± 2.04^{c}	8.40 ± 0.45^{b}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c” across the rows differ significantly ($p < 0.05$).

Table 14
Phosphorus level in (mg/kg) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	3.00 ± 0.72^{a}	8.20 ± 0.95^{b}	9.10 ± 1.20^{b}	15.40 ± 2.42^{c}	8.10 ± 0.81^{b}
Abelmoschus esculentus	3.00 ± 0.80^{a}	8.20 ± 1.04^{b}	10.30 ± 0.63^{c}	11.40 ± 1.17^{c}	7.60 ± 0.80^{b}
Cucurbita pepo	3.00 ± 0.26^{a}	8.20 ± 1.45^{b}	9.00 ± 1.02^{b}	14.50 ± 1.74^{c}	9.15 ± 0.34^{b}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c” across the rows differ significantly ($p < 0.05$).

Table 15
Potassium level in (mg/kg) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	3.60 ± 0.88^{a}	9.70 ± 1.25^{b}	10.30 ± 2.40^{b}	12.40 ± 2.60^{c}	9.30 ± 1.42^{b}
Abelmoschus esculentus	3.30 ± 0.75^{a}	9.50 ± 1.50^{b}	8.40 ± 1.43^{b}	16.30 ± 1.37^{c}	9.35 ± 1.30^{b}
Cucurbita pepo	3.20 ± 0.58^{a}	9.85 ± 1.67^{b}	8.90 ± 1.52^{b}	13.30 ± 1.45^{c}	9.50 ± 0.98^{b}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c” across the rows differ significantly ($p < 0.05$).

Table 16
Zinc level in (mg/kg) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	1.20 ± 0.24^{a}	2.20 ± 0.85^{b}	1.90 ± 0.60^{b}	3.50 ± 0.92^{c}	1.50 ± 0.31^{a}
Abelmoschus esculentus	1.00 ± 0.62^{a}	2.00 ± 0.44^{b}	1.00 ± 0.43^{a}	3.30 ± 0.27^{c}	1.20 ± 0.40^{a}
Cucurbita pepo	1.00 ± 0.20^{a}	2.10 ± 0.52^{b}	1.30 ± 0.35^{c}	3.00 ± 0.54^{d}	1.00 ± 0.24^{a}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c, d” across the rows differ significantly ($p < 0.05$).

Table 17
Manganese level in (mg/kg) of plants after 60 days planting

Plants	CS	MI	WHC	MI + WHC	NPK
Zea mays	0.90 ± 0.32^{a}	1.70 ± 0.35^{b}	1.30 ± 0.41^{c}	3.00 ± 0.62^{d}	1.68 ± 0.54^{b}
Abelmoschus esculentus	1.20 ± 0.43^{a}	1.20 ± 0.24^{a}	1.20 ± 0.43^{a}	3.00 ± 0.47^{b}	1.21 ± 0.30^{a}
Cucurbita pepo	1.00 ± 0.37^{a}	1.10 ± 0.37^{a}	1.10 ± 0.22^{a}	2.80 ± 0.94^{b}	1.10 ± 0.12^{a}

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium. Values bearing different superscript alphabets “a, b, c, d” across the rows differ significantly ($p < 0.05$).

Molecular Characteristics of Isolates

Four bacteria strains were isolated from the Genbank characteristics as shown in Table 18 while Fig. 2 reveal the 16S rRNA PCR bands from the isolates (*see supplementary file for further explanation*).

Table 18
Genbank characteristics (strain, name of organism, closest GenBank, percentage identity and accession number) of the bacterial isolates

S/N	Strain	Organisms (isolated)	Closest GenBank Match	Similarity (%)	Accession No
1	PA4	*Bacillus cereus*	*Bacillus cereus* strain JCM 2152	99.64	OR105044
2	PA6	*Bacillus cereus*	*Bacillus cereus* strain IAM 12605	100	OR105045
3	PA7	*Bacillus fungorum*	*Bacillus fungorum* strain 17 SMS-01	99.64	OR105046
4	PA11	*Bacillus paramycoides*	*Bacillus paramycoides* strain MCC IA04098	99.64	OR105047

Please insert Fig. 2 here.

