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Biofertilizer production using plant growth-promoting bacteria from cassava peels and plantain leaves to evaluate the effects on growth parameters of beans (*Phaseolus vulgaris* L.) and *Groundnut* (*Arachis hypogaea* L.) seeds

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Abstract
Improper disposal of agricultural waste and soil nutrient deficiency are major issues in Nigeria, leading to a heavy reliance on chemical fertilizers for crop growth. Consequently, the objective of this paper was to produce biofertilizers using plant growth-promoting bacteria from cassava peels and plantain leaves to evaluate the effects on growth parameters of Beans (*Phaseolus vulgaris* L.) and *Groundnut* (*Arachis hypogaea* L.) seeds using appropriate standard microbial and agro-techniques. The effects of each treatment were examined on various growth parameters of beans and groundnuts for 20 days, including shoot length, leaf length, leaf number, leaf width, stem girth, and number of branches. Plantain leaves had higher concentrations of magnesium and nitrogen, while cassava peels had more phosphorus and potassium. On day 20, the highest shoot length (39 cm), leaf width (7.2 cm), and largest stem girth (2.5 cm) for beans were observed in soils treated with CP+PL+*B. pumilus*, CP+PL+*S. maltophilia*, and CP+PL+*B. pumilus*+ *S. maltophilia*, respectively. For groundnuts, the highest shoot length (22 cm), leaf width (3.5 cm), number of leaves (60), and number of branches (16) were observed in soils treated with CP+PL+*B. pumilus* + *S. maltophilia*, CP+PL+*B. pumilus*, CP+PL+*S. maltophilia*, and CP+PL+*S. maltophilia*, respectively. The results showed that there was a significant difference in the treatments and the duration of the treatments at a 5% level of significance. The growth parameters of beans and groundnut plants revealed that using *B. pumilus* or *S. maltophilia* as single-strain biofertilizers or in a consortium improved the plants' growth. The study recommends the production of commercial biofertilizers from common agricultural waste to promote sustainable agriculture and entrepreneurship among microbiologists.

Keywords: Plantain leaves, cassava peels, biofertilizer, agro-waste, *Stenotrophomonas maltophilia*, *Bacillus pumilus*

Introduction
Nigeria faces two major environmental issues - waste disposal and nutrient deficiency. One solution to these problems is to create a sustainable environment and a circular economy that recycles waste into beneficial agricultural products. Nigeria is one of the top five producers of cassava (*Manihot esculenta*) worldwide, with a production of over 63 million metric tonnes (FAOSTAT, 2021) [10]. With such a large production, much waste is released. In Nigeria, the cassava processing industries and households produce approximately 55% of waste from the peels, according to Tran *et al*. (2022) [30]. Plantain (*Musa* sp.), which is commonly cultivated in several states of Nigeria, is mainly harvested for food and food wrapping using fresh leaves (Adeniyi *et al*., 2019) [1]. The old plantain leaves that dry on the tree are generally considered waste and cut down. Unfortunately, agricultural waste from agro-industries and small farms is often not disposed of properly, leading to offensive smells, contributing to epidemics and climate change when burned, and limiting agricultural space. Nutrient deficiency in crops is also an issue in many states in the country caused by infertile soil. Plants need essential nutrients to survive, such as Nitrogen, Potassium, Phosphorus, Calcium, Magnesium, Sulphur, and Iron. These nutrients are obtained from the soil but are in a form that plants cannot uptake (Asadu *et al*., 2020) [4]. Farmers take pride in their land, and the quest for fertile soil drives them to seek means to enhance and improve the soil for better

yields. Chemical fertilizers containing Nitrogen, Phosphorus, and Potassium have undoubtedly benefited modern cropping systems (Kiprotich *et al*., 2023) [16]. However, their drawbacks are numerous. Overutilization of these fertilizers can cause environmental deterioration, such as air, water, and soil pollution (Indumathi, 2017; Devi & Sumathy, 2018) [13, 7], and impair the health of agricultural soils by disturbing the important plant growth-promoting rhizobacteria (PGPR) in the soil, resulting in lower yields (Kiprotich *et al*., 2023) [16]. Asadu *et al*. (2020) [4] reported that the problems associated with using chemical fertilizers include degradation of soil structure, soil, ground water, and surface water pollution, resulting in eutrophication. Onyia *et al*. (2020) [25] added that excessive application has resulted in algal bloom, land degradation, food and crop product contamination, adverse human health effects, ozone layer depletion, and ecosystem imbalance. Despite the known health hazards of chemicals, they are often used to boost soil fertility and protect plants from pests.

Researchers are increasingly focusing on the development and use of biofertilizers as a way to address the environmental and health concerns associated with the widespread use of chemical fertilizers (Kiprotich *et al*., 2023; Onyia *et al*., 2020) [16, 25]. With the growing demand for safe food and a sustainable environment, agronomists are exploring these eco-friendly methods to replenish soil nutrients. These efforts are crucial for meeting the Sustainable Development Goals (SDG) and Agenda 2063 goals. Biofertilizers can boost soil fertility and promote plant growth, making them a more sustainable alternative (Onyia *et al*., 2020) [25]. They are inexpensive alternatives to chemical fertilizers. They improve crop quality, soil fertility, and food safety (Kiruba & Saeid, 2022) [17]. Their use reduces the reliance on costlier and toxic agrochemicals, increases overall soil fertility, maintains soil microflora, and improves the nutrient status of the soil (Khan *et al*., 2023) [15]. Biofertilizers are a type of fertilizers classified into different categories such as carrier-based biofertilizers, liquid biofertilizers, nitrogen-fixing biofertilizers, phosphate-solubilizing biofertilizers, and potassium-solubilizing biofertilizers. These biofertilizers contain plant growth-promoting bacteria such as *Bacillus*, *Azotobacter*, *Pseudomonas*, and *Klebsiella* species. These genera are classified as plant growth promoting bacteria which are free-living microorganisms that have been proven to enhance plant growth through numerous mechanisms, whether direct or indirect (Masood *et al*., 2020) [21]. Some of these mechanism include enhancing stress resistance, increasing the availability of nutrients to the plant, in-organic phosphate solubilization, production of siderophores, amongst others (Ibiene *et al*., 2012) [12]. These plant growth promoting bacteria qualify as good biofertilizers once they are formulated into substances or trapped on suitable carrier materials that can be applied to the soil to enhance the fertility of the soil, and to plants to stimulate their growth (Aloo *et al*., 2022) [2]. The carrier material serves as a protection and preservation for the bacteria, and it also provides essential nutrients like magnesium, potassium, phosphorus, nitrate, and iron that enrich the soil and increase its fertility. When the carrier material containing the bacteria is introduced to the soil, the microorganisms make these nutrients available to the plants by converting them into forms that plants can uptake (El-Fattah *et al*., 2013) [9].

Therefore, the objective of this paper was to produce biofertilizers using plant growth-promoting bacteria from cassava peels and plantain leaves to evaluate the effects on growth parameters of Beans (*Phaseolus vulgaris* L.) and *Groundnut* (*Arachis hypogaea* L.) seeds.

Materials and Methods

Isolation of Nitrogen fixing bacteria and Phosphate solubilizing bacteria: Ashby Mannitol Media was prepared following the method described by Opokwasili and Odukuma (1996) [24] and adapted by Ogugbue *et al*. (2017) [23]. Mannitol (20 g), Dipotassium phosphate K2HPO4 (0.2 g), dipotassium sulphate K2SO4 (0.1 g), Magnesium sulphate pentahydrate MgSO4.7H20 (0.2 g), Calcium carbonate CaCO3 (5 g), Sodium chloride NaCl (0.2 g), and Agar (15 g) were added to 1000 ml distilled water in a conical flask. The Pikovskaya media was prepared by adding Glucose (10 g), Yeast extract (0.5 g), Potassium Chloride KCl (0.2 g), Tricalcium phosphate (5 g), Sodium Chloride NaCl (0.2 g), Magnesium sulphate pentahydrate MgSO4.7H20 (0.1 g), Ammonium sulphate (NH4)2SO4 (0.5 g), and Agar (15 g) in 1000 ml distilled water in a conical flask. The media were sterilized in an autoclave at 121 °C for 15 minutes at 15 psi. Nitrogen-fixing bacteria and Phosphate solubilizing bacteria were isolated from the soil sample using serial dilution following a method by Ashritha *et al*. (2021) [5], where one gram of the soil sample was diluted in 9ml sterile distilled water and mixed thoroughly to prepare a stock which was serially diluted up to 10-5. An aliquot of 0.1 ml from the 10-5 tube was inoculated onto the petri dishes. Ashby mannitol medium plates were incubated at room temperature for 5 days while the Pikovskaya medium Petri plates were incubated at 37 °C for 24 hours. The resulting colonies with distinct colours and that showed clear zones on both media were repeatedly cultured to obtain pure colonies for further analysis. Morphological characteristics such as elevation, surface, pigmentation, margin, and shape were observed. The following biochemical tests were carried out: catalase, oxidase, citrate, triple sugar iron agar test, sugar fermentation test, and Methyl Red Voges-Proskauer tests.

