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Enhancing plant growth parameters with synergistic application of biofertilizing bacteria from forest soil and *Eichhornia crassipes* compost

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Abstract

Background The purpose of this study is to enhance the growth parameters of three plants (*Zea mays*, *Abelmoschus esculentus* and *Cucurbita pepo*) with synergistic application of biofertilizers produced with bacteria, forest soils and *Eichhornia crassipes* compost.

Materials and methods 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 $0.26 \pm 4.50 \times 10^{10}$ to $1.00 \pm 2.07 \times 10^{11}$ 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 a significant improvement in the growth of the plant species as indicated in their shoots, leaves area, total chlorophyll content, germination rate, height, yield and nutrient absorption rate 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 boost plant growth parameters, nutrient levels and hormones and that treatment with MI, WHC or combination of MI + WHC offered better efficiency than the use of NPK fertilizer.

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Keywords Biofertilizers, Forests, Growth performance, Plant hormones, Soil horizons, Water hyacinth

Introduction

All over the world, there is constant increase in demand for agricultural products as a result of rapid urbanization and population increase (Bertrand et al. 2024). According to statistics, the population of people in the world is anticipated to hit ten billion in the next 50 years (Bertrand et al. 2024; Daniel et al. 2022; Mahanty et al. 2017). In order to address food scarcity and other challenges caused by continuous rise in global population, farmers deployed innovative methods for soil preparation and planting to boost agricultural yield within the shortest time (Bertrand et al. 2024).

Chemical fertilizers and pesticides have been deployed by farmers to optimize yield and quality of agricultural products. Although their continuous reliance and misuse started raising public concerns in terms of their negative impact, safety and sustainability (Wang et al. 2023). Chemical fertilizers must be applied to crops on a regular basis to replace the nutrients lost during crop growth as their continuous application degrades agricultural activities (Ammar et al. 2023). According to Gyaneshwar et al. (2002), additional application of inorganic fertilizers in excess cause plant death whereas biological fertilizers have no negative effects. Also, chemical fertilizers contaminate water bodies, degrade soil fertility, and causes imbalance in the ecosystem equilibrium (Aadesh et al. 2024; Seenivasagan & Babalola 2021). When taken up by crops, chemical fertilizers can compromise yields which in turn affect health of man and living organisms (Aadesh et al. 2024).

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 and other substitutes (Britannica, 2019; Kumar et al. 2017). 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. 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 (Ammar et al. 2023).

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. Unlike chemical fertilizers, the continuous application of biofertilizers improves soil structure and crop product quality, increases agricultural produces by 22–32%, and replaces inorganic by 27% (Ammar et al. 2023).

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 (Elemuo et al. 2019). 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).

Water hyacinth (*Eichhornia crassipes*), is a free-floating, perennial hydrophyte that is thought to have originated in South America. It is a member of the Pontederiaceae family and has been shown to be invasive in most aquatic environments in Africa (Vincent-Akpu et al. 2024). According to Ilo et al. (2022), it is considered an annoyance to aquatic life and activities that can even reduce biodiversity, ecological benefits, and socioeconomic gains. Because it can outcompete other hydrophytes in water and reduce solar radiation penetration, which in turn affects other living organisms in water and ultimately lowers the concentration of dissolved oxygen, it is well known as the "world's worst weed". It has been shown that higher temperatures and minerals like potassium, phosphorus, and nitrogen promote the growth of water hyacinth (Vincent-Akpu et al. 2024). The aim of this study is to enhance the growth parameters of three plants (*Zea mays*, *Abelmoschus esculentus* and *Cucurbita pepo*) with synergistic application of biofertilizers produced with bacteria, forest soils and *Eichhornia crassipes* compost.

Materials and methods

Description of the study 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).

Sample collection

Soil samples were collected randomly at three different depths (0–15, 15–30 and 30–45 cm) 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 three communities were dominated with forest trees and enriched with soil nutrients while the choice of control sample was based on agricultural soil that had no forest vegetation on it. 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 h. The soil samples were sieved through a 5 mm mesh size sieve and were stored at 4 °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). These crops were opted for because of their fast germination which favours this research and because they are commonly grown within those region. 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.