Impact of Biofertilizers on Plant Hormones

Table 19
Indole-3-acetic acid (IAA) in crop leaves

Treatments	*Zea mays*				*Abelmoschus esculentus*				*Cucurbita pepo*			
	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)
CS	0.1	0.6	95.15 ± 2.36^{a}	4.5	0.1	0.6	92.10 ± 4.15^{a}	7.3	0.1	0.6	81.20 ± 8.07^{a}	3.8
MI	0.4	1.8	208.50 ± 7.60^{b}	5.2	0.5	1.3	211.40 ± 5.20^{b}	3.1	0.3	1.3	158.80 ± 11.60^{b}	4.7
WHC	0.2	0.5	213.80 ± 6.50^{b}	25.6	0.3	0.4	207.30 ± 16.10^{b}	16.2	0.1	0.4	163.10 ± 9.30^{b}	12.5
MI + WHC	0.1	0.3	740.70 ± 15.20^{c}	28.7	0.2	0. 4	688.60 ± 23.40^{c}	38.6	0.1	0.5	590.40 ± 17.30^{c}	18.0
NPK	0.1	0.6	192.90 ± 12.42^{d}	7.4	0.2	0.5	209.70 ± 17.25^{b}	6.0	0.1	0.4	129.50 ± 8.13^{d}	7.2

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium, LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation, FW = fresh weight. Values bearing different superscript alphabets “a, b, c, d” down the columns differ significantly ($p < 0.05$).

Table 20
Gibberellin A_4 (GA_4) in crop leaves

Treatments	*Zea mays*				*Abelmoschus esculentus*				*Cucurbita pepo*			
	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)
CS	0.1	0.6	3.26 ± 0.25[a]	4.5	0.1	0.6	5.62 ± 0.83[a]	7.3	0.1	0.6	4.30 ± 0.61[a]	3.8
MI	0.4	1.8	3.42 ± 0.70[a]	5.2	0.5	1.3	5.60 ± 0.62[a]	3.1	0.3	1.3	4.34 ± 0.48[a]	4.7
WHC	0.2	0.5	N.D	25.6	0.3	0.4	5.71 ± 1.50[a]	16.2	0.1	0.4	4.47 ± 0.52[a]	12.5
MI + WHC	0.1	0.3	8.40 ± 0.93[b]	28.7	0.2	0. 4	8.94 ± 1.76[b]	38.6	0.1	0.5	9.60 ± 0.41[b]	18.0
NPK	0.1	0.6	3.30 ± 0.22[a]	7.4	0.2	0.5	6.92 ± 1.40[c]	6.0	0.1	0.4	4.31 ± 0.22[a]	7.2

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium, LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation, FW = fresh weight., N.D = not detected. Values bearing different superscript alphabets “a, b, c” down the columns differ significantly ($p < 0.05$).

Table 21
Abscisic acid (ABA) in crop leaves

Treatments	*Zea mays*				*Abelmoschus esculentus*				*Cucurbita pepo*			
	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)
CS	0.1	0.6	182.50 ± 4.11[a]	4.5	0.1	0.6	177.40 ± 3.20[a]	7.3	0.1	0.6	168.60 ± 5.07[a]	3.8
MI	0.4	1.8	153.60 ± 5.60[b]	5.2	0.5	1.3	146.94 ± 5.20[b]	3.1	0.3	1.3	148.20 ± 8.60[b]	4.7
WHC	0.2	0.5	147.70 ± 6.50[b]	25.6	0.3	0.4	150.06 ± 6.20[b]	16.2	0.1	0.4	151.30 ± 7.30[b]	12.5
MI + WHC	0.1	0.3	139.90 ± 15.20[c]	28.7	0.2	0. 4	132.42 ± 5.10[c]	38.6	0.1	0.5	147.20 ± 6.30[b]	18.0
NPK	0.1	0.6	216.80 ± 12.42[d]	7.4	0.2	0.5	210.10 ± 5.15[d]	6.0	0.1	0.4	199.40 ± 7.23[c]	7.2

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium, LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation, FW = fresh weight. Values bearing different superscript alphabets “a, b, c, d” down the columns differ significantly ($p < 0.05$).

Table 22
cis-Zeatin (cZ) in crop leaves

Treatments	*Zea mays*				*Abelmoschus esculentus*				*Cucurbita pepo*			
	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)
CS	0.1	0.6	79.45 ± 2.30[a]	4.5	0.1	0.6	59.22 ± 2.20[a]	7.3	0.1	0.6	63.61 ± 3.15[a]	3.8
MI	0.4	1.8	81.43 ± 4.10[a]	5.2	0.5	1.3	59.40 ± 4.33[a]	3.1	0.3	1.3	62.80 ± 1.98[a]	4.7
WHC	0.2	0.5	84.50 ± 2.90[a]	25.6	0.3	0.4	59.10 ± 5.34[a]	16.2	0.1	0.4	64.52 ± 4.20[a]	12.5
MI + WHC	0.1	0.3	87.73 ± 6.20[a]	28.7	0.2	0. 4	60.54 ± 3.25[a]	38.6	0.1	0.5	63.70 ± 6.40[a]	18.0
NPK	0.1	0.6	85.60 ± 4.55[a]	7.4	0.2	0.5	58.90 ± 2.88[a]	6.0	0.1	0.4	64.22 ± 2.23[a]	7.2

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium, LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation, FW = fresh weight. There was no significant difference ($p < 0.05$) in the comparisons made.