Identification of 16S rRNA

Bacterial DNA was extracted using the Zymo Research bacterial DNA mini-prep extraction kit, produced by the Zymo Research (ZR) Company, Irvine, California. The DNA was quantified using the NanoDrop Spectrophotometer 2000C. The primers 27F: 5`- AGA GTT TGA T CM TGG CTC AG-3` and 907R: 5`-CCG TCA ATT CMT TTR AGT TT-3` were used to amplify the gene fragment. The sequencing and sequence analysis of the DNA fragments was performed by Inqaba Biotechnological in Pretoria, South Africa with the BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 from Nimagen. The resulting labelled products were purified using the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053). The sequence data was then collected and analyzed using Chromaslite for base calling, BioEdit for sequence editing, and BLAST was performed at the NCBI database. Finally, the multiple sequence alignment software, ClustalW was employed to align similar sequences. The evolutionary relationship was obtained by the Neighbour-Joining method according to Saitou and Nei (1987) [28]. The Jukes-Cantor method (Jukes & Cantor, 1969) [14] was used in the computation of the evolutionary distances.

Collection of Waste Samples

Cassava peels (*Manihot esculenta*) were obtained from a cassava mill located in Elele, Emuoha Local Government Area, and were left to sun-dry for 15 days. The dried plantain leaves (*Musa* sp.) were sourced from plantain trees in a farm yard situated in Delta Park, University of Port Harcourt, Choba, Obio/Akpor, and were sun-dried for 3 hours.

Biofertilizer Production

The biofertilizer was produced using a modified method adapted from Anubrata and Rajendra (2014) [3]. To prepare starter cultures, inoculum was picked from respective culture slants containing the selected NFB and PSB isolates and then transferred aseptically into broth tubes. The two culture tubes were incubated at 35 °C for 4 days.

Inoculum Preparation

Nutrient broth was prepared in 250 mL conical flasks, and 10 mL was dispensed into tubes. Inocula were transferred from the respective starter cultures of Aw and PPB into the 10 mL broth tubes. A consortium culture of Aw and PPB was also prepared. The broth cultures were incubated at 35 °C for three days.

Preparation of carrier materials

The plantain leaves and cassava peels were ground separately into fine powder at a local market in Choba community. A small part of the samples were evaluated for some physicochemical parameters. The carrier materials (CP and PL) were measured separately (100g each) into sterile polyethylene bags (With air expelled out of it) and wrapped in aluminum foil and labelled accurately. Also, 50g of the cassava peels were measured and mixed with 50g of the plantain leaves to obtain a 100g weight; this gave a ratio of 1: 1. The carrier materials were sterilized in an autoclave for 60 minutes at 121 °C for 4 days. After sterilization, 1g of each carrier material was serially diluted by 10-fold serial dilution and spread on nutrient agar plates, incubated at 32 °C to confirm the sterilization effect, according to a method by Senoo *et al*. (2002).

Preparation of Inoculant-Carrier Packet

The sterilized carrier materials were placed on a sterilized bench surface and opened close to a flame. Ten (10) ml of the inoculum, both single and multi-strain (table 1) was added to the mixed carrier material (CP + PL) and mixed thoroughly by hand (Sterile hand gloves rubbed with ethanol were worn and changed after each mix). The inoculant-carrier package was partially sealed and left to cure under laboratory temperature for about 9 days. The prepared biofertilizers were transferred aseptically to sterile packages and stored in a cool place away from direct heat, to be ready for use in planting.

Table 1: Set up for inoculant-carrier package

Set-up	Summary
PL	Plantain leaves alone
CP	Cassava peels alone
CP + PL + Aw (Biofertilizer)	Cassava peels mixed with Plantain leaves and inoculated with *Bacillus pumilus*
CP + PL + PPB (Biofertilizer)	Cassava peels mixed with Plantain leaves and inoculated with *Stenotrophomonas maltophilia*
CP + PL + Consortia (Biofertilizer)	Cassava peels mixed with Plantain leaves and inoculated with *Bacillus pumilus* and *Stenotrophomonas maltophilia*

Key: PL = plantain leaves, CP = cassava peels, PPB = *Stenotrophomonas maltophilia*, Aw = *Bacillus pumilus*

Physicochemical Analysis of Samples

The physicochemical parameters of the samples were measured including magnesium, phosphorus, iron, nitrogen, potassium, pH, and temperature. The samples were analysed as follows, carrier materials separately, mixed carrier material in a ratio of 1: 1; the planting soil alone; and the soil mixed with the carrier materials.

Pot Experiment

Fourteen Polyethylene pots, measuring 24.1 cm in length and 22.2 cm in width, were filled with 900 g of loamy soil obtained from a plantain plantation in Delta Park at the University of Port Harcourt. The pots were labelled as follows for beans and groundnut seeds: Seed alone, PL + seed, CP + seed, CP + PL + PPB + seed, CP + PL + Aw + seed, CP + PL + Consortia + seed. Before sowing, the bean and groundnut seeds were rinsed with sterile distilled water and allowed to air-dry. The CP + PL mixtures with single isolate, and the CP + PL mixture containing the consortium served as the biofertilizers. The soil application method was carried out where the respective biofertilizers and carrier materials were added to the respective pots and allowed to stand for 2 days. Finally, the bean and groundnut seeds were sown in their respective pots.

Table 2: Beans and Groundnut Treatment for Pot Experiment

S/N	Treatments	Summary
1	Seed alone	Sowing of seed in soil alone
2	Soil + CP + seed	Mixture of Cassava Peel in the soil
3	Soil + PL+ seed	Plantain Leaves mixed in the soil
4	Soil + CP + PL+ Aw + seed	Cassava Peels and Plantain Leaves inoculated with *Bacillus pumilus* mixed in the soil.
5	Soil + CP + PL+ PPB + seed	Cassava Peels and Plantain Leaves inoculated with *Stenotrophomonas maltophilia* mixed in the soil
6	Soil + CP +PL + Consortia + seed	Cassava Peels and Plantain Leaves inoculated with *B. pumilus* and *S. maltophilia*

Plant Growth Parameters

Measurements of the shoot length, leaf length, leaf width, stem girth (cm), leaf number, and number of branches were taken at 4 days interval for 20 days.

Data Analysis

Two types of statistical analyses were used to analyse the data. The Two-way Analysis of Variance (ANOVA) was used to determine whether the different treatments and the

planting days had effect on the growth parameters yield; and the one-way ANOVA was used to ascertain whether there are significant differences between the means the physicochemical properties of the individual carrier materials and when they are mixed together, the soil alone and when mixed with the carrier material.

Results and Discussion

Isolation and identification of Nitrogen fixing bacteria and phosphate solubilizing Bacteria

A total of seven bacterial isolates were obtained from the rhizosphere soil and labelled as Ap, Ay, Ac, Aw, PPB, YPB, and CPB. The biochemical characteristics of the isolates in Table 3 showed that *Bacillus*, *Klebsiella*, and *Pseudomonas* sp. were isolated. The colonies labeled PPB and Aw were selected based on their ability to utilize tricalcium phosphate in the pikovskaya media and fix nitrogen in the Ashby mannitol media respectively, and based on their abundance in the soil (Fig. 1).