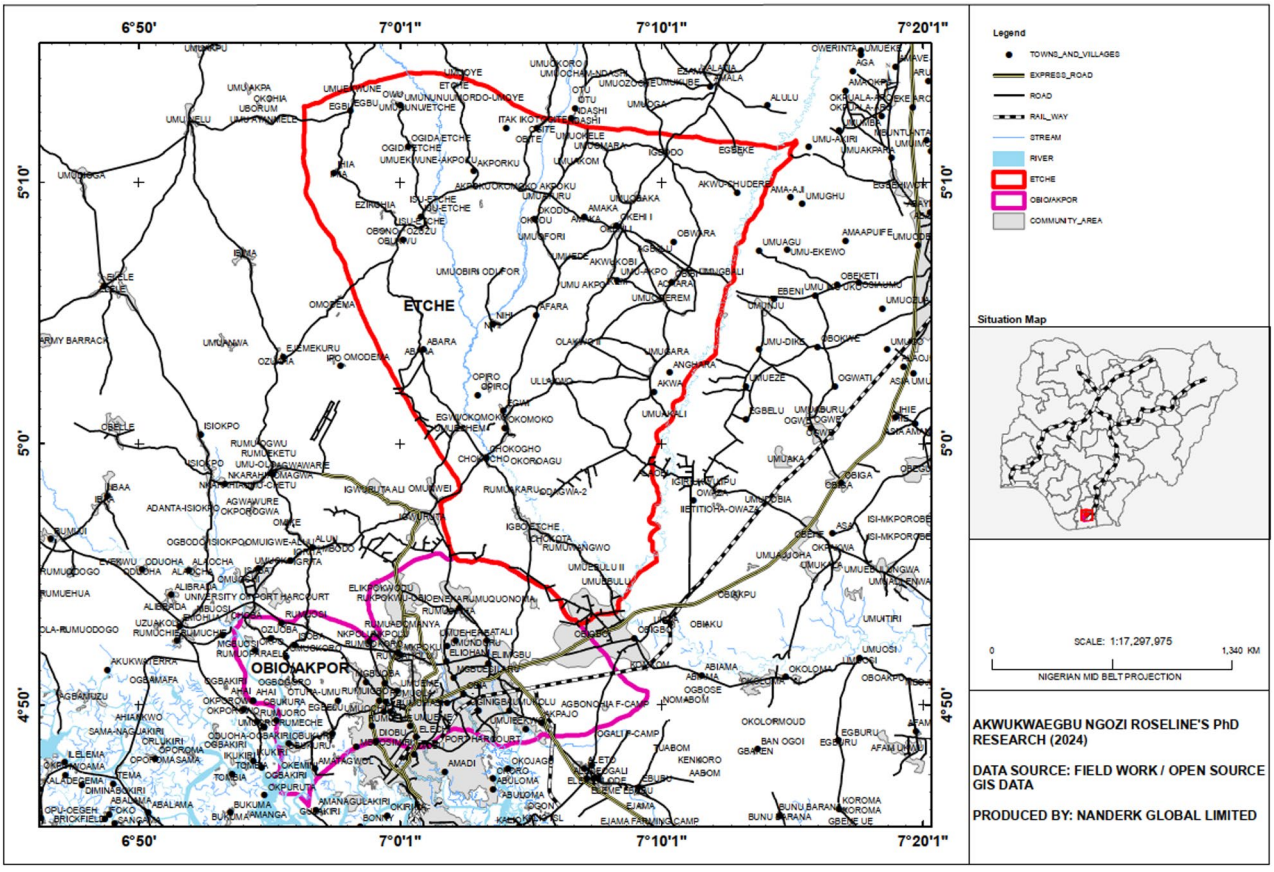


Fig. 1 Sampled Study Locations in Etche and Choba LGA in Rivers state. This is a composite map showing the geographical locations of soil sample collection points in two different local governments