Table 23
Indole-3-acetic acid (IAA) in crop shoot

Treatments	*Zea mays*				*Abelmoschus esculentus*				*Cucurbita pepo*			
	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)
CS	0.1	0.6	98.20 ± 4.53[a]	4.5	0.1	0.6	96.30 ± 8.20[a]	7.3	0.1	0.6	86.44 ± 7.17[a]	3.8
MI	0.4	1.8	212.30 ± 9.54[b]	5.2	0.5	1.3	217.22 ± 9.35[b]	3.1	0.3	1.3	152.40 ± 8.30[b]	4.7
WHC	0.2	0.5	214.44 ± 10.50[b]	25.6	0.3	0.4	207.60 ± 12.40[b]	16.2	0.1	0.4	156.70 ± 12.42[b]	12.5
MI + WHC	0.1	0.3	690.30 ± 8.95[c]	28.7	0.2	0. 4	689.70 ± 9.66[c]	38.6	0.1	0.5	598.78 ± 9.40[c]	18.0
NPK	0.1	0.6	187.40 ± 6.92[d]	7.4	0.2	0.5	211.80 ± 10.30[b]	6.0	0.1	0.4	131.65 ± 7.20[d]	7.2

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium, LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation, FW = fresh weight. Values bearing different superscript alphabets “a, b, c, d” down the columns differ significantly ($p < 0.05$).

Table 24
Gibberellin A_4 (GA_4) in crop shoot

Treatments	*Zea mays*				*Abelmoschus esculentus*				*Cucurbita pepo*			
	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)
CS	0.1	0.6	5.17 ± 0.52[a]	4.5	0.1	0.6	7.24 ± 1.27[a]	7.3	0.1	0.6	6.82 ± 0.73[a]	3.8
MI	0.4	1.8	5.34 ± 0.68[a]	5.2	0.5	1.3	7.72 ± 0.97[a]	3.1	0.3	1.3	6.88 ± 0.57[a]	4.7
WHC	0.2	0.5	2.64 ± 0.83[b]	25.6	0.3	0.4	7.80 ± 2.21[a]	16.2	0.1	0.4	6.91 ± 0.61[a]	12.5
MI + WHC	0.1	0.3	11.32 ± 0.72[c]	28.7	0.2	0. 4	12.10 ± 2.15[b]	38.6	0.1	0.5	12.77 ± 0.54[b]	18.0
NPK	0.1	0.6	5.20 ± 0.43[a]	7.4	0.2	0.5	7.81 ± 2.05[a]	6.0	0.1	0.4	6.83 ± 0.34[a]	7.2

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium, LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation, FW = fresh weight. N.D = not detected. Values bearing different superscript alphabets “a, b, c” down the columns differ significantly ($p < 0.05$).

Table 25
Abscisic acid (ABA) in crop shoot

Treatments	*Zea mays*				*Abelmoschus esculentus*				*Cucurbita pepo*			
	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)
CS	0.1	0.6	154.20 ± 6.27[a]	4.5	0.1	0.6	147.20 ± 3.61[a]	7.3	0.1	0.6	153.56 ± 3.15[a]	3.8
MI	0.4	1.8	138.40 ± 8.23[b]	5.2	0.5	1.3	126.30 ± 3.42[b]	3.1	0.3	1.3	139.42 ± 9.20[b]	4.7
WHC	0.2	0.5	139.62 ± 7.20[b]	25.6	0.3	0.4	128.40 ± 4.30[b]	16.2	0.1	0.4	137.34 ± 8.20[b]	12.5
MI + WHC	0.1	0.3	122.32 ± 7.50[c]	28.7	0.2	0. 4	123.70 ± 4.32[b]	38.6	0.1	0.5	126.85 ± 4.54[c]	18.0
NPK	0.1	0.6	184.62 ± 8.20[d]	7.4	0.2	0.5	191.30 ± 4.20[c]	6.0	0.1	0.4	176.82 ± 5.08[d]	7.2

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium, LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation, FW = fresh weight. Values bearing different superscript alphabets “a, b, c, d” down the columns differ significantly ($p < 0.05$).

Table 26
cis-Zeatin (cZ) in crop shoot

Treatments	*Zea mays*				*Abelmoschus esculentus*				*Cucurbita pepo*			
	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	Hormone conc (pmol/g FW)	RSD (%)
CS	0.1	0.6	85.60 ± 3.41[a]	4.5	0.1	0.6	64.50 ± 8.16[a]	7.3	0.1	0.6	65.70 ± 2.70[a]	3.8
MI	0.4	1.8	87.20 ± 2.63[a]	5.2	0.5	1.3	65.92 ± 7.21[a]	3.1	0.3	1.3	67.92 ± 3.50[a]	4.7
WHC	0.2	0.5	86.90 ± 3.25[a]	25.6	0.3	0.4	65.80 ± 8.22[a]	16.2	0.1	0.4	67.97 ± 2.32[a]	12.5
MI + WHC	0.1	0.3	88.12 ± 4.15[a]	28.7	0.2	0. 4	67.64 ± 7.12[a]	38.6	0.1	0.5	68.25 ± 3.25[a]	18.0
NPK	0.1	0.6	86.30 ± 3.36[a]	7.4	0.2	0.5	65.45 ± 3.60[a]	6.0	0.1	0.4	68.10 ± 4.10[a]	7.2

Keys: CS = control soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, NPK fertilizer = Nitrogen, Phosphorus, Potassium, LOD = limit of detection, LOQ = limit of quantification, RSD = relative standard deviation, FW = fresh weight. There was no significant difference ($p < 0.05$) in the comparisons made.