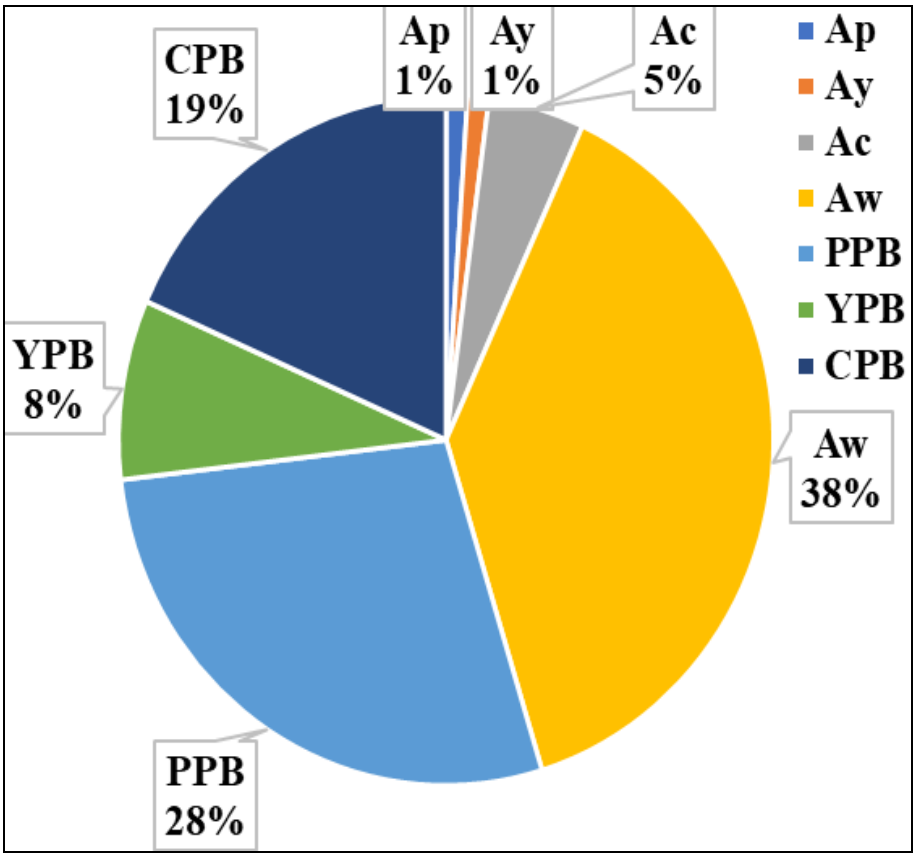


Fig 1: Percentage Abundance of NFBs and PSBs in the Rhizosphere soil

Figures 2 and 3 showed that isolate PPB, which was suspected to be a *Pseudomonas* sp. based on its biochemistry, had 70.55% similarity to *Stenotrophomonas maltophilia* SoD9b. Meanwhile, isolate Aw, which was suspected to be an *Azotobacter* sp. based on its cultural characteristics, had 77.58% similarity to *Bacillus pumilus* NCT919. This suggests that *Bacillus pumilus* can grow on Ashby Mannitol Media (AMM), which is typically used for cultivating nitrogen-fixing bacteria, particularly *Azotobacter*. On Ashby Mannitol Media, *B. pumilus* formed clear zones around its colonies, indicating that it could utilize mannitol as a carbon source and fix nitrogen. To confirm this, a stock culture was sub-cultured again on AMM and subjected to gram staining and a series of biochemical tests. The results confirmed that the bacteria was indeed a *Bacillus* sp. This finding was consistent with a study conducted by Di *et al*. (2023) [8] that aimed to identify and characterize *Bacillus strains* and assess their plant growth-promoting traits. The researchers observed transparent circles produced by the *Bacillus strain* on Ashby Mannitol medium. According to Harwood *et al*. (2013) and Logan and De Vos (2015) [19], *Bacillus* species are capable of growing in chemically defined salt media with carbon sources from glucose, malate, and other simple sugars, and with nitrogen sources from ammonium salts or certain amino acids. *B. pumilus* can also grow in the presence of sodium chloride. The bacteria was able to grow in Ashby Mannitol Media because the medium contains the necessary nutrients: agar-agar provides calcium, iron, and other essential elements (Pacific Harvest, 2023) [26], while dipotassium phosphate in the media supplies phosphate and helps maintain the pH. *Bacillus pumilus* requires sources of carbon and energy, which are provided by mannitol, a sugar alcohol present in AMM.

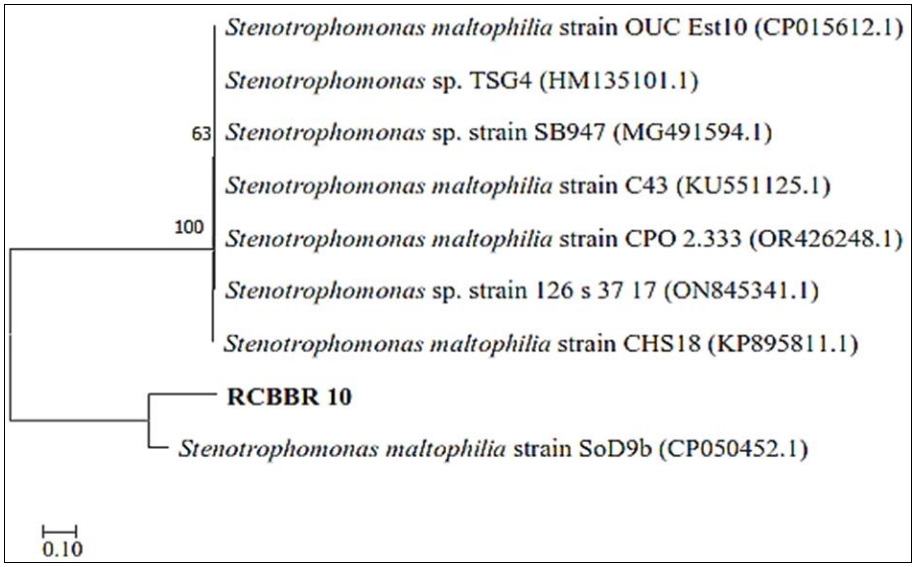


Fig 2: Neighbour-Joining Evolutionary Relationship of Isolate PPB (RCBBR_10) with the Closest Matches

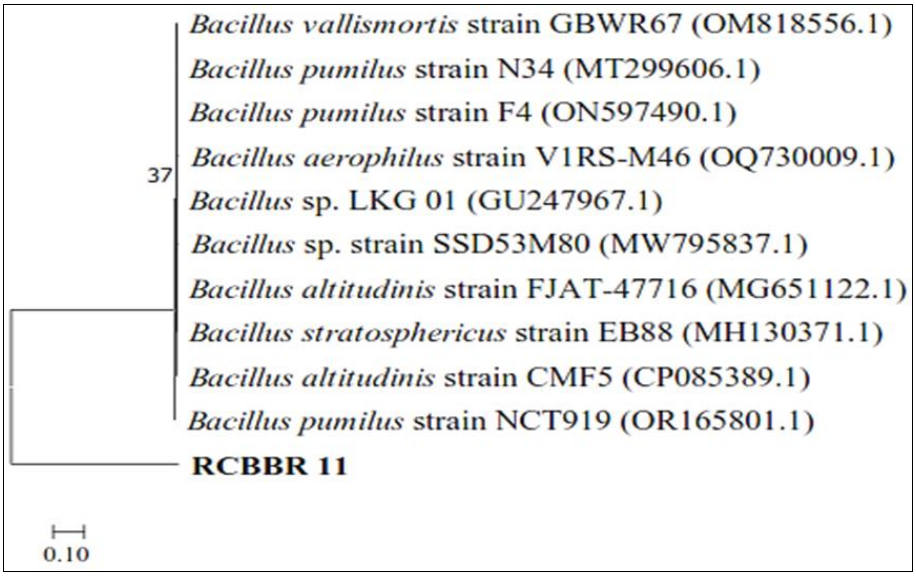


Fig 3: Neighbour-Joining Evolutionary Relation of Isolate Aw (RCBBR_11) with the Closest Matches

Table 3: Biochemical Characteristics of the Isolates

Isolates	Cultural morphology	Catalase	Oxidase	Lactose	Sucrose	Glucose	Citrate	MR	VP	Slant	Butt	Gas	H_2S	Shape	Gram	Tentative Microorganisms
Ap	Flat, pink mucoid surface, regular margin.	+	+	-	-	+	+	-	+	R	R	-	-	Rod pink	-	*Bacillus* sp.
Aw	Flat, shiny white mucoid surface, regular margin	+	+	-	+	-	+	+	-	Y	R	-	-	Rod Pink	+	*Bacillus* sp.
Ay	Raised elevation, yellow mucoid surface, regular margin	+	-	-	+	-	+	+	-	Y	Y	-	-	Rod Pink	-	*Klebsiella* sp.
Ac	Raised, creamy mucoid surface, irregular margin	+	-	-	-	-	+	+	-	Y	Y	-	-	Rod Pink	-	*Klebsiella* sp.
PPB	Flat, pink mucoid surface, regular	+	+	-	-	-	+	+	-	R	R	-	-	Rod Pink	-	*Pseudomonas* sp.
YPB	Flat, yellow mucoid surface, irregular	+	-	-	-	+	+	-	+	Y	Y	-	-	Rod Pink	-	*Pseudomonas* sp.
CPB	Raised, creamy mucoid surface, regular	+	+	-	-	-	-	+	-	R	R	-	-	Rod Pink	-	*Pseudomonas* sp.