Preparation of water hyacinth compost

Exactly 1 kg of water hyacinth was collected by hand from the New Calabar River from Rivers State. The recently collected water hyacinth plants were air and sun-dried at room temperature after being submerged in the surrounding water to remove any loose material from the leaves, stems, and roots. A common cutter was used to shred the dried plants into pieces. It was put through a 250-liter cylindrical bioreactor made of tin plates that had been galvanized. The bioreactor had baffles that were connected to a central pipe for inlet of air into the chamber. The baffles produced mixing effect as the entire set-up was positioned in a rectangular building with a corrugated roof that shielded it from the sun and rain. A suspension of consortia of microorganisms obtained from cow rumen fluids was used to irrigate the shredded material inside the bioreactor to 50% (v/w). The chain and pulley were used for sporadic mechanical spinning during the sixty-day composting process. A small, motorized industrial grinding mill was used to turn the resultant compost into a coarse powder. To increase the final product's solubility, a final grinding was carried out using a mechanical hand grinder (Abu et al. 2024).

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 analysed following the digestion of HF-$HClO_4$-HNO_3.

Microbiological analysis of soil samples

Preparation of soil sample

To remove the organism from the soil particles, one gram (1 g) 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.

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 h. After incubation, the colonies were counted using viable plate count method to measure the number of colony forming units (CFU) per gram of soil sample. Discrete colonies (colonies that clumped together) were chosen and then sub cultured (Hema et al. 2017).

Screening for phosphate solubilizing bacteria

Each sample had about 2 g 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) (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 g L^{-1}) and Jensen's media agar plates (20 sucrose, 0.1 K_2HPO_4, 0.5 $MgSO_4.7H_20$, 0.5 NaCl, 0.1 $FeSO_4.7H_20$, 0.005 Na_2MoO_4, 2.0 $CaCO_3$ and 15 agar g L^{-1}) (Pikovskaya, 1948). To get rid of the soluble phosphate impurities, the insoluble $Ca(PO_4)$ was centrifuged at 155 rpm after being cleaned with diluted water. The wet $Ca(PO_4)$ was dried using a vacuum-flask device, and the supernatant was disposed off. 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 h and 50 ml of semi-solid medium was inoculated with 1 ml of 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 to give 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 + 0.1 kg Microbial inoculant.

2 kg of Soil + 0.1 kg Water Hyacinth Compost.

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

2 kg of Soil + 0.1 kg 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 thrice. Sterile distilled water was used to revive 0.1 kg of the substrate, which was subsequently combined with 0.1 kg of the different substrate in an aseptic setting. The seeds were subjected to viability testing before they 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: 32 °C, min: 15 °C) (Akintokun et al. 2019).

Determination of shoot length

Mid-line tracking method which counts the number of points on the stem to determine shoot length was used. 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:

$$(\mathrm{L} \times \mathrm{W}) \ + 1.66$$

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.100 g^{-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 m 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 sand medium was prepared using clean, coarse (horticultural) sand sterilised at 82 °C for 30 min. The sand was allowed to cool completely, and then single sheet of paper was placed at the bottom of the pot to allow drainage and retain sand. The sand is moistened via bottom-watering to allow seed germination before seeds was planted at equidistant rows. 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 16 S rRNA genes, and gel electrophoresis of the isolates. The European Genome and Diagnostics Centre, GATC Biotech AG, Jakob Stadier-Platz 7, 78,467 Constance, Germany, received the PCR products for 16 S rRNA sequencing using the Targeted RNA sequencing method. 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 16 S rRNA genes was carried out using the primer set 27 F-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 µl 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 45 s, and extension at 72 °C for 1 min. A final extension was then performed for 10 min 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 80 V for one hour and thirty minutes.

Methods of phylogenetic tree construction and analysis

The Neighbor-Joining approach was used to infer the evolutionary history. The ideal tree is displayed. Next to the branches is the proportion of duplicate trees where the related taxa are clustered together in the bootstrap test (500 repetitions). With branch lengths in the same units as the evolutionary distances used to deduce the phylogenetic tree, the tree was scaled. The Jukes-Cantor technique was used to calculate the evolutionary distances which were measured in base substitutions per site. MEGA11 was used to perform evolutionary analysis (Ezebuiro et al. 2015).