Discussion

Microbial analysis of samples

Soil recorded highest THBC within the range of 31.0 x 10^8 -100.66 x 10^9cfu/. The outcome demonstrates that the native bacterial community can flourish and proliferate in the environment. Ibiene et al. (2011) and Eze and Okpokwasili (2011) both made a similar finding. The THBC and HUB in the present study are higher than values reported by Douglas (2018), where mean THBC ranged from 35.0 x 10^8 to 99.3 x 10^8CFU/g. However, HUB which ranged from 4.2 x 10^8 to 6.4 x 10^8CFU/g was similar in ranged to values reported in the present study. the values for NFB and PSB for Control soil, Etche, Ogbogoro and Choba soils respectively is in the ranged of values obtained by Widawati and Suliasih (2001) in their study of population of nitrogen fixing bacteria and phosphate solubilising bacteria in the rhizosphere.

These organisms' capacity to repair atmospheric nitrogen is suggested by its development on nitrogen-free substrates. The species of bacteria found in this study using 16S RNA gene sequencing are members of the phosphate-solubilizing and nitrogen-fixing bacterial groups. This data supports the argument made by Ikuesan and Fajolu (2022) that bacteria can be categorized as both nitrogen-fixing and phosphate-solubilizing due to the elevated levels of the enzymes nitrogenase and ACC deaminase, which lower ethylene production in plants.

Physicochemical composition of the soil

The physicochemical parameters of a wetland soil as shown in Tables 5 indicates that a tropical moist forest soils have a low value of Electrical Conductivity (EC); control soil 117.46 ± 0.05µS/cm, Etche: 104.65 ± 0.02 µS/cm, Ogbogoro: 108.38 ± 0.06 µS/cm and Choba 121.52 ± 0.15 respectively. The amount of accessible nutrients in the soil is measured by electrical conductivity. The ability of the soil water to transport an electrical charge is shown by the soil EC value. The soil's EC level is a reliable gauge of how much nutrition is available for crop uptake. An ideal EC level is greater than 100µS/cm. Any soil with EC level below 100 indicates that the plant is not getting adequate nutrients, and it may also indicate a sterile soil with limited microbial activity (Jennifer 2016). The forest soil used in this experiment fall within a good range of EC value which means the soil is highly fertile. The pH of the control soil was 7.23 ± 0.05, Etche was 5.85 ± 0.02; Ogbogoro was 6.58 ± 0.07; Choba was 5.80 ± 0.11 respectively. The soil pH slightly varies from 5.60–7.23. Control soil has a moderate neutral range while Ogbogoro soil has a slightly neutral range, Choba and Etche forest have a moderate acidic soil, and thus the pH shows acidic in nature. As a result, the soil is neither too alkaline nor acidic, but rather has an average degree of alkalinity that promotes microbial development. This pH result differs from that of Horsfall et al. (2015), who concluded that forest soils are extremely productive but defined them as having a mean pH range of 4.5, which is acidic. All soil treated samples had alkaline pH which supports microbial activities except soil treated with NPK (Table 6). This finding is in line with the report of Zhang et al. (2023) who reported improvement of acidity to alkalinity in biofertilizer treated soils used in cultivating spinach. According to Zhang et al. (2023), acidic soil has adverse effect on planted crops as it suppresses growth and increase production of reactive oxygen species which negatively affect plant metabolism.

Additionally, the soils' organic carbon values range from 2.63% to 3.87% (0–15% and above) (Table 5), suggesting that their organic content is naturally fruitful and this finding is comparable to Horsfall et al. (2015), whose results range from 2.40% to 3.0%. Generally, soil treated with combination of Microbial Inoculant and Water Hyacinth Compost had highest pH, nitrate, potassium, phosphate, calcium, zinc and manganese levels when compared to other treatments (Table 6). The electrical conductivity, organic matter, total organic carbon, nitrogen, phosphorus, zinc and manganese concentrations were significantly improved in soils treated with biofertilizers than soils without biofertilizer (Table 6). The results on the physicochemical analysis of soil samples agree with other studies (Anli et al. 2020; Bertrand et al. 2024) who all reported that soil prior to biofertilization were acidic and had poor mineral nutrients which does not support growth and performance of date palm and okra cultivars respectively.