Key: R = Red (Base), Y = Yellow (Acid), + = positive, - = negative

Preparation of carrier material and biofertilizer

The cassava peel carrier material had a greyish-brown or taupe color, it was powdery and light-weight (plate 2). While the dried plantain leaves had a brownish appearance; it was lighter in weight than the cassava peels (plate 4). When mixed together with the inoculum, the produced biofertilizer had a distinctive smell and the color of loamy soil as shown in plate 5.

Plate 1: Dried cassava peels

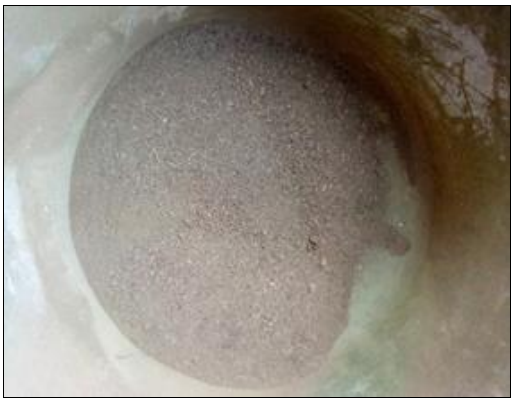


Plate 2: Grinded cassava peels

Plate 3: Dried Plantain leaves

Plate 4: Grinded plantain leaves

Plate 5: Packaged product

Physicochemical Properties of the Carrier Materials and Planting Soil

According to Table 4, the loamy soil contained higher levels of Nitrogen, Phosphorus, Potassium, Magnesium, and Iron prior to the addition of the mixed carrier material (M CM). Upon adding the M CM, these properties decreased. The soil temperature was initially measured to be 28.6 °C, which reduced by 0.3 °C after the addition of the M CM. Ludovici (2004) [20] explains that groundnut and beans grow best in soil temperatures ranging from 26 – 35 °C, thereby indicating that the variation in temperature is suitable for plant growth. The pH value of the soil was measured to be 2.99, indicating that the loamy soil collected from the farm yard in Delta Park at the University of Port Harcourt was ultra-acidic. The pH value further reduced to 1.87 after adding the mixed carrier material. The concentration of phosphorus and potassium in cassava peels (13.678 µg/kg and 1570.43 g/100g) was observed to be higher than that of plantain leaves (8.505 µg/kg and 41.98 g/100g). This indicates that cassava peels are rich in these two nutrients than plantain peels. On the other hand, the concentration of Nitrogen and magnesium was higher in plantain leaves (4.29% and 50.3%) than in cassava peels (3.76% and 44.7%). This suggests that N and Mg are in abundance in plantain leaves. However, it was observed that when these two carrier materials were mixed, the concentration of these four nutrients decreased, indicating that using them alone without mixing is better. Mixing cassava peels and plantain leaves together with the planting soil reduced the concentration of essential nutrients that the plant needed.

Table 4: Physicochemical Properties of the Individual Carrier Materials, Mixed Carrier Material, Soil and Soil Mixed with the both Carrier Materials

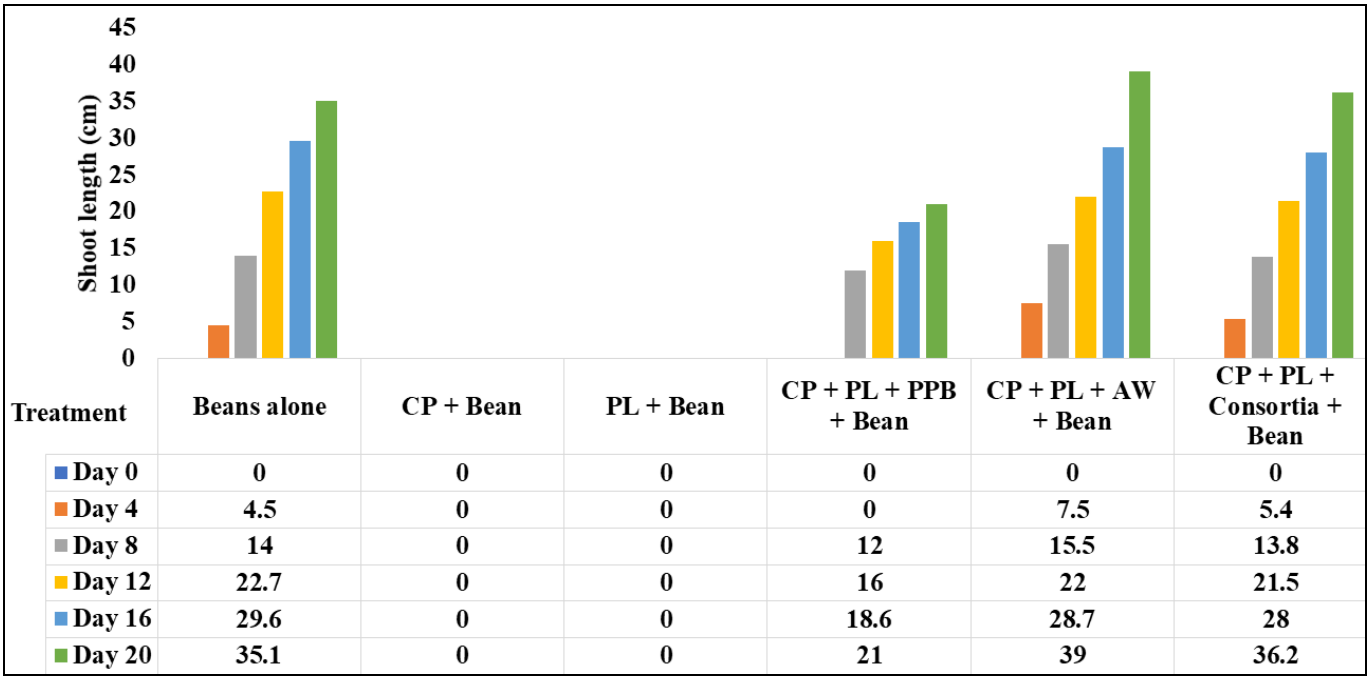
Samples	N (%)	P (µg/kg)	K (g/100g)	Mg (%)	Fe (mg/L)	pH	T (°C)
CP	3.76	13.678	1570.43	44.7	0.037	3.96	28.4
PL	4.29	8.505	41.98	50.3	0.037	3.79	29.1
CP + PL	4.17	0.182	0.028	13.5	0.036	2.36	28.6
Soil	8.34	0.1498	375.17	21.06	0.036	2.99	28.6
Soil + CP + PL	3.63	0.115	0.019	18.33	0.028	1.87	28.3

Keys: CP: Cassava Peels, PL: Plantain Leaves, N: Nitrogen, P: Phosphorus, K: Potassium, Mg: Magnesium, Fe: Iron

Effect of the treatments on the growth parameters of bean plants

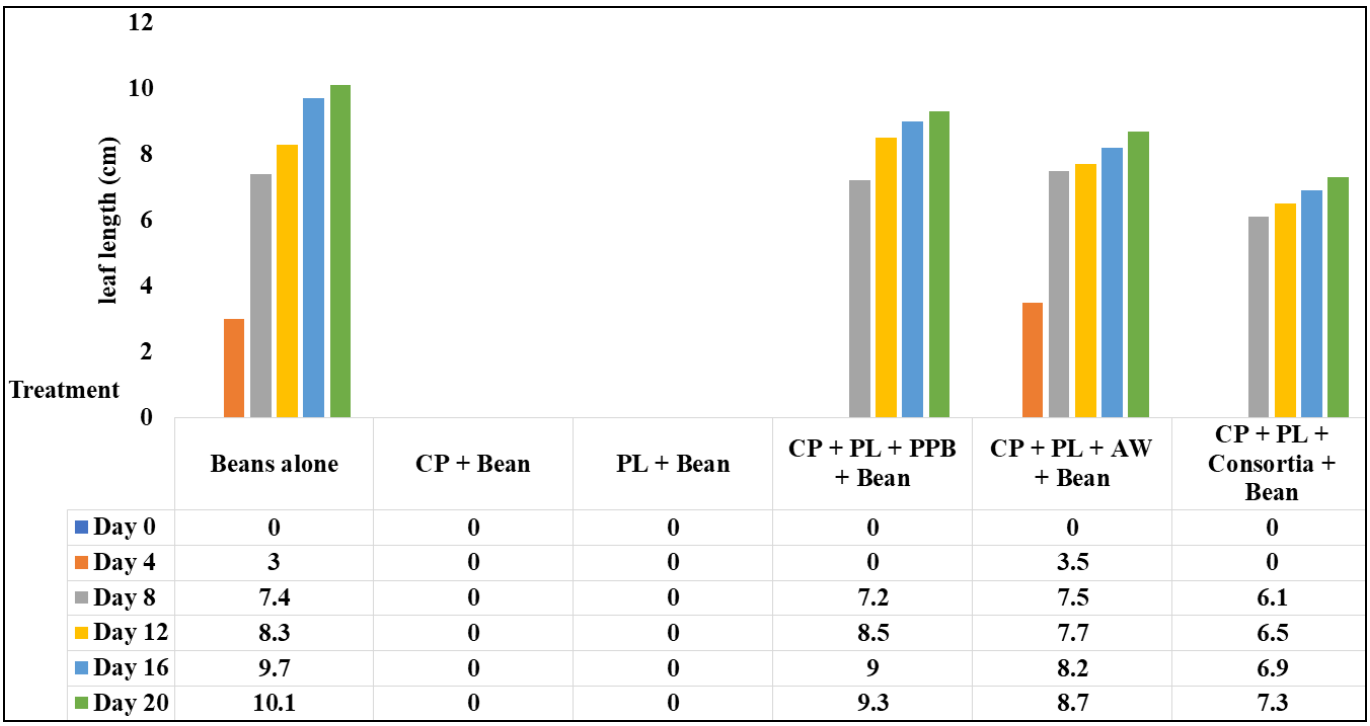
The investigation into the effect of different treatments on the growth of bean plants has been presented in Figures 4 to 9. The treatments CP + beans and PL + beans had no