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

the procedure previously described by Simura et al. (2018) was used for the sample preparation and plant hormone extraction. 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 et al. 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 min 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,

Table 1 Total heterotrophic bacterial (THB) count (cfu/g)

Soil depth	Control soil	Etche forest	Ogbogoro forest	Choba forest
0–15 cm	$0.75 \pm 1.30 \times 10^{11a}$	$0.07 \pm 3.50 \times 10^{11b}$	$1.00 \pm 2.07 \times 10^{11c}$	$0.07 \pm 8.88 \times 10^{11b}$
15–30 cm	$0.05 \pm 4.00 \times 10^{11a}$	$0.06 \pm 7.50 \times 10^{11b}$	$0.81 \pm 1.37 \times 10^{11c}$	$0.06 \pm 8.14 \times 10^{11b}$
30–45 cm	$0.03 \pm 2.08 \times 10^{11a}$	$0.04 \pm 3.50 \times 10^{11b}$	$0.04 \pm 7.09 \times 10^{11b}$	$0.04 \pm 3.51 \times 10^{11b}$

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 (THUB) count (cfu/g)

Soil depth	Control soil	Etche forest	Ogbogoro forest	Choba forest
−15 cm	$3.52 \pm 6.08 \times 10^{10a}$	$0.32 \pm 1.52 \times 10^{10b}$	$0.55 \pm 5.50 \times 10^{10c}$	$0.44 \pm 3.21 \times 10^{10d}$
15–30 cm	$0.44 \pm 9.53 \times 10^{10a}$	$0.36 \pm 1.15 \times 10^{10b}$	$0.44 \pm 5.13 \times 10^{10a}$	$0.42 \pm 2.50 \times 10^{10c}$
030–45 cm	$0.32 \pm 1.00 \times 10^{10a}$	$0.33 \pm 2.64 \times 10^{10a}$	$0.34 \pm 3.60 \times 10^{10b}$	$0.35 \pm 4.50 \times 10^{10b}$

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 (PSB) count (cfu/g)

Soil depth	Control soil	Etche forest	Ogbogoro forest	Choba forest
0–15 cm	$5.00 \pm 4.58 \times 10^{10a}$	$0.34 \pm 4.10 \times 10^{10b}$	$0.69 \pm 4.35 \times 10^{10c}$	$0.37 \pm 6.65 \times 10^{10b}$
15–30 cm	$0.38 \pm 9.16 \times 10^{10a}$	$0.34 \pm 4.93 \times 10^{10b}$	$0.38 \pm 11.00 \times 10^{10a}$	$0.35 \pm 7.23 \times 10^{10b}$
30–45 cm	$0.32 \pm 2.08 \times 10^{10a}$	$0.33 \pm 3.21 \times 10^{10a}$	$0.36 \pm 2.51 \times 10^{10b}$	$0.32 \pm 3.40 \times 10^{10a}$

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 (NFB) count (cfu/g)

Soil depth	Control soil	Etche forest	Ogbogoro forest	Choba forest
0–15 cm	$3.20 \pm 3.50 \times 10^{10a}$	$0.27 \pm 2.03 \times 10^{10b}$	$0.30 \pm 5.20 \times 10^{10a}$	$0.40 \pm 1.00 \times 10^{10c}$
15–30 cm	$0.30 \pm 2.50 \times 10^{10a}$	$0.30 \pm 4.93 \times 10^{10a}$	$0.27 \pm 3.50.x\ 10^{10b}$	$0.35 \pm 5.20 \times 10^{10c}$
30–45 cm	$0.28 \pm 2.20 \times 10^{10a}$	$0.23 \pm 3.21 \times 10^{10b}$	$0.26 \pm 4.50 \times 10^{10a}$	$0.31 \pm 3.30 \times 10^{10c}$

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 ($p < 0.05$)

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 of plant growth parameters, nutrient levels and plant hormones. Means were separated using Duncan's tool while a value (P) was deemed statistically significant if it was less than 0.05. i.e., $P < 0.05$.

Results and discussion

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. The THB in the soil ranged from $0.03 \times 10^{11} - 1.00 \times 10^{11}$ cfu/g while THUB in the soil ranged from $0.32 \times 10^{10} - 3.52 \times 10^{10}$ cfu/g. There was a significant difference ($P < 0.05$) in the THB count in the three forest soils obtained at 0–15 cm, 15–30 cm and 30–45 cm when compared to their controls (Table 1). Similarly, all the THUB in the three forest soils except Etche forest soil obtained at 30–45 cm differed significantly ($P < 0.05$) when compared to their controls (Table 2).