The effect of biofertilizer on growth parameters

The effects of 0.1 g substrate of various biofertilizer treatments (Mixed soil MI = Microbial Inoculant, WHC = Water Hyacinth Compost, MI + WHC = Microbial Inoculant + Water Hyacinth Compost, and NPK = Nitrogen, Phosphorus, Potassium Fertilizer) on growth parameters of *Cucurbita pepo*, *Zea mays* and *Abelmoscus esculentus* are shown in Tables 7–12. Results showed significant increase in the shoot length, leaves areas, total chlorophyll contents, germination rate, height and yield in these three crops planted in biofertilizer treated soils than control. This implies that the biofertilizer treatments deployed in this study enhanced growth parameters positively. Generally, biofertilizer treatment with combination of microbial inoculum and water hyacinth compost gave the best results on the growth parameters followed by treatment with water hyacinth alone while treatment with microbial inoculum gave the least effect on growth parameters of the three planted crops. The efficiency displayed by the combined treatment of microbial inoculant and water hyacinth compost could be linked to high organic matter content in soil treated with it as well to availability and accessibility of soil nutrients such as nitrogen, phosphorus, potassium, and magnesium that are required for growth of vegetative parts (Djoufack et al. 2025). Even while chemical fertilizers offer easily accessible nutrients (Ano and Ekefan 2023), a sizable amount may be lost as a result of rainfall, which could cause leaching and lower the efficiency of nutrient uptake. Chemical fertilizers have the potential to impede soil compaction by decreasing soil porosity. Furthermore, although having a high nutritional content, inorganic fertilizers lack the growth-promoting substances found in water hyacinth compost and microbial inoculant, which are what increase fertility and production (Sanwal et al. 2007). The growth performance of these three crops increased steadily with increase in period of planting with best growth performance obtained after 60 days, .*Abelmoscus esculentus* had the best performance from the biofertilizer treatments which probably tells of its excellent response to soil nutrients (Djoufack et al. 2025).

This result agrees with the study of Al-Hadethi et al. (2017), Zhang et al. (2023) and Anli et al. (2020) who reported significant increase in the growth parameters (leaves area, chlorophyll contents, plant height, leaf length, shoot length and stem diameter) of Peento peach cultivar, spinach and date palm respectively cultivated with different soil biofertilizer treatments when compared with control. Both of them equally reported that combination of biofertilizer treatments produced better results on the growth parameters of Peen to peach cultivar and spinach plant. The result further aligns with the findings by Al-Hadethi (2015), Kumar et al. (2013), Ebrahimi et al. (2023) and Dawood et al. (2019) who reported significant increase in stem diameter, leaves areas, shoot length and germination rate of apricot trees, peartrees, *Zygophyllum eurypterum* and flax cultivars respectively cultivated in biofertilized soils. Chlorophyll is the green pigment that facilitates photosynthesis (Dawood et al. 2019). The significant increase in chlorophyll contents of the three crops treated with biofertilizer could be because of bio-regulator role which altars photo-respiratory and photosynthetic balance in plants (Olaiya 2010). More so, high chlorophyll contents contribute to delay in aging of leaves while supporting RNA and protein synthesis (Shalaby & El-Nady 2008).

Cucurbito pepo, Zea mays, and *Abelmoscus esculentus* have all shown a notable increase in height, which indicates that their responses vary based on the fertilization regime used. This could be attributed to a substantial and essential supply of nutrients, including nitrogen, which the plant can directly absorb for vegetative growth, and other nutrients (potassium, phosphorus, magnesium, etc.) that are involved in root development.

The type of crop and the quantity of fertilizer used are typically correlated with production yield. The study's findings demonstrated that the three crops' (*Cucurbito pepo, Zea mays*, and *Abelmoscus esculentus*) production yield varied, which may have been caused by physiological reactions brought on by variations in the genetic information in their genomes that ensured their growth and development by absorbing nutrients from the soil and responding favorably to climatic constraints. Its greater ability to adapt to climatic circumstances, its efficient photosynthesis, and its improved nutrient assimilation may be the reasons for the increased output yield of *Zea mays* and *Abelmoscus esculentus* treated with a mix of microbial inoculant and water hyacinth compost. In reality, *Zea mays* and *Abelmoscus esculentus* would have more readily absorbed the nutrients from the soil treatments than *Cucurbito pepo*, as nitrogen, phosphorus, potassium, and water are the limiting factors in achieving high yields (Agbede et al. 2017). Our findings are consistent with a study by Djoufack et al. (2025) that found that the use of organic fertilizers produced the highest production yield values.