significant effect on the growth of the bean plant. On day 20, the plant inoculated with *Bacillus pumilus* + mixed carrier material had the highest shoot length of 39 cm, while the plant inoculated with *Stenotrophomonas maltophilia* had the lowest shoot length of 21 cm. The treatment with beans only had the longest leaf length of 10.1 cm, while the treatment with *Bacillus pumilus* + *Stenotrophomonas maltophilia* + mixed carrier material had the shortest leaf length of 7.3 cm. The plant inoculated with *S. maltophilia* had the highest leaf width at 7.2 cm, while the plant treated with *B. pumilus* had the lowest leaf width at 5.2 cm. The treatment with only beans had the highest number of leaves (17) on day 20 compared to the treatment with the consortium, which had 12 leaves. The plant treated with *Bacillus pumilus* + *Stenotrophomonas maltophilia* + mixed carrier material had the largest stem girth of 2.5 cm, while the plant treated with *Stenotrophomonas maltophilia* had the smallest stem girth of 2.1 cm. The treatments with *Bacillus pumilus* increased the shoot length and number of branches, while *Stenotrophomonas maltophilia* increased the leaf width and number of branches of the bean plant. An increased leaf width enhances the plant's ability to trap more light and produce more chlorophyll.



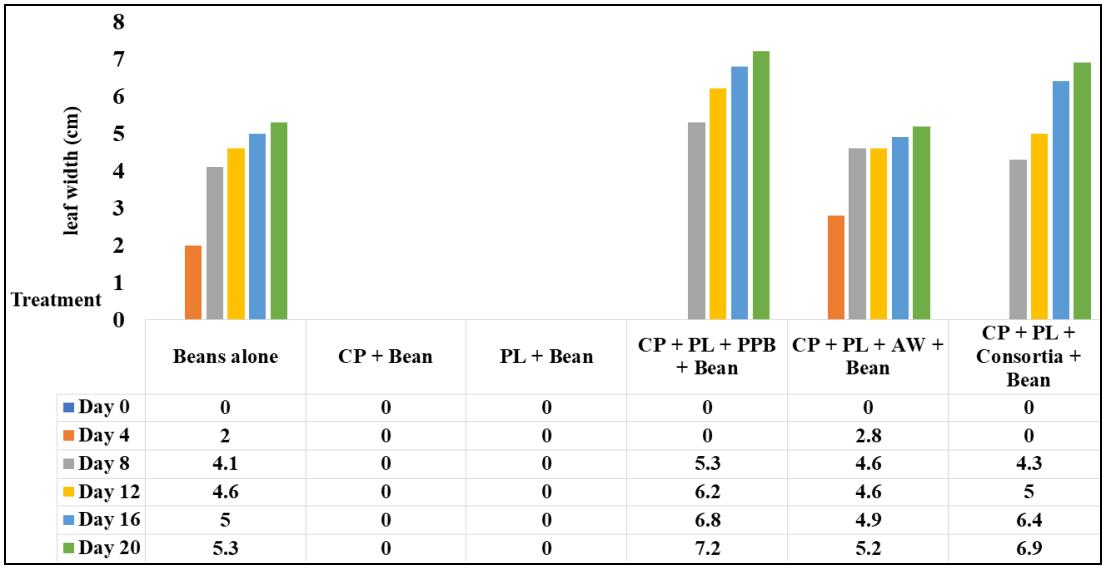
Treatment	Beans alone	CP + Bean	PL + Bean	CP + PL + PPB + Bean	CP + PL + AW + Bean	CP + PL + Consortia + Bean
Day 0	0	0	0	0	0	0
Day 4	4.5	0	0	0	7.5	5.4
Day 8	14	0	0	12	15.5	13.8
Day 12	22.7	0	0	16	22	21.5
Day 16	29.6	0	0	18.6	28.7	28
Day 20	35.1	0	0	21	39	36.2

Figure 4: Effect of the various treatment on the shoot length of bean plant



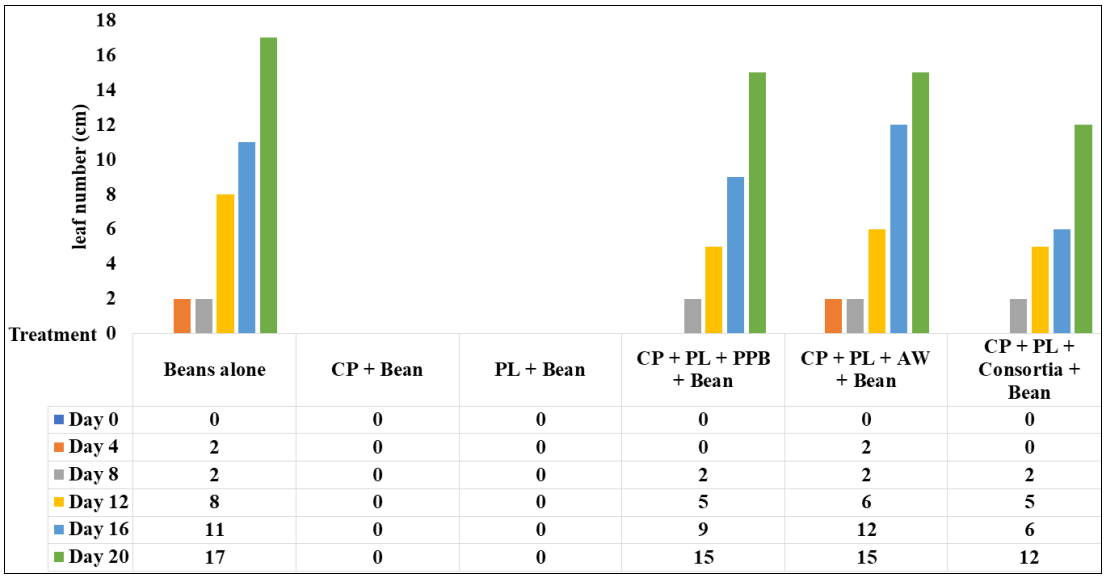
Treatment	Beans alone	CP + Bean	PL + Bean	CP + PL + PPB + Bean	CP + PL + AW + Bean	CP + PL + Consortia + Bean
Day 0	0	0	0	0	0	0
Day 4	3	0	0	0	3.5	0
Day 8	7.4	0	0	7.2	7.5	6.1
Day 12	8.3	0	0	8.5	7.7	6.5
Day 16	9.7	0	0	9	8.2	6.9
Day 20	10.1	0	0	9.3	8.7	7.3