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. There was a significant difference ($P < 0.05$) in the PSB count in the three forest soils at 0–15 cm, Etche and Choba forest at 15–30 cm, and Ogbogoro forest at 30–45 cm when compared to

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}	$1.55 \pm 0.0.50^{a}$
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}

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

their control (Table 3). Also, aside Ogbogoro forest soil at 0–15 cm and 30–45 cm, and Etche forest soil at 15–30 cm, the remaining forest soils had significantly different ($P < 0.05$) NFB count when compared to their controls (Table 4).

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 to 7.23 while- electrical conductivity ranged from 104.65 to 121.52 µS/cm. From trace elements, mercury is the least with 0.01 mg/kg in all the soil samples tested.

Table 6 showed the physicochemical characteristics of the soils in the proportion mixture of 1:1:1 with various substrates (MS, MI, WHC, NPK Fertilizer) before and after adding the treatments. The 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 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}

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}

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 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}

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 9 Total chlorophyll content in (mg.100 g^{-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}

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}

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 ($p < 0.05$)

Effect of biofertilizers on crop growth parameters

Table 7 showed significant increase ($P < 0.05$) in the shoot length of the three crops planted in all the soil treatments except *Z. mays* planted on MI when compared with control soil. Similarly, there was a significant increase ($P < 0.05$) in the leave area and yield of the three crops planted in all the soil treatments when compared with the control soil (Table 8 and 12 respectively). The chlorophyll content significantly increased ($P < 0.05$) in the three crops only on MI + WHC and in *C. pepo* planted on MI and NPK fertilizer when compared with control soil (Table 9). The germination rate was significantly higher ($P < 0.05$) in the three crops grown on all the soil treatments except *A. esculentus* planted on NPK fertilizer when compared with control soil (Table 10). Also, there was a significant increase ($P < 0.05$) in the height of the three crops grown on all the soil treatments except *Z. mays* planted on MI and *A. esculentus* planted on NPK fertilizer when compared with control soil (Table 11). The crop growth parameters of the three crops were highest on MI + WHC treatment when compared to others Table 12.

Effects of biofertilizer on nutrient levels

The nitrogen, phosphorus and potassium levels in the three crops grown on all the soil treatments were significantly higher ($P < 0.05$) when compared with control soil (Tables 13, 14 and 15 respectively). There was a significant increase ($P < 0.05$) in the zinc level of the three crops planted on all the soil treatments except *Z. mays* and *C. pepo* planted on NPK fertilizer, and *A. esculentus* planted on WHC and NPK fertilizer when compared with control

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}

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 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}

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}

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}

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}

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}

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 ($p < 0.05$)

soil (Table 16). Similarly, there was a significant increase in the manganese level of *Zea mays* planted on all the soil treatments and on both *A. esculentus* and *C. pepo* planted on MI + WHC treatment when compared with the control soil (Table 17). The nutrient levels of the three crops were highest on MI + WHC treatment when compared to others.

Molecular characteristics of isolates

Four bacteria strains were isolated from the Genbank characteristics as shown in Table 18 while Fig. 2 reveal the 16 S 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 12,605	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

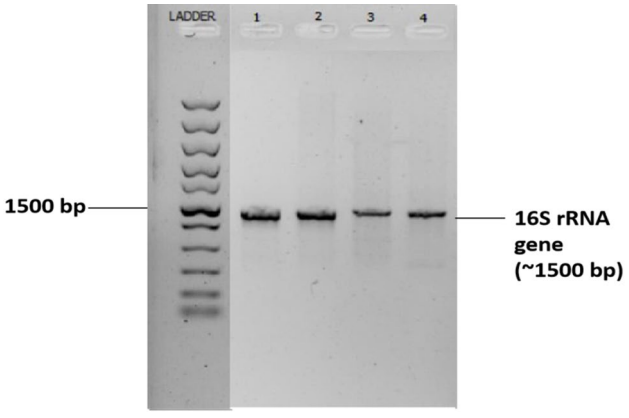


Fig. 2 PCR amplification image of the 16 S rRNA gene. (1: PA4; 2: PA6; 3: PA7; 4:PA11). The DNA 1 kb molecular ladder is given in the first lane