According to Barker and Pilbeam (2007), the better performance of crop planted on biofertilizer treated soil could be attributed to cumulative impact of biofertilizers applied which in turn enhanced biochemical processes and photosynthesis in planted crops by enabling them to utilize plant nutrients like potassium, nitrogen and phosphorus through the help of enzymes. The second possible reason for the significant increase in growth parameters of these crops by biofertilized treated soils might be because of presence of micro and macro nutrients, proteins, vitamins, and

plant growth regulators that facilitate the building up of dried matter in plants (Mirabal-Alonso et al. 2008). Biofertilizers equally promote the release and availability of soil nutrients in treated soils thereby facilitating physiological processes and all the biochemical procedures (Yu et al. 2014).

Effect of biofertilizers on nutrient content and absorption by crops

The nutrient levels in soil samples after 60 days of planting period are shown in Table 13–17. Results showed that the concentrations of N, P, K, Zn and Mn were generally lower in soils planted these three crops after 60 days of planting period which implies that the crops must have utilized these nutrients in boosting their performance. These results are quite similar with earlier reports (Aedesh et al. 2024; Demir et al. 2023; Al-Hadethi et al. 2017; Sundari and Gandhi 2017; Kumar et al. 2017; Radhakrishnan and Lee 2016; Kumar et al. 2013; Dutt et al. 2013; Sharma et al. 2008) who all reported higher levels of nutrients in crops planted on biofertilized soils. The mechanism of action of biofertilizers include restoration of nutrient cycles in soil, fixation of nitrogen, solubilization of phosphorus and supporting the synthesis of plant growth promoting factors through the help of organisms present in them which leads to increase in nutrient absorption (Aadesh et al. 2024; Al-Hadethi et al. 2017). Also, nitrogen and phosphorus are made available for better utilization through the help of biofertilizers (Regar et al. 2018) with the help of bacteria like *Azotobacter, Bacillus, Pseudomonas and Rhizobium* organism which aid in dissolving phosphate. These organisms are capable of converting non-absorbable phosphorus into forms that can be absorbed (Tosic et al. 2016). According to Chen et al. (2006), these bacteria can breakdown up to 50% of non-absorbable phosphorus in the soil. *Bacillus* and *Pseudomonas* sp found in the control and test soils utilized in this study might have converted phosphorus into absorbable forms for easy uptake by crops thereby diminishing their concentrations in soils after period of planting.

More so, the metabolism of carbohydrates, the promotion of growth and early root development, and the quickening of plant maturity, fruiting, and development are all aspects of plant growth that depend on phosphorus. However, after being absorbed by the plant, nitrogen is needed for optimal growth because it is transformed into nitrate ions, which act as a substrate for assimilation and signaling molecules that partially control the pattern of growth and development by regulating the expression of different genes (Ali et al. 2003; Takei et al. 2002).

Molecular identification of functional gene

Agrorose electrophoresis showed the Amplified 16s rRNA gene. Lane 1 PA4 *Bacillus cereus* (OR105044), Lane 2: PA6 *Bacillus cereus* (OR105045), Lane 3 PA7 *Bacillus fungorum* (OR105046) and Lane 4 6A11 *Bacillus paramycoides (*OR105047) (Table 18) while ladder at the first lane represents the 1500 pb molecular ladder, identification of isolate showed bacterial isolates as *Bacillus cereus*, *Bacillus fungorum* and *Bacillus paramycoides* with accession number OR105044, OR105045, OR105046 and OR105047 respectively (Fig. 2). This agree with the findings of Amal and Heba (2023) and Agu et al. (2021) who screened and identified species as *Bacillus cereus* (OP970172), *Bacillus licheniformis* (OP970169), *Pseudomonas fluorescens* (OP970170) and *Azotobacter chroococcum* (OP970171) to be useful in enhancing plant growth through mineralization such as solubilization of phosphate and nitrogen fixation in biofertilizer production study.

Effect of biofertilizer on plant hormones

In the field of agricultural production, plant growth regulators have become widely used because they may effectively influence crop growth and development while improving crop output and quality (Jiang et al. 2020). The findings showed that IAA was evenly distributed in shoot and leaf of the three crops, GA_4 and cis-zeatin were found more in shoot than leaf while ABA were mostly higher in leaf than shoot of crops which suggest the presence of plant hormones capable of regulating activities within the tissues of these crops (Tables 19–26). Additionally, the study showed that the content of IAA and GA_4 in crop shoots and leaves was higher in soil treated with a combination of MI and WHC than in other treatments (Tables 19–26). Consistent with earlier research, it was clear that applying organic soil treatments increased the amounts of GA_4 and IAA while lowering ABA (Shi et al. 2022). However, as compared to other groups, the NPK-treated group had higher levels of ABA in both the crop's leaves and shoots (Tables 21 and 25). Djoufack et al. (2025) obtained similar results.