Fig 5: Effect of the various treatment on the Leaf length of Bean plant



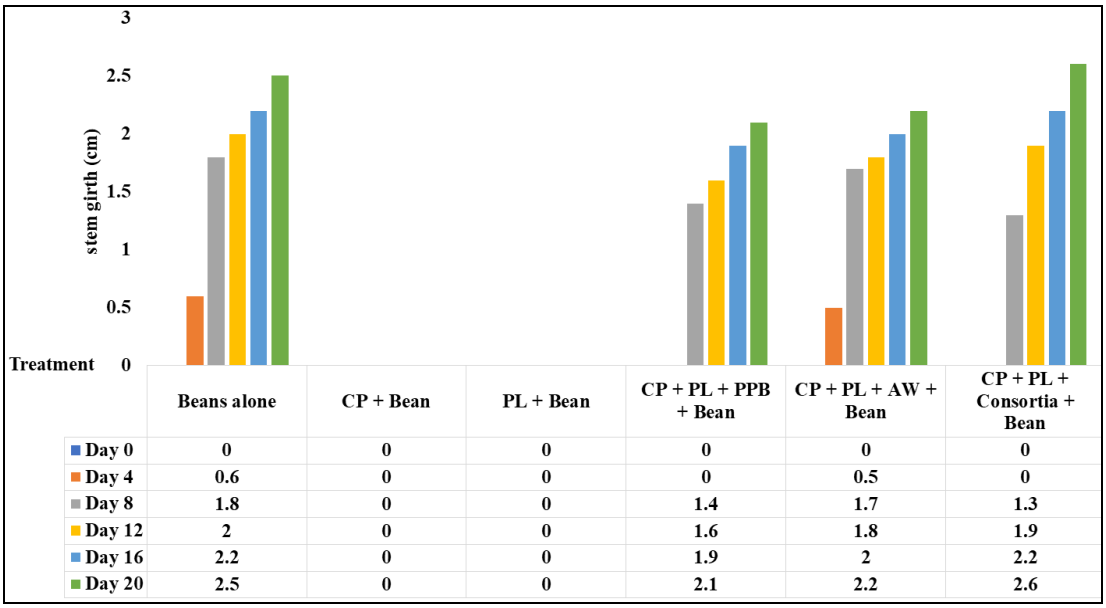
	Beans alone	CP + Bean	PL + Bean	CP + PL + PPB + Bean	CP + PL + AW + Bean	CP + PL + Consortia + Bean
Day 0	0	0	0	0	0	0
Day 4	2	0	0	0	2.8	0
Day 8	4.1	0	0	5.3	4.6	4.3
Day 12	4.6	0	0	6.2	4.6	5
Day 16	5	0	0	6.8	4.9	6.4
Day 20	5.3	0	0	7.2	5.2	6.9

Fig 6: Effect of various treatment on the Leaf Width of Bean plant



	Beans alone	CP + Bean	PL + Bean	CP + PL + PPB + Bean	CP + PL + AW + Bean	CP + PL + Consortia + Bean
Day 0	0	0	0	0	0	0
Day 4	2	0	0	0	2	0
Day 8	2	0	0	2	2	2
Day 12	8	0	0	5	6	5
Day 16	11	0	0	9	12	6
Day 20	17	0	0	15	15	12

Fig 7: Effect of various treatment on the Leaf Numbers of Bean plant

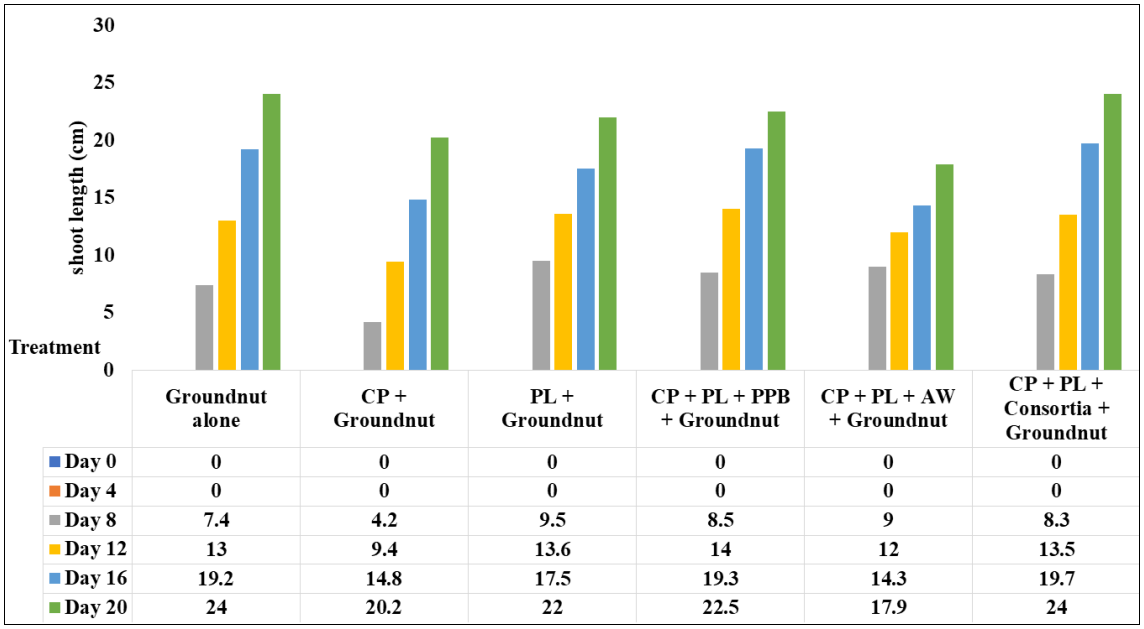


	Beans alone	CP + Bean	PL + Bean	CP + PL + PPB + Bean	CP + PL + AW + Bean	CP + PL + Consortia + Bean
Day 0	0	0	0	0	0	0
Day 4	0.6	0	0	0	0.5	0
Day 8	1.8	0	0	1.4	1.7	1.3
Day 12	2	0	0	1.6	1.8	1.9
Day 16	2.2	0	0	1.9	2	2.2
Day 20	2.5	0	0	2.1	2.2	2.6

Fig 8: Effect of various treatment on the Stem Girth of Bean plant

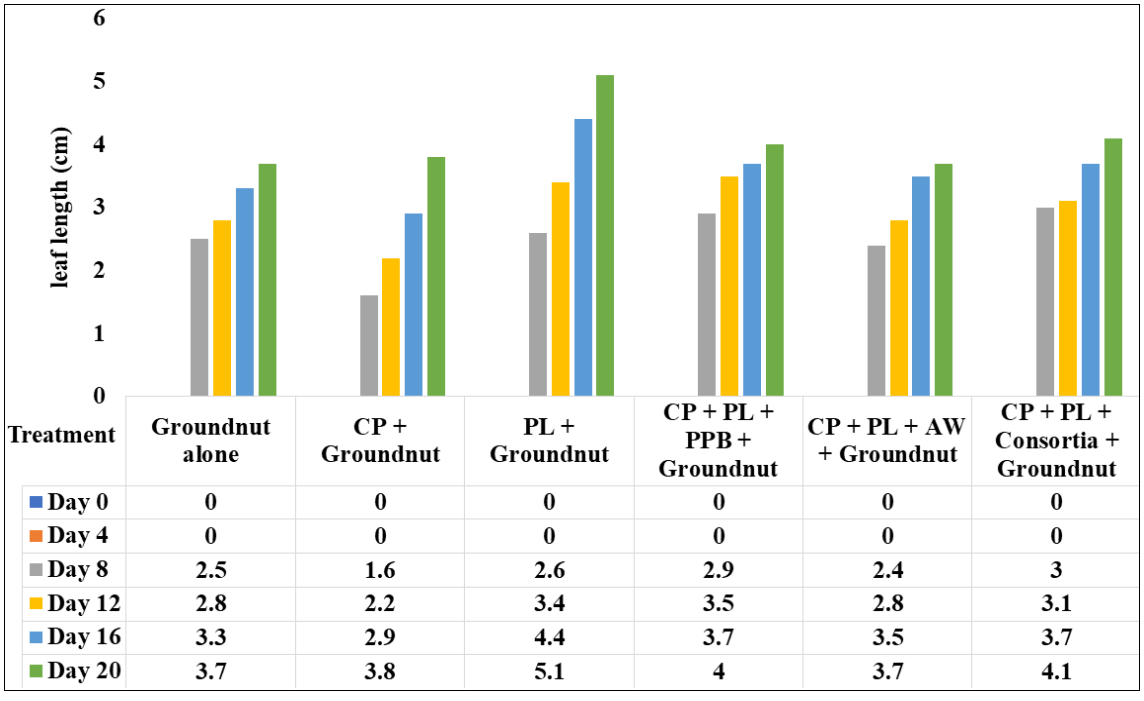
Legends: CP + Bean: Cassava peel + Bean seeds**;** PL + Bean: Plantain leaves + Bean seeds; CP + PL + PPB + Bean: Cassava peels + Plantain leaves + *Stenotrophomonas maltophilia* + Bean seeds; CP + PL + Aw + Bean: Cassava peels + Plantain leaves + *Bacillus pumilus* + Bean seeds; CP + PL + Consortia + Bean: Cassava peels + Plantain leaves + *Bacillus pumilus* + *Stenotrophomonas maltophilia* + Bean seeds