Impact of biofertilizers on plant hormones

The IAA in the leaves and shoot of the three crops grown on all the soil treatments were significantly higher ($P<0.05$) when compared with control soil (Table 19 and 23 respectively). There was a significant increase ($P<0.05$) in GA_4 in the leaves and shoot of the three crops grown on MI + WHC, leaves of *A. esculentus* grown on NPK and shoot of *Z. mays* grown on WHC when compared with control soil. GA_4 was below detection limit in the leaves of *Z. mays* planted on WHC (Table 20 and 24 respectively). There was a significant increase ($P<0.05$) in ABA in the leaves and shoot of the three crops grown in MI, WHC and MI + WHC and a significant decrease ($P<0.05$) in ABA in the leaves and shoot of the three crops grown in NPK when compared with control soil (Table 21 and 24 respectively). There was no significant difference in the cis-zeatin levels in the leaves and shoot of the three crops when compared with one another (Table 22 and 26). Tables 23 and 25.

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

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 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

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 ($p < 0.05$)

Discussion

Microbial analysis of samples

Soil recorded highest THB count within the range of 0.03×10^{11} -1.00×10^{11}cfu/g. The outcome demonstrates that the native bacterial genus can flourish and proliferate in the environment. Ibiene et al. (2011) and Eze and Okpokwasili (2011) both made a similar finding. The THB count and THUB in the present study are higher than values reported by Douglas (2018), 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 16 S RNA gene sequencing are members of the phosphate-solubilizing. 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 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase), which lowers ethylene production in plants.

Physicochemical composition of the soil

The physicochemical parameters of a forest soil as shown in Table 5 indicates that a tropical moist forest soils have a low value of Electrical Conductivity (EC); The amount of accessible nutrients in the soil is measured by knowing the amount of electrical conductivity involved. 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 soil pH slightly varies from 5.60 to 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

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 ($p < 0.05$)

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 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 on growth parameters of *C. pepo*, *Z. mays* and *A. esculentus* are shown in Tables 7, 8, 9, 10, 11 and 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

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

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 ($p < 0.05$)

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,.*A. 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 and El-Nady 2008).

C. pepo, *Z. mays*, and *A. 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' 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

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

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

Z. mays and *A. esculentus* treated with a mix of microbial inoculant and water hyacinth compost. In reality, *Z. mays* and *A. esculentus* would have more readily absorbed the nutrients from the soil treatments than *C. 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 Tables 13, 14, 15, 16 and 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 (Aadesh, 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 (Tošić, 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 signalling 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

The species identified 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) as shown in (Table 18) are excellent selections for biofertilizers that increase plant growth, promote soil health, and lessen dependency on chemical fertilizers for environmentally friendly farming because they develop vital relationships with root systems, improving nutrient cycling (N-fixation, P/K solubilization) and protection.This agree with the findings of Amal and Heba (2023) and Agu et al. (2021) who screened and identified species as *Bacillus cereus* (OP970172) and *Bacillus licheniformis* (OP970169), 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, 20, 21, 22, 23, 24, 25 and 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, 20, 21, 22, 23, 24, 25 and 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,

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

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

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 ($p < 0.05$)

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. This is not surprising having stated some of the negative impact of chemical fertilizers on crop and man. Considering ABA to be a potent growth inhibitor, it implies cell division and elongation will be markedly inhibited in the three crops due to their high levels. This 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 *C. pepo*, *Z. mays*, and *A. esculentus* 60 days after planting by enhancing GA_4 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 *C. pepo*, *Z. mays* and *A. esculentus* after 60 days of planting. The study equally discovered species with biofertilizing potentials. 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.

Therefore, it is recommended 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 since it has proven efficient at reducing side effects associated with chemical fertilizer usage and cost while increasing growth benefit.

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			

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

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

Supplementary Information

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Supplementary Material 1

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Institutional review board

Not applicable.

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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Data availability

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

Declarations

Ethics approval and consent to participate

Not applicable.

Informed consent

Not applicable.

Competing interests

The authors declare no competing interests.

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