Cell division and elongation are markedly inhibited by ABA, a strong growth inhibitor. It may hinder the growth of leaves, embryos, embryo sheaths, stems, hypocotyls, and roots, among other plant elements. Additionally, ABA promotes stomatal closure and dormancy, which further suppresses plant growth (Chen et al. 2018). According to earlier studies, elevated IAA levels speed up organ abscission and promote the synthesis of ethylene (Mao et al. 2014). According to Zhu's research, growth hormone increases GA production, which in turn stimulates the creation of fiber (Zhu et al. 2022). Similar to the findings of Djoufack et al. (2025), soil treatments had no discernible impact on the amount of cis-ZT. This ensures cell viability and delays plant senescence when applied in combination (Jiang et al. 2020). Together, these findings imply that different plant growth regulator compounds are essential in regulating the growth and development of *Cucurbito pepo, Zea mays*, and *Abelmoscus esculentus* 60 days after planting by affecting GA4 and IAA levels, lowering ABA content, and preventing these crops from senescence.

Conclusion

This study have shown that the type of soil treatment had a significant impact on growth performance parameters, yield, nutrient levels and hormones of *Cucurbito pepo*, *Zea mays* and *Abelmoscus esculentus* after 60 days of planting with treatment administered in combination (Microbial Inoculant + Water Hyacinth Compost) producing the best performance. The study equally discovered species with biofertilizing

potentials. More so, as much as the addition of the soil with NPK chemical fertilizer increased leave area and germination rate in crops; it however increased the acidity of the soil and soil nutrients from NPK was lost rapidly than in other soil treatments which can be detrimental to the microbial activities and health.

We therefore recommend that in order to increased food security, create jobs, and strengthen the country's agro-economy, the Nigerian government and private investors should collaborate in the industrialization of biofertilizer manufacturing and application of biofertilizer since it is an efficient and feasible way of reducing chemical fertilizers rate, cost and increased growth benefit and more. Biofertilizer should be continuous in use to increase our production and fight hunger to enable the nation actualize United Nations Sustainable Development Goals (SDGs) 1 (No poverty), 2 (zero hunger) and 3 (Life on land).

Abbreviations

MI Microbial Inoculant

WHC Water Hyacinth Compost

ABA Abscisic acid

IAA indole-3-acetic acid

GA_4 gibberellin

cZ cis-Zeatin

PSB phosphate solubilizing bacteria

THB total heterotrophic bacteria count

THUB total hydrocarbon utilizing bacteria

NFB nitrogen fixing bacteria

NPK nitrogen, phosphorus, potassium

Declarations

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Author contributions

RNA, PIA and HOS developed the study’s concept and design. RNA and PIA participated in data collection. RNA, PIA and GOA performed data cleaning, analysis, and interpretation. RNA, PIA, GOA and IFV drafted the manuscript. All authors reviewed the manuscript and agreed to the final version of the manuscript.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author upon reasonable demand.

Ethics approval and consent to participate

Not applicable.

Conflict of interest

The authors declare no competing interests.

Informed consent statement

Not applicable.

Institutional review board statement

Not applicable.