Effect of the treatments on the growth parameters of groundnut plant: The investigation of the effects of different treatments on the growth parameters of groundnut is presented in Figures 9 to 14. At day 20, the treatment with *B. pumilus* + *S. maltophilia* + mixed carrier material, and the groundnut-alone treatment both had the same shoot length of 22 cm, while the lowest shoot length of 17.9 cm was observed in the *B. pumilus* treated plant. The plant treated with plantain leaves had the highest leaf length of 5.1 cm, while both groundnut-alone and *B. pumilus* treated plants had the lowest leaf length of 3.7 cm. The widest leaf width of 3.5 cm was noted in the *B. pumilus* treatment, while the least was observed in the plantain leaf-alone treatment (2.9 cm). The groundnut treated with *S. maltophilia* had the greatest number of leaves (60), while the ones treated with cassava peels alone and *B. pumilus* respectively had the lowest number of leaves (32). In terms of stem girth, plantain leaves-alone treatment (3 cm) had a greater stem girth as compared to the cassava peel treatment (2.4 cm). The treatment with *S. maltophilia* had more branches than that of *B. pumilus*, which had the least number of branches. Overall, the consortia plus mixed carrier material increased the shoot length of the groundnut plant, *S. maltophilia* increased the number of leaves and branches, and *B. pumilus* increased the leaf width.



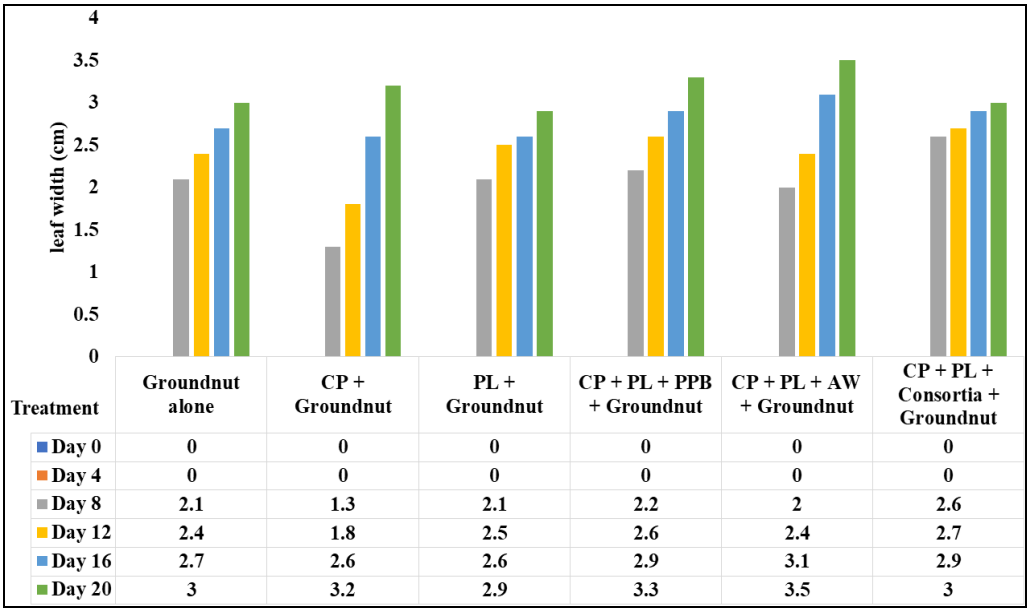
Treatment	Groundnut alone	CP + Groundnut	PL + Groundnut	CP + PL + PPB + Groundnut	CP + PL + AW + Groundnut	CP + PL + Consortia + Groundnut
Day 0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 8	7.4	4.2	9.5	8.5	9	8.3
Day 12	13	9.4	13.6	14	12	13.5
Day 16	19.2	14.8	17.5	19.3	14.3	19.7
Day 20	24	20.2	22	22.5	17.9	24

Fig 9: Effect of various treatment on the shoot length of groundnut plant



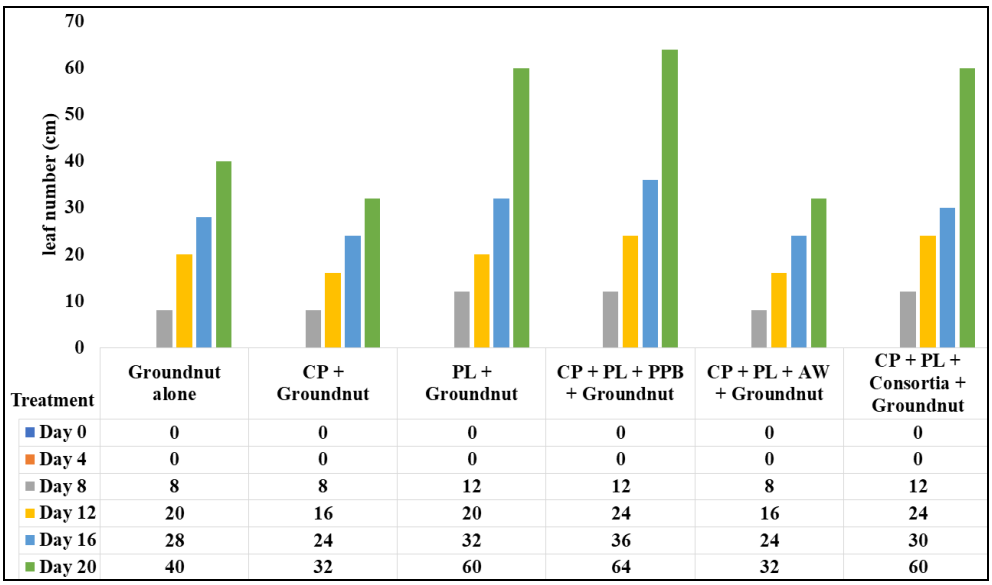
Treatment	Groundnut alone	CP + Groundnut	PL + Groundnut	CP + PL + PPB + Groundnut	CP + PL + AW + Groundnut	CP + PL + Consortia + Groundnut
Day 0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 8	2.5	1.6	2.6	2.9	2.4	3
Day 12	2.8	2.2	3.4	3.5	2.8	3.1
Day 16	3.3	2.9	4.4	3.7	3.5	3.7
Day 20	3.7	3.8	5.1	4	3.7	4.1

Fig 10: Effect of various treatment on the Leaf length of groundnut plant



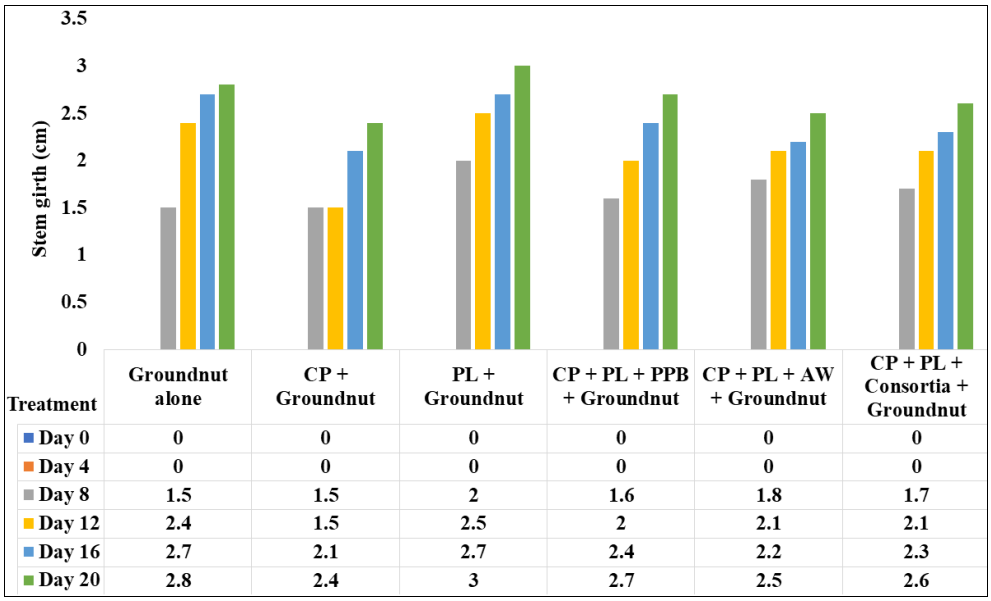
Treatment	Groundnut alone	CP + Groundnut	PL + Groundnut	CP + PL + PPB + Groundnut	CP + PL + AW + Groundnut	CP + PL + Consortia + Groundnut
Day 0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 8	2.1	1.3	2.1	2.2	2	2.6
Day 12	2.4	1.8	2.5	2.6	2.4	2.7
Day 16	2.7	2.6	2.6	2.9	3.1	2.9
Day 20	3	3.2	2.9	3.3	3.5	3

Fig 11: Effect of various treatment on the Leaf Width of groundnut plant



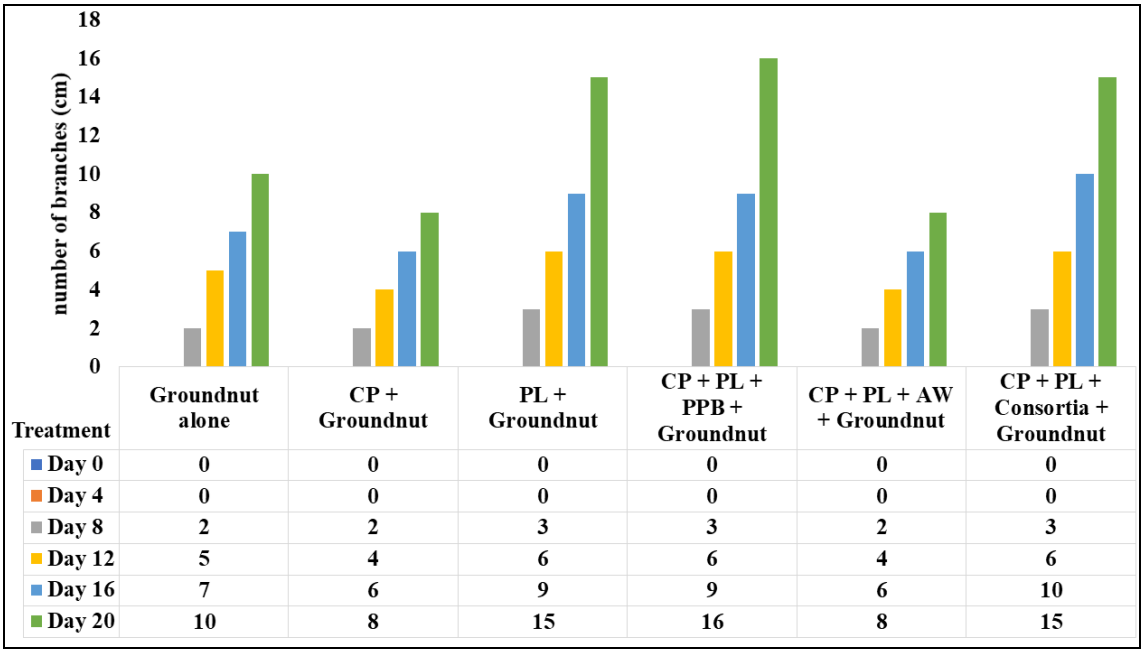
Treatment	Groundnut alone	CP + Groundnut	PL + Groundnut	CP + PL + PPB + Groundnut	CP + PL + AW + Groundnut	CP + PL + Consortia + Groundnut
Day 0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 8	8	8	12	12	8	12
Day 12	20	16	20	24	16	24
Day 16	28	24	32	36	24	30
Day 20	40	32	60	64	32	60

Fig 12: Effect of various treatment on the Leaf Numbers of groundnut plant



Treatment	Groundnut alone	CP + Groundnut	PL + Groundnut	CP + PL + PPB + Groundnut	CP + PL + AW + Groundnut	CP + PL + Consortia + Groundnut
Day 0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 8	1.5	1.5	2	1.6	1.8	1.7
Day 12	2.4	1.5	2.5	2	2.1	2.1
Day 16	2.7	2.1	2.7	2.4	2.2	2.3
Day 20	2.8	2.4	3	2.7	2.5	2.6

Fig 13: Effect of various treatment on the Stem girth of groundnut plant



Treatment	Groundnut alone	CP + Groundnut	PL + Groundnut	CP + PL + PPB + Groundnut	CP + PL + AW + Groundnut	CP + PL + Consortia + Groundnut
Day 0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 8	2	2	3	3	2	3
Day 12	5	4	6	6	4	6
Day 16	7	6	9	9	6	10
Day 20	10	8	15	16	8	15

Fig 14: Effect of various treatment on the Number of branches of groundnut plant

Legends: CP + Groundnut: Cassava peel + Groundnut seeds; PL + Groundnut: Plantain leaves + Groundnut seeds; CP + PL + PPB + Groundnut: Cassava peels + Plantain leaves + *Stenotrophomonas maltophilia* + Groundnut seeds; CP + PL + Aw + Groundnut: Cassava peels + Plantain leaves + *Bacillus pumilus* + Groundnut seeds; CP + PL + Consortia + Groundnut: Cassava peels + Plantain leaves + *Bacillus pumilus* + *Stenotrophomonas maltophilia* + Groundnut seeds

Bacillus pumilus and *Stenotrophomonas maltophilia* are two types of bacteria that have been shown to be excellent for promoting plant growth. Researchers have found that *Stenotrophomonas* is capable of solubilizing phosphate, producing phytohormones, and facilitating the uptake of iron in plants. In addition, *Stenotrophomonas* has been shown to increase shoot and root length in plants (Kumar *et al*., 2023) [18]. *Bacillus pumilus*, on the other hand, has been found to produce gibberellins (Chakraborty *et al*., 2023) [6] which are plant hormones responsible for promoting seed germination, flowering, and stem elongation. Gibberellins also enhance the calcium, phosphorus, and potassium content in plants (Niharika *et al*., 2021; Wang *et al*., 2019) [22, 31]. Moreover, PGPBs (Plant Growth Promoting Bacteria) like *Bacillus pumilus* and *Stenotrophomonas maltophilia* can help plants withstand stressful environments such as acidic soil, salinity, and drought. These bacteria work by reducing the levels of ethylene, which is a plant hormone that triggers stress responses. By reducing the levels of ethylene, PGPBs help alleviate stress in plants, allowing them to grow and thrive even in harsh environments (Weil & Brady, 2016; Poria *et al*., 2022) [32, 27].

Table 5 indicates that days 4, 8, 12, and 16 are significant based on the different alphabet. Table 6 shows that the treatments CP + groundnut, CP + PL + *S. maltophilia* + beans, CP + PL + *B. pumilus* + groundnut, and CP + PL + *B. pumilus* + *S. maltophilia* + beans are significant. The two-way ANOVA test was conducted to evaluate the effect of treatment and days, and it revealed that there was a significant difference in the treatments (F [5, 432] = 3.788, $p < 0.05$ at 5% level of significance) and the time period the treatments were subjected to (F [11, 432] = 24.260, $p < 0.05$ at 5% level of significance). However, there was no significant difference in the interaction effect between the treatment and days (F [55, 432] = 0.593, P > 0.05 at 5% level of significance). The one-way ANOVA was also conducted to examine the effect of the mixed carrier material on the physicochemical parameters of the soil, and it showed that there was a significant difference when the mixed carrier material was added to the soil (F [6, 27] = 2.775, $p < 0.05$ at 5% level of significance).

Table 5: Descriptive and Post Hoc Test for the Days

Days	Row mean ±Standard deviation
Day 0	0.00±0.00a
Day 4	0.47±1.36a,b
Day 8	3.85±4.024b,c
Day 12	6.16±6.74c,d
Day 16	8.31±9.40d,e
Day 20	11.51±14.72e

Key: Different alphabet = significance

Table 6: Descriptive and Post Hoc Test for the Treatments

Treatment	Row mean ±Standard deviation
Groundnut Only	5.99±9.25a
Beans Only	6.24±8.28b
CP + Groundnut	4.88±7.53a,b
CP + BEANS (No growth)	0.00±0.00a
PL + Groundnut	7.09±11.82b
PL + Beans (No growth)	0.00±0.00a
CP + PL + PPB + Groundnut	7.45±12.81b
CP + PL + PPB + Beans	4.86±5.80a,b
CP + PL + AW + Groundnut	5.14±7.42a,b
CP + PL + AW + Beans	6.35±8.49b
CP + PL + Consortia + Groundnut	7.20±12.0b
CP + PL + Consortia + Beans	5.40±8.08a,b

Key: Different alphabets = significant

Conclusion

Biofertilizers can be produced by combining plant growth-promoting bacteria with recycled agro-wastes like cassava peels and plantain leaves. This biofertilizer is an excellent

option for improving soil fertility and boosting nutrient uptake by beans and groundnut plants, even in low-nutrient soil conditions. The growth parameters of beans and groundnut plants showed that using both *B. pumilus* and *S. maltophilia* either as a single strain bioinoculum or as a consortium results in improved plant growth. With the use of biofertilizers, farmers can improve their crop yields while also promoting sustainable agricultural practices.

Conflict of Interest
Not available

Financial Support
Not available

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