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Figures

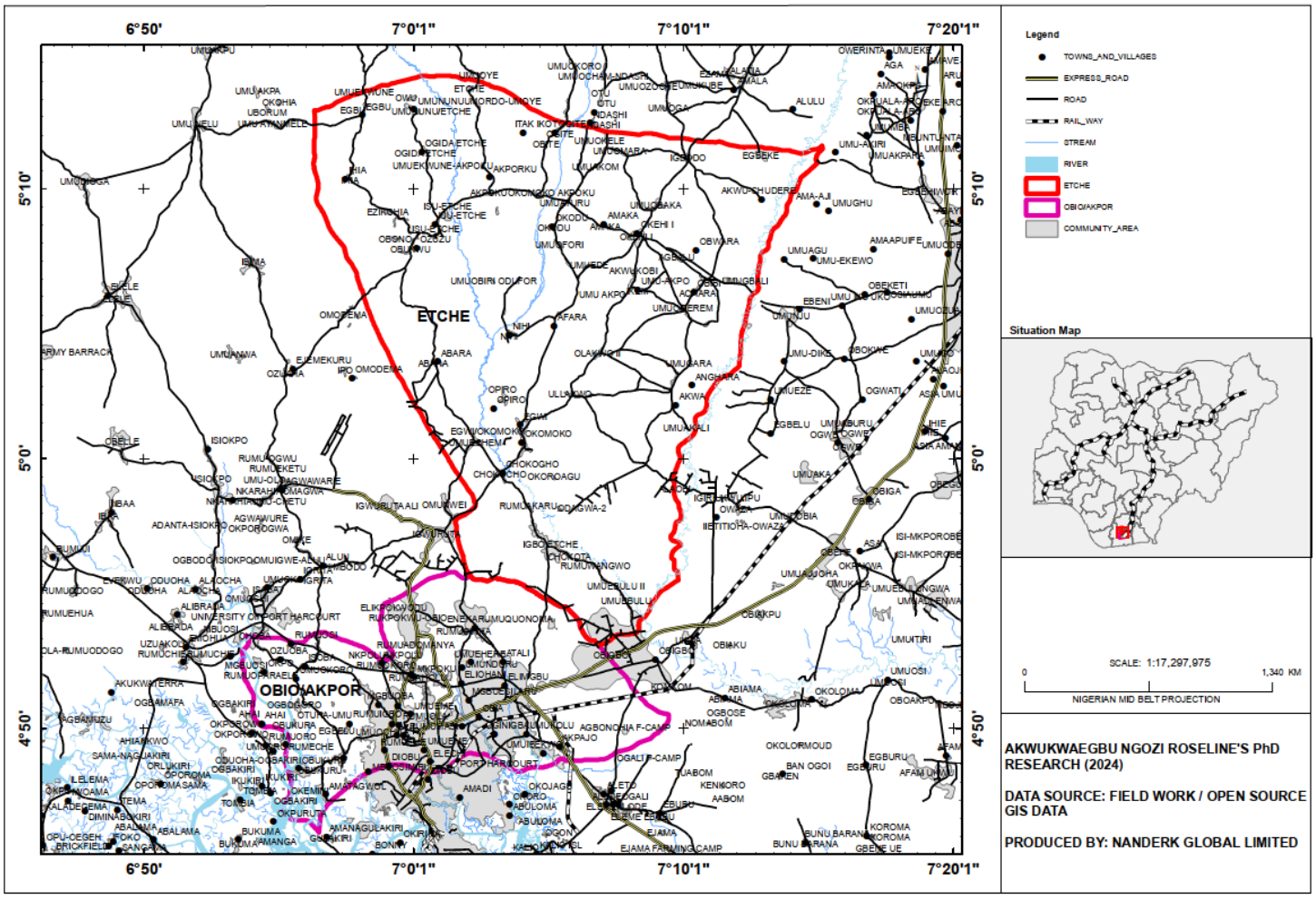


Figure 1

Sampled Study Locations in Etche and Choba LGA in Rivers state.

This is a composite map showing the geographical locations of soil sample collection points in two different local governments.

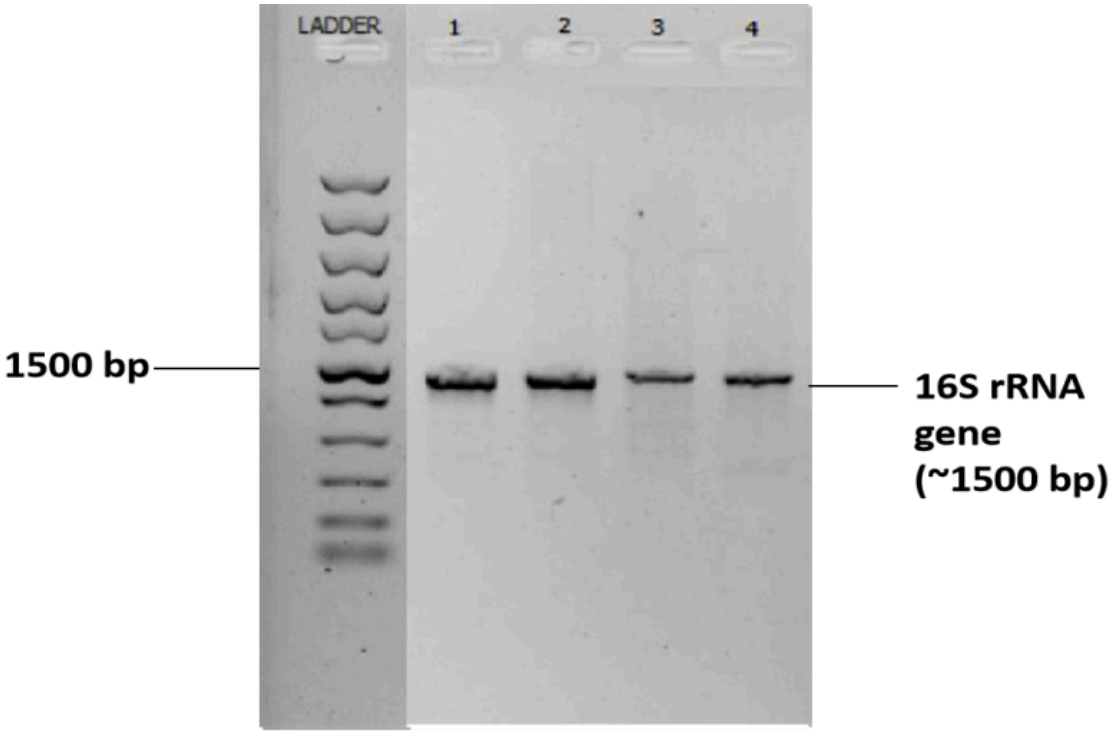


Figure 2

PCR amplification image of the 16S rRNA gene. (1: PA4; 2: PA6; 3: PA7; 4:PA11). The DNA 1kb molecular ladder is given in the first lane.

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